Medicinal Plant Monographs

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Pictures on the front cover: *Silybum marianum* (L.) Gaertn., Asteraceae (milk thistle), *Borago officinalis* L., Boraginaceae (borage), *Malva sylvestris* L., Malvaceae (common mallow), *Matricaria recutita* L., Asteraceae (German chamomile). Photos by C.L. Quave.

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COMMON PLANT NAMES

Wormwood

Yarrow

Yerba maté

PREFACE

Cassandra L. Quave, Ph.D.

Throughout history, mankind has utilized plants as a primary source of food, medicine, and shelter. This tradition continues today. In 2001, the World Health Organization estimated that approximately 80% of the world's population was directly dependent on plant-based medicines (Cox and Heinrich 2001). In contrast with most other organisms, plants have the ability to undergo a process of secondary metabolism, in which unique compounds are produced for the purposes of defense, pollinator attraction, and coloration, among others. Examples of these important secondary metabolites usually fall within the following natural product classes: alkaloids, non-protein amino acids, cyanogenic glycosides, coumarins, glucosinolates, monoterpenes, sesquiterpene lactones, diterpenoids, saponins, liminoids, carotenoids, phenols, flavonoids, and quinones (Dewick 2001).

These same compounds, which serve important roles in plant survival, are also useful in mammalian systems. For example, upon entry into the human body and an environment of physiological pH, the very structure of alkaloid compounds takes on a quaternary form, through which the compound can interact with human receptors – inhibiting or stimulating activity of the targeted site. This may have a medicinal, or even a toxic, effect on the body. For instance, the alkaloid strychnine acts as a glycine mimic, binding to glycine receptor sites in the spinal cord, and exhibiting a toxic effect. On the other hand, the alkaloid atropine can exert a medicinal anticholinergic effect by competing with acetylcholine for the muscarinic receptor site in the parasympathetic nervous system (Dewick 2001).

Some notable plant-derived drugs still in use today include vincristine (*Catharanthus roseus* (L.) G. Don; Apocyanaceae), physostigmine (*Physostigma venenosum* Balf. f.; Fabaceae), pilocarpine (*Pilocarpus jaborandi* Holmes; Rutaceae), reserpine (*Rauvolfia serpentina* (L.) Benth. ex Kurz; Apocyanaceae), and digitalis (*Digitalis purpurea* L.; Scophulariaceae) (Cox and Balick 1994; Cox and Heinrich 2001; Dewick 2001; Solomon 2004). Today, the search for new drugs coming from vascular plants continues with particular focus on plants exhibiting antimicrobial, anti-cancer, and antiviral effects. The need for new, structurally unique antimicrobials is of utmost importance in this era of widespread microbial resistance to existing therapies (Levy 2002; Smith and Coast 2002; Walsh 2003).

Many plants that are used for their antimicrobial activity contain polyphenolic compounds, which commonly act as antiviral agents, or essential oils, which often act as antiseptics (Heinrich et al. 2004). One of the best known antimicrobial plants is garlic, *Allium sativum* L., Amaryllidaceae. Garlic contains a wealth of sulphur compounds, glycosides, and monoterpenoids. These sulphur compounds have shown remarkable *in vitro* antibacterial, antiviral, and antifungal activity (Blumenthal, Goldberg, and Brinckmann 2000; Srinivasan et al. 2001; Zenner et al. 2003).

Another well-known plant with broad-spectrum antimicrobial activity is the tea tree, *Melaleuca alternifolia* Cheel., Myrtaceae. The monoterpenoids present in tea tree oil have demonstrated activity against a broad spectrum of microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, *Leishmania major*, and *Trypanosoma brucei* (Heinrich et al. 2004).

Secondary metabolites, or natural products, manufactured by plants as a means of defense against herbivory or microbial infection, have demonstrated potent activity against human pathogens. Yet, less than 0.5 % of the more than 265,000 flowering species that exist on earth have been exhaustively studied for their chemical composition and medicinal potential (Cox and Balick 1994). It is certain that a wealth of novel structures wait to be studied, and likely that some of these will, in fact, exhibit potential for medicinal applications.

EXPLANATION OF MONOGRAPH SECTIONS

In this e-book, I have compiled the medicinal plant monographs written by my students as their final paper for "Botanical Medicine and Health" course taught at Emory University and "Medical Botany" web-based course taught at the University of Arkansas at Little Rock. At the beginning of the semester, students were asked to select a medicinal plant and research its uses in CAM and traditional medicine, the mechanisms of action of its constituents, and assess the data reported in laboratory and clinical trials. Using this information, they each wrote a plant monograph which focuses on the medicinal applications of their selected species. Each monograph has been formatted following these specifications:

Introduction

This section gives an introduction to the plant by listing its scientific name and family, common names, and a brief overview of how it is used, and what its main constituents are.

Description

This section contains a general description of the morphological characteristics of the plant. This includes its habit (tree, shrub, herb, etc.), its habitat (marshy areas, arid plains, etc.), and its flower and fruit characteristics (color, smell, shape).

Traditional Uses

This section pertains to the ethnomedical and other ethnobotanical applications of the species, including descriptions of its use as a food, decorative, construction, clothing, and etc.

Chemistry and Pharmacology

A description of the principle chemical constituents of the plant are given here.

Biological Activity

In this section, *in vitro* and *in vivo* laboratory studies conducted on extracts or fractions or purified compounds from the plant is described. Mechanisms of action are also discussed and issues with drug resistance are addressed.

Clinical Studies

When applicable, results from clinical studies are discussed in this section.

Contraindications

Drug contraindications (interactions with other CAM or allopathic medicines), side effects, toxicity and toxicology of the plant products are discussed here.

Current Use in Allopathic and CAM Therapies

In this section, the applications of the plant products in both CAM and allopathic medicine are discussed. Products on the market, including dietary supplements, OTC, and prescription medications are discussed.

Discussion

In this section, a summary of the utility of this medicinal species is given and the importance of its role in human health is discussed.

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Achillea millefolium L., Asteraceae

Tianjiao Chu

Introduction

Achillea millefolium L., commonly known as yarrow, belongs to the family Asteraceae. Due to its global distribution it goes by many names and is often also referred to as the milfoil, thousand-leaf, or old man's pepper because of its aromatic smell. The genus name *Achillea* originates from a Greek legend in which the hero Achilles applied Yarrow leaf to stop the bleeding wounds of his soldiers. As suggested by the mythology, *A. millefolium* heals wounds and treats blood-related illnesses giving it the names of bloodwort, soldier's woundwort, herb militaris, sanguinary, nosebleed, or knight's milfoil. Numbering in eight species, *A. millefolium* was the most widely used medicinal plant used by indigenous tribes in North America (Yaniv, et al 2005), although it was possibly confused with and used interchangeably with the nearly identical *Achillea lanulosa* (Chandler, et al 1982). Yarrow was also one species of pollen found at Shanidar IV, a Neanderthal burial cave in Iraq dating 60,000 years ago, suggesting its early ritual or medicinal use (Yaniv, et al 2005). Hence for centuries, cultures around the globe have recognized the many therapeutic effects of yarrow that is still sold as panacea in herbal supplements and ointments today.

Figure 1. *Achillea millefolium* L. illustrated diagram, (Source: http://botanical.com/botanical/mgmh/y/yarrow02-l.jpg)

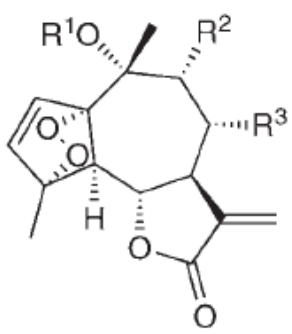
Botanical Description

Achillea millefolium propagates from a small rhizome and is often recognized by weedy scientists in their compendia. It is a perennial with a circumpolar distribution across Europe, Asia, South America and North Africa. Since its introduction in

colonial times, it has naturalized throughout temperate zones in North America with ranges across all fifty U.S. states, Canada, as well as Greenland, demonstrating its ability to survive in a wide variety of soil types (Yaniv, et al 2005). Typically, Yarrow can be found in un-shaded, open areas ranging from cliffs and lowland meadows, to roadsides and waste grounds (Chandler, et al 1982).

Generally growing 12 to 24 inches (30 to 60 cm) in height, *A. millefolium* appears gray-green in color from the numerous small, silky white hairs that covers it. Its species name *millefolium,* or thousand-leaves, refers to its highly segmented foliage that vary in size from 3-20 cm in length and 1-6 cm in width (**Figure 1**). Leaves are arranged in a feathering or fern-like way with clusters at the base of flowering stems and smaller leaves alternating upwards. When crushed or dried, the leaves give off a characteristically spicy aroma and have a bitter and astringent taste when consumed (Chandler, et al 1982).

A. millefolium's stem is angular instead of round, and has a rough texture. Its flowers bloom in clusters at the ends of stalks between May to October, peaking in quality during July, and are known to attract bees and many other insects. Tiny and daisy-like, the flowers may be white, pink, or red in color (Libster 2002). Orange or yellow-blooming yarrow are often confused as *A. millefolium* but in actuality belong to other species of *Achillea*. Evolution in the *A. millefolium* group has been recorded due to this mechanism of polyploidy, with certain species having a particular number of chromosomes. As such *A. millefolium* is widespread as a hexaploid, with greatest diversity has been found in Europe where diploid, tetraploid, and octoploid species have also been reported (Chandler, et al 1982).



	R^1	R^2	R^3
48	Ac	OH	H
49	H	OAc	H
50	Ang	OH	H
51	Tig	OH	H
52	H	H	H
53	H	H	OH

Figure 2. Terpenoids from *A. millefolium* (Si, et al 2006)

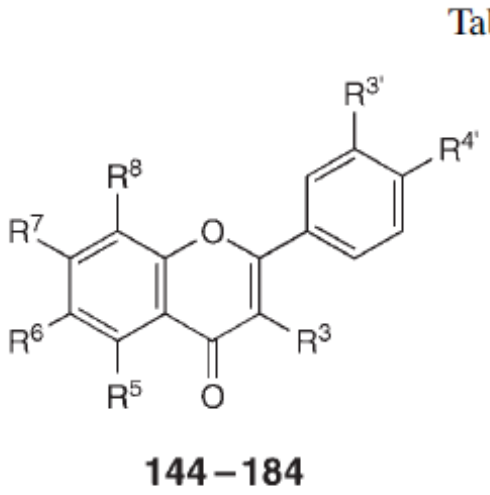


Figure 3. Flavonoids from the *A. millefolium* (Si, et al 2006).

Traditional Uses

Ritual uses

Perhaps due its strong healing abilities, yarrow has been considered across many cultures to possess special spiritual or magical properties. In East Anglia yarrow, or 'nosebleed,' was employed as a plant which can divine future love by placing the leaf inside the nose and reciting the rhyme, '*Yarroway, yarroway, bear a white blow, if my love love me, my nose will bleed now*' (Grigson 1996). In ancient China, yarrow was often used as a sacred ritual plant that symbolized the balance between yin and yang. Its stems are still used in to make divination sticks of the I Ching. In North America, Native American tribes, among their prodigious healing uses for the plant, also burned the yarrow flowers to use as ceremonial smoke.

Commercial uses

In Iran yarrow is found to be a good source for natural dyes for wool, due to the presence of flavonoids luteolin V and apigenin VI. As an aromatic and editable herb, in Sweden yarrow, called "field hop," is sometimes used in the production of especially potent beer. Elsewhere in Europe, yarrow is an approved additive to foods and beverages as a flavor agent, as long as the thujone content, a ketone and monoterpene, does not exceed 0.5 mg/kg. In the US however, yarrow is only approved for flavor uses in alcoholic beverages, with products that remain thujone-free (Libster 2002).

Preparation

Yarrow may be boiled in water as tea, with or without an infusion of other herbs, to be drunk as part of a sweating treatment. Other oral therapeutic applications include fluid extracts or tinctures. For topical applications, Yarrow is soaked in oil, which can be rubbed as a massage. For external treatments, Yarrow may be burned and inhaled, or soaked into baths (Libster 2002).

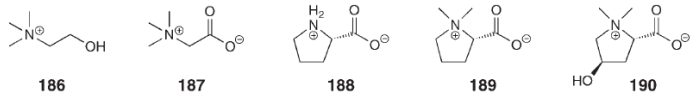
Therapeutic applications

Due to its world-wide distribution, cultures around the world in China, East India, Russia, across Europe in Serbia (local name "Hajduˇcka trava"), Germany, Great Britain and Hungary (where it's known as 'kitten tail'), and even as far as Argentina where it grows in the Falkland Islands, have recognized the variety of therapeutic uses for *Achillea millefolium.* In many countries, yarrow is used as a panacea drug for all sorts of aliments (Jaric et al 2007). The most common and widespread uses for yarrow in folk medicine, cross-culturally, is to take it orally as a tea to treat internal blood-related illnesses, digestion, cramping, and other gastrointestinal conditions. Yarrow is also commonly applied topically to slow-healing skin conditions to stop bleeding and inflammation.

For example in China, fresh yarrow is applied topically to treat wounds and sores, while the dried herb is used for internal bleeding, hemorrhoids, and menstrual disorders. Recorded as far back as the fourteenth century, Russian herbalists also used yarrow as the plant of choice to stop all sorts of internal bleeding (Libster 2002).

Among the common uses found in other cultures, early Anglo-Saxons also applied yarrow for snake and dog bites, and ate the roots on an empty stomach for toothache. Know as 'gearwe' the medicinal uses of *A. millefolium* has been documented in three of the oldest Anglo-Saxon texts: the *Old*

Table 4. *Amino Acid Derivatives from the Genus* Achillea



186 187 188 189 190

No.	Compound Name	Source	Ref.
186	Choline	*A. collina, A. milleforium*	[60]
187	Betaine	*A. collina, A. milleforium*	[60]
188	Proline	*A. collina, A. milleforium*	[60]
189	Stachydrine	*A. collina, A. milleforium*	[60]
190	Betonicine	*A. collina, A. milleforium*	[60]

Table 1. Amino Acid from *A. milleforium* (Si, et al 2006)

English Herbarium (950 AD), Bald's *Leechbook* (900-1000 AD), and the *Lacnuga* (1000 AD). (Watkins et al 2011).

Some culturally specific and different uses for *A. millefolium* have also been recorded. In ancient times, Aztecs were known to use yarrow to cure coughs. Modern Hispanics in the southwestern U.S. know yarrow as *plumajillo* and currently use it treat colds, anemia, diarrhea, flatulence, and as a diuretic. In both Italy and East India, yarrow is recognized for its antipyretic abilities. In India especially it is used as a medicated vapor bath for fevers, or powered and drank as a tonic to dispel internal gas and cramping. In Russia, yarrow was also given to nursing mothers to increase milk supply, reduce high blood pressure, stimulate bile flow from liver and gallbladder, and used to prevent the formation of kidney stones and gallstones. And in Germany, yarrow is sometimes used instead of iodine for children's minor skin lacerations, as well as to treat mood-swings or depression. German herbalists also recommended Yarrow to be used with fennel as a hypnotic, to help with post-surgical recovery (Libster 2002).

Native American tribes perhaps made the most extensive use of *A. millefolium*. Yarrow was most commonly used among different tribes to treat bruises, sprains and inflammation,

Chemical composition of the essential oil from *Achillea millefolium* subsp. *millefolium*

Compound[a]	R_t[b]	Composition (%)
Thujene	9.412	0.1
α-Pinene	9.669	2.4
Camphene	10.323	2.4
Benzaldehyde	10.948	0.2
Sabinene	11.523	2.8
β-Pinene	11.592	4.2
2,3-Dehydro-1,8-cineole	12.355	0.6
α-Terpinene	13.545	0.5
Eucalyptol	14.496	24.6
γ-Terpinene	15.666	1.0
Terpinolene	17.123	0.2
Linalool	17.807	0.6
Camphor	20.166	16.7
Borneol	21.117	4.0
Terpinen-4-ol	21.692	2.8
α-Terpineol	22.515	10.2
Myrtenol	22.663	0.3
Fragranol	23.486	0.5
7-Methyl-3-methylene-6-octen-1-ol	23.694	0.2
3,7-Dimethyl-3,6-octadien-1-ol	24.408	0.6
Chrysanthenyl acetate	25.716	0.8
Bornyl acetate	26.866	0.1
Myrtenyl acetate	28.888	0.1
Eugenol	30.196	0.2
α-Copaene	31.019	0.1
Caryophyllene	32.991	0.4
β-Farnesene	34.002	0.3
γ-Curcumene	35.588	0.2
Zingiberene	36.262	0.3
Nerolidol	39.464	0.1
Caryophyllene oxide	40.604	0.7
γ-Eudesmol	43.647	1.8
β-Eudesmol	44.945	1.6
Bisabolol oxide II	45.381	3.8
Bisabolone oxide	47.354	3.3
α-Bisabolol	47.443	2.1
Others not identified (33)		9.2
Total		100

[a] Compounds listed in order of elution from a HP-5 MS column.
[b] Retention time (as minutes).

Table 2. Chemical composition of the essential oil of *A. millefolium* (Candan et al 2003).

wound healing, and to provide itch relief from rashes and other causes (Chandler et all 1982). Among the approximately 450 species used medically by the Haudenosaunee (Iroquois) peoples in Upstate New York, *A. millefolium* was considered as one of the four most powerful herbs. Often considered a fix-all, the Haudenosaunee used yarrow as an analgesic, antidiarrheal, antiemetic, antihelmintic, antipyretic, antirheumatic, for blood illnesses, gastrointestinal problems, and for venereal disease (Fray et al 2010).

Chemistry and Pharmacology

In 1961, the first flavonoids, cynarosides and cosmosiin, which displayed spasmolytic activity, were isolated from *A. millefolium.* In 2006, the two terpenoids were isolated from *Achillea millefolium*: Angeloyloxy) artabsin 1,4-endoperoxide and 8a-(Tigloyloxy)artabsin 1,4-endoperoxide (**Figure 2**). The following eight flavonoids have been isolated from *A. millefolium*: Luteolin**,** Cynaroside**,** Luteolin 7-malonylglucoside, Apigenin, Cosmosiin**,** Apigenin 7-malonylglucoside**,** Rutin and 5-Hydroxy-3,6,7,4'-tetramethoxyflavone (**Figure 3**) (Si et al 2006). In addition the flavonoids casticin and isorhamnetin were also found in a separate study (Chandler et al 1982). The amino acids derived from *A. millefolium* include: choline, betaine, proline, stachydrine, and betonicine (**Table 1**) (Si et al 2006).

The essential oil of *A. millefolium* is characterized by a high number of monoterpenes: Eucalyptol (24.6%), camphor (16.7%), α-terpineol(10.2%), β-pinene (4.2%), and borneol (4.0%). These five monoterpenes are the principal components, comprising the 59.7% of the essential oil (**Table 2**). Changes in the composition of *A. millefolium* essential oil are due to the maturation of the plant, with increasing ratios of monoterpenes to sesquiterpenes (Candan et al 2003). Inactive compounds from Yarrow may be seen in (**Table 3**) (Chandler et al 1982)**.** This list is by no means exhaustive and further studies of the species may yield additional minor chemical constituents not mentioned.

Biological Activity

Anti-inflammatory

Recent pharmacological studies of plant extracts from all parts of *Achillea millefolium* have shown anti-inflammatory properties (Watkins et al 2001). A variety of *A. millefolium*'s compounds contribute to its anti-inflammatory and antipruritic activities: azulene, chamazulene, the sesquiterpene lactones, some of the other constituents of the volatile oil like menthol and camphor, tannins, and possibly the sterols and triterpenes. Some of these active agents may account for the plant's effectiveness in treating some of the other skin afflictions. Salicylic acid derivatives, eugenol, menthol and a number of other components present in the plant's volatile oil can be responsible for the local analgesia results. The antipyretic activity may also be a consequence of the presence of salicylic acid derivatives, chamazulene or similar agents. Thujone, a known abortifacient, may be the active ingredient responsible for the use of these plants to treat a wide range of problems associated with the female reproductive system (Chandler et al 1982). Known *A. millefolium* constituents include matricin and other proazulenic sesquiterpene lactones known to yield chamazulene carboxylic acid, a propionic acid analogue, which can inhibit cyclooxygenase-2 and has been used for the semi-synthesis of analogues as potential anti-inflammatory drugs. (Watkins et al 2001).

TABLE 6. MISCELLANEOUS COMPOUNDS FROM YARROW.

Compounds		References[a]
Acids		
	Amino	1, 2
	Fatty	3–9
	Aconitic (Achelleic)	3, 10
	Ascorbic	11
	Caffeic	12, 13
	Folic	14
	Salicylic	2–4
	Succinic	2, 3
Carbohydrates		
	Alditols	2, 5
	Cyclitols (Viburnitol)	15
	Saccharides	2, 9
Carotenoids		16
Coumarins		9
Fatty alcohols		2, 5, 8, 17
Hydrocarbons		2, 3, 5, 9, 18–20
Proteins		21–23
Protein-Carbohydrate complex		24
Resins		9
Tannins		2, 9, 23, 25
Miscellaneous		
	Aldehydes	3, 4
	Bitter principle (Ivain)	26
	Cyanogenic compound	27
	Minerals	11, 28, 29
	Polyacetylenes	19, 30, 31
	Polyamines	32
	Thiophene amides	30

Table 3. Miscellaneous compounds from Yarrow (Chandler et al 1982).

Antimicrobial

In vitro studies have shown that the essential oil of *A. millefolium* has great antioxidant and antimicrobial properties. Extracts from the aerial parts of *A. millefolium* have been found to exhibit a broad spectrum of antimicrobial activities against five bacteria: *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Salmonella enteritidis*. The extracts also demonstrated activity against two fungi: *Aspergillus niger* and *Candida albicans* (Si et al 2006). In a separate study, *A. millefolium* also demonstrated antibacterial activity against *S. typhimurium* and *S. aureus*, with predicted MICs of 10 s of µg/mL or 10 s of mg/mL. Two of the studies found that ether-hexane-methanol extracts of *A. millefolium* caused inhibition zones against *E. coli* in disc diffusion assays, whereas studies with aqueous extracts of flower, leaves, roots, and shoots and in a separate study of essential oil and methanolic extracts did not. These differing results may be due to the different extraction methods used or the regional variation in the chemical constituents of the plants (Frey 2010).

Gastrointestinal effects

In gastrointestinal research, strips of stomach antrum were taken from six patients who underwent total or subtotal gastrectomy for gastric carcinoma and treated with a standardized water extract obtained from the flowering tops of *A. millefolium*. Results showed that the extract is able to contract mice and humans gastric atrum and could provide a pharmacological basis for the traditional use of *A. millefolium* in the treatment of dyspeptic ailments (Borrello et al 2012). In a separate study, tests conducted on mice with gastric ulcers induced by 80% acetic acid showed significant regeneration and increased cell proliferation after oral treatment with 1 and 10mg/kg hydroalcoholic (HE) *A. millefolium* extract. The study confirmed the antioxidant and gastroprotective activities of the HE extract, although the precise mechanism in the effect remains to be understood (Potrich 2010).

Cardiovascular

Studies on rats under anaethesia with extracts of *A. millefolium caused a* dose-dependent (1–100 mg/kg) fall in

arterial blood pressure. In spontaneously beating guinea-pig atrial tissues, the extracts exhibited negative inotropic and chronotropic effects. The results demonstrate that *A. millefolium* exhibits BP-lowering, cardio-suppressant, vasodilator and bronchodilator activities possibly mediated through Ca^{2+} antagonism in addition to an endothelium-dependent relaxant component and justifies its application in cardiovascular and congestive airway disorders. (Khan et al 2010).

Studies on *A. millefolium* extract *in vitro* effects on the growth of primary rat vascular smooth muscle cells (VSMCs) as well as the potential involvement of estrogen receptors (ERs) has demonstrated the ability to modulate the NF-kB pathway. Results show that the tested *A. millefolium* extract, containing flavonoid glycosides and CQA derivatives, is able to enhance VSMC growth partly by acting through ERs and impair NF-kB signaling in endothelial cells. These studies justify the traditional use of the plant, by explaining its activity through the modulation of the inflammation pathway and demonstrating that *A. millefolium* may induce novel potential actions in the cardiovascular system (Dall' Acqua 2011).

Phytoestrogens

Results show that apigenin can stimulate ERs-dependent biological pathways, although with a smaller potency as compared with the endogenous hormone and can activate both subtypes of human estrogen receptors, *α* and *β*. Luteolin appears to have a very slight effect on the *β* receptor and does not seem to activate *α* at all. The study concludes that the role of apigenin in the emmenagogic effects of *A. millefolium*, as traditionally reported, cannot be defined (Innocenti 2007).

Skin rejuvenation

In the superoxide radical inhibition cases, *A. millefolium* was found to be more effective than ascorbic acid (Candan et al 2003). *In vivo*, a 2-month treatment with *A. millefolium* extract at 2% significantly improved the appearance of wrinkles and pores compared with placebo. Results were also directionally better than those of glycolic acid that was chosen as a reference resurfacing molecule. *A.millefolium* extract thus represents a relevant cosmetic ingredient useful for rejuvenating the appearance and feeling of epidermal surfaces. Its properties as a natural water plant extract also represent a gentle alternative to alpha-hydroxy acids that require acidic formulation to preserve their functionality and efficacy (Pain et al 2011).

Tumor growth

In cancer studies, antiproliferative assays demonstrated that centaureidin is the most effective constituent of the aerial parts of *A. millefolium*. High cell growth inhibitory activities were reported on HeLa and MCF-7 cells. Casticin and paulitin were also highly effective against all three tumour cell lines (IC50 1.286–4.76 μM), while apigenin, luteolin and isopaulitin proved to be moderately active (IC50 6.95–32.88 μM). Artemetin, psilostachyin C, desacetylmatricarin and sintenin did not display antiproliferative effects against these cell lines (Csupor-Loffler, et al 2009). In another case, there is a recently discovered antitumoural effect of casticin by the induction of G2/M cell cycle arrest and apoptosis (Watkins et al 2011)

Clinical Studies

A recent clinical study on kidney disease was conducted with *Achillea millefolium* and plasma nitric oxide. Increased plasma

nitric oxide concentration has been understood as one of the possible mechanisms of bleeding tendency in patients who suffer chronic kidney disease. In a randomized controlled trial, thirty-one chronic kidney disease patients were tested. 16 patients received 1.5 g of powdered *A millefolium* flower 3 days a week for 2 months, and 15 received placebo for the same period. Plasma samples were collected before and after the study period to estimate the effect of *A. millefolium* on plasma nitric oxide metabolites. The results showed that the plasma nitrite and nitrate concentrations decreased after 2 months administration of *A. millefolium*. However with the placebo group, plasma nitric oxide metabolites were only slightly lowered after *A. millefolium* administration in chronic kidney disease patients. The study suggested that higher doses or a longer duration of plant administration might make these changes more significant. Ultimately the study failed to yield statistically significant results on the effect of *A. millefolium* and potential therapeutic effects on chronic kidney disease (Vahid et al 2012).

A different study in Iran was carried out to evaluate the effect of topically applied *A. millefolium* in conjunction with intralesional glucantime on acute cutaneous leishmanial lesions, a common parasitic skin infection. Sixty patients with confirmed acute cutaneous leishmaniasis were randomly divided into two groups to receive topical gel of Achilles millefolium 5% with 5% polyphenol, or a placebo, twice a day for four weeks along with weekly injection of intralesional Glucantime. The results from the study showed no significant difference between the two groups according to age, gender, and duration of the infection. Neither was there significant difference in the complete or relative cure rates between the group which received the placebo or the A. *millefolium* gel (p=0.35). However, some patients in the group which received the gel reported severe reactions and itching at the application site. The study concluded that there was ultimately no significant difference in cure rates of lesions between yarrow and placebo topical gels as an adjuvant drugs with intralesional glucantime in treatment of acute cutaneous leishmanial lesions (Jaffary et al 2012).

In the last decades more intensive pharmacological studies have been conducted on *A. millefolium*; however most of these studies have been conducted *in vivo*. Published results on human clinical investigations of *A. millefolium* still remain rare or with results that are mostly inconclusive.

Contraindications

Achillea millefolium is considered a nonpoisonous plant and is not listed in literature on human poisoning. Long term trials with *A. millefolium* extracts on rats have only demonstrated the gastroprotective activity of the extract and was accompanied by no signs of toxicity in any relevant areas linked to liver, kidney or hematological systems for periods up to 90 days, in either female or male rats (Cavalcanti et all 2007).

However, due to documentation of the death of a calf following its consumption of yarrow it is listed among plants to avoid for livestock feed. Dermatitis has also been reported in various studies in conjunction with yarrow application, suggesting it's a sensitizer at nonirritant concentrations, despite no known compounds of α-methylene-α-lactone, that are believed to induce dermatitis, have been found in yarrow (Chandler et al 1982).

Anti-spermatogenic effects have also been observed in lab studies with mice that were treated with an EtOH extract *of A. millefolium*. Treatment with the extract showed an increased

Figure 4. Example of a Yarrow supplement sold online (Source: http://www.pennherb.com/milfoil-herb-capsules-60-vcaps-467x)

number of metaphases in the mice germ epithelium, which might be due to cytotoxic substances or substances stimulating cell proliferation (Si et al 2006).

Pregnant women should avoid taking yarrow, because its ability to relax the smooth muscle of the uterus may cause a miscarriage. Yarrow should also be avoided as a means to treat fevers over 102°F, as it may create a dangerous, temporary increase in the fever. Those with allergies to plants in the daisy family should also avoid using Yarrow as it may cause a similar allergic reaction (Libster 2002).

Current Use in Allopathic and CAM Therapies

A great variety of yarrow supplements and oils can be found sold through health-food marketing websites for both humans and for pets. It is marketed primarily for its wound-healing abilities, with tag lines that highlight the herb's mythological names ("military herb"). Nature's Wonderland Yarrow Herb supplement highlights uses which include: "maintaining liver and gallbladder function, immune health, digestion, blood cleansing, and easing menstrual cramps." Recommended doses includes up to 2100 mg per day (**Figure 4**). Although extracts and tinctures of *Achillea millefolium* are more concentrated than the dried powdered plant, their bitterness and pungent odor may repel the consumer (Vahid et al 2012). Due to the current lack of human clinical trials, over the counter or prescription drugs derived from *A. millefolium* remains to be widely marketed. The majority of current yarrow-drugs are sold in their natural forms as teas or herbal supplement pills.

Discussion

Achillea millefolium's therapeutic applications seem to be as widespread as its geographic range. The occurrence of several genotypes of *A. millefolium*, each with its unique chemical composition, might explain why ethnic groups from different geographical areas used yarrow for considerably different reasons. Phytochemical studies on *A. millefolium* flowers from wild populations have revealed a considerable variation in these phenolic compounds that seems to arise from the influence of different growing habitats (Benetis et al 2008). Furthermore, many of these components that are highly bioactive (Si et al 2006), such as flavonoids and sesquiterpenoids, have demonstrated anti-proliferation effects against leukemia (Fray 2010). This suggests the importance of considering genotypes as well as the age of the yarrow, geographical location, season of harvest, and plant parts when collecting plants for phytochemical investigation (Chandler et al 1982). For modern drug discovery, there remains a wealth of opportunities to research *A. millefolium* for minor constituents against current diseases not mentioned in ancient texts or already practiced in current ethobotanical approaches. Clearly the common yarrow holds great potential as a source for many new drugs.

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Allium sativum L., Amaryllidaceae

Shannan Healy

Introduction

Allium sativum L., commonly known as garlic, is a member of the Amaryllidaceae family. More commonly known for its culinary role, *A. sativum* has many properties that are useful in medicine. Originally cultivated in central Asia, garlic is now cultivated throughout the world, but heavily in the Mediterranean and Asia (Brewster 2008). *A. sativum* is largely composed of sulfur compounds; the enzyme alliinase reacts with alliin upon crushing or chewing of the clove which results in the compound allinin (Block 2010). Allicin is a diallyl sulfinate that is recognized as the constituent responsible for many of garlic's medicinal properties, such as its preventative role in cardiovascular disease, antioxidant activity, and activity as a broad-spectrum antimicrobial. As people are more commonly seeking natural health care products, there has been extensive research done on *A. sativum* for its roles as a broad-spectrum antimicrobial, antioxidant, and for its overall effects on the cardiovascular system (Block 2010). Unfortunately, many trials report conflicting results and are not reliable because they do not specify the form of garlic used, which has tremendous effects on the release of active compounds.

Botanical Description

Other members of the onion genus, *Allium*, include *A. cepa* (onion), *A. schoenoprasum* (chives), *A. ascalonicum* (shallots), and *A. porrum* (leeks). *Allium sativum* is further divided into two subspecies, *A. sativum var. sativum*, also known as softneck garlic, and *A. sativum var. ophioscorodon*, also known as hardneck garlic. Both varieties are composed of an underground bulb made up of cloves, which are prophylls enclosed by dry membranous skins and held together by a basal plate. A bulb with some cloves removed to reveal these skins can be seen in **Figure 1**. Flat leaves that point at the ends reach about two feet in length and arise from underground; they do not originate from the stalk (Brewster 2008).

Figure 1. A bulb of *Allium sativum* with some cloves removed to reveal membranous skin. (Image source: http://www.123rf.com/photo_3369595_a-white-clove-of-garlic-broken-open-and-isolated-on-a-white-back-ground.html)

The variations differ in that hardneck garlic's bulb is

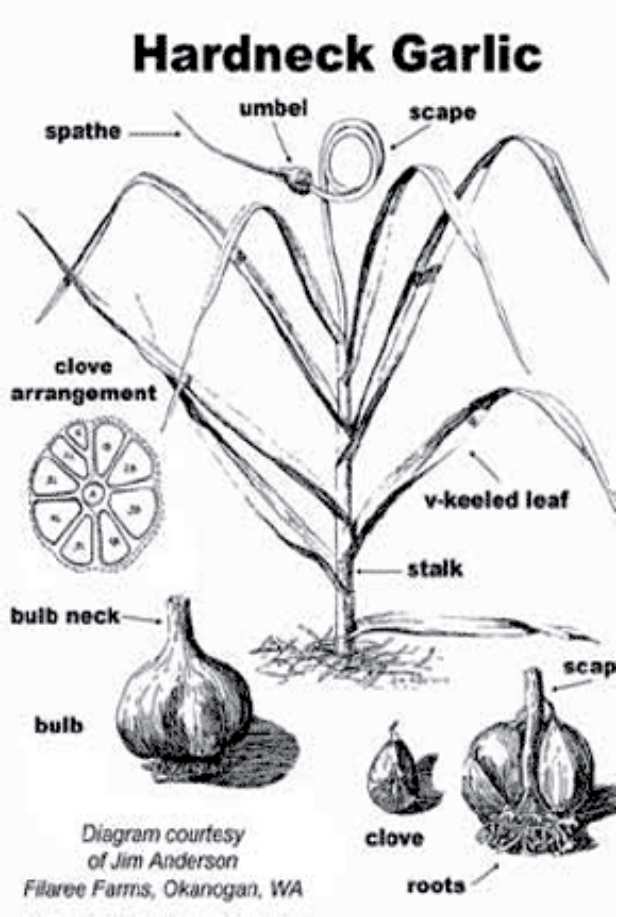

Figure 2. Diagram of *Allium sativum spp. ophioscorodon.* (Image source: http://saknoffel.co.za/garlic_cultivation.html)

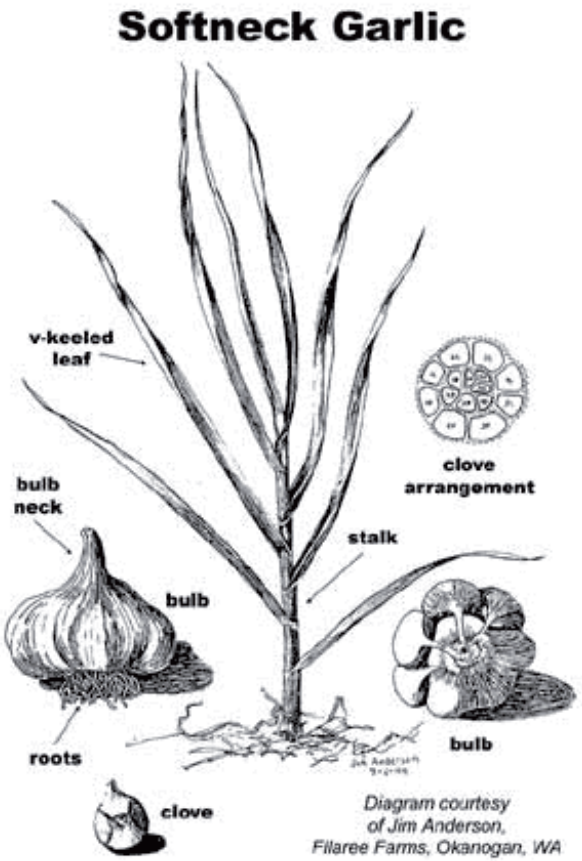

Figure 3. Diagram of *Allium sativum spp. sativum.* (Image source: http://saknoffel.co.za/garlic_cultivation.html)

composed of six to eleven cloves, circled around a centralized woody stalk (Block 2010). This variety of garlic has a scape that curls at the top, but it is generally removed after it curls one to three times. This is because if it continues to grow, less energy can be utilized towards the bulb (Block 2010). Eventually, the scape would give rise to bulbils, containing miniature cloves. The bulbils are occasionally accompanied by white or light purple flowers, although these are sterile. Softneck garlic does not have a flowering top and contains up to twenty-four cloves per bulb (Block 2010). The stem is central and soft, hence the name, and the cloves are layered with larger ones on the outside (Block 2010). **Figures 2 and 3** illustrate the differences between the two subspecies and **Figure 4** shows an example of a flowering bulbil. While *A.*

Figure 4. Diagram of flowering bulbils. (Image source: http://www.wegrowgarlic.com/7422.html)

sativum sativum is the more common variation, many studies involving garlic do not specify which subspecies is used, but chemical and biological action are assumed to be similar.

A. sativum is sterile and hence is grown asexually from cloves, not requiring a pollinator (Block 2010). It grows best in mild climates, through hardneck varieties are better adapted to colder environments. *A. sativum* is a perennial species, as are most members of the genus. Garlic is composed of very strong organosulfur compounds that serve as secondary metabolites as described in the section entitled chemistry and pharmacology. These compounds are responsible for the very pungent smell and taste of raw garlic and act as defenses against predators (Block 2010).

Traditional Uses

Traditional Medicinal Uses

Medicinal uses of garlic are very prevalent in history, not only for the treatment of disease but also for the maintenance of health. Records date back to ~1550 BC: 22 of 875 therapeutic formulas in Ebers Papyrus contain garlic, including heart disease and abnormal growths, implying cancer (Block 2010). In Dioscorides' *De Materia Medica*, *Allium sativum* is mentioned 23 times and is listed as a remedy for driving out intestinal parasites, diarrhea and other gastrointestinal disorders and animal bites (Block 2010). Dioscorides also recommended garlic because it "cleans the arteries," and currently it is being investigated to help treat atherosclerosis, known as hardening of the arteries (Rivlin 2001). Hippocrates advised its use as a purgative agent and for pulmonary complaints (Moyers 1996).

It is suspected that historically, garlic was given mostly to laboring classes, as upper classes avoided the pungent smell. When King Tutankhamen's tomb was excavated (dating to ~1500 BC), garlic cloves were identified, although it was not evident what their use was (Kahn 1996). *A. sativum* was consumed by Egyptian slaves to increase their strength when building the pyramids (Kahn 1996). For similar reasons, it was given to Roman soldiers. In Ayurvedic medicine, garlic was used to warm the body, likely a result of improved blood circulation (Moyers 1996). In Traditional Chinese Medicine, garlic was used as an ailment for respiration and digestion discomfort, diarrhea, and worm infestation (Moyers 1996). In World War II, it was deemed Russian penicillin because it was used to fight infection and gangrene (Petrovska and Cekovska 2010). The Cherokees traditionally used it as a diuretic, expectorant, and for scurvy due to its high vitamin C content (Hamel and Chiltoskey 1975). It was also used to fight

Nutrient	Per Clove (3g)
Water	1.76g
Energy	4 kcal
Protein	0.19 g
Total lipid (fat)	0.02 g
Carbohydrate	0.99 g
Fiber, total dietary	0.1 g
Sugars, total	0.03 g
Calcium	5 mg
Iron	0.05 mg
Magnesium	1 mg
Phosphorus	5 mg
Potassium	12 mg
Sodium	1 mg
Zinc	0.03 mg
Vitamin C, total ascorbic acid	0.9 mg
Thiamin	0.006 mg
Riboflavin	0.003 mg
Niacin	0.021 mg
Vitamin B-6	0.037 mg
Vitamin K (phylloquinone)	0.1 mg

Table 1. Nutrient data in raw *Allium sativum* (USDA 2012)

infection during the Black Plague (Woodward 1996). Other traditional uses include infection, colds, diabetes and heart disease (Tsai et al. 2011). It is evident that ancient uses of *A. sativum* inspired many uses today in complementary and alternative medicine.

Traditional Culinary Uses

Allium sativum is a very nutritious food, but unfortunately this is not reflected since it is generally eaten in such small quantities. Nutrient information per clove of garlic can be seen in **Table 1**. It is believed to have been a regular part of the diet in China since 2000 BC (Kahn 1996). Due to the overpowering taste, garlic does not have an extensive history of culinary use until the 20th century (Block 2010). In fact, monks, widows and children were forbidden from eating it in the Far East due to its aphrodisiac affects and the resulting odor was considered unsuitable for upper classes (Hospodar 2004). Perhaps the rise in consumption is correlated with cooking garlic, as heat mellows the flavor when it destroys the enzyme allinase so it cannot react with allin to form allinin, the sulfur compound responsible for the strong taste and smell (Block 2010). The role of these compounds will be further investigated in the section entitled chemistry and pharmacology.

Traditional Spiritual Uses

Allium sativum is involved in many religious and superstitious beliefs, and today is referenced in pop culture as a means to ward off vampires. In the Bible, the Jews are said to miss garlic when they leave Egypt: “we remember the fish we did eat in Egypt, the cucumbers, the melons, leeks, onions and garlic.” An ancient Islamic myth states that when Satan left the Garden of Eden, garlic sprouted from his left footprint and onion from his right (Block 2010). According to the Bowers Manuscript, in ancient India, garlic is believed to have originated in the blood of a demon (Block 2010). In Greece, midwives used garlic when delivering babies to protect them against evil eye and often advised mothers to sew it into their clothing to protect them (Block 2010). Although some of these tales have negative connotations, these references all indicate garlic as a very powerful substance.

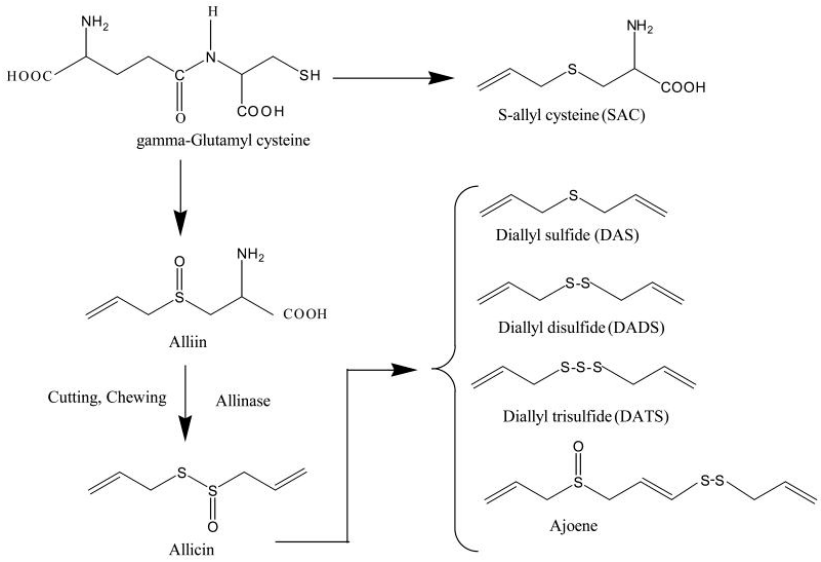

Figure 5. Chemical structures of organosulfur compounds involved in reactions with allicin. (Image source: Powolny and Singh 2008)

Chemistry and Pharmacology

Sulfur compounds are the main chemical constituents responsible for *Allium sativum*'s taste, smell, and likely for its biological effects. When a garlic clove is intact, glutamyl cysteins are the primary sulfur components (Powolyny and Singh 2008). These are hydrolyzed to form alliin. When garlic is crushed by chewing, chopping, etc., the alliin promptly reacts with the enzyme alliinase to form allicin; after 30 seconds the reaction is 97% complete (Block 2010). Allicin is a diallyl thiosulfinate that accounts for 70-80% of the thiosulfinates present in *Allium sativum* (Harunobu et al. 2001). Allicin is also highly unstable and quickly decomposes to yield sulfur compounds when oxidized such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene, and hydrogen sulfide (Banerjee and Maulik 2002; Harunobu et al. 2001; Mukherjee et al. 2009, Powolyny and Singh 2008). The pathways of this reaction can be seen in **Figure 5.** DAS, DADS, DATS and ajoene are all oil soluble (Miroddi et al. 2011).

Chemical Manipulation

When garlic is manipulated to produce alternate forms, the unstable sulfur compounds react and hence alter active chemical constituents. It is important to note that these changes in chemistry can alter the bioavailability of the compounds. Unfortunately, many studies do not specify the actions taken, which could possibly account for inconsistencies in reported data. Traditionally used in its raw form, garlic is now often heated, dehydrated, and aged. Only freshly crushed garlic has hydrogen sulfide, which is suspected to have significant cardioprotective effects as a vasodilator (Mukherjee et al. 2009).

Garlic powder can be made through dehydrating the plant with heat, but when high temperatures are applied to garlic, alliinase is deactivated and hence cannot react with alliin to form allicin (Tsai et al. 2011). This explains why cooked garlic has a mellower flavor than raw garlic. Powder can retain some allicin content if the cloves are frozen before being pulverized; acetone removes the water and alliin and alliinase remain separate yet intact until water is added, at which point allicin is formed (Block 2010). While dehydration during the powdering process does not destroy alliinase like heat does, more than half of the alliin is lost (Banerjee and Maulik 2002; Harunobu et al. 2001). While levels are lower than with raw garlic, dried garlic does contain alliin and alliinase, as explained above. Alliinase is deactivated by the acidic environment of the stomach (Touloupakis and Ghanotakis

2011). Freeman and Kodera (1995) also came to this conclusion by exposing dehydrated garlic powder to simulations of the gastrointestinal fluids; allicin production decreased by 99%, presumably due to the lack of allinase. This suggests that it could be beneficial to further research the affects of dehydrated garlic powder when taken in a capsule with an enteric coating to protect it from stomach acid. These studies also show that manipulating garlic's form leads to changes in the active constituents and could lead to data inconsistencies in studies.

Garlic can also be aged by soaking it in aqueous ethanol and then extracting and concentrating essential compounds. When allicin is dissolved in oils, the major compound in the final product is S-Allylcystein (SAC) while ajoene, the most stable component of garlic, is also present (Rahman 2002). Kodera et al. (2002) suggest that SAC could pass through the gastrointestinal tract without decomposing and successfully be absorbed. Kodera et al. (2002) also suggest that SAC might be stable in blood, whereas allicin is unstable in blood and cannot reach target organs via circulation (Harunobu et al. 2001).

Biological Activity

Cardiovascular Activity

In a review of *in vitro* and clinical studies, Rahman and Lowe (2006) found an inverse correlation between garlic consumption and the progression of cardiovascular disease with the most significant effects being the inhibition of platelet aggregation, reduction of blood cholesterol, reduction of blood pressure and increase of antioxidant status.

Banerjee and Maulik (2002) found similar results regarding the cardioprotective effects of garlic. This was attributed to suppressed low-density lipoprotein oxidation, which reduces lipid content in arterial walls, leading to protection against atherosclerosis, also known as hardening of the arteries. This anti-aggregatory response is attributed to the presence of allicin and thiosulfinates (Cavagnaro et al. 2007).

In a study of rats, Ali et al. (2000) fed all subjects a diet with increased serum cholesterol. Those who also had a garlic powder supplement experienced a reduction in serum cholesterol and in systolic blood pressure. Diallyl sulfide (DAS), Diallyl disulfide (DADS), and diallyl trisulfide (DATS) are the oil-soluble sulfur compounds responsible for reducing cholesterol (Harunobu et al. 2001). DAT's presence in garlic oil has also been attributed to anticoagulation in rats. Chan et al. (2006) found that DATS inactivated thrombin when given in a dose of 5mg per kg of body weight. Ajoene, a component of aged garlic extract, stops blood from clotting, acting as an antithrombotic (Block 2010).

Mukherjee et al. (2009) conducted a study comparing the effects of freshly crushed and processed garlic in rats. While both exhibited cardioprotective effects, the freshly crushed garlic's effects were more significant, likely due to the presence of hydrogen sulfide (H_2S), which is absent in processed garlic. Red blood cells convert diallyl disulfide and diallyl trisulfide to hydrogen sulfide, which then acts as a vasodilator and reduces blood pressure.

Anti-cancer Activity

In looking at multiple studies, Powolny and Singh (2008) conclude that organosulfur compounds such as DAS, DADS, and DATS act by arresting the cell cycle of cancerous cells. Data from Miroddi et al. (2011) and Omar and Al-Wabel (2009) support this, showing that these allyl derivatives act as

antioxidants and arrest the cell cycle. DATS was found to have the most significant role by Powolny and Singh (2008), and is even a potential skin cancer fighting compound (Wang et al. 2010). More specifically, garlic acts as anti-inflammatory agent by altering cytokines and inhibiting NF-kB activity in surrounding tissues (Keiss et al. 2003). In a study using human promyeloleukemic cells, Dirsch et al. (1998) found that ajoene prompted apoptosis in cancerous cells but not healthy ones; this might be due to peroxide production. In an in-vitro study involving rats, Jastrzebsk et al. (2007) found that raw garlic had the strongest antioxidant activity. Tsai et al. (2011) reviewed animal and cell studies and found an inverse correlation between consumption of garlic and presence of cancerous cells, suggesting it has anticancer effects. Eleischur and Arab (2001) conducted a review of in vitro studies, and while multiple reports concluded that garlic prevents cancer of the stomach, colon and rectum, the analyses were not thorough and did not adjust for potential cofounders and hence are not definitive.

Anti-Microbial Activity

While *Allium sativum* only has about 1% of the antibacterial activity of penicillin, it is able to act upon both gram-positive and gram-negative organisms (Ankri and Mirelman 1999). While it does act as a bactericidal agent, *A. sativum* is primarily a bacteriostatic agent that destroys sulfhydryl groups necessary for bacterial growth (Block 2010). Feldberg et al. (1988) found that allicin affects RNA synthesis and inhibits DNA protein synthesis in *Salmonella typhimuriam.* Allicin can also act as an antibiotic by reacting with thiol-containing enzymes such as L-cysteine to form S- allylmercaptocysteine, which inhibits cysteine proteinases (Ankri and Mirelman 1999). As an anti-fungal, garlic inhibits mycotoxin formation, presumably through thiol enzymes, however this mechanism of action is not fully known (Ankri and Mirelman 1999). In an in-vitro study, the effectiveness of ajoene at its minimal inhibitory concentration, 15 µg/ml, was tested against *Candida albicans*. Ajoene inhibited 98.2% of fungal growth (Carrero et al. 2009). Ajoene is also known to have more antiviral properties than allicin (Ankri and Mirelman 1999). In another study involving *C.* albicans, allicin was not found to have any antifungal effects on its own, but enhanced the effects of the common treatment Amphotericin B both in vivo and in vitro (An et al. 2009). The wide-spectrum antimicrobial effects of garlic are due to the presence of allicin and ajoene, which are suspected to inhibit multiple thiol-dependent enzymatic systems (Ankri and Mirelman 1999). Allicin is effective against microbial cells that do not have an intracellular thiol content that can offset its thiol oxidation (Harris et al. 2001).

Other Activity

Eidi et al. (2006) conducted a study on both normal and diabetic rats, comparing the treatment effects of Glibenclamide, a known antidiabetic drug, and garlic. They found that garlic increases serum insulin. Liu et al. (2005) also found that garlic oil and DATS increase insulin sensitivity and section, hence improving glycemic control. Both studies suggest the need for further investigation into *A. sativum*'s antidiabetic role.

Ankri and Mirelman (1999) suggest that the short reactive time of allicin is an evolutionary adaptation. A larger production of allicin could be toxic for the entire plant, so the actions of allicin in a confined area prevent any self-damage. Allicin is not bioavailable and cannot reach target organs by circulating through the blood (Harunobu et al. 2001).

However, it is able to penetrate biological membranes. Shortly after injection into the bloodstream it is no longer in circulation (Block 2010). This has made it difficult to study allicin's antibiotic effects outside of in vitro applications. Many studies focusing on the role of garlic as an antibacterial, antiviral, and antifungal are only clear in vitro, perhaps due to allicin's short presence in the blood stream.

Clinical Studies

Some clinical studies report data that differs from data collected through *in vivo* and *in vitro* studies. These differences might be accounted for by the synergistic effects of compounds in *Allium sativum*, as the concentration of these compounds and their effects in vivo and in vitro may not determine effectiveness without considering their roles in relation to one another (Harunobo et al. 2001). The different forms of garlic must be considered in clinical studies, as this affects the bioavailability of various compounds. It is also important to note that blinded trials are difficult to conduct when using raw garlic due to its strong and recognizable scent and flavor.

Cardiovascular Clinical Studies

In a review of clinical studies, Banerjee and Maulik (2002) note reports of both positive and negative results in relation to the hypolipidemic role of garlic, possibly due to different levels of sulfur compounds in the forms of garlic used. Most studies involving the effects of garlic on hyperlipidemic patients use garlic powder rather than raw garlic. Powdered garlic has less allicin available than raw garlic. As far as cardiovascular studies are concerned, results on hypolipidemic patients are the least conclusive.

Steiner et al. (1996) conducted a double-blind study in hypercholesterolemic men in which aged garlic was used. It was found that aged garlic led to a significant total serum cholesterol reduction, low-density lipoprotein (LDL) cholesterol reduction, and systolic blood pressure reduction. Aged garlic has strong levels of SAC, which could account for these results (Rahman 2002). Ali and Thomson (1995) found that after consuming a single clove of fresh garlic daily for 26 weeks serum cholesterol levels dropped 20% and serum thromboxane levels dropped 80%. However, Gardner et al. (2007) compared treatments for adults with moderate hypercholesterolemia using raw garlic, powdered garlic, and aged garlic (each the equivalent of an average-sized clove six days per week for six months) but no significant effects on LDL levels were found. In a meta—analysis comparing the effects of garlic on serum lipid profiles, Zeng et al. (2012) note that garlic powder and aged garlic were more effective in the reduction of serum total cholesterol levels whereas garlic oil was more effective in the reduction of triglyceride levels. These are only a few examples of conflicting results presented by clinical studies. However, the majority of studies conclude that fresh garlic can slow atherosclerosis development and moderately reduce blood pressure, which is caused by the production of hydrogen sulfide (Mukherjee et a. 2009)

Bordia et al. (1996) note that while garlic components leave the body quickly, active ingredients may build up slowly. This was concluded because a single high dose of garlic did not affectively inhibit platelet aggregation, but long-term administration of low doses was found to be affective. This suggests that including garlic in the diet regularly could have more beneficial effects than consuming it only during times of compromised health.

Cancer Activity Clinical Studies

Most studies involving *A. sativum* and cancer are carried out in animal models because there is not enough information to advise a proper dose for a clinical trial (Thomson and Ali 2003). Ahmend et al. (2001) did find that ajoene enhanced the activation of two chemotherapeutic drugs, Cytarabine and Fludarabie, both used in leukemia treatments. Lamm and Riggs (2000) suggest that regular consumption of aged garlic extract may reduce the risk of stomach cancer. Thomson and Ali (2003) also note that aged garlic extract exhibits radical scavenging activity; these antioxidant properties are thought to inhibit tumor growth. While there are not many studies testing the clinical effects of *A. sativum* on cancer, research suggests that eating garlic as a regular part of the diet serves as a form of preventative medicine. More research is also suggested to investigate the effects of garlic on enhancing modern drugs.

Other Clinical Studies

Historically, *A. sativum* was used to treat the common cold. Lissiman et al. (2009) analyzed previous studies and clinical trials and found that garlic might prevent occurrences but the evidence is not conclusive; this also warrants future studies.

Borhan-Mojabi et al. (2012) tested the effect of garlic on salivary microbial populations and found it significantly decreased colony counts. This suggests the potential for use of garlic in a mouthwash, however most participants complained about taste and resulting breath, implying that the product would not be successful commercially.

Ledezma et al. (2000) compared the effects of ajoene (0.6% and 1.0%) against the topical drug Terbinafine (1%) in the

treatment of *Tinea pedis*, more commonly known as athlete's foot. They found that ajoene (1%) was more effective than Terbinafine (1.0%), which was only slightly more effective than the 0.6% ajoene.

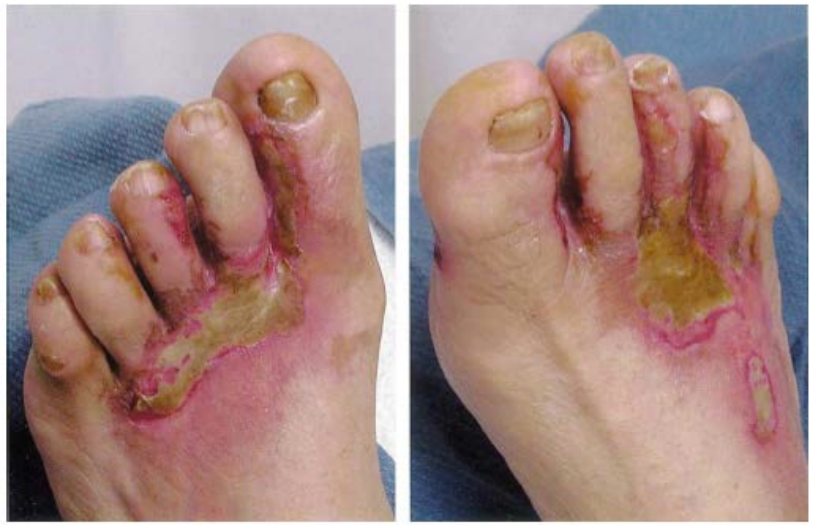

Figure 6. Burn wounds from the topical application of *A. sativum.* (Image source: Friedman, T., Shalom, A., and Westreich, M. (2006). Self-inflicted garlic burns: our experience and literature review, *Int J Dermatol 45,* 1161-1163.)

Contraindications

A common side effect of garlic consumption is halitosis, more commonly known as bad breath. Allyl methyl sulfide (AMS) is a component of garlic that cannot be digested and is hence absorbed into the blood where it travels to the lungs and skin, causing consumers to emit "garlic breath" and "garlic sweat" (Tatteman 2005). An overconsumption of garlic can cause gastrointestinal upset, heartburn, diarrhea and flatulence. Because garlic inhibits platelet aggregation and acts as a vasodilator, garlic consumption should be avoided two weeks

Figure 7. One example of the many garlic supplements available for purchase. (Image source: http://www.aragonproducts.com/theproducts.cfm?master=7434)

before scheduled surgery and before childbirth due to risk of bleeding (Stanger et al. 2012). For the same reasons, excessive garlic should not be used in combination with anticoagulants and antiplatelets such as Aspirin and Warfarin (Chen et al. 2011). Garlic decreases the effectiveness of some non-nucleoside reverse transcriptase inhibitors used for the treatment of HIV/AIDS such as Ritonavir and Saquinavir (Chen et al. 2011; Berginc et al. 2010). It is also thought to increase the break down of estrogen present in many oral contraceptives and might decrease their effectiveness, although much more research is needed (Pinto and Rivil 2001). Dietz et al. (2004) and Friedman et al. (2006) investigated the effects of garlic burns. They both cited patients who applied crushed garlic topically. If there is too much pressure from wound wrapping, diallyl disulfide can cause severe local inflammation that can develop into chemical burns. Some cases included applications to treat headaches, *T. pedis*, and self-mutilate. An example of garlic burns can be seen in **Figure 6.**

Because garlic is more commonly recognized as a food than as a medicine, it is not often acknowledged that it can interact with prescription drugs. *Allium sativum* and its commercial products might not be regarded as a drug, but merely as a form of functional food, which could lead consumers to not share their use with their physicians. Elmer et al. (2007) conducted a study evaluating medication use among the elderly and found that of 393 participants combining medications, 379 of these involved a risk of bleeding due to ginkgo, garlic, or ginseng use and its effect on prescription medications. This further indicates the need to educate people that natural products are not necessarily safe.

Current Use in Allopathic and CAM Therapies

While *Allium sativum* is generally recognized as safe and continues to be used as a food across the globe, many studies on garlic supplements are inconclusive, yet these are also commonly used for medicinal purposes. There are no prescription drugs derived from garlic, but herbal supplements are available over the counter. An example of a product can be seen in **Figure 7**. When the supplements are formed, the manipulation from heating, dehydrating and aging alters the active constituents. Studies use so many different variations of garlic, making the effects of each specific form not easily accessible to potential consumers. Garlic-derived products have a lot of variation among them and can be confusing to consumers. Harunobu et al. (2001) advise careful reading of products because garlic oil does not contain allicin and amounts of garlic essential oils are often minimal because customers do not like the smell. Something else to consider is

whether or not garlic supplements in pill form have an enteric coating. Without an enteric coating, all compounds will dissolve in the stomach and will not be present in the gastrointestinal tract (Kannar et al. 2001). Tattleman (2005) recommends the consumption of one to two cloves of raw garlic per day for adults. While their effects are not entirely confirmed, many use supplements for presumed cardiovascular, antioxidant, and anti-microbial effects.

Discussion

Allium sativum has long been recognized for its traditional ethnobotanical and ethnomedical uses. Many traditional uses have given rise to its modern uses as a functional food. *A. sativum* has been recognized for its role in maintaining normal blood cholesterol levels and blood pressure along with supporting immune function. While there is an extensive amount of research on garlic's potential role in many diseases, there is an absence of conclusive and reliable results, specifically involving the use of garlic supplements. Garlic has a high potential for continuing research because it is suspected to have effects on very relevant medical conditions, specifically cardiovascular disease and cancer. As populations are becoming resistant to western medicines, the development of new remedies or discovery of traditional ones is necessary. Many also express the desire to rely on natural medicine. Nonetheless, it is important to recognize that not all natural products are safe. There is a need for studies that consider the different forms of garlic; this specificity will allow for better understanding of the mechanisms of action of each component and better application of these, leading to an improving continuum of research.

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Aloe vera (L.) Burm. F., Xanthorrhoeaceae

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Introduction

Aloe vera (L.) Burm. F. belongs to the *Aloe* genus, which contains about 500 species of flowering succulent plants, the most well-known and common being *Aloe vera.* Aloe has many different common names even within countries, but it is most commonly *Aloe* in English speaking areas. It is known as *Sabila* in Central and South America and *Ghikumar* in India. Known under many families over the years, *Aloe vera* is currently listed under the Xanthorrhoeaceae family, and also under the subfamily Asphodeloideae. In the past, it has been categorized under the Liliaceae family and was also recognized by the scientific name *Aloe barbadensis* until it was discovered that the name *Aloe vera* given by Burman pre-dated the name *Aloe barbadensis.* The first record of *Aloe vera* is dated to Pedanuis Dioscorides' *De Materia Medica*. Dioscorides was an authority on herbal plants around 60 AD (S. Carter, 2011). Aloe has been used for thousands of years to topically treat burns and other skin abrasions, as well as for its laxative properties when taken orally; it is also used to treat diabetes, asthma, epilepsy, and osteoarthritis, among other conditions (Medicine, 2011). The latex, or inner leaf, of the aloe plant contains strong laxative compounds such as aloin, aloe-emodin, and barbaloin, and the gel from inside the leaf may help to heal burns and abrasions, though more research is needed to verify these claims (Medicine, 2011).

Botanical Description

The origin of *Aloe* vera (**Figure 1**) is unknown, but it is native to North Africa, the Mediterranean region of southern Europe, and

Figure 1. *Aloe vera* leaves and flowers. (Image source: http://www.tropicos.org/Name/18403421)

to the Canary Islands (S. Carter, 2011). Now, however, it is found to be cultivated throughout the West Indies, tropical America, and in the tropics in general (Ross, 1999). The *A. vera* plant is a short-stemmed or entirely stem-less succulent plant with leaves that bunch together to form large groups of up to 20 leaves (Ross, 1999). The leaves are thick and fleshy, grayish-green with a brownish tinge in color, have spots when they are young and can grow to be 20-60 centimeters long and 6-7 centimeters wide at the base(S. Carter, 2011). The leaves are also 1 to 2.5 centimeters thick and have spiny edges (S. Carter, 2011). *A. vera* produces yellow flowers that grow on central stalks that can be up to 100 centimeters tall (Ross, 1999). The yellow outer part of the flower is divided into 6 different lobes with 6 stamens and a three-celled ovary in each flower (Ross, 1999). The fruit is a capsule that flowers then fruits from July through January (Gowda, 2004).

Traditional Uses

According to the National Center for Complementary and Alternative Medicine (NCCAM), *Aloe vera* has been traditionally used as a topical treatment for burns, wounds, and various other skin conditions, and has also been used orally as a laxative. Typically, the leaf extract is treated in hot water and then used for the treatment of burns, sores and other wounds (Ross, 1999). In Argentina, however, the hot water extracts from the leaves is taken orally to both alleviate painful menstruation and to induce abortion (Ross, 1999). It is also used as an abortifacient in Switzerland and in India (Ross, 1999). *A. vera* is used extensively in India: it is often taken orally to treat inflammation and pain (Ross, 1999). The leaves are also applied externally to treat wounds and guinea worm infection sites (Ross, 1999). In the Cook Islands in the South Pacific Ocean, fresh latex from the leaves is also taken orally, on a regular basis, to prevent high blood pressure, cancer, and diabetes (Ross, 1999). It is also used as a diabetes prevention method in Haiti, Mexico, Saudi Arabia, and Tunisia (Ross, 1999). Most peoples that use *A. vera* agree that it should be avoided during pregnancy, as it does appear to have possible abortifacient effects. For a more extensive list of traditional uses around the world, see **Table 1**.

Chemistry and Pharmacology

Aloe vera contains numerous chemical constituents that are shown to have significant activity. Alanine, for example, is found in the leaf in amounts of about 15,769 parts per million (Duke, 1996). Alanine is an amino acid and is known for displaying antioxidant and cancer-preventive activity (Duke, 1996). There are also significant levels of Alocutin-A, which is an analgesic and cancer-agglutinator (Duke, 1996). Aloesin has purgative and sunscreen effects, and small amounts (about 20 parts per million) of aluminum and amylase (an enzyme) have antimicrobial and

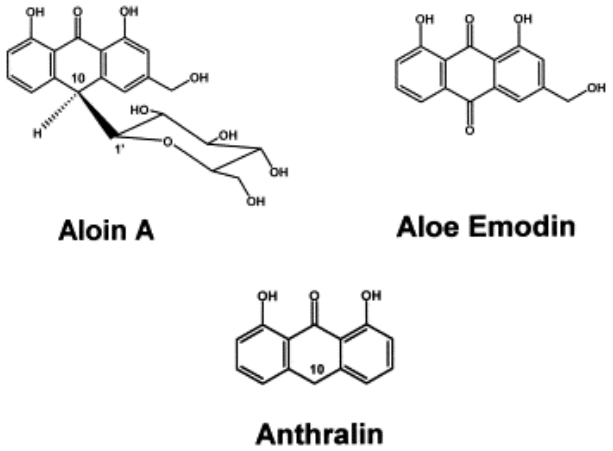

Figure 2: Chemical structures of the anthraquinones Aloin A, Aloe emodin and Anthralin (Wamer et al., 2003).

sedative effects, respectively (Duke, 1996). Another major group of chemical constituents in aloe include the anthraquinones, which are aromatic organic compounds that have antiithic, carcinogenic, and laxative effects (Duke, 1996). Aloe emodin, anthralin and aloin A are the better-known anthroquinone constituents, and have significant antimicrobial activity as well as laxative, cathartic, anti-leukemic, antiseptic, cytotoxic, estrogenic, fungicide, pesticide, genotoxic, mutagenic, and tuberculstatic effects (Duke, 1996). To see the structures of the above anthraquinones, see **Figure 2.**

Biological Activity

In vitro

Antioxidant properties

Although the leaves are traditionally used, there are also possible beneficial compounds in the flowers of *Aloe vera.* A high polyphenolic content is associated with significant antioxidant

Argentina	Hot water extract of leaves is taken orally to induce abortion and to facilitate menstruation.
Bomini	Leaf juice is used externally for skin irritations, cuts, boils, and sunburn.
Bolivia	Fresh leaf juice is used as an analgesic topically for burns and wounds. Orally, the juice is used as a laxative.
Brazil	Fresh leaf juice is taken orally as an anthelmintic and febrifuge. Infusion of dried root is taken orally to treat colic.
Canary Islands	Fresh fruit juice (unripe) is taken orally as an antiasthmatic and purgative. Infusion of fresh leaf juice is taken orally as a laxative, for dental caries, and as a teniafuge.
China	Hot water extract of leaf juice is taken orally as an emmenagogue.
Cook Island	Fresh sap in water is taken orally, regularly, to prevent high blood pressure, cancer and diabetes. Externally it is used to treat burns and cuts.
Cuba	Water extract of leaf pulp is taken as an emmenagogue.
Egypt	Fresh leaf juice administered intravaginally is used as a contraceptive before or after coitus. Data was obtained as a result of questioning 1200 puerperal women about their knowledge of birth control methods. 52.3% practiced a method, and 47.6% of these depend on indigenous methods and/or prolonged lactation.
England	Hot water extract of dried leaves with a mixture of *Zingiber officinale, Mentha pulegium* (essential oil), *Ipomoea purga*, *Glycyrrhiza glabra* and *Canella alba* is taken orally for amenorrhea.
Guatemala	Hot water extract of dried leaves is used externally for wounds, ulcers, bruises and sores, skin eruptions, erysipelas, dermatitis, inflammations, burns, abscesses, and furuncles, and scrofula.
Haiti	Hot water extract of dried leaves is taken orally both as a purgative and against diabetes and worms
India	Decoction of dried leaves is taken orally to induce abortion, for sexual vitality, and the dried leaf juice is taken as an emmenagogue Decoction of root is taken orally for venereal disease and externally it is used to treat wounds. Fresh fruit juice (unripe) is taken orally as a laxative, cathartic, and for fever. Fresh leaves are crushed and applied locally for guinea worms. Hot water extract of dried entire plant is taken orally as an emmenagogue, purgative, anthelmintic, stomachic, for liver enlargement, spleen enlargement, and piles. Hot water extract of fresh plant juice is taken orally for inflammation and amenorrhea. The pulp of the plant is mixed together with salt and fermented sugar cane juice and taken orally to treat pain and inflammation of the body. Hot water extract of leaf juice is taken orally as a cathartic; it should not be used by pregnant women. Leaf pulp is taken orally regularly for 10 days by women to preventconception. Leaf juice is taken orally to treat viral jaundice. The juice is taken twice daily for three days.
Malaysia	Hot water extract of leaf juice is taken orally as a cholagogue and emmenagogue. Hot water extract of leaves is taken orally as an emmenagogue
Mexico	Fresh stem juice is taken orally for diabetes. Infusion of dried leaves is taken orally to treat ulcers.
Nepal	Fresh leaf pulp is taken orally to relieve amenorrhea. 10–15 gm of leaf pulp is given with sugar or honey once a day. Hot water extract of dried entire plant is taken orally as a purgative and to terminate pregnancy.
Panama	Fresh leaves crushed with egg white is taken orally as a laxative and demulcent. Sap is taken orally for stomach ulcers and externally for erysipelas and to treat swellings caused by injuries.
Peru	Hot water extract of fresh leaves is taken orally for asthma, as a purgative, and antivenin. Externally, the extract is used as an antiseptic for washing wounds.
Puerto Rico	Drink made from fresh leaf pulp plus fruit pulp of *Genipa americana* is a popular remedy for colds.
Saudi Arabia	Hot water extract of dried aerial parts is taken orally for liver complaints, piles, as an emetic, antipyretic, against tumors, for enlarged spleen, as a cooling agent, purgative, for diabetes, skin diseases and asthma. Hot water extract of dried leaves is taken orally for functional sterility, amenorrhea, piles, thermal burns, constipation, flatulence, intestinal worms, diabetes, to treat functional sterility, amenorrhea, to treat constipation, and piles. Externally used for burns.
South Korea	Hot water extract of whole dried plant is taken orally as a contraceptive, an abortifacient, and emmenagogue. Use is contraindicated during pregnancy.
Switzerland	Hot water extract of leaves is taken orally as an abortifacient.
Taiwan	Decoction of dried leaves is taken orally to treat hepatitis.
Thailand	Fresh leaf juice is used on burns. Hot water extract of dried resin is taken orally as a cathartic.
Tunisia	Hot water extract of dried leaves is taken orally for diabetes and to treat problems of venous circulation. Externally, the extract is used for eczema.
USA	Fresh leaf juice is taken orally for stomach ulcers and used externally to heal wounds. Fluid extract of leaf juice is taken orally as an emmenagogue. Hot water extract of dried leaves is taken orally as a cathartic. Hot water extract of gum is taken orally as an emmenagogue to promote and stimulate menstruation. Water extract of leaves is used externally for insect bites, myopathies, arthritis, topical ulcers, and other skin conditions. Hot water extract is taken orally to increase menstrual flow; should be avoided during pregnancy.

Table 1. Comprehensive list of the traditional uses of *Aloe vera*. Adapted from (Ross, 1999)

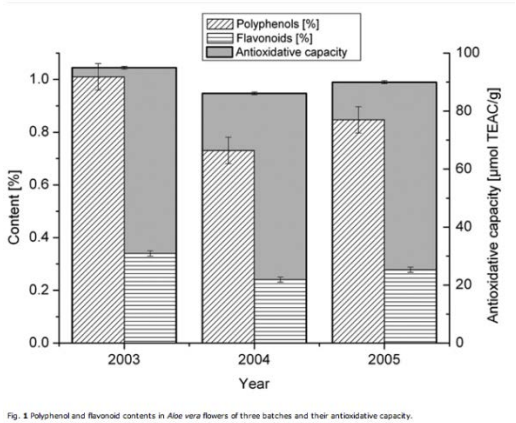

Figure 3. Results of a study examining the correlation between polyphenol and flavonoid content in *A. vera* flowers and the antioxidant activity (Keyhanian & Stahl-Biskup, 2007).

activity (Keyhanian & Stahl-Biskup, 2007). Aloin A and B, anthraquinones associated with the laxative properties of aloe, were not found in the dried flowers, but the antioxidant activity measured correlated positively with the amount of flavonoid and polyphenol content (Keyhanian & Stahl-Biskup, 2007)(**Figure 3**).

Photobiological properties

Aloe emodin and aloin A are two important anthraquinones in aloe. Since aloe is now often included in topically applied cosmetics because of its moisturizing properties, it is important that any adverse effects are identified. A recent study indicated that the constituent aloe emodin may make the skin more sensitive to UV light, so a follow-up study was conducted to examine the events that follow photoexcitation of both aloin A and aloe emodin (Wamer, Vath, & Falvey, 2003). Human skin fibroblasts that were treated with aloe emodin then irradiated with UV light showed dimished survival, while cell survival was not effected by aloe emodin alone (Wamer et al., 2003). The oxidative damage to both RNA and DNA occurred even at low levels of photocytotoxicity (Wamer et al., 2003).

Anticancer activity

Aloe emodin may also exhibit antineuroectodermal tumor activity. Researchers at the Universities of Padova and Genova in Italy devoted their attention to natural compounds in plants that have been used traditionally to treat numerous diseases for hundreds of years and focused on aloe emodin (Pecere et al., 2000). Neuroblastoma cells that were grown in a complete medium and treated with aloe emodin displayed cytotoxicity; the growth of the cells was inhibited and cells showed signs of having undergone apoptotic cell death, as identified by cell shrinkage, membrane blebbing, and nuclear fragmentation (Pecere et al., 2000). It was also determined that, unlike other cancer drugs that are toxic at the necessary therapeutic levels, the aloe emodin did not inhibit the proliferation of hematopoietic progenitors; that is, it displayed selective toxicity where necessary (Pecere et al., 2000). To further address this topic, the same researchers conducted follow-up studies in 2003; aloe emodin induced apoptosis in p53 mutant cells, p53 being a tumor suppressor gene (Pecere et al., 2003).

In vivo

Abortifacient effects

In one study, aloe that was extracted with water was given to pregnant rats during the first nine days of gestation ("Final report on the safety assessment of AloeAndongensis Extract, Aloe Andongensis Leaf Juice,aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Arborescens Leaf Protoplasts, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe

Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice,aloe Barbadensis Leaf Polysaccharides, Aloe Barbadensis Leaf Water, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract," 2007). It was found to be an abortifacient and produced significant skeletal abnormalities in the fetal rats ("Final report on the safety assessment of AloeAndongensis Extract, Aloe Andongensis Leaf Juice,aloe Arborescens Leaf Extract, Aloe Arborescens Leaf Juice, Aloe Arborescens Leaf Protoplasts, Aloe Barbadensis Flower Extract, Aloe Barbadensis Leaf, Aloe Barbadensis Leaf Extract, Aloe Barbadensis Leaf Juice,aloe Barbadensis Leaf Polysaccharides, Aloe Barbadensis Leaf Water, Aloe Ferox Leaf Extract, Aloe Ferox Leaf Juice, and Aloe Ferox Leaf Juice Extract," 2007).

Immunostimulatory effects

A study was conducted on industrial broiler chickens to test the effects of *A. vera* against coccidiosis, a parasitic infection. Results showed that pulp from the leaves stimulated both humoral and cellular immune responses in the chickens (Akhtar et al., 2011). Higher antibody activity can hinder the development of the parasites in the intestines, and it is stipulated that the anthraquinones, saponins, and sterols of the plant may have inhibited the multiplication of the parasites as well (Akhtar et al., 2011). It has potential to be used as a low cost alternative to allopathic medicines to control coccidiosis.

Hepatoprotective effects

In investigating how to remedy the improper handling of petroleum products that leads to possible hepatotoxicity and carcinogenic effects, researchers found that mice treated with *A. vera* gel exhibited reduced γ-glutamyl transferase (γGT) levels, indicating hepatoprotective qualities (Gbadegesin, Odunola, Akinwumi, & Osifeso, 2009).

Hypoglycemic and hypolipidemic effects

In a study by researchers at ShamYook University in Seoul, South Korea, a mouse model was used to test the effects of processed *Aloe vera* gel (PAG) on diet-induced non-insulin-dependent diabetes mellitus (NIDDM). This is especially relevant to humans because of the dramatic increase in rates of NIDDM and the associated complications of the disease (Kim et al., 2009). Processing the *A. vera* gel involves incubating the gel with cellulase (the enzyme that catalyzes the hydrolysis of cellulose) followed by passing the gel through a charcoal column in order to remove the anthraquinones, responsible for the laxative effects of aloe (Kim et al., 2009). To induce the NIDDM state in the mice, they were fed a high fat diet for 21 weeks, followed by the treatment of different doses of PAG for 8 weeks whilst continuing the high fat diet (Kim et al., 2009). After 2 weeks, the hypoglycemic effects were apparent: the fasting blood glucose levels of the diet-induced obesity (DIO) mice were not significantly different from those of regular diet-fed mice (Kim et al., 2009). In addition to a decrease in blood glucose and insulin levels, improved blood glucose homeostasis was observed; that is, when given high doses of glucose, the mice that had been treated with PAG showed high tolerance and a faster return to homeostasis than the control mice (Kim et al., 2009). While there have been other reports that indicate that aloe may actually increase plasma glucose levels, it is speculated that this may be due to the parts of the plant used, as well as the difference in animal models – the mouse model reasonably models NIDDM in humans (Kim et al., 2009).

Another study was conducted to determine the constituents of *A. vera* that normalize hyperglycemia in a mouse that has been induced into the NIDDM state. Fresh *Aloe vera* gel was divided into five separate extracts and administered separately to the mice (Tanaka et al., 2006). Only one of the five extracts, T1, significantly reduced blood glucose levels of the mice, and was further divided into fractions until five phytosterols were isolated via chromatography and detailed C-NMR spectroscopy, which allows for the identification and elucidation of chemical structures (Tanaka et al., 2006). Phytosterols are structurally similar to cholesterol, and in the 1950s it was recognized that plant sterols lower the serum concentrations of cholesterol (Tanaka et al., 2006). Mice that were treated with the five phytosterols showed decreases in both fasting blood glucose levels and random blood glucose samples (Tanaka et al., 2006).

Burn care

While aloe is generally regarded as beneficial in the treatment of minor burns, a study in 2008 found that the application of *Aloe vera* to the burn wound of a large white juvenile pig was no more beneficial than the control group in terms of re-epithelialisation, cosmetic appearance or histology of the scar (Cuttle et al., 2008). The aloe did, however, decrease the subdermal temperature of the wound, indicating possible cooling and soothing purposes, which cannot be overlooked (Cuttle et al., 2008).

Immunomodulatory activity

To test the immunomodulatory activity of processed *Aloe vera* gel (PAG) in a diabetic mouse model, the mice were infected with *Candida albicans*, to which the kidney is particularly susceptible (Im et al., 2010). The mice that were given PAG had significantly reduced renal strain, though the mechanisms could not be indentified (Im et al., 2010).

Clinical Studies

Periodontal Effects

In a study conducted by the Jaipur Dental College in India, 15 subjects were evaluated for periodontitis and treated by applying *Aloe vera* gel to periodontal pockets (Bhat, Kudva, & Dodwad, 2011). After three months, subjects showed a significant reduction in pocket depths as compared to the control group, as well as a reduction in gingival index, which is attributed to the anti-inflammatory and antibacterial effects of *A. vera (Bhat et al., 2011)*. Improved healing was attributed to increased oxygenation because of the *A. vera,* as well as the presence of vitamins A, C, E, B_{12} and folic acid (Bhat et al., 2011). Vitamin C especially dilates blood vessels, thus increasing oxygen concentration. No adverse effects were found, though a longer study time and larger sample size is necessary (Bhat et al., 2011). These promising results, as well as the ease of application and low cost of *Aloe vera* show the potential for its use as a means to improve periodontal conditions.

Anti-hyperglycemic/Anti-hypercholesterolemic effects (Anti-diabetic)

In a randomized double-blind placebo-controlled clinical trial at the Research Institute of Medicinal Plants in Karaj, Iran, aloe leaf gel powder was prepared was evaluated for efficacy in the treatment of patients with type II diabetes. Just as in the mouse model trial described above, the aloe gel was treated to remove aloin and other anthraquinones, known to have laxative effects (Huseini, Kianbakht, Hajiaghaee, & Dabaghian, 2012). The gel was then freeze-dried and made into 300 mg capsules (Huseini et al.,

2012). One variant from the mouse model is that the patients were recommended to decrease their intake of carbohydrates and fatty foods, while the mice continued to eat a high-fat diet. The results of this study showed that the aloe gel improved glycemic control and lowered both total cholesterol and low-density lipoproteins, but did not effect any other blood lipid levels and did not seem to have any adverse effects (Huseini et al., 2012). The only bioactive compound that was indentified in this trial was the mucopolysaccharide acemannan, but the mechanisms involved in these hypoglycemic and hypocholesterolemic effects were not investigated in this study (Huseini et al., 2012).

Burn care

Aloe vera is generally considered safe for topical use (Maenthaisong, Chaiyakunapruk, Niruntraporn, & Kongkaew, 2007). In some studies aloe has been shown to reduce healing time for burns and other wounds, while other studies have been inconclusive or show that aloe has no great effect on healing (Maenthaisong et al., 2007). Some studies indicate that the aloe may simply be used for its cooling and soothing effects, but it is generally agreed upon that 100% aloe is most effective at treating topical wounds, and anything less than 50% has no effect (Korac & Khambholja, 2011). One study found that application of *A. vera* increased the production of collagen, thus increasing the rate of epithelialization (Korac & Khambholja, 2011). In directing the care of outpatient burns, the application of aloe to the affected area was noted to be successful in treating the pain associated with superficial wounds or burns (Lloyd, Rodgers, Michener, & Williams, 2012).

Ulcerative colitis

Since *Aloe vera* is known for its anti-inflammatory activity, a double blind, randomized trial was used to examine the effectiveness of aloe in the treatment of mild to moderate ulcerative colitis. Subjects took 100mL of *A. vera* gel orally twice daily for four weeks and the control group were given a placebo (Ke, Yadav, & Ju, 2012). Subjects who had been taking the *A. Vera* gel were nearly four times as likely to show response, improvement, or complete clinical remission as compared to those taking the placebo (Ke et al., 2012).

Contraindications

Aloe vera is known to contain compounds that have been documented to be cytotoxic. In a study by Patna University in India, researchers found that while there was not an increase in structural abnormalities of the chromosomes in the bone marrow of mice injected with the crude leaf extract of aloe, there was a significant increase in chromosome number anomalies and in the mitotic index, or proliferation status, of the cells (Verma, Gupta, Kumar, & Khan, 2012). There have also been reports of hepatotoxicity induced by aloe. Patients who had been taking aloe preparations and had been diagnosed with acute hepatitis showed the return of liver enzymes to normal levels after the aloe use had been discontinued (Yang et al., 2010). Although still unclear, there are also some findings that indicate that the compound aloe-emodin induces apoptosis in human kidney-2 (HK-2) cells, though these possible toxic effects are unclear and need further research (Zhu et al., 2011). There was clear evidence of carcinogenic activity in rats from oral consumption of the whole leaf of the aloe plant, but once again, more research is needed (Medicine, 2011). Also with the oral use of aloe, especially as a stimulant laxative, there have been reports of abdominal cramps and diarrhea, which can further lead to a

decrease in the ability of other drugs to be absorbed (Medicine, 2011). Since some compounds in *A. vera* are known to lower blood-glucose levels, people with diabetes are warned to be cautious, as they are generally already prescribed an alternate glucose-lowering medication (Medicine, 2011).

Current Use in Allopathic and CAM Therapies

Aloe vera supplements are not FDA approved, and thus are used at the patient's own risk. There are many people who do choose to supplement biomedicine with an herbal supplement such as aloe, but drug-herb interactions may be overlooked and can lead to serious, sometimes fatal consequences. There is anecdotal evidence of older patients taking aloe preparations for months and developing acute hepatitis that returned to normal after discontinuing the use of the oral aloe (Yang et al., 2010). This is discordant with the *in vivo* study mentioned above that indicates hepatoprotective properties in aloe, and it is clear that more clinical trials need to be conducted before people continue to use *A. vera* in complementary and alternative medicine without consulting a healthcare professional. There is further risk of using aloe as complementary medicine if it is used in combination with antiretroviral drugs for the treatment of HIV, as the pharmacokinetic interactions between the antiretrovirals and herbal medicine is unknown; only antiretrovirals have proven efficacy in treating HIV (Lamorde, Byakika-Kibwika, & Merry, 2012). There were several studies conducted on complementary and alternative medicine usage in Nigeria. In hypertensive patients who visited a tertiary care center, 4.5% used *Aloe vera* in addition to conventional medical treatments (Amira & Okubadejo, 2007). At a multi-specialist hospital in Nigeria, aloe was also found to be used by 28% of patients with osteoarthritis (Obalum & Ogo, 2011). In Germany, 7.3% of children with type I diabetes mellitus are given an aloe supplement as part of a complementary medicine regimen, with 77.8% of parents stating that they assumed aloe had fewer potential side effects (Dannemann et al., 2008). It generally accepted that aloe gel is generally regarded as safe for the treatment of minor burns – there are many topical aloe gel treatments available on the market, but anything less than 100% *Aloe vera* gel is thought to have little to no efficacy in treating burns, so one might be best served by having the plant itself for usage.

Discussion

Aloe vera has been used for its medicinal properties all over the world. The gel inside the leaf has been used to treat burns and other minor wounds topically, and it can also be ingested orally; the anthraquinones make it a very effective laxative. Aloe has also been employed traditionally for its possible abortifacient effects, hypoglycemic and hypolipidemic effects, and hepatoprotective activity. Though many studies have been conducted to determine the efficacy of *A. vera* in regards to these potential beneficial effects, there are enough discrepancies that the results are generally inconclusive, and it is clear that further studies are required, especially since determining the exact mechanism of action could help to identify how the chemical constituents in aloe help or inhibit certain qualities. Since aloe can be cultivated in a wide range of areas it is not in danger of being overharvested, so more studies can be conducted in the future without fear of extinction of the species. *Aloe vera* has the potential to have many advantageous medicinal properties, even anticancer activity, and further research will help to solidify findings now that support aloe in the treatment of diabetes mellitus, among other diseases. Since rates of obesity have increased, rates of diabetes have followed, and finding new, effective treatments that have no adverse effects may prove to be where *Aloe vera* is needed.

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Ananas comosus (L.) Merr., Bromeliaceae

Anum Dhukani

Introduction

Ananas comosus, otherwise known as the pineapple, belongs to the family Bromeliaceae. It is also known as the *piña* by the Spanish, *abacaxi* by the Portuguese, *ananas* by the Dutch and French, as well as *nanas* in Southern Asia and the East Indies (Morton, 1987). While the family Bromeliaceae includes approximately 2,000 species, the genus *Ananas* consists of 2 species: *A. macrodontes* and *A. comosus*. Of these 2 species, the more common species is *A. comosus* (Sanewski 2011) (**Figure 1**). It is native to Central and South America but is grown in other areas such as Hawaii, South Africa, China, India, Kenya, Thailand, Malaysia, and the Philippines (Tochi et al. 2008). Bromelain is a proteolytic enzyme obtained from the stem of the pineapple that exhibits therapeutic properties such as preventing malignant cell growth and platelet aggregation, thrombus formation, anti-inflammatory and wound-healing action, and increased permeability of drugs (Maurer 2001). Other constituents besides stem bromelain include fruit bromelain, ananain, and comosain (Rowan, Buttle, and Barrett 1990).

Figure 1. The 5 most common pre-Columbian cultivars: 'Perola' (A), 'Queen' (B), 'Manzana' (C), 'Red Spanish' (D), and 'Cayenne' (E). (Source: Sanewski 2011).

Botanical Description

Pineapple is indigenous to the tropical and subtropical regions, especially southern Brazil and Paraguay. It is essential for the pineapple to grow in these regions due to the favorable temperatures ranging from 65°F to 95°F; low temperatures can potentially delay growth as well as cause the fruit to taste acidic. In addition, high elevations can also induce acidity. The

Volatile compound	Aroma
4-hydroxy-2,5-dimethyl-3(2H)-furanone	Sweet, caramel, fruity, burnt. Strawberry and pineapple at low conc
4-methoxy-2,5-dimethyl-3(2H)-furanone	Sweet, caramel, fruity, musty, savoury
Ethyl 2-methyl butanoate	Green, apple, fruity
Methyl 2-methyl butanoate	Fruity, apple
Methyl butanoate	Fruity, apple
Ethyl 3-(methyl thio) propanoate	Pineapple
Methyl 3-(methyl thio) propanoate	Sulfurous, pinapple
Ethyl 2-methyl propanoate	Sweet, fruity
Methyl 3-propanoate	Fruity, pineapple
Methyl propanoate	Fruity, rum-like, apple, banana, strawberry
octalactone	Creamy, coconut
decalactone	Sweet, coconut, peachy
hexalactone	Sweet, creamy, coconut, herbaceous
vanillin	Vanilla
1-(E,Z)-3,5-undecatriene	Fresh, green, pineapple
octanol	Citrus
Methyl hexanoate	Fruity, pineapple
Ethyl hexanoate	Fruity, pineapple
3-methylbutyl acetate	Fresh, fruity. Banana and pear at low conc

Table 1. Major compounds in pineapple aroma. This is a list of the compounds that give the pineapple its sense of aroma and flavor (Source: Sanewski 2011).

plant can be grown on small plots or steep slopes, but since the pineapple's roots are shallow, the land must be prepared on the surface to avoid damage (Morton 1987). The Bromeliaceae family consists of two branches: terrestrial and epiphytes. While the terrestrial plants are rooted in soil, the epiphytes grow on other plants and objects. The pineapple belongs to the terrestrial branch (Okihiro 2009, 76).

The pineapple plant is an herbaceous perennial ranging from 2.5 to 5 ft high with a short stem of pointy, waxy green leaves that are 20 to 72 in. long (Pineapple 1996). The stem begins to elongate near the apex during blooming time and small purple or red flowers appear. Known as the "crown" of the pineapple, this clump of firm, short leaves grows after the stem develops. Developing from the flowers is the cone shaped fruit that is 12 or more in. in height (Morton 1987).

The pineapple fruit is composed of many small fruitlets, which determine the pineapple's size. In general, fruit size can be as little as 100 g to as much as 7 kg. Most pineapples range from yellow to orange skin; however, there are also cream, pink, and red skinned pineapples. The yellow, green, and red skin color is due to carotenoids, remaining chlorophyll, and anthocyanins, respectively. The yellow flesh color is also a result of carotenoids similar to the skin. The sweet flavor of the pineapple is because of the sucrose content. However, it is important to note that the smaller the fruit, the sweeter it is. Flesh and skin volatile compounds establish the pineapple's distinctive aroma (Sanewski 2011). **Table 1** lists some of the major compounds that contribute to the pineapple's aroma and flavor.

The primary pollinator of the pineapple plant is the hummingbird. Hard seeds can be present in the fruit if the flowers are pollinated; however, if they are not, few seeds will be present (Morton 1987). There are several pests and diseases that can infect the pineapple including mealy bugs and rats. Bud rot, fruit core rot, and heart rot are diseases that are also common. Bud rot is rotting of the stem and the eventual death of the plant while fruit core rot is seen in fruits that turn brown in the inside and are smaller in size. Heart rot

is when the leaves begin to redden and the edges become brown and wilt. To avoid bud rot, the plant should not be cut in a way as to allow fungus to infect it. Controlling mealy bug invasion and removing the crown in the rainy season can prevent fruit core rot. In addition, proper drainage and elevation of the plant can ensure safety from heart rot (Pineapple: Pests and Diseases 2011).

Five common pineapple cultivars include the 'Perola', 'Queen', 'Manzana', 'Red Spanish', and 'Cayenne' illustrated in **Figure 1** (Sanewski 2011). The 'Perola' is a yellow, large, and cylindrical pineapple with no spines and is popular in Venezuela and Columbia. The 'Queen' is prevalent in South Africa, Queensland, and the Philippines. It is cone-shaped, golden-yellow with more fragrance and flavor than other cultivars. However, it matures early, requires thinning, and has a low yield. A minor cultivar in Columbia is the 'Manzana.' In addition to these is the popular cultivar in Florida, the West Indies, Venezuela, and Mexico known as the 'Red Spanish.' Resistant to rotting, it ranges from having orange to red skin and represents 85% of commercial planting in Puerto Rico. Due to its cone-shaped form, orange skin, mildly acidic flavor, and canning properties, the 'Cayenne' is known worldwide despite its lack of ability to withstand disease (Morton 1987).

Traditional Uses

Traditional medicinal uses

The pineapple has been used traditionally for a variety of ailments particularly in Central and South America (Taussig and Batkin 1988). The juice was not only consumed as a diuretic but also gargled for sore throats and to prevent seasickness. Because unripe pineapples are poisonous, it was traditionally taken for abortion and removal of intestinal worms. The pineapple root in the form of dried powder was used to heal edema in Africa while the rind was topically applied to fractures and hemorrhoids. Even today, compounds in the pineapple are commonly used to treat edema as well as to reduce pain and inflammation. In Panama, the leaf juice was used to promote menstruation, remove worms, and cleanse the intestine (Morton 1987).

Food uses

Pineapples are also commonly used in food. The flesh of the fruit can be eaten alone, in desserts, salads, pies, cakes, puddings, or made into sauces. Malayans add the pineapple in their curries while the Filipinos use extracted pulp in a dessert called *nata de piña*. The pineapple cannot be frozen because the flavors are subsequently extinguished. Because pineapples contain a proteolytic enzyme called bromelain, either the pineapple or the enzyme is commonly used as a meat tenderizer to break down protein. The canning of pineapples has significantly increased its demand and made it one of the leading fruits worldwide. Hawaii alone supplied 70% of the world's canned pineapple for about a decade starting in 1970 until the company, Dole, transferred its operation to the Philippines due to increased production costs (Morton 1987).

Ethnobotanical uses

Native Americans cultivated the pineapple in Mexico and the West Indies before the arrival of Christopher Columbus. Columbus and his crewmembers saw the pineapple for the first time in 1493 in Guadeloupe. Inhabitants of the Caribbean set pineapples outside their homes to represent friendship and hospitality. As a result, Europeans in Spain and England

Figure 2. **Decorative fruits.** The pineapple's flowers were commonly used as a source for flower arrangements (Source: Sanewski 2011).

illustrated the fruit in carvings on doorways (Morton 1987). Pineapple ware such as pots, mugs, cups, and bowls were even sold in colonial America (Okihiro 2009, 164).

In addition to its medicinal value, the pineapple was used for many other applications. The juice was used as a cleanser for knife blades and scrubbing boat decks (Morton 1987). The flowers of the plant could be used as an ornamental or flower arrangement illustrated in **Figure 2**. The fruit itself was also used as a means to decorate the home. For example, George and Martha Washington adorned their wall lamp with a pineapple on top illustrated in **Figure 3** (Okihiro 2009, 166). In the Philippines, the natives extract fiber from the leaves in order to make fabric (Pineapple: Pests and Pesticides, 2011).

Harvesting

Pineapple harvesting requires an enormous amount of experience; in addition to size and color, other factors must be

Figure 3. The pineapple as a common ornamental. A pineapple sits as an ornamental on top of a wall lamp in George and Martha Washington's home. (Source: Okihiro 2009).

taken into account. Before the pineapple reaches maturity, starch is converted into sugars in a span of a few days. During the summer, crop is harvested when the skin shows a light green color because sugar content and volatile flavors begin to develop. However, the winter crop takes approximately one month longer to reach maturity; therefore, fruits are harvested when the base is covered with a light yellow pigment. In spite of this, winter pineapple is more acidic and has lower sugar content than summer pineapple. In addition, if the fruit is overripe, it is not as flavorful and is more likely to perish. Usually after the stem is cut, a 3% solution of benzoic acid is applied to the stem in order to protect the fruit from rain and dew. Pineapples should not be stored for more than six weeks although storage life can be extended by immersing the fruit in a wax solution with fungicide (Morton 1987).

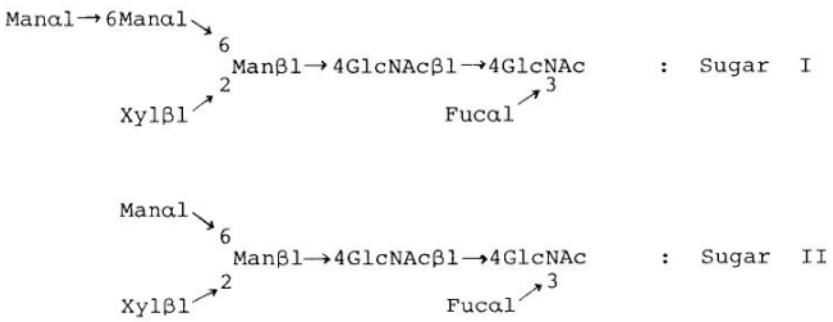

Figure 4. Structure of bromelain. This illustrates the complete structure of the 2 oligosaccharides in bromelain. (Source: Ishihara et al. 1979)

Chemistry and Pharmacology

One of the main constituents in the pineapple is a cysteine protease known as stem bromelain (**Figure 4**). It is an aqueous extract from the stem and sometimes fruit of pineapples that is prepared by centrifugation, ultrafiltration, and lyophilization. This complex process yields a yellow powder. However, when in an aqueous solution, bromelain can degenerate due to self-digestion; therefore, α_2-macroglobulin, a plasma protein, is added to prevent deterioration (Maurer 2001). The majority of research has been conducted on bromelain and many of the therapeutic effects are attributed to it. However, there are also other cysteine proteases such as fruit bromelain also known as bromelin, ananain, and comosain. Ananain and comosain are found in the stem along with stem bromelain. Bromelain is a proteolytic enzyme comprised of a mixture of components including peroxidase, acid phosphatase, protease inhibitors, and organically bound calcium (Orsini 2006). Used as complementary medication to glucocorticoids, antirheumatics, and immunomodulatory agents, proteolytic enzymes' low toxicity provides the capability for the treatment of inflammatory conditions. They consist of a broad range of therapeutic effects such as antiedematous, anti-inflammatory, antithrombotic, and fibrinolytic activities. These proteolytic enzymes also help regulate immune cells and cytokine production as well as functions of adhesion molecules on blood and endothelial cells (Maurer 2001). **Table 2** lists the components found in *Ananas comosus*.

Biological Activity

In vitro

Pineapple's main therapeutic component, bromelain, has been studied in many *in vitro* and *in vivo* studies. *In vitro* studies have found that not only is bromelain degraded in blood plasma by protease inhibitors, but oral administration may facilitate bromelain in preserving its proteolytic activity (Orsini 2006).

Researchers stimulated mouse ileum to induce contractions and then administered bromelain, resulting in the inhibition of contractions. This suggests bromelain inhibits intestinal motility. This discovery could help bromelain become a leading drug in regulating intestinal motility in intestinal inflammation as well as diabetes (Borrelli et al. 2011). Bromelain also hinders tumor growth by 90% in Lewis lung carcinoma, YC-lymphoma, and MCA-1 ascitic tumor even after destruction of the proteolytic activity by heat. This indicates that another factor is responsible for the anticancer effect observed in pineapple (Taussig and Batkin 1988). Bromelain also reduces platelet adhesion to endothelial cells as well as platelet aggregation. In addition, it has the ability to stimulate plasminogen to produce plasmin, which can then cleave fibrin and decrease fibrin levels (Maurer 2001).

Components of *Ananas comosus*

Plant

2,5-dimethyl-4-hydroxy-3(2H)-furanone (1.2 ppm)
5-hydroxytryptamine
acrylic acid
ananasic acid
beta-methyl-thiopropionic-acid-ethyl-ester
beta-methyl-thiopropionic-acid-methyl-ester
ergosterol peroxide
ferulic acid (200-760 ppm)
p-coumaric acid (330-730 ppm)
stigmast-5-ene-3-beta-7-alpha-diol
tetrahydro-alpha-alpha-5-trimethyl-5-vinylfurfuryl-alc

Fruit

Acetic acid (.49 ppm)
Acetoxyacetone
Alpha-linolenic acid (620-4592 ppm)
Amyl caproate
Asparagine (1251 ppm)
Biacetyl
Bromelain
Bromelin
Butyl formate
Chavicol (.27 ppm)
Delta octalactone (.3 ppm)
Dimethyl malonate (.06 ppm)
Esters (1-250 ppm)
Ethyl acetate (3-120 ppm)
Ethyl acrylate (.77 ppm)
Ethyl alcohol (60 ppm)
Ethyl-beta-acetoxyhexanoate Ethyl-beta-hydroxyhexanoate Ethyl-beta-methylthiopropionate Ethyl butyrate
Ethyl caproate (.77 ppm)
Ethyl caprylate
Ethyl formate
Ethyl isobutyrate
Ethyl isovalerate (.39 ppm)
Ethyl lactate
Ethyl proprionate
Gaba
Gamma-butyrolactone
Gamma-caprolactone (.12 ppm)
Gamma-octalactone (.3 ppm)
Hexosans (1000-1500 ppm)
Indole acetic acid oxidase
Isobutanol
Isobutyl acetate
Isobutyl formate
Isocapronic acid
Isopropyl isobutyrate
L-malic acid
Malid acid (1000-4700 ppm)
Methanol
Methyl acetate
Methyl-beta-acetoxyhexanoate Methyl-beta-hydroxybutyrate Methyl-beta-hydroxyhexanoate Methyl-beta-methylthiopropionate Methyl butyrate
Methyl caproate
Methyl caprylate (.75 ppm)
Methyl-cis-4-octenoate (.001 ppm)
Methyl isobutyrate
Methyl isocaproate (1.4 ppm)
Methyl isovalerate (.6 ppm)
Methyl-n-propyl ketone
N-valerianic acid
Nitrate (1200 ppm)
Oxalic acid (50-58 ppm)
P-aminobenzoic acid (1 ppm)
P-coumaric acid (330-730 ppm)
Pentanol
Pentosans (3300-4300 ppm)
Phosphatase
Phytosterols (60-444 ppm)
Propanol
Propyl acetate
Propyl formate
Serotonin (19-60 ppm)
Vanillin
Vit-b-6 (.9-6 ppm)

Table 2. Components in *Ananas comosus* (Duke 2010).

Bromelain also has an antisecretory effect. Diarrhea is caused by the activation of the cAMP, cGMP, or calcium-dependent signaling pathways in the large intestine. Using rabbit ileum, researchers found that bromelain prevents changes in these pathways (Mynott et al. 1997).

In vivo

A common question is whether bromelain is absorbed after oral ingestion; researchers found that 40% of labeled bromelain is absorbed from the intestine (Maurer 2001). Bromelain's anticancer effect was tested *in vivo* as well in mice subjected to ultraviolet radiation. Those fed bromelain showed retardation of the growth of lesions (Goldstein et al. 1975). Baez et al. also studied a number of tumor lines *in vivo* including P-388 leukemia, sarcoma (S-37), Ehrlich ascitic tumor, Lewis lung carcinoma, MB-F10 melanoma, and ADC-755 mammary adenocarcinoma. All were administered bromelain intraperitoneally and all had an increase in survival rate besides MB-F10 melanoma (Baez et al. 2007).

Bromelain also has the capability of reducing existing edemas as well as preventing the formation of new edemas (Maurer 2001). Oral bromelain not only reduces swelling and pain but also reduces the time it takes to heal by half. Experiments explored the anti-inflammatory effect of bromelain on edemas in rats along with eight other drugs, including aspirin, and found that bromelain was the most effective in terms of a reduction in inflammation (Taussig and Batkin 1988). Further experiments found similar results of bromelain's potency in comparison to other drugs even when applied intraperitoneally (Smyth, Brennan, and Martin 1962).

Topical bromelain is used for debridement of third degree burns without removing unburned tissue; a study was done on rats with experimental burns in which topical bromelain helped remove damaged tissue with no side effects (Klaue, Aman, and Romen 1979). Bromelain is especially beneficial because it prevents bacterial growth, contamination, and infection to the healthy tissue by removing the burnt tissue (Rosenberg et al. 2004).

Mechanism of action

Bromelain has a number of therapeutic uses implicating that it must have multiple mechanisms (Taussig and Batkin 1988). The anti-platelet and anti-inflammatory effects are related to bromelain's proteolytic activity. However, the anticancer and burn debridement properties are due to some other unknown factor in bromelain. A possible mechanism of action has been proposed for the anticancer property of bromelain which stimulates differentiation of leukemic cells possibly leading to apoptosis of tumor cells (Maurer 2001).

Bromelain's mechanism of action is partially due to the activity of two types of prostaglandins: pro-inflammatory prostaglandins and anti-inflammatory prostaglandins. Pro-inflammatory prostaglandins stimulate inflammation, platelet aggregation, and vasoconstriction. Anti-inflammatory prostaglandins have the opposite effect. Bromelain is a pro-inflammatory prostaglandin inhibitor that is similar to aspirin but weaker so it does not promote bleeding. Bromelain activates plasminogen, which induces plasmin production. Plasmin subsequently cleaves fibrin, which inhibits pro-inflammatory prostaglandins. Consequently the ratio between pro-inflammatory and anti-inflammatory prostaglandins shifts in favor of anti-inflammatory prostaglandins. This leads to the activation of adenylyl cyclase and the production of c-AMP. Anti-inflammatory prostaglandins generally lead to increased c-AMP production, inhibited platelet aggregation, inhibited inflammation, dilated blood vessels and smooth muscles, and increased tissue permeability; this mechanism is illustrated in **Figure 5** (Taussig and Batkin 1988).

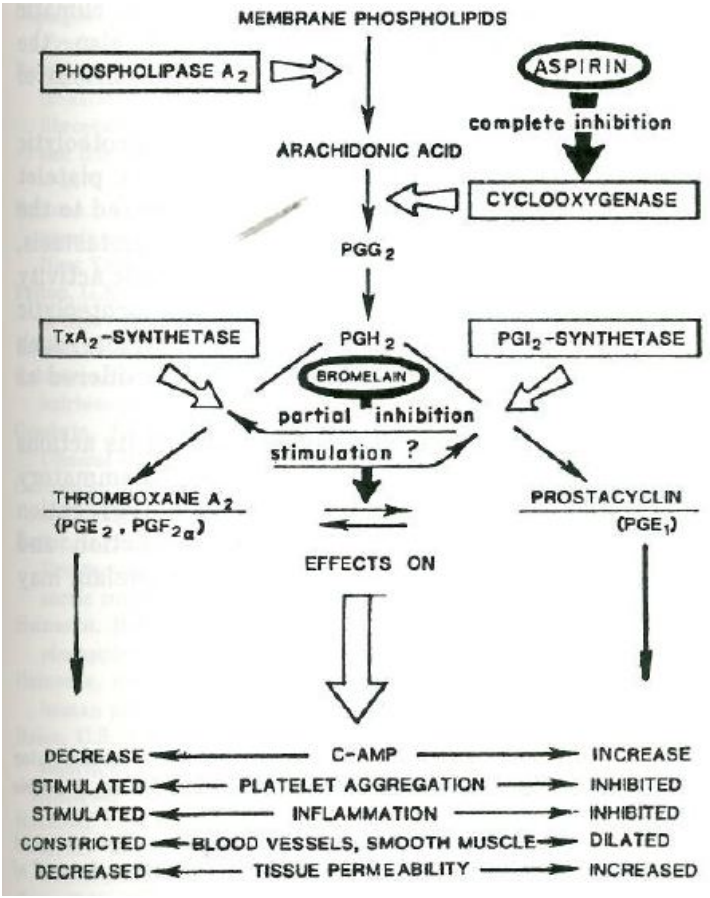

Figure 5. Biosynthesis of prostaglandins and bromelain effect. This diagram illustrates how bromelain and aspirin affect prostaglandins (Source: Taussig and Batkin 1988).

Clinical Studies

Pain and Inflammation

Bromelain demonstrates both analgesic and anti-inflammatory properties (Brien et al. 2004). Patients given bromelain felt less pain linked to mediolateral epistomy (Zatuchni and Colombi 1967) and bradykinin (Bodi 1966). Furthermore, bromelain reduces mild knee pain and helps improve general well-being in patients given 200 or 400 mg of oral bromelain (Walker et al. 2002). It has been widely used for inflammation ever since its discovery and is commonly used for rheumatoid arthritis, thrombophlebitis, hematomas, oral inflammations, diabetic ulcers, rectal and perirectal inflammations, oral and plastic surgery (Taussig and Batkin 1988). Researchers found that patients suffering from arthritis with joint swelling when administered bromelain report reduction in swelling, pain, and soreness (Walker et al. 2002). Previous studies have indicated that bromelain may serve as an alternative treatment to NSAIDs for patients suffering from osteoarthritis (Brien et al. 2004). However, the required dosage is unclear; while one study used between 60 and 160 mg of oral bromelain per day (Cohen and Goldman 1964), another study administered 1890 mg of oral bromelain (Tilwe et al. 2001) per day both of which reported reduced pain and swelling. Adverse reactions must also be taken into consideration when assigning dosage. While one study using 945 mg of oral bromelain per day reported gastrointestinal problems and subsequent drop-outs (Singer, Singer, and Oberleitner 2001), the previously mentioned study administering 1890 mg of oral bromelain per day reported no drop-outs or safety issues (Tilwe et al. 2001). Bromelain's use in the treatment of osteoarthritis is promising; however, more studies must be conducted in order to identify an optimum dosage.

Cancer

Bromelain is shown to have an anticancer effect; however, studies verifying this have primarily been *in vivo*. Nevertheless, its activity was tested in a few clinical studies. Twelve patients with different tumors were treated with oral bromelain and were found to have a decrease in ovarian carcinoma, breast cancer, and metastases. Another study found tumor regressions in patients administered high doses of bromelain simultaneously with chemotherapeutic agents such as vincristine. Both studies found that at least 2.4 g per day of bromelain was necessary and dosages less than 1000 mg were insufficient (Taussig and Batkin 1988).

Antibiotics

It has been shown that bromelain can enhance the absorption of antibiotic drugs. It increases tissue permeability yielding enhanced diffusion of the antibiotic after subcutaneous or intramuscular application (Maurer 2001). Researchers found that patients treated with bromelain and antibiotics had an increase in serum and tissue levels of amoxicillin (Tinozzi and Venegoni 1978). Twenty-three patients with pneumonia, bronchitis, staphylococcus infections, thrombophlebitis, as well as other conditions were administered bromelain with antibiotic therapy. These patients previously did not react to antibiotic therapy but after simultaneous bromelain treatment, 22 of the patients responded (Neubauer 1961).

Burn debridement

One of bromelain's few topical treatments is its effectiveness in the debridement of third degree burns. Patients with second and third degree burns were treated with Debridase, a multienzyme combination containing bromelain. Debridement

of the eschar was achieved after only one to two applications (Rosenberg et al. 2004). Eschar is burnt and traumatized tissue that can prevent proper assessment of the burn's depth and lead to bacterial growth, contamination, and infection. Surgical removal is not only painful and results in significant bleeding but it also requires frequent doses of anesthesia (Tochi et al. 2008). Bromelain can achieve the same end result with no blood loss and few side effects (Rosenberg et al. 2004).

Circulation

By inhibiting platelet aggregation, bromelain can serve as an important treatment in cardiac medicine. Patients with a high platelet count or history of heart attack and stroke were treated with oral bromelain leading to a decrease in platelet aggregation (Heinecke, Waal, and Yokoyama 1972). Researchers suggest the activity is due to the activation of plasminogen followed by an increase in fibrinolytic activity, therefore blocking fibrin production (Orsini 2006). Bromelain also inhibits thrombus formation due to its inhibition of platelet aggregation and decreased fibrin production (Tochi et al. 2008).

Contraindications

According to the U.S. Federal Drug Administration, bromelain is generally recognized as safe (Orsini 2006). It has low toxicity and few side effects associated with it (Maurer 2001). Possible adverse effects of bromelain include gastrointestinal problems, headache, fatigue, dry mouth, skin rash, and allergic reactions (Orsini 2006). However, these effects are uncommon in the general population; a study found a 1.8% prevalence of side effects after bromelain administration (Maurer 2001). It is important to note that higher doses of bromelain will lead to a greater likelihood of exhibiting side effects (Orsini 2006). Since unripe pineapples were used as an abortifacient in the past, pregnant women should limit intake unless used for that purpose (Morton 1987). Consumption of pineapples can also cause allergic reactions in which symptoms include itching, rashes, abdominal pain, vomiting, and diarrhea (Kabir, Speelman, and Islam 1993). The blood glucose response in diabetic patients after pineapple consumption is significantly higher than other fruits such as mango and chico. Due to the increased glucose response and pineapple's identification as a fruit with a high glycemic index (GI), it is suggested that diabetic patients limit intake to moderate amounts (Guevarra and Panlasigui 2000).

Bromelain can potentially increase bleeding when taken with aspirin and warfarin due to its anticoagulant properties. In addition, there is very little research conducted on the drug safety of bromelain in children; most clinical studies focus on adults. Therefore, children should not be given bromelain supplements due to the lack of studies in children. There is also little information on bromelain's effects at high doses, taken long term, or taken in combination with other medication (Orsini 2006). More research is needed for bromelain's use as an established compound for medicinal purposes.

Current Use in Allopathic and CAM Therapies

Bromelain is an extract that yields a yellow powder (Maurer 2001) available in the form of a powder, tablet, cream, or capsule (Orsini 2006). Bromelain is also available in multienzyme combinations in Debridase, Phlogenzym, Wobenzym, and Traumanase (Orsini 2006) (**Figure 6**). Other trade names include Ananase, Bromelain, Resolvit, Extranase,

Figure 6. Examples of bromelain multienzyme herbal supplements. (Source: http://www.apomio.de).

and Inflamen (Sittig 2007). The German Commission E recommends 80-320 mg of bromelain two to three times per day. However, if used for arthritis, 500-2000 mg per day should be consumed; for injuries, 500 mg should be consumed four times per day. In order to use bromelain for its anticancer activity, at least 2.4 g per day must be consumed (Taussig and Batkin 1988). Bromelain is a popular supplement in European countries such as Germany; the German Commission E approved bromelain to be used simultaneously with other therapeutic agents in the treatment of inflammations of the nose and sinuses due to surgery (American Cancer Society 2011). It is also recommended to consume the bromelain herbal supplements on an empty stomach to reduce reactions with other foods (University of Maryland Medical Center 2011).

Discussion

Ananas comosus is an important plant with many ethnobotanical and even more medicinal uses. Its primary constituent, bromelain, is not only an anti-tumor agent, but prevents platelet aggregation and hence thrombus formation. It is an analgesic as well as an anti-inflammatory agent used in a number of conditions such as rheumatoid arthritis, oral inflammations, ulcers, and plastic surgery. Although it can promote antibiotic drug absorption, one of the most important capabilities of bromelain is its use in burn debridement of second and third degree burns. Bromelain is generally recognized as safe and there are very few if any side effects due to it, which can possibly occur at very high doses. However, research is limited in bromelain's use in children as well as the required dosage to view its effects. With more research, *A. comosus* can serve as an important plant for the treatment of dermatological disorders, inflammatory conditions, as well as cancer treatments in the future.

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Angelica archangelica L., Apiaceae

Jessica Elinburg

Introduction

Known by a variety of names including wild celery, Holy Ghost, Norwegian angelica, and garden angelica, *Angelica archangelica* L., of the Apiaceae (parsley) family is a plant useful for a vast variety of therapeutic remedies. Sprinkled throughout the damp soil of Northern Europe and Asia, angelica was once (and in some cultures still) considered to be a precious source of holistic medicine as well as a delicious snack and when dried, a pungent flavoring agent. Historically, *A. archangelica* has been used for a plethora of therapies. From superstitiously wearing the flowers of angelica as jewelry to instituting the plant as the "official" remedy of the infamous Plague, society's awe of angelica's mysterious beneficial properties can be traced back to pre-medieval Europe. The odd towering herb displays antioxidant properties and possesses anxiolytic (anxiety inhibiting), hepatoprotective (liver protecting), and anticarcinogenic (cancer preventing) effects; it also inhibits acetylcholinesterase, an enzyme that activates acetylcholine, a neurotransmitter crucial in muscle function and relaxation. Although only one formal clinical research study has been performed on *A. archangelica*, many *in vitro* and *in vivo* studies display promising results for the active compounds in the plant. Angelica archangelica is truly a diamond in the raw, a herb largely undiscovered by the Western world that just may hold the key to obtaining holistic health.

Figure 1. Compound umbel of *A. archangelica* http://apps.rhs.org.uk/plantselector/plant?plantid=136

Figure 2. Leaves of *A. archangelica*
http://www.mygourmetconnection.com/reading/ingredient-guides/fresh-herbs-a-to-z-part1.php

Botanical Description

Angelica archangelica L. is a surprisingly tall herb, reaching heights of up to eight feet. The cylindrical, hollow stalk of the plant is a dim greyish purple color and eventually branches into a globular compound umbel. The umbel may span up to five inches in width, donning small vibrantly colored flowers (**Figure 1**). The color of the flowers exists on a continuum ranging from vivid yellow green to bright white. At the base of the plant, green pinnate leaves extend. Lobes branch from both sides of the stem's axis. The leaves are long, slender, and ovular, typically serrated down the sides. Hanging from tender stalks, they may measure up to three inches in length. The terminal leaflets exist in triads (**Figure 2**). *A. archangelica* produces a green pod-shaped fruit that contains two seeds.

The fragrance of *A. archangelica* resembles that of celery with sweet earthy undertones, much like a combination of citrus, mint, and earth. A. archangelica has an appealing taste and is thus used in a variety of culinary pursuits.

A. archangelica originated in Europe and Asia, though it has expanded to many other areas, including the United States, for agricultural purposes. The herb favors moist environments near water sources such as marshes, lakes, streams, and even saltwater bodies (though this is less common). In fact, angelica favors waterfront cultivation sites to the extent that domestically cultivating the herb is quite a challenge. *A. archangelica* grows best in partial shade (Kowalchik, Hylton, Carr, & Press, 1987). Surprisingly, insects and parasites rarely feed on angelica, with the exception of a few species of leaf-munching worms. Angelica is also a relatively hearty plant, able to withstand unseasonably cold periods (Bhat, Kumar, & Shah, 2011).

A. archangelica's maturation process is unique. In its first year of growing, only leaves are present among the plant's thick stalk. By the second year, the plant has a canopy of serrated leaves teaming from its stems. Finally, but not until the third or fourth year of existence, does the angelica plant flower and produce fruit. This rather lengthy cultivation period often leads to overharvesting and decimation of wild angelica populations.

The global demand for *A. archangelica* is great. However the prized plant is becoming rapidly scarcer each year as overgrazing, unfavorable environmental conditions, habitat degradation, and overharvesting take place over time. Although angelica is cultivated all over the world, like many

plants, the wild herb is generally more desirable than the domesticated. Angelica populations in Asia are especially at risk for endangerment. Conservation efforts are currently being undertaken in hopes that the wild populations of angelica can be replenished. If conservation efforts are successful, not only will exploratory pharmaceutical endeavors may profit, but the ethnobotany of traditional subartic cultures may be preserved through the continued use of sacred indigenous plants (Bhat, et al., 2011).

Traditional Uses

The history of *Angelica archangelica* L. is rich with superstitious folk tales and traditional remedies. The herb receives its name from the biblical figure Saint Michael the Archangel. Reportedly, the plant bloomed faithfully every year on May 8, the day of the feast of Saint Michael, a celebration in Christian tradition. Curiously, today, the plant is more commonly apt to flower in the early months of summer, June and July.

For centuries, *A. archangelica* has had a pagan association with healing powers; the plant was commonly thought to ward off malevolent spirits and demonic forces. Parents crafted chains of *A. archangelica* leaves to garb their children with spiritual protection as a sort of precautionary jewelry (Bhat, et al., 2011).

A well-known legend that may well have begun *A. archangelica's* association with the divine originates from a lakeside Latvian town. In the early medieval age, a horde of Latvian peasants marched into town with the intent of peddling bundles of *A. archangelica*. As they made their way through the countryside, they chanted ancient mantras that no one, not even they, could understand. This occurrence prompted the townspeople to propose that perhaps the herbal product the peasants were carrying, which happened to be *A. archangelica*, somehow had a divine connection or some bizarrely idolatrous spiritual significance.

Soon, the reputedly magical plant was cultivated into daily life in European culture, most commonly being prepared as a type of Carmelite water. Extracts from the plant were made into an herbal tonic rumored to serve as an effective diuretic, and more importantly a sweet-tasting treatment for respiratory and digestive ailments. The herb was commonly used to soothe persistent tussis and resultant chest pain. Digestively speaking, *A. archangelica* eased digestive upset and lulled gripe. The herb was also interestingly used to treat rabies. There have also been reports of using A. archangelica to keep deafness at bay by pouring juice from the plant directly into the ear canals.

A. archangelica grows mainly in the grasslands and mountainous terrain of sub-arctic Europe. Historically, most of the arctic population settled in coastal areas, long distances away from the prized plant, in order to have assured access to food, travel, and trade. Records indicate the settlers of Greenway and Norway would travel cross-country to obtain A. archangelica. On the journey back home to the arctic coast, the sweet plant was eaten raw in rather impressive quantities (Porsild, 1953).

Perhaps *A. archangelica's* most historically prominent use occurred in the mid-1600s during the time of the infamous bubonic plague. Legend has it that an English monk experienced a vision in which an angel guided him to use *A. archangelica* as a cure for the plague. Once word of the divine dream had rounded the community, London's College of Physicians included it as an official published treatment for the plague, boasting it to be "the King's Majesty's Excellent

Recipe for the Plague". The treatment called for the herb to be heated alongside nutmeg and sugar into a sweet syrup and the syrup be taken orally twice per day. A rather confident seventeenth century once wrote than angelica was "the forefront of all medicinal plants" ((Bhat, et al., 2011).

By the end of the seventeenth century, widespread use of *A. archangelica* in Europe was becoming increasingly rare, other than for the occasional treatment of acute colic. However, the plant was reintroduced into alternative therapies in European medical culture in the early twentieth century when it was included in the British Pharmaceutical Codex (1934).

Across the Atlantic, Native Americans had also been using *A. archangelica*, like their European counterparts, for centuries. Native Americans had therapies similar to those contrived in Europe and Asia. For example, the herb was used for respiratory ailments such as tuberculosis and as a mucous expectorant. On the digestive spectrum however, instead of soothing stomach upset, *A. archangelica* was used in concentrated doses as an emetic (Sarker & Nahar, 2004). Interestingly, when combined with Artemisia canadensis L., commonly known as Canada wormwood, *A. archangelica* could be prepared into a topical remedy useful for relieving pain and swelling. Similar to therapies in Europe, the herb was also often incorporated into herbal drinks intended to replenish the body after illness.

Historically speaking, *A. archangelica* has also been used as a flavoring agent in both tobacco and alcoholic beverages such as gin and vermouth. Culinarily, the leaves of plant were used in salads and the stems of the leaves candied into a sweet confection (Sarker & Nahar, 2004). One particularly interesting Scandinavian record reports that angelica was often used to flavor reindeer milk as a festive beverage (Bhat, et al., 2011).

Chemistry and Pharmacology

The composition of the essential oils of the fruit seeds of three native A. archangelica samples was determined using gas chromatography and mass spectroscopy by chemical researchers studying in Lithuania. From the study, sixty seven chemical compounds were identified in the essential oil (83.9% to 90.0% of the total constituents) and of the compounds identified, 63.5% to 76.6% were determined to be monoterpene hydrocarbons. Of the monoterpenes present, β-phellandrene, an aromatic compound responsible for the slightly minty, citrusy scent of the *A. archangelica* plant, makes up 33.6% to 63.4% of the content. The second most abundant constituent in angelica's essential oil is α-pinene (4.2% to 12.8%). This compound is responsible for the characteristic aroma of pine, turpentine, and rosemary; it also contributes mildly to the unique aroma of angelica. Some of the minor terpenoids present in the oil include α-phellandrene (7.4%), sabinene (3.3%), and germacrene D (3.0%). Although many parts of the plant contain essential oil, the oil if found to be most concentrated In the roots (Nivinskiene, Butkiene, & Mockute, 2005). Monoterpenes serve as important antioxidants for the body (Xi, Gandhi, Lai, & Kublaoui, 2012).

The main chemical compounds responsible for *A. archangelica's* bioactivity are coumarins and the activity of those coumarins depends greatly on their concentration within the plant, which may vary from seed to root to flower. Typically, coumarins concentration is highest in the fruit of the plant. The two most common types of coumarins found in *A. archangelica* are imperatorin and xanthotoxin. Imperatorin is approximately three times more abundant within the plant than xanthotoxin. Some other minor coumarins found in the plant include osthal, psoralin, bergapten, and angelicin (Patra, Ghosh, & Mitra, 1976),

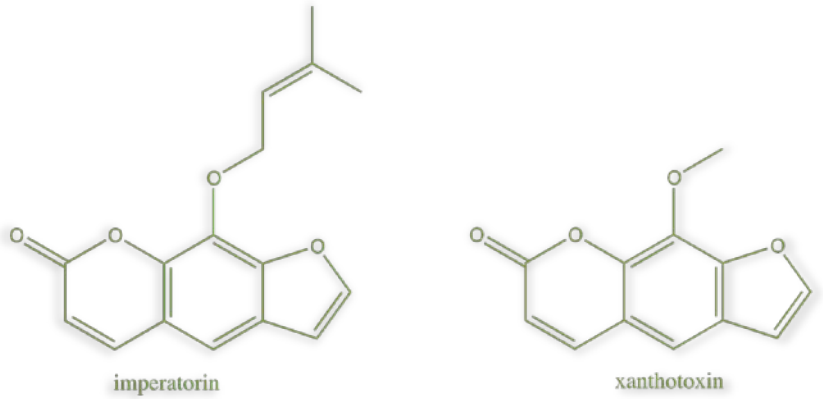

Figure 3. Imperatorin and xanthotoxin, important coumarins in *A. archangelica.* (Source: ChemDraw)

Biological Activity

Anticarcinogenic effect

In vitro tests of organic extracts from *A. archangelica* on cancerous pancreatic cell lines proved that angelica displayed dose-dependent anticarcinogenic activity towards mutagenic cells. The impressively strong bioactivity of the herb was determined to be sourced from the two main coumarins in angelica: imperatorin and xanthotoxin (**Figure 3**). It should be noted that the terpenoid content of angelica, although responsible for the appealing aroma and taste of the plant, contributed very little, if at all, to the antiproliferative effects of the plant extract.

In a similar test upon a cancerous Crl cell line, results proved that leaf extract from the plant was only mildly effective in preventing the proliferation of cancer cells, quite a decrease from the aforementioned study. To explain the unexpected results, *in vivo* testing in mouse models was performed. However, the in vivo tests proved quite successful: out of eleven test mice treated with extracts of *A. archangelica*, 82% developed no tumors or insignificantly small tumors compared to unrestrained tumor growth observed in untreated control mice. Interestingly, the antiproliferative effects of the *A. archangelica* leaf extract upon the cancerous cells could not be attributed to the presence of furanocoumarins present in the plant, a hypothesis previously well-accepted (Wojcikowski, Stevenson, Leach, Wohlmuth, & Gobe, 2007). Further research is being conducted to determine *A. archangelica's* anticarcinogenic activity against varied cancer cell lines. Also, new proposals for the plant's promising bioactivity are being explored after the previously-mentioned study concluded that the coumarins present in the plant may not always be responsible for angelica's bioactivity.

Hepatoprotective effect

In vitro studies in mice with ethanol-induced hepatotoxicity were performed to study the potential defensive effect of A. archangelica against oxygen free radical formation in the liver. The study found that varying doses (ranging from 10 to 50 mg/kg) of *A. archangelica* of "indirectly protected the liver from oxidative stress" (Yeh, Liu, Huang, & Huang, 2003).

Inhibition of acetylcholinesterase

A study conducted at the University of Iceland examined the acetylcholinsterase inhibition potential of several herbal species native to the area. Although many of the plants displayed little inhibitory activity, ethanolic extracts from the seeds of *A. archangelica* contained two primary coumarin compounds that displayed promising behavior: imperatorin and xanthotoxin. Both of these compounds were shown to inhibit acetylcholinesterase by competing with the compound for nicotine receptors in the body. Of the two coumarins,

xanthotoxin displayed almost twice the activity of imperatorin. However, it was decided that the total inhibitory activity of A. archangelica could not be solely attributed to the activity of the two primary coumarins. Instead, researchers proposed that synergistic activity between the chemical constituents of the plant most likely occurred when consumed in vivo. Additionally, researchers concluded that optimum acetylcholinesterase inhibition occurred when extracts from *A. archangelica* were combined with those of *G. sylvaticum*, a vibrantly-hued geranium (Sigurdsson & Gudbjarnason, 2007). Acetylcholinesterase inhibition is important in promoting relaxation and a stable emotional state within the body, especially within patients facing mental debilitation (such as Alzheimer's or dementia).

Prophylactic effects on gastric ulcers

Therapeutic doses of *A. archangelica* were administered to rats afflicted with gastric ulcers resulting from indomethacin (a non-steroidal anti-inflammatory pain killer known to inflict peptic ulcers when given high doses) exposure. Lessened gastric acid production and heightened secretion of protective mucins of the stomach were observed. Overall, anti-ulcer activity was displayed by A. archangelica (Bhat, et al., 2011).

Affect on anxiety and anxiety-like conditions

Researchers at the University of Kashmir recently examined the cerebral effects of the A. arcangelica A. archangelica was extracted with petroleum ether to obtain a solid yellow precipitate. Within the precipitate, six separate furanocoumarins were present. The anxiolytic activity of both the solid yellow precipitate as a whole and each individual component of the precipitate was tested in vivo in mice models. The study concluded that although each of the individual components of the organic precipitate of A. archangelica were effective in reducing anxiety symptoms in mice, the anxiolytic activity of the precipitate as a whole was much more effective, most likely due to synergistic mechanisms of action between the constituents within the body (Dinesh Kumar, Bhat, Kumar, & Shah, 2013) (Sigurdsson & Gudbjarnason, 2007).

Influence on Alzheimer's patients

In a small herbal study aimed at understanding the effects of bioactive botanicals on Alzheimer's patients, researchers from the Royal Botanical Garden in the United Kingdom compiled data from a variety of sources to understand the potential effects of angelica (as well as several dozen more herbs) on patients suffering from dementia. In the study, it was noted that *A. archangelica* increases cerebral blood flow, relaxation, and cognition enhancement. Although no clinical study was performed, the British researchers involved in this project served as a vital catalyst in promoting the further study of an angelica not only as an anticarcinogenic agent, but also a cerebral enhancer (Howes, Perry, & Houghton, 2003).

Protection against lead poisoning

In a study examining the healing potential of *A. archangelica* on rabbits with induced lead poisoning, it was determined that angelica had an overarching positive effect on the health of the poisoned rabbits. The study orally administered high doses of lead to the rabbits for a total of 15 days. Tests to assure an initial rise of bodily lead levels were undertaken and it was confirmed that indeed the rabbits had dangerously elevated lead levels. After dosing the test group rabbits with *A.*

archangelica, it was found that the herb not only prevented premature death in the rabbits, but actually served as a healing therapeutic agent, returning the poisoned rabbits' bodily lead levels back to a level similar to those of the unharmed control group of rabbits. Researchers believe that angelica's healing properties are resultant of an unknown, yet potent antioxidative mechanism of action, quite plausibly due to angelica's active furocoumarins: imperatorin and xanthotoxin.

Clinical Studies

So far, only one formal clinical trial has executed to study the effects of *A. archangelica*. The recent study examined the cerebral mood-stabilizing effects of geriatric patients suffering from dementia:

In a 2011 study by the Japan Geriatrics Society, twenty patients diagnosed with dementia, ranging in age from 72 to 92, were treated with a therapeutic dose of ferulic acid and A. archangelica, known as Feru-guard. At the end of four weeks, 95% of patients saw reduced symptoms of dementia (including delusions, anxiety, and hallucinations, and irritability). Though the outcome of the study was quite positive, further studies with a larger sample population must be conducted before any definite conclusions concerning A. archangelica's efficacy and safety may be made (Kimura, Hayashida, Murata, & Takamatsu, 2011).

Contraindications

Caution should be taken by those seeking to collect *Angelica archangelica* L. It is important to beware of other species that appear very similar to the herb. For example, the water hemlock, *Cicuta virosa* L., is a very poisonous plant that grows in similar moist habitats as those of A. archangelica and has a similar appearance (**Figure 4**). Only those with botanical expertise and experience should scavenge for potent herbs.

Figure 4. *Cicuta virosa*, a poisonous plant similar in appearance to angelica.

The U.S. Food and Drug Administration has declared *A. archangelica* to be generally regarded as safe (GRAS), however this label applies only to the product's culinary use. Women who are breastfeeding or pregnant are not encouraged to use the herb due to the unknown mechanism of action the prime constituents in *Angelica archangelica* undertake when consumed in the body. Also, diabetics should exercise caution when consuming angelica as it tends to raise blood sugar

levels (as measured by urine analysis) (Bhat, et al., 2011). Caution should always be taken when considering any herbal therapeutic remedy.

Current Use in Allopathic and CAM Therapies

Angelica archangelica is used widely today in allopathic and complementary alternative medicine. Its uses are endless: distinct cultures seem to utilize the plant in a variety of ways, alluding to the generalized antioxidant activity occurring on the phytochemical level of the plant. Different parts of the plant (the roots, seeds, leaves, stalk, flower) may be used for different purposes and may be prepared in various manners.

Root

The oil of the root is commonly used in incense and aromatherapy treatments as a relaxant. Roots may be dried, ground, and ingested to relieve digestive problems including eating disorders and peptic ulcers. Targeting the female reproductive system, angelic is used to relieve painful cramping associated with menstruation and labor pains. The grounds of the root may be steeped into a tea to relieve flatulence and general stomach upset. The root may also be chewed as a dentifrice to relieve mild tooth pain.

Leaves

The leaves of angelica may be boiled into a tea to be ingested after meals to reduce the occurrence of colic. In Chinese medicine, the plant is used to treat cerebral disorders and ailments including anxiety, headaches, and eating disorders (D. Kumar, Bhat, & Shah, 2012). Topically applied, the leaves are often used to relieve symptoms stemming from bronchitis, cough, and the common cold. Angelica's respiratory activity is largely due to its activity as an expectorant.

Stalks and stems

In many European cultures, the stalks and stems of the plant may be cooked alongside other vegetables. Chewing on the stalks and stems of angelica will stimulate one's immune response and induce a temporary anesthetic effect. Overall, use of *A. archangelica* tends to produce an overarching antidepressant and emotionally-stabilizing effect on the user, stemming from the strong inhibitory of activity of the plant towards acetylcholinesterase in the body.

Discussion

It's no wonder than *Angelica archangelica* was thought to be a magical plant by early users. The herb, impressive in both stature and remedy, serves traditionally as a digestive and respiratory aid. In current medical culture, the plant's anticarcinogenic and cerebral enhancement properties are being studied and so far, appear promising. It is interesting to note that although there are over thirty species belonging to the Angelica genus, Angelica archangelica is the only species considered useful for medicinal purposes (Bhat, et al., 2011). Conclusions from studies to date imply that instead of having a solitary active metabolite, the many terpenoids and coumarins present in A. archangelica work together in a synergistic fashion to produce many wonderful health benefits for the body. On a more solemn note, as a society, we must remember to be as environmentally responsible as possible. Due to a variety of biological factors, the population of the wild *A. archangelica* is become more at risk everyday. If research and pharmaceutical development is to be continued

on such a promising plant, we must rethink how we, as humans, are interacting with the most precious gift Mother Earth has given us: medicinal plants.

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Arctostaphylos uva-ursi (L.) Spreng., Ericaceae

Marston Jones

Introduction

Arctostaphylos uva-ursi (L.) Spreng. is a well-known plant in the Ericaceae family. It goes by many names due to its thorough distribution across the northern hemisphere, and because it has been widely used from North American and Canada, through Europe, and into Russia. Kinnikinnick, or bearberry, as it is most often referred to in the English language, grows in North America from northern California stretching up into Alaska, across Canada and the northern U.S., and even to New England and Newfoundland. On the western portion of the states, it follows the Rocky Mountains down into New Mexico, and can be found throughout the Appalachian mountains, even as far south as Georgia (Crane, 1991). The berries are a well known food source for bears, grouse, and deer (Kuhlein and Turner, 1991). This may be due to the fact that the fruit spoils slowly, so it is available throughout winter and into spring, which means it can be a constant food source when others are no longer available. It can withstand low summer moisture, salt-spray, and can grow slowly in semi-shady conditions. In some places it may also be a good indicator of areas that are moisture deficient, and grows rapidly in areas following moderate disturbances (Crane, 1991).

It was first reported for medicinal use in the thirteenth century, and throughout time has made its way into many pharmacopeias because of its wide range of uses. In 1601, it had reported use as a hemostatic, and by the time Western medicine began to flourish, beginning with Spanish and Italian physicians, bearberry found use for calculus complaints,

Figure 1. *Arctostaphylos uva-ursi* flowers. (Source: http://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+0506+1197)

bladder and kidney disorders, and urogenital disorders, though since then many more uses have been recorded (Kay, 1996; Radulovic et al., 2010).

Botanical Description

Arctostaphylos uva-ursi is a prostrate, evergreen shrub with dark green, leathery leaves and twisted trunks. The leaves are

obovate and smooth edged while the bark is thin and reddish brown. The plant flowers with white-pink urn shaped clusters, and is followed by red berries (**Figures 1 & 2**). The berries themselves are noted to be very dry and mealy when eaten. The plant can be found at altitudes from 3000 to 9000 feet, and often in open, rocky slopes and sandy areas. (Kuhnlein and Turner, 1991; Kay, 1996; Crane, 1991) Bearberry can be confused with both cowberry (*Vaccinium vitis-idaea* L., Ericaceae) and box (*Buxus sempervirens* L., Buxaceae), but while box does not share the same pharmacological constituents as bearberry, cowberry could potentially be used as a suitable substitution in certain cases (Radulovic, 2010).

Traditional Uses

Bearberry has been used over time for many different reasons. Much of the information available pertains to its use by Native Americans of North America, though its use is certainly not limited to one group of indigenous people or a single area. It's been noted to have appeared in the thirteenth century pharmacopoeia of the Welsh, "Physicians of Myddfai", in addition to being mentioned by Gerhard in Berlin around 1763 (McKenna et al., 2002). Its use continued to be recorded in many different locations for a variety of applications. One of the most widely mentioned uses of *A. uva-ursi* comes from its leaves. The leaves of the plant were used in smoking mixtures, either to be mixed in with tobacco to lessen the harsh taste, or to be toasted in an oven to be smoked alone (Kuhlein and Turner, 1991; Crane, 1991, Duke, 1985). Another name by which bearberry is known, Kinnikinnick, means smoking mixture, but the leaves of *A. uva-ursi* are generally used in conjunction with other plants and not just smoked by themselves (Willard, 1992). The smoke from the

Figure 2. *A. uva-ursi* fruits. (Source: http://calphotos.berkeley.edu/cgi/img_query?enlarge=9189+3301+3549+0113)

leaves was also used by some tribes to attract game and to treat earache (Pennachio et al, 2010). Some of its earlier uses were based on its astringent properties, and it was used as a wash for sores, to treat kidney pain, and as a tea for cold, chest, and lung conditions (Kay, 1996).

The fact that the berries of the plant are edible gave way to another simple use: food. In some instances it could be used as an "emergency food" because of its nature of lasting through winter, but it was also eaten in general by many North American tribes. The berries can be eaten raw, but because of their dry mealy texture, many recorded instances involve individuals roasting and cooking the berries, and even mixing them with animal grease (Kuhlein and Turner, 1991; Duke, 1985). There are many more advantages of the plant, such as using the leaves for tanning in Sweden and Russia, and using different methods for the treatment of backache,

bladder ailments, bronchitis, diabetes, diarrhea, dysentery, dysurea, fever, hemorrhoids, hepatitis, nephritis, pancreatitis, rheumatism, ulcers, urinary disorders and many other issues (Duke, 1985; McKenna et al. 2002).

Bearberry's long history of use as an anti-inflammatory and antiseptic has led to specific application that helps aid in strengthening the tone of urinary passages in response to inflammatory diseases. Many different Native American tribes would prepare an infusion for the urinary tract and also for venereal disease, with some even using the leaves as a poultice to for swelling and sprains (McKenna et al., 2002). Its use has continued up through the 19th and 20th centuries, being used widely now in homeopathic remedies for many of the aforementioned maladies (Duke, 1985).

Chemistry and Pharmacology

Bearberry contains phenols, tanning agents and flavonoids. The flavonoids can be used to chemically differentiate the taxa within the genus, and are may be different depending on the geographical origin of the plant (McKenna et al., 2002) Its main phenolic constituent is arbutin, its most notable compound (**Figure 3**), with a content level of about 5-18%. Its main flavonoid is quercetin-3-0-galactoside. *A. uva-ursi* also contains 6-40% tannin, methyl arbutin, ericinol, ericolin, allantoin, isoquercetin, gallic acid, malic acid, quinic acid, ursolic acid, ursone, ellagic acid, uvaol, hyperin, myricetin, myricitrin, corilagin, and pyroside, to name a few (Kay, 1996; Kenndler, 1990; Duke, 1985)

Biological Activity

For the current uses of bearberry, arbutin is the chemically active compound of importance. It has antimicrobial activities

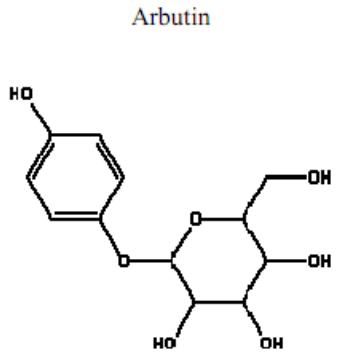

Figure 3. Structure of arbutin. (Source: http://ntp.niehs.nih.gov/files/Arbutin.pdf)

which is why, nowadays, the plant is used primarily as a treatment for urogenital diseases, or at least marketed as such. Many different things can be natural antioxidants: phenolic compounds such as flavonoids, phenolic acids, and tannins, nitrogen containing compounds such as alkaoids, chlorophyll derivatives, amino acids, peptides, and also carotendoids, tocopherols or ascorbic acid and its derviatves. Epidemiological evidence shows that eating items containing a large number of these antioxidant phytonutrients is very healthy (Amarowicz et al. 2004). Bearberry contains several compounds that fall under these categories, but another pharmacologically active compound of medical importance in this plant is hydroquinone, which is the result of *in vivo* glucoside cleavage of arbutin (Kenndler, 1990). The antimicrobial activity of the plant is dependent on arbutin being hydrolysed by β-glucosidase to yield hydroquinone which is an antiseptic and an astringent (Heinrich et al., 2004).

In a study performed by Radulovic et al. (2010) using GC and GC/MS analyses, 243 different constituents were identified in *Arctostaphylos uva-ursi* oils. Within the *A. uva-ursi*, α-

terpineol, linalool, hexdeconioc acid, and (E)-geranyl acetone were predominant. There were high levels of terpenoids, in addition to fatty acid derived compounds, and fatty acid ester and carotenoid derived compounds also made up a large portion of the oils. The study stressed that the essential oils contained in this plant are vital to the contribution of any medicinal properties, even though they may be found in very small amounts.

A study performed by Dykes et al. (2003) looked at the biological effects of antioxidants extracted from *A. uva-ursi* on food-related bacteria. The antioxidant activity in extracts is beneficial to human cells, and will hopefully have the potential to serve as a natural replacement to synthetic antioxidants for foodstuffs. Dykes et al notes that ethanolic extracts have already been found to have "potent antioxidant activity" in model and meat systems, and while this plant has been used traditionally as antiseptics in European countries, the use as an antioxidant in foods will be a new non-medical use for the plant. The study showed that the extract used affected the hydrophobicity of a very wide variety of bacteria, and hydrophobicity can be associated with increased pathogenicity and ability to invade human cells. While some bacteria actually increased in hydrophobicity, others, such as *S. aureus*, saw a substantial decrease. The authors caution against use of plant extracts as nutraceuticals, but support that bearberry does show antibiotic effects against various strains of bacteria.

Clinical Studies

In a clinical study performed by Larsson et al (2003), women who had been through multiple bouts of cystitis (at least three) before the study were treated with UVA-E or a placebo for a month. UVA-E contained a hydroalcoholic extract from the leaves of *Arctostaphylos uva-ursi* as well an extract from *Taraxacum officinale*, which provided mild diuretic effects. After being given three pills a day for a month of either UVA-E or the placebo, there was a significant effect on the recurrence of cystitis between the two groups. None of the women in the UVA-E group experienced a recurrence, whereas 5 of the women in the placebo group did. The researchers concluded that UVA-E had a significant prophylactic effect on recurrent cystitis, but also noted that it may only act on the most common microorganisms to be isolated in cases of urinary tract infections.

While there are not many clinical studies directly relating to the use of *A. uva-ursi,* there are studies looking at potential effects that arbutin, one of bearberry's primary constituents has on the human body, and the ways that the two interact. In a study by Blaut et al (2006), hydroquinone, a mutagenic and carcinogenic substance that is an aglycone of arbutin, was shown to be present in fecal slurries from several human subjects. The release of this aglycone due to glycosylation by intestinal species was determined to pose a potential risk, even though a large majority of arbutin (65-75%) is excreted in urine. Any potential dangers would also be directly related to large consumption of foods containing arbutin as well.

Contraindications

If making a tea from the leaves of *Arctostaphylos uva-ursi*, one should be careful not to extract using hot water to minimize the tannin content and avoid upsetting the stomach. Prolonged use is also cautioned against because of the high tannin content and the hydroquinones which can damage the liver in high doses (Kay, 1996). Very large doses containing hydroquinone are oxytoxic and can lead to collapse, convulsion, delirium, nausea, tinnitus and possibly death

(Duke, 1985). For the compounds to have antiseptic properties on the urinary tract, urine must be alkaline, so acidic foods should not be consumed during treatment (Heinrich et al., 2004).

Current Use in Allopathic and CAM therapies

Bearberry is recommended in homeopathy for cystitis, dysuria, hematuria, incontinence, pyelitis, urethritis, and urogenital disorders. Because arbutin shows antiseptic affects on the urinary mucous membrane, it is also used for cervical ulcerations, cystitis, blennorrhea, enuresis, gallstones, gonorrhea, gout, and nephritis (Duke, 1985). Willard (1992) even suggests soaking feet in a strong decoction of the plant for one hour the night before a long hike or walk to "toughen up the feet" and to avoid blisters. Other than its advantages in treating urinary tract infections, many of the uses of *A. uva-ursi* are yet to be fully investigated scientifically (McKenna et al. 2002). There are plenty of available products that contain bearberry ranging from simple supplement and vitamin items to skin care products (**Figure 4**).

Figure 4. Uva ursi products. (Source: http://www.herbalremedies.com/uva-ursi-standardized.html ; http://www.herbalremedies.com/17600.html ; http://www.johnmasters.com/osc.htm)

Discussion

For a small shrub, *Arctostaphylos uva-ursi* has been utilized in an incredibly large variety of ways for centuries. This is due in part to its abundance in the northern hemisphere, and as a result of record keeping of its use throughout history. Its many different names are evidence of it widespread influence and use, as well as its efficacy. Other than research showing its ability to aid against urogenital problems, few studies have been done to verify the many different ways that the plant has been utilized in the past, but the fact that so many uses exist stands as a testament to the versatility of this plant. It continues to be used in homeopathic remedies, and can still be easily found in many of the regions where it has grown for years. Some characteristics about the plant, such as its astringent, anti-inflammatory, and antiseptic properties have been well studied, but there is still much to learn, and ways to make sure its use either as a strictly homeopathic or maybe even allopathic remedy are safe and viable.

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Armoracia rusticana G. Gaertn., B. Mey. & Scherb., Brassicaceae

Andrew Mongue

Introduction

Armoracia rusticana, more commonly known as horseradish, is a member of the Brassicaceae family which includes broccoli, turnips, and mustard (Sampliner & Miller, 2009). The name *Armoracia* comes from Celtic and roughly means "near the sea." Another old name, searadish, furthers the idea that this plant was traditionally found near the coast. The German for searadish was 'meerettich' and it has been proposed that the name horseradish resulted as a mistranslation confusing 'meer' (sea) for 'mare' (a female horse) (Courter & Rhodes, 1969). However the common name came to be, it is rather ironic because horseradish is toxic to horses, pigs, and cattle. Despite the widespread occurrence of *A. rusticana* in the United States however, reports of livestock poisoning are rare, perhaps do to the pungent taste of the plant (DiTomaso).

This plant derives its famous smell and taste from a class of compounds known as isothiocyanates, which are also responsible for much of its biological activity and therefore important in its medicinal uses (Nedorostova et al., 2011). These chemicals are natural antibiotics with broad ranging applications from the treatment of sinus infections to the preservation of food.

The other major class of chemicals is the peroxidase enzyme family found in horseradish. These compounds have a range of uses in both laboratory and industrial settings. They can be used in everything from waste treatment to chemotherapy (Govere et al., 2005; Greco, Folkes, Wardman, Tozer, & Dachs, 2000).

Figure 1. Whole view of a horseradish plant. Image source: http://www.finecooking.com/assets/uploads/posts/5645/ING-horseradish-2_sql.jpg

For centuries *A. rusticana* has been recognized as a source of food and medicine, though its uses have changed through time. During its history of use the plant we know today was created through directed selection (commonly known as

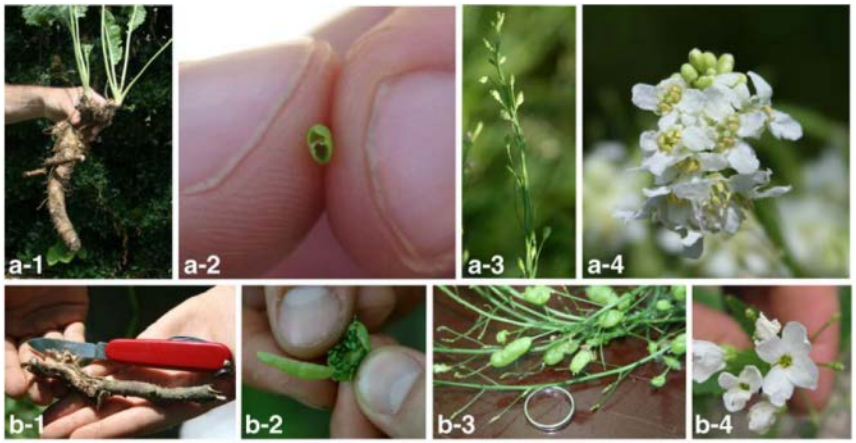

Figure 2. Fe**atures of *A. rusticana* (a) compared to the closely related *A. macrocarpa* (b).** Note the lack of seeds in a-2 compared to b-2 and the increased root size in a-1 compared to b-1. Image adapted from Sampliner & Miller, 2009.

domestication); however its status is now in doubt because of its apparent inability to produce seeds (Courter & Rhodes, 1969). As discussed below, despite the continued apparent success of horseradish as a crop, the method of its cultivation could lead to problems in the future. This cannot be allowed given all of the applications botanists are just now discovering for this versatile plant.

Botanical Description

Though *Armoracia rusticana* is now grown on several continents worldwide, it is believed that horseradish most likely originated in Europe. Wild populations in America began to grow in Boston in 1840 and were likely the result of previously cultivated plants being abandoned and left to propagate on their own (Courter & Rhodes, 1969). Outside of cultivation it grows best in wet, disturbed areas such as fields and along roadsides.

A. rusticana, like all members of the *Armoracia* genus, is a perennial herb. Horseradish has smaller flowers than other members of its genus, but like all *Armoracia* species, its flowers are four-merous and white in color. Its hairless, crenulated leaves are arranged in a rosette and grow basally (**Figure 2**). Despite continued fruit production, it is unclear whether this species has retained its non-vegetative reproductive ability. Since its domestication it has been propagated almost exclusively from root cuttings and many *A. rusticana* fruits in domestication lack seeds entirely (Sampliner & Miller, 2009). One suggested reason for this apparent sterility is the possibility that domestic horseradish is an interspecific hybrid of two true species. The observation that chromosome count varies between individuals and even within an individual plant (leading to partial chromosomal pairing) supports the hybrid theory (Courter & Rhodes, 1969).

Alternatively, some researchers now believe that horseradish as we know it, *A. rusticana*, is nothing more than a domesticated form of other wild *Armoracia* species. Evidence for this is mostly negative, that is to say, despite surveying efforts no *A. rusticana* specimens have been recorded in the wild. They can be found only in areas associated with current or previous human cultivation. Some possible positive evidence is the relative root size of horseradish in comparison to other *Armoracia* species. *A. rusticana* roots are much larger than those of related herbs, suggesting that this trait may have been cultivated by human rather than natural selection (Sampliner & Miller, 2009).

The sterility phenomenon was examined by experiments conducted on seed production in various American cultivars

of horseradish. Plants from various backgrounds (i.e. commercial farming and untended "wild" locations) were collected and allowed to flower. In the overwhelming majority of cases the plants could not produce functional pollen grains. In those that did, no more than 50% of pollen was viable, and those plants were effectively incapable of producing viable seeds through self-fertilizing. Outcrossing between the two varieties collected (Bohemian and common) had the greatest success in securing seed but even then only one to two seeds were obtained per fruit. When planted, these seeds successfully germinated and grew, suggesting that there is hope for restoration of sexual reproduction (Weber, 1949).

Traditional Uses

References to *Armoracia rusticana* by Dioscorides place its first confirmed use to roughly two thousand years ago. While Europe has a long history of using this plant, cultivation in America can only be traced back to 1806. The now robust agricultural production of horseradish can be specifically linked to the Sell and Sass families who immigrated from Germany to Chicago in the 1850's (Courter & Rhodes, 1969). To this day, the plant is a successful commercial crop and is still grown throughout Illinois (Walters & Wahle, 2010). Typically, only the taproot is sold commercially while the smaller offshoot roots are used as propagates for next year's crop (Courter & Rhodes, 1969).

The now gigantic condiment business started by Henry Heinz also has its roots with *A. rusticana*. The would-be condiment king grew up in Pennsylvania, which by this time was recognized as a fertile ground for horseradish. At the beginning of his career in the mid-eighteen hundreds, foods like horseradish were either grown by individuals or by small vendors who then bottled and sold preserves locally in green or brown glass jars. Anecdotal reports of other plants and even wood chips used to pad out the contents of these jars speak to a general lack of quality in the condiment market at the time.

The key then to Heinz's success was in the way he preserved the condiment. By boiling the preserves in clear glass jars in salt water after packing he could boast a superior quality that customers could clearly see. Additionally, this sterilizing process meant he could ship goods to more distant markets and eventually become a national and then international name brand. Though Heinz today is synonymous with tomatoes and ketchup it is important to note that it all started with horseradish (Koehn, 1999).

In cooking, horseradish has been used as a flavoring condiment on everything from meats to salads. German recipes including *A. rusticana* can be found as early as the 1500's (Courter & Rhodes, 1969). The root can be grated directly onto food or mixed with ketchup, vinegar, or oil to form a sauce. In some cases the root is boiled, squeezed, washed, and mixed with salt and sugar to remove some of the isothiocyanates which cause its pungent taste.

Leaves and roots of *A. rusticana* are used in the process of pickling other vegetables, which allows them to be stored for much longer periods of time. In some places it is still the preferred method of generating a stock of food for the winter. The plant is also fermented to produce a Romanian brandy called Tuica (Sampliner & Miller, 2009).

Horseradish has a long tradition of ritualistic use as well. It is one of the traditional plants eaten during Jewish Passover.

English herbalist John Gerard studied horseradish in the sixteenth century. His medical uses included treatment of colic and sciatica. It was also reported as a diuretic and an

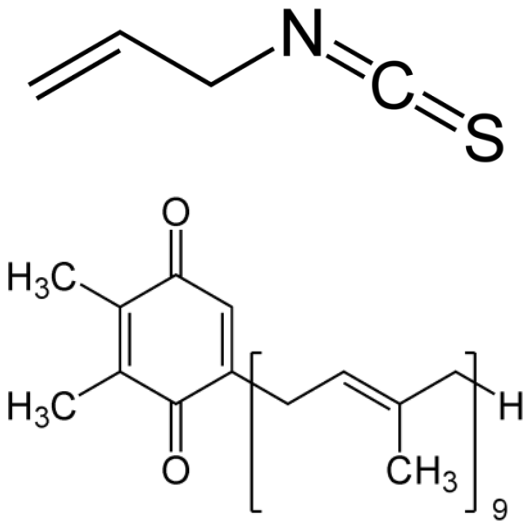

Figure 1. **Structure of allyl isothiocyanate (top) and plastiquinone-9 (bottom).** Images source: http://upload.wikimedia.org/wikipedia/commons/9/98/Allyl-isothiocyanate-2D-skeletal.png and http://upload.wikimedia.org/wikipedia/commons/3/3b/Plastoquinone.png

aphrodisiac. *A. rusticana* was also used to kill parasitic worms in children and promote the expulsion of afterbirth (Courter & Rhodes, 1969). While its agricultural uses have remained largely unchanged, today's medical uses are quite different from those described above.

Chemistry and Pharmacology

The essential oils of *Armoracia rusticana* consist almost entirely (i.e. nearly 98%) of allyl isothiocyanate (Chen, Akinkurolere, & Zhang, 2011). This compound, along with butyl isothiocyanate and phenylethyl isothiocyanate are responsible for the distinct aroma of the plant (Courter & Rhodes, 1969; Nedorostova et al., 2011).

The peroxidase enzymes found naturally in *A. rusticana* can be used to screen for the presence of phenolic compounds in water samples (Gonzalez-Sanchez, Laurenti, Rubio-Retama, Valero, & Lopez-Cabarcos, 2011). Additionally peroxidase has been proposed as part of the treatment for bladder, breast, and nasoesophageal cancer (Greco et al., 2001).

Three more compounds from horseradish (plastoquinone-9, see **Figure 3**; 1,2-dilinolenoly-3-galactosylglycerol; and 6-O-acyl-β-D-glucosyl-β-sitosterol) have been implicated in cancer inhibition (M. J. Weil, Y. Zhang, & M. G. Nair, 2005).

Biological Activity

Enzymes in the peroxidase family have a range of applications as oxidizing agents and can be efficiently recovered (up to 72% yield) from relatively simple extraction and purification methods applied to *Armoracia rusticana* roots (Lavery et al., 2010). There have also been studies showing that certain genetically modified moth larva can be made to produce peroxidase, thus providing an alternative source with similarly high yields (Romero, Targovnik, Wolman, Cascone, & Miranda, 2011).

One example of horseradish peroxidase use is in the farm industry. Here it has been proven effective as a deodorant when added to livestock manure. It removes malodorous phenolics for up to three days per application (Govere et al., 2005).

Allyl isothiocyanate (**Figure 3**), an essential oil of *A. rusticana*, has shown promise as a natural fumigant that is lethal to at least two common crop pests, weevils and meal moths. It has been proposed that this gaseous compound suffocates insects

by blocking their spiracles and thus preventing oxygen from reaching their internal tissues. This hypothesis is supported by the observation that allyl isothiocyanate is effective against adult weevils but not to their pupae, which are less permeable to gases due to their structure (Chen et al., 2011).

Essential oils from *A. rusticana*, composed of allyl isothiocyanate along with the related phenylethyl isothiocyanate, were tested as gaseous antibiotics in an *in vitro* study alongside extracts from several other plants, including garlic. Researchers found the isothiocyanates to be the most effective oils tested (as determined by the minimum inhibitory concentration), effective against even MRSA *Staphylococcus aureus*. As such, it could have applications as a nontoxic disinfectant spray pending clinical trials (Nedorostova et al., 2011).

Finally with regard to isothiocyanates, there is some evidence that these phytochemicals may have applications as a preservative. One study found that adding no more than 300 parts per million of isothiocyanates, distilled from *A. rusticana* root, to commercial grade tofu was enough to significantly decrease bacterial growth and increase shelf life of the product (Shin et al., 2010). This idea is particularly appealing since isothiocyanates are organic and nontoxic to humans at such concentrations.

Laboratory studies suggest that the roots of *A. rusticana* are quite effective at removing harmful substances from the soil. By adding phosphate to soil containing uranium, the roots were able to absorb 98% of the heavy metal and even without the phosphate assistance the plants removed more than 80% of the uranium (Soudek, Petrova, Benesova, & Vanek, 2011). In another experiment, horseradish root completely depleted soil concentrations of the pharmaceutical acetaminophen, making it a good candidate for use in phytoremediation, the use of plants to deal with environmental contamination (Kotyza, Soudek, Kafka, & Vanek, 2010).

The aforementioned anti-cancer potential of horseradish comes from two different mechanisms. First are the inhibitory effects of plastoquinone, sitosterol, and galactosylglycerol on the cyclooxygenase-1 (COX-1) enzyme in cancer cells. When purified, these compounds showed 50 to 75% inhibition of the COX-1 enzyme and growth inhibition in both lung and colon cancer cell lines (Marvin J. Weil, Yanjun Zhang, & Muraleedharan G. Nair, 2005).

The second approach to cancer treatment uses the nascent technique of gene therapy. Researchers found that by inserting the gene for the already discussed peroxidase enzyme, also known as horseradish peroxidase (HRP), into bladder cancer cells, these cells became susceptible to the otherwise harmless plant hormone indole-3-acetic acid (IAA). Cancer cell DNA became condensed and fragmented, ultimately leading to cell death (Greco, Dachs, Tozer, & Kanthou, 2002). This treatment shows promise because IAA is a long lasting chemical that can cross cell membranes, but it is not inherently toxic. Thus it would not produce the deleterious side effects of many chemotherapies (Greco et al., 2000).

A follow up study expanded the treatment using HRP to treat two other cancer cell lines, with more promising results. This study also noted that successful treatment could be obtained through proportionally low successful integration of the target gene. Even when only 5% of cells expressed HRP, up to 90% of cancer cells were killed by IAA treatment. Additionally, researchers found that by modifying IAA slightly, they could achieve better results, indicating great potential for this treatment. Like most gene therapies however, HRP therapy is

not yet in clinical trials and has to undergo more testing before reaching public use (Greco et al., 2001).

Clinical Studies

A double blind study in Germany examined the use of *Armoracia rusticana* as an ingredient in an herbal treatment for urinary tract infections. Participants took two doses a day for a three month period. At the end of the study it was found that patients on the *A. rusticana* derived treatment, marketed as Anti-Infekt N, had a significantly lower recurrence of urinary tract infections with no adverse side effects thus making it a safe and effective treatment option (Albrecht, Goos, & Schneider, 2007).

Another study using the same drug, Anti-Infekt N, and a much larger pool of participants also found it to be as safe and effective in treating sinusitis and bronchitis as traditional antibiotic regiments. That is, both treatment groups saw comparable reductions in symptoms (around 80% in both treatments) at the end of the study (Goos, Albrecht, & Schneider, 2006). It is worth noting that both the respiratory and urinary tract infection treatments are traditional applications as well, demonstrating that traditional knowledge has much to offer modern medicine.

Contraindications

While the human trials above showed no harmful side effects, in vitro studies involving rats suggest that consuming more than eight milligrams per kilogram of the isothiocyanates found in *Amoracia* rusticana on a regular basis can cause bladder lesions (Hasumura et al., 2011).

Since Hasumura et al.'s study found no other side effects and the dosages were much higher than the dosages in Albrecht et al.'s study, the results of the Hasumura study should not be seen as evidence against the therapeutic use of *A. rusticana* taken at typical or traditional quantities.

Of note for the cancer inhibition research, the same scientists who found cancer inhibiting effects also found that another horseradish compound, desulfosinigrin, actually promoted proliferation of colon and lung cancer cells (Weil, Zhang, & Nair, 2004). As mentioned in their later study with COX-1 inhibitory enzymes, *A. rusticana* possesses cancer inhibiting chemicals as well. The authors suggest that in typical culinary use these two effects balance each other out and result in no effect on cancer risks (M. J. Weil et al., 2005).

Finally, as a culinary and supplement note, like most members of the Brassicacae family, horseradish has been classified as a goitrogen. Consumption of raw goitrogens leads to decreased thyroid function which can cause goiters over a period of time (Robbins, 2007). The chemical culprits here are the abundant isothiocyanates which are part of a broader class of known goitrogens: thiocyanates (Keshteli, Hashemipour, Siavash, & Amini, 2010). Additionally as discussed with relation to the origin of the common name, horseradish is toxic to some domestic animals and should thus be separated from livestock if grown in their presence (DiTomaso).

Current Uses in Allopathic and CAM Therapies

Armoracia rusticana has both internal and external historical use as a remedy and continues to be used as such in some European countries. For rheumatic pain and headache, grated horseradish roots or leaves are wrapped in cloth and applied to the skin at the site of pain. The same preparation is used in

treating sinusitis with the cloth being placed on the throat. For internal use it is often mixed with other ingredients like honey or vinegar and sugar and ingested to treat cough or high blood pressure, respectively. In some countries, like Romania, it is common to eat a teaspoon of *A rusticana* daily prophylactically (Sampliner & Miller, 2009).

As a western dietary supplement, horseradish is sometimes sold as a powder or pill, made by grinding up roots which can sell for over seventy dollars per pound. It is marketed in much the same ways it has been historically used. It is a purported to be beneficial to the lungs and sinuses as well as kidneys and bladder. In addition, *A. rusticana* supplements are said to increase blood flow to extremities. Warnings attached to this product include specific mention that individuals with thyroid problems should not take horseradish supplements (Plus, 2011). As a liquid extract horseradish root is dissolved in water and alcohol; it can sell for seven dollars per ounce and is marketed as a treatment for urinary and respiratory tract infections. Additional claims include relief from gout, indigestion, rheumatism, and influenza (Creek).

Discussion

Human domestication of plants like *Armoracia rusticana* has significantly altered them, both phenotypically and genotypically. Most immediately obvious is the increase in size of consumable plant parts like flowers, fruits, leaves, or roots depending on the plant (compare the roots of the now predominantly if not exclusively domestic *A. rusticana* with the still wild *A. macrocarpa* in **Figure 2**), but domestication also has the effect of bottlenecking the gene pool for the targeted species. Domesticated crops show a decrease in genetic diversity compared to wild plants (Doebley, Gaut, & Smith, 2006).

As mentioned above, the apparent decrease in fecundity in *Armoracia rusticana* may be a result of a drastic loss of genetic diversity. In cultivation, this problem is circumvented by root propagation with great success (Sampliner & Miller, 2009); however, it should be noted that this method essentially creates clones of the main plant and can only ideally preserve or more realistically decrease, but not increase, genetic diversity. From an evolutionary point of view, this implies that as an agricultural crop horseradish is highly vulnerable to disease since any disease able to infect one plant would necessarily be adapted to infect all the plants propagated from that plant. Indeed there are reports that this is the case; some estimates claim effectively 100% of one *A. rusticana* variety is susceptible to mosaic viruses. It follows that increased efforts to use sexually produced plants (i.e. those from seeds rather than root cuttings) would lead to greater disease resistance and benefit the farming industry (Weber, 1949) , but given the current state of the species, this method of farming would not be profitable for some time.

With less variation now it may be harder to select for preferable medicinal or culinary traits. Luckily, the plant in its current state already has a wide range of applications from food preparation to pain relief. Up until now, horseradish has been grown largely for food and minor remedies. However recent research shows that it has great untapped potential.

The essential oils in *A. rusticana* may become another useful tool in the arsenal used to fight drug resistant bacteria and insects. This idea is particularly appealing because the extract of *A. rusticana* is topically nontoxic, making it well suited for use in situations where it could come into contact with people. Isothiocyanate sprays could be used as hospital disinfectants and organic pesticides on food crops in the field. After harvest, perishables could be treated with isothiocyanates to increase their shelf life, leading to greater distribution possibilities and

potentially less wasted food. The issue of thyroid suppression linked to thiocyanates means that the isothiocyanate products would have to be regulated and monitored but their potential should not be left unexplored.

The live plant itself has proven to be a versatile clean-up tool, absorbing heavy metals and pharmaceuticals from the soil. Given this, horseradish could be introduced in industrial accident sites to help dispose of contaminants. In addition, it could be planted downstream from medical facilities to limit the spread of antibiotics and therefore antibiotic resistance to the surrounding ecosystem. The odor blocking activity of the peroxidases combined with the antibiotic properties of the isothiocyanates make *A. rusticana* a good choice for managing animal waste in farm settings. While it is true that horseradish is toxic to some farm animals, using it in a cleaning capacity would be safe as long as the animals were kept away from the waste treatment, which they should be anyway.

There is no doubt that horseradish will continue to enjoy popularity as a condiment; see, for instance, the twenty five year history of the International Horseradish Festival (Davis). Additionally, as mentioned above, it has yet unexploited uses that could benefit a variety of fields, both medicinally and agriculturally. For both of these reasons it is important that this plant is maintained as a viable species, with enough genetic variation to reproduce on its own and survive attacks from disease and changing environmental conditions.

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Artemisia absinthium L., Asteraceae

Michael Clanahan

Introduction

Artemisia absinthium L., commonly known as wormwood, green ginger, or wermot, has been used for thousands of years in a variety of fashions. Although most famous for its role in the production of the alcoholic drink Absinthe, wormwood has played a major part in traditional medical practices in multiple cultures. A few main components of the plant that have been isolated include thujone, absinthin, caffeoylquinic acids, phenols, and select flavonoids, all of which have different physiological effects and in some cases, beneficial health properties.

Traditionally, this plant has been used to treat many different ailments from diabetes to stomachaches to tuberculosis. Used all over the world, this powerful plant has faded in and out of popularity, but continues to have significant medicinal value in many areas.

Botanical Description

Morphologically, wormwood is very similar to other plants in the Asteraceae family. This herb has a perennial root system, and has characteristic firm, branched stems that are woody at the base (Grieve, 2011). Typically, the plant reaches no more than two to three feet, as it is found mainly in arid climates (Arnold, 1989). The leaves of the plant are whitish on both sides, but also contain silvery gray sheen due to short, fine hairs on the indented outside. This sheen can be seen in **Figure 1**. Wormwood is a flowering plant, identifiable by its small globular flower bundles of greenish-yellow tint, mainly

Figure 1. Characteristics sheen of *Artemesia absintium* L. leaves. (Source: http://www.flowertropes.com/index/tag/kevin-kline)

between the months of July and October. (Grieve, 2011). Unlike many other plants of the Asteraceae family, the small wormwood fruits do not contain a crown of hairs, also called "pappus" (Grieve, 2011). Although the leaves and flowers of the plant taste very bitter, the roots have a characteristic aromatic taste. The flowers smell of thujone, an important compound with medicinal values will be discussed. (Grieve, 2011).

Closely related plants include Roman wormwood (*Artemisia pontica*), *A. judaica*, Sea Wormwood (*A. maritima*), as well as

the herb Tarragon (*A. dracunculus*) which is also known commonly as dragon's-wort (Grieve, 2011). Tarragon is found in many spice gardens, and Southernwood (*A. abrotanum*) is found primarily in border habitats between fields. The entire family is characterized not just by physical likeness, but most importantly the intense bitterness contained in all parts of the plant (Grieve, 2011).

Traditional Uses

Wormwood is one of the oldest known herbs to be used in traditional medicine, with evidence tracing its usage back to ancient Egyptians. Wormwood's appearance in the Ebers Papyrus dating from 1550 B.C. support this claim and indicate not only medicinal value, but also religious significance (Arnold, 1989). Another important historical medical document, *Historia Naturalis*, written by Pliny in the first century A.D. mentions wormwood in a medical context that shines light on the common name of the plant. The encyclopedia describes wormwood extract as having powerful effects on gastrointestinal worms, often ridding the human of a large part of the parasitic load (Arnold, 1989). Dioscorides' *De Materia Medica*, completed around 65 A.D., also mentioned wormwood's anthelmintic properties, but included many more applications. These include applications such as a natural insect repellant and a natural antidote to poisoning by hemlock and toadstools (Grieve, 2011). The leaves were commonly dried and stored with garments and furs to protect them from moths (Arnold, 1989). *De Materia Medica's* authority for over 1500 years is illustrated by the fact that John Gerard, a highly revered Elizabethan herbalist in the 1500s, mentions in his book *Herball* that "wormewood voideth away the wormes of the guts" (Mann, 2009).

Wormwood was utilized by other cultures, such as traditional practices in modern day Turkey. In the Kirklareli Province, wormwood is known locally by many names, including pelinotu and pelin. People in this particular area would use almost all of the parts of the plant to treat different ailments. For example, the arial parts, when prepared as an infusion could be used as an abortifacient, a cure for stomach ache, and appetizer, or a blood depurative. However, when prepared as a decoction and consumed 1-2 times a day for 7-8 days, it could be used to treat diabetes, uterine cysts, and tuberculosis. Also, by cutting the shoots into small pieces and eaten two times a day for 8 days, it is believed to treat malaria (Kültür, 2007). In traditional Caribbean medicine, the plant is used primarily for women's health. This includes treatment for menstrual cramps and vaginitis (Lans, 2007). Wormwood even plays a part in traditional Chinese medicine (TCM) as a treatment for bacillary dysentery (Zhang, 2005).

Wormwood is traditionally most famous for its role in the production of the alcoholic beverage, absinthe. Although used medicinally all over the world for centuries, its narcotic effects did not become significant until around 1790 in Switzerland. Pierre Ordinaire, a French refugee and physician, created an extract of wormwood for use as a stomach tonic. Due to its characteristic green tint and reported hallucinogenic properties, the drink quickly became known as *La Fée Verte*, or The Green Fairy (Lainer, 1995). Early in the 19th century, the drink recipe was acquired by a Frenchmen named Major Dubied and his son-in-law, Henry-Louis Pernod. Pernod would eventually take over the company, and his name became historically attached to absinthe (Lainer, 1995). At this point, the drink became associated more with social functions than the medical world. Because of its high price originally, only those in certain circles could afford it, and this included the world of the artists (Lainer, 1995). A few artists known to

have partaken in the consumption of absinthe were Baudelaire, Rimbaud, Picasso, and most famously, Vincent Van Gogh (Ashcraft, 2011). Van Gogh may have been addicted to absinthe in his lifetime. Evidence also suggests that this addiction aggravated his psychosis and that the hallucinations he witnessed stemming from thujone may have caused him to commit suicide (Arnold, 1989). Other artists couldn't get enough. Oscar Wilde once wrote, "What difference is there between a glass of absinthe and a sunset?" (Ashcraft, 2011).

As the drink became more accessible, the general public began to take part. The French public consumed an estimated 700,000 liters of absinthe in 1874. However, due to increased popularity and a severely decreased wine-grape harvest (phylloxera infestation), by 1900, the number of liters consumed surpassed 36 million (Ashcraft, 2011).

Absinthism emerged as a serious disease around this time due to excessive public consumption. Characterized by dazed victims, as well as incredibly vivid hallucinations, absinthism became a serious problem towards the end of the 19th century, and also became a point of attack for those opposing the absinthe business (Arnold, 1989). Some suggested raising the taxes on absinthe to reduce addictions within the population. When this proposal failed, another bill was brought forward and ultimately passed in 1908 that increased the amount of alcohol in absinthe. The explanation for this was that absinthe with a higher degree of alcohol was healthier and that the requirement for higher alcohol content would eliminate those producers who used artificial essences with lower standards of purity," as this was thought to be the reason for some of the addictions (Lanier, 1995). It would not be for another 6 years until the sale absinthe was banned on August 16th, 1914. This was done as an "emergency measure" to stem alcoholism, but absinthe continued to circulate in the public. In January 1915, a presidential decree was issued, explicitly banning absinthe

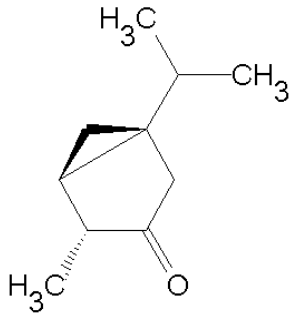

Figure 2. Chemical structure of thujone. (Source: http://www.rdchemicals.com/chemicals.php?mode=details&mol_id=8286)

(Lanier, 1995). Although the fear of alcoholism and absinthism was greatest in France, several countries banned absinthe before France, including Switzerland in 1908, The United States in 1912, and Italy in 1913 (Lanier, 1995).

In 2007, the effective ban was lifted when the Alcohol and Tobacco Tax and Trade Bureau (TTB) redefined the restrictions on the drink. Today, one can buy absinthe in liquor stores, but with one major difference: it is thujone-free. The FDA requires that all thujone be filtered out of the final product before distribution to the public. In order for absinthe to be "thujone-free," it must test for lower than 10ppm (CFR, 2007).

Chemistry and Pharmacology

Wormwood has a rich variety of present compounds. The major constituent is thujone (absinthol), a terpene, which is toxic if ingested in sufficient quantities. Thujone, characterized by its menthol-like odor, is commonly credited with causing the convulsion episodes anecdotally reported to be associated with Absinthe consumption. The structure of thujone can be seen in **Figure 2**.

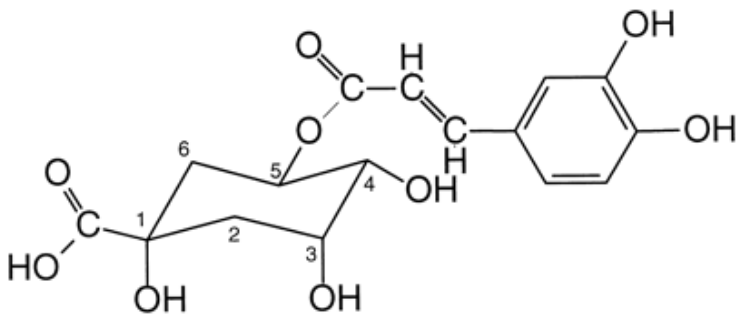

Figure 3. Chemical Structure of 5-caffeoylquinic acid.
(Source: http://www.39kf.com/cooperate/qk/American-Society-for-Nutrition/037804/2008-12-28-551072.shtml)

Wormwood's overpowering bitter taste can be directly attributed to its absinthin content. This sesquiterpene lactone has a bitterness threshold of one part per 70,000. In other words, a single ounce can be detected in 524 gallons of water (Arnold, 1989). Interestingly, the King James Bible mentions the bitterness of wormwood a few times. This occurs a few times in the Old Testament, most notably in the book of Proverbs: "But her end is bitter as wormwood, sharp as a two-edged sword" (AV Prov.5:4). Wormwood is again specifically mentioned by name in The Book of Revelations as part of the rapture: "And the third angel sounded, and there fell a great star from heaven, burning as it were a lamp, and it fell upon the third part of the rivers, and upon the fountains of waters; And the name of the star is called Wormwood: and the third part of the waters became wormwood; and many men died of the waters, because they were made bitter" (AV Apoc. 8:10-11). Although the biblical source is accurate in depicting wormwood's intrinsic bitterness, there is no evidence to support its lethality.

Wormwood also contains important compounds called phenols, which have been linked to antioxidant activities. According to Mahmoudi group, wormwood contains 194.9 ± 9.7 mg gallic acid equivalent/g of extract. Polyphenols, or more specifically flavonoids, have also been shown to exhibit antioxidant activities. Total flavonoid levels in *A. absinthium* are 12.4 ± 0.6 mg quercetin equivalent/g of extract, a promising value (Mahmoudi, 2009). It is thought that the antioxidant properties of many phenols and flavonoids may decrease incidence of certain human diseases when the consumption of foods rich in these compounds is increased (Mahmoudi, 2009).

Caffeoylquinic acids contained within wormwood are a relatively new discovery in the scientific community. 5-caffeoylquinic acid is one of the most abundant within *A. absinthium*, and also goes by the name of chlorogenic acid (Fiamegos, 2011). **Figure 3** depicts the structure of 5-caffeoylquinic acid. Studies suggest 5-caffeoylquinic acid is a potent antimicrobial compound, as well as an effective biofilm reducer (Fiamegos, 2011).

Biological Activity

Thujone may be the most important, as well as controversial compound contained within wormwood. Its method of action is thought to be a reduction of GABAA receptor activity, thereby reducing serotonergic responses (Deiml, 2004). Because thujone reduces GABAA receptor activity, neuron-firing inhibition is reduced, allowing neurons to fire more frequently. This is thought to cause the convulsions anecdotally and scientifically reported (Höld, 2000). As stated before, wormwood was traditionally used as an anathematic. Thujone is the compound responsible for this anti-parasitic activity. Although thujone is a toxic substance, and can be

lethal as high doses, ethanol appears to protect against lethal effects of thujone, which may have implications for thujone content in the popular drink, Absinthe (Höld, 2000).

As discussed before, the phenolic and flavonoidic contents of *A. absinthium* exhibit important antioxidant properties. Oxidizing compounds, such as H_2O_2 (hydrogen peroxide) and NO (nitric oxide), can cause severe damage within the human body due to the propagation of free radicals. Although not fully understood, flavonoids exhibited strong reducing potential. When compared to a known reducing (antioxidant) compound, in this case Vitamin C, a methonalic extraction of wormwood flavonoids showed no significant difference in absorbance levels (Mahmoudi, 2009).

Phenolics, including quercetin, may have a direct effect on H_2O_2 scavenging, thereby resulting in the termination of the free radical chain reaction (Mahmoudi, 2009). The structure of quercetin, a polyphenolic, is shown in **Figure 4**. Quercetin showed high antioxidant activity, or high H_20_2 scavenging activity, as its IC_{50} was 17.01 ± 0.03μg/ml. Hydrogen peroxide as a compound does not have a high rate of activity, but can sometimes create hydroxyl radicals within the body (Mahmoudi, 2009). Wormwood phenolics were also extremely effective in chelating Fe^{2+}, a transition metal capable of free radical generation from peroxides, with an IC_{50} value of 419 ± 20.95μg/ml (Mahmoudi, 2009).

A study from the Ahmad group showed that methanolic wormwood extractions exhibited strong analgesic properties using the tail immersion method in mouse model. Analgesic properties were detectable in all three tested dose levels (300, 500, and 1000 mg/kg), of which the results were dose dependent (Ahmad, 1992). When compared to the standard acetylsalicylic dose of 300 mg/kg, the wormwood extract showed a significantly faster onset of analgesic effects, but

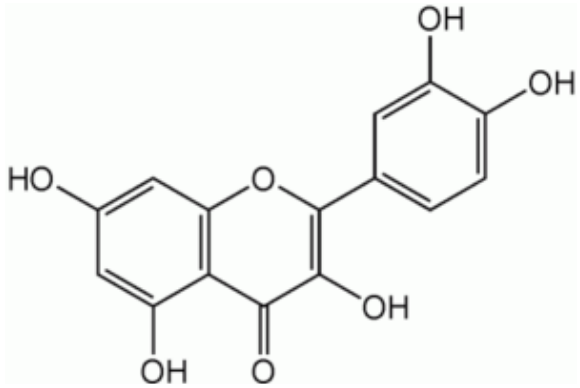

Figure 4. Chemical structure of quercetin. (Source: http://www.worldofmolecules.com/antioxidants/quercetin.htm)

ultimately this analgesia was less potent overall (Ahmad, 1992). This same group then studied anti-inflammatory effects of the methanolic extract to conclude that it was effective, but also dosage-dependent. The 1000 mg/kg dose was tested using the hind paw edema method in a mouse model, and again exhibited significant activity. Results showed a peak activity at 3 hours after administration, and a resulting 41% inhibition in increased paw volume (Ahmad, 1992). Again, however, this activity was much less intense and lasted a shorter period of time, as well as a delayed response in anti-inflammation (Ahmad, 1992). Although these effects were exhibited, the exact compounds responsible for the medicinal actions are unknown.

A method of action is unknown for the methanolic wormwood extraction resulting in antidepressant effects in the mouse model. For this experiment, a forced swimming test (FST) was used. The results showed a remarkable decrease in duration of immobility, and were dose dependent (Mahmoudi, 2009). These results can be seen in **Table 1**. The results for the 1000 mg/kg wormwood extraction dose are very similar to the 5

Table 1. Effect of methanol extract of *Artemisia absinthium* L. on the duration of immobility during forced swimming test and tail suspension test.

Group	Dose (mg/kg)	Duration of immobility (s), FST	Duration of immobility (s), TST
Control	-	150.2 ± 8.9	159.6 ± 12
Artemisia	125	130.8 ± 8.2*	93.4 ± 1.2*
Artemisia	250	111.6 ± 5.1*	83.2 ± 1.4*
Artemisia	500	49.2 ± 2.3*	71.2 ± 2.4*
Artemisia	1000	21.0 ± 0.9*	---------
Imipramine	5	21.6 ± 0.7*	---------
Imipramine	10	14.4 ± 0.5*	73.8 ± 4.2*

*ANOVA followed by Newman–Keuls multiple comparisons test shows that all test groups are significantly different from control group (P<0.001). Values are mean ± SD (n = 10).

Table 1. Effect of methanol extract of *Artemisia absinthium L.* on the duration of immobility during forced swimming test and tail suspension test (Mahmoudi, 2009).

mg/kg dose of the antidepressant imipramine, a known seratonin (HT-5) and norepinephrin reuptake inhibitor (Mahmoudi, 2009).

As mentioned before, *A. absinthium* extracts contain a significant amount of 5-caffeoylquinic acid (5-CQA). Recent developments in antimicrobial studies have pointed towards bacterial efflux pumps. Efflux pump inhibitors (EPIs) effectively stop bacteria from expelling antibiotics taken up from the surroundings, therefore effectively allowing the antibiotics to kill the bacteria. Although the effective compounds from *A. absinthium* extracts have not been completely identified, 5-CQA plays a key part in the antimicrobial actions as an EPI by docking with the efflux pump and changing its conformation, effectively cutting off any flow (Fiamegos, 2011).

Clinical Studies

One of the most interesting clinical studies conducted on wormwood involves its usage in treating Crohn's Disease. This disease, characterized by intense abdominal pain and frequent diarrhea, is thought to be an autoimmune disorder, but there is existing evidence pointing towards a viral cause (Smith, 1993). Wormwood extracts contain caffeoyl and dicaffeoylquinic acids that have been shown *in vitro* to inhibit HIV-1 integrase. HIV-1 integrase is responsible for the integration of reversibly transcribed viral DNA into the host genome (Fiamegos, 2011). Another study conducted by Karim et al. (1996), produced evidence suggesting that aqueous extracts of *A. absinthium* are capable of protecting cells against herpes virus (Karim, 1996). The Omer group (2007) conducted a study administering wormwood capsules to Crohn's Disease patients being regularly treated with 5-aminosalicylates and select steroids. For standardization, the wormwood capsules contained 250mg wormwood powder, and a filler consisting of 100mg rose, 40mg cardamom, and 10mg mastic resin, whereas the placebo capsule only contained the filler (Omer, 2007). The results showed that at week 10, 65% of the test group exhibited little to no symptoms of the disease. Even more interesting is that these patients did not need to restart steroid treatment, as there is no remission of the disease (Omer, 2007). Another interesting point is that those patients that benefited from the treatment also experienced a higher quality of life and better mood than those who didn't, according to Hamilton's Depression Scale (Omer, 2007).

Contraindications

As stated before, because thujone reduces GABAA receptor activity, neuron-firing inhibition is reduced, allowing neurons

to fire more frequently. This is thought to cause the convulsions anecdotally and scientifically reported (Höld, 2000). These convulsions were also reported in other sources, along with a possible connection to kidney failure (Ashcraft, 2005). Another source reported thujone as hepatotoxic, as well as damaging to the brain. Other reported side effects include anxiety and sleeplessness (Naser, 2005). Thujone's interactions with other drugs has not been well documented.

Current Use in Allopathic and CAM Therapies

Artemisia absinthium is not used currently in allopathic medicine the United States. However, it is commonly used in CAM therapies as a tea or tincture, and there are claims that it aids in many common ailments, including appetite promotion, anti-anxiety, as well as hastening the child birthing process. Wormwood has even been reported as an effective agent in treating dropsy, as it is believed to be a powerful diuretic (Grieve, 2011). However, because of the negative aura surrounding wormwood from previous centuries, natural supplements and use is relatively low.

Discussion

Artemisia absinthium is an incredibly important historic herb, used for thousands of years for its medical benefits, abused for its hallucinogenic and euphoric properties, and in modern times, studied for its beneficial compounds that aid in fighting disease. Although in past centuries it was abused and consequently received negative light, current applications of incredibly important compounds contained within wormwood (phenols, flavonoids, caffeoylquinic acids, etc.) show a promising future for the plant.

Currently, wormwood's promise in treating Crohn's Disease and other viral diseases is attracting the attention of the scientific community. As stated previously, *A. absinthium* contains large amounts of 5-caffeoylquinic acid (5-CQA), which is being investigated as a compliment to antibiotics. This may have larger implications in the medical community's struggle to keep up with antibiotic-resistant strains of bacteria. If 5-CQA is investigated further in its mechanism and effectiveness against bacteria, it may grant the medical community more time to develop stronger antibiotics.

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Asparagus officinalis L., Asparagaceae

Sandy Jiang

Introduction

The genus *Asparagus* contains more than 200 species, of which *Asparagus officinalis* L., (**Figure 1**) is the most well-known; it can be found both at the supermarket and on our dinner tables. Asparagus belongs to the Asparagaceae family. "Asparagus" is supposedly of Persian origin (Hexamer, 1901), and it is believed to have originated in the eastern Mediterranean region, but can now be found growing in many parts of Europe. Naturalized in the Americas and even New Zealand, it is now a common crop grown for its young edible shoots that resemble spears. The lower fibrous parts of the plant are usually peeled off, leaving the tips to be consumed (Grubben, 2004). *A. officinalis* is high in nutrients, particularly carotenoids and sulfur containing compounds which both give the plant its distinctive taste. *A. officinalis* is also high in saponins, which have been shown to have antifungal, antioxidant, hypolipidaemic, hypoglycemic, and hepatoprotective properties. Recent studies have shown that compared to the stem, the often discarded bases actually contain more bioactive compounds. More research is being conducted on this exciting aspect.

Figure 1. ***A. officinalis.*** Image Source: http://2.bp.blogspot.com/-wchTZKfSOHY/T-odkzJN9tI/AAAAAAAAIVo/p8qaIvATDUI/s1600/asparagus+draw.jpg

Botanical Description

The asparagus plant (**Figure 2**) is a dioecious perennial herb with erect stems up to 2 meters tall. The thick stalks have bud clusters with leaves that resemble prickly brownish scales. The true leaves usually become spines at the base of the branches while the false leaves, termed cladolia, are actually the same as modified branches (Hexamer 1901). The flowers are unisexual, small, greenish-yellow and differ in size depending on of the gender of the plant. The fruit produced are red berries up to 1 cm in diameter that produce black, round, and flattened-on-one side shaped seeds (Grubben 2004).

Figure 2. *A. officinalis* plant structure. Image Source: http://www.mdidea.com/products/proper/asparagus-officinalis.jpg

Asparagus germination is slow even at optimum temperatures of 25-30°C. Flowering, which relies on insect pollination, is continuous and begins in the plant's first year of life. An adequate amount of nutrients is necessary, and fertilizers are usually administered annually to these crops. Good balance of fertilizers and appropriate water drainage systems can protect the plant against certain *Fusarium* infections which can cause devastating results to crops. Most diseases that attack asparagus are fungi including *Stemphylium botryosum, Cercospora asparagi,* and *Phoma asparagi* (Grubben 2004).

In warmer climates, the asparagus plant is green throughout the year, but in temperate climates, it senesces during autumn and resumes growth during spring. The seeds are usually grown in situ within a green house rather than directly sown into the field. The choice of the asparagus species is crucial as it is a long-term plant (Grubben 2004). The soil must be well-drained, sandy, naturally fertile, silicious, and deep so that the roots can penetrate deeply and absorbed all the nutrients from that soil (Ilot 1901). Older asparagus roots become hollow over time as young roots form above them. Thus, asparagus plants gradually rise above the original soil level and it is often necessary to cover up the exposed crowns with additional soil (Ilot, 1901).

Traditional Uses

History

It is believed that asparagus was first cultivated by the Egyptians or Greeks. In his book *De Re Rustica,* Cato lauded the plant as a valuable garden vegetable for the Romans. Cultivated asparagus went back to being wild after the fall of

the Roman Empire before it was brought back into the monastic gardens of the Middle Ages. It has been said that the Italians cultivated the finest asparagus, and therefore many societies compared their asparagus to that of the Italians'. Also, the French Huguenot refugees brought asparagus to England, improving the delicious but not as robust English variety. The English colonists then brought asparagus to North America, where it became a commercial crop in the 19th century (Chiffolo & Hesse, 2006).

Traditional medicinal uses

The Romans prized the asparagus highly and it was one of their oldest and most valued medicines. The fresh roots were used as a diuretic and as a sedative while syrups made from the young shoots and extract of the roots were used as a sedative for heart problems. Mixtures of asparagus, celery, parsley, holly, and fennel were used for the treatment of dropsy and gravel. Also, a liniment of asparagus and oil was believed to protect the user from bee stings, and the root was used for tooth aches (Hexamer 1901).

Since *A. officinalis* is a cultivated plant, most traditional uses in medicine came from *Asparagus racemosus* Willd as it was the wild variety. *A. racemosus* was used in traditional Indian medicine to increase fertility and to cure bodyaches. Women would take half a cup of root extract early in the morning for seven days to increase fertility and conception while tribal ladies took the root powder orally to cure bodyaches and leucorrhea. *A. officinalis* was used to increase sexual potency for both men and women (Jain et al., 2004).

The asparagus species has been used in India and China for its diuretic properties as it flushes the kidneys and helps prevent formation of kidney stones. An interesting folk belief is that Chinese pharmacists save the best asparagus roots for their close friends and families, believing it will promote feelings of compassion and love. *A. officinalis* roots have been used as a remedy for schistosomiasis, tuberculosis, dropsy, and cardiac medicine in these societies as well as Western medicine (Negi et al., 2010).

Food uses

Asparagus is usually eaten cooked. The tips are usually eaten, with chefs cutting off most of the base. Asparagus can also be eaten raw, though thinner stalks are preferred as they tend to be tenderer compared to the fibrous thicker stalks Fresh asparagus must be consumed immediately as asparagus have high respiration rates and will become hard and more fibrous and thus, not consumable. Asparagus can be bottled, canned, or frozen. The seeds have been reported to be used to make a coffee like beverage while germinating seedlings have been used as vegetables (Ong, 2008). Asparagus, because of its buttery taste can be used in casseroles and almost any other dish including cream of asparagus soups, fried dishes, broths, and stir fries (Shulman,2010). *A. officinalis* is usually recognized for two varieities, green and white. The white asparagus is grown by covering the plant in dirt to keep it growing in the dark. This process, termed "etiolation" gives it a more bitter taste, but a tender, fiberless, soft, and more delicate flavor than the green variety.

Ethnobotanical Uses

Asparagus are high in saponins which give it its characteristic taste. Saponins have been shown to have spermicidic and antifungal properties. In India, asparagus has been used for its medicinal purposes for centuries, and it is believed that

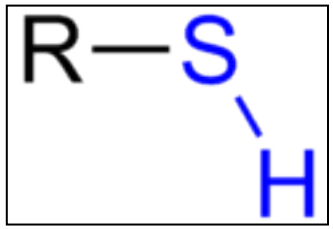

Figure 3. Thiols are also known as mercaptans which give asparagus its famous odor.

saponins are the main active ingredients behind these physiological activities. The bottom parts that are usually chopped off and discarded are found to actually possess high amounts of saponins (Shimoyamada et al., 1990).

Pest Control/ Cultivation

The common asparagus beetle *Crioceris asparagi* was a huge pest in the early United States in both its larval and adult forms (Ilot 1901). The insect preys on the soft parts of the asparagus plant rendering it unfit for human consumption. The insect however was very susceptible to sudden changes in temperature and had a wide variety of predators. Chickens and ducks were efficient predators employed by farmers to curb disease. Prominent asparagus farmers used to cut down all plants in early spring to force the beetles to lay eggs on new shoots which were cut before the eggs could hatch. Other stalks were designated as sacrificial to lure the beetles before air slacked lime was dusted on the plants in the early morning to kill the pests (Hexamer 1901). Paraffin and soap were described by Charles Ilot to be efficient insecticides. It is interesting that such organic methods kept the shoots still safe to consume while efficiently removing the pests compared to modern chemicals.

Harvesting

Asparagus is perennial plant and can be harvested annually. It is typically not economical to harvest asparagus plants that are over 10-15 years of age, but asparagus plants over a hundred years of age have been located in western Europe. The spears of the plant are the most marketable and require high nutrition to ensure quality, meaning that there must be sufficient nutrients in the base of the plant to fuel its growth. Thus, most asparagus are not harvested until two years after planting to ensure proper nutrition for the plant. The spears are cut and washed before being sorted and packed for marketing. Asparagus have high respiration rates and will shrivel without water. Thus, they have short shelf lives and must be kept under high humidity conditions (Grubben 2004).

Chemistry and Pharmacology

Like many plants, asparagus is high in many allelochemicals and other nutritious chemicals, including minerals and carotenoids. *A. officinalis* also contains sulfur compounds, which are responsible for its distinctive taste and smell.

Both the green and white varieties of *A. officinalis* are edible, though the green variety is more abundant in micronutrients. The characteristic odor of asparagus is derived from dimethyl sulphide, which is formed from the degradation of the amino acid S-methyl methionine (Grubben, 2004). Sulphur containing compounds are known as thiols or mercaptans (**Figure 3**). The pungent urinary odor produced by many individuals after the consumption of asparagus has been shown to be due to six mercaptans: methanethiol, dimethyl sulphide, dimethyl disulphide, bismethyl methane, dimethyl sulphoxide, and dimethyl sulphone (Waring, Mitchell,

Nutrient	n=100g sample	Percentage of 100g sample
Water	75 g	75%
Energy	25 kcal	-
Protein	2.9 g	2.9%
Fat	0.6 g	0.6%
Carbohydrate	2 g	2.0%
Fiber	1.7g	1.7%
Calcium	27 mg	0.027g%
Magnesium	13 mg	0.013%
Phosphorus	72 mg	0.072%
Iron	0.7 mg	0.0007%
Zinc	0.7mg	0.0007%
Carotene	315 µg	0.000315%
Riboflavin	0.06 mg	0.00006%
Niacin	1 mg	0.001%
Vitamin C	12 mg	0.012%

Table 1. Breakdown of nutrient components of *Asparagus officinalis.* Source: Grubben, J.G.H., (Ed.)(2004). *Vegetables: plant resources of tropical Africa.* (Vol.2). Wageningen, Netherlands: PROTA foundation/Backhuys Publishers.

Fenwick, 1987). *A. officinalis* also contains a wide variety of nutrients, including calcium, phosphorus, and protein (**Table 1**).

A. officinalis is high in carotenoids, especially in its fruits. The ripe fruits contain large amounts of capsanthin, β-carotene, and zeaxanthin and small amounts of capsorubin, cryptoxanthin, cryptocapsin, antheraxanthin, violaxianthin, and capsanthin isomers. Interestingly, the biosynthesis pathway of carotenoids in *A. officinalis* was found to be similar to that in paprika (Deli, Matus, Tth, (2000).

It is interesting to note that once old asparagus plants die, the field cannot be used to replant new asparagus crops because the leftover tough roots release hytotoxic compounds and may have *Fusarium oxysporum (FOA)* infections (Grubben, 2004). Asparagus plants and their decaying root tissues have been shown to release allelochemicals such as autotoxins, which can make the plants more susceptible to FOA infections or exacerbate pre-existing FOA infections (Nair et al., 1990).

Of the two varieties, the white asparagus is grown by covering the plant in dirt to keep it growing in the dark. This process, termed "etiolation" gives it a more bitter taste, but tender, fiberless, soft, and more delicate flavor than the green variety. Saponins are primarily responsible for the asparagus's bitter taste. In 2012, Hofmann and Dawid isolated five other compounds that contribute to the bitter taste. **Table 2** lists the names and the human taste thresholds for these compounds. Hofman and Dawid also found that asparagus plants have a buttery mouth-coating effect that was attributed to the two 1,2-dithiolan-4-carboxylic acid 6-D-glucopyranose esters.

Biological Activity

The edible portions have been shown to possess antifungal, antioxidant, hypolipidaemic, hypoglycemic, and hepatoprotective activies. During the industrial processing of *A. officinalis,* around 30-40% of the spear is discarded as waste which wastes food and leads to significant environment pollution. However, it has recently been shown that the base is rich in bioactive phytochemicals such as steroids, and in particular, saponins (Wang et al, 2012). Saponins have a large variety of biological properties including antioxidants, immunostimulants, antihepatotoxic, antibacterial, therapy for diabetic retinopathy, anticarcinogenic, antidiarrheal, antiulgerogenic, antioxytocic, and reproductive agents. Saponin rich plants, such as *A. officinalis* have been shown to improve growth, feed efficiency, and health in cattle such as

sheep and cows (Negin et al., 2010). This biological activity section explains the in vitro and in vivo studies performed by various studies on the bioactive properties of *A. officinalis.* **Table 3** summarizes the plant parts, isolated compounds, and the activities of the compounds mentioned before and in this section.

In vitro

Proteolytic Properties

Proteolytic enzymes break down proteins and thus can tenderize meats for culinary purposes. Ha et al., (2012) performed a study comparing kiwifruit and asparagus extracts and their proteolytic activities in tenderizing meats. Although purer cysteine enzyme extracts are needed for further analysis, they found that asparagus had noticeable effects in tenderizing meats. Even though asparagus was not as effective as kiwifruit, ester hydrolysis in asparagus occurred over the entire assay pH range from 2-14 and between temperatures 45-75°C, whereas the same reaction in kiwifruit extracts was restricted to a pH range of 5.5-7 and a narrower temperature range. The asparagus enzyme hydrolyzed myofibrillar proteins slower than kiwifruit. Although it only targeted myosin heavy chains, after an extended incubation, the myofibrillar proteins, except troponin C were degraded.

Alcohol Metabolism

Another study was on the alleviation of alcohol hangover in which *A.officinalis* leaves and shoots extracts increase the activities of two key enzymes that metabolize ethanol, alcohol dehydrogenase (ADH) and CYP2E1. Chronic alcohol use can cause ethanol induced fatty liver as well as oxidative stress

Compound no.	Compound	Threshold (μmol/L)
1	3-*O*-[α-L-Rha*p*-(1→2)-{α-L-rha*p*-(1→4)}-β-D-glc*p*]-26-*O*-[β-D-glc*p*]-(25*R*/*S*)-22-hydroxyfurost-5-ene-3β,26-diole	65.9
2	(*25R*/*S*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rha*p*-(1→4)-β-D-glc*p*]-26-*O*-β-D-glc*p*	10.9
3	(*25R*/*S*)-furostane-3β,22,26-triol-3-*O*-[α-L-rha*p*-(1→4)-β-D-glc*p*]-26-*O*-β-D-glc*p*	25.5
4	3-*O*-[{α-L-Rha*p*-(1→2)}{α-L-rha*p*-(1→4)}-β-D-glc*p*]-(25*R*/*S*)-spirost-5-ene-3β-o	70.6
5	3-*O*-[{β-D-Glc*p*-(1→2)}{β-D-xyl*p*-(1→4)}-*β*-D-glc*p*]-(25*S*),5β-spirostan-3β-ol	199.7

Table 2. Five saponins found in *Asparagus officinalis* that give its bitter taste and the human taste recognition thresholds. Source: Dawid, C., &Hofman, T., (2012). Structural and sensory characterization of bitter tasting steroidal saponins from asparagus spears (*Aspragus officinalis* L.). *Journal of Agricultral of Food Chemistry.*

caused by the production of cytochrome P-450E1. ADH metabolizes ethanol to acetaldehyde and then acetaldehyde dehydrogenase (ALDH) before becoming acetate. Acetaldehyde at high concentrations can cause toxic effects such as sweating, vomiting, and increase pulse rate and thus, there is a need to find an efficient and quick way to remove excess alcohols and their metabolites from the body. Kim et al.(2009) demonstrated that the leaves which are usually discarded have the most therapeutic use compared to the stem for alcohol hangover treatment especially on the HepG2 cell line. *A. officinalis* had significant effects on ADH and ALDH by providing strong antioxidant activity and acting as a potent

Aerial parts	2-hydroxyasparenyn 4'trans-2hydroxy-1methoxy-4-5(4methoxyphenoxy)-3-penten-1-ynyl-benzene	Inhibitory against cyclooxygenase -2
Fruits	Capsanthin, capsorubin, capsanthin 5,6 epoxide 3-O-[a-L-rhamnopyranosyl (1-2);] beta-o-glucopyranosyl](25S)spirost-5-ene-3beta-ol	Antifungal
Seeds	Methyl protodioscin and protodioscin	Cytotoxic
Fruits	Spirostanol glycoside	Immobilization of human spermatozoa
Roots	Sucrose-1-fructosyltransferase	Cytotoxic
Roots	Sarsasapogenin and nine asparagosides A,B,C,D,E,F,G,H, and I	Cytotoxic
Roots	Steroids	Cytotoxic
Leaves	Flavonoids and rutin	Cytotoxic
Base	Saponins	Antitumor and anticancer
Base	Saponins	Antifungal

Table 3. Chemical compounds found in *A. officinalis* and their activities.

catalytic factor to stimulate the enzymatic activities required to break down alcohol. The amino acid and inorganic mineral content was higher in leaves than in the shoots.

Antifungal Properties

Shimoyamada et al.(1990) have isolated saponins that have antifungal activity from *A.officinalis* extracts. They have determined that the antifungal properties of the saponins come from the waste products of asparagus processing. The compound was shown to have antifungal activity against fungi including *Candida albicans, Crytococcus albidus, Epidermophyton floccosum, Microsporum gypseum,* and *Trichophyton spp* but was ineffective against other fungi such as *Rhizopus* and *Chaetomium.*

Antitumor Activity

Cancer occurs when harmful mutated cells experience uncontrolled growth. Cells that experience uncontrolled growth usually form tumors, but those that mutate and then divide uncontrollably cause cancer. The global burden of cancer has made it necessary to find ways to inhibit the growth of malignant cells. Most cancers can be cured surgically before metastasis, but once metastasis has started, it is almost impossible to treat. Metastasis occurs when malignant cells escape from the original tumor and spread via blood and lymph vessels to other sites. It has been shown by Wang et al. (2012) that *A. officinalis* saponins (SSA) inhibit the viability of various cancer cells including breast, colon, and pancreatic cancer cells in a dose dependent manner. SSA also induces cancer cell apoptosis, which is the self destruction of cancer cells. SSA also inhibits cancer cell migration better than blocking cancer cell growth. It thus also inhibits cancer cell invasion because migrating cells' cytoskeletal networks were seriously disrupted by them. Normal cells are usually round in shape, but they take on a sword shape after being disrupted; this shape reduces cell motility. Cell migration is regulated by Rho GTPases, and it has been shown that SSA targets the Rho GTPase signaling pathway. Wang et al., argue this potential use of asparagus waste can reduce environmental pollution and also potentially be used as anticancer and anti tumor medicines.

In vivo

Anti-diabetic Properties

Diabetes occurs when the body cannot efficiently break down sugar. This usually occurs when the body does not break down insulin or does not have insulin sensitive receptors, and thus there is a need to find treatments for this illness. Metabolism and metabolic studies were conducted by Hafizur et al. (2012) that demonstrated *A. officinalis* extracts can control blood glucose by improving beta cell function in type 2 diabetes induced rats. Streptozotocin was injected into rat pups to induce non obese diabetes. After the thirteenth week, the rats were treated with an extract of *A. officinalis* seeds before blood glucose, serum insulin, and total antioxidant status were measured. It was shown that the treatment with these extracts suppressed the elevated blood glucose level that would have been high in diabetic mice without insulin. The efficicacy of this extract depended on the dosage with a 500 mg/kg having higher efficacy than 250 mg/kg. The study thus shows that *A.officinalis* extract can have anti-diabetic effects by improving insulin secretion and beta cell function and antioxidant status.

Hypolipidaemic Effects

Hyperlipidaemia includes hypercholesterolaemia and hypertriclyceridaemia, and is a major risk factor in the development of cardiovascular diseases. There has been an emphasis on dietary plants that show the potential to lower cholesterol levels in plasma. *A. officinalis* has been shown to reduce levels of body weight gain, serum total cholesterol, and serum LDL cholesterol in mice with high cholesterol when they were administered a daily dose of 200 mg/kg for 8 weeks. HDL cholesterol levels were increased in mice treated with aqueous extracts. Qu et al.(2009) found that the ethanolic (EEA) and aqueous (AEA) extracts of asparagus have strong hypolipidaemic and hepatoprotective properties that can be used in complementary and alternative medicinal treatments in combination with other hypoipidaemic drugs.

Clinical Studies

It has been found that *Asparagus officinalis* has been used as a medicinal plant for the treatment of several diseases. *A. officinalis* is one of the many plants including broccoli, butterhead lettuce, chickpea, dry beans, and spinach that are high in folic acid. Folic acid is important for the methylation of amino acids, DNA and RNA, cell division, differentiation, and regulation. It also regulates the central nervous system, mood, sleep, and appetite. Women who have folate deficiencies at child bearing age have a high chance of having neural tube defects and spina bifida in newborn babies (Lester, 2006).

Besides being consumed for health purposes mentioned before such as fertility, diuretic, and sexual potency, most trials are still *in vitro* or *in vivo* for *A. officinalis* since there has only been a recent discovery that the usually discarded base holds more compounds than was suspected.

Contraindications

Allergies

It has been found that some individuals may be allergic to asparagus. *A. officinalis* has been shown to cause delayed cell mediated reactions and IgE mediated reactions. The most common allergy is skin contact allergies and only a few IgE mediated reactions have been observed. IgE reactions are further categorized into food allergy and reactions due to

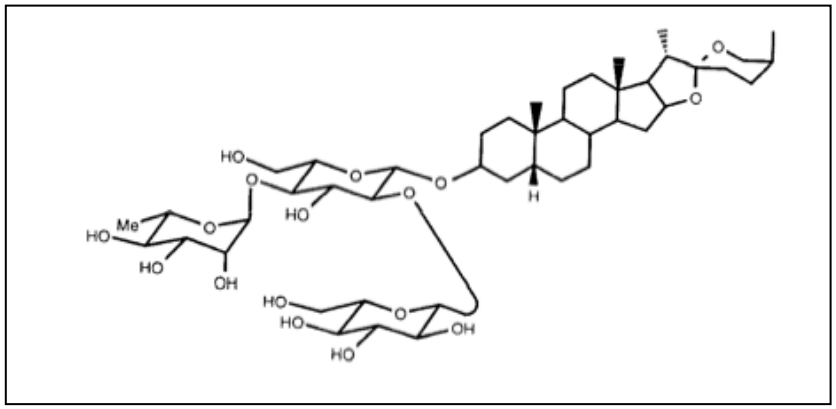

Figure 7. An LB positive spirostanol glycoside that was shown to have potential spermicidal activity.

cutaneous or respiratory exposure with anaphylaxis being the most clinical symptom of allergy. Asthma, rhinitis, and uriticaria are other less reported reactions (Tabar et al, 2003). Asparagus is usually rarely encountered as a cause of IgE mediated disease, as only two reports of allergy to asparagus have been published. Usually food allergies are connected to pollen allergies, but the individuals who reported allergies were tested and it was found that it could be an onion specific IgE that leads to the sensitization (Escribana et. al, 1998). Although most responsible allergens are unknown, there is work being done in isolating the responsible compounds. One of them is 1,2,3-trithiane-5-carboxylic acid, which is a plant growth inhibitor secreted by asparagus in the early phase of the season (Hausen&Wolf, 1996).

Gout

Gout is a rheumatic disease that involves chronic hyperuricemia or the buildup of uric acid from the end product of purine metabolism. About 66 percent of the purine load comes from the cell cycle, and the other 33 percent come from diet. Thus, treatment for gout requires a diet that is low in purines or can break down uric acid better. Products that are high in purines include animal meats, seafood, and some plants including asparagus (Gordon, 2005). Individuals with gout, therefore should not consume asparagus and other high purine products.

Current Use in Allopathic and CAM Therapies

It has been shown that saponins can be used as potential effective spermicidal agents. The methanolic extracts of fruits of *A. officinalis* has been shown to have glycosidic activities that may have such spermicidal activity (Morris et al., 1988). When established methods were used, the extracts caused 100% immobilization of human spermatozoa at a 1.5% level (**Figure 4**).

As stated earlier, *A. officinalis* is high in saponins which have been shown to have many biological effects. In complementary and alternative medicines, saponin rich compounds have been used for a variety of treatments such as being antidiarrheal, antioxytocic, antibacterial, antihepatotoxic, antioxidants, and reproductive agents. It is also used still in India as a sexual potency drug and in Chinese culture it is used for diuretic and kidney stone prevention (Negi et al., 2010).

Asparagus is highly recommended to women in child bearing ages as it is high in folic acid which is important for the central nervous system, moods, and development of children. In CAM and food therapy, asparagus is highly promoted with other high folic acid vegetables such as spinach, brussel sprouts, and broccoli for pregnant women (Lester, 2006).

Discussion

A. officinalis is a common vegetable found around households and restaurants that are high in bioactivity. It is found in two kinds of varieties including the white and green varieties, with the green variety possessing more micronutrients. It is high in folic acid and carotenoids, which are crucial for physiological processes such as neural development and antioxidant properties. *A. officinalis* like other asparagus species has a famous odor, especially in the urine of some individuals after consumption. The smell and odor derives from many of the sulfur compounds, also known as mercaptans. Many studies have found the plant to have wide ranging activities notably antitumoral, anti diabetic, anti bacterial, hypolipidaemia, antifungal, and spermicidal use. It has also been shown to exhibit alcohol metabolizing enhancing effects. *A. officinalis* is used in traditional Indian medicine for fertility treatments as well as preventing body aches. It has a long history that dates back to the Romans, Greeks, and Egyptians who prized the plant so highly. The plant fell into wildness during the fall of Rome and did not reappear until the Middle Ages in monastic gardens. The Chinese highly laud the plant for its prevention in kidney stones and treatment of diabetic kidney failure. The Chinese and Indian use *A. officinalis* for its diuretic properties to flush out toxins. *A. officinalis* is one of the edible cultivated species of the 200 species of asparagus. It is interesting that it has a dual identity in both increasing fertility but also killing sperm. Although it is high in nutrients, it is not as dense as nutrients as the wild variety *A. racemosus. A. officinalis* has highly nutritious stalks but surprisingly has much more powerful compounds in its bases which are usually discarded and not consumed. Scientists are advocating for more research in the bases to isolate more useful compounds that can be used in clinical trials and more in vitro and in vivo studies. More work must be done on the saponins which are the densest in the base. *A. officinalis* holds a bright potential in his bioactive compounds as scientists have only begun to realize the bulk of its nutrients, which are in the base that usually requires removing 40% of the stalk, have been discarded for years and are looking into traditional medicines for clues of its activity.

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Atropa belladonna L., Solanaceae

Kristen Cross

Introduction

Atropa belladonna, commonly know as deadly nightshade, belladonna, devil's cherries or death cherries, belongs to the Solanaceae family, which is also home to other well-known plants such as tobacco and potatoes (*Nicotiana* and *Solanum*, respectively). As implied by its common names, the toxicology of this plant is well known and it has been used as a poison as well as a cosmetic and anesthetic throughout its extended and interesting tale with the human culture. This 'femme fatale' has been incorporated into human history since before the Middle Ages, making a name for itself as a temptress due to the neurological and muscarinic effects as conveyed by its tropane alkaloids scopolamine and atropine. These drugs are still used today as an antidote for cholinergic crises and have been implicated in helping those with mental illnesses such as depression and bipolar disorder. These constituents are also responsible for the plant's notorious use a recreational drug in order to induce hallucinogenic episodes. The fascination with this plant stems from its alluring appearance as well as the mystery and taboo surrounding it.

Figure 1. *Atropa belladonna* in natural habitat. Source: www.plants.usda.gov

Botanical Description

Atropa belladonna is native to Western Europe and North Africa, where it commonly grows in disturbed woodlands as a result of it being a somewhat shade-intolerant species. The herbaceous perennial is often found cultivating the soil on rocky hillsides and other regions where the soil contains an adequate amount of calcium carbonate. *A. belladonna* is nitrophilous and requires a sufficient amount of nitrogen present in the soil in order to grow. Due to the plant's importance as an anticholinergic, studies were conducted to

find the ideal growing conditions that produce the highest concentrations of these alkaloids. They found that an increase in nitrogen in the soil would increase the concentrations of atropine and scopolamine, especially if water is limiting (Baricevic et al. 1999). This factor could be a large contributor to the fact that *A.belladonna* is rarely found growing in grass communities, but rather in largely open and uncultivated areas.

It appears as an erect, weed-like plant, standing 50-200 cm high depending on the amount of sunlight available (**Figure 1**). The stem is densely covered in fine, short hairs while the broad, ovular leaves branch out in unequal pairs starting about 25cm from the ground. These leaves are green, smooth and often asymmetric. A single purple flower droops from each leaf-axil and in the fall season a smooth, dark purple berry is produced (Butcher 1947). It is important to note that all parts of the plants are poisonous upon ingestion, as the alkaloids are ubiquitous throughout.

The plant was initially confused with mandrake (*Mandragora*), as they are closely related members of the Solanaceae family, but belladonna conveys a higher toxicity than the former. Dioscorides was the first to recognize that *Atropa* was a distinct genus and should not be used in place of mandrake for medicinal purposes (Lee 2007).

Traditional Uses

Atropa belladonna gets its namesake from Atropos, the Fate responsible for cutting the thread of life. This christening was based on the plant's dark tie to 11th century black magic, especially related to its use in love potions and enhancing the appearance of female allure via dilation of the pupils (Müller 1998). Witches and wizards of the olden days incorporated it into their pharmacopeia as a flying ointment known as 'sorcerers pomade'. This ointment included nightshade, henbane, mandrake and hemlock mixed together and pounded with bear grease. The ointment was then applied all over the skin and the users claimed to experience hallucinations and sensations of flying. Divination practices also included the consumption of nightshade in small amounts in order to experience visions that may foretell events to come (Lee 2007). Many superstitions surround this mystic plant, including the placement of cuttings of the belladonna plant in the household in order to ward off evil spirits. Conversely, the witches and wizards took a liking to the plant because they believed that it was a favorite of the Devil himself.

The practice of using plant extracts (particularly those from the Solanaceae family) to dilate the eye in order to enhance physical appeal was first recorded by Matthiolus in 1565 (Matthiolus , Feinsod 2000). This activity was recognized as being generally dangerous, but as the doses were relatively small, few fatalities were recorded. It was this practice of Venetian women that lead to the discovery of the mydriatic effects of atropine, as well as what led Linnaeus to give the plant its species name, *belladonna.* This translates to "beautiful lady" in Italian, referring to the enhanced appearance of the women after their pupils were dilated by drops extracted from the plant.

Pre-dating these medieval witches, *A. belladonna* was included in tinctures during the Bacchanalian festivals celebrated by the Greeks and Romans (Lee 2007). In larger doses, the plant was indicated as a deadly poison and is thought to have made an appearance in *The Odyssey* as the poison administered to the sailors by the lovely yet malicious Circe (Campbell 2007, Lee 2007). Tropane alkaloids have also been used to bolster beers of the ancient world in order to give them more potent effects upon consumption.

During the Roman and Byzantine empires, the drug began to lose its tie to religious and prophetic rites and instead became an important soporific used for medicinal purposes. (Ramoutsaki et al. 2002). From the mid-nineteenth century into the 1950s, *A. belladonna* was incorporated into plasters and liniments for the treatment of neuralgia, chronic rheumatism, lumbago, myalgia, pleurisy, pulmonary tuberculosis and acute mastitis. These plasters were taken off the market when pharmacists realized that some of them contained extracts from monkshood (*Aconitum napellus*), which produces an alkaloid called aconitine. This alkaloid was implicated in respiratory and cardiac failures and could be used in combination with atropine to poison people (Lee 2007).

Based on this long history of the plants use for recreational purposes, herbalists and apothecaries began to examine the mechanisms behind its - in some cases deadly - effects on the body and they found that in lower doses it was useful for medicinal purposes. Andrew Duncan of the Edinburgh Dispensatory recommended it as a treatment for the plague as well as nervous disorders such as epilepsy and mania (Duncan 1803). He also recorded the first use of mydriatic drugs in ocular surgery by Professor Reimarus, an eye specialist in the 1800s. The professor used an infusion of nightshade to dilate the pupil while he removed cataracts.

A. belladonna has had a dark and fascinating tie to European culture ever since its first recorded use in cosmetic applications in the 1500s (Matthiolus). It has even worked its way into modern media in Tim Burton's movie *A Nightmare Before Christmas*. The movie featured the dried flowers implicated in a potion used for its soporific effects. The intrigue induced by this plant stems from its use in taboo, sexual practices as well as the fear of its lethal properties. Though it is dangerous to handle without proper care, this mystic past is what led to the investigation of it for medicinal practices.

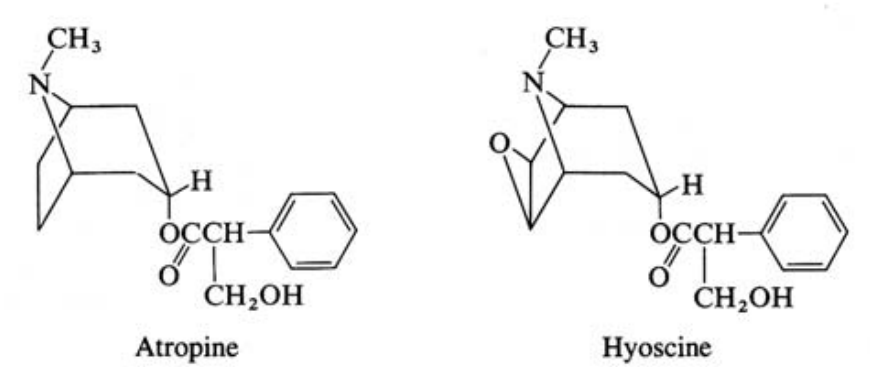

Figure 2. Structures of atropine and hyoscine (scopolamine). Source: Lee 2007

Chemistry and Pharmacology

The main chemical constituents of this plant include atropine and scopolamine (**Figure 2**). Atropine, a tropane alkaloid, was first isolated from *Atropa belladonna* in 1831 by German pharmacist Mein (Sneader 1985). The compound exists as l-hyoscyamine in the plant and is the only active isomer, but it isomerizes upon extraction into the dextro compound. The racemic mixture of these two isomers is called dl-hyoscyamine or, more commonly, atropine. Its unique tertiary-amine structure allows penetration of the central nervous system (CNS). It is the important characteristic of its structure that makes it so potent as an antidote, or in cases of overdose, a lethal poison.

Hyoscine (also called scopolamine) is another tropane alkaloid that has been isolated from *A. belladonna.*

Chemical	Part	Lo PPM	High PPM
Atropine	Root		
Hyoscyamine	Root	2000	8712
Scopolamine	Root	40	88

Table 1. Average concentrations of chemicals in *Atropa belladonna*. Source: http://www.ars-grin.gov/duke/

Scopolamine is 10 times more potent than atropine, but it works in the same pathway. The only difference in the structure of the two alkaloids is in incorporation of an oxygen atom into one of the 6-membered rings. Like atropine, scopolamine is able to penetrate the central nervous system (CNS) and act on the muscarinic receptors. It is interesting to note, however, that these two compounds act only on the muscarinic receptors and not nicotinic receptors.

The average concentrations of the two compounds found in *A. belladonna* are reported in **Table 1**.

Biological Activity

Atropine has significant effects on the central nervous system. Its properties as a stereotypical anti-muscarinic xenobiotic are well-known and given in the right dosages, it can be a vital antidote following exposure to muscarinic agonists, such as pilocarpine and physostigmine (Wills 1963, Greenblatt and Shader 1973). In the 1970s, atropine was recognized as having the ability to reverse the effects of the cholinergic crisis as it competes with muscarinic agonists and acetylcholinesterase inhibitors at both central and peripheral muscarinic receptors. This activity was extremely beneficial for military personnel, as it could mitigate the cardiac effects of exposure to physostigmine as well as prevent the effects if given preemptively, of up to two to three times the lethal dose (Greenblatt and Shader 1973).

The isolation of l-atropine from *A. belladonna* in the 1830s also marked an important venture into the study of neurotransmitters in mammals, specifically the in activity of acetylcholine and its effects in the body. It was used to map this pathway because it allowed for a better understanding of what effects particular neurotransmitters have on the body (Lee 2007).

Laboratory studies on mice showed that atropine extracted from *Atropa belladonna* has immuno- and gastroprotective effects in the event of stress-induced alterations on behavior. Although the mechanism of action was not elucidated in this study, the authors attribute the behavioral inhibition to the "anxiolytic-like effects" of *A. belladonna* (Cromwell 1943).

Studies of the effects of atropine extracted from *A. belladonna* as applied to the eyes of rabbits confirmed the mechanism by which atropine is absorbed in the iris and results in dilation of the pupils. This is achieved by blocking the innervation to the sphincter pupillae and ciliary (North and Kelly 1987). Reversal of the mydriasis can take up to 10 days in humans and can also lead to blindness and other complications when dosed inappropriately (Salazar et al. 1976). The effect is responsible for the plant's use in the Middle Ages in order to dilate the pupils for cosmetic appeal. In humans, the drug works on the M_3 muscarinic receptor on the iris sphincter muscle. The effect is almost immediate and it extremely powerful, lasting for 7 to 10 days depending on the administered dose. This is due to the ease by which it is absorbed into the body, showing a systematic absorption of up to 65% in some cases (Howland 2011).

A review of the general effects of atropine was conducted using 250 laboratory mice. Each of the subjects was injected with 10μg of atropine and the resulting behaviors were then observed. The authors noted that there was an increase in respiration, a relaxing of the tail and the ears flattened against the head. Increased sensitivity to touch and sound was also noted, though the animals were fatigued for 2-3 hours after the injection. After 24 hours, all of the symptoms had ceased (Haley and McCormick 1957). This study was conducted as a general survey of the effects of atropine in order to potentially expound upon previous findings and perhaps better understand the mechanism of action. The results of this study have been used to further investigate the mechanisms of action behind the atropine-induced behavioral changes.

Hyoscine has an anesthetic effect and unlike atropine, it does not have negative effects on the electrical activity of the brain. This was used to calm patients with mental illnesses and it was later found useful in the treatment of major depressive disorder and anxiety disorders. These were observed in mice initially as a having the ability to increase "engagement" and awareness of seemingly disinterested mice. Animal studies such as these have been effective in the past in determining which drugs may act on the receptors of neurotransmitters in such a way as to regulate mood and behavior. Hyoscine is currently undergoing clinical trials in humans with promising results (Katz and Hersh 1981, Drevets and Furey 2010).

The primary uses of atropine and scopolamine involve its potent effects as an anticholinergic. Its ability to work on neurotransmitters has led to recent studies, which are looking into its ability to alleviate the symptoms of depression as well as inhibition of short-term memory recall (Aigner and Mishkin 1986, Drevets and Furey 2010). Its use in modern medicine has been decreased due to the infamous cases of poisoning by some doctors who misused their access to the drug, but it is still an important component in opthamalic therapies.

Clinical Studies

The most common symptoms of *A. belladonna* poisoning are tachycardia and mydriasis, as indicated by the anticholinergic effects of the drug. Other observed symptoms include increase in body temperature, flushing of the face, repressed salivation, bizarre mental state described as manic and paralysis of the detrusor muscle of the bladder, resulting in urine retention (Lee 2007).

Most studies of the effects of *A. belladonna* on humans come from toxicology reports wherein the plant was accidentally ingested and the symptoms were severe enough to warrant medical attention. The amount ingested from eating the berries, which are sometime mistaken for blackberries, was not enough to kill any of the 23 children in the reported cases. Common symptoms of belladonna intoxication include meaningless speech, tachycardia, mydriasis, and flushing. In some cases the anticholinergic effects were so severe that physostigmine had to be administered to restore homeostasis (Çaksen et al. 2003).

Scopolamine was administered transdermally to 16 patients with nausea induced by calorization of the ear in a randomized, double blind study. Nausea was reduced significantly compared to the effects of the placebo and it was also noted that introduction of scopolamine into the body 6 to 8 hours before exposure to a known motion sickness-inducing stimulus will prevent the onset of symptoms. Side effects were negligible (Pyykkö et al. 1985).

One of the most important uses of atropine came about during the 1930s and 40s when OP insecticides that were later

developed into nerve agents in chemical warfare due to their ability to inhibit acetylcholinesterase. Upon exposure to these agents, one would experience increased levels of acetylcholine, as the enzyme to metabolize it was no longer functioning due to inhibition. Atropine was then administered due to its muscarinic antagonist effects and it worked effectively and in small enough doses to be safe (Howland 2011).

Few clinical studies have been conducted using these drugs due to the well-known cases of poisoning and the generally infamy the plant has earned throughout its long, dark history. The most recent interest with these muscarinic antagonists comes in the field of mental health, where a new theory is circulating that Alzheimer's and dementia may be related to the cholinergic system. Scopolamine has been used to induce short-term memory inhibition in order to help elucidate the mechanism by which dementia develops. (Aigner and Mishkin 1986). This new field has shown some promising results and may potentially provide just retribution for atropine and scopolamine and import and effective treatments in western medicine.

Contraindications

Allergic reactions to topical application of atropine have been described as causing swelling of the eyelids, followed by itchy and stinging sensations. In patients who are prone to developing narrow angle glaucoma, application of the drug can lead to an acute attack, but this risk has been evaluated as minuscule.

'Hot as a hare, blind as a bat, dry as a bone, red as beet and mad as a hen' was an aphorism widely used to describe the symptoms of belladonna poisoning. These symptoms are fairly unique to the effects of this particular plant, but can sometimes be confused with other plant poisons in the Solanaceae family, such as henbane (*Hyoscyamus niger*). Dilation of the eyes, as induced by atropine, results in photophobia until the pupils return to their normal circumference, which is usually no longer than a few hours. Dryness of the skin, mouth and throat can occur due to decreased secretion from the mucous membranes. Restlessness, irritability or delirium can also occur, as atropine stimulates the central nervous system. Use of atropine for any condition in elderly patients is not recommended, as it can cause confusion and may cause brain damage. Fatalities, though rare, can occur upon ingestion of this drug due to depression of circulatory and respiratory functions. This is more dangerous in children than adults, as the fatal dose in children is 10mg versus 100mg (North and Kelly 1987). 90-130 mg is considered to be a toxic dose (Lee 2007). Death is rare in the modern age because the symptoms are so well known and a cleansing of the stomach as well as use of artificial ventilation and diazepam can control convulsions and reduce the risk of mortality. In most cases, atropine is eliminated from the body in 72 to 96 hours without lasting effects on the patient.

When atropine was first isolated and brought to the medical market the medicinal knowledge about the drug's effects was incomplete. The plasters and eye drops could be incorrectly prepared and as a result, many accidental deaths occurred (Lee 2007, **Figure 3**). These treatments have since been banned from the market. Atropine can also be absorbed through the GI tract, so if an animal consumes the berries and the meat from said animal is ingested by a human, it can cause unusual and sometimes toxic symptoms (Polson et al. 1983).

One of the most notorious incidences of atropine poisoning was the attempted murder of Mrs. Agutter by her husband in

Figure 3. Plaster containing atropine that used to be sold in drug stores. Source: Lee 2007.

1994. By the 1990s, atropine was only available for the use of physicians and nurses, so it was relatively easy for him to acquire as he worked in a hospital. Dr. Agutter served his wife a gin and tonic laced with atropine, as the quinine in the tonic water disguised the bitter taste. Within five minutes she felt an agonizing pain in her throat and she started to experience hallucinations. It was later found that she had only consumed 50mg of atropine before the symptoms hit and she stopped drinking. Due to her husband's miscalculations, she survived and he was found guilty of attempted murder and spent the next twelve years in prison (Lee 2007).

Current Use in Allopathic and CAM Therapies

Atropine sulfate drops are one the main therapies prescribed for the treatment of amblyopia, a condition of the eye that can lead to vision impairment. A clinical study evaluating the efficacy of the atropine drops over a 6-month and two year period showing 49% improvement in the condition of the affected eye (Repka et al. 2005). Atropine can be administered via oral, inhalation and intramuscular routes and it can last for 24 hours within the body depending on the dose. When given a 2mg dose via autoinjector, heart rate increased to maximal level in 16 minutes. These autoinjectors are used as an immediate response for terrorist attacks, especially when they involve exposure to nerve gas. The atropine stops muscle spasms and allows heart rate to return to normal (Howland 2011).

Atropa belladonna is commonly used to combat local inflammation that, if untreated, can lead to sepsis and death. A study on the various homeopathic remedies that used *A. belladonna* outlines the major drug used in homeopathic treatment of infection. These include *Belladonna—Injeel®, Belladonna—Injeel Forte®* and *Belladonna Homaccord®*. Preparations containing *A. belladonna* constituents were effective in regulating what types of cells migrate during the inflammatory process. This activity helps lower the presence of proteolytic enzymes, free radicals and chemical mediators that may infer with the immune processes (Pedalino et al. 2004). Another medicinal use of atropine is to lower blood pressure via competition for the norepinephrine receptor, thus mitigating the effects of hypertension (Abraham et al. 1981).

Hyoscine is currently undergoing clinical trials as a treatment for major depressive disorder. Its efficacy in relieving symptoms of depression relative to the effect of placebo is promising and the efficacy improves with continuous dosing. The mechanism of action is currently unknown, but the late onset of the antidepressant effects (past the time of the anticholinergic effects) suggest that the drug may work on transcription of later onset genes as opposed to working on the muscarinic receptors (Drevets and Furey 2010). This alkaloid has also been found to alleviate motion sickness

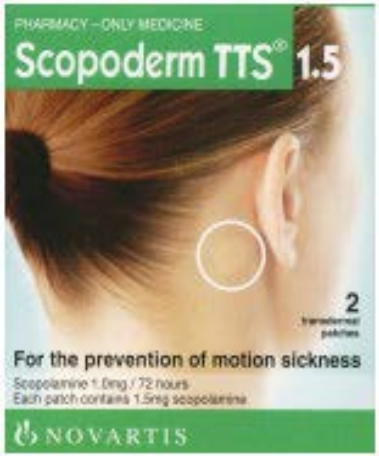

Figure 4. Scopolamine patches used to prevent the onset of motion sickness. Source: pharmacydiscounter.com

through transdermal administration (Price et al. 1981). Current uses of scopolamine also include its use in anti-vertigo drugs, as well as a preventive against motion sickness (Pyykkö et al. 1985), **Figure 4**. Major adverse effects of this drug include dryness of the mouth and throat, both common symptoms of taking anti-nauseates.

Discussion

Atropa belladonna has fascinated and intrigued people for centuries with its mystical yet deadly allure. From a notorious past as the plant of the devil to its vital role in modern medicinal practices, it has become irrevocably entwined in human culture. Although atropine is considered too dangerous to be incorporated into modern medicinal practices, its ability to act on muscarinic receptors has lead to important discoveries about human physiology and it is still critical as an antidote for cholinergic crises. Hyoscine, another important alkaloid extracted from belladonna, has aided in the mitigation of mental illnesses and helped to enhance our understanding of depression and its causes. Ultimately, the powerful and potent biological activity of *Atropa belladonna*, initially only recognized by its ability to poison people or induce hallucinogenic stupors, is what makes it such a powerful tool to the future of drug discovery. We have known about some of the effects of belladonna for centuries, and we are just now discovering some its new applications. The story of deadly nightshade is an important tale, as it clearly illustrates the mercurial temperament of western medicine and how important it is to understand that we do not yet know all there is to know about the effect of these compounds on the human body.

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Avena sativa L., Poaceae

Kylee Martens

Introduction

Avena sativa L., commonly known as oat, is a part of the Poaceae family of grasses. *A. sativa* is also called avoine in French, hafer in German, avena in Spanish, and javi or jai in Hindi (Butt et al., 2008). *A. sativa* was first discovered in ancient Egyptian ruins that date back 4,000 years and the first documented medicinal use of oats was recorded by Dioscorides in *de Materia Medica*. Though oat is believed to be Asiatic in origin, it is now predominately grown in moist, temperate regions in the United States, Canada, Russia, and Europe. Oats rank sixth in world cereal production, following wheat, maize, rice, barley and sorghum (Stevens et al., 2000). Oats contain high nitrate content largely due to the presence of phenolic compounds known as avenanthramides, which are unique to oats. They also contain soluble dietary fiber, in the form of β-glucans. Both avenanthramides and β-glucans are responsible for most of the biological and pharmacological activity of oats. Though oats were first used as food for animals and scorned for their bland taste, they are now a valued source of antioxidants and contain anti-inflammatory, antipruritic, emollient, and anxiolytic properties. The study of the medicinal value of *A. sativa* is gaining popularity as more health benefits of this functional food are being discovered.

Figure 1. *Avena sativa,* commonly known as oat. (Source: http://ddr.nal.usda.gov/bitstream/10113/3559/1/IND43886716.pdf)

Botanical Description

Avena sativa L. grows in cool, moist regions throughout the United States, Canada, Russia, and in the Mediterranean countries in Europe (Heywood, 1982). As mentioned above, *A. sativa* is in the Poaceae family and is an annual grass that typically grows and matures rapidly during the spring and summer. Oats are hardy crops that can generally withstand the cold (Vavilov, 1992). There are three main cultivated forms of oats that differ based on the number of chromosomes, including diploid *strigosa-brevis*, tetraploid *abyssinica*, and hexaploid *sativa-byzantina-nuda* (Heywood,

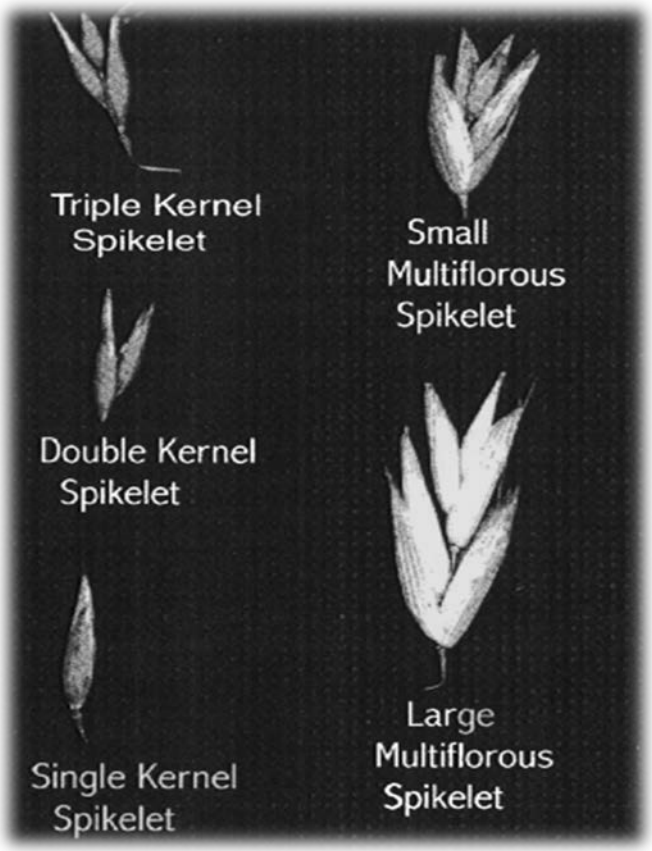

Figure 2. Spikelets of varieties of oats, which contain the oat groat. (Source: http://books.google.com/books?hl=en&lr=&id=h7coAAAAYAAJ&oi=fnd&pg=PA1&dq=history+of+oat&ots=4rjyPduEf1&sig=D1i10Ut1F1VuVPqJNnPbsM7kilg#v=onepage&q=history%20of%20oat&f=false)

1982). The hexaploid form is the most common type of subspecies.

A. sativa **(Figure 1)** consists of leaves and up to thirty tillers, or lateral shoots, projecting from each stem. The main stem and tillers can reach a height of up to two or more feet (Butt et al., 2008). Each tiller terminates in a panicle that bears spikelets (**Figure 2)**, which contain one to two kernels, also known as groats, which are small and spindle shaped. When oat plants mature into a yellow color, the groats are ready to be harvested and processed to the desired consistency. The groats are heated and dehulled and can then be processed further (Sides et al., 2001). Some of the varieties of processed oats are rolled oats, flaked oats, or steel-cut oats, often heated and eaten with water in the form of oatmeal. Colloidal oatmeal, a recognized natural skin product, is prepared through a fine-mill process, which produces a powder of oats with concentrated starch and protein (Cerio et al., 2010).

Oats in general have a rather bland taste and a tendency to spoil (Butt et al., 2008). This occurs because the grinding process activates a lipolytic enzyme system in oats, which causes rancid properties (Sides et al., 2001). Ideally, the heating process stabilizes the oat from spoiling, but doesn't affect its antioxidant content. The typical nutty, "oaty" flavor associated with *A. sativa* often presents itself after the oats have been heated.

Oats use self-pollination, in which pollen grains settle on the stigma of the same floret (Thapa, 2007). Pollen advance occurs from the uppermost floret towards the basal spikelets in about 10 to 11 days (Rajala & Peltonen-Sainio, 2011). Pollen is most often distributed by wind, but oats also benefit from insect pollination (Thapa, 2007).

Traditional Uses

Use as fodder

The origins of *Avena sativa* L. are mostly unclear, but this species is believed to have originated in Asia and may have

spread throughout Europe with barley and wheat and been mistaken as a weed (Vavilov, 1992). *Avena* most likely comes from the Latin word, *Aveo*, which means to desire (Coffman, 1977). Before oat was desired as a food source for humans, it was first used as fodder for animals in central Europe and west Asia (Pieroni, 2009). Oats are not indigenous to North America, but were instead introduced by the Spanish to the southern part of North America in the 16th century as food for their horses (Coffman, 1977). The first oats introduced in northern North America were planted by English colonists in Jamestown in the early 1600s, primarily as fodder.

Use as food

A. sativa eventually evolved into a food source for people, mainly in Ireland and Scotland beginning in the sixteenth century. English author Samuel Johnson defined oats as "eaten by people in Scotland but fit only for horses in England" to which James Boswell responded, "that's why England has such good horses and Scotland has such fine men" (Maunsell, 2011). Due to their bland taste, oats were not highly reputed and mainly eaten only in Scotland and Ireland. Oats were even thought to be a diseased form of wheat. Today, oats have evolved from a food for animals to a desired and important food for humans throughout the world. Processed oats are now found in many different kinds of foods including oatmeal, different varieties of cereal and granola, breads, and oat flour. Oats are often combined with other food condiments to enhance their bland taste.

Traditional ethnomedicinal uses

Not only were oats used for fodder and for food, but they also have a long history of use for skin treatment, especially by the ancient Greeks and Romans. Medical texts written by Pliny suggest the topical application of oatmeal for the treatment of a variety of dermatologic conditions (Sur et al., 2008). The topical application of oats was used in facial masks and baths to relieve itching and irritation, as well as eczema (Aburjai & Natsheh, 2003). Ayurvedic practitioners also used a tincture of oats to cure the opium addiction, and oats were also reputed to reduce nicotine cravings (Lewis & Elvin-Lewis, 2003). Oats were most likely selected for this use because of their sedative properties and ability to act as a depressant on the central nervous system. *A. sativa* has also traditionally been used by the ancient Greeks and Romans to treat nervous anxiety, stress, and excitation as well as to promote mental health and cognitive function (Abascal & Yarnell, 2004).

The ancient traditional uses of *A. sativa* for skin disorders have largely remained the same, and much of the knowledge of the ethnomedicinal uses of oats have come from ancient medical texts like *de Materia Medica*. While whole or rolled oats were traditionally used in baths for their soothing properties, oats are used today in the form of finely ground colloidal oatmeal (Kurtz & Wallo, 2007). Colloidal oatmeal is more effective than whole or rolled oats because it is more easily dispersed in bath water, and thereby applied more thoroughly.

Other uses

The Spanish settlers in the 16th century combined *A. sativa* with other materials to make adobe brick buildings (Coffman, 1977). Oats have not only been used as fodder, but also traditionally used as bedding for animals. Oats also were distilled for the production of alcohol, which is still a method used today (Pereira, 1855).

Chemistry and Pharmacology

Avena sativa contains about 65-85% starch and soluble polysaccharides, 15-20% proteins, 3-11% lipids, and 5% fiber (**Table 1)** (Sur et al., 2008). Polyphenols, saponin glycosides, flavonoids, and vitamin E represent the remaining compounds found in oats (Heinrich, 2004). The major medicinal chemical components of *A. sativa* are discussed below.

β-glucans

The dietary fiber in oats mainly consists of the compound β-glucan (**Figure 3)**. β-glucan is a linear-nonbranched polysaccharide with high viscosity, which is responsible for its exceptional nutritional value. Its high viscosity contributes to many of oat's medicinal properties, including its low glycemic response and its emollient properties. Oat groats contain about 2.3 to 8.5% β-glucan content, mainly present in the outer layers of the groat (Butt et al., 2008).

Phenolic Compounds

Oats contain many different forms of phenolic compounds, such vanillin, sinapic, ferulic, and caffeic acids (Bratt et al., 2003). Perhaps the most important phenolic components unique to oats and responsible for their flavor are the low molecular weight, soluble polyphenolic compounds known as avenanthramides (Meydani, 2009). Oats contain over twenty avenanthramides produced by the oat plant in response to pathogens. They are present in oats at about 300 parts per million, or 0.03%, and are available in three major forms: A, B, and C (Sur, et al., 2008).

Major Compounds	Percent Composition in *A. sativa*
Starch	65-85%
Proteins • **Avenin** • **Albumen**	15-20%
Lipids	3-11%
Fiber • β-glucan	5%
Polyphenols • Avenanthramides A, B, C • Vanillin acid • Sinapic acid • Ferulic acid • Caffeic acid	0.03%
Flavonoids • Apigenin • Luteolin • Tricin	0.05%

Table 1. Major chemical compounds found in *A. sativa*.
(Source: Sur et al., 2008)

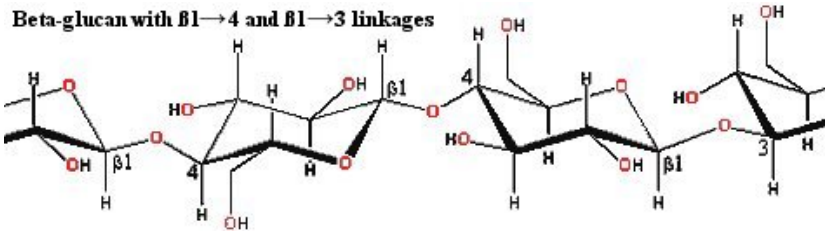

Figure 3. Structure of β-glucan, main component of soluble fiber in *A. sativa*
(Source: http://www.oatsandhealth.org/index.php?option=com_content&view=article&i d=14&Itemid=38)

Flavonoids

Three major flavonoids have been identified in oats, apigenin, luteolin, and tricin, which also are responsible for *A. sativa's* antioxidant properties and its resistance to the generation of reactive oxidative species (Cai et al., 2012). Though avenanthramides have been shown to have greater antioxidant activity than flavonoids, flavonoids are still an important medicinal component to oats.

Biological Activity

Results of *in vitro and in vivo* studies have demonstrated that *Avena sativa* has antioxidant, anti-inflammatory, antipruritic, emollient, and anxiolytic properties.

Antioxidant

The antioxidant activity of *A. sativa* was studied by analyzing the concentrations of phenolic compounds of oat fractions *in vitro*. The total phenolic content of the pearling fractions, which contain a greater percentage of the outer layers of the oat groat, was almost double the total phenolic content in other oat fractions (Emmons et al., 1999). This suggests that the outer layers of the groat, as opposed to the inner content, contain more phenolic compounds, which have been linked to increased antioxidant activity. Antioxidant activity was evaluated by β-carotene bleaching, which showed that the cinnamic acids, like ferulic acid and caffeic acid, inhibit low-density lipoprotein (LDL) oxidation (Emmons et al., 1999). Avenanthramides have also been analyzed for their antioxidant activity and have been found to have 10-30 times the antioxidant activity of the other phenolic compounds found in oats (Emmons et al., 1999). Though there are many different kinds of phenolic compounds present in oats, avenanthramides appear to be the most medicinally beneficial, present in highest amounts in the outer layers of oats.

An *in vitro* study using two different tests to determine antioxidant activity, β-carotene bleaching and DPPH assay, confirmed that avenanthramides have antioxidant properties (Peterson et al., 2002). *In vivo,* the antioxidant activity of avenanthramides was also analyzed by feeding rats an avenanthramide-enriched diet of 0.1g/kg for 50 days (Li Ji et al., 2003). The results of this study concluded that the rats that were fed this diet had decreased reductive-oxidative species (ROS). Specifically, it was found that avenanthramides increased the level of superoxide dismutase (SOD) and glutathione peroxidase (GPX). Both SOD and GPX are tissue antioxidant enzyme systems.

Anti-inflammatory and anti-irritant

Avenanthramides have also been shown to have anti-inflammatory properties. It was found in an *in vitro* study that avenanthramides inhibit NF-κB signaling in cells treated with these polyphenols (Sur et al., 2008). NF-κB is an important regulator of expression of pro-inflammatory proteins. By inhibiting this signaling, avenanthramides inhibited the production of interleukin-8 and thus prevented inflammation in cells.

The anti-irritant properties of oats have also been studied and attributed to the effects of avenanthramides. Specifically, these phytochemicals have been shown to decrease the ionophore-stimulated liberation of arachidonic acid and can inhibit prostaglandin biosynthesis (Sur et al., 2008). By

inhibiting these processes, oats can naturally relieve skin irritation through this topical application of *A. sativa*.

Antipruritic

The antipruritic property of avenanthramides was also evaluated in an *in vivo* study in mice. Avenanthramide-treated mice scratched 40.7% less than the control mice when injected with pro-itch compound 48/80 (Sur et al., 2008). Because avenanthramides can inhibit the production of interleukin-8, they can thereby inhibit the release of inflammatory cytokines shown to be elevated in pruritic skin disorders. Avenanthramides may also inhibit histamine signaling, which suggests another mechanism for the role of these compounds in the treatment of chicken pox and other pruritic skin diseases (Sur et al., 2008).

Emollient

In vitro studies on the effects of β-glucan on the skin have concluded that this dietary soluble fiber is able to penetrate the dermis layer of the skin. Pillai et al. concluded that β-glucans interact directly with macrophages to produce interleukin-1 (IL-1), which promotes the production of procollagen (2005). The conversion of procollagen to collagen explains oat's emollient properties, as well as its cosmetic uses for the reduction of wrinkles in the skin. β-glucan's high viscosity also contributes to oat's emollient and moisturizing properties.

Anxiolytic Properties

Oats have also been proven to act as a mild sedative to alleviate anxiety, stress, and excitation (Abascal & Yarnell, 2004). A recent *in vivo* study was performed on rats to access the effects of oats on behavioral responses in situations that would produce anxiety (Schellekens et al., 2009). Rats given oat extract showed friendly social behavior and less aggressive behavior in agonistic interactions, supporting the claim that oats provide anti-anxiety and calming effects. The oat extract showed inhibition of the neurotransmitter metabolizing enzyme monoaminoxidase B and of the cAMP degrading phosphodiesterase 4. These enzymes play a role in anxiety and depression, so this inhibition may account for the anxiolytic properties of *A. sativa*.

Clinical Studies

Various clinical studies have been conducted to study the medicinal benefits of *A. sativa* in the prevention and treatment of heart disease, diabetes, skin disorders, gastrointestinal disorders, and libido.

Heart Disease

Avena sativa has been linked to cholesterol-lowering effects, which reduce the chance for developing coronary heart disease. In a study conducted to analyze this property of oats, it was shown that there was an 8.7% reduction in low-density lipoprotein (LDL) cholesterol levels in subjects who ate two oat-bran muffins a day for a month (Gold & Davidson, 1988). Another study demonstrated that there was a similar reduction of total and LDL cholesterol levels in patients with an increased coronary heart disease risk (Berg et al., 2002). Soluble fibers, like β-glucan, are able to bind to bile acids and cholesterol, which increases bile acid excretion (Berg et al., 2002). This excretion increases lipid excretion and upregulates the amount of hepatic cholesterol receptors,

Figure 4. Aveeno skin lotion contains 1% colloidal oatmeal
(Source: http://www.aveeno.com/skincare/products/daily-moisturizing-lotion)

which help to clear cholesterol from the blood. In 1997, the FDA issued a statement that declared that diets low in saturated fat and cholesterol that include soluble fiber from whole oats may reduce the risk of developing heart disease (Wood, 2006). This statement recognized the beneficial effects of soluble fiber from β-glucans in *A. sativa* in the prevention of heart disease. There are also a few studies that support the blood-pressure lowering effect of β-glucans. Specifically, Maki et al. studied the effects of a β-glucan diet in obese patients (2007). The blood pressures of these patients dropped significantly when they followed this diet, suggesting a strong correlation between β-glucan and lowered blood pressure in overweight patients. This correlation may be related to insulin responses, which may have indirectly lowered blood pressure in these patients, but additional research is necessary to define the mechanism of lowered blood pressure in response to the intake of *A. sativa*.

Hyperglycemia

Oats have been associated with the prevention of diabetes by lowering blood-glucose levels, which prevents the development of insulin insensitivity. Specifically, β-glucans in oats have been studied for their blood-glucose-lowering capabilities. In one study, blood-glucose levels of both healthy and type 2 diabetic subjects were reduced after consuming oat bran as compared to the blood-glucose levels of subjects in the control group who received no oat products (Wood, 2006). The primary factor responsible for oat's low glycemic response and reduced blood glucose levels is β-glucan's high viscosity.

Skin Disorders

A. sativa has been used to treat eczema and repair UVA/UVB damaged skin (Aburjai & Natsheh, 2003). Oats have even been evaluated for their efficacy in the reduction of wrinkles. In one study by Pillai et al., 27 subjects used gel formulations of β-glucan twice daily for 8 weeks, applying the gel to the face (2005). A significant reduction of wrinkles was observed on the faces of the subjects, and it was also concluded that β-glucan penetrates the dermis layer of the skin and is able to stimulate the production of collagen.

Using the sodium lauryl sulfate irritation model, Vie et al. studied the anti-inflammatory effects of oat extract on the upper arms 12 healthy subjects (2002). The oat extract was able to significantly counteract the inflammation caused by this irritation model, suggesting the use of oat products in the reduction of skin irritation and inflammation. Avenanthramides were shown to have these anti-inflammatory and anti-irritant effects, which explains the use of oats in the treatment of dermatitis and eczema.

Celiac Disease

Celiac Disease is an autoimmune disease that generates intolerance to gluten. Oats have been studied in patients with Celiac disease to determine if they can be tolerated (Butt et al., 2008). Oats do not contain the intolerable protein gliadin that is found in wheat, but instead contain the protein avenin. Oats are safe to eat by those with Celiac disease, but they must be pure because often, processed oats contain some gluten from other grains. Pure oats provide an alternative food source for celiac patients who follow a gluten-free diet.

Gastrointestinal Disorders and Colon Cancer

Soluble dietary fiber in oats, found in the form of β-glucans, has also been shown to have numerous beneficial effects on the gastrointestinal system. The high viscosity of β-glucans have been shown to increase fecal wet-weight, and thereby relieve constipation (Malkki & Virtanen, 2001). There may also be a correlation between the soluble fiber in oats and the prevention of colon cancer. This is mainly due to the fermentation of the fiber in the intestine, which stimulates cell proliferation in normal epithelium, but prevents the growth of carcinoma cells (Malkki & Virtanen, 2001). This potential anti-cancer effect is still being reviewed and researched.

Libido and impotence

The phrase, "sowing ones wild oats," was coined in the 16th century to describe destructive sexual liaisons. The correlation between sex and oats has been widely marketed in the past few years, and oats have been suggested as a treatment for male erectile dysfunction. However, a few studies have confirmed its effectiveness in treating erectile dysfunction and promoting libido (Moyad et al., 2004). Though oat is a well-known aphrodisiac, more research must be conducted to determine its effectiveness in treating impotence.

Contraindications

Avena sativa has no known contraindications, adverse effects, or interactions (Abascal & Yarnell, 2004). It is generally regarded as safe (GRAS). Colloidal oatmeal is one of the few natural products that has been recognized and approved by the FDA in the treatment for dermatologic conditions (Cerio et. al, 2010). In 1997, the FDA also recognized that oats rich in soluble fiber may reduce the risk of heart disease (Wood, 2006).

Current Use in Allopathic and CAM Therapies

Avena sativa ranks sixth in world cereal production and has gained much popularity since they were first used solely as fodder. Not only are they eaten, but they are also widely used for their emollient properties. Oats have long been used to provide antipruritic relief, skin hydration and smoothness. In

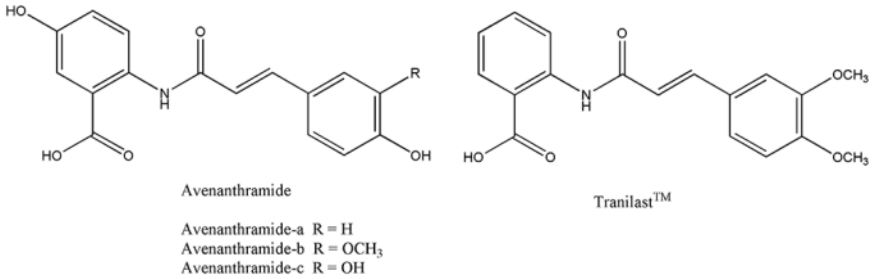

Figure 5. Structure of drug Tranilast compared to an avenanthramide, polyphenol compound unique to *A. sativa.*
(Source: http://onlinelibrary.wiley.com/doi/10.1111/j.1753-4887.2009.00256.x/full)

1945, colloidal oatmeal became available by finely grinding the oat and extracting the colloidal material (Kurtz & Wallo, 2007). Today, colloidal oatmeal is included in shampoos, bath powders, and in moisturizing creams like Aveeno **(Figure 4)**. The use of oats in skin products for cosmetic purposes provides a cheaper and safer alternative to cosmetic procedures. In 2003, the FDA approved colloidal oatmeal as a safe and effective skin protectant based on its moisturizing and anti-irritant properties (Aburjai & Natsheh, 2003). Colloidal oatmeal is also commonly used to treat the itching and irritating symptoms of chicken pox, due to its soothing and antipruritic effects. It is also currently used to topically treat diseases like dermatitis and psoriasis (Cerio et. al, 2010).

Currently in Japan, the synthetic drug Tranilast, with a structure similar to an avenanthramide, is used as an antihistamine (Meydani, 2009). Both avenathramides and Tranilast (**Figure 5**) were discovered to have anti-proliferative effects on vascular smooth muscle cells, which could explain the mechanism by which oats reduce the risk of colon cancer. Avenanthramides may also inhibit histamine signaling and play a similar role as the drug Tranilast, which could also explain the antipruritic effects of avenanthramides (Sur et al., 2008).

A. sativa is currently sold as a supplement and is marketed as an aphrodisiac to improve sexual libido. There are few studies that confirm these properties of oats, and these herbal supplements have not yet been proven to be effective. Some oat extract supplements also claim to improve nervous system health, though this claim also needs to be further studied.

Discussion

Avena sativa L. has evolved throughout the centuries, since it was first used in ancient Egypt almost 4,000 years ago. Because of its rich content of avenanthramides and β-glucans, oat is a rich source of antioxidants and it contains anti-inflammatory, anti-irritant, antipruritic, emollient, and anxiolytic properties. The traditional medicinal knowledge of oats for the skin, discovered over 2,000 years ago by the ancient Greeks and Romans, is still valued today, and continued research of oats has helped to discover both CAM and biomedical treatments for heart disease, diabetes, gastrointestinal ailments, and impotence. *A. sativa* provides a natural prevention to many of the prevalent diseases that humans are succumbing to throughout the world. Oat is a cheap and healthy plant that is easily accessible and can be added to the diet with no known contraindications. For these reasons, the medicinal future of *A. sativa* appears promising as this plant gains more popularity as a healthful food source, and continued research leads to the discovery of more of the medicinal benefits of this plant.

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Banisteriopsis caapi (Spruce ex Griseb.) C.V. Morton, Malpighiaceae

David Nardo

Introduction

The term ayahuasca refers to a hallucinogenic brew used in many of the healing practices and religious rituals of the natives of the Amazon Basin region. The word stems from Quechua and means "vine of the souls" (aya = spirit or ghost, huasca = vine or rope). The hallucinogenic brew is composed of a myriad of plants and varies in recipe from person to person, the only constant being the use of *Banisteriopsis caapi. B. caapi* is known colloquially by many names, among them are ayahuasca, yaje (also yage), caapi, and huasca (Dobkin de Rios, 1971; Rivier & Lindgren, 1972). *Banisteriopsis caapi* (**Figure 1**), of the Malpighiaceae family, is a vine that has been the source of several political arguments over the last few decades as it has made its way from the heart of the Amazon into 'civilized' areas and has been exploited by thrill seekers under the pretext of achieving a higher state of mind and a more personal relationship with god.

Although little is known about the origins of its use, the role of the plant in indigenous cultures dates back to ancient times. There is an abundant body of archeological evidence affirming its use as early as 1500, although much of this archeological evidence seems to attest more to the use of other pants, like tobacco and coca, rather than ayahuasca (Dennis J, 2004). Knowledge of the plant and its use have been passed down through generations, from shaman to shaman, and continues to take part in many of the practices today. Non-natives that also use the brew in their religious practices are the UDV, Barquinia and Santo Daime sects in Brazil. These, although not part of native tribes, have partaken in many of the customs

Figure 1. Botanical drawing of *Banisteriosis caapi* (Image source: http://jornadasdamente.blogspot.com/p/ayahuasca.html)

and religious practices of the native, including the consumption of ayahuasca. It is also because of these groups that the plants used to concoct ayahuasca have made it into mainstream culture. Despite the cultural roots of the beverage, however, it seems likely that the use of ayahuasca will fade from society, namely because of people who use the cover of religion to partake in the use of ayahuasca. As more and more people join religious communities, like the Santo Daime, for the sole purpose of usingthe drug, the public opinion will over time become worse and most likely, the plants that are used in making ayahuasca will be banned from every country.

Figure 2. *B. caapi* vine. (Image source: http://interactivetimeline.com/824/timeline-of-entheogen-use/57.php?w=580)

Figure 3. Young leaves of *B. caapi*. (Image source: http://en.wikipedia.org/wiki/File:Caapi.jpg)

Botanical Description

Banisteriopsis caapi (**Figure 2**) is endemic to the entire Amazon basin area and can be found anywhere from Brazil, to Peru, to Ecuador (Tupper, 2008). The plant grows naturally in the moist climate of South America but can also be cultivated through the cuttings: the cuttings are allowed to sit in water until they develop roots, at which point they can be transplanted to soil (Luna, 1984). It should be noted, however, that most of the plants used in making the ayahuasca brew are taken from the wild and not cultivated (De Alvarez De Toledo, 1960; Dobkin de Rios, 1971; Flores & Lewis, 1978; Kjellgren, Eriksson, & Norlander, 2009).

The ayahuasca plant takes the shape of a large vine and grows only in moist tropical climates. In its early stages, the plant stems are green and the leaves are small (**Figure 3**). However, as it reaches maturity, the vine becomes long and woody and branches significantly. The woody stems of the vine can reach several inches in diameter, giving the appearance of thin trees or tree branches. In adult vines, the leaves are green and appear only on the smaller branches. The leaves have an oblong shape that points at the ends and can reach about 7 inches in length. On the more distal branches grow the inflorescence holding the flowers, which are white or pale pink and reach about a half inch in length (**Figure 4**). Polinators include bees and other forest insects that help carry pollen (Dennis J, 2004; Rätsch, 2005). The seeds, due to their "helicopter shape" (see **Figure 5**) are able to travel great distances being carried by the wind.

With pertinence to the plant's use in making the ayahuasca brew it should be noted that two varieties that have been distinguished, *B. caapi* var. *caupari* and var. *tukonaka.* The latter of these two is the better known one, which has a

Figure 4. Flowers of *B. caapi.* (Image source: http://upload.wikimedia.org/wikipedia/commons/f/f6/Banisteriopis-caapi-flowers-lg.jpg)

smooth stem. The former, said to be more potent, has knotty stems (Gates, 1982; Kjellgren et al., 2009; Rätsch, 2005). Aside from the differences in stem shapes and in chemical concentrations, there seem to be no other notable differences in the varieties.

Traditional uses

Although the plant leaves are sometimes smoked, caapi is most commonly consumed in a brew. The shamans believe that, while other plants are responsible for visions, it is *B. caapi* that allows them to connect to the spirit world and to escape their corporeal forms. They consider the plant to be a connector to the realm of the spirits where they can communicate with the dead, or with the spirits of the forest, from whom they can derive knowledge on healing, etc

Figure 5. "Helicopter-shape" seeds of *B. caapi.* (Image source: http://www.shamanic-extracts.com/xcart/shamanic-products/banisteriopsis-caapi-seeds.html)

(Dobkin de Rios, 1971; Flores & Lewis, 1978; Kjellgren et al., 2009; Luna, 1984; Reichel-Dolmatoff, 1987). The plant is used for spiritual, rather than physical healing. Although it does not produce physical healing, *B. caapi* is believed to confer a sense of completeness and a connection with the spiritual. It is used by shamans as a guide when they are trying to find answers to problems, and it is used by laymen in trying to resolve conflicts.

In preparing the ayahuasca brew, the stems, bark and leaves of the vine can be employed, although the stems are used in most cases (**Figures 6 and 7**) (De Alvarez De Toledo, 1960). The stems of the plant are taken and crushed and placed in a vessel and other plants, such as *P. viridis*. The vessel is the filled with water and the mixture is boiled for at least one hour, after which the brew can be strained and allowed to cool (Rivier & Lindgren, 1972).

On her treatise of the plant, Dr. de Alvarez de Toledo mentions that the plant is used by Peruvian natives 'when a person is in love and the family knows that the other person is bad for them and they take them to a healer'. It is believed that the plant can help a person find out another person who has wronged you and can also work to make you confess to

Figure 6. Ayahuasca vine. (Image source: http://upload.wikimedia.org/wikipedia/commons/5/51/Banisteriopsis-caapi-vine2.jpg)

wrongdoing. The natives also mentioned that, while under the influence of ayahuasca, a healer can also make a person commit crimes. Upon taking the beverage, the first symptoms felt are usually nausea and vomiting. Once these started, it was only necessary to open one's eyes and see, by focusing the mind, what it was that one wanted to see, whether it was those who wished us ill, or a dead person's spirit. The overall experience described by de Alvarez de Toledo, however, is extremely disappointing since, according to her, the experience was not at all what the natives described and

Figure 7. Preparation of Ayahuasca. (Image source: http://fbgamestipsandtricks.com/wp-includes/Text/ayawaska-buy)

rather she perceived but mild pseudohalucinations. Finally, she adds that the natives of the High Amazon use the plant in the treatment of mental illnesses, although she was unable, herself, to presence any of these (De Alvarez De Toledo, 1960).

Chemistry (pharmacology and toxicology)

Despite what one might assume, none of the chemicals in *B. caapi* are responsible for causing hallucinations in humans. Instead, *B. caapi* contains high amounts of β-carboline MAOIs (uses discussed later), namely banisteriopside A and B, harmine, tetrahydroharmine, harmaline, harmol and harmamol (also called h-carbolines) (Riba et al., 2003). The stems of the plant contain up to .83 percent of these alkaloids,

Plant Part	Yield,[a]	Compounds Quantified (Content, %)								
	%	1	2	3	4	5	6	7	8	9
Fresh leaves	0.49	0.002	0.0004	0.0004	0.000	0.004	0.0001	0.002	0.021	0.002
Fresh leaves[b]	5.28	0.072	0.015	0.011	0.003	0.114	0.002	0.042	0.333	0.090
Dried leaves	10.06	0.166	0.027	0.005	0.005	0.101	0.004	0.031	0.070	0.030
Fresh young stem	0.85	0.009	0.002	0.0004	0.0003	0.005	0.0003	0.001	0.002	0.002
Fresh young stem[b]	11.70	0.129	0.032	0.007	0.005	0.055	0.005	0.020	0.070	0.070
Dried matured stem	12.74	0.270	0.572	0.004	0.003	0.148	0.042	0.233	0.166	0.624
Dried matured stem[b]	9.27	0.075	0.046	0.002	0.003	0.030	0.005	0.007	0.195	0.185
Fresh bark of matured stem	2.16	0.085	0.198	0.005	0.004	0.012	0.005	0.089	0.151	0.093
Dried bark of matured stem	14.29	0.314	0.809	0.019	0.014	0.041	0.017	0.280	0.672	0.486
Fresh debarked young stem	2.67	0.087	0.058	0.001	0.002	0.107	0.015	0.063	0.112	0.096
Dried debarked young stem	9.76	0.267	0.188	0.007	0.002	0.340	0.058	0.226	0.527	0.703
Fresh leaves	0.60	0.005	0.001	0.001	0.0002	0.010	----	0.003	0.014	0.005
Fresh young stem	0.80	0.005	0.002	0.002	0.001	0.006	----	0.002	0.011	0.002
Fresh bark of large branch[d]	3.0	0.117	0.322	0.003	0.007	0.020	0.004	0.098	0.051	0.018
Dried bark of large branch[d]	18.1	0.711	1.926	0.018	0.040	0.103	0.029	0.672	0.434	0.163
Fresh debarked large branch[d]	5.2	0.120	0.279	0.002	0.010	0.089	0.018	0.177	0.062	0.036
Dried debarked large branch[d]	7.6	0.154	0.378	0.002	0.010	0.103	0.020	0.229	0.205	0.114
Fresh leaves	3.71	0.080	0.049	0.007	0.003	0.126	0.007	0.062	0.063	0.056
Fresh young stem	2.50	0.027	0.041	0.005	0.001	0.016	0.004	0.040	0.008	0.010
Dried young stem	8.20	0.158	0.125	0.003	0.007	0.157	0.030	0.103	0.098	0.148
Fresh leaves	2.17	0.018	0.007	0.004	0.002	0.039	----	0.011	0.254	0.156
Dried leaves	8.33	0.107	0.021	0.008	0.006	0.147	----	0.025	0.017	0.025
Fresh matured stem	2.9	0.062	0.103	0.002	0.003	0.050	0.010	0.075	0.078	0.099
Dried matured stem	9.56	0.210	0.382	0.004	0.008	0.207	0.046	0.350	0.057	0.115
Dried matured stem (BCEx-1)	15.43	0.150	0.074	0.002	0.006	0.103	0.017	0.043	0.046	0.031
Dried matured stem (BCEx-4)	9.80	0.170	0.153	0.002	0.008	0.159	0.024	0.133	0.245	0.382

[a]Yield of the aqueous plant extract measured by weight.
[b]Extracted with water by automated ASE-200 extractor.
[c]Plants from this collection are 3 years old.
[d]Large branches: diameter: 3-8 cm. NT = Not tested. NA = No antioxidant activity up to 31.25 μg/mL.

Table 1. Quantification of markers banistenoside A (**1**), banistenoside B (**2**), harmol (**3**), THNH (**4**), THH (**5**), harmaline (**6**), harmine (**7**), epicatechin (**8**) and procyanidin B2 (**9**) of various extracts of *B. caapi*. Adapted from: http://ukpmc.ac.uk/articles/PMC2878139?table=T1/.

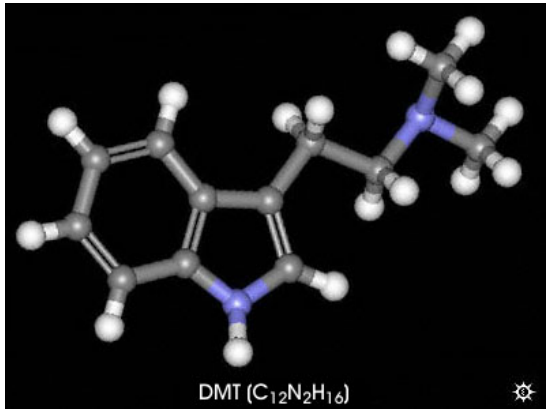

Figure 8. DMT, found in Ayahuasca brew (derived from other species – not *B. caapi*). (Image source: http://www.erowid.org/chemicals/dmt/dmt_chemistry.shtml)

while the branches contain up to .37, the leaves up to .70 and the roots contain up to 1.95 percent (**Table 1**) (Riba, Anderer, Jane, Saletu, & Barbanoj, 2004; Riba & Barbanoj, 2005; Riba et al., 2003; Rivier & Lindgren, 1972; Wang et al., 2010; Winkelman, 2005). Interestingly, in dried plant material, the concentration of these chemicals does not seem to diminish, but rather increases, indicating that they are not lost due to evaporation.

In addition to the great variety and quantity of β-carboline MAOIs found in *B. caapi*, there are also two proanthrocyanidins, epicatechin and procyanidin 2B, which exhibit a great deal of antioxidant activity. There are also several other compounds in the plants that have yet to be classified, but exist in very small amounts (Wang et al., 2010). It should be noted that several sources indicate that DMT (discussed later) is present in *B. caapi*. According to all the literature cited here, this is not the case. While DMT (**Figure 8**) *is* present in the ayahuasca brew, it does not come from *B. caapi*, but rather from other sources.

Biological activity

The procyanidins mentioned in the previous section, epicatechin and procyanidin 2B, belong to the flavonoid class of compounds. Flavonoids have the distinct ability that they are able to rid the body of reactive oxygen species (ROS). ROS are oxygen containing compounds that can be found in the body as a response to pathogens, as a result of oxidative phosphorylation, or simply due to intake in certain foods. These compounds contain unpaired electrons with extremely high levels of energy that cause them to react with nearby molecules, resulting in damage to tissues and potentially causing cancer. Flavonoids, as do other antioxidants, interact with these ROSs and absorb the damage, thus preventing any harm to the body. Because of the composition of these compounds, they are able to deal with the stress created by ROS much better than say, DNA or cell phospholipids. Drinking ayahuasca has also been linked with improved immunity and several of the β-carbolines have shown effectiveness in getting rid of protozoal, bacterial and helminthic pathogens (Dennis J, 2004).

For the making of the brew, however, it is the β-carboline MAOIs that prove useful. One key aspect of digestion is the ability to break down nitrogen containing compounds. One class of nitrogen containing compounds are monoamines, compounds containing a single nitrogen atom. Via a process called monoamine oxidation, spearheaded by molecules called monoamine oxidases (MAOs), single-nitrogen containing molecules are broken down by the body. This results in the formation of a complex carbohydrate and an ammonia molecule, which is then expelled along with other wastes. The β-carbolines found in *B. caapi*, such as harmaline, act as monoamine oxidase inhibitors (MAOIs). By performing this duty, MAOIs are able to prevent the breakdown of single-nitrogen containing molecules (MB & PF, 2004).

One of the uses of MAOIs has been in the treatment of depression. Depression is caused by a low level of serotonin in the brain. Serotonin is normally released from one neuron to the next and is reabsorbed from the neuronal synapse. As a monoamine, however, serotonin is sometimes broken down by MAO and the resulting drop in serotonin levels eventually leads to depression. Later studies also found that in patients with Parkinson's disease, who are also afflicted by low levels of serotonin, MAOIs have a significantly positive effect, which has led to research on the use of MAOIs for this condition (MB & PF, 2004). MAOIs also affect the concentrations of epinephrine and norepinephrine in the body. Like serotonin, these two molecules are monoamines and can be up-regulated by administering MAOIs. This can help regulate several conditions that are dependent of these neurotransmitters (Dennis J, 2004).

Indeed the happy feeling of those who take ayahuasca is likely due to the effects of MAOIs in the brew (Dennis J, 2004). However, what makes *B. caapi* so interesting is the interaction that it has with another chemical present in the brew. The compound in the ayahuasca brew that produces hallucinations N,N-Dimethyltryptamine (DMT), a monoamine that is not actually found in *B. caapi*, but in *Psychotria viridis*, *Diplopterys cabrerana* and the other plants used in the ayahuasca brew.

Upon oral ingestion, a drug has to go through the stomach where it is broken down and eventually absorved in the intestine, this is also true of DMT. However, upon reaching the stomach DMT is broken down by monoamine oxidases and it is unable to reach the brain, where it causes its hallucinogenic effects. DMT acts by interacting with serotonin and dopamine receptors in the brain, which lead to the euphoric and hallucinogenic properties of the chemical. When inhaled or injected, DMT can act on the body to induce these properties and it abilities have been properly documented by researches in the field. However, when taken orally, DMT is broken down and procures no reaction (de Araujo et al., 2011).

By combining *B. caapi* with other plants that contain DMT the stomach is unable to break down DMT and it can be absorbed into the bloodstream and make its way to the brain. What makes ayahuasca so interesting is that it provides such a finely balanced mixture of chemicals, coming from a people who haven't the slightest knowledge of chemical. The ayahuasca brew exemplifies the complexity and the knowledge of the natives of South America (Morton, 1931; Riba et al., 2004; Riba et al., 2003; Rivier & Lindgren, 1972; Wang et al., 2010).

Contraindications

B. caapi alone seems to confer the MAOI effect in the brew. When an extract of the vine is used alone, it produces mood-enhancing and sedative properties, however, the β-carbolines in the plant can induce nausea and vomiting (Dobkin de Rios, 1971; Luna, 1984; Rivier & Lindgren, 1972). Aside from the nauseating feelings caused by ayahuasca consumption, the brew also seems to have other side effects. Among the more significant ones is an increase of diastolic pressure. According to several studies, however, this might actually be caused by DMT and not by any of the chemicals found in *B. caapi* itself (Riba et al., 2003).

Several studies seeking to test the potentially adverse effects of the plants showed no adverse side effects in pregnant women, or their children (dos Santos, 2010; Labate, 2011). There is also no evidence to suggest that consumption of the plant has negative effects on teenagers (Doering-Silveira et al., 2005). Frequent use of the plant seems to have a higher rate of illicit drug use, although ayahuasca itself does not seem to

have any particularly harmful or addictive properties (Fábregas et al., 2010).

In interesting case mentioned by McKenna is that the β-carbolines in *B. caapi* can induce epileptic form seizures in rat and in chickens homozygous for the epileptic gene. In addition, rats fed with *B. caapi* extract showed decreased fertility (Dennis J, 2004).

Current Uses in Allopathic and CAM Therapies

Most studies performed on *B. caapi* focus on trying to determine whether the plant is safe to use, or not. Some research has been done regarding its potential use in treating substance abuse, alcoholism, serotonin deficiency, as well as immunity enhancing, but these studies have been uncertain and have ahd difficulty progressing, often because of legal issues with the plant (Dennis J, 2004).

Today, *B. caapi* is still used by South American natives in pretty much the same manner that it has always been used. In addition, several groups throughout the world use the plant in their so called religious practices. Among these are the UDV, Barquinia and Santo Daime sects in Brazil, who use the plant in religious rituals in trying to become enlightened, but have been in part responsible for bringing the attention of authorities to the plant (Dennis J, 2004).

Another important aspect of ayahuasca use is the artistic visions it produces. Several artists, like Peruvian native Pablo Amaringo and New Yorker Robert Venosa, have helped popularize ayahuasca through awing portrayals of their visions (**Figure 9**). These paintings range from simplistic geometric patterns to very complex symbolist imagery of the spiritual ideas associated with ayahuasca. Vines and serpents

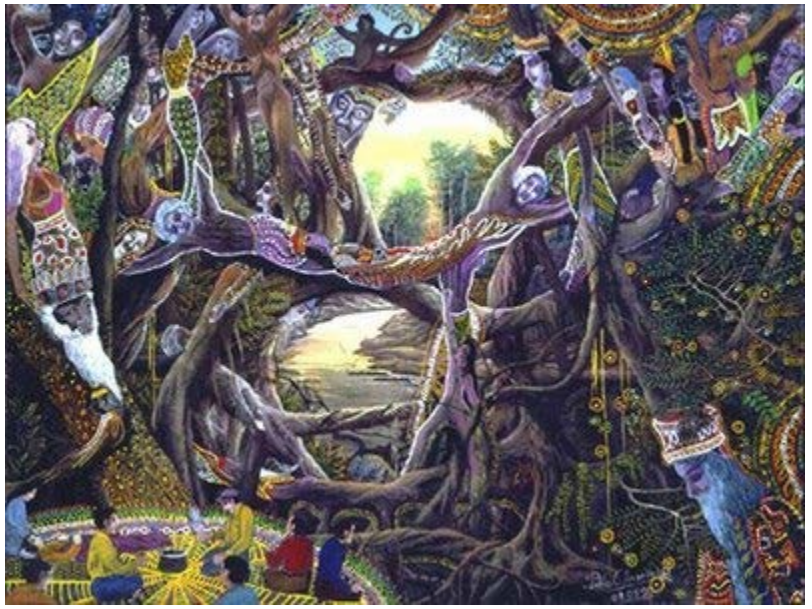

Figure 9. Ayahuasca vision art. (Image source: http://inyectamemas.blogspot.com/)

are often seen in the paintings as well as other images that are imbedded in the psyche of the drinker (Shanon, 2010).

Legal Status

During the twentieth century, media and government attention was brought to ayahuasca. This was due in part to the previously mentioned religious groups, many of which have a negative light in the public's eye. These religious groups, like Santo Daime are based on syncretic beliefs, taking from African, Native American and European Judeo-Christian cultures. Their goal in using ayahuasca is to achieve a connection with God. As these groups have made their way out of Brazil into Europe and the United States, they have

gained popularity among those who wish to "acheive enlightenment". In addition to those who seek enlightenment, there are many who have learned to use ayahuasca as a recreational drug. This has brought the plants used in the brew, *B. caapi* included, to be questioned by politicians and mass media (Kjellgren et al., 2009; Winkelman, 2005).

In the United States and in most other countries, DMT is a Schedule I drug. However, the right to use plants containing DMT varies from country to country and there are no international laws prohibiting the growth and sale of these plants by the public (Bullis, 2008; Dennis J, 2004; Winkelman, 2005). This means that those who wish to purchase *B. caapi* or *P. viridis* can do so freely in most countries and they can do with these plants as they desire.

In the Unites States, those who believe the plant is harmful and those who use it in their spiritual practices are debating the use of plants containing DMT. The law currently allows for the importation of many of the plants used to make ayahuasca for the purpose of religious ceremonies. Brazil has had similar issues since the 1980s; they have concluded that so long as the plant is being used for religious purposes, then the consumption of the plants is allowed. However, the Brazilian government bans the use of ayahuasca for recreational purposes. The legal status in the Netherlands is a bit more permissive, as people are allowed to buy the plants needed for the brew as they please (Bullis, 2008; Dennis J, 2004).

Conversely, in France and the UK the law forbids the sale and use of any plant containing DMT. Several public articles online explain how a French supreme court ruling in 2005 allowed the use of ayahuasca plants so long as no extractions were performed on these to obtain pure DMT. This decision was overturned four months later because of another case that made all of the ingredients in ayahuasca illegal to use or possess.

Discussion

Ayahuasca's healing properties exist for those who believe in them. The natives continue to use the plant for healing rituals that focus on healing the soul and the mind. In those cases where they use the plant to search for lost causes to illnesses, the healer, or the "patient", interprets the hallucinations created by the plant to discover a cause or cure. Again this requires a great deal of belief in the spiritual something that is not found in many "civilized cultures." As far as Western medicine is concerned, ayahuasca conveys no actual medicinal purpose and like many other plants used by indigenous people, its useful for nothing other than sooth saying and spiritual psychobabble. What little evidence is reported on the potential use of the plant in treating diseases is unconvincing and the current legal issues regarding the plant make it barely worth the effort.

While the Native Americans who use ayahuasca do so in order to heal, whether the mind or some physical state through spiritual means, the newer groups that have spread ayahuasca to the rest of the world propagate it as purely religious plant. In the religious ceremonies of groups like Santo Daime, ayahuasca takes the brew to connect with God. This has led thrill seekers to join these religions for the sheer purpose of "getting high". What is worse, people have started to use it for recreational purposes. Since the spread of Santo Daime, drug tourism has caused a negative light to be shed on ayahuasca (Bullis, 2008).

In reality, the brew seems to convey little harm to those who take it, yet many countries, like France, have chosen to ban the

plants used to make ayahuasca. Because of the exploitation of the plant by tourists and by those who have close constant with natives, as well as by natives themselves, who choose to sell their knowledge for monetary gain, the banning of the ayahuasca plants from public opinion is imminent. Looking at the history of plants like cannabis, it seems inevitable that ayahuasca will eventually become one of the many plants that are controlled by government regulations globally.

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Calendula officinalis Linn. Asteraceae

Paula Tyler

Introduction

The Common Marigold or Pot Marigold, is formally known as *Calendula officinalis* Linn. of the Asteraceae family (formerly Compositae) (Gilman & Howe, 1999). This small and unassuming but brightly colored herb is also known as the Bride of the Sun, Mejorana or flaminquillo in Spanish, fior d'orgni in Italian, gauche-fer or souci des champs in French, goldblume or Ringelblume in German, and the golden flower of Mary (Basch et al., 2006). Native to Europe and parts of Asia, *C.officinalis* has been cultivated since the 12th century for medicinal use as an antiseptic, anti-inflammatory, cicatrizing agent, and antibacterial (Khalid & da Silva). *C.officinalis* is also used for culinary purposes, and for ethnoveterinary medicine. It contains many types of secondary metabolites, including terpenoids, flavonoids, carotenoids, volatile oils, quinones, lipids, and coumarins. Many of these compounds have potent pharmacological and biological activities that support its history of ethnomedicinal use.

Figure 1. *Calendula officinalis.* Note the characteristic yellow-orange color and the ovate leaves. (Image Source: http://pharmacology.georgetown.edu/urbanherbs/calendula.htm)

Botanical Description

Calendula officinalis is a hardy cool season annual herb. Its habit is round, with single or double showy white, orange, or yellow flowers (**Figure 1**). It grows 12-18 inches in height and width, and it is dense with simple ovate green leaves. It grows in full sun in acidic clay or sand and is known to attract butterflies (Gilman & Howe, 1999). *C. officinalis* is indigenous to Europe, and is cultivated in North America, Europe and the Balkans, and India (Khan et al., 2011).

Traditional Uses

Ethnomedical

Bangladesh

From an ethnomedicinal survey of the Kavirajes of the Kushtia District of Bangladesh, *Calendula officinalis* is known as Ganda. The leaf juice is applied topically for ear aches, skin infections, and insect bites (Rahmatullah et al., 2009).

India
For medicinal uses in India, *Calendula officinalis* cream is often combined with other herbs to treat hemorrhoids, burns, and abrasions. The florets in particular are made into ointments for wounds, herpes, ulcers, and other skin damage. Varicose veins are treated with an infusion of the leaves. Other ethnobotanical applications include the use of dried flowers as an insect repellent (Khalid & da Silva).

Italy
In a phytotherapy study conducted in the Peninsula Sorrentina of southern Italy, *Calendula officinalis* was a constituent of mixtures used for the treatment of unspecified varices. It is known locally as Calendula or sciure'e Sant'antonio (De Feo et al., 1992). *C.officinalis* teas are also used for eye infections, throat inflammation or pharyngitis, gingivostomatitis, and inflammatory skin conditions (Khalid & da Silva).

Portugal
Trás-os-Montes is a small area in Northern Portugal. *Calendula officinalis* is known there as Calêndula, Mariana, or Maravilha. The dry flowers are popularly used for problems with anxiety (nervousness and insomnia), the liver (jaundice), the eye (inflammation), digestion (ulcers), the skin (callus and warts), and incontinence (Neves et al., 2009).

Serbia
Calendula officinalis is locally known as Neven in the Kopaonik Mountian area of central Serbia. An ointment of the plant is used for foot fungus, wounds, burns, and frostbite. Flowers are combined with boiled fat and then filtered after 24 hours. This is used to treat oedema of the leg and painful veins. A tea is also prepared as a vermifuge (Jarić et al., 2007).

United Kingdom
In the UK, psoriasis, leprosy, measles, and smallpox were treated with a decoction of *Calendula officinalis* flowers, while the juice could be used for jaundice, constipation, and menstrual flow suppression (Khalid & da Silva).

United States of America
Similar to the Portuguese ethnomedicinal use, *Calendula officinalis* was used in the US for ulcers, liver problems, wounds, and conjunctivitis in the 19th century (Khalid & da Silva).

Ethnobotanical

Culinary

As an alternative to saffron, *C.officinalis* is occasionally used in India as a colorant and for flavoring. (Khalid & da Silva).

Veterinary

In a survey of the Lower Mainland of Canada, Thompson/Okanagan Region and South Vancouver Island of British Columbia, livestock farmers reported using the flower oil of *Calendula officinalis* for wounds on their ruminants. The farmers specified that it is not generally used for deep wounds because the cicatrizing action of the herb can close the wound too quickly and seal infection inside. *C.officinalis* is also combined with *Plantago spp., Urtica dioica* and *Symphytum officinale* for diarrhea and a tea is given orally for the relief of a sore stomach (Lans et al., 2007).

Chemistry & Pharmacology

The flowers of *Calendula officinalis* contain various terpenoids, volatile oils, 15 amino acids, and fatty acids. Inflorescences contain carbohydrates, coumarins, and flavonoids. Terpenoids are found in the roots, while the leaves and stems contain carotenoids. Pollens and petals also contain carotenoids. Quinones are found in the leaves, cellular chloroplasts, and mitochondria. Seeds contain many neutral lipids in addition to various fatty acids. Loliolide is a main bitter constituent. Other compounds include calendulin, and n-paraffins (Muley et al., 2009). **Table 1** contains an adapted summary of the chemical classes, names, and locations of these chemical compounds. Flavonoids and carotenoids are powerful antioxidants, while triterpenoids are efficacious anti-inflammatory agents. The structures of some of these terpenoids are demonstrated in **Figure 2**.

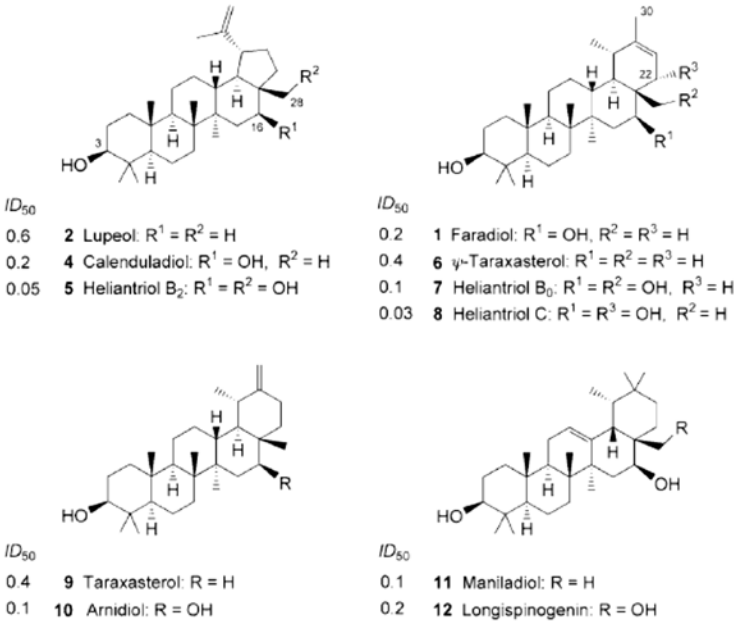

Figure 2. A summary of the structures of anti-inflammatory triterpenoids in *Calendula officinalis.* *(*Image Source*:* Neukirch *et al.,* 2005*)*

Biological Activity

In Vivo Studies

Spasmolytic and Spasmogenic

The use of *Calendula officinalis* for gastrointestinal problems is less documented than some other uses, but there are spasmolytic and spasmogenic constituents in the flowers that could elucidate the plants effect on cramps and constipation. Fresh flowers were powdered and extracted in distilled water, dichloromethane, and ethyl acetate. The spasmolytic and spasmogenic activity was analyzed in 2 cm long segments of rabbit jejunum or guinea pig ileum, respectively. As a spasmolytic, *C.officinalis* inhibited the contraction of free Ca^{++} induced jejunum contraction in a dose-dependent fashion. A pretreatment dose of 0.3 mg/mL exhibited 80-90% inhibition against acetylcholine and histamine induced contraction in the guinea-pig ileum. The mechanism may be an interference of calcium release or influx.

The dichloromethane extract contained more spasmolytic constituents than the ethyl acetate or aqueous extracts. However, the aqueous extract had a dose-dependent spasmogenic effect on the guinea-pig ileum at 1-10 mg/mL but was partially blocked with pretreatment of atropine. This suggests that the spasmogenic mechanism as potentially similar to acetylcholine, which is important for regulation of peristalsis. By acting as a calcium channel blocker or cholinergic, *C.officinalis* is capable of either relaxant or stimulant actions (Bashir et al ., 2006).

Chemical Class	Compounds	Plant Part	Activities
Terpenoids	1) Sitosterols, stigmasterols, diol diesters, taraxasterol monoesters, erythrodiol, brien, ursadiol, faradiol esters, arnidiol esters, calenduladiol esters, oleanolic acid saponins, calendulosides, calendulaglycosides, glucuronides, and cornulacic acid 2) Oleanolic acid glucosides	1) Flowers 2) Roots	Antioedematous Anti-inflammatory
Flavanoids	Quercetin, isorhamnetin, isoquercitin, narcissin, calendoflaside, calendoflavoside, calendoflavobioside, rutin, isoquercetirin, neoheperididosides, and isohamnetin and quercetin rutinosides	Inflorescence	Proteolytic Enzyme Inhibition Antiviral
Coumarins	Scoplotetin, umbelliferone, esculetin	Inflorescence	
Quinones	1) Plastoquinone, phylloquinone 2) α-tocepherol 3) Ubiquinone, phylloquinone, α-tocepherol	1) Leaves 2) Chloroplasts 3) Mitochondria	
Volatile Oil	Monoterpenes and sesquiterpenes: α-thujene, α-pinene, limonene, 1,8- cineol, geraniol, Essential oil: α-cadinene, α-cadinol, tmuurolol, lionene, and 1,8-cineol	Flowers	Allergen
Carotenoids	1) neoxanthins, violaxanthins, luteoxanthins, auroxanthin, favoxanthin, luteins, cryptoxanthins, lycopene, α-carotene, β-carotene 2) neoxanthins, violaxanthins, luteoxanthin, antheraxanthin, mutatoxanthins, luteins, cryptoxanthins, and β-carotene	1) Pollens and Petals 2) Leaves and Stems	Anti-inflammatory Choleretic-Cholagogue Antioxidant Increase Tumor Latency
Amino Acids	alanine, arginine, aspartic acid, asparigine, valine, histidine, glutamic acid, leucine, lysine, proline, serine, tyrosine, threonine, methionine, and phenylanlanine	Flowers	
Carbohydrates	Polysaccharides and monosaccharides	Inflorescence	
Lipids	1) Neutral lipids: phospholipids, glycolipids and Fatty acids: conjugated trienic acid and dimorphecolic acid 2) Fatty acids: lauric, myristic, palmitic, stearic, oleic, and linoleic monols, sterol esters, 3-monoestersm and 3-monoester diols 3) oxygenated fatty acid	1) seeds 2) flowers 3) seed oil	
Other	Loliolide Calendulin n-paraffins		Bitter constituents

Table 1: Summary of Phytochemical Constituents of *Calendula officinalis L.* (Asteraceae) and examples of known bioactivity (adapted from Muley et al., 2009).

Antidiabetic & Antihyperlipidaemic

The search for natural products efficacious in the treatment of diabetes mellitus is worth pursuing because synthetic and semi-synthetic drug products can have undesirable side effects. Chakraborthy *et al.* (2011) examined the ability of *Calendula officinalis* to affect the metabolism of glucose and lipid peroxidation in rats with induced diabetes. The study

used an 8.7 % extract of the leaves in ethanol and water. Alloxan and glucose were administered intraperitoneally to induce diabetes in rats, and 36 rats with a resulting blood glucose range of 200-270 mg/dL after two weeks were given either 2 mL saline as a control, 25 mg/kg, 50 mg/kg, or 100 mg/kg of *C.officinalis,* or 6 U/kg of insulin orally for 42 days. Blood glucose and urine sugar were measured at 2 weeks, 4 weeks, and 6 weeks. The response of blood glucose and urine sugar to *C.officinalis* extract was determined to be dose dependent. The 100 mg/kg dose actually restored the blood glucose/urine sugar to normal levels, while the 25 and 50 mg/kg dose still significantly lowered these indicators. There was an overall significant increase in haemoglobin and body weight when given *C.officinalis.* The lipid levels in blood serum were also lowered. The proposed mechanism of the extract is the activation of increased insulin secretion from the pancreas. Because the effects of the extract are so similar to that of administered insulin, the potential of *C.officinalis* for antidiabetic and antihyperlipidaemic use at 100 mg/kg body weight (BW) should be explored further for human application (Chakraborthy et al., 2011).

Hepatoprotective

Potential hepatoprotective properties of *Calendula officinalis* were examined using induced hepatic toxicosis in rats. Carbon tetrachloride (CCl_4) produces the free radical CCl_3, which affects lipid peroxidation and enzyme activity. CCl_4 was given to induce the toxicosis, and the animals were treated with an ethanol extract of powdered flowers every 24 hours for 7 days beginning 30 minutes after intoxication. *C.officinalis* showed a positive effect by decreasing hepatocytolysis by 28.5% and reducing enzyme and steatosis changes. The carotenoids, flavones, saponins, and phenylpropanoids are believed to be responsible for the anti-inflammatory and choleretic-cholagogue activity (Rusu et al., 2005).

Antioxidant

Oxidative stress, resulting from an excess of free radicals and lack of antioxidant action can result in damage to lipids, proteins, and DNA. The carotenoid and polyphenol content of *Calendula officinalis* is considered high and indicative of antioxidant activity, so Frankič *et al.* (2009) explored the effectiveness of *C.officinalis* extract for protection against DNA damage and lipid peroxidation in pigs. The extract was prepared from flower tops or petals in propylene glycol and experimental groups received either 3mL/day of petal extract or 3 mL/day of flower top extract in the feed. Oxidative DNA damage was induced by high polyunsaturated fatty acids (PUFA) in the feed, and both *C.officinalis* extracts significantly decreased the amount of DNA damage. High PUFA content also raises the marker of lipid peroxidation in urine, but the amount of *C.officinalis* extract was not sufficient enough to reduce the lipid peroxidation in this study. In summary, these therapeutic amounts of *C. officinalis* are considered efficacious enough in antioxidant activity for the protection of DNA, but are not sufficient for inhibiting lipid peroxidation (Frankič et al., 2009).

Anticancer

Calendula officinalis contains lutein, which is a potent carotenoid antioxidant. High levels of lutein have previously been shown to correlate with survival rates and the expression of estrogen receptors in breast cancer cells. In order to examine the potential of low dietary lutein as an inhibitor of mammary cancer cells, a murine mammary tumor cell culture was prepared and infused into female mice. After

the amount required to induce 65% tumor incidence was determined, 30 mice were given a semi-synthetic diet with 0.002, 0.02, and 0.4% extract from *C.officinalis* and inoculated with the tumor cells after two weeks. At 70 days post-inoculation the solid tumors were excised and lipid peroxidation activity was determined. Mice fed 0.02 and 0.4% lutein had a tumor incidence of only 20-37% compared to 70% in non-supplemented mice, and final tumor incidence was greater at day 70 for the untreated mice. Mice fed 0.002% had a tumor latency of about 50 days, while the tumor latency was delayed by 15 days with other concentrations. Lipid peroxidation was not dose dependent on lutein. Though lutein from *C.officinalis* did not prevent tumor growth, it may prevent the initial establishment of tumor cells as indicated by the increased tumor latency. The proposed mechanism of action is the antioxidant activity of lutein by quenching singlet oxygen and possibly additional immunomodulation, cell-cell communication, and prostaglandin production (Park, Chew, & Wong, 1998).

Antioedemous

Three of the most powerful compounds in *Calendula officinalis* include the tripterpenoid alcohols faradiol-3-myristic acid ester, faradiol-3-palmitic acid ester and $^{\varphi}$- taraxasterol. Their efficacy as antioedemic agents was investigated by Zitterl *et al.* (1997). The compounds were extracted from the powdered flower heads with dichloromethane. The irritant, Croton oil, was applied to the right inner ear surface of mice and the left ear was left untreated for comparison. Experimental mice received a mixture of Faradiol monoesters, Faradiol-3-myristic acid ester, Faradiol-3-palmitic acid ester, $^{\varphi}$-taraxasterol, and Faradiol, or the control Indomethacin while control groups only received the irritant. After 6 hours, plugs used to measure the size of the ear canal in both ears of each animal were removed and the difference in plug weight indicated the amount of swelling. Faradiol-3-myristic acid ester and Faradiol-3-palmitic acid ester exhibited 50% oedema inhibition at 240 µg/cm^2 and 65-66% at 480µg/cm^2. On a molar basis, 21 µmol/cm^2 of $^{\varphi}$- taraxasterol is needed to achieve the same effect. It was proposed that the limited activity at higher doses may be due to the difficulty of lipophilic compounds moving through the epidermis. It was concluded that Faradiol was as effective as Indomethacin and more active than the esters or $^{\varphi}$- taraxasterol (Zitterl-Eglseer et al., 1997).

Anti-inflammatory

Reducing inflammation is perhaps the best known activity of *Calendual officinalis* and most common ethnomedicinal application, so Preethi *et al* (2009) studied this particular property in a multi-pronged study. Similar to the antioedematous activity, the combination of caratenoids, flavanoids, and triterpenoids in *C. officinalis* was found to mediate acute and chronic inflammation in mice by cytokine and macrophage inhibition, and free radical scavenging.

Flower tops were extracted with ethyl alcohol to a yield of 1.1 g/100 mL, dried, and dissolved in distilled water for experimental use, and 8 groups of 6 mice received an injection of carrageenan and Dextran in the right paw to model acute inflammation. The control groups orally received only carageenan, distilled water, Dextran, or Diclofenac. The experimental groups orally received either 250 or 500 mg/kg BW of *Calendula officinalis* extract. Caliper measurements of the paw thickness were taken before and after injection and every hour. Conversely, Formalin was used to model chronic inflammation in 4 groups of 6 mice. These groups received no

treatment, 250 or 500 mg/kg BW of extract, or Diclofenac 1 hour before an injection of Formalin and for 6 days afterward. Calipers were used to measure the paw every day. The extract significantly reduced acute and chronic edema with 50.6-65.9% and 62.3% inhibition, respectively.

The effect of *Calendula officinalis* on TNF-α, a tumor necrosis factor from macrophages, was also examined in this study. Macrophages were induced by sodium caesinate in experimental groups of mice. The mice were treated with the extract alone at 100 or 250 mg/kg BW or with lipopolysaccharide (LPS) + extract at 100 or 250 mg/kg BW for 5 days. LPS was injected on the 5th day and after 6 hours the macrophages were collected for the TNF-α activity assessment. Treatment with the extract resulted in minimal macrophage cytotoxicity and normal cell growth.

The extract's effect on proinflammatory cytokines was determined by treatment of mice with LPS, or 50, 100, or 250 mg/kg BW LPS and *Calendula officinalis.* The LPS was administered after 5 days, and the levels of cytokines were determined after 6 hours. The cytokinase level in response to inflammation was also inhibited by *C.officinalis.*

Inflammation was also induced by LPS after 5 days of receiving 100 or 250 mg/kg BW of extract to determine the expression of the cyclooxygenase-2 gene. RNA was isolated from the spleen, and cDNA was prepared by RT-PCR and amplified. Band intensity of the Cox-2 gene was lower in *C.officinalis* treated animals, indicating expression inhibition.

In summary, the proposed mechanism for the anti-inflammatory action of the extract is the modulation of cytokines and Cox-2 gene inhibition in addition to antioxidant action against activated macrophages (Preethi, Kuttan, & Kuttan, 2009).

Cutaneous Wound Healing

Calendula officinalis has long been used in topical applications for wound healing because of its anti-inflammatory, cicatrizing action, and anti-microbial properties. The effect of a gel containing *C. officinalis* on collagen production and wound healing in rats was studied by Naeini *et al.* (2012). The oily product was extracted from fresh flower tops with ethyl alcohol, dried, and a 5%, 7%, and 10% gel were created. The rats received a square 2x2 cm surgical skin incision under anesthesia, and the gel was applied daily for 14 days. Control rats received no gel or a gel base placebo, and experimental rats received 5% gel, 7% gel, or 10% gel and they were observed daily. The wounds were investigated by biopsy at days 14, 21, and 45. At day 21, the collagen and hydroxyproline content were higher for the 7% and 10% groups, but on days 14 and 45 the 7% group was the highest. It was concluded from statistical evaluation that the 7% gel was more effective that the other concentrations because it showed significant collagen synthesis. Furthermore, the *C.officinalis* gel was most effective at day 14 (Naeini et al., 2012).

In Vitro Studies

Antimicrobial& Antifungal

One aspect of *Calendula officinalis* wound healing action may be the antimicrobial and antifungal properties of the plant. These activities were studied by Efstratiou *et al* (2012). Petals from the flowers were isolated, dried, and extracted in methanol or ethanol. Each extract was tested against *Bacillus subtilis, B. cereus, B. pumilis, Pseudomonas aeruginosa,* three strains of *Escherichia coli* and ampicillin-resistant *E.coli, Staphylococcus aureus, Klebsiella aerogenes, Enterococcus faecalis,* two strains of *Candida albicans, C. krusei, C. glabrata,*

C. parapsilos, Aspergillus flavus, A. fumigates, A. niger, and Exophiala dermatiditis. The extracts were dissolved to 300 mg/mL in their respective solvents, and 15 µL were used to soak paper discs. Bacterial controls were Ciprofloxacin and solvents. 10 mg/mL extracts were used for the fungal test and the controls were Fluconazole and the solvents. The antimicrobial activity, indicated by growth inhibition, was observed by disc diffusion. *C.officinalis* was comparable against both Gram-positive and Gram-negative samples, and methanol was generally more effective. However, *S. aureus and E. faecalis* were more susceptible to the ethanol extract. Both methanol and ethanol extracts were comparable in antifungal activity to Flucanazole (Efstratiou et al., 2012).

Immunomodulatory

It is possible that *Calendula officinalis* regulates inflammation by regulating the immune response on a cellular level. To test this hypothesis, Amirghofran *et al* (2000) prepared ethanol extracts of *C.officinalis* and other herbs. Human lymphocytes and thymocytes were isolated and assayed with the extract. Proliferation was significantly inhibited by *C.officinalis* and dose-dependent. The potential mechanism is through interaction with growth factors or cell surface molecules (Amirghofran, Azadbakht, & Karimi, 2000).

Antiviral

Calendula officinalis was investigated for antiviral activity by Kalvatchev *et al* (1997). The dried flowers were extracted as organic and aqueous stock solutions. The aqueous extract did not show inhibition of HIV-1 replication in infected cells, but the organic extract was an effective inhibitor at 10-30 µg/mL. Again, the aqueous extract was less effective than the organic extract, which also inhibited HIV-1 mediated cell fusion and delayed cell death up to 24 hours. The organic extract also reduced HIV-1 reverse transcriptase activity dose-dependently. The mechanism is proposed to be some action of the flavonoids blocking virus adsorption, potentially by interfering with binding sites on the membrane of the cell or virus (Kalvatchev, Walder, & Garzaro, 1997).

Inhibition of Proteolytic Enzymes

In addition to the anti-inflammatory triterpenoids, carotenoids and antioxidants, *Calendula officinalis* contains the flavonoid quercetin, which can inhibit proteolytic enzymes like matrix metalloproteinases (MMPs). MMPs are expressed by fibroblasts and are implicated in periodontal disease. The efficacy of *C.officinalis* was tested against collagen degradation and MMP activity from human gingival fibroblasts (HGF). The fibroblasts were incubated with either *C.officinalis* or quercetin in ethanol or aqueous Doxycycline, and the assay did not indicate statistically significant cytotoxicity. Collagen degradation was completely inhibited by *C.officinalis,* but dose-dependently inhibited by quercetin alone. In regards to MMP activity, *C.officinalis* exhibited 86% and 98% inhibition at 1.0% and 1.5% concentrations, and complete inhibition at 2.0% and 3.0% concentration. The extract containing quercetin was more efficacious than quercetin alone, suggesting that there are other active compounds acting on the proteolytic enzymes. In conclusion, 1-3% *C.officinalis* was not cytotoxic to fibroblasts, and prevented collagen degradation completely by mediation of MMPs (Saini et al., 2012).

Clinical Studies

Mouth Rinse

The use of mouth rinse as a complement to daily brushing and flossing for the prevention or elimination of plaque biofilm could be instrumental to oral health, and the active components of some botanicals like *Calendula officinalis* have been shown *in vivo* to exhibit antigingivitis and antimicrobial properties. One prepared mouth rinse containing 0.67% melaleuca oil (*Melaleuca alternifolia)*, 0.33% manuka oil (*Leptospermum scoparium),* 1% *Calendula officinalis* flower extract, 0.5% *Camellia sinensis* extract, and 12.8% ethanol in water was subjected to a Phase II double blind randomized placebo-controlled study against 12.8% ethanol. Twice a day, participants rinsed with 15 mL of their assigned liquid for 30 seconds. Each tooth was examined at baseline, 6 weeks, and 12 weeks for Gingival and Plaque Index, and plaque samples were collected along with vitals and blood for monitoring. Genomic DNA from the plaque samples was used to determine the abundance of *Actinobacillus actinomycetemcomitans* and *Taneralla forythensis*. Two women reported adverse events and withdrew from the trial, and others left the study for unrelated reasons for a final total of 17 out of 20 original subjects. Statistical analysis did not show a significant reduction in average Plaque Index, Gingival Index, or abundance of *A. actinomycetemcomitans* or *T. forsythensis*, but this small scale study did support the safety of the product and warranted further study of efficacy. Limitations cited were the aqueous nature of the rinse (due to solubility issues) and the less than optimal concentrations of the ingredients. However, great interest is shown in botanical agents like *C.officinalis* for oral health applications and potential for further trials. (Lauten et al., 2005).

Radiation Dermatitis

Postoperative irradiation for breast cancer can often induce dermatitis of varied severity. It is usually treated with topical corticosteroids, *Aloe vera* gel, or Trolamine. A Phase III randomized trial of a commercialized *Calendula officinalis* extract ointment manufactured by Boiron Ltd in comparison to Trolamine was conducted between 1999 and 2001 on women aged 18-75 who were undergoing postoperative radiotherapy for non-metastatic breast adenocarcinoma in France. This particular ointment was known to be well-tolerated for the management of second and third- degree burns compared to a proteolytic ointment or petroleum jelly. *C.officinalis* was randomly assigned to 126 while the remaining 128 were assigned Trolamine. The assigned ointment was applied topically twice a day or as needed after the onset of radiotherapy. Once a week the acute dermal toxicity, pain, and reactions to the ointment of each patient were recorded. Patient treatment with *C.officinalis* was only interrupted once with no allergic reactions compared to 15 interruptions and 4 allergic reactions with Trolamine. The acute skin toxicity of grades 2-3 occurred in 41% of *C.officinalis* patients compared to 63% of Trolamine patients. Less pain and more overall satisfaction with erythema prevention and pain was reported for the *C.officinalis* group than the Trolamine group. However, *C.officinalis* was considered difficult to apply by 30% of patients. *C.officinalis* ointment was significantly superior to Trolamine by statistical analyses for the prevention of acute skin toxicity above grade 2 as well as incidence of allergic reactions, treatment interruption, pain relief, and patient satisfaction. Based on the results of this study, *C.officinalis* ointment can be proposed as an alternative preventative treatment for irradiation induced dermatitis after surgery for breast cancer (Pommier et al., 2004).

Contraindications

Allergy & Topical/Cosmetic Use

Since *Calendula officinalis* is a member of the daisy family, Asteraceae (Compositae), individuals with known allergies to other members of this family should avoid the using this herb. The Asteraceae family contains sesquiterpene lactones that may trigger acute allergic reactions or delayed hypersensitivity. This hypersensitivity may manifest as either chronic or gradually progressive reactions. Barring known allergy incidence, *C. officinalis* is generally recognized as safe for culinary use and does not raise concern as a cosmetic ingredient (Reider et al., 2001).

Reproductive

There have been no reports of reproductive or developmental toxicity from *Calendula officinalis* itself. However, further study may be warranted because similar compounds are found in coriander oil, which one study found to decrease rat litter size in high doses. (Reider et al., 2001) Additionally, *in vitro* studies reported mild uterotonic effects and the safety of use during pregnancy and lactation is unknown (Basch et al., 2006).

Additive Effects

Some *in vivo studies* that administered high doses of *Calendula officinalis* resulted in sedative and hypotensive effects, so there may be reason for concern regarding additive effects when used alongside sedative or antihypertensive agents. As implicated by the aforementioned antidiabetic study by Chakraborthy *et al.* (2011), *C. officinalis* may have additive effects when administered with hypoglycemic drugs, antihyperlipidaemic drugs or herbs, or insulin (Basch et al., 2006).

Figure 3. Traumeel® offers a range of anti-inflammatory products containing *Calendula officinalis* extract as one of the active ingredients. (Image source: http://www.traumeel.us/)

Current Use

In addition to the biological activities under current study, research is ongoing for the applications of *Calendula officinalis* in allopathic medicine. The terpenoids responsible for anti-inflammatory activity are of particular interest, and more effective synthetic derivatives are in development. The addition of polar groups to $^{\varphi}$- taxarenes strengthened the anti-inflammatory action, and may serve as a springboard for further modification (Neukirch et al., 2005). Current use is most prevalent in complementary and alternative medicine as there are many topical products available as tinctures, lotions or creams, and also teas for internal use. Products like

Traumeel® for musculoskeletal injuries and Otikon Otic or NHED eardrops for otitis media are compounded solutions that contain *C. officinalis.* Traumeel® ointment contains 450mg/100g *C. officinalis* and has undergone extensive clinical trials (**Figure 3**). It is well tolerated and available as an ointment, tablet, or injection. It is a viable anti-inflammatory and analgesic alternative to NSAIDs (Schneider, 2011). Studies of Otikon Otic eardrops suggest that they are well tolerated and may be efficacious alternatives after further study (Basch et al., 2006). With such a wide range of biological activity, the potential conventional medicinal uses of *C. officinalis* are still being explored.

Discussion

It is little wonder that *Calendula officinalis* has such a long and varied history of use. Its biological activity includes antimicrobial, antiviral, anti-inflammatory, antioedematous, immunomodulatory, spasmolytic, spasmogenic, antidiabetic, antihyperlipidaemic, antitumor, antioxidant, and hepatoprotective mechanisms that are just beginning to be understood. These activities support its traditional use as a wound healing agent, for treatment of liver problems, for relief of cramping and diarrhea, and as an analgesic. *C.officinalis* is generally well tolerated and popular in CAM therapeutic remedies. It has potential for incorporation into allopathic therapies for diabetes, cancer, and topical wound healing in particular. The future of research for this "golden child" of an herb includes safety and long term toxicity studies, standardization of dosages for various treatments, isolation and/or modification of specific compounds and combinations for drug development, and further determination of mechanisms of action.

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Camellia sinensis (L.) Kuntze, Theaceae

Nina Patel

Introduction

The scientific name for tea is *Camellia sinensis*, and it belongs to the Theaceae family ("Tropicos," 2012). It is one of the oldest drinks in the world, and only water surpasses it in consumption (Chow & Hakim, 2011). Other common names of tea used by different cultures include thee, cha, chai, shai, and O cha (Saberi, 2010). Archaeological evidence suggests that the origins of tea date back to China over 5,000 years ago, and from there it diffused into India, Japan, Thailand, Korea, and Sri Lanka (Meltzer, Monk, & Tewari, 2009). Tea was native to the ancient kingdoms of Shu, which was located in the "four rivers" and the neighboring kingdom of Ba in China. The Chinese book *Chajin* by Daoist Lu Yü, is the first book written that discusses tea, and it dates back to 780 CE (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009). The medicinal use of tea was also discussed in the ancient Chinese pharmacopoeia "Ben Cao Gang Mo" (Lin, Tsai, Tsay, & Lin, 2003).

There are three main types of tea originating from the same plant: green, oolong, and black (Ferrara, Montesano, & Senatore, 2001) . Green tea is consumed mostly in China, Japan, India, some North African and Middle Eastern countries whereas black tea is consumed mostly in Asian and Western countries (Cooper, Morre, & Morre, 2005). Green tea is prepared from fresh leaves and buds that are heated by pan frying or steaming to inactivate the enzymes and stabilize the monomeric catechins, and then these leaves are dried (Ferrara et al., 2001); (Hara, 2011). To produce oolong tea, fresh leaves are wilted in the sun, bruised a bit, and then finally fermented. However, if the wilted leaves are fermented, black tea is produced instead (Ferrara et al., 2001).

Tea is composed of three main constituents: caffeine, catechins, and theanine (Saberi, 2010). Tea also contains other compounds, such as volatile oils, vitamins, and minerals. The active compounds in tea are the polyphenols, mainly epigallocatechin, gallic acid, and bioflavonoids. These catechins may help to fight various types of cancers, such as skin, esophageal, stomach and colon cancer. Tea can also be used as a topical agent to help control bleeding that has resulted from cuts and scrapes. *C. sinensis* can also be used to relieve insect bites and help control blood sugar and insulin levels (Ferrara et al., 2001). Overall, tea has many medicinal uses.

Apart from its medicinal uses, it is also a historically, economically, and culturally significant plant. For example, one of the reasons that the colonization of India occurred was to control the tea trade (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009). *C. sinensis* is an important botanical plant that still continues to have relevance 5,000 years after it was first discovered.

Botanical Description

Camellia sinensis is grown in thirty countries (Cooper et al., 2005). It is grown 1,000-7,000 feet above sea level, in temperatures between 50-85ºF, and in areas with annual

Figure 1. ***Camellia sinensis.*** Source (Hamilton, 2010)

rainfall between 80 to 90 inches (Saberi, 2010). It is grown best in humid climates (Cooper et al., 2005). The ideal geographical area for tea to be grown ranges from Java in the south to Japan in the east and the north and India to the west. This area also includes Formosa, China, Sumatra and outer neighboring countries. It is grown best on terraced hillsides and open fields (Shalleck, 1972). It can be grown as a tree or a shrub (Mahmood, Akhtar, & Khan, 2010). It is an evergreen, perennial plant (Mondal, Bhattacharya, Laxmikumaran, & Singh Ahuja, 2004).

For tea growth, warm weather with an even distribution of rainfall throughout growing season is needed. The leaves will grow more if the weather is wetter and the rainy season is longer. The colder the weather, the slower the growth of the tea bushes, which will result in fine tea with small supple, leaves. However, frost will blacken the leaves thereby marring the tea (Shalleck, 1972).

Tea can reach a height of 10-15 meters when grown in the wild, and 0.6-1.5 meters when cultivated. The leaves are green, short stalked, coriaceous, alternate, lanceolate, serrate margin, glabrous or pubescent beneath, and vary in length from 5-30 cm and are 4 cm in width. Mature tea leaves are bright green, smooth, and leathery whereas the young tea leaves are pubescent. The flowers are white fragrant and range from 2.5-4 cm in diameter. The flowers either occur by themselves, or they can occur in clusters of two or four. They have stamens with yellow anthers, and they make brownish red capsules. The fruit is a flattened, smooth, round trigonous three celled capsule, and there are seeds the size of a small nut in each fruit (Mahmood et al., 2010). The seeds ripen on the plant, and then they germinate in damp sand pits. When the seed shell opens, the seeds are planted in either nurseries or in fields. Proper shading is crucial for tea bushes. The tea plants are prevented from developing into trees by pruning and hand-picking (Shalleck, 1972). A picture of the plant can be seen in **Figure 1.** Tea is a self-sterile and cross-pollinating crop (Abrol & Abrol, 2012).

There are four varieties of plants: the Assam (Indian) plant; the Chinese plant (Bohea); a relative of the Indian plant which is grown in Ceylon, Java, and Sumatra; and a relative of the Chinese plant which is cultivated in Formosa and Japan (Saberi, 2010). These types are mainly distinguished by their leaves (Mondal et al., 2004). Bohea is a small and stunted bush that is compactly branched containing small stiff leaves and purple leaf buds (Saberi, 2010). The relative of Bohea is similar in appearance, but it is larger and contains bigger leaves. Assam tea can reach up to a tree height of thirty feet when grown in its wild state. This Indian tea plant contains huge leaves and few flowers. The relative of Assam is similar, but it is composed of smaller, thicker, and more precisely serrated flowers (Shalleck, 1972). The Chinese plant can live

for hundreds of years whereas the Assam variety can live for about 40 years (Saberi, 2010).

Traditional Uses

Traditional Medicinal Applications

Tea has been used an ethnomedical remedy for centuries. For example, in the Chinese book *Kissa Yojoki* (ca 1191), tea was described as a medicine to help control bleeding, heal wounds, regulate blood sugar, help with digestion, and regulate body temperature (Meltzer et al., 2009). During the Zhou dynasty in China, tea was used as a stimulant to promote positive moods. Tea was consumed to calm and clear the mind, sharpen mental acuity, and relax smooth muscles. It was also used as a diuretic, antitoxin, mild disinfectant, and as an efficacious rinse to soothe strained, tired eyes and to alleviate skin ailments (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009). The remedy used to treat many of these discussed problems was drunk as a bitter medicinal drink. This drink was prepared by infusing tea leaves in hot water. Salt was often added (Saberi, 2010). When rinsed in the mouth, tea cleansed the palate and since it contains trace minerals like fluoride, it promoted dental health and aided digestion (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009). In the ancient world, tea was thought to increase blood flow, act as an immunostimulant, speed up metabolism, help with anemia, fight the effects of summer heat, and increase the number of years a person lived (Blofeld, 1985).

For the Daoists, tea was thought to promote health and increase life span. It was prescribed as a tonic and remedy by apothecaries and Daoist healers. These healers worked with chefs of noble houses to infuse tea into healthy recipes and tasty dishes. It was a bitter herb that was used in cooking and took on many forms: fresh leaves, pulp, pastes, and gels in season, dried loose leaves or "bricks" of compresses leaves, and "wafers" and "cake" of dried paste (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009)

Many of these ancient uses still have relevance in modern CAM applications as well as modern day traditional medicines. Powerful infusions of tea are used often for bathing the feet and to treat fungoid infections. Tea (in the drinking form) can be used as cleanser for the face to help get rid of pimples and skin rashes. In rural China, strong tea is used as a mild disinfectant for new skin lacerations. Hair can become soft and glossy when washed with tea. It is also used for halitosis in the leaf form by placing the leaves in the mouth. In North China, individuals rinse their mouths with green tea after eating. Toothaches can be relieved by chewing tea leaves (Blofeld, 1985). Green tea is consumed between meals or after meals in Japan and Asian countries (Lee et al., 1997). In Japan, tea can be found in foods, such as *kanten* (tea jelly) and a green tea ice cream called *matcha.* It is also found in *O-chazuke*, which is a rice dish (Saberi, 2010)

Oolong tea has hypocholesteromic properties that become active after a fatty meal, and this can help with hypertension and arterial disease by being an anti-clot agent. Black tea has a high content of tannins, which can help with diarrhea and headaches. Damp black tea bags can help with tired, red eyes as well and relieve itching and redness caused by insect bites (Ferrara et al., 2001). Many of the ancient day uses of tea still apply today.

Tea Drinking

Around the world, many forms of drinking tea occur, and this is intricately tied into the cultures of many societies. In Central Asia and Tibet, the oldest form of tea drinking still remains. This ancient practice consists of boiled tea that is made with compressed tea, which is either shaved off a brick or broken off a cake. Then, it is boiled with water and usually other ingredients. Indian chai is made in a similar manner. During the Song Dynasty (960-1279), whisked tea arose in China and diffused into Japan, where it was integrated into ceremonial practices. Whisked tea is made from leaves that are grounded into a fine powder, and then whipped with bamboo sticks and hot water in separate bowls. Steeped tea, in which tea leaves are steeped in the tea pot, became a common practice during the Ming dynasty in China (1368-1644). It was steeped tea that maritime traders introduced into Europe (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009).

Tea drinking is intricately tied into the Japanese culture as it has influenced the fine arts, including garden design, flower arrangement, architecture, calligraphy, painting, lacquer, and ceramic arts. The tea ceremony in Japan is called *chado, 'The Way of Tea,'* and it was originally a Buddhist ritual. This tea ceremony still continues on today in Japan (Saberi, 2010).

In Korea, nconsumption of tea is tied with the *Panyaro Seon* (Zen) of tea. Tea ceremonies are seen as a spiritual activity that results in inner awakening and even total enlightenment. Temples in Korea also serve tea to visitors (Saberi, 2010).

In England in the 1850s, tea began to become the focus of social visits. It was served with sandwiches, biscuits, cakes, and pastries. Fancy silver or fine bone china tea-ware arose to accommodate this important social event. Afternoon tea became a unique event. It became common among the high, middle, and lower classes in Great Britain. Although today this tradition has declined, tearooms can be found across Great Britain, and they are very popular placaces (Saberi, 2010).

The Economics and History of Tea

Camellia sinensis is a historically important plant and is closely interlaced with the histories and economies of China, India, and Britain. China used to dominate the international tea trade till the mid-nineteenth century. China lost control because Great Britain started to compete with them, as they were eager to make their own tea. To do this, Britain turned to other regions. For example, the colonialization of India started in 1757 in part because the British wanted to make their own tea, and India presented the perfect opportunity with its huge population of peasant cultivators. As in other British islands, the indenture system was eventually established in India. This system recruited and constrained laborers to tea plantations under a penal system. Indian indentured laborers also supported the British plantation economy in other places besides India, such as Fiji and East Africa and the lives of these laborers were like those of the African slaves. This indenture system eventually caused Indian tea to surpass Chinese tea in the global market (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009).

The history of tea is also closely interlaced with the history of sugar because sweetened tea was a common beverage. Both tea and sugar entered the British market as rare and expensive items during the mid-seventeenth century that only the elite could afford. Two hundred years later, sweet tea replaced both beer and malt liquor as the top beverage. The United Kingdom drank 40 percent of the tea worldwide by 1900. This was due to industrialization—it helped the new

industrial workers meet their caloric needs and helped to keep them awake by acting as a stimulant—and the growth of the British Empire. In the British Caribbean colonies, over a million African slaves worked under arduous conditions to produce sugar. These workers were overworked, malnourished, and disease ridden and their death rate far exceeded their birth rate. This cheap land and labor increased the demand for tea by making it cheap and readily accessible to the lower classes in Britain (*Steeped in history : the art of tea / Beatrice Hohenegger, editor ; with essays by Terese Tse Bartholomew ... [et al.]*, 2009).

Tea has a rich history that was tied to the exploitation of native populations. It has changed the world, and it still is relevant today. As a highly consumed beverage, tea is still an economically significant commodity. For example, in the United Kingdom, around 165 million cups of tea are consumed daily (Saberi, 2010). Also, 2/3rd of the world population consumes tea daily as a morning drink, and about 2.2 millions tones of it are produced annually (Mondal et al., 2004).

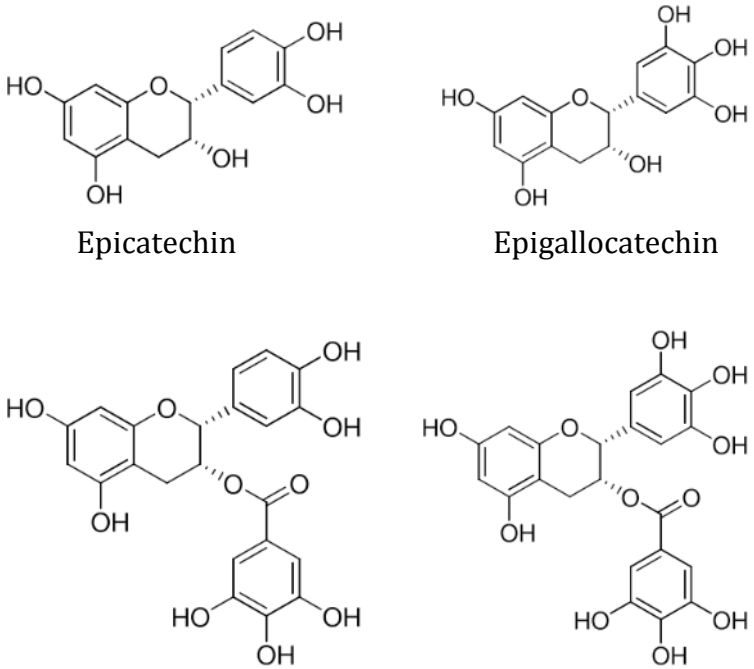

Figure 2. Chemical structures of the four major catechins found in *Camellia sinensis*. These catechins are EC (epicatechin), EGC (epigallocatechin), ECG (epicaetchin-3-gallate), and EGCG (epigallocatechin-3-gallate). Source (Kim et al., 2010)

Chemistry and Pharmacology

The constituents of tea possess biological properties that are beneficial for one's health as they have been shown to be possess antiallergenic, antioxidant, antimutagenic, anticancer, antiartherosclerotic, and antibacterial properties (Ferrara et al., 2001). Many of these biological activities can be traced back to the three main components of tea: caffeine, catechins, and theanine. Catechins are polyphenolic flavonoids (tannins), and they are responsible for the briskness and astringent flavor of tea. Caffeine, a mild stimulant, also brings about the bitter flavor of tea. In contrast to caffeine, theanine is an amino acid that is responsible for the sweetness of tea, and it acts to suppress the stimulant activity of caffeine (Saberi, 2010).

Catechins are made by the tea plant/shrub during the day and stored in cell vacuoles in the shoot of the plant. As the temperature gets higher, the rate of production of these catechins increases These catechins may be useful secondary metabolites that may serve to protect leaves from bacteria, viruses, fungi, and vermin bites. Catechin oxidation forms

Active Ingredient	Content (g/kg dry leaves)
Caffeine	36
Catechins	
Epicatechingallate	15.2
Epigallocatechin	46.0
Epigallocatechin gallate	129
Epicatechin	0.9
Major catechins	191
Flavonols	
Myricetin	0.8
Quercetin	1.8
Kaempherol	2.6

Table 1. Major constituents of green tea by g/kg of weight. Source (Perva-Uzunalic et al., 2006)

dimers and polymers named theaflavins and thearubigins, which retain many of the properties of catechins. Catechin oxidation is the main chemical reaction involved in the synthesis of black tea (Shalleck, 1972).

Catechins comprise 30% of the dry weight of tea leaves (Chyu et al., 2004). They can be categorized as either free catechins or galloyl catechins. The free catechins include (+)-catechin (+C), (+) galocatechin (+GC), (-) epicatechin (EC) and (--) epigallocatechin (EGC). Galloyl catechins include (-) epicatechin gallate (ECG), (-)-epigallocatechin gallate (EGCG), and (-)-galoocatechin gallate (GCG) (Hara, 2011). EGCG is also the main antioxidant catechin found in tea, and is responsible for many of the attributed health effects of tea (Chyu et al., 2004). The structures of some of these major catechins are shown in **Figure 2**. They consist of polyphenolic aromatic rings that contain hydroxyl groups (Hara, 2011).

In a cup of tea, the following percentages of compounds are found: caffeine constitutes 2-4% tea; amino acid constitute 4% of tea; lignins constitute 6.5%; organic acids constitute 1.5%

Components	Green Tea	Black Tea
Catechins	30-42	3-10
Ravanols	5-10	6-8
Other flavonoids	2-4	-
Theogallin	2-3	-
Ascorbic acid	1-2	-
Gallic acid	0.5	-
Quinic acid	2	-
Other organic acids	4-5	-
Theanine	4-6	-
Other amino acids	4-6	13-15
Methyxanthines	7-9	8-11
Carbohydrates	10-15	15
Minerals	6-8	10
Volatiles	0.02	<0.1
Theaflavins	-	3-6
Thearubigins	-	12-18
Caffeine	3-4	3-4

Table 2. Major constituents of green and black tea by weight % of extract solids. Source (Chow & Hakim, 2011).

of tea; proteins constitute 15% of tea; chlorophyll constitutes 0.5% of tea; the polyphenols, such as EGCG and EGC constitute 25-35% of tea. A cup of green tea can contain anywhere from 300-400 mg of polyphenols or 10-30 mg of EGCG The polyphenolic compounds present in tea are essentially colorless (Meltzer et al., 2009). **Table 1** shows the percent composition of the constituents of tea as present in green and black tea.

Table 2 shows the content by weight of active ingredients in dry tea leaves. Caffeine weighs 36g/kg; the major catechins weigh 191 g/kg; the flavonols weight 5.2 g/kg (Perva-Uzunalic et al., 2006).

Among the three different types of tea, there is also a difference in chemical content as the they undergo different

levels of fermentation. Green tea has the most antioxidant content due to a higher EGCG content with black tea having the least EGCG content (Cheng, 2006). Black tea has the most caffeine content, and green tea has the least (Lin et al., 2003).

Biological Activity

Many of the health benefits of tea can be attributed to its EGCG content. EGCG has strong antioxidant properties, and it also accounts for more than 65% of the catechin content of tea and more than 10% of the extract dry weight of tea (Kaedei et al., 2012).

In Vivo

Atherosclerosis

Many of the potential protective effects of catechins found in *Camellia sinensis* may not work once a disease or illness is well established. For example, many of the human clinical trials that administer antioxidants may fail because the therapy begins long after atherosclerosis is already established in the patients. In contrast, the positive results seen in laboratory animals may occur because the early initiation of antioxidant therapy occurs when atherosclerosis is still developing.

This study investigated the preventive potential of tea catechins in hypercholesterolemia apolipoprotein E-null mice. Mice with either evolving or established atherosclerotic lesions were injected daily with either intraperitoneal injections of EGCG (10 mg/kg) or PBS. These lesions were produced by periadventitial cuff injury to carotid arteries. Results were taken after 21 and 42 days of treatment. It was found that this therapy decreased evolving atherosclerosis plaque sizes at 21 days by 55% and 42 days by 73% compared with PBS treatment ($P<0.05$). Also, the sizes of the plaques were the same in both day 42 and day 21 treated mice that had been administered EGCG. This suggests that treatment may halt the development of these lesions. EGCG treatment did not reduce established lesions in the aortic sinuses or the rest of the aorta. EGCG injections may have reduced evolving lesions because it increased antioxidant capacity in local vascular tissue as well as systemic circulation. It also decreased vascular smooth muscle cell proliferation and redox sensitive gene activation (Chyu et al., 2004).

Diabetes

Hyperglycemia can lead to diabetic complications, such as neuropathy, retinopathy and nephropathy in diabetes mellitus. This study investigated the effect of oral administration of green tea (GT) on kidney tubules of diabetic rats, and it found that diabetic rats treated with green tea showed a reduction in serum levels of glucose, glycosylated proteins, creatinine, and blood urea nitrogen levels in comparison to untreated diabetic rats. Glucose, protein, urea nitrogen and creatinine excreted in the urine of GT treated diabetic rats also decreased. A decreased number of glycogen-filled proximal tubules were also seen in diabetic rats treated with GT, which means that GT has a positive effect on renal histochemical parameters. Overall, GT was found to improve serum levels and urine excretions as well as reduce glycogen levels in the kidneys (Renno, Abdeen, Alkhalaf, & Asfar, 2008).

In Vitro

ECGC and motility of Sperm In Vitro

Catechins can inhibit some of the effects of ROS (reactive oxygen species), hydrogen peroxide, hydroxyl radicals, and

nitric oxide. They have also been shown to have higher antioxidant properties than both vitamin C and E (Kaedei et al., 2012; Zhao, Li, He, Cheng, & Xin, 1989).

For motility, sperm require the production of ATP. The synthesis of ATP in the mitochondria results in the formation of ROS, which can decrease the motility of sperm. In this study, it was found that the motility and penetrability of boar spermatozoa improved with co-incubation of 50 and 100 μMs of EGCG. EGCG could potentially protect spermatozoa from oxidative stress and improve energy production, which would result in improved sperm movement (Kaedei et al., 2012).

Breast Cancer

A long-term exposure to low doses of environmental carcinogens may cause most human cancers. For example, short term exposure to low doses of NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone) and B[a]P (benzo[a]pyrene) results in higher levels of ROS (reactive oxygen species). NNK is a potent lung carcinogen in tobacco products and B[a]P is an environmental, dietary, and tobacco carcinogen. This study looked at the preventive aspects of catechins found in *Camellia sinensis* in preventing breast cancer.

Immortalized, non-cancerous human breast epithelial MCF10A cells in culture were exposed to 100pmol/l of NNK and B[a]P. This exposure resulted in carcinogenesis of human breast epithelial cells due to an increase in ROS levels, which activated the Raf-independent extracellular signal-regulated kinase (ERK) pathway and led to successive cell growth and DNA damage.

Green tea catechins (GTCs) stopped ROS elevation, ERK activation, cell growth, and DNA damage in non-cancerous breast cells. EC, ECG, EGC, and EGCG are some of the main catechins found in green tea. It was shown that EC decreased cell proliferation and apoptosis at 100 μg/mol. ECG, EGC, and EGCG decreased cell viability and proliferation and increased cell apoptosis. EGCG was more toxic than EGC, which was more toxic than ECG, which was more toxic than EC. GTCs have preventive activity as they were shown to suppress chronic cellular carcinogenesis from exposure to environmental carcinogens (Rathore, Choudhary, Odoi, & Wang, 2012).

Melanoma

This study tested whether a physiological concentration of EGCG at .1-1μM inhibited human metastatic melanoma cell lines, 1205Lu and HS294Y, and they found that it did in a dose dependent manner. The research group showed that EGCG depresses NF-kB activation in a dose dependent manner. IL-1beta signaling led to activation of NF-kB. The secretion of IL-1 beta also decreased because NLRP1 (a part of the inflammasomes) was suppressed and this led to subsequent capase-1 activation depression. They also found that turning off NLPR1, an inflammasome that may be involved in tumor inhibition, reversed these results (Ellis et al., 2011).

Mechanism of Action

EGCG is involved in several cellular pathways (**Figures 3 and 4)** that result in inhibition of apoptosis, cell cycle arrest, growth inhibition, inflammation, antiangiogenesis, and inhibition of metastasis. EGCG inhibits receptor tyrosine kinases, such as epidermal growth factor receptors (EGFRs), insulin-like growth factor-I receptor and vascular endothelial growth factor (VEGF) receptor (VEGFR), and some of their

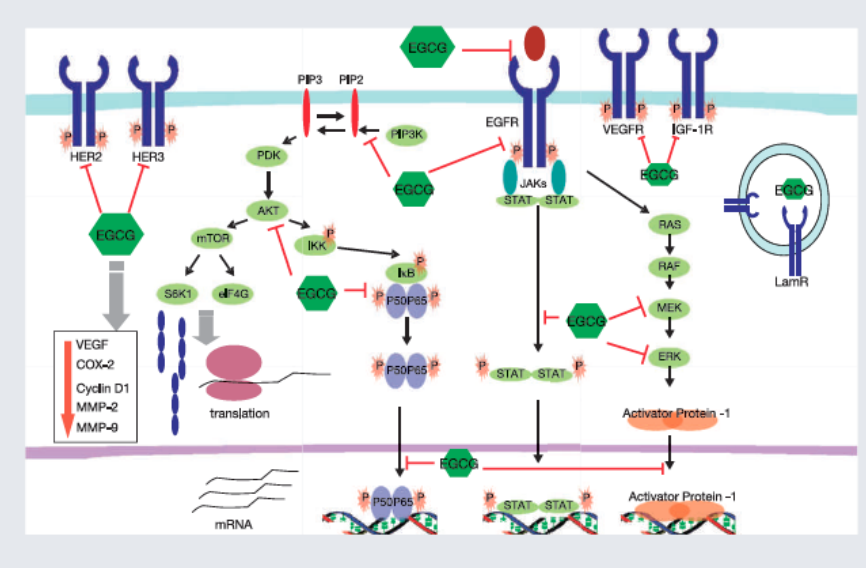

Figure 3. Some of the cellular pathways that EGCG targets. Source (Kim et al., 2010)

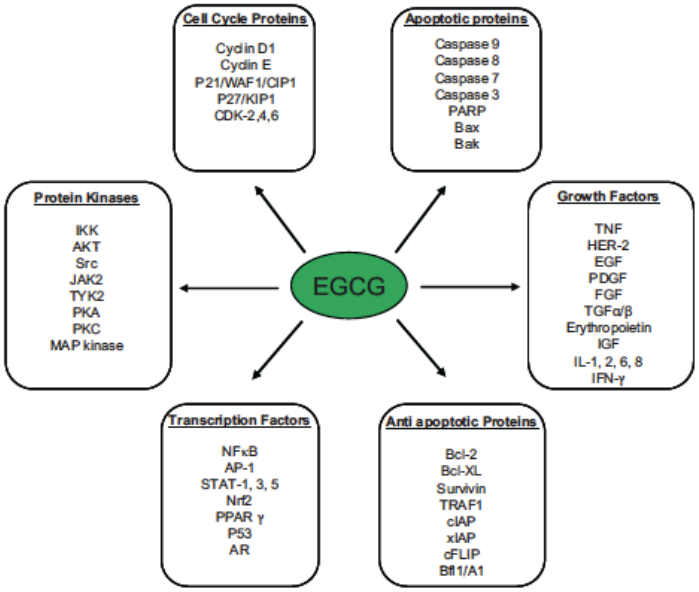

Figure 4. Some of the cellular pathways that EGCG targets. Source (Singh, Shankar, & Srivastava, 2011)

downstream effectors, such as pAkt and pERK by preventing ligand binding and phosphorylation/activation of the receptor tyrosine kinases. It also inhibits cytoplasmic signaling molecules, such as Akt, extracellular signal-related kinase (ERK) ½, and mitogen-activated protein kinase or ERK kinase (MEK). EGCG inhibits intracellular signaling pathways , such as phosphoinositide 3-kinase/Akt/mammalian target of rapamycin (mTOR), Janus-activated kinase (JAK)/signal transducer and activator of transcription (STAT), RAF/mitogen activated protein kinase or ERK kinase (MEK)/ERK/AP-1, and Akt/NF-kb. NF-kB is involved in DNA transcription of genes, and these genes are involved in inflammation, immunity, and carcinogenesis. ECGC also activates p53 and its downstream targets p21, p57, and Bax, which results in cell death and G_0-G_1 arrest. It also inhibits the binding of effector transcription factors—NF-kb, AP-1, and the signal transducer and activator of transcription 3 (Kim, Amin, & Shin, 2010).

In the atherosclerosis study already mentioned, in vitro studies showed that treatment with EGCG 30 µmol/L reduced the activation of redox-sensitive transcription factor AP-1 and TNF-a induced iNOS expression. iNOS is a source of reactive oxygen species and is a part of the tissue proliferative repair process (Chyu et al., 2004).

Clinical Studies

Obesity

In this clinical study, AR25, which is a green tea extract, was found to inhibit lipases and at the same time stimulate thermogenesis and fat oxidation. AR25 is an 80% ethanolic

dry extract that is standardized at 25% EGCG catechins. This study involved seventy patients aged between 20 to 69 years that were given an AR25 extract. During the course of the study, a decrease of 4.6% in mean body weight and a reduction of waist circumference by 4.48% was observed. After three months of treatment, no significant decreases in plasma cholesterol levels or reduction in blood pressure was seen. The increased thermogenesis and fat oxidation was not linked to a heart rate increase. This is promising since obese patients tend to have underlying cardiovascular problems and hypertension. This extract can influence both body weight and composition by influencing body energy expenditure and the utilization of various substrates in the body (Chantre & Lairon, 2002).

Cancers

Recent research shows that 30% of men with high-grade prostate intraepithelial neoplasia (HG-PIN) develop prostate cancer (CaP) within a year after repeated biopsy. Several epidemiological studies have shown that Asian countries where green tea is a popular beverage have a lower incidence of CaP, and that when these Asian immigrants migrate to the United States and adopt new dietary habits, the prevalence of CaP increases. The green tea catechins capsules used in this clinical trial consisted of 5.5% (--)-epigallocatechin, 12.24% (--)-epicatechin, 51.88% epigallocatechin-3-gallate, and 6.12% (--)-epicatechin-3-gallate. Less than 1% caffeine was used. Sixty male volunteers with HG-PN underwent this double blind, placebo-controlled trial, and they were given three daily green tea catechin (GTCs) capsules, 200 mg each for one year. The incidence rate of tumors among the 30 GTCs-treated men was 3% (1 person developed a tunor), and it was 30% (9 individuals developed tumors) among the 30 placebo treated individuals. There were no significant side effects. This study shows that GTCs are both safe and effective as a preventive therapy for the development of CaP. These capsules also reduced lower urinary tract symptoms associated with benign prostate hyperplasia (Bettuzzi et al., 2006).

A study examined the chemopreventive effects of green tea among cigarette smokers of 52 healthy male aged 20-51 years of age. Smoking increases the frequency of sister-chromatid exchange (SCE) in mitogen stimulated peripheral lymphocytes by 35%. This increase may be due to smoking-induced DNA damage. It was found that the frequency of SCE in smokers who consumed green tea (3 cups a day for 6 months) was similar to that of non-smokers suggesting that consumption of green tea may help to prevent lung cancer (Lee et al., 1997).

Contradictions

Camellia sinensis should be avoided if on anticoagulants, such as Warfarin as it decreases the anticoagulant effect through an antagonism mechanism. Warfarin inhibits the production of vitamin-K dependent clotting factors. Green tea contains vitamin K, and therefore acts in opposition to the drug (Izzo, Di Carlo, Borrelli, & Ernst, 2005).

Products containing green tea have been found to be hepatotoxic. A study examining this found that no chronic liver damage occurred. All of the patients recovered within 4 months after stopping to use supplements containing *C. sinensis* (Bonkovsky, 2006).

Tea herbal supplements should also be checked for their caffeine content. Caffeine levels can reach a high level in these products and may cause thermogenic, cardiovascular, and other health effects in humans (Seeram et al., 2006). Too much

consumption of tea may also result in insomnia, and this may be due to the caffeine content of tea (Izzo et al., 2005).

Since EGCG and polyphenols in green tea may be proteasome inhibitors, they should not be taken with other proteasome inhibitors, such as boranic acid-based inhibitors or Bortezomib. If taken in conjunction with these drugs, then tumor cell death may not occur thereby preventing these drugs from working (Shord, Shah, & Lukose, 2009).

The catechins in *Camellia sinensis* are metabolized by catechol-O-methyltransferase, and therefore should not be taken in conjunction with monoamine oxidase inhibitors or dopamimmetic drugs due to a potential adverse interaction (Shord et al., 2009).

Current Use in Allopathic and CAM Therapies

Allopathic Medicine

The green tea sinecatechin, Polyphenon E (*Veregen*) ointment is the first FDA approved botanical drug. Polyphenol E is administered for the treatment of anogenital warts because of its antiviral, immunostimulatory, and antioxidant properties (Meltzer et al., 2009). It is sold as a 15% ointment. Each gram of ointment is composed of 150 mg of sinecatechins ("Veregen: a botanical for treatment of genital warts," 2008). Over 85% of this drug consists of catechins that are derived from a partially purified fraction of water extract of green tea leaves from *Camellia sinensis* (Meltzer et al., 2009). The tea leaves are extracted with water followed by ethyl acetate. The resulting product undergoes column chromatography using water/alcohol to elute the catechins. The resulting catechin powder has the following composition: EGCG>65%, EC<10%, EGC<10% and other trace catechin derivatives (Hara, 2011). Gallic acid, caffeine, and theobromine compose another 2.5 % of the drug ("Veregen: a botanical for treatment of genital warts," 2008).

A 0.5 cm strand of *Veregen* ointment should be dabbed on each wart, covering it with a complete layer, 3 times each day. Treatment should continue until the warts completely disappear, but should not last longer than 16 weeks ("Veregen: a botanical for treatment of genital warts," 2008).

CAM Therapies/ Modern Day Products

Camellia sinensis is found in many herbal supplements on the market (Seeram et al., 2006). For example, EXOLISE® is a capsule containing 375mg of green tea extract AR25, and it has been found to have anti-obesity properties (Chantre & Lairon, 2002). Many of these other supplements are available in capsule form with a recommended dosage of 100mg to 150mg a day (Shord et al., 2009).

C. sinensis is also available in iced and brewed teas, and no more than 2.25g of tea per 6 ounces of water is recommended per serving (Shord et al., 2009). Many skin care products sold by corporations, such as Avon Products Inc, Estee Lauder Inc, Elizabeth Arden, L'Oreal, Carter-Wallace Inc, Aubrey Organics, and Schwartzkopf & Dep Corp. contain green tea. These merchandises include toothpastes, shampoos, depilatory creams, facial cleansing lotions, moisturizing creams, moisturizing lotions, scented sprays, body lotions, and bath and shower gels. Due to the presence of catechins, these products may have anticarcinogenic, antioxidant, and anti-inflammatory activity. Clinical trials should be conducted to investigate these potential biological activities (Katiyar, Ahmad, & Mukhtar, 2000).

Apart from being used in supplements and skin care products, *C. sinensis* has been found to be incorporated into the

treatments of patients suffering from illnesses and chronic diseases. For example, a study conducted in Jordan found that 20.5% percent of diabetes patients used some form of *C. sinensis* in their treatment. Most of these patients used this plant to relieve the symptoms (Wazaify, Afifi, El-Khateeb, & Ajlouni, 2011). In another study, it was found that some cancer patients undergoing radiation therapy for various neoplasms at rural cancer centers used green tea. Of the 153 patients surveyed at these rural centers, 23 people or roughly 15% reported using green tea (Rausch et al., 2011).

Discussion

The origins of *Camellia sinensis* began in China, but today it has diffused into all regions of the world. It is a culturally, historically, and economically relevant botanical plant. For example, the plant is used in food, cosmetics, tea ceremonies, and traditional and allopathic medicinal therapies. Many of the biological and clinical studies discussed in this paper focused on the preventive aspects of tea in preventing cancer, diabetes, obesity, and atherosclerosis. Many of these activities are due to the major catechin found in tea, which is EGCG. EGCG has numerous cellular targets, and therefore may be an effective product to consume to prevent diseases and illnesses. EGCG is also a rich source of antioxidants. The first FDA approved botanical drug was also derived from tea catechins. Due to its relevance in many diverse areas, *C. sinensis* will continue to play an important role in human history.

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Cannabis sativa L., Cannabaceae

Anh-Phuong Pham

Introduction

Cannabis sativa, the most popular plant in the Cannabaceae family (Linnaeus, 1753), grows annually in warm climates (**Figure 1**). There are two species closely related to *C. sativa*: *Cannabis indica* and *Cannabis ruderalis*. However, taxonomists continuously argue over whether or not these plants may just be the subspecies of *C. sativa* or the same species adapted to different environments. *C. sativa* is more commonly known as marijuana, pot, weed, ganja, and hundreds of other nicknames among alternative cultures. Marijuana is a mind-altering hallucinogen that contains hundreds of chemicals. Cannabinoids, the main class of chemicals found in marijuana, are unique to the plant. The main cannabinoid found in marijuana is Δ-^{9}-tetrahydrocannabinol (tetrahydrocannabinol, Δ-^{9}-THC, or THC), which contributes to most of the psychoactive behavior of the drug. Two other active cannabinoids in marijuana are cannabidiol (CBD) and cannabinol (CBN). CBD makes up about 40% of marijuana extracts and has been found to have a wide scope of medicinal properties, while CBN is a metabolite of THC and a psychoactive agent in *C. sativa*. Marijuana can be cultivated in many ways depending on its use, which include industrial, recreational, medicinal, and spiritual purposes. The seeds are most commonly used to make oil or food, while the flowers are where the psychoactive compounds are found. The flowers and leaves can be dried or made into resin or oil. The main methods of marijuana use consumption through inhalation, mixed with foods, or in tea (Booth, 2003). Currently, marijuana is considered a Schedule I substance

Figure 1: *Cannabis sativa* flowers grow hairs that produce psychoactive cannabinoids. (Source: http://psychotropia.co/?attachment_id=3623).

under the Controlled Substances Act (CSA) in the USA, meaning it is an illicit drug that is not federally accepted for medical treatment (ElSohly & Slade, 2005).

Botanical Description

Cannabis sativa is an herb that blooms in the spring and summer. Of all the plants in the *Cannabis* genus, *C. sativa* is the

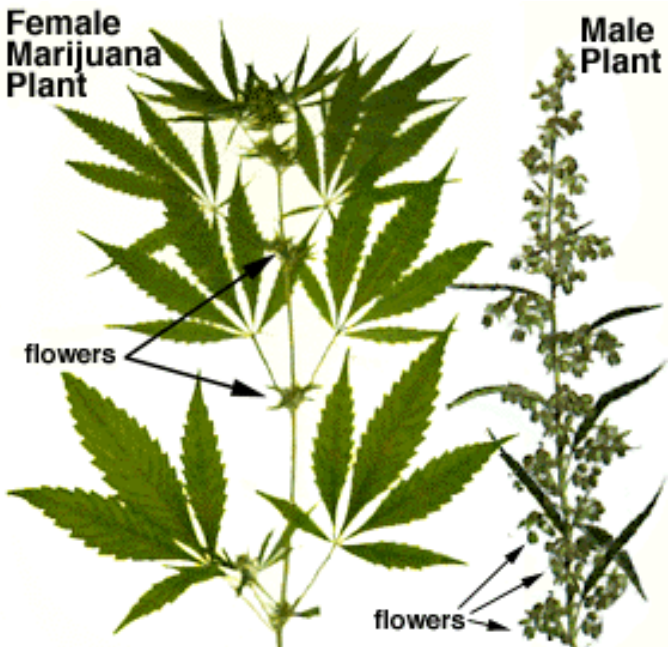

Figure 2. The flowers of male *Cannabis sativa* plants are much smaller than the flowers of their female counterparts (Source: http://life.nthu.edu.tw/~g864204/hemphist.html).

most widespread, capable of growing up to 6 m in height. This plant is very adaptable; it can be cultivated up to 8000 ft in altitude, in poor soils, and with only a small amount of water. However, direct sunlight, open ground, loamy soils, and tropic or temperate areas are best for the plant to thrive. Originally cultivated in central Asia, *C. sativa* has since spread to many other areas of the world, including India, northern Europe, and the Americas (Booth, 2003).

Marijuana plants are usually either male or female, but monoecious plants are not completely unknown. The male plants, which produce the pollen, are taller than the female

Figure 3. A chunk of Lebanese resin, also known as "hashish" (Source: http://www.erowid.org/plants/show_image.php?i=cannabis/hashish_chunk_i2006e1398_disp.jpg).

plants. The female plants are able to produce a sticky resin and flowers even if it has not been pollenated. The resulting plants are called *sin semillas* ("seedless" in Spanish) and are popular among marijuana cultivators today for the psychoactive properties provided by the high amount of THC produced from the continually growing buds. Successful pollination of *C. sativa* in the wild depends on wind strength and air currents. The flowers of the male plants are small, and can be shades of green, yellow, and purple. They grow in dense bunches and wither and die after they release their pollen. The female flowers are more complex, consisting of a pair of white stigmas which join an ovule a small green pod formed by bracts and bracteoles (**Figure 2**). The hairs on the

anthers of the male stamens and the perianths of the female flowers produce an amber-colored resin (**Figure 3**). The resin has also been speculated to help seeds from suffering from water loss during transpiration, protect seeds from ultraviolet radiation, or to trap pollen, but no primary function has been verified (Booth, 2003). Females tend to grow more hairs than males, with three distinct types: bulbous, capitate-sessile, and capitate-stalked. These three glandular hairs vary in their developing bracts, and although not much research has been done on the glands, there exists a direct correlation between the amount of glands and THC in the plants (Hammond & Mahlberg, 1973). The stalks from cannabis plants have been used for centuries as a natural fiber because of their incredible strength. Cannabis seeds enclose oils that contain many unsaturated fatty acids that are rich in nutrients and can also be used to make lamp fuel, soap, varnish, and paint (Booth, 2003).

Within the *C. sativa* plant species are two subspecies: *Cannabis sativa* L. ssp. *indica* and *Cannabis sativa* L. ssp. *sativa* (USDA). While THC is the main cannabinoid found in all cannabis plants, CBD is the next most common. The var. *indica* plant appears to have a higher CBD to THC content ratio than its close relative. The wide difference between members of the *C. sativa* species has resulted in some taxonomists speculating that the different subspecies and varieties may just be botanically adaptable versions of the same plant (Graham, 1976). The specific chemotype of individual cannabis plants and observable distinctions, differentiated by the amount of THC they produce may be more important when comparing plants than focusing on taxonomic classification. The "drug type" contains high concentrations of THC and no CBD, the "intermediate type" has moderately high concentrations of THC and CBD, and the "hemp type" has low THC and low CBD concentrations (Lewis & Elvin-Lewis, 2003).

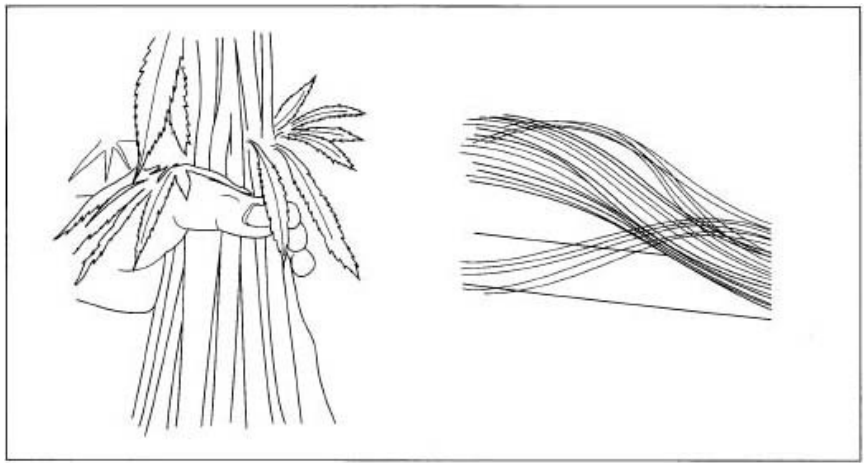

Figure 4. A drawing of hemp and hemp after cultivation, harvesting, and cutting (Source: http://www.madehow.com/Volume-6/Industrial-Hemp.html).

Traditional Uses

Hemp fiber

Hemp fibers are tall, grow in warm climates, and contain very little psychoactive properties. The extremely strong fiber is made from the stalks of *C. sativa* plants (**Figure 4**). Domestication of *Cannabis sativa* as hemp began in China around 4500 BP, and was the only fiber available at that time. Historical evidence of hemp fabrics can be found in Anatolian, Sarmatian, Scythian, and Russian cultures (Zohary, Hopf, & Weiss, 2012). Before silk was discovered and woven, hemp cloths were very popular because of their enduring nature. Pottery shards with pieces of impressed hemp cord from the Tapenkeng culture in Taiwan, dating back to between 10000 and 3000 BC, are one of the earliest recorded records of the usage of *C. sativa* as a fiber. The extraction process begins with soaking the stems in water until they break down and cause

Hemp Seed Macrocomposition	
Components	**Results (% w/w)**
Fatty Acids	
Linoleic Acid (18:2ω6)	**52-62**
α-Lineoleic Acid (18:3ω3)	**12-23**
Oleic Acid (18:1ω9)	**8-13**
Palmitic Acid (16:0)	**5-7**
Stearic Acid (18:0)	**1-2**
γ-Linoleic Acid (18:3ω6)	**3-4**
Eicosanoic Acid (20:0)	**0.39-0.79**
Eicosenoic Acid (20:1)	**0.51**
Eicosadienoic Acid (20:2)	**0.00**
Natural Products	
Cannabidiol	**10 mg/kg**
Δ^9-tetrahydrocannabinol	**not detectable**
Myrcene	**160 mg/L**
ß-Caryophyllene	**740 mg/L**
ß-Sitosterol	**100-148 g/L**
α-Tocopherol	**trace amounts**
γ-Tocopherol	**468 mg/L**
Methyl salicylate	**trace amounts**

Table 1. *Cannabis sativa* plants are rich in fatty acids, making them high in nutritional value for human consumption. Table adapted from (Leizer, Ribnicky, Poulev, Dushenkov, & Raskin, 2000)

the non-fibrous tissue to fall off. Afterwards, the fibers can be separated for use by bending the stems (Booth, 2003). England began importing hemp from Russia in the early 1600s, eventually demanding the American colonies to cultivate the plant. Hemp became an important crop in America in its early days, but production decreased after laws were implemented against the plant. Most ropes are made from nylon or cotton today, but some ropes made from hemp fiber are still available (Earleywine, 2002).

Hempseed oil

Seeds produced by *Cannabis sativa* have a wide array of uses, including food for animals and humans, bait for fishermen, and as a source of oil (Graham, 1976). The seeds have great nutritional value, including minerals, vitamins, amino acids, unsaturated fats, and protein. Most of the fatty acids in hempseed oil are linoleic, α-linolenic, and oleic acids (**Table 1**). The presence of α-linoleic acid is effective in preventing coronary heart disease and cancer, while γ-linolenic acid is known for its effects against inflammation. Hempseed oil is also associated with a reduction of serum cholesterol and heart diseases (Oomah, Busson, Godfrey, & Drover, 2002). Vegetable oil can be extracted from hempseed, which can then be turned into diesel fuel, flammable fuel oil, or non-viscous lubricant. Hempseed oil can be used as a cheaper alternative to almond, coconut, and olive oils in cosmetics as an emollient. However, the US government requires sterilization of hemp products before they are put on the market, which greatly reduces their important properties (Booth, 2003).

Psychoactive properties

Historically, *Cannabis sativa* has been used for its psychoactive properties recreationally, medicinally, and spiritually in many societies. *C. sativa* grown as a psychoactive agent can be prepared herbally, as cannabis resin, or in the form of hashish oil. Herbal cannabis is prepared by drying the flowers and leaves, cannabis resin requires separating the hairs from the plant, and hashish oil requires a sophisticated, hot extraction process, but is the most potent (Oomah et al., 2002). Long

after being cultivated as a fiber, *C. sativa* began being used medicinally around 2737 B.C. in China. The emperor, Shen Neng, used cannabis, ephedra, camellia sinensis, and ginseng for their medicinal properties (Earleywine, 2002). Shen Neng's book, *Pen Ts'ao Ching* is the earliest known Chinese pharmacopoeia and lists hundreds of herbs he tasted with grade and rarity ratings. In particular, marijuana was noted as an anti-inflammatory agent and painkiller (Li, 1975). Shen Neng would advise patients to drink a 'hemp elixir' made of cannabis leaves and flowers to treat many different types of ailments including gout and malaria (Booth, 2003). Marijuana was also considered a holy plant in the *Atharvaveda*, a Hindu text; ancient Indian healers prescribed *C. sativa* for asthma, congestion, fevers, or inflammation in the mucuous membranes. These medicines were most commonly consumed as a tea. Other known applications include easing pain during childbirth and alleviating postpartum depression. Reports of early use of marijuana as an intoxicant are not as prevalent as its other purposes. While China's Taoist movement first condemned recreational cannabis use, it was eventually used in religious rituals by the Taoists. A group of writers in the 19th century known as the Hashish Club used marijuana orally and through inhalation for its creative elements. Baudelaire, a member of this club, documented changes in thoughts, sensations, euphoric and dysphonic reactions, and intoxication that resulted from marijuana use. *C. sativa* as an intoxicant continued to spread, and finally reached the Americas in the 16th century. However, laws against cannabis arose in the United States in the early 1900s, most likely due to discrimination against certain minority ethnic groups including African and Mexican immigrants who were the most common marijuana users of that time. Its popularity quickly increased again in the 1960s. This time, the enthusiasts were "hippies" and college students. The alternative culture soon spread throughout the Western world, promoting social marijuana use through inhalation, study and acceptance of other religions and philosophies, and psychedelic films and books. Many accounts of marijuana use still exist today, despite its illicitness (Earleywine, 2002).

India

Traditional herbal medicine passed down orally from generation to generation is the most important form of healthcare for rural populations living in India. Today, the Monpa group in Arunachal Pradesh still use *Cannabis sativa* to cure dysentery and diarrhea in cattle and goat (Namsa, Mandal, Tangjang, & Mandal, 2011) while the Khetawas in the Jhajjar district of Haryana use it to treat coughs (Panghal, Arya, Yadav, Kumar, & Yadav, 2010). For both of these cultures, *C. sativa* is consumed orally.

Indians also use cannabis for recreational purposes. Hindus in north Indian use three different forms of cannabis. Bhang refers to the dried leaves of the herb, charas are the hashish form, and ganja is what they call the flowering tops. Bhang is usually eaten or used as a drink called thundai, while ganja and charas are smoked through pipes. Hindu castes do not condemn cannabis; it is sometimes used in religious rituals, ceremonies, and festivals (Hasan, 1975).

Jamaica

The hemp plant has been present in Jamaica since the late eighteenth century, but it became a popular smoking commodity among the working class around the mid-nineteenth century. When Indian indentured laborers came to the West Indies, they introduced cannabis and "the ganja complex." This consisted of cultural beliefs about *Cannabis*

Classes of Chemical Compounds in *C. sativa*	
Classes	**Amount Known**
Cannabinoids	61
Nitrogenous compounds	20
Amino acids	18
Proteins, glycoproteins, & enzymes	9
Sugars & related compounds	34
Hydrocarbons	50
Simple Alcohols	7
Simple Aldehydes	12
Simple Ketones	13
Simple acids	20
Fatty acids	12
Simple esters & lactones	13
Steroids	11
Terpenes	103
Non-cannabinoid phenols	16
Flavanoidglycosides	19
Vitamins	1
Pigments	2

Table 2. There are hundreds of compounds that are found in *Cannabis sativa*. Some of these constituents have never been isolated, while others, especially cannabinoids, have been gone through many research analyses. Table adapted from (Turner, Elsohly, & Boeren, 1980)

sativa, methods and preparation, and Hindu names that are still common terms for Jamaicans today. Jamaicans use ganja to treat all their maladies. This culture still exists in Jamaica today, where children begin smoking the drug recreationally at around 8 years old. While use is most predominant in the lower class, it has spread to the middle class, which focuses more on psychoactive reactions. One well known group of cannabis smokers in Jamaica are a religious group called the Rastafarians. They advocate a "back to Africa" movement and worship Haile Selassie I, the emperor of Ethiopia from 1930-1974. Rastafarians consider marijuana a biblical weed and use the herb while chanting hymns during religious ceremonies (Rubin & Comitas, 1976).

Nepal

Cannabis sativa has been used traditionally in Nepal for centuries for ritual, social, and medicinal purposes. *Ayurvedic* medical systems use cannabis orally to treat a variety of physiological and psychosomatic conditions. One treatment uses cannabis with around 15 other ingredients to treat diarrhea. Cannabis can also be used as a tranquilizer to put children to sleep. Another common cannabis practice is recreational use and pain relief among older people (Fisher, 1975). Since the majority of the population lives in areas where health care facilities are lacking, traditional healing systems are responsible for passing down knowledge about medicinal plants. The people of the Manang district in central Nepal continue to cultivate many medicinal plants in the *Ayurvedic* tradition, including *C. sativa*. Many parts of the plants are used medicinally and can be made into powders, pastes, decoctions, and infusions. Powders and decoctions are the most popular forms used by the Nepalese and are most often prepared by dissolving in hot and cold water, but milk, honey, oil, and ghee can also be used (Bhattarai, Chaudhary, & Taylor, 2006).

Chemistry and Pharmacology

Currently, there are over 420 known compounds in *Cannabis sativa* (**Table 2**), which is considered a psychoactive drug. Psychoactive agents are either psychotropic or

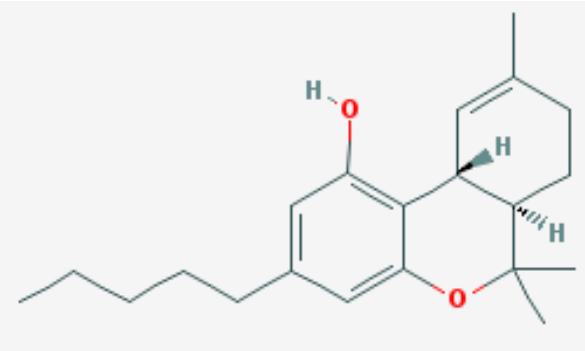

Figure 5.Chemical structure of Δ-^{9}-THC, the most active isomer of THC, a psychoactive compound found in *Cannabis sativa* (Source: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=16078).

psychotomimetic. *C. sativa* falls under the latter category, but rather than containing alkaloids like other hallucinogens, its psychoactive properties are attributed to the presence of cannabinoids, which are unique to the plant. The most common cannabinoid is THC (**Figure 5**), but CBD (**Figure 6**) is also very active, and CBN is present in the resin (Booth, 2003). Cannabinoids are found in both male and female parts of cannabis plants, but are most concentrated in the female tops. Besides cannabinoids, there have also been reports of terpenes, phenols, sugars, flavonoids, *trans*-cinnamic acid, choline, trigonelline, piperidine, alkaloids, and cannabamines found in cannabis plants. The interactions between these compounds and the cannabinoids may enhance or decrease the psychotomimetic effects of cannabis. The full pharmacological activity of *C. sativa* is still unknown because most cannabis research focuses on the effects of THC (Graham, 1976). Researchers attribute most of cannabis' effects to Δ-^{9}-THC because of its abundance in the plant. When it reaches the liver, it is broken down into 11-OH- Δ -^{9}-THC (11-hydroxy- Δ-

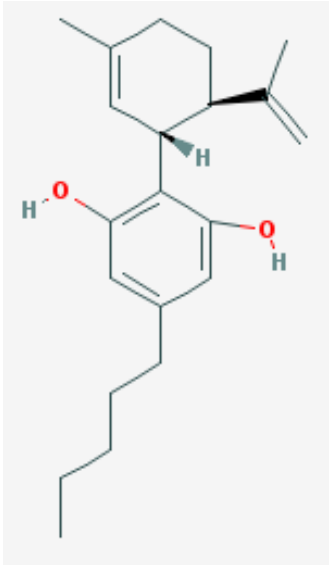

Figure 6. CBD is a major cannabinoid found in the plant. Although it is a psychoactive compound, it is not intoxicating and has a wide range of medical applications (Source: http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=136351015&loc=es_rss).

^{9}-THC), which may be almost three times as potent. The cannabinoid composition in marijuana leaves may be different than in the smoke. Over 60% of the original THC content appears in the smoke after normal smoking conditions, but with a negative side effect of containing 70% more benzopyrene (**Figure 7**) than tobacco smoke (Petersen, 1980).

Biological Activity

The active cannabinoids in *Cannabis sativa* act on specific cannabinoid receptor sites. Two receptors have been cloned, CB_1 and CB_2, and more receptors are suspected to exist. The CB_1 receptors are found on C-fibers and are expressed in

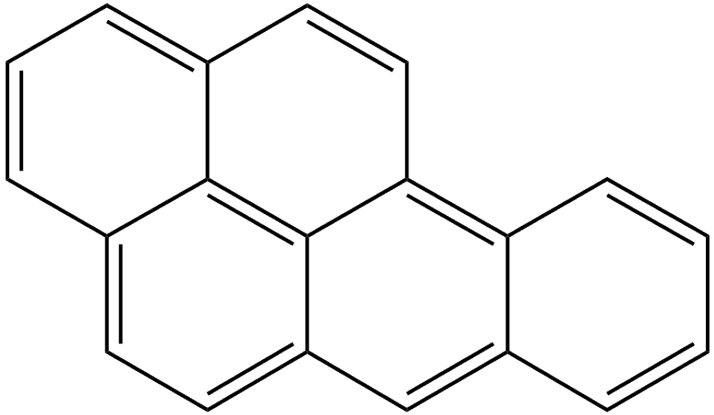

Figure 7. Benzopyrene is an organic compound that can be found in coal tar, after volcanic eruptions, in cigarette and wood smoke, and in burnt foods. Inhalation, ingestion, and dermal contact of benzo(a)pyrene are related to lung cancer (Source: http://wtt-pro.nist.gov/wtt-pro/index.html?cmp=benzo~a~pyrene).

neurons found in the nervous system, while CB_2 receptors are found in non-neuronal tissues. Animal studies have led to the belief that the main role of the endocannabinoid system is to suppress pain. In this case, the cannabinoids found in marijuana are able to act as neurotransmitters. CB_1 receptor agonists have been observed to produce antinociception in response to acute pain through peripheral nervous system, spinal cord, or brain injection, or through the usage of crude marijuana extract. Other studies suggest that cannabinoids can diffuse from stimulated cells to act as presynaptic neuron receptors. Furthermore, two natural cannabinoids in the body, anandamide and methanandamide are able to activate vanilloid receptors expressed in kidney cells (Calixto, Scheidt, Otuki, & Santos, 2001). These compounds are less potent and short-lived than THC, but imitate some of its effects. Cannabinoid receptors can also be found in a variety of other species including mollusks, protozoa, and many mammals, which suggests they play important functional roles in the body. Human and rodent cannabinoid receptors are very similar which is helpful in applying animal study results to people. Overall, there is definitive evidence that CB_1 receptors are involved with memory and motor control, while CB_2 receptors are associated with the immune system (Earleywine, 2002).

Structural MRI and post mortem examinations of the brain have been used in *in vivo* studies of human patients to investigate the relationship between cannabis use and brain morphology. Cannabis use in these studies showed a decrease in specific brain structures in psychosis or at-risk for psychosis patients. Brain regions rich in CB_1 receptors, and patients with mental illness were the most affected (Rapp, Bugra, Riecher-Rossler, Tamagni, & Borgwardt, 2012).

Interest in the consequences of prolonged *C. sativa* use can be attributed to its popularity in parts of the world, especially lower socioeconomic groups. *In vivo* and *in vitro studies* were conducted to investigate the cytogenetic effects of *C. sativa*. The *in vitro* studies consisted of rat embryonic fibroblasts treated with cannabis resin and colcemid (Ciba) after 4-14 days of culture, processed, and then examined for chromosomal irregularities, and the addition of cannabis resin to the blood of rat and human subjects. The *in vivo* studies were comprised of pregnant rats that had daily injections of cannabis resin for the six days of gestation. The embryos were then removed from each rat on days 11-13 and homogenized in cell suspension. After 3-6 days of culturing, the cells were processed for chromosomal examination. Blood was also taken from the pregnant rats when they were killed and cultured for two days to examine the chromosomes. Another *in vivo* experimental group involved chronic cannabis users, who had been smoking at least three "spliffs" a day for at least

10 years. These individuals were admitted to a hospital for five days of clinical study, and had peripheral blood drawn the first morning of admission. The blood was cultured for two days for chromosomal examination. Unlike the other experimental groups, there was no treatment for these cultures. The control groups for this study received a Tween-saline solution instead of cannabis resin (Martin, Thorburn, & Bryant, 1974).

Cannabis resin given to the experimental groups in the *in vitro* studies resulted in a lowered mitotic rate. Higher concentrations of cannabis showed a relation to greater mitotic depression, with 200 μg/mL of resin resulting in complete inhibition. However, because cells that received cannabis resin *in vivo* did not show any mitotic inhibition, there is no confirmed evidence that exposure to cannabis resin is related to chromosome abnormalities. It may be possible that very high concentrations of *C. sativa* in frequent users may inhibit the proliferation of embryonic and germ cells. If a large amount of the drug passes through the placenta, mitotic arrest can occur, interfering with normal embryonic development (Martin et al., 1974).

Clinical Studies

Multiple sclerosis (MS) is an inflammatory disease that results in demyelination, scarring, and many poorly controlled symptoms. Spasticity is common in individuals suffering from MS, and many of these individuals respond adversely to anti-spasticity medication. Evidence that cannabinoid receptors may be able to control spasticity led to the belief that *Cannabis sativa* could be used as an effective treatment against neurological conditions. One randomized, placebo-controlled trial examined the effects of smoking cannabis on spasticity. Patients that received cannabis instead of a placebo had a reduction of 2.74 points on the Ashworth scale, and an average of 5.28 points in reduction in pain scores on a visual analogue scale was also seen in the experimental group (Corey-Bloom et al., 2012).

Smoked cannabis also seems to be effective in relieving pain and improving sleep and mood according to patients with chronic neuropathic pain. The pain is usually a result of pharmacotherapy and not many treatment options are known. One randomized controlled trial concluded that inhaling 25 mg of 9.4% THC three times a day for five days effectively and tolerably reduces pain and improves sleep (Ware et al., 2010).

Epidemiological studies in Sweden, New Zealand, The Netherlands, and Israel show that cannabis abusers have an increased likelihood to developing schizophrenia. Other studies suggest that the cannabis use precedes the onset of symptoms of schizophrenia in subjects with cannabis use disorders (CUD). However, no significant association was found between the onset of schizophrenia and CUD in subjects in a study that controlled demographic and clinical variables (Sevy et al., 2010).

Contraindications

Cannabis sativa is the most commonly used illegal drug. About a third of all Americans have used marijuana at least once, but less than 5% report using it at least once a week. It is difficult to truly measure the extent of marijuana use because some users lie due to its legal status or forget about their use. Marijuana addiction is also difficult to measure because there is no universal definition. To some, addiction refers to a physical or biological process, whereas dependence is a collection of three of the following symptoms: tolerance, withdrawal symptoms, use exceeding initial intention, failed

attempts to decrease use/constant desire for the drug, loss of time related to use, reduced activities because of drug use, and continued use despite problems. A small portion of frequent cannabis users also appear to suffer from marijuana substance abuse. The current diagnosis requires one of the following symptoms: interference with major obligations, intoxication in unsafe situations, legal problems, and continued use despite problems. Overall, only about 6-23% of cannabis users report difficulties with marijuana use (Earleywine, 2002).

The toxicity of cannabinoids, cannabis, and cannabis products are varied because many different compounds exist in the plant and resin. In one study, two comparable male subjects smoked cannabis, with the experienced user enjoying himself, and the less experienced user becoming disoriented and suffering adverse side effects, including dizziness, tremors, and muscular rigidity. Immediate toxic effects of ingested or smoked marijuana include nausea, vomiting, thirst, and dryness of the mouth. Nevertheless, these symptoms are comparable to the effects of tobacco smoke and may not be due the active compounds of *Cannabis sativa*. Other side effects that have been reported include ataxia, cold extremities, and precordial pain due to shortness of breath. Typically, a single administration of smoked or swallowed cannabis is not very toxic, but intravenous injection done by misguided persons have resulted in disastrous effects, including tachycardia, peripheral circulatory failure, vomiting, diarrhea, enlargement of the liver and spleen and pulmonary edema. However, because cannabinoids are insoluble in water, these effects are most likely due to the act of intravenous administration. The development of an allergy a *C. sativa* constituent is another possible method leading to toxicity (Graham, 1976). In general, a lethal dosage of THC requires 125 mg of the drug per kilogram of body weight. Most marijuana cigarettes only contain about 20 mg of THC, and at least 50% of the THC is lost while smoking (Earleywine, 2002).

While there are no recorded documents of fatal marijuana overdose, it is well known that cannabis users often ingest other illegal drugs. These drugs may be detrimental to their health, as well as having adverse interactions with *C. sativa*. This common practice among drug users has prompted researchers to conduct experiments on both animals and humans on the consequences of combining *C. sativa* with other drugs, especially alcohol which is most commonly combined with cannabis. Acute depressant effects due to cannabis and alcohol combination in animal models appear to be greater than the use of either drug alone. THC also appeared to enhance the impairing subhypnotic effects of ethanol. The effects of these two drugs appeared to be additive, resulting in greater body temperature, depressed heart rate, and impaired rotarod performance. Similar results were also found in studies done on human subjects -- reduced standing steadiness, reaction speed, and cognitive performance. Besides their mental and psychomotor effects, combining marijuana and ethanol also increases pulse rate and conjunctival reddening. Cannabis also appears to enhance the depressant effects of sedatives and hypnotics in both animals and people (Petersen, 1980).

Current Use in Allopathic and CAM Therapies

Despite several US states passing legislation for medical marijuana and two states passing legislation for recreational use, possession of cannabis in the United States, even as a medicinal agent, is still a federal offense. However, medical marijuana usage rates are still high. There is evidence from controlled studies that suggest the cannabinoids found in *Cannabis sativa* are effective in reducing pain and vomiting,

Figure 8. Canasol eye drops are used all around the world for treating eye pressure in glaucoma patients (Source: http://www.hambaarst.ee/artiklid/702/).

Figure 9. Cesamet is the trade name for a synthetic cannabinoid called nabilone that is used for the treatment of nausea and vomiting due to chemotherapy in cancer patients (Source: http://www.cesamet.com/patient-about-cesamet.asp).

promoting weight gain and appetite, decreasing eye pressure in individuals with glaucoma, and minimizing spasticity and involuntary movements. Other therapeutic effects were also found for individuals with asthma, insomnia, anxiety, seizures, tumors, menstrual cramps and PMS, Crohn's disease, and other medical and psychological disorder(Earleywine, 2002).

Since 1987, a cannabis derivative has been marketed by researchers in the West Indies for reducing intraocular pressure in glaucoma patients. Canasol eye drops (**Figure 8**) have been used in many parts of the world to manage glaucoma, especially in the Caribbeans. These eye drops can lower pressures up to 50% in 15 minutes or less. They are also inexpensive and have little to no side effects, including psychoactive ones. Canasol is also compatible with other topical treatments for glaucoma (Earleywine, 2002).

Dronabinol, a synthetic version of THC, is still on the market today under the name, Marinol. It is usually suspended in sesame oil and ingested orally, and it can be used to enhance appetite and reduce nausea and vomiting. Other studies suggest its effectiveness as treatment for spasticity in MS patients and disturbed behavior in Alzheimer's patients (Earleywine, 2002). Furthermore, more recent research has shown statistically significant reductions in hair-pulling in individuals diagnosed with trichotillomania that were given dronabinol (Grant, Odlaug, Chamberlain, & Kim, 2011) and increases in food intake and improved mood and sleep in HIV-positive marijuana smokers (Haney et al., 2007). However,

Marinol has been argued to be less effective than smoked marijuana. Some of these arguments criticize that the oral ingestion of Marinol may be difficult for patients who find swallowing difficult due to their nausea. Also, the effects do not appear as quickly as smoked marijuana. The pills are extremely expensive as well; some patients may have to spend up to $1000 on the pills in one month. To put this into perspective, a 10 mg pill of dronabinol costs as much as $13 or $8 a pill if bought in bulk, whereas the same amount of THC can be found in half a marijuana cigarette, which can be purchased for less than $5 if bought in bulk illegally (Earleywine, 2002).

Nabilone, marketed as Cesamet (**Figure 9**), is another example of synthetic THC that is available in some countries today. It is an orally ingested drug available that has been approved for treating nausea and vomiting during chemotherapy since 1985 (Skrabek, Galimova, Ethans, & Perry, 2008). Nabilone is typically administer in single doses ranging from 1 to 5 mg. and the effects can take between 1 and 1.5 hr to become evident and continue for up to 12 hr (Lemberger & Rowe, 1975).

The newest cannabis inspired drug on the market is called Sativex (**Figure 10**), which is the trade name for Nabiximols. Sativex is currently the only non-synthetic cannabinoid pharmaceutical product. Currently, it is used as an oromucosal spray for MS patients suffering from spasticity. There is definitive evidence that continual use of Sativex results in long-term improvement of spasticity (Notcutt, Langford, Davies, Ratcliffe, & Potts, 2012). Currently, it is also going under research as a possible treatment for cancer patients experiencing chronic pain (Portenoy et al., 2012).

When administering THC to patients, it is important to control dosage because the therapeutic dose is variable in different

Figure 10. Sativex is a new drug developed by GW Pharmaceuticals for treating spasticity in multiple sclerosis patients (Source: http://clarkfrench.wordpress.com/2011/06/06/access-sativex/).

individuals. Overall, THC can act as an analgesic, muscle relaxant, and appetite stimulant. Patients are often advised to deliver small doses throughout the day in order to avoid the intolerable side effects while still being effectively treated for whatever pain they may be experiencing (Barnes, 2006).

Discussion

There is a lot of evidence from clinical, *in vivo* and *in vitro* studies that the active ingredients in *Cannabis sativa* are effective in a variety of treatments including spasticity and chronic pain. The cannabinoids in marijuana have also been found to be effective bronchodilators, which makes it a good

alternative treatment for asthma and allergies (Ziment & Tashkin, 2000). The US Drug Enforcement Administration (DEA) currently lists marijuana as a Schedule I drug under the CSA. Schedule I drugs, which also include heroin and LSD. Schedule I drugs are considered to have a high potential for abuse and no accepted medical purposes. They are the most dangerous group of drugs under the CSA because of the potential dependence. Since it has been outlawed in the USA since 1937 and the UK since 1971, clinical advances for cannabis have been hindered (Wingerchuk, 2004). However, marijuana prohibition was not based off of any scientific basis. Historically, prohibitions often stem from some form of bigotry towards a minority group. The Mexican (discrimination) hypothesis helps explain how penalties for marijuana were actually decreased in the 1970s after a large amount of white teenagers were arrested for possession. Marijuana prohibition may also be in part due to the benefits that the chemical industry and companies that produce artificial fibers gain due to the prohibition of hemp (Thornton, 1991). Due to its legality, it is difficult to market drugs based off of cannabinoids.

While marijuana has a high potential for dependence and abuse, there has never been a death due to lethal overdose of the herb because it can only become toxic in extremely high doses. One area of concern regarding marijuana use is its interaction with alcohol and other depressants. Studies show that mixing marijuana with other drugs can reduce cognitive and psychomotor functions as well as enhancing the depressant effects (Graham, 1976).

Currently, most of the known information about the psychoactive properties of marijuana is related to the effects of THC. Subsequent research should focus on the effects of other cannabinoids and their possible medical applications. *C. sativa* has been a historical tradition in many cultures, and if the Western world were more willing to accept it as a possible alternative medicine rather than a forbidden drug, more treatments for cancer, HIV, and other chronic diseases could be researched and marketed. Medical marijuana is a strongly debated topic, and will probably remain in the dark until it is finally accepted by governments.

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Capsicum annuum L., Solanaceae

Ezinne Nwankwo

Introduction

Capsicum annuum L. is also known by a variety of names. The numerous subspecies include bell peppers, chili peppers, paprika, and banana peppers to name a few. It is a member of the nightshade family, Solanaceae (Andrews, 1995). These peppers are native to Central and South America but have been cultivated worldwide and are essential members of many cultural cuisines. Other members of this family are the tomato and *Atropa belladonna*, or deadly nightshade (Andrews, 1995).

The fruit of this plant is rich in vitamins A, B complex C, E, and P. Additionally; it serves as a source of polyphenols. The chemical constituent, capsaicin, gives the fruit its well-known pungent characteristic (Andrews, 1995) and confers many of its medicinal properties. Capsaicin is anti-carcinogenic and anti-inflammatory (Surh, 2002), (Reddy, Ravinder, Prasad, & Kanjilal, 2011). Peppers have been used to combat diseases as diverse as inflammation, diabetes, and evil eye (Aggarwal, Surh, & Shishodia, 2007), (Ishtiaq, Hanif, Khan, Ashraf, & Butt, 2007). *C. annuum* varieties have other uses also. They have been used as a coloring agent for processed food, drugs, and cosmetics.

a.

b.

Figure 1 a and b. Flowering *C. annuum*
(Source: http://www.g6csy.net/chile/var-ann.html)

Botanical Description

Capsicum annuum is herbaceous when young and woodier with age. The woodiness only occurs in areas that do not freeze when grown as a perennial. There is dichotomous branching, with the main stem ending in a flower with several leaves branching off of the same main stem. There are also two or three more stems branching off of the first, main stem

Figure 2. *C. annuum* fruits
(Source: http://www.beautifulbotany.com/STOCK%20C/Capsicum%20annuum%20'Garda%20Tricolor'.jpg)

that also end in flowers. The height of the plant varies with the variety. It can be tall and erect, compact, or prostrate. The leaves vary in shape and texture. They can be smooth or wrinkled. The colors of the leaves range from green to purple to white. The roots can be deep or shallow depending on the variety.

The corolla of the plant (all of the petals of the flower) tapers to a point. This means that they are acuminate. On average the corolla is five lobed but this number can also change. There are three veins in each lobe. The color of the corolla can be anything from cream to purple to greenish white (**Figure 1a and 1b**). There are also some varieties with greenish yellow spots. The pistil can be longer than the stamens, which is characteristic of more primitive plants, or shorter than the stamens. The shorter variety is more favorable for self-fertilization. Sexual reproducing plants are frequented by honey bees, who are attracted to the scentless nectar. The nectar is secreted and accumulates at the base of the lobes of the corolla and eventually presents at the surface of the petals. Other insects such as aphids, butterflies, and ants also transfer pollen (Andrews, 1995).

The fruit formation depends on genetic factors (size and shape) as well as environmental factors (growth). The apex of the fruit can be sunken and lobed with a depression, as in the boxy bell peppers. In other varieties it can be pointed or blunt. The base of the pod, the attachment point to the peduncle or stalk, may bulge or be nonbulged. In domesticated versions, a hard to detach stem is present. This feature was selected on the basis of ease of storage. Wild versions have a deciduous or easily detachable stem. Many cultivators prefer this version to use with machine harvesting and to decrease the amount of waste generated. The pericarp, or fruit wall, ranges in thickness from thin to thick. This has an impact on preservation technique. The fruit with a thin pericarp can be dried readily but those with thicker pericarps must be pickled, canned, or smoked. The placenta and vein of the plant are also included in the pericarp and contain the active ingredient, capsaicin. The color of the fruits is usually green when unripe but is known to be yellow-green, yellow, orange, bright or deep purple (**Figure 2**). When the fruits reach maturity, the colors range from yellow, orange, and red or brownish red. Red, however, is the most common color. (Andrews, 1995)

Traditional Uses

Traditional Medical Uses

West Indians use a mixture of ground bird peppers combined with onions, cucumber, lemon juice and Madiera as a stomachic, or stomach strengthener (Andrews, 1995). The

people of the Samahni valley in Pakistan use *Capsicum annuum* as a rubefacient as well as sore throat, dyspepsia, yellow fever and snake bites. It is treated by moving three, five, or seven chilies over the head. These same chilies are then burned in a fire. Chili smoke is also used as a ghost repellent in the treatment of giant. Giant is a cultural disease in the middle east (Ishtiaq et al., 2007).

The Mayans used chilies for similar ailments that include sore throats, asthma, cough and respiratory disorders. They also used the peppers topically to heal infected gums. Swedes use a salve made from Chiltepine peppers to sooth sore muscles. In Germany, the peppers are an ingredient in a warming bandage used for muscle pain (Andrews, 1995).

Many cultures use peppers for other medicinal purposes*. C. annuum* has been used as an aphrodisiac, carminative, central nervous system stimulant, and a tonic. Interestingly, it has been used to treat gonorrhea (Dr. Duke Ethnobotanical Uses).

Food Uses

The use of *Capsicum annuum* as a condiment and spice is spread worldwide. It is a constituent of Indian curry powder and Texan chili powder. The dried, crushed peppers of the red and cayenne varieties are used in America and Turkey. The ground form of cayenne pepper is grown and used in Africa, India, Japan, and Louisiana as a flavoring. Red peppers are grown in the southern and western regions of the United States as well as Turkey. Other pepper varieties are used in the whole from, either dried or fresh. This form is popular in Central and South America as well as southern North America. (Andrews, 1995)

Paprika is a ground mixture of many capsicum varieties. It has a characteristic red coloring and can be mild or more pungent. Sweet paprika contains mostly fruit wall with the seeds removed. The hotter versions contain more of the placenta, seeds and stalks. Interestingly, there is a Hungarian version that is very pungent and uses the long, dried pods. Conversely, the Spanish version contains a more tomato shaped pepper. Morocco and Bulgaria are two other countries that have their own versions of paprika. Many Balkan growers brought paprika seeds to South Carolina after World War II and California is one of the leading producers of paprika. This increase in the United States industry may be due to the decline of the Soviet Union, which caused a decrease in the European paprika industry. (Andrews, 1995)

Pepper sauce is another way that *C. annuum* is consumed. The peppers are either pickled whole in brine or vinegar or mashed to make the sauce. The sauce is then used to flavor other foods. Chili peppers are the variety used in this method. Peppers can also be canned or processed to make dips and sauces. (Andrews, 1995)

Use as a Coloring Agent

The peppers have been used by Central and Southern Americans to enhance the color of a bland diet. They are also used to replace red coloring lost in food preparation. Carotenoids from the peppers are also used to color drugs and cosmetics (Andrews, 1995).

C. annuum has been used extensively in the food industry. Foods from sausages to salad dressings to gelatin are colored with the extractable colors of capsicums. The waste from pimento pepper processing is used to influence the color of egg yolks. The capsanthin present in the pimento waste gets

deposited in the egg yolk and gives it a dark reddish yellow color. The color of the chicken itself is also influenced, with the addition of pimento assuring a yellow skin color in the body of the chicken. An unintentional influence is that the hatched chicks are stronger and offspring of pimento fed chicken have a higher rate of hatchability. This "improved" color is also manifested in faded pink flamingos and trout that are fed pimento waste. (Andrews, 1995)

Other Uses

Italians in the high Molise region of central-southern Italy use a pungent variety of *Capsicum annuum* to protect food products from parasites (Guarrera, Lucchese, & Medori, 2008).

Chemistry and Pharmacology

Capsicum annuum contains a large variety of chemicals that have a huge impact on its pharmacology. It is to be noted that there are multiple varieties of this species that differ in their chemical makeup. The major difference in these varieties is the amount of capsaicin. More pungent varieties such as the red chili pepper have more capsaicin content that less pungent varieties like bell peppers with have a minimum amount of capsaicin (Andrews, 1995).

Capsaicin or CAPS is the major pungent ingredient in *C. annuum.* CAPS is a homovanillic acid derivative (see **Figure 3**) (Aggarwal et al., 2007). It has effects on many organ systems in humans. For example, it causes peristalsis in the stomach stimulating the mucous membranes. This movement aids in digestion. Stomach acid secretions are increased with *C. annuum* consumption. These increased secretions also increase appetite along with aiding digestion. The increased

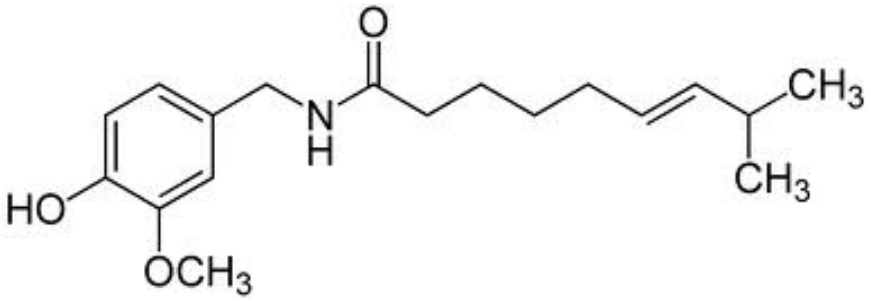

Figure 3. Capsaicin structure

mucous production in the mouth from ingestion protects stomach ulcers from harsh stomach acids (Andrews, 1995).

Mammals are unique in that they have a specific capsaicin receptor on their sensory neurons called the vanilloid receptor-1 (VR1) or the transient receptor potential of vanilloid 1 (TRPV1). Administration of CAPS to peripheral nerve endings results in an initial reaction of pain and neurogenic inflammation. However, desensitization occurs with repeated application of CAPS to the same nerve endings may have indications for its use as an analgesic for many painful neuralgic conditions. The nerve desensitization from CAPS is most likely from the effect on the neuropeptide Substance P. Substance P is involved in pain transmission to the brain and is inhibited by CAPS. The inhibition with increased, repeated exposure to CAPS has the effect of moderating pain (Aggarwal et al., 2007).

As shown above, *C. annuum* has effects on the nervous system. Similar to other members of the Solanaceae family, more pungent varieties of *C. annuum* can alter the conscious state, albeit momentarily. The effect of *C. annuum* differs from other members of the Solanaceae family in that it is nonhallucinogic and nonaddictive. It is also generally safe for consumption

although. *annuum* has stimulant activities that can activate the vagal reflex and cause vomiting (Andrews, 1995).

In hot climates, *C. annuum* can cause psychological gustatory sweating. This is a form of thermoregulation that is confined to the face and scalp. Evaporation of this sweat can facilitate a cooling effect. Cooling the body can relieve this gustatory sweating from consuming the peppers (Andrews, 1995).

Other chemicals in *C. annuum* include many nutrients, including Vitamins A (beta-carotene) and C (ascorbic acid). They also contain capsanthin and many capsianosides. Carotenoids and phenolic acid compounds are also found in these peppers. Minerals, amino acids and sugars occur in abundance in *C. annuum* fruits (Dr. Duke Phythochemical and Ethnobotanical Database).

Biological Activity

In vitro

It has been found that the CaBtf3 NAC subunit in *Capsicum annuum* is involved in the hypersensitive response of plants to viral infection (Huh, Kim, & Paek, 2012). The hypersensitive response facilitates plant cell death after viral infection. Plants use it as a strategy for preventing viral movement and gaining systemic acquired resistance against further viral infection (Huh et al., 2012). It is not known whether this is a transcription factor but knowledge of its involvement in the plant viral response could have implications for potential therapies. In 2011, researchers isolated 28 polyphenols from three *C. annuum* bell pepper varieties and determined that these compounds had both anticancer and antioxidant effects in vitro (Jeong et al., 2011). Many studies conducted confirmed that the polyphenols found in *C. annuum* do exhibit antioxidant activity in vitro (Lee et al., 2009), (Materska & Perucka, 2005).

A study was conducted on effect of Indian spices on esophageal squamous carcinoma cells. In the study, an aqueous chili pepper extract induced a 88% apoptotic cell demise and cell death within 24 hours of exposure (Dwivedi, Shrivastava, Hussain, Ganguly, & Bharadwaj, 2011).

In a similar vein, a study was done on human umbilical vein endothelial cells in which extracts from *Capsicum* species and capsaicin were assessed for cytotoxicity to the endothelial cells and protection from lipopolysaccharide (LPS) induced cellular apoptosis (Chularojmontri, Suwatronnakorn, & Wattanapitayakul, 2010). It was shown that the extracts and capsaicin improved endothelial cell function and conferred protection against the LPS induced apoptosis. It was also shown that the extracts where not cytotoxic to the endothelial cells. This protection indicates that consumption of the fruits of *Capsicum annuum* species could promote endothelial health (Chularojmontri et al., 2010).

In addition to promotion of cellular health, *C. annuum* may be a potential antibacterial agent. A Japanese study showed that a methanol extract of capsaicin inhibited cholera toxin production in *Vibrio cholera*. (Chatterjee et al., 2010) This discovery may have implications for the use of natural compounds to combat increasingly antibiotic resistant bacterial pathogens (Chatterjee et al., 2010).

In Vivo

Many rat and mice studies have been conducted on the effects of capsaicin on pain reduction, weight reduction, and cancer treatment and prevention along with a host of other topics. In one study it was shown that capsaicin may confer protection

from autoimmune diabetes. This study was conducted by researchers at the University of Connecticut School of Medicine. The vanilloid receptor 1 or VR1 is present on sensory neurons as well as immune cells (Nevius, Srivastava, & Basu, 2012). This receptor can regulate gut events in response to its ligand, capsaicin. In mice, when capsaicin is administered orally it stops the growth and activation of autoreactive T cells, which attack the cells of the body. This attenuation is specific to lymph nodes in the pancreas, which is the area of insulin production. The attenuation is how the protection from type 1 diabetes is conferred (Nevius et al., 2012) because capsaicin prevents the autoreactive T cells from attacking the insulin producing cells. The researchers also found that the binding of capsaicin to the VR1 receptor activates pancreatic macrophages that express anti-inflammatory factors (Nevius et al., 2012). The influencing of nutrient-immune interactions by VR1 through inhibiting the autoreactive T cells and activating anti-inflammatory macrophages could have implications on gut-mediated immune tolerance (Nevius et al., 2012).

Capsaicin has been implicated as an anti-carcinogenic. Female mice treated topically with capsaicin exhibited lowered multiplicity of VC-induced skin tumors (Aggarwal et al., 2007). When CAPS was applied prior to induced mutagenesis there was less skin tumor formation in the treated mice (Aggarwal et al., 2007). Several studies conducted showed that capsaicin inhibited metabolic activation of carcinogens via various mechanisms. One such mechanism was the modulation of CYP-dependent monooxygenase activities (Aggarwal et al., 2007). Specifically, a rat study showed that capsaicin inhibited the activity of epidermal arylhydrocarbon hydroxylase. This inhibition blocked the activation and binding of the carcinogen benzopyrene to DNA which prevented it from mutagenizing the DNA (Aggarwal et al., 2007).

As stated above, capsaicin can decrease sensation of sensory neurons. One study measured the effects of capsaicin mediation denervation on bone metabolism in adult rats (Ding, Arai, Kondo, & Togari, 2010). It was shown that high doses of capsaicin decreased trabecular bone volume because of increased bone resorption. This increase in bone resorption led to weaker bones in the rats. The study concluded that sensory innervation of bone helps to maintain trabecular bone volume by inhibiting bone resorption (Ding et al., 2010). There was no indication of whether the amount of capsaicin administered to the rats was comparable to levels commonly consumed by humans.

Several *in vivo* studies have shown that capsaicin has an effect on weight. One such study focused on rabbits (Yu et al., 2012). It was shown that the capsaicin specific receptor, TRPV1, was expressed in many organs. The organs were the brain, kidneys, spleen and adrenal gland. The rabbit TRPV1 receptor amino acid sequence was more similar to humans than to rats, which makes the rabbit a great model system for the effects of capsaicin on humans. In the study, two groups of rabbits were fed a high fat diet. One group's high fat diet also included 1% hot pepper. This group had a significantly lower body weight than the high fat control group. This study concluded that capsaicin could potentially decrease diet-induced obesity (Yu et al., 2012).

Another study fed a spice cocktail including ginger, curcumin, piperine and capsaicin to high fat rats to measure the influence on bile secretion and fat digestion (absorption of dietary fat) in the rats (Prakash & Srinivasan, 2011). This study found that fat digestion was enhanced by the spice cocktail because of increased secretion of bile salts and stimulation of other pancreatic enzymes. It was also found that the spice cocktail increased energy expenditure in the rats, which prevented the accumulation of the digested fats

(Prakash & Srinivasan, 2011). This study differs from the first in that it shows a mechanism of action for the capsaicin induced weight loss.

Clinical Studies

Many of the *in vivo* studies about energy expenditure and weight control effects of *Capsicum annuum* focused on capsaicin. A clinical study showed that capsinoids, which are non-pungent capsaicin analogs, activate brown adipose tissue in humans. This activation increases energy expenditure, which could have implications in the use of capsaicin as a weight loss aid (Yoneshiro, Aita, Kawai, Iwanaga, & Saito, 2012). In this same study, participants ingested an oral dose of the capsinoids under warm conditions. The energy expenditure was measured under both warm and cold conditions. The subjects in the negative control and placebo group did not show the same amount of energy expenditure (Yoneshiro et al., 2012).

Capsaicin has demonstrated anti-carcinogenic activity in both *in vitro* and *in vivo* studies. One study completed in 2002 showed that the vanilloid compounds that make up capsaicin induce oxidative stress and contributes to the death of tumor cells from human cutaneous squamous cell carcinoma, which is a form of skin cancer (Surh, 2002).

A crossover placebo controlled study was conducted that included nineteen health male athletes with experience (Opheim & Rankin, 2012). The purpose of the study was to assess the influence of capsaicin on inflammation and exercise performance. The same group was used for two trials, a placebo study and a capsaicin study. After each supplementation period, the subjects completed repeated sprint tests. Additionally, blood was drawn before supplementation, 45 minutes before the repeated sprint test and immediately after the repeated sprint test. Several perceived symptoms were also recorded which included perceive exertion, gastrointestinal distress, and muscle soreness. This data was collected one minute before the test, during the test, and one minute after the test. Muscle soreness was also measured three days after the test. The capsaicin increased the amount of gastrointestinal symptoms 6-fold. It did not influence performance on the sprint test. The study concluded that capsaicin should not be recommended for athletes involved in repeated sprints (Opheim & Rankin, 2012).

Another recent study tested the tolerability of a capsaicin patch on human patients for treatment of neuropathic pain. In this case, three other topical anesthetics were used as a pretreatment in the patients. It was generally safe and well tolerated in the patients (Webster et al., 2011). Two clinical trials also conducted in 2011 compared Qutenza, a new high potency capsaicin patch, to existing capsaicin patches (Jones, Moore, & Peterson, 2011). It was found that the Qutenza patch reduced the numeric pain rating scale (NPRS) score at higher rates than the control capsaicin patch (Jones et al., 2011). However, the Qutenza must be administered under a doctor's care and comes with a risk of application site pain and erythema (Jones et al., 2011).

Contraindications

The contraindications found in *Capsicum annum* other than the risks of application site pain and erythema associated with the Qutenza pain patch (Jones et al., 2011). *C. annuum* is also an emetic (Andrews, 1995). Further research into this area may prove otherwise.

Figure 4. Capsaicin Pain Cream
(Source: http://drugster.info/drug/medicament/4073/)

Current Use in Allopathic and CAM Therapies

Topical capsaicin in complementary and alternative medicine is used in the treatment of various ailments like rheumatoid arthritis (Richards, Whittle, & Buchbinder, 2012) and peripheral neuralgia (Webster, Peppin, Murphy, Tobias, & Vanhove, 2012). It can come in a cream form or as a patch (**Figure 4**) and is also used in allopathic medicine (Jones et al., 2011).

Discussion

Capsicum annuum L. is a very diverse species. There are many varieties under the *C. annuum* umbrella that have many unique properties in terms of the botanical description, capsaicin content and traditional uses. Additionally, there are not a lot of scientific studies that describe the ethnobotanical uses of *C. annuum* as it relates to specific cultures and uses. The peppers included under this umbrella are widely used as a food and flavoring source worldwide, even though they are native to South America. There is evidence that *C. annuum*, like many members of the Solanaceae family as well as other brightly colored fruits contain polyphenols, which have antioxidant properties. The fruits have a rich plant chemistry that contributed a promising drug in capsaicin. Interestingly, this fruit has been used for similar reasons in different cultures across the globe. This is a nod to the knowledge of indigenous peoples that embrace the natural world. Though it is consumed primarily as a food source, there may be other traditional medical uses for it. For example, my mother and many other West Africans use a pepper soup as a normal food as well as a treatment for colds and respiratory ailments. This plant is very influential worldwide and deserves to be the subject of more ethnobotanical research.

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Carica papaya L., Caricaceae

Apollonia Wenting Kang

Introduction

Carica papaya, an arboreal, herbaceous, and monosexual plant from the small Caricaceae family, is of Central America origin (Doughari, Elmahmood, & Manzara, 2007). Of the four species within the *Carica* genus, *Carica papaya* L. is the most commonly cultivated species among others (Krishna, Paridhavi & Patel, 2008). Other names of papaya include papali in India, tepaya used by Kadazan Dusun community in Malaysia, betik in Peninsular Malaysia, pawpaw in Sri Lanka, lechosa in Venezuela, Ibebe in Yoruba-Nigeria, or Okroegbe in Igbo-Nigeria, all speaking volumes of its widespread availability in a diversity of regions worldwide (Anuar, Zahari, Taib & Rahman, 2008). Although it originates from Central America, specifically, Costa Rica, Mexico and Panama, nowadays it grows and is cultivated in almost all tropical and sub-tropical regions around the world.

Major chemical compounds responsible for papaya remedies include vitamins, minerals, polysaccharides, proteolytic enzymes, citric acid, alkaloids, flavonoids, and proteins like chemopapain, papain, and carpain. Different concentrations of those bioactive compounds render different parts of papaya a wide spectrum of medicinal uses. It has been widely cultivated and consumed as a fruit containing a wide array of nutrients, and is also commonly used in daily diets as vegetable. Its medicinal properties have been exploited in therapeutic remedies, both traditionally and currently, mainly because it is thought to contain some antimicrobial, immune-stimulating and anti-oxidant agents that are beneficial for wound healing (Canini, Alesiani, D'Arcangelo, & Tagliatesta,

Figure 1. *Carica papaya* L. trees (source: http://www.henriettesherbal.com/pictures/p03/pages/carica-papaya-2.htm)

2007). In a related vein, immature fruits and roots are famous for their abortifacient properties (Cherian, 2000); consequently, its seeds are now strong candidates for anti-fertility drugs with great efficacy and negligible side effects (Lohiya et al., 2000). The latex and the seeds are also used in the treatment of gastrointestinal nematode infections called *Heligmosomoides polygyrus*, because of its anthelmintic activity (Doughari, Elmahmood, & Manzara, 2007). The leaves relieve gastrointestinal upsets, serving as an expectorant. Papaya leaves also dispel asthma, fever, and amoebic dysentery. Methanolic leaf extracts demonstrated vasodilatory and anti-oxidant effects, lowering cardiovascular risks (Runnie et al., 2004).

Botanical Description

C. papaya is a popular choice of edible fruit because of its palatable, juicy taste, nutritive value and easy digestion (Monti et al., 2004). Its physical appearance resembles palm trees. The height of its tree may range from 3 to10 meters (Krishna et al., 2008). A scar marks every spot where leaves have fallen off on the fleshy stem. On the top of the tree there stands a canopy made of long petioles with leaves and five to seven lobes (**Figure 1**). Male flowers are fragrant, trimorphic, and unisexually dioecious, with tips of pendulous, hollow rachis covered by dense pubescent cymes (Krishna et al., 2008). By contrast, nurturing large berries in various sizes, female flowers are large, solitary, and shaped in few racemes with a short thick rachis (Krishna et al., 2008). It is worth pointing out that the polygamous attribute of papaya seeds pose a challenge to identify whether a plant is male, female or hermaphrodite. The racemes extend to form a globular structure with a large cavity in the middle, where the black seeds of papaya are lumped together and enfolded in a transparent aril (**Figure 2**). Usually only trees younger than 18 months old inoculate. Papain-containing milky juice will leak out if leaves and unripe fruit are cut. *C. papaya* plants are omnipresent in tropical and subtropical regions worldwide, after being distributed from Central America to Malaysia, India, China, Sri Lanka, and Africa. Papaya is commonly grown for commercial purposes, but it is grown in home gardens as well.

Figure 2. Flowers and fruits with seeds of *Carica papaya* L. (Source: http://botanical.com/botanical/mgmh/p/papaw-02.html)

Traditional Uses

Dietary supplements

Given their richness of nutrients and anti-oxidative components, papaya leaves also serve culinary functions as a vegetable. Papaya leaves are also popular as medicinal drinks in manufacture of teas and infusions (Canini et al., 2007). The black seeds are known for a spicy, sharply bitter taste, to the extent that sometimes they replace black pepper in cooking in India (Krishna et al., 2008). In south Asia, the young, green leaves of papaya are steamed and consumed like other vegetables (Krishna et al., 2008).

Chronic diseases

Aborigines from Central America are aware of the anti-oxidant properties effective in preventing several chronic diseases such as cardiovascular disease, cancer, and diabetes (Murakami et al., 1994). Researchers agreed with this traditional use of papaya, because they found out that unripe pulp of *C. papaya* treats congestive heart failure effectively due to its abundant carbohydrate and starch (Oloyede, 2005).

Expectorant and antibacterial agents

The immature fruit and seeds are known to inhibit growth of human enteric pathogens, hence unripe papaya fruits are often remedies for gastrointestinal upset in tropical regions (Osato et al., 1993). Moreover, Africans also recognized the bacteriostatic activity of papaya, so African hospitals use unripe fruit to cure skin trauma and burns (Starley et al., 1999).

Contraceptives and abortifacient agents

Papaya seed extracts have been traditionally used as a promising male contraceptive in various parts of Assam in northern India (Tiwari et al., 1982). The Kadazan Dusun community in east Malaysia uses the decoction of papaya root as an effective tool of birth control by stopping menstruation (Fasihuddin & Ghazally, 2003). Indians use papaya roots assuredly because papaya fruits work effectively without known effect on the reproductive tract morphology (Bitto et al., 2006).

Indian traditional beliefs dictate that unripe fruits of plants such as papaya, banana and pineapple are “hot, fiery” food that promotes rapid blood flow in uterus and thus inducing miscarriages (Tiwari et al., 1982). In India and Assam, the combination of *C. papaya* and resin from *Ferula narthex* are used in abortive procedures (Tiwari et al., 1982).

Skin diseases and infections

In Cambodia, Laos and Vietnam, *C. papaya* latex is used to treat genital wounds, eczema and psoriasis (Amenta et al., 2000). Topical application is the most common traditional use of *C. papaya* (Gurung & Basnet., 2009). Ethnobotanical practitioners mention that fully mature but unripe fruit of papaya contains more latex than that in ripe papaya, thus containing more wound-healing constituents (Krishna et al., 2008).

Respiratory and intestinal complications

Carica papaya L. is used for the treatment of bronchitis in the Atlantic Forest coastal region in Brazil (Figueiredo, Leitao-Filho, & Begossi, 1997). In Ayurvedic medicine, different parts

of papaya are utilized frequently to treat constipation, diarrhea, and gonorrhea (see **Table 1**).

Part	Main Bioactive Compounds	Medicinal Uses
Fruits	Protein (papain etc.), fat, fiber, polysaccharides, minerals (calcium, phosphorous, iron), vitamins A and C, carotene, riboflavin, thiamine, amino acids, citric and malic acids, volatile compounds (linalool, benzylisothiocyanate), alkaloid, carpaine, bezyl-β-D-glucoside	Ripe fruits: digestive, stomachic, carminative, diuretic, dysentery and chronic diarrhea, expectorant, sedative and tonic, obesity-relieving, bleeding piles, urinogenital wounds, ringworm and skin infections, psoriasis Unripe fruits: Laxative, diuretic, dried fruit reduces enlarged spleen and liver, antidote for snakebite, abortifacient, anti-impalantation activity and antibacterial activity
Juice	N-butyric, n-hexanoic and n=octanoic acids, lipids; myristic, palmitic, stearic, linoleic, linolenic and oleic acids	Seed juice treats bleeding piles and enlarged liver and spleen
Seeds	Fatty acid, crude protein, crude fiber, papaya oil, carpaine, carpsemine, benzylisothiocyanate, myrosin, caricin, β-sitosterol	Carminative, emmenagogue, vermifuge, abortifacient, counter irritant, as paste in the treatment of ringworm and psoriasis, male contraceptives
Roots	Carposide, myrosin, saponins, tannins, alkaloids, glycosides, phenols, benzylglucosinolate	Abortifacient, diuretic, antifungal, checking bleeding from the uterus and piles
Leaves	Flavonols (kaempferol and quercetin), caffeic acid, alkaloids carpain, pseudocarpin and dehydrocarpain I and II, carposide, vitamin C and E	Young leaves as vegetable, jaundice, urinary complaints & gonorrhea (infusion), dressing wounds (fresh leaves), antimicrobial activity, vermifuge, in colic, fever, beriberi, abortion (infusion), ashma (smoke)
Flowers	Not known	Jaundice, emmenagogue, febrifuge and pectoral properties
Stem Bark	β-sitosterol, glucose, fructose, sucrose, galatose	Jaundice, anti-haemolytic activity, sore teeth (inner bark), antifungal activity, STD
Latex	Proteolytic enzymes (papain, chemopapain, glycyl endopeptidase and caricain), chinitase, linamarase, chymopapains A, B and C, peptidase A and B and lysozymes	Anthelmintic, relieves dyspepsia, cures diarrhea, pain of burns and topical use, bleeding haemorrhoids, stomathic, whooping cough

Table 1. Chemical constituents in different parts of *Carica papaya* L. and their corresponding therapeutic uses
(adapted from table 2 and 3 in Krishna, Paridhavi and Patel, 2008)

Miscellany

In Kelantan, a province of Malaysia, the latex of unripe fruit of papaya is used as a poison for criminal purposes (Wiart, 2006). However, *C. papaya* juice is traditionally known as an antidote (Wiart, 2006). In India, papain, a prominent compound extracted from fruit and stem latex is used in

brewing and fermentation of wine, and in the textile and tanning industries (Krishna et al., 2008). Generally, fermented papaya acquire antioxidant properties related to both hydroxyl scavenging and iron chelating properties targeting at superoxide and hydroxyl radicals (Imao et al., 1998). Seed juice treats bleeding piles, enlarged liver and spleen (Imao et al., 1998). Stem bark relieves sore teeth (Krishna et al., 2008). For more uses, please refer to Table 1.

Chemistry and Pharmacology

A considerable amount of chemical compounds and biochemical activities in *Carica papaya* were identified, but very little has been published on the percentages of the chemical compositions of different compounds in papaya. As alluded to previously, different structures of papaya contain different bioactive components (T**able 1**).

Leaves

Phytochemical analysis identified secondary metabolites, particularly phenolic compounds, from the leaves of *C. papaya*, using the technique of gas chromatography-mass spectrometry (Canini et al., 2007). The majority of the free phenolics are flavonols, such as kaempferol and quercetin, which have shown anti-oxidant and anti-carcinogenic activities. Caffeic acid, also one type of phenolic acids, is the most abundant of all the identified compounds.

Seeds

Alkaloids like carpain and carpsemine, which render papaya seeds a bitter taste, have been reported as a depressant, calming agent to the heart, thus lower risks of cardiovascular disease (Canini et al., 2007).

Latex

The papaya latex is famous for providing four cysteine endopeptidases, namely papain (see **Figure 3**), chemopapain, glycyl endopeptidase and caricain (Azarkan et al., 2003). Chemopapain in latex after purification has shown immunological properties (Krishna et al., 2008). The latex from unripe papaya fruits contains a mixture of antifungal chinitases, protease inhibitors, linamarase, and proteins without known functions (Azarkan et al., 2004).

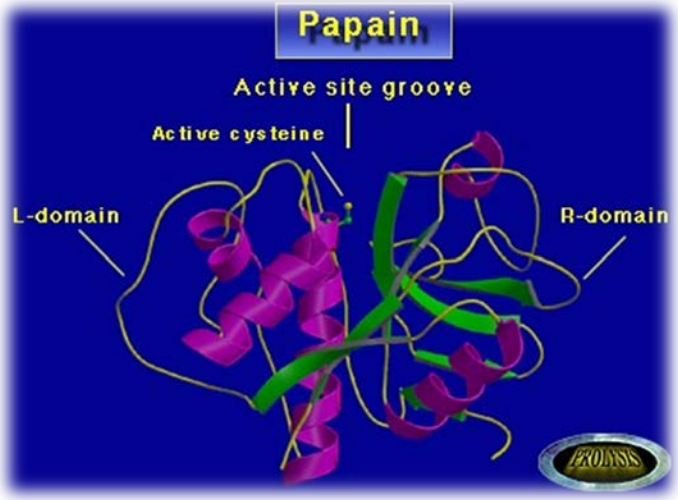

Figure 3. Molecular structure of papain (source: http://linus.chem.ku.edu/hewlett/Chem188/Enzyme/enzyme_background.htm)

Roots

The phytochemical analysis of root extracts of *C. papaya* is indicative of the presence of the following phytoconstituents: saponins, alkaloids, tannins, glycosides, and phenols (Doughari et al., 2007).

Biological Activity

In vitro

Antimicrobial. The agar cup plate method showed that the seed and pulp of both ripe and unripe papaya effectively kill parasitic enteropathogens such as *Bacillus subtilis, Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris and Pseudomonas aeruginosa* (Krishna et al., 2008). Gram-negative bacteria were more susceptible to the extracts, yet both gram-negative and gram-positive bacteria, including *E. coli, S. aureus, B. cereus and S. pyogenase*, were inhibited by *C. papaya* root extracts (Doughari et al., 2007). This result does not only validate the topical use of *C. papaya* extracts in traditional treatment of ailments, but also gives rise to possibilities of developing therapeutic substances against multidrug-resistant organisms.

Anthelmintic. The air-dried papaya seeds, cheap, natural, easily accessible, and generally regarded as safe, significantly eliminate harmful human intestinal parasites. Benzylisothiocyanate, a main component in seeds, plays a key role in determining its anthelmintic properties. Ethanol extracts of papaya possess a promising potential of anti-parasitic action, directly impacting eggs, infective larvae and adult *Haemonchus contortus* (Hounzangbe-Adote, Paolini, Fourastel, Moutairon, & Hoste, 2005). Organic extracts were more effective than aqueous extracts in defending against drug resistance, because the active components dissolve better in organic solvents (Olafsdottir et al., 2002).

Anti-amoebic. The diarrhea-causing intestinal ameobiasis is now a dangerous disease in tropical regions. Fully mature papaya seeds macerated in aqueous solutions exhibited anti-amoebic activity against *Entamoeba histolytica* (Tona, Kumbu, Ngimbi, Cimanga, & Vietinck, 1998).

Anti-malarial. The petroleum ether extract of the rink of raw papaya fruit has shown noteworthy antimalarial property. The rinds of papaya are normally discarded after consumption as waste; if the rinds can be exploited for antimalarial activity, there may be impressive commercial and health benefits (Bhat & Surolia, 2001).

Antifungal. Partnered with Fluconazole, papaya latex demonstrated synergic actions in the suppression of *Candida albicans* growth by degrading fungal cell wall (Giordani, Siepaio, Moulin-Traffort, & Regli, 1991). The fungistatic effect is accomplished by breaking down the polysaccharides components of the outmost wall of defense in fungal cells and forcing cell debris into the culture medium. Another advantage is that only minimal protein concentration is required for papaya latex to be antifungal (Giordani et al., 1991).

Antioxidant. The contribution of L-ascorbic acid (AA), a measure of antioxidant capacity, to the scavenger hunt of free radicals was 48% in solo papaya and 62.3% in foot long papaya, indicating the extraordinary potential in slowing down cell aging and cellular damage due to oxidation (Leong & Shui, 2002).

Immunomodulatory. Fermented papaya preparation exerted immunomodulatory actions on the macrophage cell line called RAW 264, which initiates the damaging actions of macrophage cells, thereby intensifying nitric oxide synthesis and TNF-alpha secretion to benefit the growth and repair of the organism (Rimbach et el., 2000).

In vivo

Antimicrobial. Papaya seeds demonstrably have bacteriostatic actions on both Gram-positive and Gram-negative organisms, which may potentially become a valid treatment of chronic skin ulcers (Krishna et al., 2008). In diabetic rats, the aqueous extract of fruit still exhibited antimicrobial activity and enhanced wound healing process (Nayak, Pereira, Pinto, & Maharaj, 2007).

Abortifacient. Varying doses of papaya latex extract differentially strengthened the uterotonic contractile activity in rats during the proestrus and estrus stages of the estrous cycles, eliciting prolonged uterine contraction and thus facilitating parturient delivery, due to the harm induced by benzylisothiocyanate on the myometrium (Adebiyi, Ganesan, & Prasad, 2003).

Contraceptive. Speaking of male antifertility properties of papaya, the pronounced hypertrophy and hyperplasia of pituitary gonadotrophs have been identified in papaya seed extracts. Genital epithelium cells were degenerated post-treatment, leading to confirmation of the antifertility activity of papaya seeds (Uche-N, Ezeokoli, Adogwa, & Offiah, 2001). Besides, after 3 weeks of treatment, the cavities of the seminiferous tubules were significantly emptier in the experimental mice, indicating ceased spermatogenesis (Uche-N et al., 2001). The benzene chromatographic portion of the chloroform extracts from the seeds elicits revocable male contraceptive possibility without apparent toxicity, by immobilizing sperms, diluting sperm concentration and lowing viability of sperms in male langur monkeys, male adult rabbits and male albino rats (Lohiya, Manivannan, Goyal, & Ansari, 2008). The sharp decline in numbers of sperm is called oligospermia, which can be reached after treatment of 75 consecutive days (Lohiya, Mishra, Pathak, & Maniyannan, 1999). Chloroform extracts of papaya seeds are extremely anti-fertile, because they selectively target new-born germ cells; in other words, prolonged treatment can develop into azoospermia and reversible sterility (Lohiya, Goyal, Jayaprakash, Ansari, & Sharma, 1994). Toxicological assessments of papaya seed extracts have reached consistent conclusions: the drug was free of adverse side effects (Lohiya, Pathak, Mishra, & Maniyannan, 2000). Papaya seed extract also changes the biochemical operations and the contractile pattern of vas deferens, thus even a short term administration of an aqueous extract of papaya seed yielded a shortage of androgen, leading to anti-fertility effects (Chinoy & George, 1983).

In terms of female antifertility, papaya root extract has been suggested as containing bioactive compounds in the induction of morphological changes in the endometrial surface epithelium in albino rat uterus (Sharma & Mahanta, 2000). The disorientation of cells and diminishing microvilli on epithelium account for the anti-fertile properties of the root. Additionally, the organic and aqueous extracts of papaya seeds restrain ovulation in female rabbits (Kapoor, Garg, & Mathur, 1974). Aqueous seed extract exhibited abortifacient actions on female Sprague Dawley rats, whereas interestingly the extracts were shown to have neither anti-zygotic nor anti-implantation activities on female rabbits (Bodhankar, Garg, & Mathur, 1974).

Anthelmintic. Heligmosomoides polygyrus was inhibited by the anthelmintic latex of papaya in experimentally infected mice, proposing a potential anthelmintic agency against stubborn intestinal nematodes in most mammalian hosts (Satrija, Nansen, Murtini & He, 1995). Papaya latex has also shown 100% anthelmintic efficacy against a common infection of *Ascaris suum* found in pigs (Satrija, Nansen, Bjorn, Murtini &

He, 1994). Papaya extracts also possess does-dependent, anthelmintic activity on the egg, larvae and adult worms of Triochostrongylus *colubriformis* (Hounzangbe-Adote, Fouraste, Moutairou & Hoste, 2005).

Diuretic. The root extract of papaya has shown potent efficacy in treating dysuria because it exerts diuresis in treated rats (Spripanidkulchai et al., 2001). Orally taken extracts of papaya root yielded increased frequency of urination in rats (Bungorn, Varima, Pisamai, Jamsai & Dusit, 2001).

Antioxidant: As free-radical scavengers, fermented papaya preparation is able to repair oxidative DNA damage due to hydrogen peroxide in rat cells (Marotta, Yoshida, Barreto, Naito, & Packer, 2007). It may also facilitate the reversal of inflammation in cirrhosis due to oxidation by *hepatitis C.* virus (Marotta et al., 2007). By and large, yeast fermented papaya may have a potentially supportive role in regulating any neurological diseases that involve the damages caused by free radicals (Imao, Wang, Komatsu, & Hiramatsu, 1998).

Hepatoprotective. The ethanol extracts of papaya fruit significantly alleviate induced hepatotoxicity, but the mechanism of action remains unknown (Krishna et al., 2008).

Muscle relaxant and antihypertensive. Ethanol extract of papaya seeds have dose-dependent, irreversible deactivation of jejunal shrinks in rabbits (Adebiyi & Adaikan, 2005). Moreover, pentane extract of papaya seeds were found to have muscle-weakening effects on the carotid arteries of dogs (Wilson, Kwan, Kwan, & Sorger, 2002). Ethanol extract of unripe papaya fruit bears a calming effect on arterial pressure, much stronger than that of hydralazine in rats with hypertension. Hence, fruit juice of papaya potentially involves antihypertensive components (Abeywardena et al., 2000). Papaya leaves also afford potent depressant actions on aortic ring contractions (Runnie, Salleh, Mohamed, Head, & Abeywardena, 2004).

Wound healing. Wound healing entails three phases in sequence: inflammation, proliferation and maturation (Diegelmann & Evans, 2004). *C. papaya* latex aids all three phases. Extracts of green papaya accelerated healing of wounds in mice, due to the antimicrobial and antioxidant properties (which reduce any potential oxidative damage to the tissues) from the proteolytic enzymes, chymopapain and papain in the epicarp (Osato et al., 1993). Mahmood et al (2005) replicated the same results that the extracts speeded up wound-healing process in rats. Papaya latex significantly increased collagen synthesis, as indicated by incremental hydroxyproline content (Gurung & Basnet, 2009). Growth in hydroxyproline content suggests faster collagen synthesis turnover, thus promoting more rapid wound healing. Wound contraction, another variable measuring wound healing process, was more intense and frequent, after the application of papaya latex (Dawkins, Hewitt, Wint, Obiefuna, & Wint, 2003). Higher levels of wound contractions indicate that the tissues have acquired greater reparative properties to shrink the wound more quickly. The epithelialization time, the third parameter quantifying wound healing, was largely reduced than that in untreated groups (Dawkins et al., 2003).

Clinical Studies

Systematic studies evaluating the pharmacological and therapeutic properties of *C. papaya* are limited. Unripe papaya extracts were shown to have antisickling activity that puts off the sickling of hemoglobin on sickle cell patients (Oduola et al., 2006). Oral ingestion of *C. papaya* extracts during different stages of pregnancy exerted various effects on embryonic development, such as anti-implantation activity, increased

post-implantation loss and embryotoxicity (Oderinde et al., 2002). In terms of wound-healing, topical treatment of mush pulp of *C. papaya* containing papain and chemopapain on human skin facilitates desloughing of necrotic tissue, granulation and healing, and reduces odor in chronic skin ulcers (Hewitt et al., 2000). The mechanism of action may include proteolytic enzymes such as chemopapain and papain exerting antimicrobial activities (Starley, Mohammed, Schneider, & Bickler, 1999). Most importantly, *C. papaya* demonstrated purported potential in improving immunity. In fact, *C. papaya* seed extract enhanced the phytohemagglutinin responsiveness of lymphocytes *in vitro*, indicating the immunostimulatory actions (Mojica-Henshaw et al., 2003). Nevertheless, researchers have yet to replicate this finding on humans.

Contraindications

Bioactive compounds such as papain and chymopapain lead to intense uterine contractions, accounting for the abortifacient properties of papaya. However, papaya latex only causes such voluntary uterine contractions if other enzymes or alkaloids have induced increased estrogen levels (Cherian, 2000). Crude papaya latex may play a similar role as oxytocin in causing spasmodic contraction of the uterine muscles via the suppression of progesterone. Progesterone is crucial because it affirms that the fertilized ovum will not be dislodged and that no more ova will be released. However, Schmidt (1995) suggested that pure papain itself up to a dose of 800 mg/kg (in rats) neither intoxicated the maternal organism nor jeopardized the embryonic development. Due to the different chemical contents between the ripe and the unripe, reasonable intake of ripe papaya during pregnancy should not impose any hazardous consequence, whereas the unripe papaya could be dangerous in pregnancy (Adebiyi et al., 2002).

Of note, papaya should be used with caution in the treatment of urinogenital disorder like trichomoniasis to avoid overdose toxicity. Higher concentration of papaya seed extracts is cytotoxic, given the increased membrane permeability to calcium ions (2+; Wilson, Kwan, Kwan, & Sorger, 2002).

Current Use in Allopathic and CAM Therapies

Burns and trauma wounds are common in both developing and developed countries. But because of the heavy financial burden on citizens of developing countries, burn wounds impose a bigger challenge to local healthcare systems (Gurung & Basnet, 2009). The promising wound shrinking capacity of papaya offers people in developing countries an economical approach to relieve financial burdens.

Papaya is praised for its nutritional value as it is low in calories and rich in natural vitamins and minerals (Krishna et al., 2008). Papaya is ranked in the top tiers among all fruits for vitamin C, vitamin A, riboflavin, folate, calcium, thiamine, iron, niacin, potassium and fiber (Krishna et al., 2008). Papaya notably increases iron absorption in rice meals. Ripe papaya fruit is highly recommended for obese people on diet due to its relatively low concentration of calories (32 kcal/100g; Krishna et al., 2008). Papaya has high carotene content in comparison with other common fruits such as organs, apples, guavas and plantains, lending hands to its excellence at stopping free radicals from oxidizing body tissues. Unripe green papaya differs in nutritive contents from ripe papaya the most in terms of the lack of carotene. As a popular choice of vegetable in salads, juices, pies and confections, green

unripe papaya ensures sufficient supply of vitamins to maintain good eyesight. Papaya is rich in a diversity of enzymes with papain being the major one. Papain, vegetable pepsin, works well under different pH environments (acidic, alkaline, or neutral). Papain comforts the digestive system and slows down bowel movement. Adding raw papaya to recipes with meat prevents people from dyspepsia. It also stretches people's tolerance of certain proteins which would not be digested without treatment of crude papain (Krishna et al., 2008). Papaya, if fermented, becomes a more powerful antioxidant. Another enzyme in papaya, the papaya lipase, acts like a biocatalyst that hydrolyzes the water insoluble portion of crude papain to build up a protective trench against aging oxidants in elderly patients.

Bionormalizer, an effective antioxidant health food from Japan that is mostly consisted of fermented papaya, enhances the haemorrheology in alcoholics either by direct activation of the lipoperoxidation and xanthine oxidase system, or by alternating red blood cell membrane characteristics. It also displays therapeutic actions against several pathological disorders, such as tumors and immunodeficiency, achieved by free radical scavenging as well as stabilization of an organism's superoxide level. Bionormalizer normalizes superoxide level via the inhibition of the ferrous ions, which wither organisms. Currently, papaya seed extracts are propelled as a nutritional supplement pertaining to revitalize the body (Mojica-Henshaw, Francisco, De Guzman, & Tingo, 2003).

Moreover, papaya fruit extracts are contained in the topical ulcer ointment used by nurses in the Spanish Town Hospital, Kinston Public Hospital, as well as the University Hospital of the West Indies in Jamaica, for its desloughing, granulating and healing properties (Hewitt, Whittle, Lopez, Bailey, & Weaver, 2002). It is especially effective and cost-efficient for chronic skin ulcers patients who have developed drug resistance against most lab-synthesized drugs because its chemically diverse compounds make bacteria and parasites more susceptible to antibiotics.

Discussion

Although *Carica papaya* L. is commonly recognized for its edible and nutraceutical values throughout the world, its medicinal and therapeutic properties should not be taken lightly. Traditionally, people have been exploiting various ethnopharmacological functions of the different structures of papaya (see **Table 1**). Primarily, papaya extracts are responsible for desloughing necrotic tissue, prevention of infection, and the antimicrobial and antioxidant properties associated with hydroxyl scavenging and iron chelating. The reliance on ethnobotanical remedies such as papaya reduces the financial burden of people in developing countries where insurance and healthcare systems are not sophisticated. Besides, papaya is also frequently used in relieving gastrointestinal upset, and even as "birth control pills" in traditional medicine.

In the modern era, attention has been drawn to its capacity in weakening drug resistance. The search for alternative sources of antibiotics encompasses a wide array of challenges, among which drug resistance remains the most burdensome. Infectious diseases cost us 50,000 deaths every day worldwide (Ahmad & Beg, 2011). The development of multidrug resistance further worsened the situation. Researchers thus turn to plants in the quest of new resources of antibiotic drugs with greater efficacy. Plants contain more complex bioactive constituents that are unlikely to be synthesized in lab. In addition, chemical compounds in plants have been attached to valuable functions throughout the long

course of evolution. People in Asia, Latin America and Africa use natural plants as primary health remedies. Papaya seems to be a strong candidate for an alternative source of antibiotic to fight off multidrug resistance.

Taken together, *C. papaya* contributes a significant amount to maintain overall human health, by enriching daily diets, curing infections, combatting against drug resistance, garnering free radicals, and rejuvenating our bodies. Researchers have replicated findings consistently on a diversity of animals *in vivo*, all suggesting the effectiveness of *C. papaya* chemicals. Going forward, more human clinical trials should be implemented to investigate if the same effects hold for humans. I suggest future clinical studies on human subjects focus on the following aspects: antibacterial activity against skin diseases, birth control efficacy on humans, multidrug resistance on various bacteria, antihypertensive properties in preventing cardiovascular diseases, and anti-carcinogenic activities treating cancer.

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Castanea sativa Mill., Fagaceae

Sam Anderson

Introduction

Castanea sativa Mill., also known as sweet chestnut, belongs to the Fagaceae family. Additionally known by the common names of marron, European chestnut, Spanish chestnut, and Portuguese chestnut, *C. sativa* fruits are a popular food item on nearly every continent (De Vasconcelos, Bennett, Rosa, & Ferreira-Cardoso, 2010). A mid-sized tree that originated in the Mediterranean areas of Europe over ninety million years ago, *C. sativa* has spread throughout the rest of the continent and into parts of western Asia and northern Africa (Lim, 2012). Archeological evidence indicates that the *sativa* species surfaced in Asia Minor where it was first domesticated. The advent of the Roman Empire shortly thereafter greatly expanded the species' range into areas of western and northern Europe where it became a prominent food source for rural populations (De Vasconcelos et al., 2010; Ketenoglu, Tug, & Kurt, 2010). Thus, European populations have relied on sweet chestnut for their nutritional value for nearly 3,000 years, and its popularity has migrated across oceans and over mountains to both Asia and America where high consumption occurs. Although edible, chestnuts are infrequently eaten raw, and most often the nuts are cooked, boiled or baked in order to enhance the taste, modify the texture, and increase nutritional value (De Vasconcelos et al., 2010).

Despite this widespread use as a food source, sweet chestnut also harbors several medicinal properties that greatly benefit human health. As early as the first century, *C. sativa* was used for its astringent and antitoxic capabilities evidenced in the works of an early pharmacologist, Pedanius Dioscorides (De Vasconcelos et al., 2010). Since antiquity, all parts of the sweet

Figure 1. ***C. sativa*** **Catkins Hanging from Elliptical Leaves.** The jagged edges of elliptical leaves hanging down form a tomentose arrangement of leaves. Yellow catkins sit on top of the leaves and extend upwards ("Photo Gallery," 2013).

chestnut plant—the bark, wood, leaves, fruits—have been used in the Mediterranean and other areas of Europe to treat a wide range of illnesses, including respiratory problems, skin and soft tissue infections, inflammation, vascular problems, diarrhea, wounds, and rheumatism among others (Budriesi et al., 2010; Chiarini et al., 2013; Jarić et al., 2007; Quave, Plano, & Bennett, 2011; Zlatanov, Antova, Angelova-Romova, & Teneva, 2013). Recent studies have not only confirmed and justified many of these traditional applications of *C. sativa,* but they have also found new medicinal uses and potential applications of various parts of the plant. Due to high concentrations of phenolic compounds in nearly every part of the plant, *C. sativa* leaves, bark, fruit, spines and wood have demonstrated significant antioxidant activity and thus can be used to prevent photo-aging, cancer, diabetes, neurodegenerative diseases and other oxidative stress-associated diseases (Grdovic et al., 2012). In addition to widespread antioxidant activity, individual components of *C. sativa* have demonstrated other significant disease-reducing capabilities; various extracts have shown cardioprotective, anti-quorum sensing, antispasmodic, anticancer, anthelmintic, and antibacterial activities (Bahuaud et al., 2006; Basile et al., 2000; Budriesi et al., 2010; Chiarini et al., 2013; Frederich et al., 2009; Jedinak, Valachova, Maliar, & Sturdik, 2010; Quave et al., 2011). Coupling its rich medicinal properties with its excellent taste, *C. sativa* is a superb medicinal food and promising candidate for pharmaceutical development (De Vasconcelos et al., 2010).

Botanical Description

Castanea sativa is a mid-sized tree that reaches a maximum height of 40 meters and a diameter of 2 meters. As a deciduous tree, sweet chestnut has narrow, oval-shaped

Figure 2. **Spiny Burrs Surrounding Chestnut Fruit.** The red-brown chestnut fruit is protected by spiny burrs of female cupules or flowers lacking pedals ("Photo Gallery," 2013).

leaves with jagged edges seen in **Figure 1** that it loses and regrows seasonally. These pastel-green leaves generally reach lengths between fourteen and twenty-eight centimeters and widths of anywhere between five and nine centimeters (Ketenoglu et al., 2010; Lim, 2012). The crown of *C. sativa* expands significantly past the diameter of the trunk, and the branches lay compressed over one another in a tomentose fashion. *C. sativa* catkins are either entirely male or androgynous with a few female flowers present as well. These

catkins hang down loosely and are pale blond (Lim, 2012). Both male and female flowers lack petals, and the female flowers take the form of light green, spiny cupules shown in **Figure 2**. The fruit of sweet chestnut boasts a red-brown color and possess a diameter between 1.3 and 2.5 centimeters. (Lim, 2012).

Native to the European regions surrounding the Mediterranean, sweet chestnut is now found throughout Europe, northern Africa and the Middle East (Lim, 2012). Due to its preference of locations that receive between 750 and 1200 mm of precipitation, *C. sativa* thrives in Mediterranean climates (Ketenoglu et al., 2010; Lim, 2012). With an extensive root system, sweet chestnut favors faintly acidic and loamy soils that are well drained and allow its roots to penetrate deep into the earth. Although it prefers sunlight, the tree survives in shaded areas as well and has been found in regions with altitudes ranging from sea level to 1500 m (Alía., 2003.; Lim, 2012). Pollinated by both insects and wind, the lightweight chestnut pollen can be carried up to 100 km from its parent tree. Due to its wide distribution and expanding crown, *C. sativa* is a notable tree (Alía., 2003.; Lim, 2012).

Traditional Uses

Ethnomedical Uses

Sweet chestnut has a wide range of traditional uses in areas of the Mediterranean and neighboring regions. During the Roman Empire, Pedanius Dioscorides, a Greek physician and author of a major pharmaceutical guide, recorded the use of sweet chestnut for its astringent and antitoxic properties. Throughout the Middle Ages, the uncooked chestnut seeds were used to treat cardiovascular problems in several areas of Europe (Lim, 2012). More recent ethnopharmacological studies have found a large range of traditional uses, some of which are unique to certain cultures and others that are shared across different societies. Throughout the Mediterranean, the leaves of *C. sativa* are ground and dissolved in boiling water to create a decoction to treat asthma, coughs, colds and other respiratory diseases (Chiarini et al., 2013). In southern and eastern regions of Europe, the fruit is used for pain relief, inflammation, vascular disorders and burns (Zlatanov et al., 2013). Additionally, in southern Italy, the leaves are applied to the skin surrounding varicose veins to diminish swelling (Quave et al., 2011). In northern areas of Europe such as France, sweet chestnut leaves are dissolved into a tea that alleviates diarrheal symptoms (Budriesi et al., 2010). In regions of Turkey, the honey is used as an antibacterial to cover wounds, burns and ulcers of the skin (De Vasconcelos et al., 2010). The use of sweet chestnut in traditional Slovak medicine in the Carpathian mountains is recorded but no specific ailment or method of use is found (Jedinak et al., 2010). The most detailed record of use of *C. sativa* is from an ethnobotanical study on the usage of wild medicinal plants in central Serbia. In the area surrounding the Kopaonik Mountain, the fruit of sweet chestnut is chopped and dissolved in plum-brandy for six weeks and then internally taken as an astringent that lessens coughing and relieves varicose vein pain (Jarić et al., 2007). Thus, *Castanea sativa* has a wealth of traditional medicinal uses in various parts of Europe and the Mediterranean.

Nonmedical uses

In addition to the numerous medicinal applications of sweet chestnut, the plant is also used for many other reasons. The dependency of Europe's rural, mountainous populations on *C. sativa* as a source of nutrition cannot be overstated; the highly

nutritious content of sweet chestnut fruits was so fundamental to their survival that historians deem these societies as chestnut civilizations (Conedera, Krebs, Tinner, Pradella, & Torriani, 2004). The popularity of sweet chestnut fruit persists to this day, and chestnuts are ingredients in many food items. Often processed via boiling, roasting, or frying to improve taste, the nuts are often added to desert items such as chocolate and cakes. As well, chestnuts are used to create flour, Corsican porridge and beer (Lim, 2012). However, chestnut fruits are not the only product used non-medicinally. During Roman times, sweet chestnut wood was used as timber due to its numerous applications for use, and the use of chestnut for construction purposes continues to this day (Conedera et al., 2004; Lim, 2012). In addition to the use of the wood for construction, it is also used to tan hides and skins due to the high tannin concentration. Other products obtained from *C. sativa* include a dye extracted from the leaves, and a shampoo from the fruit husk and leaves (Lim, 2012). *C. sativa* has numerous medical and nonmedical traditional uses, some of which persist to this day.

Chemistry and Pharmacology

Sweet chestnut boasts a wide variety of biologically active chemicals that vary in their concentration depending on the part of the plant. Chestnut fruits have been extensively analyzed for nutritious and thus chemical content due to their popular consumption. Analysis of chestnut fruit composition revealed that water claimed 53% w/w, followed by carbohydrates with 42%, crude protein with 2.7%, and crude fat with 0.7% (Barreira et al., 2012). The lipid composition of the fruits was thus found to be low, but it contained high amounts of polyunsaturated fatty acids at 42% w/w of the lipid fraction, 38.7% monounsaturated fatty acids and 19.4% saturated fatty acid. Polyunsaturated and monounsaturated fatty acids demonstrate anticancer properties, and decrease risks for cardiovascular disease. Specifically, polyunsaturated fatty acids have demonstrated hypolipidemic activity by decreasing blood lipid concentration, antithrombotic activity by mitigating the thickening of arteries, and anti-arrhythmic activity by eliminating abnormal heart rhythms (Das, Bhaumik, Raychaudhuri, & Chakraborty, 2012). High amounts of these beneficial fatty acids and low amounts of saturated fatty acids promote general human health by reducing cardiovascular disease.

In addition to the high amounts of beneficial fatty acids, sweet chestnut yields high amounts of antioxidant vitamins, vitamins E and C (De Vasconcelos et al., 2010). In the sweet chestnut fruit, vitamin E or ϒ-tocopherol was found at an average concentration of 1.9mg/100g fruit. Vitamin E is a lipid-soluble antioxidant that protects lipid components of the cell—membranes, fatty acids—from peroxidation by free radicals (De Vasconcelos et al., 2010). Vitamin E's antioxidant activity is generated by its donation of a hydrogen atom to a singlet oxygen molecule, therefore preventing the oxidation of lipids by the singlet and damage to the cell (Das et al., 2012). Vitamin C or ascorbic acid is found at a concentration of 15.6mg/100g chestnut fruit (De Vasconcelos et al., 2010). Vitamin C acts as an antioxidant in the mitochondria of colon cells by donating a hydrogen atom to lipid radicals and singlet oxygen radicals; thus it responds to and prevents oxidative-stress induced damage (Das et al., 2012; De Vasconcelos et al., 2010). Additionally, vitamins E and C act synergistically to eliminate reactive oxidative species. The elimination of reactive oxygen species and the mitigation of oxidative damage stalls the development of many diseases and conditions such as cancer, stroke and heart attack (Das et al., 2012). The high levels of antioxidant vitamins E and C

Compound	mg/g
Ellagic acid	84.1
Valoneic acid dilactone, methyl ester	73.7
Methyl gallate	30.8
Gallic acid derivative	22.5
p-Methoxycinnamic acid	20.5
Ellagitannin	16.3
Flavogallonic acid	9.3
Kaempferol	7.4
Dehydrodigallic acid, dimethyl ester	6.3
Isorhamnetin	4.0
Flavogallonic acid	3.5
Protocatechuic acid	2.7
Tryptophan	0.0695
p-Hydroxybenzoic acid	0.0338

Table 1. Compounds from the Antioxidant and Phenolic-Rich *C. sativa* Spiny Burr Extract. High amounts of phenolic compounds were found in this extract that displayed high antioxidant activity due to the elimination of reactive oxygen species. (Grdovic et al., 2012)

promote health by preventing and mitigating damage caused by reactive oxygen species.

Despite the beneficial effects of vitamins and fatty acids, phenolic compounds are responsible for the majority of sweet chestnut's medicinal properties. Phenolic compounds, composed of a benzene ring with a hydroxyl group(s) attached, display antioxidant, anti-microbial, anti-inflammatory and cardioprotective capabilities (Das et al., 2012). The major phenolic compounds found within *C. sativa* extracts are ellagic acid and its derivatives at 100.4mg/g dry extract, gallic acid and its derivatives at 59.6 mg/g, and flavonoids at 24.1mg/100g; these compounds are listed in **Table 1**. Studies have found a correlation between total plant phenolic content and antioxidant activity (Grdovic et al., 2012). Phenolic compounds prevent oxidative damage to cells by many methods; the phenolic compounds in C. sativa demonstrate antioxidant activity due to their scavenging of peroxyl, hydroxyl, and superoxide radicals (Grdovic et al., 2012). These phenolic compounds in *C. sativa* are responsible for much of the plant's antioxidant activity.

Tannins, another class of phenolics, are found in relatively high amounts in *Castanea sativa*. In sweet chestnut, there are high amounts of ellagitannins, a type of hydrolysable tannin. Ellagitannins are responsible for anti-inflammatory and antioxidant activity (Chiarini et al., 2013). Another subset of tannins present in *C. sativa* are condensed tannins or proanthocyanidines. Proanthocyanadines are responsible for reducing cardiovascular disease due to relaxation of endothelium cells (Chiarini et al., 2013). Due to these high concentrations of both hydrolysable tannins and condensed tannins, sweet chestnut exhibits numerous medicinal properties.

Biological Activity

Antioxidant Activity

Castanea sativa extracts have demonstrated incredible antioxidant activities. Oxidative damage leads to many severe, debilitating conditions such as cancer, neurodegenerative diseases, diabetes, heart disease, and several more. Reactive oxygen species wreak havoc on cells by oxidizing cell components; antioxidants can prevent oxidative damage by eliminating reactive oxygen species or by improving the cell's response to reactive oxygen species by increasing enzyme activity (Grdovic et al., 2012). The antioxidant properties of *C. sativa* have been examined in several studies, both *in vitro* and

in vivo, and in several different types of cells. One study examined the formation of reactive oxygen species and cell survivorship in cultured neonatal rat cardiomyocites treated with a methanol extract of sweet chestnut bark. Following exposure to the *C. sativa* extract for 24 hours, the cells were exposed to hydrogen peroxide for half an hour, and the levels of cellular reactive oxygen species were determined. Cardiomyocites treated with 100µg/mL of extract produced only twenty percent of the control group's level of reactive oxidative species. In other words, treatment with this concentration reduced reactive oxygen species formation by 80% (Chiarini et al., 2013). Cell viability following exposure to hydrogen peroxide likewise increased with increasing extract concentration. Cardiomyocites treated with 100µg/mL of the extract survived roughly thirty percent more than control cells (Chiarini et al., 2013). Thus, the methanol extract of sweet chestnut bark decreased the formation of reactive oxygen species and improved cell viability following exposure to hydrogen peroxide. Thus, *C. sativa* exhibits significant antioxidant activity in neonatal rat cardiomyocites.

Considering oxidative stress' role in diabetes, another study examined the influence of C. sativa spiny bur extracts on pancreatic cell death following oxidative stress. Pancreatic β-cell death due to oxidative stress induces diabetes in humans (Grdovic et al., 2012). To mimic the immune system's destruction of pancreatic β-cells that leads to reactive species formation, rat pancreatic β-cells were treated with streptozotocin (STZ). Following cell exposure to STZ, the pancreatic cells were treated with differing concentrations of chestnut extract, and afterwards, levels of free radical scavenging, total reducing power, hydrogen peroxide-scavenging activity, nitric oxide radical scavenging, cell viability, DNA damage, lipid peroxidation, and antioxidant enzyme activity were examined. These measurements reveal the extent and mechanism of antioxidant activity in *C. sativa* (Grdovic et al., 2012).

Free radical scavenging, reducing power, hydrogen peroxide scavenging, and nitric oxide radical scavenging determine whether an antioxidant eliminates reactive oxygen species. Free radicals are reactive oxygen species that cause oxidative damage; free radical scavenging is an antioxidant property that reduces cell damage (Grdovic et al., 2012). Scavenging was measured by the reduction in absorbance of 2,2-diphenyl-1-picrylahydrazyl (DPPH). The *C. sativa* extract dramatically decreased DPPH absorbance and thus increased levels of free radical scavenging. Total reducing power—a measure of antioxidant activity—was measured by absorbance as well. The reducing power of the extract was likewise very high; it matched the reducing power of the control, vitamin C or ascorbic acid at a concentration of 0.5 mg/mL. Hydrogen peroxide-scavenging activity, another indicator of antioxidant activity, was found by measuring the absorbance of hydrogen peroxide solution. Again, the sweet chestnut extract exhibited total hydrogen peroxide scavenging ability at 0.75 mg/mL, a concentration lower than that of the control, ascorbic acid. *C. sativa* additionally showed impressive scavenging ability of nitric oxide, a reactive species; the extract completely scavenged nitric oxide at 1 mg/mL, which again was lower than the concentration of the control molecule curcumin. As well, lipid peroxidation, a result of oxidative damage, was 2.4 times greater in cells not treated with the sweet chestnut extract (Grdovic et al., 2012). Thus, *C. sativa*'s antioxidant activity is due to its elimination of reactive oxygen species.

Examining cell survival, DNA damage, and antioxidant enzyme activities demonstrate how the removal of these reactive oxygen species benefits the cell. When treated with the extract, β-cell survivorship increased by 19% and DNA damage was significantly reduced as well (Grdovic et al.,

2012). In order to determine whether these extracts influenced the cells' antioxidant enzymes' responses to STZ, the activity of superoxide dismutases and catalase were examined. β-cells treated with the extract showed a 28% decrease in catalase activity, and two out of three superoxide dismutases' activity levels returned to activity levels of control cells not treated with STZ. The decrease in these enzymes' activity levels signifies that the extract does not stimulate the cell's response to oxidative stress; it reveals that sweet chestnut antioxidants prevent oxidative damage and reduce the cell's burden to quell oxidative stress (Grdovic et al., 2012). Thus, the sweet chestnut spine extract demonstrated antioxidant activities through the elimination of several reactive oxygen species that lessened damage to DNA, lowered cell morality, and decreased the activity of the cell's antioxidant enzymes. Therefore, *C. sativa* extracts possess high antioxidant capabilities in vitro due to elimination of reactive oxygen species, and this elimination improves cell outcomes. Additionally, the high phenolic chemical composition of this fraction was analyzed and displayed in **Table 1**.

In addition to *in vitro* studies of cell outcomes, *in vivo* studies of *C. sativa* extracts' antioxidant activity in animals have revealed that sweet chestnut exhibits antioxidant activity in animals. In a study determining the antioxidant activity of *C. sativa* in living organisms, pigs were fed a diet of *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) to increase their vulnerability to lipid peroxidation and then orally given a *C. sativa* wood extract with high amounts (73%) of hydrolysable tannins. Antioxidant activity was then measured by determining levels of lipid peroxidation, DNA damage, and oxidative stress (Frankic & Salobir, 2011). Lipid peroxidation levels were measured by two peroxidation products, malondialdehyde and F_2-isoprostanes; only levels of malondialdehyde decreased by roughly fifty percent compared to the pigs fed the *n*-3 PUFA diet and no extract. As well, the measure of total antioxidant status did not differ among any group regardless of *n*-3 PUFA or *C. sativa* extract treatment. However, levels of DNA damage were reduced to levels of control diet pigs. As well, levels of oxidative stress were reduced to those of the control (Frankic & Salobir, 2011). Thus, the *C. sativa* extract showed significant *in vivo* antioxidant activity in several tests that measure different oxidative stress outcomes. Additionally, liver toxicity wrought by the sweet chestnut extract was determined by measuring the activity of liver enzymes aminotransferase, alanine aminotransferase and glutamyl-transferase. The three enzymes' activity levels in the experimental group treated with the extract mirrored those of the control, signifying that the extract is not hepatotoxic (Frankic & Salobir, 2011). This in vivo study shows that *C. sativa* extracts demonstrate safe *in vivo* antioxidant activity in animals and warrants further study for potential use in humans.

Cardioprotective Activity

C. sativa not only is an excellent antioxidant, but it also exhibits cardioprotective activity. To examine the cardioprotective activities of *C. sativa*, one study examined the response of guinea pig right and left atria along with the left papillary muscle to agonists and antagonists when treated with the sweet chestnut bark extract high in hydrolysable tannins (Chiarini et al., 2013). As well, the response of guinea pig aortic strips to potassium ions and noradrenaline was examined. Atria treated with *C. sativa* showed positive inotropic effects and negative chronotropic effects; thus, it slowed the heart rate and increased the potency of the heart muscle's contractions. Likewise, *C. sativa* lessened the

contraction of aortic strips (Chiarini et al., 2013). Thus, *C. sativa* exhibits cardioprotective activity by regulating heart rate and contraction strength.

Quorum Sensing Inhibition

In addition to its antioxidant and cardioprotective activities, an ethanol *C. sativa* leaf extract inhibits quorum sensing in *Staphylococcus aureus* bacteria. Quorum sensing allows bacterial cells to coordinate the release of toxins once a certain number of bacteria are present in a host. Preventing quorum sensing reduces the ability of bacteria to release these toxins and harm host cells (Quave et al., 2011). The ethanol leaf extract was applied to *S. aureus* bacteria, and quorum sensing was measured by quantifying the production of δ-toxin. Following treatment with 125 μg/mL *of* C. sativa extract, δ-toxin production decreased by 90% (Quave et al., 2011). Thus, the sweet chestnut leaf extract shows promising quorum sensing inhibition that could hinder bacteria's capability to infect a host.

Antispasmodic Activity

Not only does *C. sativa* weaken bacteria, but it also induces muscle relaxation. In order to characterize the *C. sativa* wood extract's antispasmodic effects, guinea pig ileum and proximal colon tissues were exposed to spasmodic agents such as carbachol, histamine, potassium chloride, and barium chloride and then were treated with the sweet chestnut extract (Budriesi et al., 2010). Determining the extent of antispasmodic activity was performed by quantifying the reduction in the spasmodic response. Treatment with the extract significantly reduced the spasmodic effects of the agents in the tissues. Thus, *C. sativa* exhibits antispasmodic effects on intestinal tissues, signifying possible therapeutic use for the treatment of diarrhea (Budriesi et al., 2010).

Anticancer Activity

C. sativa also displays strong anticancer activity against at least three human cancer lines. In one study, the ethyl acetate bark extract of sweet chestnut was applied to LoVo colon cancer cells, PC3 prostate cancer cells, and U373 glioblastoma cancer cells. The C. sativa extract eliminated 50% of the cancerous cells at 25 μg/mL for the colon cancer, 31 μg/mL for the prostate cancer cells, and 24 μg/mL for the glioblastoma cells (Frederich et al., 2009). Earlier studies showed that the cytotoxic activity was due to polysaccharides, but analysis of this extract showed little polysaccharides present (Frederich et al., 2009). Thus, *Castanea sativa* is an excellent *in vitro* anticancer agent for multiple cancer types and deserves further study in animal models to determine its mechanism of action.

Antibacterial Activity

C. sativa shows potent antibacterial activity. An ethyl acetate fraction demonstrated effective antibacterial activity against both gram-positive and gram-negative bacteria. Further analysis of the active fraction showed that the chemicals rutin, hesperidin, quercetin, apigenin, morin naringin, galangin and kaempferol were present in high amounts. These chemicals were then individually applied to bacteria, and quercetin, rutin and apigenin demonstrated the highest antibacterial activity. Some reactivity was lost upon dividing the fraction, suggesting a synergistic mechanism. Thus, *C. sativa* exhibits high antibacterial activity against both types of bacteria, and

the individual compounds within the fraction mirrored that same reactivity (Basile et al., 2000).

Anthelmintic Activity

In addition to its antibacterial activity, *C*. *sativa* also targets other small organisms such as parasitic nematodes. Earlier studies have demonstrated that plants high in tannins exert anti-parasitic effects against nematodes, and *C. sativa* contains significant concentrations of tannins, some of which are listed in **Table 1**. One study examined the effect of a *C. sativa* fruit extract on the exsheathment or transition of the nematode from its final larval stage to full parasite. Exposing nematode larvae to the extract for fifty minutes inhibited 100% of the worms' exsheathment. Thus, *C. sativa* exhibits extraordinary anthelmintic and anti-parasitic activity.

Clinical Studies

Antioxidant Activity

To determine whether a *C. sativa* extract could be used as a topical antioxidant to prevent photo-aging and other oxidative stress diseases, an extract of *C. sativa* leaves was applied topically to the skin of twenty volunteers to determine whether the use of the extract would induce any unpleasant effects such as allergic reactions or dermatitis and to determine whether efficacy was maintained *in vivo*. The extract used contained 238 mg of phenolic compounds/g extract, and contained cholorogenic acid, ellagic acid, rutin, isoquercitrin and hyperoside in high amounts. The extract was determined to be safe for use due to little or no dermatitis or allergic reactions. As well, analysis of the skin cells showed that DPPH scavenging activity surpassed 80% at 50 µg/mL, and iron chelating activity—another antioxidant activity—reached over 90% at 500 µg/mL (Almeida et al., 2008). Thus, the study demonstrated that the leaf extract of *C*. *sativa* is safe and its efficacy is maintained in vivo.

Contraindications

Castanea sativa does not present many contraindications; however oral allergy syndrome can be induced by plant-oral mucosa contact. The allergy generally occurs in individuals with allergies to certain pollens (Antico). Additionally, very high levels of phenolic consumption and ingestion are unhealthy, and the precise effects of phenolic compounds on health are not entirely understood (Das et al., 2012). Moreover, cytotoxic levels of extract use have been recorded for concentrations above 100 µg/mL whereas some extracts only exhibited effective activity at concentrations above 100 µg/mL. Thus, there is potential for a narrow margin between the therapeutic and toxic doses of extracts (Almeida et al., 2008; Chiarini et al., 2013).

Current Use in Allopathic and CAM Therapies

Castanea sativa is used throughout Mediterranean regions to treat a wide range of illnesses in accordance with traditional medicine practices. As mentioned earlier, these illnesses and conditions include varicose veins, vascular problems, diarrhea and multiple others (Budriesi et al., 2010; Quave et al., 2011; Zlatanov et al., 2013). No articles on contemporary medicinal uses other than the continuation of traditional practices were found. However, chestnut food products can be used as a substitute for gluten-containing foods for individuals who suffer from coeliac disease and cannot digest gluten. Chestnut does not contain gluten; thus, it can be used to make flower that substitutes for the many food items that contain gluten.

Additionally, chestnut is a healthy, nutritious food, and is a suitable substitute for grains. Thus, it is an appropriate substitute for those affected by the disease (De Vasconcelos et al., 2010).

Discussion

C. sativa has numerous medicinal uses, some of which have been in use for thousands of years whereas others have only recently been discovered. Capable of preventing oxidative stress, inhibiting bacterial quorum sensing, eliminating bacteria and parasites, protecting the heart, stopping cancer and relaxing muscles, sweet chestnut shows tremendous potential as both a medicinal food and as a source of novel medicines. Due to its high concentration of phenolic compounds, including hydrolysable tannins and condensed tannins, *C. sativa* displays extraordinary antioxidant capabilities that establish its importance as a medicinal food that promotes general health by preventing causes of disease. Numerous other properties, such as its anticancer, cardioprotective and antibacterial activities, warrant further study and show promise in the development of pharmaceuticals using chestnut compounds to improve human health and treat illnesses. Considering the wide breadth of traditional uses that have not been validated, there may be other medicinal applications of *C. sativa* found in future studies. Sweet chestnut's excellent taste and health benefits deserve a place in the diet of all seeking to maintain and improve their health, and its potent ability to treat several diseases could dramatically improve our well being and recovery from disease.

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Catharanthus roseus (L.) G.Don, Apocynaceae

MyKeya Henderson

Introduction

Catharanthus roseus, whose common name is Madagascar periwinkle or just periwinkle, belongs to the Apocynaceae. Although *C. roseus* is the accepted scientific name, this plant was once referred to as *Vinca* rosea in the West Indies from its association with folk medicine (Lewis and Elvin-Lewis 2003). Probably one of the most important pharmacological properties of *C.roseus* is its production of vinca alkaloids or bisindole alkaloids (Shams, et al. 2009). This is a native plant of the Island of Madagascar, hence the common name "Madagascar." This plant has been widely cultivated and used in traditional medicine for thousands of years. Not only was this an important plant in Europe for medicinal purposes such as treatment of diabetes, but it has also been used in several other countries such as China and the Caribbean for a variety of health conditions and diseases ranging from cough suppressants and eye infections to Hodgkin's disease and even cancer (Quave 2011). Aside from its medicinal use, Madagascar Periwinkle is also grown for its beautiful botanical appearance as an ornamental plant.

Figure 2. Catharanthus roseus, commonly known as Madagascar Periwinkle. (Source: http://digilander.libero.it/felrig/photos/catharanthus_roseus.htm).

Description

If you have ever seen any vivid beds of flowers or visited a botanical garden, you have probably seen the plant *Catharanthus roseus,* as shown in **Figure 1**. *C. roseus* is an annual evergreen perennial shrub. It usually has five petals. The flowers are typically rose pink in color, but sometimes red, purple, white, and pink. The flowers are tubular in shape and have a corolla tube that is a 1 inch long. This slender tube can expand to be 1 ½ inches wide. The leaves are not glossy and are about 2 to 3 inches wide with rigid-like stems. The plant is typically small and grows to be anywhere from 2 to 3 feet tall. The fruit are classified as follicles and are anywhere from 2 to 4 centimeters long. Madagascar periwinkle grows best in frost-free environments. Although this plant is native to Madagascar, it grows world-wide, but mostly in the tropics, subtropics, and the southern parts of the United States. It is

also grown in Australia, Africa, India, and southern Europe for commercial use (Afolayan and Sunmonu 2010). Even with its pharmaceutical interest, this plant can be found often times in the wild. The type of conditions that Madagascar periwinkle grows best in is one with full sunlight exposure or partial shade with well-drained soil. Growth is inhibited when the soil is too fertile or moist. A unique characteristic about this perennial is that the flowers fall off after blooming and the plant reseeds itself under favorable conditions. Madagascar periwinkle can even withstand hot conditions, unlike other periwinkle plants. This plant also produces "milky latex" like sap from its stem (Floridata 2010). Madagascar periwinkle is closely related to the class of true periwinkles, *Vinca*, which also belongs to the family Apocynaceae. The difference in the two is that Madagascar periwinkles can endure hot conditions, but true periwinkles cannot; the leaves of true periwinkles tend to curl until the temperature returns to normal (Floridata 2010).

Traditional Uses

As mentioned before, Madagascar periwinkle is found often as an ornamental plant in flower pots or bedding of flowers; however, it also has a variety of other uses. The usage is specific to the area and culture in which it is cultivated.

European Usage

In Europe, the primary pharmaceutical interest is for the treatment of diabetes. They sometimes used the pant to treat headaches too. Herbalists would use the juice from the leaves as a household remedy. In India, about 7 flowers or leaves were used each time, but in other countries such as the West Indies who used the roots along with whiskey, and the Cook Islands who used 18 leaves boiled in a pot a water, different variations were practiced (Nammi, et al. 2003). The extracts were also used in India for treatment of wasp stings. During the medieval period, Europeans also believed that this plant could ward off evil spirits" and thought of it as a "magical plant," while the French believed it to be "the violet of sorcerers" (Lewis and Elvin-Lewis 2003).

Chinese Usage

The Chinese, on the other hand, used Madagascar periwinkle as an astringent and cough remedy, as well as for its diuretic properties (Lewis and Elvin-Lewis 2003). These forms of treatment were made using extracts from the plant.

Caribbean Usage

The traditional use for this plant in the Caribbean was for eye infections and diabetes (Lewis and Elvin-Lewis 2003). However, using extracts of the whole plant, the plant was used for treating asthma and flatulence in the Bahamas. Among the other islands such as Cuba, Puerto Rico, and Jamaica, the flower of the plant was used as general eye wash (Mishra 2009). Although there is very limited research; Madagascar periwinkle has also been used to treat "unspecific male problems" such as those related to reproduction issues in Trinidad and Tobago (Lans 2007).

Other Usage

Recently, the pharmaceutical properties of Madagascar periwinkle have drawn interest for the treatment of cancers such as lung or leukemia (Ueda, et al. 2002). There are several other usages for this plant such as for the treatment of Malaria

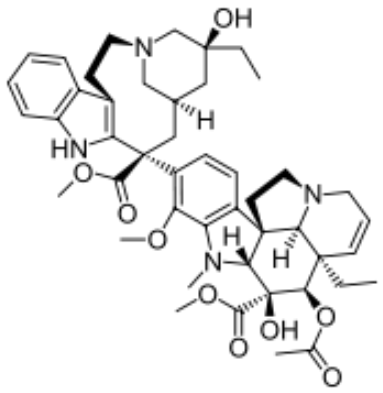

Figure 3. Chemical structure of vinblastine (Source: http://www.thefullwiki.org /Alkaloid).

in Vietnam, a remedy for sore throat in South America, for controlling hypertension in Curacao in Bermuda, controlling bleeding in patients in Hawaii, for treatment of dyspepsia and indigestion in Mauritius and for treatment of dysmenorrheal in Indo-Chinese (Mishra 2009). Other indications of the plant's usefulness in medicine include treatment of hypertension and tuberculosis and for regulation of menstruation. (Nazif, et al. 2009) Because of the wide-variety of medicinal purposes that Madagascar periwinkle has, it has become of the most interesting plants in the pharmaceutical industry.

Chemistry and Pharmacology

For several years, *Catharanthus roseus* had been studied for its anti-diabetic properties, which was influenced by folk medicine. The discovery of vinca alkaloids was by Robert Laing Noble (Wright 2002). Noble had obtained some leaves of the Madagascar periwinkle plant from his brother, Clark Noble, who is best known for his studies on insulin. Robert Noble then tested leaf extracts and discovered that there were little effects on blood glucose levels. However, the extracts did cause a noticeable effect on bone marrow and the white blood cell count. About 2 years later, Robert Noble and an organic chemist joined forces and began to isolate and characterize vinca alkaloids. These alkaloids soon became one of the most important drugs to cancer chemotherapy (Wright 2002).

C. roseus is the source of two important chemical compounds that are also classified as anticancer agents. The compounds vinblastine and vincristine, whose chemical structure is shown in **Figure 2**, are well known vinca alkaloids that are derived from this plant (Roepke, et al. 2010). Vinblastine, which is marketed as by Eli Lily, is used to treat lymphoma, advanced breast and testicular cancer, Hodgkin's disease, and Kaposi's sarcoma. Likewise, vincristine is marketed as Oncovin and is used to treat acute leukemia, Hodgkin's disease, as well as other types of lymphomas (Lewis and Elvin-Lewis 2003).

The fractions and percentages of the alkaloids are shown in **Table 1**. Although vinblastine and vincristine are the primary alkaloids, a study by The Phytochemistry Department in Europe showed another alkaloid, catharanthine, to be present (Shams, et al. 2009). These alkaloids were discovered over a course of five different methods.

Biological Activity

Vinca alkaloids of the plant *Catharanthus roseus* are sone of the most widely studied anti-mitotic drugs because of their biological activity as it relates to treating certain forms of cancer. The alkaloids act by disrupting the microtubules in cells. First, they bind to tubulin monomers. At low concentrations of the alkaloids, the formation of microtubules is inhibited. If the concentration of the alkaloids is high enough, they can cause the protofilaments of the microtubule

Method	Frations	* % of alkaloidal fractions	*HPLC % of Vb & Vc rich fractions	
			Vb	Vc
I	Vindoline rich fraction (A) Vinblastine rich fraction (B) Vincristin rich fraction (C)	0.748 0.00274 0.0075	- 0.00399 0.00014	- 0.00012 0.00772
II	vinblastine rich fraction (D) Vincristin rich fraction (E)	0.002 0.0015	0.00253 0.0009	0.0007 0.00184
III	vinblastine rich fraction (F)	0.1153	0.1417	0.00017
IV	Vindoline and catharanthine rich fraction(G) Vinblastine rich fraction (H)	0.0087 0.0035	- 0.0041	- 0.0004
V	Vinblastine & Vincristin rich fraction (I)	0.1033	0.0950	0.0110

Table 1. Alkaloids and the percentage present in *Catharanthus roseus*.

walls to peel. If the microtubules fail to form, the assembly of the mitotic spindle cannot take place, which therefore blocks mitosis (Jordan, Himes and Wilson 1985). Once mitosis is blocked, the affected cells begin to die.

Over the past few years, there have been lots of studies regarding the biological activity of *C. roseus*. One study explored the chemical composition and activity that occurs through the leaf. The results showed indications of the leaf secreting varying amounts of catharanthine (Roepke, et al. 2010). This is an important feature because catharanthine has antifungal and antiseptic properties, among others. In fact, the complexity of the leaf is so dynamic that the monoterpenoid indole alkaloids (MIAs) regulate the transport mechanism and the rate of biosynthesis within the plant (Roepke, et al. 2010).

Another study explored the vast gene-to-metabolite networks that are inhibited by the alkaloids. The results showed the varying branches and metabolic pathways within the planet. According to the review, the purpose of the networks is to "identify a select number of genes and metabolite likely to be involved in the biosynthesis of terpenoid indole alkaloids" (Rishcer, et al. 2006). The results are important for understanding the metabolic rate and mechanism within the plant.

There have been several innovating studies conducted concerning *Catharanthus roseus* and its medicinal contribution. In a study by the Department of Pharmaceutical Sciences at the Andhra University, results indicated there are notable juices produced by the plant the exhibits hypoglycemic activity. These juices showed a significant decrease in blood glucose of normal and diabetic rabbits (Nammi, et al. 2003). These results thus support the belief of traditional herbalists who used the plant a remedy for diabetes.

Another study showed the healing power of *C. roseus.* This experiment was conducted on lab rats, rather than rabbits as in the previously mentioned study. Results showed that extracts from the plant "increased the wound breaking strength in the incision wound model" (Nayak and Pinto

Pereira 2006). This study, too, supports the beliefs incorporated from traditional medicine that *C. roseus* can be used as an antiseptic.

One other study focused on the biochemical specialization of the plant. The plant is composed of several classes of metabolites. Among that was about different types of cells as well as numerous genes and gene networks (Murata, et al. 2008). All of these components contribute to the plants ability to multifunction for different medicinal purposes.

Clinical Studies

Aside from the *in vivo* studies, there have also been clinical studies conducted. One study focused on the treatment of Hodgkin's lymphoma with BEACOPP. BEACOPP is a regimen composed of the vinca alkaloid, vincristine, and a host of other drugs such as bleomycin, etoposide, doxorubicin, cyclophosphamide, procarbazine, and prednisone. A random selection of patients that had untreated Hodgkin's lymphoma were given BEACOPP, while another group were given ABVD. ABVD is composed of a vinblastine, doxorubicin, bleomycin and dacarbazine. The study showed that patients with the regimen that included vincristine (BEACOPP) showed a greater progression than the patients who received ABVD (Simonetta, et al. 2011).

One of the newest vinca alkaloids is vinflunine. It was developed through the process of fluorination. The alkaloid is most studied for its anticancer properties as well. Studies have shown that like most vinca alkaloids, vinflunine has "mitotic-arresting and tubulin-interacting properties" (Kruczynski 2001). However, this alkaloid is easily distinguishable from other vinca alkaloids because it "induced smaller spirals with a shorter relaxation time" (Kruczynski 2001). In clinical studies, vinflunine is most noted for its antitumor activity (Kruczynski 2001).

In addition to the ongoing clinical trials has begun approval of some vinca alkaloids and their derivatives. For instance, vincristine has been approved and is available as an injection by prescription only (Drugs.com n.d.). Something interesting to note about this drug is that although it has received approval, the mechanism of action is still under investigation.

Contraindications

As with any medicinal plant or drug, there are always cautions associated with the usage. *Catharanthus roseus* has displayed toxic effects when exposed to cattle (Lewis and Elvin-Lewis 2003). For humans, it should not be taken by mouth because the vinca alkaloids present can be highly poisonous. Some common side effects associated with consumption include nausea and vomiting, the loss of hearing, as well as liver damage and death (Lewis and Elvin-Lewis 2003).

Current Use in Allopathic & CAM Therapies

Currently, there are several studies being conducted to verify the safe usage of the drug as an anticancer agent. However, several semi-synthetic derivatives of the drug are available for clinical usage. Aside from Velbe and Oncovin, as mentioned before, *Catharanthus roseus* is also marketed as Eldisine to treat leukemia and lung cancer and as Navelbine to treat ovarian cancer (Lewis and Elvin-Lewis 2003).

Discussion

Catharanthus roseus is a complex plant that has a wide variety of medicinal uses. Because of the vinca alkaloids that are produced, cancers such as leukemia and lymphomas can be treated (Lewis and Elvin-Lewis 2003). Minor health conditions such as the common cough can also be treated by this plant. *C. roseus* is so dynamic that is cultivated in countries across the world to treat anywhere from everyday wasp stings to more serious conditions such as diabetes. Extracts of this plant has linkages to traditional medicine dating all the way back to folk medicine in the West Indies. Even today, *C. roseus* still proved to be an important plant in the pharmaceutical industry.

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Chondrodendron tomentosum Ruiz & Pavón, Menispermaceae

Kimberly Beck

Introduction

Chondrodendron tomentosum Ruiz & Pavón is a member of the Menispermaceae family, also known as the Moonseed family, and is found primarily in South American rainforests. The Moonseed family consists of over 78 genera, mainly woody vines, and is commonly found in the tropics (Wiart, 2006). *C. tomentosum* was first identified by Hippolyto Ruiz and Joseph Pavón in 1793 after they brought it back from a South American expedition to be identified and classified. *C. tomentosum* is primarily utilized to make curare, a poison used in hunting and previously in tribal warfare, in the western Amazon. The root, known as the *Pareira brava*, is the primary source of curare from *C. tomentosum*; however many times due to shortage the stem of the vine is used as a substitute (Bentley, 1902). Locally known amongst indigenous Amazonian tribes as *curare, pareira* or *amphihuasca*, the name amphihuasca comes from the name "ampi" the term for the macerated bark product used to extract the poison. The Tikuna tribe of the Peruvian Amazon is generally considered amongst Amazonian tribes, as well as the outside world, to produce the highest or best quality of curare poison to be used on poison darts or arrows. Curare is found throughout the western Amazon basin, leading to different storage techniques. Some groups would store the curare in terracotta pots in the soil while others would store it in dried gourds. The most influential manner of storage, though, was in bamboo tubes leading to the name of the derivative tubocurarine, meaning "tube of curare." Now curare is stored in cans or whatever storage containers can be found

Figure 1. *Chondrodendron tomentosum* leaves, stem, and fruit.

(Birmingham, 1999). Curare comes from both *C. tomentosum,* common to the forest parts of South America in Ecuador and Peru, and *Strychnos toxifera,* found in other regional areas. D-tubocurarine and other muscle relaxants that have anticholinergic effects at the neuromuscular synapse were developed from these two plants. *C. tomentosum* was initially used as an anesthetic and muscle-relaxant during surgeries in the 18th century paving the way for increased development in the field of surgery allowing advancements that would otherwise not have been possible (Booij, 2000).

Botanical Description

Chondrodendron tomentosum is a liana, a strong woody-stemmed vine that is rooted in the soil and grows to a thickness of three-quarters of an inch to over two inches, climbing up trees into the forest canopy in order to reach a well-lit area. Given the name "*Pareira brava*", signifying "wild vine" in Portuguese, the liana grows slowly and extensively in the western Amazon region climbing to the peak of high trees. The bark is generally dark brown or black, thin and loosely attached with deep furrows and ridges in a longitudinal pattern as well as diagonal ridges and channels. The wood itself is porous, concentrically marked, separated into wedge portions and is of a yellow or a brown-grey color. The wood is fibrous when broken; however, when shaved or cut, it has a waxy consistency (Bentley, 1902). Like other plants of the Menispermaceae family, *C. tomentosum* has large heart-shaped three-veined leaves that alternate and typically exhibit a wiry pulvinar flexion where the leaf connects to the stem (see **Figure 1**). The bottoms of the leaves of the *C. tomentosum* are densely white and tomentose, as well as broadly ovate and veined (Gentry, 1996). It is a woody, branchless, twisted vine

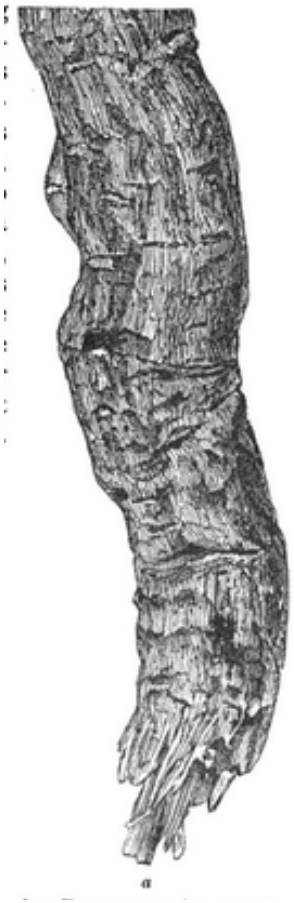

Figure 2. Twisted *Chondrodendron tomentosum* vine.

found in South American rainforests (see **Figure 2**); it has been specifically used in western Amazonian tribes in Peru and Brazil (Birmingham, 1999). *C. tomentosum* has small flowers, as do all menisperms, with each containing six or more tan or grayish, pubescent hard monocarps (Gentry, 1996). Fruits spread and radiate from the thick woody gynophores stalk to which they are attached by short stalks. The fruit are of a purplish black color, measure to nearly one-

inch long and are oblong-ovoid shaped. When fully ripe the oblong fruit resemble a bunch of grapes in appearance, this can be seen in **Figure 3** (Bentley, 1902).

Traditional Uses

Traditional Uses in Hunting

Chondrodendron tomentosum is the source of curare for western Amazonian tribes and its origins are rooted in traditional Tiriós folklore. The story goes that the world began with one man and three animals; a howler monkey, a yellow-headed parrot and a yellow-headed vulture. The howler monkey is really a woman in disguise and through a series of events the man and her marry. The Tiriós man is then shown a liana and the recipe for curare by a harpy eagle that tells him the curare are to help him in his hunting. The Tirió story demonstrates the power and importance curare is traditionally believed to possess; as the man gains knowledge he is too quick to try his newfound weapon and hastily kills all the howler monkeys in the region including his wife disguised again as the howler monkey (Plotkin, 1993). This tragedy serves as a warning to respect the poison and not use it in excess. The added cultural component of a sacred folk tale adds to the obvious importance of the tradition of how curare is prepared and what it is used for. The methods by which curare is prepared are ritualistic in nature, and of such importance that its preparation was shrouded in secrecy for years. It took a long time for ethnobotanists to gain the trust of the tribal elders and learn how various tribes prepared curare from *C. tomentosum* (Plotkin, 1993). Traditionally, "curare" or "ampi" is prepared by native Amazonians by crushing and boiling the roots and stems, adding in other herbaceous plant or animal venoms, and mixing it until it reaches a light syrup consistency. They then use this concentrate to poison the tips

Figure 3. Botanical drawing featuring coloring, leaves, stem, vine, flowers, and fruit of *Chondrondendron tomentosum*.

of their darts and arrows by creating grooves with piranha teeth and then dipping the darts and arrows into the syrupy

substance. (Duke, 1994). Each tribe's recipe varies by the different venoms that are added; the one constant ingredient throughout the western Amazon is the base of *C. tomentosum* (Plotkin, 1993). The storage of the curare also varies; some tribes store the decoction in bamboo tubes while others store it in terracotta pots. The name tubocurarine (an alkaloid derivative of *C. tomentosum* utilized in modern medicine) literally comes from the curare stored in the tubes. The usage of curare derived from *C. tomentosum* for hunting is consistent throughout the western Amazon, even though indigenous tribes' methods of creating the concoction vary. In the western Amazon region, curare is still used in hunting (Plotkin, 1993).

Traditional Medicinal Uses

In native Brazilian culture, the root of *Chondrodendron tomentosum* is used to treat a variety of internal ailments, including as a febrifuge, an emmenagogue and a diuretic. It is common in the treatment of dropsy, madness, bruising, orchitis and kidney stones (Duke, 1994).

In addition, *C. tomentosum* has been used as a diaphoretic to cure snakebites through rapidly sweating out the venom in Amazonian culture. The root extract affects the mucus membrane of the genito-urinary organs by acting as an astringent and sedative upon them, thus, decreasing the secretion of the ropy mucus during bladder inflammation as well as reducing the inflammation. A decoction is made from adding small pieces of the root to water and filtering the mixture to get a dose of 30-100 grams (de Tavera, 1901).

In addition, *C. tomentosum* has been traditionally used as an antiseptic, anti-inflammatory, in the treatment of rheumatism, jaundice, dropsy, and gonorrhea (Gonzalez-Coloma, 2011).

Chemistry and Pharmacology

Chondrodendron tomentosum has several different active crystalline alkaloid compounds, one of which is d-Tubocurarine, the alkaloid responsible for inhibiting nerve impulse transmission to muscles. (Panda, 2002) The tubocurarine contained in *C. tomentosum* is that of tubocurarine chloride which contains a bisbenzyl isoquinoline structure in a quaternary compound as its principle alkaloid. Tubocurarine only occurs naturally in *C. tomentosum* and acts as a muscle-relaxant utilizing a non-depolarizing mechanism at the neuro-muscular junction. Muscular paralysis is caused by a competitive blockade of the post-synaptic nicotinic acetylcholine receptors. In this respect it is similar to the alkaloid toxiferine found in the *Strychnos* species but was found to be less active (Bisset, 1989). It also contains four non-quaternary bases: isochondrodendrine, isochondrodendrine dimethyl other, berberine and chodrocurine. Berberine is the alkaloid responsible for the bitter principle in *C. tomentosum* (Daniel, 2006). After initially being used by Benjamin Brodie in a patient with tetanus curare was then used topically as well as in the treatment of a variety of ailments including hydrophobia, epilepsy and cholera (Booij, 2000).

Mechanism of Action

Curare derived from *Chondrodendron tomentosum* works rapidly to block nerve impulses to the muscles resulting in paralysis from flaccid muscles. The muscle paralysis induced by curare is temporary but still has the potential to lead to death through failure of respiratory muscles to function and, thus, asphyxiation (Panda, 2002). In terms of paralysis, curare first acts on the muscles of the eyes, followed by the nose and the neck and then the limbs. The diaphragm is the final muscle

to become paralyzed leading to probably asphyxiation and death. Curare acts on the motor endplate of a nerve by depressing the potential of the endplate in the neuromuscular junction, but does not affect the pre-junctional release of acetylcholine. Curare competitively inhibits acetylcholine and also interferes with the feedback control of acetylcholine by occupying the pre-synaptic receptors involved in acetylcholine release. It was demonstrated that between 70% and 95% of acetylcholine receptors must be occupied by curare to inhibit muscle contraction, this margin is know as the margin of safety of neuromuscular transmission. At 70% occupation of acetylcholine receptors, muscle contraction begins to decrease while at 95% occupation full blockade exists. (Booij, 2000). Curare can be ingested as it is inactive unless injected directly into the bloodstream. This is because the compounds are so large and highly charged that they are unable to pass through the digestive tract lining and be absorbed into the bloodstream (Nasiripourdori, 2011).

Biological Activity

Chondrodendron tomentosum and the Heart

In the early 19th century, Benjamin Brodie used rabbits as a model to show the potential for artificial ventilation of the lungs as a means to maintain life after curare-induced paralysis. He observed that curare did not have an effect on the heart and proceeded to use curare on a human being to treat tetanus in 1811. Subsequently, in 1850 Claude Bernard demonstrated the effect of curare on neuromuscular transmission utilizing frog nerve-muscles. Through this experiment he showed that direct muscle stimulation resulted in muscle contraction while nerve stimulation had no resulting contraction. This proved that curare acts on the nerves rather than on the muscle leading to the discovery that motor function, not sensory function, was affected by curare (Booij, 2000).

Chondrodendron tomentosum & Leishmania

Extracts of *C. tomentosum* were tested to see if it is a possible new treatment for Leishmaniasis, a protozoan parasitic disease with a high associated morbidity and mortality rate that is endemic in 88 countries, and Chagas disease, a tropical parasitic disease. The current treatment for Chagas disease, nifurtimox and benzinidazole, have severe side effects and their distribution and production are problematic. Current treatments for both diseases are at extremely high dosages with high potentials for resistance leading to the necessity for the development of a new treatment. After interviewing locals from the city of Iquitos, Peru on the subject of the most powerful traditional medicines, *C. tomentosum* was selected as a possible new treatment for both Leishmaniasis and Chagas disease. A crude alkoloidal extract was evaporated from *C. tomentosum.* 700 g of dry bark extract of *C. tomentosum* was macerated with ethanol to yield an extract of 54.6 g (7.8%). After testing *C. tomentosum* along with other local traditional medicines it was concluded that *C. tomentosum* bark is one of the most effective of the medicines tested against Leishmaniasis as well as Chagas disease. *C. tomentosum* exhibited strong anti-parasitic effects exhibiting selective activity against *L. infantum* promastigotes. Additionally the Menispermaceae family produced alkaloid extracts that exhibited antimalarial activity and in vitrotoxicity against the tumoral cell lines KB-3, KB-V1 and P-38878. The study continued to subject *C. tomentosum* to bioassay-guided fractionation. Chondrocurine (**Figure 4.4**), (S,S)-12-O-methyl(+)-curine (**Figure 4.5**), and cycleanine (**Figure 4.6**) are all bisbenqylisoquinolines that were isolated from *C.*

Figure 4. Bioassay-guided fractionation of *C. tomentosum*. Chondrocurine (Figure 4, 4), (S,S)-12-O-methyl(+)-curine (Figure 4, 5), and cycleanine (Figure 4, 6).

tomentosum. Chondrocurine and (S,S)-12-O-methyl(+)-curine exhibited activity against both parasites. It was concluded that these compounds are responsible for the activity of *C. tomentosum* and that *C. tomentosum* is active against Leishmaniciasis as well as Chagas disease (Gonzalez-Coloma, 2011).

The Effect of Sepsis on d-Tubocurarine as seen in Rats

The effects of sepsis on the lateral cricoarytenoid (LCA) and posterior cricoarytenoid (PCA) muscles were evaluated as they underwent the neuro-muscular blocking actions of d-tubocurarine *in vitro.* The study was conducted in order to demonstrate the importance of understanding the differential paralysis of the laryngeal muscles caused by d-tubocurarine and the best method by which upper airway obstruction may be avoided during anesthetic/sedative induction. Cecal ligation and puncture was used to induce sepsis and educe panperitonitis in rats in order to record electromyograms and endplate potentials from the LCA and PCA muscles. It was noted that in normal rats the PCA muscles are more sensitive the d-tubocurarine than the LCA muscle. Sepsis is thought to decrease laryngeal muscle sensitivity to non-depolarizing neuro-muscular blockers such as d-tubocurarine. Sepsis was induced in the rats by anesthetizing them with isoflurane and making an incision mid-abdomen to puncture the cecum filled with feces. The incisions were then closed and the rats were injected with saline solution subcutaneously in the back during the operation in order to resuscitate them. The rats were observed for two hours post surgery and given free access to water but deprived of food. 10 to 16 hours post surgery isoflurane anesthesia was used to kill the rats in order for the larynx and attached recurrent laryngeal nerves could be dissected. The preparations were maintained in a modified Kreb's solution (NaCl, 135; KCl, 5; $CaCl_2$, 2; $NaHCO_3$, 15; Na_2HPO_4, 1; glucose, 11: in $mmol^{-1}$) bubbled with 95% O_2 and 5% CO_2 and maintained during oxidation at a pH of 7.40 ± 0.05. Before beginning the experiment, electromyographic response and visual inspection were used to confirm the contraction of the LCA and PCA muscles by supramaximal stimulation of the recurrent laryngeal nerves. Extracellular microdes measured the compound action potentials' amplitudes of the LCA and PCA muscles' action potentials. In order to assess neuromuscular transmission intracellular microelectrodes recorded the EPPs of the muscles. Currents were measured as several concentrations of d-tubocurarine were applied through superfusion into the Kreb's solution.

The data was then statistically analyzed. The study showed that d-tubocurarine induced neuromuscular blocking was weakened by sepsis. Sepsis inhibits the reduction of transmitter release induced by d-tubocurarine in the LCA and PCA muscles with the LCA muscles showing more d-tubocurarine resistance. The study indicated that sepsis blocks the neuromuscular transmission by stage-dependently inhibiting the d-tubocurarine and, thus, depressing action potentials. The study acknowledged that the contraction force of the laryngeal muscles is reduced by the inhibition of d-tubocurarine as a neuro-muscular block by sepsis. The study concludes that d-tubocurarine induced neuromuscular blocking is weakened by panperitonitis-induced sepsis in both the LCA and PCA muscles. It is noted that the differential sensitivities of the LCA and PCA muscles to d-tubocurarine was not significantly influenced by the induced sepsis, the PCA muscle remained more sensitive than the LCA muscle to d-tubocurarine (Nishikawa, 1999).

Clinical Studies

Change in Histamine Levels as a Result of d-Tubocurarine Administration

As a result of an allergic reaction experienced by a twenty-five year old patient undergoing a tendon transfer to his left arm. Swelling was noted in the eyelids of the patient immediately after tracheal intubation using d-tubocurarine anesthesia. Other allergic symptoms included swelling of the face and neck and the appearance of giant hives on most of the body. The symptoms were so severe that the surgery was postponed and the patient was removed from anesthesia where the patient's symptoms subsided and he was discharged. The patient's history revealed urticarial reactions as a result of a variety of things including ingesting strawberries or green onions, and emotional upset. The study was conducted when the patient was asymptomatic; ten days after the reaction, a blood sample to determine histamine was drawn. In addition to blood samples, direct intradermal tests were conducted on the patient's right forearm of 0.75% d-tubocurarine, 2.5% thiopental, and 0.4% gallamine each injection of 0.05 ml volume. The histamine levels of the patient's blood were analyzed using a spectrophotometer. The study inferred the histamine response was a result of d-tubocurarine administration. The histamine levels in the patient at the time of the reaction were nearly twice the patient's normal value.

Various Historical Clinical Studies and Usages

Initially, curare derivatives from *C. tomentosum* were tested and used as a muscle relaxant to relieve stiff muscles caused by polio as well as to treat a variety of conditions such as lockjaw, epilepsy, and chorea (a disorder leading to irregular and uncontrollable muscle movements). These efforts proved fruitless as other more effective and safe treatments were found for these ailments. Efforts were successful, however, in extracting the compound d-tubocurarine from *C. tomentosum* and injecting it as a muscle relaxant for use during throat, rectal and abdominal surgeries in addition to using it to lessen the burden of tense flexed muscles on the spines of patients receiving shock therapy (Plotkin, 1993).

Contraindications

The danger associated with the derivative tubocurarine when it was used as a muscle relaxant during surgery was possible asphyxiation; however, this worry was reduced with technological breathing support during surgery. The effects of tubocurarine as an active constituent are temporary; with

artificial ventilation of the lungs to ensure that the patient is still breathing the risk of asphyxiation is minimized.

When prepared in liquid extract to act as a diuretic or tonic, *C. tomentosum* is considerably less powerful. Curare must be injected directly into the bloodstream in order to reach its full toxicity; it is not harmful when taken orally in recommended doses. This also explains why people of the Amazon are able to hunt with it and then eat the "poisoned" meat; the animal meat is not poisonous to them due to the nature of the compounds in curare. The highly charged and large nature of the compounds causes them unable to cross through the digestive lining in the gut (Booij, 2000).

Allergic reactions to d-tubocurarine have also been documented in patients with injection of d-tubocurarine resulting in increased histamine output in the body inducing swelling and the appearance of hives on the body (Westgate, 1961).

Current Use in Allopathic and CAM Therapies

When traditionally prepared in the western Amazon regions of Brazil and Peru as a diuretic or tonic, the root of *C. tomentosum* is dried and then used to create a liquid extract. The powdered root is seeped in boiling water and then a small portion is evaporated to get an approximation of how much of the plant was extracted. The liquid is then concentrated until it thirty three and a half percent of it is made up of the extractive. Alcohol is then added to bring it up to dosage. This method is rather ineffective, however, and leads to a product that lacks potency and produces inconsistent results and yields (Gadd, 1904).

There are not currently drugs on the market, as the traditional natural derivatives have been replaced with synthetic drugs. The synthetics include steroidal and benzylquinolone derivative classes as well as the drug Fazadinium. These synthetic drugs are not as complex or as multi-dimensional as *C. tomentosum*, but they are much safer and more controlled than the tubocurarine derived from *C. tomentosum* (Booij, 2000).

Discussion

It is amazing that something in nature is potent enough to paralyze a monkey with the prick of one dart. *C. tomentosum* is the source of an incredibly powerful drug that enabled surgery to advance. The development of modern medicine was incredibly impacted by *C. tomentosum,* muscle relaxants developed from derivatives like curare and d-tubocurarine allowed surgery to advance in ways that it could not have otherwise. Although its use has been replaced with synthetics such as Fazadinium and other compounds belonging to the steroidal and benzylquinolone derivative classes, curare holds an unforgettable place in medical history and can never be fully recreated in a laboratory (Booij, 2000). The importance of *C. tomentosum* was only realized through working with native Amazonian tribes and having an open mind about a so-called poison; there are always multiple ways of looking at things and this happened to be more than just a poison. Without innovative minds in the early twentieth century, tubocurarine might never have been derived from this notorious poison. Even though *C. tomentosum* may not play as influential of a role in the future of medicine as it did in the past, it still has potential in many facets of medicine that it has not previously been tested in. The tribes of the Amazon possess knowledge of countless plants and animals in the region and could hold the key to a number of illnesses modern medicine has yet to cure. It is the combination of traditional

medicinal knowledge and innovative and open minds that truly hold the key to unlocking cures to diseases that plague the world, it is necessary to look into the rich history of traditional medicine and consider the knowledge they can provide when looking for chemicals and ingredients that can truly make all the difference in medicine.

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Cimicifuga racemosa (L.) Nutt., Ranunculaceae

Aerhealle Hampton

Introduction

Native to North America, *Cimicifuga racemosa* (syn. *Actaea racemosa*) has long been a plant of many debated names. Its common names include black cohosh, black snakeroot, bugwort, bugbane, rattleweed, and squawroot, though the latter is also used to refer to the unrelated blue cohosh (*Caulophyllum thalictroides*). Of the Ranunculaceae family and originally recognized by Linnaeus as belonging to the genus "*Actaea*," in 1818, Nuttall re-classified the plant's genus as being that of "*Cimicifuga*." This was largely accepted until recent years in which taxonomists have called for the return to its original genus classification (Compton 1998). To reduce conflicts with cited literature, however, C. racemosa will here be used as the recognized scientific name.

Black cohosh has traditionally been used as an herbal remedy in women's health as it was believed to ease childbirth and stimulate menstruation. It continues to be marketed toward women and sold as a reliever of menopausal symptoms. The exact method of action is still unknown, but many important compounds have been isolated from *C. racemosa* including triterpene glycosides and phenolic acids. Antioxidant, anti-inflammatory, and anti-HIV properties have also been documented in black cohosh.

Figure 1. Racemes of *C. racemosa* displaying showy, white flowers. Source: treefrogfarm.com

Description

Cimicifuga racemosa is a perennial herbaceous plant that is harvested from the wild, almost exclusively (Thomas et al 2007). It thrives in sunny woodland pastures, as well as shaded, wooded hillsides that stretch across the mountainous regions of the midwest to eastern United States and Canada (Li et al 2002). These wooded areas provide the well drained soils that allow black cohosh to be an understory plant in deciduous forests (Thomas et al 2007). Since it is an herbaceous plant, the leaves and stems of black cohosh die down at the end of each growing period. During the next growing period, however, new flowering stalks 1-2.5 meters

in length grow and produce a crown of large, green, tri-ternate leaves. Smaller, ovoid leaflets (about 5-8 centimeters wide) are also produced with doubly serrate edges. Presumably the "black" in *C. racemosa's* common names has its origins in the dark brown to black coloring of its rhizome, from which the flowering stalks sprout (Lloyd and Lloyd 1884).

C. racemosa begins to bloom in late June and continues throughout August. It is during this time that the large flowering racemes are produced, containing between fifteen to twenty flowers (Lloyd and Lloyd 1884). The showy flowers themselves, shown in **Figure 1**, are comprised of a small pistil surrounded by multiple stamens, creamy white in color. The clustered flowers lack noticeable petals and produce abundant pollen as well as a strong, unpleasant odor that attracts pollinators (Wood 2000). The fruiting bodies of *C. racemosa*, which brought about one of the major conflicts in the designation of an appropriate genus for the plant, are a little over six millimeters in length, long, dry, oval in nature, and covered with thick, leathery sides. When the pod, containing 8-10 brown and angular seeds, dies, it remains on its stalk throughout the winter and produces a rattling sound when struck by coming winds, providing insight as to why this plant is sometimes called "rattleweed" and "rattlesnake root" (Lloyd and Lloyd 1884). In the following warm season (following a warm-cold-warm seasonal pattern of temperate regions), the seeds reach ripen and reach maturity (Wood 2000).

Traditional Uses

Traditionally, black cohosh was used as a very important and popular remedy in Native American cultures. In the instance of snakebite, the chewed root was thought to be a sure cure for the onset of the poison when applied directly to the wound. For other ailments, a decoction of the root was rubbed onto affected areas, as in a common treatment for rheumatism. Especially important was the use of *Cimicifuga racemosa* in Native American women's health. The use of this plant by Native American women was so prevalent that pioneers designated it with yet another nickname: "squawroot," using the Algonquian word for "woman". The females of these native cultures used black cohosh for ailments such as painful childbirth and to stimulate menstruation (Sattler 1884).

In the 1800s, many doctors began to include *C. racemosa* in their own reports and materia medica. Benjamin S. Barton (1801) heralded the astringent properties of the root. He noted that the gargling of a strong decoction made from the rhizome of black cohosh was useful as a treatment for an ailment known as "putrid sore throat," as well as a cure for "the itch." T.S. Garden later reported in *The American Medical Recorder* (1823) that he self-medicated with *C. racemosa* to treat what he referred to as "Phthisis pulmonalis." Though he was most likely suffering from what would now be recognized as a case of bronchitis, Garden still reported that doses of an extract from this plant lessened his night sweats, improved his coughing, and lowered his pulse. In addition to this, he claimed that the black cohosh extract also calmed him with its sedative properties and may have even possessed possible vasodilatory properties. Described under the name of *Botrophis* serpentine, C. R. Rafinesque also referred to the use of *C. racemosa* as an inducer of both sweat and menstruation, a treatment for joint pains, as well as a remedy in the treatment of hysteria. Rafinesque also mentions veterinary uses of the plant in which he suggests that the consumption of black cohosh by cattle purged them of worms. Chapman, in *Elements of Therapeutics and Materia Medica* (1831), classified C*. racemosa* as an expectorant and sited uses of it being used as a narcotic (in the sense of it being a sedative and analgesic),

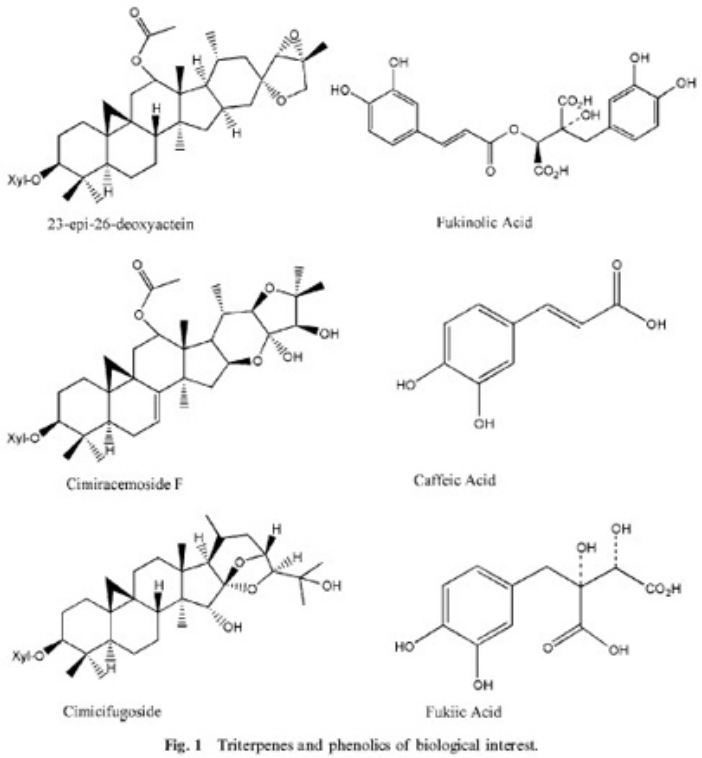

Figure 2. Major compounds found in *Cimicifuga racemosa*. Source : http://nutrition.perfectskillset.com/main/dietary-supplements/supplements-a-c/700-black-cohosh-cimicifuga-racemosa-.html

an antispasmodic, a diaphoretic, and also an emetic when taken in large enough doses. Around this time, *C. racemosa* was also a popular treatment for pulmonary ailments, including asthma and what we now know to be tuberculosis, as it relieved cough, and reduced fever and heart rate (Sattler 1884). *C. racemosa* was also documented as being a useful treatment for St. Vitus Dance chorea, a disease believed to cause sporadic movement and twitching of the limbs, loss of appetite, and possibly permanent decrease in brain function in a matter of months (Young 1832).

Chemistry and Pharmacology

Cimicifuga racemosa contains an abundant number of secondary metabolites and compounds, varying in composition during the lifecycle of the plant and dependant of from where a sample is taken. That being said, many of these compounds remain undiscovered. Due to this heterogeneity, and lack of uniform results in research, the active components in black cohosh also remain unknown. An example of two secondary metabolites that vary greatly throughout the plant and season are the terpenoids 23-epi-26-deoxyactein and cimiracemoside A. It was found that about ten times the amount of 23-epi-26-deoxyactein was found in inflorescence tissues than the amount found in the rhizome of black cohosh. The drying of this matter, however, lowers this amount to a mere fourth of that found in the rhizome (Thomas et al 2007).

A large number of the main major constituents that have been identified in *C. racemosa* are its triterpene glycosides. Of the cyclolanostane triterpenes found in *C. racemosa*, the largest group contains cimigenol as an aglycone (Zhou et al 2008). Cimicifugosides, cimiracemosides, and actein are also important compounds in the chemical makeup of this plant. Though a uniform standard for purity markers have not been created for black cohosh, these compounds have been found in varying levels in liquid extract, powdered extract, and milled plant material. Phenolic compounds, such as ferulic acid, isoferulic acid, and caffeic acid, as well as the alkaloid cImipronidine have also been found in *C. racemosa* (Li et al 2002). Structures of some of these compounds can be seen in **Figure 2**.

Biological Activity

Though extensive testing has been performed, the method of action for *Cimicifuga racemosa* is still unknown (Li et al 2002). A common misconception posited for many years that black cohosh acted as a phytoestrogen, mimicking the effects of estrogen in the body. However, contrary to popular belief, when tested alongside 7 other extracts from botanical sources *in vitro* for estrogenic activity, *C. racemosa* exhibited did not exhibit any estrogenic activity (Liu et al 2001). In 2003, Burdette et al tested rhizome extracts from *C. racemosa* to try to determine the mechanism of action for the alleviation of hot flashes using ovariectomized Sprague- Dawley rats. The rats were given a 40% propanol extract of black cohosh for two weeks with and without the presence of the compound estradiol. These researchers looked for clues of estrogenic and antiestrogenic properties by observing the unterine weight of the rats as well as recording their vaginal cellular makeup. This team's results also showed that *C. racemosa* does not possess any antiestrogenic nor estrogenic properties. Instead, the results of their experiment indicated that the mechanism responsible for the decrease in hot flashes may actually involve the 5-HT_7 serotonin receptor pathway, which is involved in thermoregulation, through possible serotonin receptor ligands (Burdette et al 2003).

In 2008, Powell et al performed *in vitro* experiments to uncover more about this possible mechanism of action by testing for receptor binding activity and cyclic AMP production. They found that, like many serotonin selective reuptake inhibitors (SSRIs), N_ω-methylserotonin may be the compound responsible for relieving hot flashes by working through the serotonin transporter (SERT) by binding to the 5-HT_7 serotonin receptor and blocking serotonin re-uptake (Powell et al 2008). In an experiment to determine possible hepatotoxicity, 300mg/kg of dry root extract of *C. racemosa* was given to male Wistar rats each day for thirty days. Their liver morphology and other hepatic functions were closely watched and samples were taken and examined for damge and abnormal cells. It was concluded that there was no correlation between *C. racemosa* and liver damage because an absence of affected liver enzymes were found (Mazzanti et al 2008). Possible uses of *C. racemosa* in the treatment of HIV have also been pursued *in vitro*. The compound actein was tested alongside twelve sapponins has been tested for anti-HIV activity. By inhibiting the replication of H9 lymphocytes, this triterpenoid displayed great effectiveness and anti-HIV in experiments (Sakura et al 2003).

Clinical Studies

In a randomized controlled study comparing the effect of exercise in changes on bone mineral density and 10 year risk for coronary heart disease, *Cimicifuga racemosa* was tested to see if it could aid in bone mineral retention. Over one hundred women were assigned to three different groups and subjected to different exercise routines involving high intensity resistance and high impact workouts with low intensity programs interspersed. Overall the study showed a positive correlation between exercise and dcreased risk of coronary heart disease, but the addition of black cohosh showed no added benefits when paired with this exercise (Bebenek et al 2010). Phase III double-blind and randomized trials were performed between 2003 and 2004 in which the experimental group of postmenopausal women who had either completed or were undergoing breast cancer treatment. Given a single capsule of 20 mg black cohosh, the women were to report their incidence of hot flashes in the form of a diary. The results of this study showed a mean decrease of 20% in hot flashes in comparison to the placebo-controlled group (Pojak et al

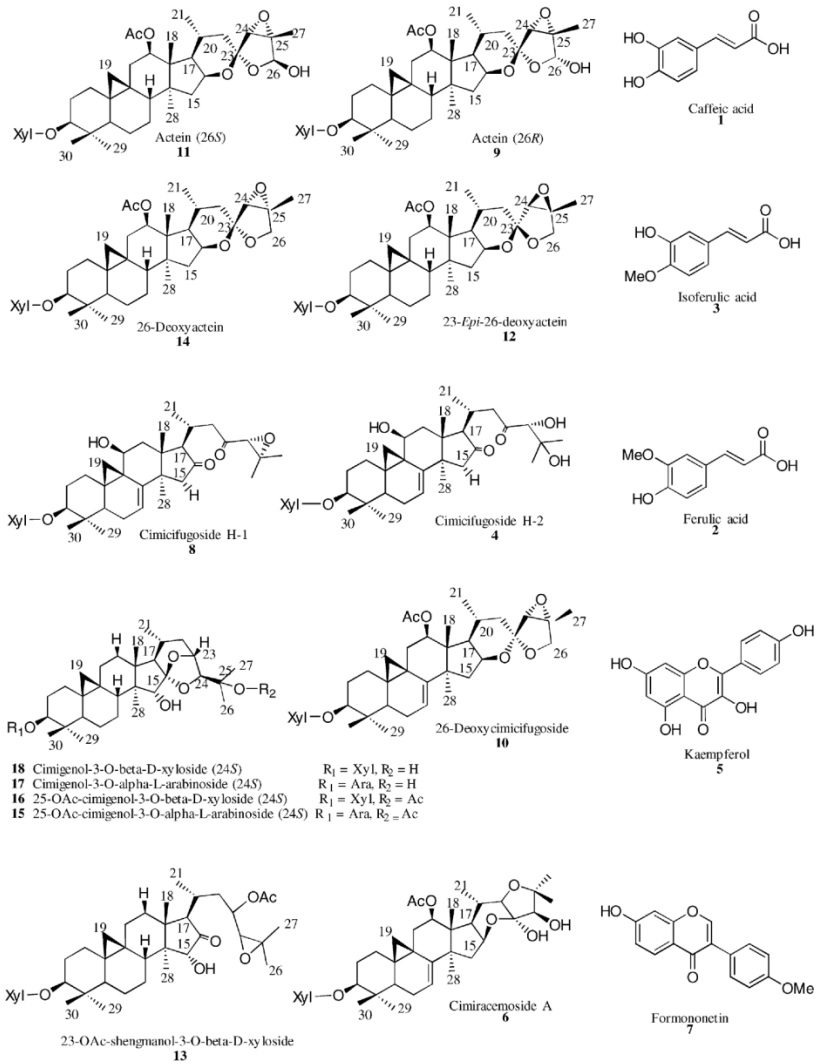

Figure 3. Marker compounds used for *Cimicifuga racemosa.*

2006). In a 2009 longitudinal clinical trial, no evidence was found to support hepatotoxic effects acting on postmenopausal women who continued use of *C. racemosa* for twelve months (Nasr and Nafeh 2009).

Contraindications

Though *Cimicifuga racemosa* is seen as relatively safe, there was an instance of forty-two possible cases of hepatotoxic effects being reported and investigated by the European Medicines Agency (Mazzanti 2008). A follow-up was conducted on four of the patients with the highest possibility of *C. racemosa* related hepatotoxicity. The results of this follow-up indicated no causality between the liver disease present in the patients and the continued usage of supplements from this plant (Teschke and Schwarzenboek 2009). Gastrointestinal upset and rash have also been reported. A possible association between *C. racemosa* extract performed in ethanol and hepatic mitochondrial toxicity (*in vivo* and *in vitro*) has been indicated, but this possible relationship is thought to be clinically irrelevant unless the patient taking supplements has underlying risk factors for liver disease (Lude et al 2007).

Current Use in Allopathic and CAM Therapies

Since the method of action is still unknown, marketed products that advertise *Cimicifuga racemosa* content typically consist of the powdered dried root or powdered root extract of this plant with no uniform composition across product lines. No single compound is recognized as a tell-tale sign of purity in the commercial black cohosh products, and as the supplements fall under the DSHEA act of 1994, they are not subject to quality assurance standards. Of the products that have made attempts at quality control, many limit still their focus on triterpene glycoside content. However, with this

limited basis of standardization, the large number of unknown and undocumented compounds being ignored in the process may be lacking and nowhere near the original content levels of the plant, itself (Zhou et al 2008). Though the search for the active compound of *C. racemosa* continues, steps are being taken to bring about some sort of standardization of products using 16-18 of the most common constituents of *C. racemosa* listed in **Figure 3** (Li et al 2002). Despite the lack of certainty in quality, sales of *C. racemosa* amounting to $79 million were reported in 2003 (Pojak et al 2006).

With many of the active compounds in black cohosh remaining a mystery, correct dosing for various ailments remain tricky as well. Experiments conducted by Powell et al in 2008, however, suggest that a 120 mg daily dose of *C. racemosa* could contain a therapeutic amount of N_{ω}-methylserotonin (3.7µg), sufficient enough to battle hot flashes and increase serotonin in postmenopausal women (Powell et al 2008).

Discussion

Though the scientific information on black cohosh is steadily developing, the long history that it has maintained as a helpful aid in women's health as well as other illnesses is sure to be a guide in future discoveries for the uses of this plant. Much more research is necessary to discover the definite method of action as well as a certainty of active chemical constituents, but the growing demand for black cohosh as a modern supplement for menopausal women as well as possible anti-HIV treatments and cures will also surely spearhead more investigative studies. Safety and purity also remain a concern, but with more research will come more knowledgeable and responsible uses of *Cimicifuga racemosa* supplements and products.

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Cinnamomum verum J. Presl, Lauraceae

Leah Demakovsky

Introduction

The recognizably spicy flavor of ethnic foods is often attributed to *Cinnamomum verum*, one of the major spices of the ancient world and one that is still commonly used today. There are several synonyms; *Cinnamomum zeylancium, Camphora mauritiana,* and *Laurus cinnamomum* are all recognized scientific names (Tropicos.org). In English the most common colloquial name for *Cinnamomum verum* is cinnamon, although it is also referred to as true cinnamon or Ceylon cinnamon, where the latter describes cinnamon grown specifically in Sri Lanka (Ravindra 2009). Cinnamon is a member of the Lauraceae family, or the laurel family (Tropicos.org). It has been used both in culinary practices and medicinally for centuries due to its cinnamaldehyde-containing volatile essential oils. It is a perennial tree that was first recorded by Redgrove in the 1200's (Ravindran, 2004). Cinnamon is invasive in many areas of the world, but because owning a cinnamon tree is a source of pride in places like the Union of the Comoros, many people are happy to have it grow (Vos, 2004). The spice is derived from the inner bark of the tree, and the oils are distilled from both the leaves and bark (Parthasarathy, 2008).

Figure 1. The *Cinnamomum verum* tree.
Source: (Yake, 2009)

Botanical Description

The *Cinnamomum verum* plant, shown in **Figure 1**, is an evergreen tree with green, oval shaped leaves, purple berry fruit and flowers, which are usually found in clusters (Parthasarathy, 2008). The bark is made up of quills, which are the source of the traditional ground spice. It is 0.2 -0.8mm thick and is darker on the inside of the bark than on outside. The leaves have a leathery texture and grow in an opposite pattern on the stem. They are typically 3.8-7.5 cm long and

Figure 2. The buds and flower of *Cinnamomum verum.*
Source: (Ambepitiyage, 2009)

7.5-20 cm wide and have more shine on the top than underneath the leaf. The flowers are silky in texture and yellow in color, as seen in **Figure 2**. The perianths can grow up to 5-6 mm and are longer than the inner tube of the flower, which only grow to about 2.5 mm in length. Even smaller are the fruit, which are only 1.3-1.7 cm in length and contain a single seed inside. They are both enclosed in an 8 mm purple bell shaped perianth (Cepae, 1999).

When ground, cinnamon is reddish brown in color. Cinnamon sticks are somewhat lighter than the ground plant, and are derived from peelings of the inner bark, which curl when dried This utilization of the cinnamon plant is the common form seen in culinary arts (Cepae, 1999).

Cinnamon is indigenous to Sri Lanka and parts of India but was introduced to Seychelles and Madagascar by the Dutch and British (Ravindra, 2009). However, the plant is invasive in other areas of the tropics, and can survive in areas that are more greatly affect by human disturbances (Vos, 2004). It has been especially invasive in the Combani in Mayotte and on the Mohéli mountains, as wells as some humid forests (Vos, 2004).

In Sri Lanka, locals have developed subclasses of the Ceylon cinnamon based on specific plant properties. *Pani kurundu* describes sweet, *pani miris kurundu* describes sweet and hot, *rasa kurundu* describes tasty, *suwanda kurundu* describes fragrant, and *tita kurundu* describes bitter cinnamon. Although they are all scientifically classified as the same *Cinnamomum verum*, differences in the soil and environment of Sri Lanka gives the cinnamon that is grown there a unique flavor (Ravindra 2009). Currently, cinnamon imports are primarily from Sri Lanka, but the plant is also grown in Africa, India, Indonesia, the Seychelles, South America and the West Indies (Cepae, 1999).

Traditional Uses

Medicinal Uses

Cinnamomum verum has been used extensively as a traditional medicine throughout the world. It was used as an anti-diuretic, a digestive aid, and as a treatment for eye infections, menstrual symptoms, inflammation of the kidney and intestines, stomachaches, and as a topical for stings and bites throughout Asia and Europe (Ravindran, 2004). The extracts of cinnamon have been used in India for the treatment of diabetes (Subash, 2007). It was a common part of Traditional Chinese Medicine, with uses in treating fever and diarrhea (Heinrich, 2004). Sho-seiryu-to, or Xian Qing Long Tang, is a mixture of several plant species including cinnamon bark that was used in the treatment of perennial allergic rhinitis, asthma, and other upper respiratory infections. Sairei, another

concoction used for the treatment of these illnesses, also contained cinnamon bark (Preedy, 2008).

Cinamomum verum had a wide use in Ayyurvedic medicine. In "The Indian Materia Medica" it is referenced as a treatment for flatulence and stomachic and was used as an antispasmodic, stimulant, astringent, antiseptic and antimicrobial. Each specific grouping of cinnamon has a different use; the names of the characteristics of cinnamon are similar to the Ceylon groupings. *Tikarasm* increases appetite and produces dry mouth, *ushnaveeryam* raises body temperature, increases circulation, increases appetite and increases digestion and *laghu* increases digestion. Each class was used in the treatment of a variety of ailments including anorexia, cardiac diseases, bowel dysfunctions, and helminthic infections (Thampuran, 2003).

Cinnamomum verum is also used in "folk" medicine for impotenence, frigidity, dysponea, eye inflammation, leucorrhoea, vaginitis, rheumatism, neuralgia, wounds and toothaches, although there are no studies to show that it is effective for any of these ailments (Ravindran, 2004).

Culinary Uses

Cinnamomum verum has been used as a spice in cooking for centuries. The oils extracted from the bark and leaves are used to flavor numerous foods, drinks, sauces, baked goods, and tobacco products (Flavours and Fragrances of Plant Origin, 2012). In Latin America, as well as many other places in the world, the quills are boiled in water and are drunk as a tea. In the United States it is used as a flavoring in the home as well as in the food industry (Ravindra, 2009). Cinnamon is commonly used in soaps, toothpastes, dentifrices and liquor flavoring, and is also used in the synthetic production of vanillin (Parthasarathy, 2008). It is an integral part of Indian curry and other traditional ethnic foods. Due to its antioxidant properties, cinnamon was a common food preservative before refrigeration was available (Parthasarathy, 2008).

Other Traditional Uses

Cinnamomum verum was extensively used as a fragrance by the ancient Egyptians in their embalming rituals (Heinrich, 2004). Cinnamon oils were commonly found in the perfumes worn by Egyptian men, and was also added to oils used as light to prevent harmful or bad smelling vapors. The spice was so highly prized by the Egyptians that Nero, the Emperor of Egypt in 66AD, was said to have burned a year's supply of cinnamon at his wife's funeral as a demonstration of his grief and sorrow (Ravindran, 2004).

Cinnamomum verum is mentioned several times in the bible and is one of the plants used in the holy anointing oil. It is also described in Rev. xviii. 13, "cinnamon, and odours, and ointments, and frankincense" (Grindon, 1883), giving light to some of the uses of cinnamon during the time period.

Traditional Importance

Cinnamomum verum has been traded extensively for centuries; there are records of its trade history during the Roman era. It was passed to the Arabs who tried to keep its original location a secret from other traders, causing European explorers to search for the plant during the 1400s (Ravindra, 2009). Herodotus, the "father of history", described the cinnamon dilemma, and stated that the Arabs did not know of its origins. The secrecy caused it to be one of the first plants which oriental traders searched extensively (Grindon, 1883). When it was found in Sri Lanka, the country was

eventually taken over by the Portuguese, Dutch and British who propagated cinnamon cultivation (Ravindra, 2009).

Chemistry and Pharmacology

While *Cinnamomum verum* has over one hundred chemical components, cinnamaldehyde is the chemical responsible for the strong smell and distinct flavor. Cinnamaldehyde, shown in **Figure 3** alongside eugenol, is an alkaloid with an aromatic ring that acts as a strong antioxidant by inhibiting amino acid decarboxlyase activity (Gupta et. al., 2008). Eugenol is a phenylpropene that is found in cinnamon leaves, giving them a less appealing odor than the bark which contains much higher concentrations of cinnamaldehyde (Flavours and Fragrances of Plant Origin, 2012).

The bark contains 6,000-30,000 parts per million of cinnamaldehyde (Duke, 1996), which is its primary constituent. Mucilage is another constituent in high amounts; there are 20,000-37,000 parts per million found in the bark (Duke, 1996). Volatile components are found in every part of the plant and consist of monoterpenes, sesquiterpenes and phenylpropenes (Parthasarathy, 2008). More specifically, these volatile oils include B-caryophyllene, linalool, safrole, cinnamic aldehyde, cinnamyl acetate, cinnamyl alcohol and benzly benzoate (Ravindran, 2004). The primary constituents of the extracted volatile oils are cinnamaldehyde and eugenol, which make up 65-80 % and 10% of the oil, respectively (Cepae, 1999).

In the United States, the Fragrance Materials Association (FMA) monograph uses percent eugenol solubility in potassium hydroxide to regulate the standard of cinnamon; it must be 80-88% soluble (FMA, 1992).

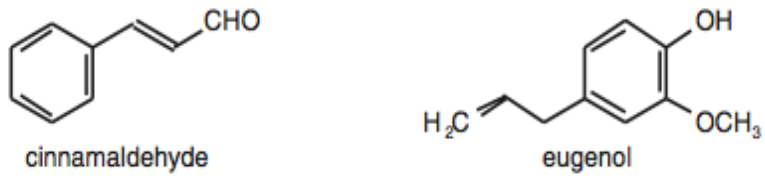

Figure 3. The chemical structure of cinnamaldehyde, the primary constituent of *Cinnamomum Verum* and Eugenol, one of the main chemical components of cinnamon oils.
Source: (Cepae, 1999).

A recent study used matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry to determine the proanthocyanidins in cinnamon in order to better understand the chemical components, which yield cinnamon's medicinal properties. It was found that the proanthocyanidins contain (epi)catechin, (epi)catechingallate, (epi)gallocatechin, and (epi)afzelechin (Mateos-Martin, 2012).

The chemical components of *Cinnamomum verum* (**Table 1**) can aid with the identification of the plant when compared to other species of *Cinnamomum. Cinnamomum verum* contains less than 2% foreign organic matter, less than 6% ash, with less than 4% of this ash being insoluble in acidic solutions. There also must be less than 6% sulfated ash due to government regulations on the treatment of plant products with sulfides. Lastly, *Cinnamomum verum* extracts have a 14-16% solubility rate in 90% alcohol solutions (Cepae, 1999). Other species of *Cinnamomum* have different percentages of these components, which may affect their different chemical properties.

Chemical Constituent in Bark	Amount	Chemical Constituent in Bark	Amount	Chemical Constituent in Bark	Amount
1,8 Cineole	165- 800ppm	Fiber	270,000 ppm	Trans-Linalol-Oxide	5-20 ppm
2-Vinylphenol	3-12 ppm	Furfural	3-12 ppm	Zinc	11.4-20 ppm
3-Phenyl-Propyl-Acetate	13-52 ppm	Gamma- Terpinene	3-12 ppm		
Acetoeugenol	16-64 ppm	Geraniol	6-24 ppm	**Chemical Constituents in Leaf**	**Amount**
Alpha-Pinene	20-236 ppm	Humulene	20- 124 ppm	Chromium	14.4 ppm
Alpha-Terpineol	40-264 ppm	Hydrocinnamaldehyde	40-160 ppm	Cobalt	1.1ppm
Alpha-Ylangene	31-124 ppm	Iodine	3 ppm	Copper	10.9 ppm
Ascorbic-Acid	309 ppm	Iron	60- 421 ppm	Manganese	101.6 ppm
Barium	60 ppm	Isoeugenol	2-8 ppm	Nickel	4.2 ppm
Beta-Carotene	1-2 ppm	Limonene	46- 184 ppm	Zinc	34 ppm
Beta- Pinene	14-76 ppm	Linalol	230- 956 ppm		
Borneol	2-8 ppm	Manganese	66-140 ppm	**Chemical Constituents in Plant**	**Amount**
Bornyl-Acetate	10-20 ppm	Mucilage	20,000- 37,000 ppm	2-Phenylacetaldehyde	
Boron	7-15 ppm	Myrcene	5-20 ppm	Alpha-Terpinene	42-168 ppm
Bromine	10 ppm	Niacin	8 ppm	Benzaldehyde	26-104 ppm
Calcium	5,329-6,000 ppm	Nickel	1.1 ppm	Peperitone	7-28 ppm
Camphene	18-72 ppm	P-Cymene	55-448 ppm		
Caryophyllene	135-1,316 ppm	Phellandrene	63-252 ppm		
Chlorine	300 ppm	Phenyl-Ethyl-Alcohol	41- 164 ppm		
Chromium	2-10 ppm	Phenylethyl-Acetate	7-28 ppm		
Cinnamaldehyde	6,000-30,000 ppm	Phosphorus	674- 1,100 ppm		
Cinnamyl-Acetate	510- 2,040 ppm	Potassium	5,525-6,000 ppm		
Cinnamyl- Alcohol	26- 104 ppm	Protein	35,000- 43,000 ppm		
Cis-Ocimene	3-12 ppm	Riboflavin	1 ppm		
Cobalt	0.6ppm	Rubidium	20 ppm		
Copper	4.9-9 ppm	Sabinene	2-8 ppm		
Cumene	66-264 ppm	Sodium	287 ppm		
Cuminaldehyde	4-100 ppm	Strontium	80 ppm		
Delta-3-Carene	3-12 ppm	Sulfur	1,900 ppm		
Eo	40,000 ppm	Terpinen-4-Ol	36-144 ppm		
Eugenol	220-3,520 ppm	Terpinolene	11-44 ppm		
Farnesol	3-12 ppm	Thiamin	1 ppm		
Fat	14,000 ppm	Titanium	40 ppm		

Table 1. The most abundant chemical components of *Cinnamomum verum* bark, plant, and leaves. Source: (Mathew, 2006; Parthasarathy, 2008)

Figure 4. A study examining the antimicrobial activity of *Cinnamomum verum* against common food borne bacteria demonstrated cinnamon's effectiveness across a broad range of bacteria. The essential oils were found to be particularly effective against *Bacillius cereus*, as shown above. Adapted from Gupta et al., 2008.

Biological Activity

Cinnamaldehyde, the primary constituent in *Cinnamomum verum,* has been found to have antiallergenic, antiulcerogenic, antipyretic, antimicrobial, anesthetic and antimutagenic properties (Mathew, 2006). There are also numerous antibiotic uses; the essential oils have been found to act as an antibacterial *in vitro* against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa (Ravindran, 2004).* The oil has also been found to act as an antifungal *in vitro* against *Aspergillus* spp., *Cladosporium werneckii, Geotrichum candidum, Kloechekera apivulata, Candida lipolytica* and *C. albicans (Ravindran, 2004).* Additionally, a study examining the effects of cinnamon oil and extracts found both to increase the zone of inhibition of several food born bacteria. Cinnamon had the greatest effect against *Bacillus cereus*, as shown in **Figure 4** (Gupta et al., 2008).

With the increasing problem of antibiotic resistance causing a need for new therapies, cinnamon has been studied for its antibacterial effects on resistant species of bacteria. The essential oils of cinnamon were found to decrease the minimal inhibitory concentration of amikacin needed to kill resistance species in the *Acinetobacter* genus (Guerra, 2011).

Many of the biological activities seen when utilizing cinnamon have been attributed to cinnamaldehyde, methoxycinnamaldehyde, and other compounds found in high concentrations in the bark and oil (Ravindran, 2004). Cinnemaldehyde's phenolic compounds act as an electron donor and catalyzes the reaction of hydrogen peroxide to water (Gupta et al., 2008). Other studies have attributed many of the beneficial properties of cinnamon to the phenolic proanthocynidins also found in the essential oil (Cabello, 2009). The cinnamic aldehyde found in the essential oil contains phenloic proanthocyidins, which have been found to inhibit growth of melanoma *in vitro* and *in vivo* (Cabello, 2009).

Cinnamaldehyde also acts as an antispasmodic and has been found to decrease histamine and barium induced smooth muscle contractions in guinea pig trachea and ileum (Reiter 1985).

The methanolic extracts of cinnamon have been shown to have antioxidant properties and act as a hydroxl radical scavenger, act as a reducing agent, and have demonstrated metal ion chelating activity. The cinnamon extracts act as a transition metal stabilizer which prevents the perpetuation of the formation of new free radicals in the body (Mathew, 2006). The phenolic compounds can act as electron donors to catalyze the reaction of H_2O_2, a toxic substance, to H_2O (Mathew, 2006).

Clinical Studies

Several species of *Cinnamomum* have been used as medicine throughout history. Cinnamon and its active ingredients are generally regarded as safe by the 'Flavor and Extract Manufacturers' Association (FEMA no. 2286, 2201) (Cabello, 2009). Although many studies fail to distinguish which sub-species within the *Cinnamomum* genus were used, there are numerous studies indicating *Cinnamomum verum* can have health benefits. Similarly, there have been\many clinical studies involving the efficacy of using cinnamon as a treatment for several diseases, especially type 2 diabetes and metabolic syndrome. Unfortunately, there are no current studies examining just *C. verum* in humans.

Sairei, the traditional Chinese remedy containing cinnamon bark used to treat upper respiratory infections has been found to inhibit basophil growth and histamine content *in vivo* (Preedy, 2008), however, there is some uncertainty regarding the species of *Cinnamomum* used and if the results can be directly attributed to the components of the cinnamon rather than the other ingredients added. This finding explains the results that people have been using for centuries, and although it is not a human clinical trial, has indications for use in humans.

A study conducted in India found that cinnamaldehyde reduces blood glucose levels in steptozotocin induced diabetic rats and thus has hypoglycemic and hypolipidemic effects (Subash, 2007). This study was important for diabetes research because it identified cinnamaldehyde as the component of cinnamon that decreased plasma glucose levels, or acted as an antidiabetic.

Although this study did not directly involve humans, the rat model is a fairly accurate indicator. Studies similar in nature have been conducted with humans. However, these studies often do not specify the type of species used, or more commonly or use a mixture of more than one species. Over all, the lack of causal evidence indicates that more clinical studies are needed to determine the specific effects of *C. verum* in humans.

Contraindications

Although *Cinnamomum verum* has numerous health benefits, Italy and Germany blocked some importation of cinnamon from Sri Lanka due to high sulfur content. Cinnamon does not have a high sulfur content naturally, but is often fumigated with sulfur dioxide during processing as a preservative which coats the cinnamon in a sulfur residue. The acceptable maximum amount of sulfur is now regulated under the Codex Alimentarius Commission (CAC). Until 2006 the CAC did not have a standard maximum residue limit, and the level acceptable in Sri Lanka was more than the level acceptable in

the European countries, leading to the block of importation in certain parts of Europe (Ravindra, 2009). Although there are limits in place currently, it is not known how the sulfur fumigation affects the medicinal properties of the plant.

Few cases of fever of unknown origin, pregnancy, stomach ulcers, and duodenal ulcers have been indirectly associated with the use of cinnamon (Bisset, 1994; Ravindran, 2004). Reactions to cinnamon have occurred in individuals who have allergies to active ingredients in cinnamon or Peru balsam (Ravindran, 2004). The "Indian Materia Medica" states cinnamon can cause irritation and can be poisonous in large quantities (Thampuran, 2003). When used in food as a flavor additive, the daily intake should not exceed 700 μg per kg of body weight per the World Health Organization and Food and Drug Administration guidelines (Thampuran, 2003). Occasionally the cinnamaldehyde can cause skin or mucus membrane irritation when used as a perfume, or in toothpastes. Oral lesions after chewing cinnamon gum are rare but have been reported in those with sensitivity to cinnamaldehyde or cinnamon (Thampuran, 2003).

The use of cinnamon in children has not been extensively studied. There are no studies indicating that *C. verum* is unsafe for use during pregnancy, nursing, or with certain other drugs. However, studies have indicated other species of *Cinnamomum* do have associated risks (Ravindran, 2004). Since *C. verum* has unique chemical properties, it does not mean that all *Cinnamomum* has these risks, although one study did find cinnamaldehyde to be teratogenic in chick embryos (Keller 1999).

Current Use in Allopathic and CAM Therapies

The use of *Cinnamomum verum* as a complementary and alternative medicine (CAM) therapy may be its most well known medicinal use. There are few drugs on the market involving cinnamon or the active ingredients in cinnamon in the United States however clinical trials are investigating the indigenous use of cinnamon to treat diabetes as well as a variety of other diseases (Ravindran, 2004), Currently, there is an herbal treatment for inflammation of the eye called *Ophthacare* which contains 0.5% cinnamon. When tested in rabbits, it was found to be effective (Thampuran, 2003). The bark and oil are included in drug cocktails as a carminative or flavoring in several countries including India, Britain, China, Australia, Belgium, Europe, France, Germany, Hungary, Japan, the Netherlands, Portugal and Switzerland (Thampuran, 2003).

Several studies have identified cinnamon as a CAM therapy throughout the world. One study in Australia surveyed diabetics and found that one-fourth of individuals with diabetes mellitus who used CAM therapy used cinnamon. However their sample size was small providing the need for a larger study to confirm results. There were no significant correlations between gender, age, income and severity of diabetes and using cinnamon as a CAM treatment. (Manya, 2012). Although the study uses the scientific name *C. verum* in its report, the common name cinnamon was used on the questionnaire, meaning there was no way to know exactly what species of cinnamon was used. There was also no question regarding the amount of cinnamon taken or the frequency. It is assumed that the powdered (ground) form of cinnamon was used as the supplement.

A larger study found that thirty percent of individuals in Bahrain with diabetes mellitus who used CAM therapies used cinnamon for the treatment of their diabetes (Khalaf, 2010). This study also did not have a way to identify the species of cinnamon used or the amount taken by the patients, but its

results were similar to studies previously done on several world populations including the United States. Another even larger study conducted in Israel found 10.8% of diabetics used cinnamon as a CAM remedy, but most individuals were from refugee camps (Ali-Shtayeh, 2012). A study using a random sample is needed to confirm results.

The World Health Organization estimates the average daily dose of cinnamon taken by those who use cinnamon for medicinal purposes is about 2- 4 grams per day when used ground, or 0.05-0.2 grams when the volatile oil is used (Ravindran, 2004).

Throughout Africa and other developing countries, the lack of legal, safe and affordable abortion or birth control services lead to the development of systemic abortifacients. Boiled wine, raisins and cinnamon is drunk as an oral abortifacient, but is not considered to be safe and the efficacy has not been studied (Warriner, 2006).

Discussion

Cinnamomum verum has a vast variety of uses and the potential for even more applications. There is an immense cultural value associated with the plant due to its significance in earlier centuries and its importance in many ethnic foods around the globe. Originating in Sri Lanka, the plant has become invasive in many areas as it can outcompete other species in a variety of climates. Cinnamaldehyde, the primary chemical constituent in cinnamon, has shown great promise in the treatment of diabetes and other chronic illnesses. There is, however, some controversy regarding the use of cinnamon. In ancient writings the exact species of cinnamon was not always stated, so the traditional benefits derived from the use of the plant may have been caused by the chemicals unique to other species of *Cinnamomum.* Even today, there are numerous studies that fail to properly ensure that the substance they are using is *C. verum* and not a cheaper species labeled as such. Studies also fail to mention which species of *Cinnamomum* they used and simply refer to the plant as "cinnamon" despite the numerous differences in chemical constituents.

Further, due to the many methods of extracting the oils of the bark and leaves, there is a high chance that one sample of oil may not match another sample chemically. Though they will still contain the same basic constituents, the varied concentrations of such can yield entirely different effects. Also, since the plant is grown in regions of the world with differences in environment, the concentration of chemicals may also vary from region to region (Flavours and Fragrances of Plant Origin, 2012). These differences are roadblocks in research involving cinnamon, but one that can be overcome as technology advances.

Currently, scientists are attempting to cultivate cinnamon with increased concentrations of cinnamaldehyde and eugenol in order to use cinnamon more widely in the treatment of disease. The major problem with cinnamon is that it is widely available but 68% of patients in the Israeli diabetes study did not report using cinnamon to their doctor, although this is far too common in many CAM remedies (Ali-Shtayeh, 2012). This has the potential for unknown medical complications. More research will be necessary before these plants can be utilized, but the future for cinnamon looks promising (Flavours and Fragrances of Plant Origin, 2012). Until then, it will be grown and used as the spice the world has come to love.

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Coffea arabica L., Rubiaceae

Alison Yarp

Introduction

Coffea arabica L., known to most of the world as coffee, is a member of the Rubiaceae family. It is also known as the "coffee shrub of Arabia" (Weinberg & Bealer, 2001), café, and multiple other regional names (I. A. Ross, 2005). *C. arabica* is the oldest known cultivated species of coffee (Weinberg & Bealer, 2001). Originally from the Ethiopian mountains, it is now cultivated in the subtropics and high altitude tropics and now grows naturally in many of these environments. There are 25 to 100 species in the genus *Coffea*, but *C. arabica* is one of the two main species currently being used in coffee production – the other being *C. canephora* (Rieger, 2006). However, *C. arabica* makes up about 75% of the worldwide coffee consumption (Weinberg & Bealer, 2001). One of the main derivatives, caffeine, is a potent stimulant, and is responsible for most of the effects, which includes central nervous system stimulation, as well as stimulation of other body systems (Kraft & Hobbs, 2004).

Figure 1. *Coffea arabica.* Source: http://www.gutenberg.org/files/28500/28500-h/28500-h.htm

Botanical Description

Coffea arabica is a small evergreen tree and can grow up to 30 feet in the wild, but is generally maintained at 6 to 8 feet (Rieger, 2006) for easier harvesting (Weinberg & Bealer, 2001). It is cultivated primarily in tropical and subtropical countries including Brazil, Colombia, Mexico, and Guatemala (Khan & Abourashed, 2010). They are considered an "upland" species, so the ideal places for growth are at 5000 to 7000 feet at the equator. In the subtropics, it grows well at sea level (Roecklein & Leung, 1987). *C. arabica* growth can be hindered above 77°F, and frosts can kill the leaves and fruits (Francis). However, for a tropical plant, *C. arabica* is still resilient at lower temperatures (Weinberg & Bealer, 2001). The appropriate amount of rainfall is about forty to sixty inches throughout the year (Weinberg & Bealer, 2001).

For a picture of *Coffea* arabica, see **Figure 1**. C*. arabica* can grow as a single shoot, but it will often branch off into multiple

stems towards the bottom (Francis). The bark of the tree is an ash white (*PDR for herbal medicines*, 1998). The leaves are dark green with an oval shape (Francis). They are about half a foot long and 1 to 2 inches wide (*PDR for herbal medicines*, 1998). They grow in pairs opposite each other on the stems. The dorsal sides have a "smooth and shiny" appearance with obvious veins (Francis) and resemble leather (*PDR for herbal medicines*, 1998). These leaves can live about 2 to 3 years.

The flowers on *C. arabica* are white and fragrant (Francis), with 5 petals coming out of a slender tube shaped base (Rieger, 2006). Up to 20 flowers will grow on an axil, and they each contain two ovules, allowing for possibly two seeds to form (Rieger, 2006). Flowers that open when it is dry and sunny generally produce more fruit than those that open on a wet day because of the increased pollination by wind and insects (Weinberg & Bealer, 2001). *C. arabica* is self pollinating and can even pollinate itself when the flowers are closed (Rieger, 2006). However, it also benefits from cross pollination by bees (Klein, Steffan-Dewenter, & Tscharntke, 2003).

The fruit, also known as berries or drupes, of *C. arabica* is elliptical and is flat when observed by cross section. They are about 12 to 18 mm long and 12 to 15 mm wide. They are green until they become ripe, at which point they are yellow and dark red (*PDR for herbal medicines*, 1998). Because of the color of the berries after they mature, farmers and processors commonly refer them to as cherries. Under the skin is a sweet and moist fleshy pulp, which is commonly eaten (Weinberg & Bealer, 2001). The fruits generally contain two seeds, or beans, which are between 8 and 12 mm long. They have one rounded and flattened end and have a groove on one of the sides (Francis). Depending on the individual characteristics and the climate conditions, a single tree can yield from one to twelve pounds of dried beans in a year (Weinberg & Bealer, 2001).

Traditional Uses

Medicinal

Depending on the region, *Coffea arabica* has many uses. As seen by **Table 1**, these uses range from inducing labor to treating headaches (I. A. Ross, 2005). In Brazil, the seed is decocted and taken orally to treat influenza. In Cuba, a hot water extract of the seed is taken orally as an aphrodisiac for males (I. A. Ross, 2005). Haitians have multiple uses of the plant: grilled decoction of the fruit and leaf is taken orally for anemia, fruit is eaten for hepatitis and liver troubles, soaked fruit is applied externally for nervous shock, and the leaf is decocted and taken orally or applied externally for headache (I. A. Ross, 2005). In Mexico, the leaf is made into a poultice and applied externally for fever, and the hot water extract of a roasted seed is taken to increase milk production in nursing mothers. Nicaraguans also have many ways of using coffee. They use the leaf externally to treat headache and orally as a hot water extract to help with stomach pain. They also use the decoction of the seed orally for fever and externally for cuts and hemorrhage (I. A. Ross, 2005). In Uganda, a decoction of the leaves is used to treat aerophagia and a cough (Lacroix et al., 2011). In Peru, a hot water extract of dried fruit is used orally for sleepiness and drunkenness, while the leaf is used to induce labor or as an antitussive in flu and lung sicknesses. The Thai use the hot water extract of the dried seed orally as a cardiotonic or neurotonic. Lastly, in the West Indies, the hot water extract of the seed is used orally for asthma, and juice from the root is taken orally for scorpion stings (I. A. Ross, 2005).

While not necessarily just used to treat ailments, coffee has also been used by many cultures as a general stimulant, to

Country	Plant Part	Preparation	Route	Use
Brazil	Seed	Decoction	Oral	Influenza
Cuba	Seed	Hot water extract	Oral	Aphrodisiac (males)
Haiti	Fruit and leaf	Grilled Decoction	Oral	Anemia, edema, asthenia, rage
Haiti	Fruit	-	Oral	Hepatitis, liver trouble
Haiti	Fruit	Soaked	External	Nervous shock
Haiti	Leaf	Decoction	Oral, External	Headache
Mexico	Leaf	Poultice	External	Fever
Mexico	Seed	Roasted Hot water extract	Oral	Increase milk production in nursing mothers
Nicaragua	Leaf	-	External	Headache
Nicaragua	Leaf	Hot water extract	Oral	Stomach Pain
Nicaragua	Seed	Decoction	Oral	Fever
Nicaragua	Seed	Decoction	External	Cuts, Hemorrhage
Peru	Fruit	Dried Hot water extract	Oral	Sleepiness, drunkenness
Peru	Leaf	Infusion	Oral	Induce labor
Peru	Leaf	Hot water extract	Oral	Antitussive in flu and lung ailments
Thailand	Seed	Dried Hot water extract	Oral	Cardiotonic, neurotonic
West Indies	Seed	Hot water extract	Oral	Asthma
West Indies	Root	Juice	Oral	Scorpion sting

Table 1: Traditional Medicinal Uses of *Coffea arabica*
Source: (I. A. Ross, 2005)

increase alertness and improve productivity (Chevallier, 1996). For example, the average daily consumption of a coffee drinker in the United States is 3.1 cups (Bisht & Sisodia, 2010).

Food

Coffee has been consumed as a beverage for about 1000 years (Chevallier, 1996), and it has become the most consumed functional food around the world (Bisht & Sisodia, 2010). In the United States, 68% of people over the age of 10 consume coffee, and in 1969, 2.8 billion pounds of coffee, which equals approximately 150 billion cups (Lewis & Elvin-Lewis, 2003). The main importers of coffee are the United States, Germany, and France (Roecklein & Leung, 1987), while the main producers are Brazil and Columbia (Bisht & Sisodia, 2010).

C. arabica has become a competitive crop in Costa Rica and is important to the nation's livelihood. To maintain this, they have to produce enough of it in a reasonable amount of time. Small farm owners did this traditionally, but today this is moving towards large plantations (Weinberg & Bealer, 2001).

Gourmet coffees are generally high quality varieties of *C. arabica* without the harsh or hard taste. They mainly come from Latin America, except Brazil (Weinberg & Bealer, 2001). However, they can also come from Kenya, Tanzania, Ethiopia, Cameroon, and Yemen. Fine coffee used to come exclusively from Yemen and would be shipped globally through the port of Mocha. After World War I, the wells in Mocha dried up, so the coffee cultivation there was almost completely abandoned. Small amounts of coffee still come from Yemen through the port of Hodeida, but it is still referred to as Mocha, as this name is still engrained in people's minds as the best coffee

beans (Weinberg & Bealer, 2001). Regarding Brazil, most coffees coming from this country are *Coffea arabica*, but they

are known to have a less refined flavor and aroma than the milder forms (Weinberg & Bealer, 2001).

Other

Other uses have included various parts of the tree. The bark can be made into parchment, mulch, manure, and pulp, which is occasionally fed to cattle in India (Roecklein & Leung, 1987).

Chemistry and Pharmacology

A list of the chemical constituents of *Coffea arabica* can be seen in **Table 2** (Duke). However, the more important ones out of the list are the purine (or also known as xanthine) alkaloids, theobromine, theophylline, caffeic and ferulic acid esters of quinic acids, no-diterpine glycoside esters, trigonellines, and diterpenes (Kraft & Hobbs, 2004). Among these, the notable ones are caffeine, chlorogenic acid, theobromine, and theophylline, especially for their medicinal and physiological effects. Caffeine, a purine alkaloid, can be seen in **Figure 2** and can range in concentration from 600 to 32000 parts per million (ppm) (Duke). In an even greater concentration is chlorogenic acid, with a range of 50000 to 80000 ppm (Duke). Because of the high concentration of chlorogenic acid, estimates have been made that state that the intake of chlorogenic acid is many times higher for people who consume coffee on a regular basis, especially when compared to non coffee consumers (Bisht & Sisodia, 2010). Theobromine and theophylline are present as well in 35 to 40 and 7 to 23 milligrams per kilogram, respectively (Weinberg & Bealer, 2001). Regarding how some of the other constituents affect *C.* arabica, 2-furfurylthiol, 4-vinylguaiacol, alkyl pyrazines, furanones, acetaldehyde, propanol, methylpropanol,

Chemical Constituents in *Coffea arabica*	
Plant Cyanidin, P-coumaric Acid, Sinapic Acid **Fruit** Caffeic Acid, L-Asparagine, L-Aspartic Acid, Oxalic Acid **Leaf** Allantoic Acid, Allantoin, Beta-Carotene, Caffeic Acid, Calcium, Carbohydrates, Fat, Fiber, Iron, Niacin, Phosphorus, Protein, Riboflavin, Theobromine, Theophylline **Seed** 2,3,5-trimethylphenol, 2-ethylphenol, 2-methoxy-4-ethylphenol, 24-methylene-cycloartenol, 24-methylenephenol, 3,4-dicaffeoyl-quinic-acid, 3,5-dicaffeoyl-quinic-acid, 4,5-dicaffeoyl-quinic-acid, 4-ethylphenol, 4-methoxy-4-vinylphenol, 5-avenasterol, 7-stigmasterol, Acetaldehyde, Adenine, Alpha-tocopherol, Arabinogalactose, Arabinose, Arachidic Acid, Ash, Asparagine, Aspartic Acid, Atractyligenin, Beta-sitosterol, Beta-tocopherol, Cafesterol, Cafestol, Caffeine, Caffeol, Caffeoyl-3-Quinic Acid, Caffetannic Acid, Cahweol, Calcium, Campestanol, Campesterol, Caprinic Acid, Carbohydrates, Carnaubic Acid, Cellulose, Chlorogenic Acid,	Cholestanol, Cholesterol, Choline, Citric Acid, Citrostadienol, Caffeoyl-3-Quinic Acid, Caffetannic Acid, Cahweol, Calcium, Campestanol, Campesterol, Caprinic Acid, Carbohydrates, Carnaubic Acid, Cellulose, Chlorogenic Acid, Cholestanol, Cholesterol, Choline, Citric Acid, CitrostadienolCoffeasterol, Cycloeucalenol, Cysteine, Cystine, Daturic Acid, Dextrins, Dihydrolanasterol, Dihydrositosterol, EO, Eugenol, Fat, Fiber, Fufuryl Alcohol, Furfuraldehyde, Galactan, Galactomannan, Gamma-sitosterol, Gamma-tocopherol, Glucogalactomannan, Glutamic Acid, Guaiacol, Guanosine, Hemicellulose, Homocellulose, Hydrogen Sulfide, Hypoxanthine, Iron, Isochlorogenic Acid, Isoeugenol, Kahweol, Lanosterol, Lignoceric Acid, Linoleic Acid, Linolenic Acid, M-Cresol, Mannan, Mannose, Methionine, Myristic Acid, N-Nonacosane, Niacin, Nitrogen, O-Cresol, O-Xylenol, Obtusifoliol, Oleic Acid, P-Cresol, P-Xylenol, Palmitic Acid, Pectin, Pentosane, Pentosans, Phosphorus, Phytate, Phytosterols, Polyamines, Protein, Putrescine, Raffinose, Rhamnose, Riboflavin, Saccharose, Salicylates, Scopoletin, Spermidine, Spermine, Squalene, Stachyose, Stearic Acid, Stigmasterol, Sugar, Tannic Acid, Tannin, Tetracosic Acid, Theobromine, Theophylline, Thiamin, Trigonelline, Water, Xanthine, Xylan

Table 2. Chemical Constituents in *Coffea arabica*
Source: (Duke)

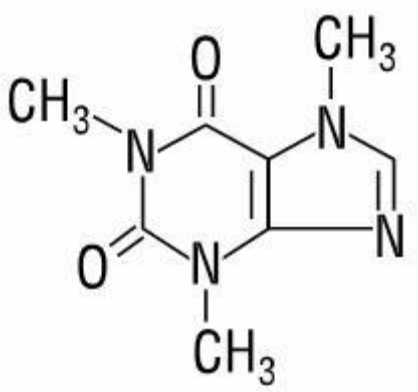

Figure 2 Structure of Caffeine. Source: http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=16514

2-methylbutanol, 3-methylbutanol have greatest impact on coffee flavor (Czerny, Mayer, & Grosch, 1999). Also, caffeine is actually a defense mechanism for *C. arabica*. It has the capability of destroying harmful bacteria and fungi and sterilizing certain dangerous insects. If it has inhabited an area for a long period of time, it could possibly inhibit weed growth as well (Weinberg & Bealer, 2001).

Biological Activity

Most of the biological activity in *Coffea arabica* stems from the presence of caffeine (Khan & Abourashed, 2010). It's stimulant properties are responsible for stimulating the central nervous system, respiratory system, skeletal muscles, and performing cardiac stimulation, arterial dilation, smooth muscle relaxation, and acting as a diuretic (Khan & Abourashed, 2010). Also, it has been observed that within 1 hour of having 250 mg of caffeine, non-coffee drinkers tend to have an average increase of 10 mmHg in systolic blood pressure (Kraft & Hobbs, 2004).

Caffeine works by binding to adenosine receptors and blocking phosphodiesterase (Wyk & Wink, 2004). As stated previously, it can affect many parts of the human body. In the cardiovascular system, it has a positive inotropic effect on the heart and a positive chronotropic effect on the sinoatrial node, which causes increased heart rate, contraction force, and a greater cardiac output (Walter & Maheswari, 2006). It can also cause vasoconstriction in the cerebral vasculature, which decreases the blood flow and the oxygen tension of the brain (Walter & Maheswari, 2006). For some patients, this could provide relief from headache pain (Walter & Maheswari, 2006). In the gastrointestinal system, caffeine initiates the secretion of pepsin and gastric acid from the parietal cells

(Walter & Maheswari, 2006). In the renal system, caffeine causes an increase in renal blood flow and the glomerular filtration rate, while decreasing the reabsorption of sodium and water by the proximal tubules. This can create mild diuresis (Walter & Maheswari, 2006). Caffeine also impedes uterine contractions and transiently increases plasma glucose with stimulation of glycogenolysis and lipolysis (Walter & Maheswari, 2006).

Studies have shown that the methylxanthines in coffee can help with respiratory issues. Theophylline, an example of a xanthine, is present in *C. arabica* at about 7 to 23 milligrams per kilogram, and has been used as a prescription medication for the treatment of asthma (Bisht & Sisodia, 2010).

In vitro & ex vivo

One study performed on *Coffea arabica* was done both in vitro and ex vivo and was looking at the antioxidant and protective

properties of green and roasted coffee. For the in vitro part of the study, the model system was β-carotene-linoleic acid (Daglia, Papetti, Gregotti, Berte, & Gazzani, 2000). According to the study, the green coffee had a higher antioxidant activity than the roasted (Daglia et al., 2000). However, when looking at the protective activity against rat liver cell microsome lipid peroxidation, the roasted coffee performed better than the green coffee (Daglia et al., 2000).

Clinical Studies

Blood Pressure

A clinical study observing the amount of coffee consumed and increase in blood pressure was performed on 522 subjects. The average amount of coffee consumed per day over a median period of 56 days. After linear regression analysis, a positive relationship between amount of coffee consumed and systolic blood pressure appeared (Jee, He, Whelton, Suh, & Klag, 1999).

Caffeine and Asthma

The methylxanthines in coffee have the capability of functioning as bronchodilators. This idea was tested by looking at data from the Second National Health and Nutrition Examination Survey (NHANES II), which was taken from a sample representing the civilian United States population. The possibility of relationships between normal coffee and tea intake to asthma and wheezing symptoms was determined. After comparison to non-coffee drinkers, the results showed that people who consumed coffee regularly saw a 29% decrease in the odds of presenting with current asthma symptoms. An inverse relationship appeared between the cups of coffee consumed per day and the occurrence of asthma, while tea consumption did not have a significant relationship to asthma prevalence (Schwartz & Weiss, 1992).

Caffeine and Analgesics

Over the last twenty years, thirty clinical studies with over 10,000 patients have been performed, and the data from these was studied to see if caffeine was useful as an analgesic adjuvant. Most of the studies involved patients with postpartum uterine cramping or episiotomy pain, but some had patients with pain from a headache or oral surgery. The results showed that to get the same response from an analgesic without caffeine, one would have to take about 40% more than one with caffeine (Laska et al., 1984). Based on this study, it was determined that a reasonable conclusion would be to add 65 milligrams of caffeine to an analgesic tablet to create a more effective analgesic. According to the study, the analgesic with caffeine has the capabilities of being an important option for therapy and management of a patient's pain (Laska et al., 1984).

Caffeine Consumption and Spontaneous Abortion

A population based study of early spontaneous abortion was performed with 562 Swedish women who had a spontaneous abortion, or miscarriage, between six and twelve weeks gestation and 953 Swedish women who did not have an abortion. The goal of the study was to see if caffeine consumption could increase the risk of an early spontaneous abortion in non-smoking women carrying fetuses with normal karyotypes. Among the non-smoking women, there were an increased number of occurrences of spontaneous abortions in the women who consumed at least 100 milligrams of caffeine per day than in women who ingested less than that. With the

smoking women, no association between caffeine ingestion and increased risk of spontaneous abortion was found. The conclusion was made that consumption of a moderate to high amount of caffeine correlated with an increased risk of spontaneous abortion when the fetus had a normal or unknown karyotype (I. A. Ross, 2005).

Mental Workload and Caffeine

Many studies have been performed and shown that caffeine is associated with the excretion of catecholamines and their metabolites. They have also shown that urinary levels of norepinephrine and epinephrine increase after the consumption of caffeine. This was seen in the levels of adrenaline and noradrenaline, as well, along with a dose dependent effect of caffeine (Papadelis et al., 2003). The possibilities of correlations between caffeine and cognitive performance, blood pressure, and catecholamines were observed under resting conditions and with a mental workload. The research subjects took the test after consuming one cup of coffee and then three cups of coffee. Blood pressure was taken at the beginning and end of each step of the experiment, and the root mean square error was watched continuously. After the three stages - at rest, under mental stress, after one dose of caffeine under stress, and after a triple dose of caffeine under stress - catecholamines were collected and measured (Papadelis et al., 2003). The only group that showed a statistically significant difference was the smokers and coffee drinkers. The study saw an increase in the urine adrenaline after consumption of one cup of coffee and a significant increase of noradrenaline in the urine. After an intake of three cups of coffee, both catecholamines significantly increased. They also increased with a mental workload. In the end, the conclusion was made of causation between caffeine and catecholamines (Papadelis et al., 2003).

Coffee and Parkinson Disease

Affecting about 3% of the population over 65 years of age, Parkinson disease is a large source of morbidity in the United States, and this statistic is expected to grow doubly over the next 3o to 40 years. One study began looking at the possible association of coffee and caffeine consumption and the incidence of Parkinson disease. The subject population consisted of 8004 Japanese-American men between the ages of 45 and 68 who were a part of the Honolulu Heart Program from 1965 to 1968. The incidence of Parkinson disease, amount of coffee consumption, and total dietary caffeine intake were measured. The coffee consumption was measured at the time of enrollment and at a 6-year follow-up, while the caffeine intake was observed at enrollment. The results showed the incidence of Parkinson disease significantly decreased with the increase of coffee and caffeine consumption. The conclusion of the study was that caffeine intake was related to the decline of risk of Parkinson disease and not the other nutrients in coffee (G. W. Ross et al., 2000).

Contraindications

When consumed in large doses, the caffeine in *Coffea arabica* can be harmful. One gram or more can cause "headache, nausea, insomnia, restlessness, excitement, mild delirium, muscle tremors, tachycardia, and extrasystoles" (Khan & Abourashed, 2010). These symptoms have also been observed in people who consume more than 1.5 grams of caffeine a day for a long period of time or with 300 to 500 mg per day for individuals with more sensitivity (Kraft & Hobbs, 2004).

Consumption of over six cups of coffee per day can lead to a negative fluid balance, due to the increased urine production caused by caffeine (Dorea & Costa, 2005). Also, for susceptible groups like the elderly, drinking more than six cups of coffee is one of the factors related to short sleep (Dorea & Costa, 2005).

Some side effects coming from the chlorogenic acid concentration in *C. arabica* are an increase in gastric acid, gastric irritation, diarrhea, and a depressed appetite (Kraft & Hobbs, 2004). Two signs of poisoning are vomiting and cramps in the abdomen (Kraft & Hobbs, 2004).

Nursing mothers should not ingest caffeine because it can cause the infant to have a sleeping disorder (*PDR for herbal medicines*, 1998). Pregnant women should also avoid caffeine, and if they consume it, they should not have more than 300 mg per day (*PDR for herbal medicines*, 1998).

The regular intake of caffeine can actually lead to "caffeinism," the psychic and physical dependency to caffeine. Withdrawal from this can present itself with headache and sleeping disorders (*PDR for herbal medicines*, 1998).

Current Use in Allopathic and CAM Therapies

"Coffee charcoal" or Coffea carbo is created from carbonized, or roasted, seeds of *Coffea arabica* (Wyk & Wink, 2004). This is generated through roasting the outer part, or exocarp, of green and dried fruit. After they become almost black, they are ground up. Coffea carbo is used for two main things: diarrhea and inflammation in the mouth and pharynx (*PDR for herbal medicines*, 1998). It is a powder that can be taken orally or applied topically. One dose of Coffea carbo is generally about 3 grams, and the daily dose is about 9 grams (for internal use) (*PDR for herbal medicines*, 1998).

Caffeine is also used by the United States Pharmacopeia in several different pharmaceuticals. These include "internal analgesics, cold and allergy products, weight control formulations (appetite depressants), and others" (Khan & Abourashed, 2010).

In traditional Ayurvedic therapies, the unripen beans of *C. arabica* are known to be used for headaches, while the ripened and roasted beans are used for diarrhea (Chevallier, 1996).

Discussion

Coffea arabica has had many uses over the years, which range from food to medicine. Because of it's beneficial effects, it has become the second largest commodity in the world after crude oil (Meletis, 2006). This originally Ethiopian plant is now cultivated in many places around the world, and exported to the others. Many people have become dependent on the effects that its main functional derivative, caffeine, gives them. While a lot of people use it for the stimulation of the central nervous system, it also can affect the renal system, gastrointestinal system, and musculoskeletal system. Before being one of the top products in the world, it was used traditionally by many cultures, and this has lead to it being used in many different pharmaceuticals, including analgesics, cold remedies, stimulants, and diuretics. Unfortunately, caffeine does have its negative effects, including the possibility of addiction, but research is being done to see what else it affects. So far, this has included the possibility of helping asthma, pain, neurologic conditions, and more. Overall, *C. arabica* has provided the world with a stimulant that is used daily, not only for general stimulation but also medicinal purposes.

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Cola nitida (Vent.) Schoot & Endl, Malvaceae

Ijeoma Okafor

Introduction

Cola nitida, also referred to as the Kola Nut, is a well-known fruit in the Malvaceae (formerly in Sterculiaceae) family and one of Africa's most valuable plants. Originally given the name *Sterculia nitida* by the French, it was later decided by the Germans that it belonged in the *Cola* family. Depending on its global location, it may also be referred to as gbanja kola, bitter kola, bese, goro, bissy or biche, kolatier, or colatero. With over sixteen different species in the *Cola* genus, the two most common edible types are the *C. nitida and C. acuminata.* Both *C. nitida* and *C. acuminata* are essential to African countries for their usage and commercial value, but the more prevalent and valuable of the two is the *C. nitida.* The kola nut coined the phrase "the kola of the caravan trade", as it is used for a variety of functions (Lovejoy, 1980). Having been transmigrated by the slave trade, the kola nut is quite versatile and has been used as a mild stimulant, antidote for specific diseases, a hunger-suppressant, gift to royalty, a token of peace or war, and even as a dye for decorating cloth (Abaka, 2005). Used in conjunction with herbal plants such as ma huang and in well-known commodities like Coca-Cola soda, the kola nut has become a major cash crop for its native African countries. Although *C. nitida* has medicinal and recreational uses, the kola nut is commonly recognized as a part of the stimulant substance group. Known for its astringent taste and high caffeine content, the kola nut produces a mild stimulating and sustaining effect once consumed (Brooks, 1980).

Figure 1. *Cola nitida* carpel and flowers: The kola nut can be found inside the carpels, which are the seeds. Image source: http://english.xtbg.cas.cn/rs/ma/201112/t20111206_79718.html

Botanical Description

Cola nitida is an understory, evergreen tree that produces the kola nut fruit. The tree can reach up to 40-60 feet tall, 1.5 meters in diameter, and is capable of producing light brown carpels that can contain anywhere from one to fifteen seeds; allowing a single tree to produce well over a thousand nuts per year (Brooks, 1980). The carpels are accompanied by flowers that are normally greenish-yellow or white with

Figure 2. Kola Nuts ranging in colors: The variety of color between kola nuts can be found in every fruit. Image source: http://www.shamanaustralis.com/forum/index.php?showtopic=2747

purple tracing along the edges of the petal (**Figure 1**). The kola nut, which is not a true nut but a seed from a follicular fruit, is roughly the size of a Brazil nut or a chestnut ranging approximately from 1 to 2 inches in size. The kola nut can also be found in a wide range of colors varying from a cream/white when found fresh to a dark reddish brown when dried (**Figure 2**). The red kola nut is the likely choice for trade because of its long-lasting qualities, while the white and pink kola nuts are generally more valued (Abaka, 2005).

The genus *Cola* is mainly located in the African tropics. More specifically, *C. nitida* is primarily indigenous to the Western Africa regions (**Figure 3**). Originally a tropical rainforest tree,

Figure 3. Western African Tropics: The location for indigenous *C. nitida.* Image source: http://www.mnzoo.com/animals/animals_dwarfCroc.asp

C. nitida can be found in hot and humid climates located in places such as Cote d'Ivoire, Ghana, Liberia, Nigeria, Sierra Leone, and the confluences of the Niger and Benue rivers. However, due to the frequent traveling by man and the rising of trade routes, the kola nut can now be found in locations such as the West and East Indies, Brazil, Jamaica, Mali, Zimbabwe, Sudan, Ethiopia, Guinea, Somalia, Chad, Congo, and several other countries (Abaka, 2005). Low elevations, well-drained soil, and moderate rainfall are needed for the successful growth of *C. nitida*, which cannot withstand flooding (Abaka, 2005). Because most African countries experience a dry season (December to March), it is important to grow *C. nitida* in areas where soil retains sufficient moisture during this season. However, *C. nitida* has even been found in altitudes over 300 meters high, where there is rich soil due to heavy rainfall (Lovejoy, 1980).

Within six to seven years after planting, *C. nitida* is able to bear fruit that can be harvested seasonally between October and February. After 5 days of fermentation, the ripe fruits are

harvested before the follicles are able to split open. The kola nuts that are chosen for cultivation are left to fully mature and ripen. *C. nitida* can then be spread by cuttings or aerial layering. However, the most important pollinators for *C. nitida* are insects. One method used to obtain the maximum yield of kola nuts, is to cultivate it along with other crops. One crop, *Theobroma cacao* can grow in the same climate conditions. Both species can actually be grown together, with *C. nitida* acting as an intercrop by providing top shade for *T. cacao*. Both *C. nitida* and *T. cacao* are close relatives to one another. Both species are a part of the Sterculiaceae (now Malvaceae) family, which contain flowering plants and is also known as the cacao family. The moderate leaching and acidity of the environment, in addition to 45-65 inches of rainfall, provides this climate with enough fertile soil to maintain the growth of both the *C. nitida* and *T. cacao* (Abaka, 2005).

Traditional Uses

Traditional Medicinal Uses

For centuries no other African commodity has been held to such a high importance in West Africa than the kola nut. Around the twelfth century, the Arabian doctor El-Ghafeky first discovered the importance of *C. nitida* as a drug. He reported that the use of powdered kola nut had the ability to relieve colic and stomachaches, while possessing "warming properties" (Abaka, 2005). During the same century, the kola nut was documented to help ease the pains of childbirth and as a treatment for rheumatism and dyspnea. In the fifteenth century, a report stating the existence of an African kola trade was documented in Pacheco Pereira's *Esmeraldo de Situ Orbis* (Abaka, 2005). For Muslims, kola nut was, and still is, a high demanding masticatory product because it is one of the only stimulants Islam condones. What was once a commodity for the elites gradually became available to the masses. Upon learning of its additional medicinal properties, the Islamic world further expanded the use and marketing of the kola nut. Traditionally, traders venturing on long journeys would chew the kola nut raw to reduce the effects of fatigue, hunger, and thirst, while maintaining alertness. In 1688, the historian Hans Sloane examined the effects of kola nut samples taken from Jamaica. He discovered the kola nut could be used as a diuretic and was great for treating water retention caused by diseases such as edema or dropsy (Mitchell, 2001). The kola was also known to provide cures for headaches and male sexual impotency (Lovejoy, 1980). It was believed that by submerging a minced kola nut in wine and taking it as a tonic daily would help stimulate a man's desire and sexuality (Brooks, 1980).

Ethnobotanical Uses

Even before the discovery of *C. nitida*'s medicinal uses, many African tribes were aware of its stimulant properties and integrated the masticatory process as a part of everyday life (Seeds, 2012). Now, many African societies see the kola nut as a high, valued commodity that is used in various ways in different cultures. In these societies, the social aspect encompassing the kola nut holds great importance as well. The kola nut can be offered to friend the same way tobacco products are shared in Western societies, it can either be chewed individually or in a group setting (Abaka, 2005). In certain Nigerian cultures, to be given a kola nut is a great honor. There are special occasions and ceremonies that encompass the use of or the giving of the kola nut. During wedding ceremonies, the breaking and sharing of kola nuts is considered a vital component during the wedding reception. The kola nut is also used as a way to accept or decline a

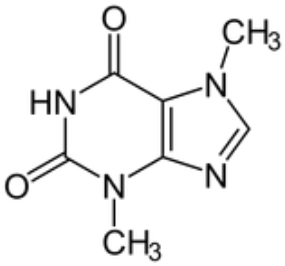

Figure 4. Caffeine. Image source: http://chemistry.about.com/od/factsstructures/a/theobromine-chemistry.htm

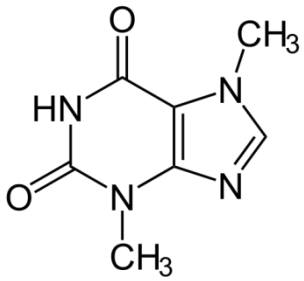

Figure 5. Theobromine. Image source: http://chemistry.about.com/od/factsstructures/ig/Chemical-Structures---T/Theobromine.htm

marriage proposal (returning a white kola means yes while a red kola means no), or could even be used to pay off part of a dowry. The color of the kola nut also plays a significant role in social and cultural communication. In many African societies, a white kola nut symbolizes friendship and could be used as ratifying treaties, while a red kola nut symbolized enmity and challenge to warfare. During a christening, a red kola represents life and the white kola purity. Also, the breaking of a kola nut is said to be a valued expression of hospitality. In Western societies the kola nut, due to its high caffeine content, was a major ingredient in the production of the Coca-Cola products. Invented by John Pemberton, the original Coca-Cola drink was made by combing coca and kola extracts with the hopes of concocting a remedy for headaches and hangovers (Burdock, 2009).

Food Source

The seeds of the *C. nitida* can be pounded into a powder and boiled in water to prepare as a tea. Due to the kola nut's strong bitter taste followed by a sweet aftertaste, it has also been used as a sweetener to get rid of bad tastes (Lovejoy, 1980). In addition to being implemented in soft drinks like Coca-Cola, the kola nut can also be combined with the coca leaves and incorporated into tonic wines, brands of cocoa, and other beverages. In Nigeria, kola nut is simply a common snack favored among the elderly (Adebola, 2011).

Other Uses

The kola nut is said to have multifarious uses. *C. nitida* can act as a water purifier in addition to dyeing clothing and as a candle. The bark is strong enough to be used for timber. It has been used for making furniture, house and boat buildings, domestic utensils, musical instruments, and wooden games and toys. The pod husk of the kola has been integrated into the production of liquid soap, and the most recent and remarkable advancement is that the kola pod husk has replaced up to 60% of maize for poultry feed formulations (Lim, 2011).

Chemistry and Pharmacology

The two active constituents present in *C. nitida* are the alkaloids, caffeine and theobromine (**Figures 4 & 5**). Both stimulate the central nervous system and the amounts of each

Stimulant	Caffeine (%)	Theobromine (%)
Kola	1.0 – 4.0	.02 - .09
Coffee	.7 – 2.3	None
Tea	1.0 – 4.7	None
Cocoa	.08 - .23	.8 – 2.3

Table 1. Caffeine and theobromine content in mild stimulants.

constituent in a kola nut fluctuate from one kola to the next. While caffeine has the stronger presence in a kola nut, theobromine has the greater responsibility in stimulating the skeletal muscles. However, there are only traces of theobromine present in *C. nitida*, while caffeine can make up anywhere from one to four percent of the kola nut. In addition to the two alkaloids, a glucoside heart stimulant, kolatin, is also present (Lovejoy, 1980). The combination of these three constituents makes the kola nut a highly effective stimulant in relations to other stimulants (**Table 1**). It is estimated that *C. nitida* has as much caffeine as 100 grams of coffee and as much theobromine as 100 grams of tea (Brooks, 1980). Because of this, one average sized kola nut is equivalent to approximately three cups of American coffee, but these effects can vary from person to person. Other chemicals found in *C. nitida* are strychnine, catechin, flavonoids, anthocyanins, quinine, theine, and tannin (Abaka, 2005).

Biological Activity

Biological activity is not only present in the kola nut, but parts of the whole tree have been reported to have significant medicinal uses. The cotyledon can be used to help treat herpes while the bark can be used for dystocia. The twigs can be taken and made into a chewing stick, which acts like a toothbrush by being able to clean the teeth and gums. A tonic can be prepared using the leaves, twigs, flowers, fruit follicles, and the bark to concoct a remedy for dysentery, coughs, diarrhea, vomiting, and chest complaints. There have also been reports about the extracts from the stem bark of *C. nitida.* It may have the potential to regulate natural fertility (Adebola, 2011).

Elastase Reduction Activity

Inflammatory diseases like asthma or cystic fibrosis are characterized by excess uninhibited neutrophil elastase, which contributes to tissue destruction. α1PI is one major inhibitor of elastase, and its presence or deficiency can also cause physiological disorders such as emphysema. A study shows that kola nut extract, depending on its concentration, can protect α1PI from HOCL inactivation by suppressing the MPO-H_2O_2 system. The suppression is caused the phenolic content in the extract scavenging oxygen species like H_2O_2 (Daels, 2003).

Aphrodisiac

During the eighteenth and twentieth century kola consumption in the Hausaland was promoted by the belief that a society's interest in sex and sexuality could be heightened (Brooks, 1980). Another study was performed on adult male rats. The study substituted drinking water with three different kola beverages for three months. At the end of the study the kola intake increased both oestradiol and

testosterone levels, suggesting that there is an additional mechanism of action beyond the effects of caffeine (Schliep, 2012).

Antimicrobial

An *in vitro* study examines four human pathogenic bacteria against *C. nitida.* The results show that the presence of secondary metabolites partially enhances the chemotaxonomic characterization of *C. nitida.* The presence of antimicrobial activity allows for the *C. nitida* extract to inhibit growth of certain bacteria and fungi. *C. nitida* showed inhibitory activity against *Aspergillus niger* and *Candida albicans* (Sonibare, 2009).

Antioxidant

The high levels of phenolic compounds found in kola nuts allow the extract to exhibit antioxidant capacities. The IC_{50} values range from 1.70-2.83 in hypoxanthine/xanthine oxidase and 2.75-4.08 mg/ml in 2-deoyguanosine HPLC-based assays. Of the secondary metabolites found in kola nut extracts, caffeine has the most antioxidant capacity proving to be an effective cancer chemopreventive metabolite (Sunday, 2007).

Cardiovascular and CNS Activity

Kola nut is reported to enhance alertness, physical energy, elevate moods, increase tactile sensitivity, and suppress the appetite. Various doses of kola nut extract were giving to male mice. The results showed that at medium doses locomotors activity increased while low doses showed no effect and high doses resulted in a depressive effect of locomotors activity (Ajarem, 1990). A later study explained the locomotors activity due to the caffeine content while kolanin is responsible for stimulating the heart (Lim, 2011).

Ocular Activity

Thirty grams of *C. nitida* was tested on visually acute and healthy volunteers to determine ocular effects. Results showed no effect on the pupil diameter of visual acuity but improvement in the near point of convergence by 43%. The amplitude of accommodation was increased by 11% while existing heterophorias were ameliorated. The kola nut extracts allows near work to done without stress. The extract could also aid the elder by relieving somnolence and ocular muscle imbalance (Lim, 2011).

Antibacterial Activity

The methanol extract that can be extracted from the root bark of *C. nitida* is shown to affect *Mycobacterium bovis* and strains of *Mycobacterium vaccae.* The aqueous and alcoholic extracts also found in the bark were show to inhibit growth of *Staphylococcus aureus, Pseudomonas aeruginosa, ß*-haemolytic streptococci, *Escherichia coli,* and *Neisseria gonorrhoeae* (Lim, 2011).

Clinical Studies

In 1888-1889, several tests were performed to examine whether the kola nut could be beneficial to soldiers in the battlefield. Ten pounds of kola nut varying in size were supplied R.H. Firth to test for four properties of a kola nut: whether a person could undergo long periods of fasting and fatigue without exhaustion, adjuvant to sustain the body

during exertion without food, restore a person in the drunken state to practical sobriety, and as a remedial agent allowing those to recover form lengthened sicknesses. The first experiment tested two patients who had venereal disease. They were administered a hot infusion of powdered kola and boiling water with their diet unchanged and urine examined twice a day. The results showed that the men's weight, pulse and respiratory capacities were unchanged, but their daily total excretion and urine increased in watery content. Those were the only physical results, however, both men stated that they had "less appetite and felt less want of food." The second test examined three soldiers performing physical activities without having eaten in 60 hours, but could be administered an infusion of 200 grams of kola powder, milk, and sugar during this timeframe. The experiment was also performed by Firth himself, and the three subjects concluded that the sense of hunger and exhaustion was less prominent. The third case used 50 men to show that the kola nut could sustain the body against fatigue and hunger. They were only allowed to chew the kola nut bit by bit for the next two days. Only 26 of the soldiers followed the rules properly and gave an accurate account of the experience. The majority stated that for the first 16 hours they felt hungry and tired, but for the reminder of the time they did not show signs of hunger or tiredness. The last experiment tested the ingestion of kola nut on a drunken person. The kola nut taken as a hot infusion was said to relieved the head of "the sense of heaviness peculiar to alcoholic excess of the previous night" (Abaka, 2005).

Branching off of the study previously mentioned, Firth was also able to determine the kola nut's direct relationship to the central nervous system. After the conclusion of the study involving the twenty-five soldiers that had eaten in two days, the majority of the men were craving fat and butter, allowing Firth to conclude that the body resorted to using fat reserves for recuperation. Therefore, the action of chewing a kola nut in addition to the absence of food is an artificial stimulant to the central nervous system (Abaka, 2005).

A clinical study was performed to show the effects of kola nut extract on the heart. The concentration of kola nut extract is directly related to the rate of metabolism and rhythmic heart activity. At low doses, the kola nut extract caused the rate of the heartbeat and the force of contraction to increase. When person consumed higher doses of the extract it resulted in the spike of metabolic rates (increasing approximately 118.76%) (Chukwu, 2006).

Contraindications

The FDA and FEMA have labeled the kola nut extract GRAS for the usage of a flavoring ingredient. However, in 1996, the FDA reported that the kola-mahuang combination for hallucinogenic effects is a deadly pair, and this brought attention to issue that more experiments involving the kola nut and kola nut combinations needs to be further explored. One study shows a linkage between the consumption of kola nut and malaria morbidity. The results revealed that eating the kola nut increases the expression of malaria symptoms by favoring parasite multiplication. *C. nitida* also contains hydrocyanic acid which, if consumed in high doses for a short period of time, is known to cause harm to the nervous, respiratory and cardiovascular systems. Also, if exposed to hydrocyanic acid for longer durations, irritation of the eyes, appetite loss, headaches, dizziness, and damages to the nervous system and thyroid gland could be potential results (Alaribe, 2003). There are also side effects to taking in too much caffeine. Problems that could arise are nausea, insomnia, headaches, and tachycardia. If caffeine is ingested in significantly large doses then muscle tumor and tinnitus could

Figure 6. Herbal supplement of kola nut: The kola nuts are crushed into a powder and encapsulated and can now be found in certain drugstores. Image source: http://www.swansonvitamins.com/SW1122/ItemDetail

be potential results. Peptic ulcer patients are advised not to kola nut since caffeine, along with theobromine, are known to stimulate gastric acid secretion. However, caffeine is still considered a GRAS but is regulated by FDA for the use in cola beverages, with .02% being the maximum concentration allowed. The FDA also regulates the use of caffeine, alone, as an OTC stimulant drug. Even with these precautions there is always the rare chance of a person becoming hypersensitivity to the active substances in *C. nitida* (Burdock, 2009).

Current Use in Allopathic and CAM Therapies

Many of the traditional uses of the kola nut are still used today. Recently in Western societies, the kola nut has been reintroduced as a flavoring for drinks. Also, the CoE, FDA and FEMA have approved the use of kola nut extract for foods (Burdock, 2009). The kola nut can also be used as a base for certain chocolates and wine. Due to its high pectin content, there is possibility that kola nut can be included in jams or jelly. The kola nut also contains high protein content that could be a useful ingredient in making fertilizers. The nuts can also be used in tinctures or made into teas. Powdered kola is now available in capsule form; however, in comparison to naturally chewing the kola nut, the strength and its effectiveness is much lower since the caffeine content is not as high (**Figure 6**) (Burdock, 2009).

Discussion

Despite its economic importance in Western and Central Africa, much still unknown about *C. nitida.* Having been exported to all parts of the world, *C. nitida* is centuries old and will most likely remain a vital resource in many cultures for centuries to come. The kola nut was already accepted amongst African societies when only the stimulant effects were known. Now that there is a worldwide awareness of the kola nut's medicinal and recreational uses, the plants significance has only gotten greater. With uses ranging from social and cultural interactions to fighting off fatigue to a component in beverages, as we begin to learn more about *C. nitida* and its diversifying effects, its demand in the world will become more prevalent. With that being said, the kola nut will continue to play a significant role as an important economic crop for internal, regional, and foreign trade (Adebola, 2011).

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Commiphora molmol (Engl.) Engl. ex Tschirch, Burseraceae

Elizabeth Giffen

Introduction

Commiphora molmol (Engl.) Engl. ex Tschirch, Burseraceae, also known as *Commiphora myrrha* var. *molmol* Engl. and commonly as the myrrh tree, ("Tropicos.Org," 2011) is an arid tree or shrub from Somalia and Ethiopia (**Figure 1**). Its main utilization is as a gum, resin or essential oil (Nomicos, 2007) and has been in perfumes and incenses since ancient times. It has religious significance in Judeo-Christian traditions and is often used ritually. Myrrh has also seen use as a wine preservative and in embalming fluids in ancient Egypt (Groom 1981). In medicine, myrrh has been traditionally used from northern Africa east through India to treat a huge range of ailments, as an anti-inflammatory, analgesic, emmenagogue, antibacterial, and for oral maladies (Groom, 1981, Walter H. Lewis, 2003). The plant produces an oleogum resin that is 2-8% essential oil (El-Shahat, et al., 2011) 23-40% resin, 40-60% gum and 10-25% bitter principles (Su, et al., 2011). Some active constituents of myrrh include the sesquiterpenes furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one, which have antibacterial, antifungal and local anesthetic activities, (Dolara, et al., 2000) and furanoeudesma-1,3-diene and curzarene, which have potent analgesic effects (Dolara, et al., 1996). The furanosesquiterpenoids lindestrene and its analogues make up 19% of the essential oil and are responsible for the famous scent associated with myrrh (Jensen). Other important constituents are terpenoids, steroids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives; these various metabolites give myrrh its manifold biological activities (Su, et al., 2011).

Figure 1. Myrrh. (Source: http://www.plantsasmedicine.com/~cleanen2/index.php?title=Commiphora_myrrha)

Botanical Description

The myrrh plant is a small, thick-stemmed tree or shrub with a succulent trunk and spine-like twigs. It grows in arid and alpine habitats in Djibouti, Ethiopia, Somalia, Kenya, Oman and Yemen (USDA). The leaves are small and sparse, generally single and grey-green, and quite variable in size and shape,

but usually oval-shaped and trifoliate (Nomicos, 2007). The leaves become yellow in autumn before falling off (Cactuspedia). The bark is silver, papery and peeling, with green bark underneath. Myrrh tree fruits are tiny and brown, with a smooth, oval shape (Nomicos, 2007). Puncturing the stems or trunk produces a pale yellow, oily myrrh resin with a bitter taste but a warm, smoky scent, which turns reddish and dusty upon hardening (Michie and Cooper, 1991).

Myrrh resin is harvested by making incisions roughly 2 inches long into the bark of the tree. The sap hardens when exposed to air and forms into pearls which are easily harvested around two weeks after the incision is made, and new incisions, or "taps" can be made over old ones after removing the resin; these usually only require an additional scratch. Tapping is done at certain times of year and specific duration depending on the region (Dharmananda 2003).

Traditional Uses

Traditions involving myrrh are well known in Western and Christian-dominated cultures primarily because of the legend of the Magi bringing the Christ-child gifts of gold, frankincense and myrrh to the nativity (Matthew 2.11). In fact, myrrh is a prominent plant throughout Biblical literature, being mentioned frequently in the Song of Solomon. It was also the last gift offered to Jesus before the crucifixion (presumably as an analgesic): "Then they offered him wine mixed with myrrh, but he did not take it," (Mark 15.23). Nicodemus is also said to have brought myrrh and aloes for the embalming of his body after his death (John 19.39). Jewish tradition utilized myrrh as a main component of anointing oil for the priests, tabernacle, ark and altar (Groom, 1981). Myrrh was once as valuable as gold and ivory, and its use as a plant-based traditional remedy is one of the oldest therapies on record (Michie and Cooper, 1991).

In a temple near Thebes, we see some of the first evidence of the myrrh trade in frescoes detailing the voyage of a fleet of Egyptians sent by Queen Hatshepsut to the land of Punt, from which they brought back, among many items, quantities of incense ('*ntyw* is believed to be myrrh, although there is some debate that it may be frankincense (Lucas and Harris, 1999)) and incense-bearing trees. Texts from centuries earlier indicate the ritual use of incense, however, and still earlier texts detail previous expeditions to Punt, although no indication of incense is made (Groom, 1981).

Medicinally, it has been said that the Greek soldiers would not go into battle without myrrh to treat wounds (Hanus, et al., 2005). The Ebers Papyrus, which dates back to c. 1550 BC, details its use as both incense and medicinal remedy (Chisholm, 1910). It is listed as being used in funerals, mummifications and cremations, but also in the treatment of wounds and skin sores (Michie and Cooper, 1991). Mesopotamian texts in Assyrian cuneiform script dating from the 10th to 6th century BC record myrrh being used medicinally, and Pliny, Theophrastus and Dioscorides have all made mention of it in various ancient texts, including the famous *De Materia Medica* (Groom, 1981).

De Materia Medica (c. 65 A.D.) in particular mentions myrrh several times as an ingredient in concoctions as an abortifacient, antiseptic ointment and a diuretic, and to treat asthma, "female ailments," dandruff, varicose veins, sleeplessness, cleaning boils, glaucoma, purging ulcers, and softening various somatic scleroses. *Stacte*, the substance made from myrrh gum resin, has an entry in *De Materia Medica*, which describes it as a precious ointment with a very sweet smell and a great deal of strength in small doses. In the

main entry on *Smyrna* (myrrh), Dioscorides even explains a method of counterfeiting myrrh by soaking gum in myrrh-infused water. When one is buying it, he recommends myrrh that is fragrant, brittle, light, and monochromatic, smooth and small when broken, and sharply bitter in taste. It is constantly referred to as 'warming' in nature, and is also drying, astringent, and retaining. As a salve it is used to soothe and open the closed vulva, as an emmenagogue, and to expedite birth. In pill form, myrrh would be taken to help a chronic cough or asthma, side and chest pains, loose bowels and dysentery, kill worms, and to calm feverish chills. It was held under the tongue like a cough drop to treat atherosclerosis and hoarseness, or chewed to treat bad breath. Myrrh could also be used in a mouthwash with wine to strengthen teeth and gums. It was used to heal broken ears, exposed bones, armpit sores, pus and inflammation in the ears, impetigo, alopecia, and smoothing rough skin (Dioscorides, 50-70).

Additionally, myrrh is mentioned in the anonymous Syriac book of medicine and the works of Avicenna (Michie and Cooper, 1991) and by Hippocrates in the 4th century BCE (Nomicos, 2007). Theophrastus says that myrrh would be added to wine to add astringency because of its bitter character. Athenaeus says that Aristotle wrote that myrrh could be boiled in water and the liquid could then be added to wine to "diminish drunkenness" (Groom, 1981).

Celsus, who lived from 25 BC to 50 AD, wrote extensively about its use to treat a huge variety of ailments: a form of malaria, dropsy, intestinal pain, as a poison antidote, to agglutinate wounds, and heal burns, for colic, throat ulcers and to treat abscesses and scabies. He also lists it among the erodents and caustics, includes it as part of ointments for liver, chest and side pain, tuberculosis infection of the lymph nodes in the neck, neuralgia, as a plaster for "broken heads," treatment of inflammation in the uvula and "for the genitals when foul," and when there is difficulty with urination, as well as to treat anal fissures, canker sores and kidney stones. As an example, to induce menstruation, the prescription is for pounded garlic with myrrh and lily ointment. Celsus writes that it is also useful for treating eye and ear afflictions, tooth pain (although it should be applied on the shoulder), swollen tonsils, and ulcerations of the mouth (Celsus, 25).

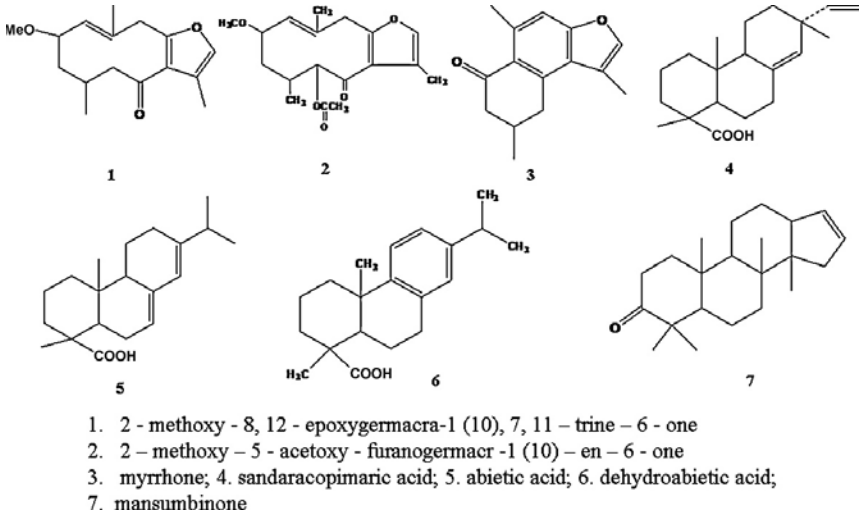
Myrrh is used in Ayurveda, although it does not come from *Commiphora myrrha* but *Commiphora mukul* (Hook ex Stocks) Engl. (C. wightii (Arnott.) Bhanol.), and is called guggul or false myrrh. Guggul has been used to adulterate myrrh, ("Efloras," 2008) and has some similar properties to *C. myrrha* but also contains many very different active compounds, such as guggulsterone (Hanus, et al., 2005).

Myrrh is first seen in traditional Chinese medicine (TCM) in Kaibao Bencao, 973 CE, under the name *mo yao*. It is still in use as an aid in promoting circulation, treating traumatic injury, and as an anti-inflammatory (Dharmananda, 2003). Myrrh has neutral, bitter properties, usually dosed at 3-10 grams in decoction (Sionneau, 1997), and is almost always used together with frankincense (Jiao, 2003). In TCM, myrrh is sometimes soaked in rice vinegar and then stir-fried or baked (Dharmananda, 2003).

Chemistry and Pharmacology

The main constituents of the myrrh oleo-gum resin are 2-8% volatile oil (El-Shahat, et al., 2011) 23-40% resin, and 40-60% gum (Su, et al., 2011). The gum is water-soluble and contains polysaccharides, proteoglycans and proteins, as well as the compounds D-galactose, L-arabinose, and 4-methyl D-glucoronic acid. The volatile oil is made of steroids, sterols and terpenes (Hanus, et al., 2005) including the

furanosesquiterpenoids lindestrene and its analogues, which account for 19% of the essential oil's character and its aromatic properties (Jensen). Close to 20 furanosesquiterpenoids have been isolated from myrrh essential oil (Su, et al., 2011), including furanodiene-6-one (0.4%) and methoxyfuranoguaia-9-ene-8-one (0.1%), (Dolara, et al., 2000) furanoeudesma-1,3-diene (15%), and curzerene (40.1%) (Dolara, et al., 1996). The essential oil can be extracted from the resin by steam distillation (Walsh, et al., 2010). The resin contains isolinalyl acetate, 3-epi-lupenyl acetate, lupeone, 3-epi-α-amirin, α-amirone, acetyl β-eudesmol, and a sesquiterpene lactone (Hanus, et al., 2005). Other metabolites of myrrh are terpenoids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives (Su, et al., 2011). Marker standards for myrrh are shown in **Figure 2**.



1. 2 - methoxy - 8, 12 - epoxygermacra-1 (10), 7, 11 – trine – 6 - one
2. 2 – methoxy – 5 - acetoxy - furanogermacr -1 (10) – en – 6 - one
3. myrrhone; 4. sandaracopimaric acid; 5. abietic acid; 6. dehydroabietic acid;
7. mansumbinone

Figure 2. Marker compounds used in myrrh (Su, et al., 2011).

Biological Activity

In an anti-inflammatory test, both an ethanol and a petroleum ether extraction of *Commiphora myrrha* significantly inhibited formalin-induced paw swelling and acetic acid-induced writhing in mice. Because no attenuation in temperature-dependent nociception was shown in this study, the authors noted that this suggests that myrrh extracts with analgesic properties act on the peripheral and not central nociceptive pathway.

A hexane (nonpolar) extraction of myrrh for analgesic activity identified furanoeudesma-1,3-diene and curzarene as having analgesic activity when injected intracerebroventricularly in mice at 1.25 mg per kg. Both significantly increased latency to pain reaction (paw licking when placed on a hot plate). Furanoeudesma-1,3-diene given 50 mg per kg orally was also effective in reducing the number of acetic acid-induced writhes (completely rescued by naxolone and morphine) and again increased latency to temperature-dependent pain reaction. The authors conclude that this suggests an interaction with opioid mechanisms in the central nervous system (Dolara, et al., 1996).

Clinical Studies

A survey done in Ezbet El-Bakly, Egypt, assessed the efficacy of myrrh extract, Mirazid, in the treatment of fascioliasis, a helminthic disease. Those with fascioliasis were given two capsules of Mirazid (600 mg) every day an hour prior to breakfast for six days. The cure rate was 88.2% and 94.1% two and three months after treatment, respectively (Abo-Madyan, et al., 2004). The same drug was assessed on a population of schistosomal infections in the same region and found to have similarly miraculous results, with the same

Figure 3. *In vitro* antimicrobial activity of myrrh
(Moussaieff, et al., 2005).

regimen showing a cure rate of 97.4% and 96.2% two and three months afterward (Abo-Madyan, et al., 2004). Unfortunately, neither of these figures stands up to rigorous repetition. Multiple experimental and clinical studies show myrrh has no significant efficacy in reducing parasite loads (Abdul-Ghani, et al., 2009). However, the anthelmintic mode of action of myrrh is still unknown. Some have postulated that myrrh causes the worms to lose muscle mass, which makes them move into the liver where they are destroyed (Abdul-Ghani, et al., 2009).

An unrelated study in India examined the efficacy of an herbal toothpaste containing myrrh compared with conventional toothpaste for the control of plaque and gingivitis. The researchers found the herbal toothpaste to be just as effective as conventional toothpaste, without any additional benefit (George, et al., 2009). Additionally, a Chinese study examined the efficacy of a number of TCM herbs for the treatment of reflex sympathetic dystrophy (RSD). It found that traditional remedies decreased both pain and swelling significantly more than placebo (Xu, et al., 2009).

Myrrh ethanolic extract in combination with aloe, mastic and olibanum (the ingredients of the historic Jerusalem Balsam, wildly popular in the early 1700s) has been shown to be strongly antiseptic. **Figure 3** shows the *in vitro* effect of Jerusalem Balsam on *Staphlococcus aureus*; disc 1 is treated with 20 µl balsam, disc 2 with 20 µl ethanol, (used to make the extraction) and disc 3 with 20 µl chlorhexidine 0.2%, (a known antiseptic). Disc 1, similar to disc 3, has almost no colonies on it; compare with the ethanol-treated disc 2 (Moussaieff, et al., 2005).

Contraindications

Myrrh is approved by the US FDA as a safe, natural flavoring substance (Abdul-Ghani, et al., 2009). However, it should not be used by pregnant women as it is an abortifacient and emmenagogue (Brinker, 2001). People with sensitive skin should avoid topical applications containing myrrh, as it has been known to cause contact dermatitis (Lee and Lam, 1993). Raw myrrh can be taken internally in very small quantities, but too much will upset the stomach, thus myrrh in the raw form is generally used externally (Dharmananda 2003). The LD_{50} value of Mirazid solution, a myrrh extract, is 3,139mg/kg in mice (Abdul-Ghani, et al., 2009) and the LD_{50} value of myrrh oil is 1650mg/kg in rats (MSDS, 2005).

Current Use in Allopathic and CAM Therapies

The People's Republic of China is currently the leading consumer of myrrh medicinal products in the world (Nomicos, 2007). It does see use elsewhere, however; a cross-sectional study looked at how diabetics in Jeddah, Saudi Arabia treat

foot disorders (open wound, chronic ulcer, skin cracks). 52.9% of respondents said they had used CAM products, among which 37.4% said they used myrrh (Bakhotmah and Alzahrani, 2010). Although there are a plethora of products containing myrrh on the market today, most of them are fragrance products. Tom's of Maine produces a toothpaste containing myrrh, which is marketed as a fluoride-free option that, along with propolis, "promote[s] a naturally clean, healthy-mouth feeling" (Bonapace, et al., 2002). Frankincense & Myrrh offers a line of homeopathic treatments for neuropathy, fibromyalgia, cold and flu prevention and sinus relief. All of them contain myrrh oil as an "inactive ingredient," and the sinus and cold & flu treatments contain "myrrha" as an active ingredient, cited as an antibacterial/anti-inflammatory. The sinus rubbing oil is dosed at 10-30 drops rubbed onto chest and throat, up to three times a day. The cold and flu prevention rubbing oil ("a topical immunity booster") is dosed at 10-30 drops massaged onto feet and ankles before bed or after waking, every day ("Frankincense & Myrrh," 2007).

Discussion

Myrrh has been an exceptionally useful plant to humans for a very, very long time. We are leaning more and more about the various mechanisms of action it can have and the benefits it confers. It is highly likely in the future that we will see it increasingly used in both CAM and allopathic treatments. It is generally safe despite some warnings, and is already beloved as a fragrance and religious symbol throughout the world. However, before we can begin to once more use myrrh as widely as someone like Celsus would have had us do, more research needs to be done into the activities of specific compounds in the resin, oil and gum components of myrrh. For example, the myrrh extract Mirazid has shown both extreme promise and equal measures of disappointment in clinical and experimental treatments of helminthic diseases like schistosomiasis. More thorough, strictly controlled evidence is needed to determine whether or not this product shows real results, but it certainly seems to have promise.

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Croton lechleri Müll.-Arg., Euphorbiaceae

Latavia Carroll

Introduction

Croton lechleri Müll.-Arg. is a member of the Euphorbiaceae family, consisting of plants that are known for the dark resins they produce. *Croton* has red latex that is released from its bark when slashed (**Figure 1**). This species is mostly found growing in Mexico, Venezuela, Ecuador, Peru, Colombia, Bolivia, and Brazil (Gupta, 2008; Jones, 2003). This species has many different uses including treatment of diarrhea, wound healing, treatment of tuberculosis, bone cancer, to treat insect bites, stomach ulcers, and it is used to promote healing after an abortion or used as a vaginal douche after childbirth (Castner, page 46). It also has other therapeutic uses as an antimicrobial, antiviral, anti-inflammatory, and antioxidant (Gupta, 2008). Some common names of this species include: Sangre de grado, sangre de drago, sangre de dragon, dragon's blood, drago, sangue de drago, and sangue de agua. In Belize it is called kush-uh-che; in Mexico: shonashe; inHonduras: pela nariz, and in Cameroon it is called ebine, enok, ndouon, or nouogui (Quattrocchi, 2000). There are more than 750 species within the genus, *Croton,* that are found all over the world (Duke, page 411). The phytochemistry of dragon's blood consists of alkaloids: sinoacutine, found in the leaves, and taspine, found in the sap; as well as proanthocyanidins, diterpenes, steroids, and lignans. All of these different chemical constituents provide the synergy that makes dragon's blood bioactive.

Figure 1. *Croton lechleri* tree. (Taken by Ricarda Riina, 2009. http://cms.herbalgram.org).

Botanical description

As mentioned previously *Croton lechleri* grows in the Amazon rainforest of Peru, Colombia, Bolivia, and Ecuador where the climate is very wet and humid. This species is a tree that grows at a fast rate of 10-15 meters in three years and is usually found at elevations of 100-600 meters (Jones, 2003). It can grow between 10-20 meters high and usually has a diameter of 30 cm (**Figure 2**). *C. lechleri* is also known as a pioneer species because it is usually one of the first plants to appear in areas that have been cleared and along roadsides (Ubillas et al., 1994). The dark green leaves of this tree are heart-shaped and are characterized by a long narrow drip-tip at the end of the leaves (**Figure 3 & 4**). The leaves tend to grow in clusters towards the ends of the branches, and can range in size from 13-30 cm long by 15-30 cm wide. There are

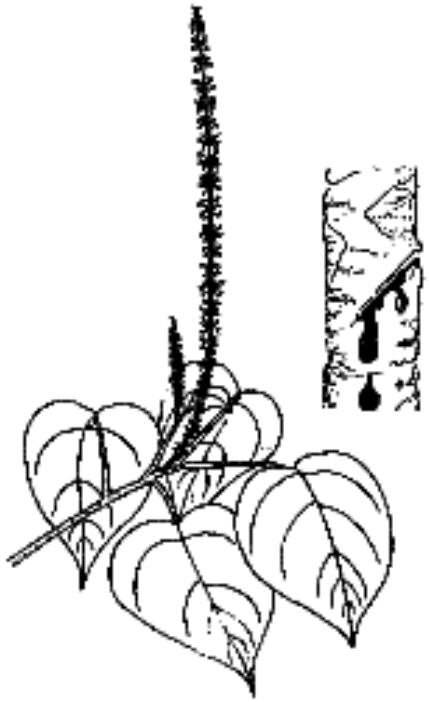

Figure2. *Croton lechleri* Müll. Arg. (Source: http://www.rain-tree.com/Plant-Images/sangre-pic.htm).

three main veins that palmate from the leaf base, as well as 6-8 obvious parallel secondary veins that diverge diagonally from the mid-vein. The larger veins and petiole are covered with tiny bumps or hairs that rub off, and the smaller veins have a dotted appearance (Castner, page 46). The greenish-white flowers bloom on a tall slender upright stalks that can be from 30-50cm long (**Figure 4**). The fruits of this tree are brown, three-parted capsules (**Figure 5**).

Traditional uses

The name "dragon's blood" dates back to the 1st century AD when a Greek sailor wrote in a shipping manual called the

Figure 3. A volunteer holding a bundle of *C. lechleri* leaves, 2008. (Source: http://cms.herbalgram.org).

"Perilous of the Eritrean Sea" about a tree that yielded drops of cinnabar on the island of Discordia (Gupta, 2008). Spanish naturalist and explorer P. Bernabe Cobo observed and recorded that *Croton lechleri* was widely used throughout the indigenous tribes of Mexico, Peru, and Ecuador in the 1600s. This information can be found in the Mateo's: Biblioteca de Autores Espanoles Series of 1956 in volumes 91 and 92 (Gupta, 2008).

Sangre de grado is used in traditional Ecuadorian and Peruvian medicine to treat diverse illnesses in adults, children, and infants (Jones, 2003). Its internal ethnomedical uses were for treatment of diarrhea, dysentery, cholera, coughs, flu, lung problems, stomach ulcers, and hives. Hives were treated by

Figure 4. Close-up picture of the flowers and leaves of *C. lechleri*. (Source: Photo taken by Leslie Taylor, 2000. http://www.rain-tree.com/Plant-Images/sangre-pic.htm).

orally taking the sap in pineapple juice at a dosage of 20 drops per 200 mL (Jones, 2003). In the upper Amazon, the sap is taken diluted in hot water to speed internal healing after an abortion, and used as a vaginal douche after childbirth (Jones,

Figure 5. Fruits of *C. lechleri*. (Source: Photo by: Brian A. Smith, 2006. http://www.discoverlife.org/mp/20p?see=I_GEO374).

2003). The sap is also used to treat tuberculosis and bone cancer, and is used externally to treat insect bites, stings, and for wound healing (Jones, 2003). The sap is also used in the healing of open sores in the mouth or on the body, as well as for herpes infection. The Quijos Quichua of eastern Ecuador soak a piece of cotton in the sap form dragon's blood and apply it to alleviate the pain of tooth extractions and cavities. The traditional internal dosage of the sap in Ecuador and Peru is generally 5–10 drops, once to twice per day for 5 days, but often the treatment is repeated for as long as 3 weeks. The sap is taken in water (cold or warm), milk, or alcohol (Jones, 2003). The yield of latex from *C. lechleri* is traditionally dependent on the age of the tree. The sap is usually harvested by slashing from a tree up to 6 years old with a diameter of about 25 cm. Felling the tree and scoring the bark produces about 5–6 L of sap, which is why is the preferred method for industrial scale production (Ubillas et al., 1994).

C. lechleri is also used in African-American folk magic or voodoo where the resin is used in mojo hands for money-drawing or love-drawing; it is used as an incense to cleanse negative entities or influences. In neopagan witchcraft, it is used to increase the potency of spells for protection, love, banishing and sexuality (Gupta, 2008).

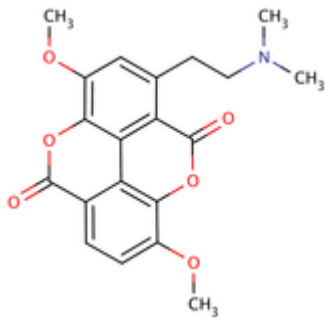

Figure 6. Taspine. (Source: http://en.wikipedia.org/wiki).

Alkaloids	Ligans	Proanthocyanidins and Flavonols	Steroids	Diterpenes
Sinoacutine	3', 4-O-dimethylcedrusin	(+/-)-galloepicate-chin	β-sitosterol-β-D-glucopyran-oside	Bincatriol
Taspine		(-)-epicatechin	β-sitosterol	Crolechinol
Magnoflorine		(+)- catechin		Crolechinic acid
Isoboldine				Hardwickiic acid
Norisobold-ine				Koberins A and B
Glaucine				
thaliporphine				

Table 1. Some of the chemical constituents found in *Croton lechleri*.

Chemistry and Pharmacology

A list of chemical constituents of *C. lechleri* were taken from the text of Jones, 2003 and typed into table format (**Table 1**). Taspine, 3', 4-O-dimethylcedrusin, and proanthocyanidins make up about 90% of the weight of dried dragon's blood (Cai et al., 1991). These chemical compounds are also responsible for the wound healing properties of dragon's blood. An *in vivo* study on female Wistar rats was conducted by Pieters et al. 1995 to determine the wound healing activity of dragon's blood. The study concluded that the proanthocyanidins stimulated contraction of wounds cut into the rats' back and formation of crust; 3', 4-O-dimethylcedrusin stimulated formation of new collagen, and regeneration of the epithelial or top-skin layer (Pieters et al., 1995).

Antimicrobial activity of chemical compounds 2,4,6-trimethoxyphenol and 1,3,5-trimethoxybenzene at amounts of 0.0003 μg showed high potent activity against the bacteria, *Bacillus subtilis*, which at this small amount was thirty times more active than chloramphenicol and penicillin (Chen et al., 1994). Crolechinic acid was also active against *B. subtilis* and *E. coli* at administered amounts of 0.2 μg and 1.0 μg, respectively. And Korberins A and B at administered amounts of 0.04 μg and 0.05 μg also showed activity against *B. subtilis* (Chen et al., 1994).

According to an *in vitro* study conducted by Chen et al., 1994, taspine, a major chemical constituent in dragon's blood (**Figure 6**), was determined to have potent cytotoxicity against KB cells (human oral epidermoid carcinoma). Taspine was able to inhibit KB tumor cell growth by 50% at a concentration of 0.39 μg/mL (Itokwa et al., 1991). The mechanism of action is not known, but it is believed to be the result of immunostimulation that cytotoxicity (Chen et al., 1994).

Biological Activity

In vitro studies

Pereira et al. 2010 conducted an *in vitro* study on the effects of de grado on cutaneous neurogenic inflammation (CNI). The procedure for how the plant extract was prepared was not mentioned, but concentrations of 1%, 0.1%, 0.01%, and 0.001% were made. These different concentrations of extract were applied to cell cultures made up of porcine or swine skin

cells and a co-culture of dorsal root ganglion neurons and keratinocytes, to examine sangre de grado's ability to reduce substance release of cutaneous neurogenic inflammation. The results yielded that there was a dose-dependent decrease in substance P release after ten minutes of incubation at concentrations of 1% with an average of 32% and at 0.1% with an average of 26%. This suggests that sangre de grado does inhibit cutaneous neurogenic inflammation. The second part of the study looked at sangre de grado's effect on substance P release when induced by capsaicin. The results yielded that after a one-hour incubation, substance P was strongly inhibited and after a 72-hour incubation, substance P was completely stopped. The study concluded "that sangre de grado is a potent inhibitor of CNI through direct inhibition of neuropeptide release by sensory afferent nerves," but more studies need to be conducted to determine which compounds in the plant were responsible for the activity (Pereira, 2010).

An *in vitro* study of SP-303 showed antiviral activity against Herpes simplex viruses (HSV-1 and HSV-2), when applied topically to infected guinea pigs. The inhibition of the thymidine kinase mutants of the viruses by SP-303 is probably done at the plasma membrane level where penetration and/ or absorption are interrupted during the early stage of viral activity (Barnard et al., 1993; Ubillas et al., 1994).

An *in vitro* study conducted by Rossi et al., 2003 examined the mutagenic, antimutagenic, and antiproliferative potential properties of sangre de drago. Sangre de drago was determined to have antiproliferative effects on the human myelogenous leukemia K562 cells. The mutagenic and antimutagenic activity of C. lechleri sap was evaluated by means of the Ames/Salmonella test. There was no mutagenic activity found on neither of the Salmonella typhimurium strains T98 and T100. On the other hand, the sap showed an inhibitory effect against the mutagenic activity of mutagen 2-Aminoanthracene in the presence of S9. And a moderate protective activity was shown against mutagens Sodium Azide and 2-Nitrofluorene. Therefore it was suggested that *C. lechleri* sap interacts with the enzymes of the S9 mix, thereby inhibiting the transformation of 2-Aminoantracene into its active forms (Rossi et al., 2003).

In vivo studies

Froldi et al. 2009 conducted a study on the activity of sap from *C. lechleri* on rat vascular and gastric smooth muscles. The sap was collected in a traditional way by cutting it from a tree growing in Napo river region of Ecuador. The voucher code No: sangre de grado 005 for the crude drug was sent to the Department of Pharmaceutical Sciences of Padua University. The study revealed "*Croton lechleri* increased contractile tension in a concentration-dependent way for both the vascular and gastric smooth muscles of rats" (Froldi, 2009). The purpose of the study was to look at how the sap increased contractile tension by observing if regulation of certain receptors and ion channels by pharmacological drugs could stimulate the activity of sangre de grado. The drugs used were atropine, a competitive muscarinic antagonist; prazosin, a selective antagonist of x1-adrenergic receptors; ritanserin, a 5-HT2a selective antagonist. All of these drugs had no effect on the vasoconstriction of sangre de grado, so therefore were not responsible for its activity.

Clinical Studies

Dragon's blood has many different traditional uses that have been shown to be beneficial through scientific studies conducted. But one particular illness that has been explored more in the potential development of drugs is diarrhea.

Diarrhea is a big problem, especially in underdeveloped areas where access to conventional medicines is limited. But another important issue that Daniel DiCesare el al. 1998 explored was traveler's diarrhea, particularly people that traveled to Jamaica and Mexico. He and his colleagues conducted a clinical trial in which 184 people from the United States with diarrhea from traveling to Jamaica and Mexico participated in a double-blind, placebo- controlled study of SP-303 or Provir, a plant-derived from *C. lechleri* with antisecretory properties. The purpose of the study was to determine the effectiveness of three doses of SP-303 given to patients four times a day for 2 days in treating and reducing acute diarrhea. Provir is a large heterogeneous natural polymer that breaks down in the presence of gastric or stomach acid and the mechanism of action of this drug is inhibition of intestinal chloride channels. The study concluded that Provir shortened the duration of diarrhea by 21% and the optimal dosage was determined to be between 125-250mg given 4 times a day for 2 days. The drug was also well tolerated with no side effects which are probably due to the fact that it is minimally absorbed by the body (DiCesare, 1998).

Another developed drug, Crofelemer, is currently in the clinical trial phase for treatment of secretory diarrheas associated with acute infections like cholera, chronic diarrhea related to HIV/AIDS, and diarrhea-predominant irritable bowel syndrome. Crofelemer is a dark reddish-brown powder that consists of proanthocyanidin oligomer extracts from the latex of *C. lechleri* (**Figure 7**). According to Lukmanee et al. 2009, Crofelemer gets its antisecretory and antidiarrheal properties by inhibition of two major apical membrane transport processes involved in intestinal fluid transport. These two apical membrane Cl- channels are

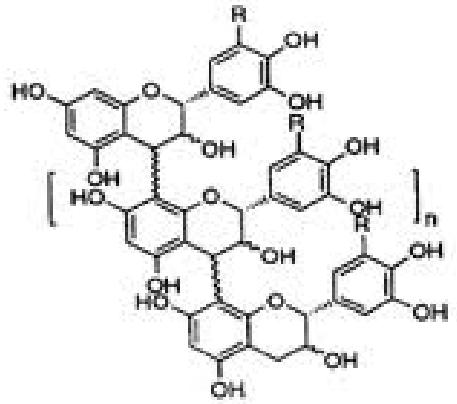

Figure 7. Chemical structure of Crofelemer. (Source: http://www.theannals.com/content/44/5/878.abstract).

cAMP-stimulated cystic fibrosis transmembrane regulator (CFTR) and calcium-stimulated (CaCC). The mechanism of action of Crofelemer in net reduction of Cl- secretion is probably the result of inhibition of one or more transporters involved in transcellular Cl- secretion such as basolateral Na+/K+ pump, NKCC symporter, or K+ channels.

Contraindications

Given the cytotoxicity of taspine, it is recommended that sangre de drago saps containing a high content of the alkaloid should not be used for wound-healing or for internal use (Chen et al., 1994). The latex on the other hand is considered non-toxic and is well-tolerated in clinical studies. According to Ubillas et al., 1994, there are no report side effects in traditional medicine of taking the sap of dragon's blood internally.

A study conducted by Bussmann et al., 2011 used a brine-shrimp assay to test the toxicity of medicinal plants used in

traditional medicine in Northern Peru. The results concluded that *Croton lechleri* was not toxic when prepared traditionally as an aqueous extract for oral or topical use, but it was very toxic when an extract was prepared with ethanol.

According to the scientific literature, *C. lechleri* is considered to be non-toxic and well tolerated, but there is no research concerning its effect on pregnant or lactating mothers.

Current us in Allopathic and CAM therapies

Published in 2010, a survey was conducted by Kazhila and Marius on plants used to manage HIV/AIDS opportunistic infections in Katima Mulilo, Caprivi region, Namibia. The purpose of the article was to collect and record knowledge of plants traditional healers used to treat illnesses related to HIV/AIDS. *Croton lechleri* was one of the 72 species used that was listed in Table 1 of article. The common name for *C. lechleri* was tassel berry, but the local name was mukena. Traditional healers use the bark of mukena to form a drink that treated diarrhea, lack of appetite, and anemia. The procedure for how the drink was prepared was not mentioned and neither was the mechanism of action. However, the article did mention that most plants used to treat AIDS-related opportunistic infections contained flavonoids, which have anti-oxidant properties that inhibit free radical production and tissue damage associated with the onset of AIDS (Chinsembu, 2010). This survey is important because it is the basis for identifying plants with anti-HIV active compounds, which could led to the development and usage of new drugs in allopathic therapies.

A study conducted by Miller et al., 2001, examined the potential soothing effect of a 1% sangre de drago balm (Zangrado Bug Bite Balm, Rainforest phytocecuticals) on itching and pain caused by insect bites. The study involved ten workers from the Terminex Pest Control Company in New Orleans, LA who applied the balm topically to insect bites of fire ants over a period of three months. The results showed that the workers experienced relief less than two minutes after applying the balm. This result provided evidence that sangre de drago inhibits sensory nerve afferent activity, but the mechanism is unknown (Miller et al., 2001).

Shaman pharmaceuticals was a company that based its development of natural products from the knowledge of traditional healers. Shaman is the creator of Provir, which at first was identified as a potential treatment for respiratory syncytial virus, but after Shaman scientists witnessed use of *C. lechleri* for a cholera outbreak in Peru of 1993, its target was switched to diarrhea treatment. As mentioned previously, Provir works by slowing down loss of water in secretory diarrhea and allows the body to get rid of toxins and bacteria at a gradual pace. Shaman is also the creator of Virend, a topical formulation of the polyphenol found in Provir, SP-303. Unfortunately this drug died in Phase III trials for herpes and Shaman did not have enough money to support its trials and had to file for bankruptcy. On August 18, 2005 Shaman pharmacuetical was no more, but it was later bought by Napo pharmaceuticals.

Discussion

Croton lechleri is an amazing plant that as many different uses in South America and the United States. Most of its uses such for wound healing, pain relief of insect bites, treatment of herpes, treatment of diarrhea, and other uses have been demonstrated in lab settings, where these properties have been shown to be effective. This plant has been important in the past to traditional cultures and it has now become

important in more developed nations where the need for new effective drugs is of constant need. As time goes on and more is learned about this plant and the compounds responsible for its activity, there will be great advancements in treatments and medicines available to humanity.

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Curcuma longa L., Zingiberaceae

Ashvi Mittal

Introduction

Plants have been used medicinally throughout history by various regions, cultures, and societies in the world. One such plant with deep historical roots is *Curcuma longa*, commonly known as Turmeric (**Figure 1**). The plant belongs to the Zingiberaceae family. The name 'turmeric' is derived from the ancient Medieval Latin name *terramerita.* This transformed into *terre merite* in French, meaning meritorious Earth (P.N. Ravindran, 2007). This was the original name that powdered turmeric was referred to in commercial practices. A few modern common names associated with the plant today include Haldi (Hindi), Saffron de India (French), acafrao de india (Portuguese), Ukon or Gajyutsu (Japanese), kurkuma (German), Daruhaldi (Arabic), Chiang husang (Chinese) Kha min chan (Thai) and Ulgeum or Gangwhang (Korean) (Aggarwal, Sundaram, Malani, & Ichikawa, 2007a; Jansen, 2005),(P.N. Ravindran, 2007).

C. longa is believed to have originated in South Asia, primarily India. Researchers believe that turmeric was introduced to India through ancient tribal groups during their migration from present day China (P.N. Ravindran, 2007). It has been used in Asian medicines since the second millennium BC. It is mainly used in South and East Asia as part of Traditional Chinese Medicine and Ayurveda (Aggarwal et al., 2007a). Turmeric is significantly known for its anti- inflammatory effects in treating muscle sprains, swelling, arthritis, and rheumatism. It also provides protection against high cholesterol levels, angina, irritable bowel syndrome, liver

Figure 1. Photograph of the rhizomes and powder of *Curcuma longa* (Image source: http://www.natural-healing-with-foods.com/turmeric.html)

diseases, and bacterial infections (Goel, Kunnumakkara, & Aggarwal, 2008; Society, 2008) Moreover, some researchers believe that turmeric has the potential to treat certain types of cancers including breast, skin, and stomach cancers (Society, 2008).

The majority of these diverse properties of the plant are due to the main compound in turmeric known as curcumin. The phytochemicals in curcumin are responsible for the yellow color of the plant (Goel et al., 2008). The compound displays a

wide array of properties such as antifungal, antiviral, anti-inflammatory, antioxidant, and anticancer activities. It is also effective against health conditions such as diabetes and arthritis (Pari L, 2008). In addition to acting as a medicine, *C. longa* is used as a spice in culinary practices, herb in religious rituals, and a coloring agent for dying clothes (Muhammed Majeed, 1996).

Botanical Description

Curcuma longa is well known to be a perennial herb which grows about 3-5 feet high. The plant is cultivated primarily in tropical areas of Asia, namely China and India (Stewart & Costerton, 2001). Its pointed, oblong leaves are about two feet long with a consistent green color surrounded by yellow-tinted funnel shaped flowers (Stewart & Costerton, 2001). The plant has tuberous roots like bulbs that produce rhizomes which are used medicinally. In its fresh condition, the roots emit a spicy and aromatic odor (Jansen, 2005). The rhizomes are commonly transformed into a yellow powder by boiling, cleaning, and drying (**Figure 2**) (Stewart & Costerton, 2001). *C. longa* generally thrives in warm and moist environmental habitats. It is usually grown in the tropical regions with about 640-4200 mm of rainfall (P.N. Ravindran, 2007). The plant is grown well in soil that is well drained, fertile, loamy, or alluvial as it cannot tolerate alkaline soil conditions (P.N. Ravindran, 2007). For the efficient growth of rhizomes, stony and bumpy areas are not preferred. The optimum temperature for the sprouting of rhizomes is 25 to 35^{o} Celsius. The plant is capable of growing in shaded areas; however, an exposure to the sun can enhance its growth. The flowers blossom about five months after planting and approximately 7-10 months after the rhizomes have matured, the yellow coloration of leaves begins to appear (Jansen, 2005)

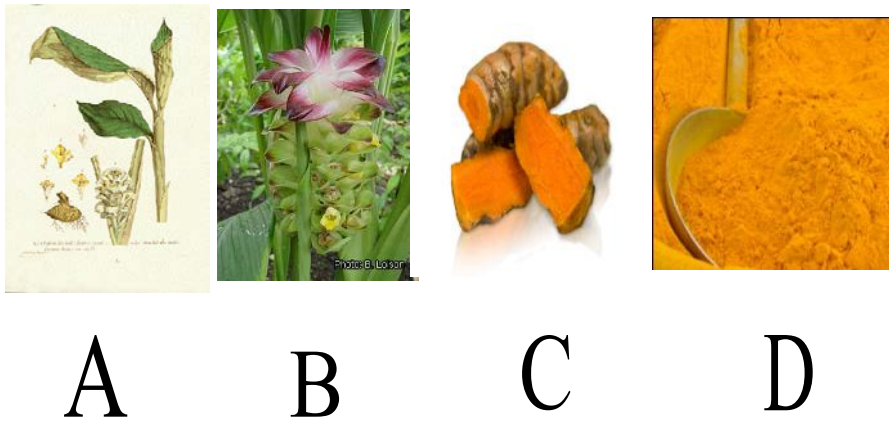

Figure 2. Illustrations of *Curcuma longa* in various forms

Turmeric as a botanical illustration **(A)** and as a full grown plant with green leaves and tinted yellow flowers **(B)**. The tuberous roots of the plants produce rhizomes **(C)**. These are further boiled, cleaned, and dried to produce a powdered form **(D)**. (Image sources: http://www.philographikon.com/botanicalsregault.html- **A**; http://database.prota.org/dbtwwpd/exec/dbtwpub.dll?ac=qbe_query&bu=http://database.prota.org/search.htm&tn=protab~1&qb0=and&qf0=Species+Code&qi0=Curcuma+longa&rf=Webdisplay **-B;** http://www.marxfoods.com/Fresh-Turmeric-Rhizomes **-C;** http://organicindia.mercola.com/herbal-supplements/turmeric.aspx **-D**)

Turmeric is also prone to certain herbivorous invaders. One such feeder is *Taphrina malucans* which is responsible for a disease called leaf spots on the plant (Jansen, 2005). The leaves of the contaminated plant are dried up by the rhizome cot, causing the rhizomes to turn brown. The best way to control this phenomenon is to burn infected plants, and sometimes, it is recommended to sanitize the soil using a fungicide(Jansen, 2005). Examples of fungicide used to treat

Figure 3. *Udaspes folus*, commonly known as the grass demon, feeds on the leaves of *Curcuma longa.*
(Image Source: http://www.butterflycircle.com/checklist%20V2/Cl/index.php/start-page/startpage/showbutterfly/283)

the soil include mancozeb and metalaxyl. In several Asian countries such as India, caterpillars such as *Udaspes folus,* commonly known as the grass demon, is the main insect that feeds on the plant (**Figure 3**). In several countries of Africa, a sucking insect named *Aspidiella harti* nourishes on the plant's rhizomes (Jansen, 2005).

Traditional Uses

The history of *Curcuma longa* in Asian medicine dates back to the second millennium BC (R.A. Sharma, 2005). Turmeric was initially cited in the *Atharvaveda* wherein it was prescribed to cure jaundice (P.N. Ravindran, 2007). It was introduced in China before the 7th century and to Africa in the 13th century. The spice was first brought to the West, essentially to Europe by Arab Sailors (Aggarwal et al., 2007a). The plant was also listed as a coloring agent in the writings of Marco Polo

Figure 4. Movement of *Curcuma longa.*
Turmeric is believed to originate in Asia, primarily in India and later spread to the West via the spice trade.
(Image source: http://ancientstandard.com/2011/02/11/how-cooking-changed-the-face-of-the-earth-the-spice-trade/)

regarding his 1280 AD journey to China and India (Aggarwal et al., 2007a). Turmeric was exposed to North America during the British colonial rule over America (**Figure 4**). It was then that turmeric combined with a variety of other spices and remained curry power in the West (Aggarwal et al., 2007a). Today, *C. longa* is primarily grown in South and East Asia and some home gardens in parts of Africa. It is used not only as medicine, but also as a beauty care agent, spice, religious herb, and a coloring agent (P.N. Ravindran, 2007). The spice is widely used as a culinary enhancement to enrich foods with its vivid yellow color and flavor. In the West, it is also used in mustard and to give color to milk products such as cheese ("Turmeric ", 2011).

Medicinal

C. longa has been traditionally used to treat a wide variety of ailments across Asia and Africa such as skin, pulmonary, gastrointestinal diseases, aches, wounds, and liver disorders (Aggarwal, Sundaram, Malani, & Ichikawa, 2007b). India is considered to be the central hub of the cultivation and application of turmeric. The spice has been used as part of Ayurvedic medicine (Ayurveda) for more than 6000 years. It was applied as a blood purifier, used internally as a tonic for stomach infections, and employed externally for skin diseases. About half to one gram of powder was taken for relieving dyspepsia and flatulence (Muhammed Majeed, 1996) . In order to cure nasal mucus, the fumes of burning *C. longa* were inhaled which led to a discharge of the mucus relieving blockage. In addition, turmeric was often boiled with sugar and milk as an ancient remedy for the common cold (Muhammed Majeed, 1996). The fresh rhizomes were used to make medicinal juices to heal skin infections, wounds, sprains, and inflammations. The powder made from these rhizomes was often mixed with olive oil to enhance and soften the skin.

In rural villages in the Northern part of India, it is common to get eye diseases (Pandeya, 2005). This is due to a lack of availability of health practitioners and antibiotic ointments to cure such diseases. In these cases, the native people use turmeric which they refer to as haldi, as a means of treatment and prevention (Pandeya, 2005). In the villages it has become a folklore that if people do not include turmeric in their daily diet then they will be infected with diseases which may kill them (Pandeya, 2005). Turmeric paste is applied to the infected regions surrounding the eye. Generally, one ounce of turmeric is added to twenty ounces of water to form a mixture. This mixture is then used as a cooling eyewash to heal an infection known as 'country sore eye.' In addition, after the neonatal period, women make a paste of turmeric with powdered ginger and mix in a hot glass of milk as a tonic drink to stay healthy. It is also believed that applying turmeric poultice is beneficial in the healing of lacerations of the birth canal (Pandeya, 2005). In addition, neem leaves and turmeric powder was used an anti-septic to treat chickenpox and smallpox. Turmeric was applied to the body and a bath was taken in boiling water with neem leaves. The ash of the rhizomes was also believed to cure the eruptions caused by the infection (P.N. Ravindran, 2007).

In many African countries such as Ethiopia and Madagascar, turmeric is grown in many residential home gardens. The rhizomes of turmeric are considered to be highly aromatic and contain antibacterial properties (R.A. Sharma, 2005). The locals commonly use the rhizomes as an ailment for bronchitis, cold and, asthma (Pari L, 2008). The juices formed from rhizomes are believed to be an effective remedy for several skin and eye infections (Jansen, 2005).

Rituals/Beliefs

Hindus believe turmeric to be sacred and auspicious. In a few tribal communities in the southern states of India such as Tamil Nadu, a piece of turmeric is tied to a string which is believed to be a sacred nuptial string (P.N. Ravindran, 2007). This practice is still prominent in many areas of South India today. In addition, a piece of turmeric is tied on the hand along with an amulet as a way to fight evil spirits (P.N. Ravindran, 2007). The upper class societies use a natural yellow rhizome in the form of a gold chain, whereas, the lower class and poor rely on turmeric alone.

Traditionally, *C. longa* was used to give yellow coloration to clothing during religious festivals. The villagers believed that the yellow color was associated with Lord Krishna who only

Figure 5. Indian yellow rice prepared with turmeric
(Image Source: http://www.lifesambrosia.com/category/recipes/cuisine/indian/)

wore yellow attires. In addition, in many rural and urban communities, turmeric is used during marriage preparations and ceremonies. Before marriage, turmeric and oil are applied on the bride and their groom by their relatives (P.N. Ravindran, 2007). In the state of Punjab in Northern India, the elders of the community place rice and an areca nut between the joined hands of the couple. Their hands are tied with dyed turmeric seven times. The couples' parents pour turmeric water from a leaf seven times over their hands to conclude the marriage ceremony.

In East Asia, *C. longa* has been an essential component of Traditional Chinese Medicine throughout history. In Indonesia, the yellow color derived from the spice is used in cooking yellow rice called nasi kuning (Aggarwal et al., 2007a). The nasi kuning has religious significance and is believed to be a sacred dish as it is sacrificed to the Gods (Aggarwal et al., 2007a).

Culinary

Turmeric remains a primary ingredient in culinary practices today. It is frequently used to give rice and Indian curry their yellow hue (**Figure 5**). It is added as a relish in authentic dishes and gives food its necessary spice and color ("Turmeric ", 2011). In the South East Asia region, the native people prefer turmeric dry. In Thailand, fresh rhizomes are used to embellish curry dishes and to make yellow curry paste (Aggarwal et al., 2007a).

Coloring Agent

In India, turmeric was believed to be used as a coloring dye by the British. They used the plant as a coloring agent for commercial purposes and monetary gain. In the city of Calcutta, the dyers make a bright yellow hue called *basanti rang* by mixing it with lemon and the carbonate of sodas (P.N. Ravindran, 2007). The plant was also used to make shades of green and indigo. First, the cloth was dyed with indigo and then exposed to a mixture of turmeric. In addition, the Indian Calico printers mixed turmeric with alum and pomegranate to make a dye (P.N. Ravindran, 2007). The mixture was later used to color silk and wool to produce different shades such as browns and olives. Currently, *C. longa* is not applied as a commercial coloring agent.

In West Africa, rhizomes of the plant are used as yellow coloring dyes for products such as cotton clothing, thread, palm fibers, and tanned leather (Jansen, 2005). In Nigeria, a paste is made from the rhizomes with water and rubbed into tanned leather hides. This is a technique which is used to color leather. In Ethiopia, the cloth for coloring is placed into a boiling decoction of turmeric paste. This method is commonly used in many regions of Ethiopia to dye clothes (Aggarwal et

al., 2007a). In addition, in many Asian nations, turmeric is used to form different color combinations of yellow in cotton and silk clothing which is subsequently sold for household earnings (Jansen, 2005).

In European nations, the ground rhizome of turmeric is employed in the food industry as well as a coloring agent in several processed foods. In addition, turmeric is considered a cheaper substitute for saffron as it is utilized in pharmaceutical companies and textile industries (Jansen, 2005) . Some European countries use turmeric in dyeing wool to make various shades of yellow and gold (Jansen, 2005). In North America, the oils of *C. longa* have been labeled as Generally Recognized as Safe (GRAS)(Jansen, 2005)

Chemistry and Pharmacology

Curcuma longa comprises of a wide range of compounds (**Table 1**). The plant contains essential macronutrients such as minerals (3.5%), fats (5.1%), protein (6.3%), moisture (13.1), and carbohydrates (69.4%) (Ishita Chattopadhyay, 2004) . The leaves are rich in various types of acids such as o-coumaric acid, protocatechuic acid, ayringic acid, and vanillic acid (Duke, 2012). The essential oils consist primarily of monoterpenes and sesqueterpenes. The root of the plant contains bisabolene and boron. The rhizomes contain the largest variety of chemical constituents which include polyphenols, monoterpenes, flavonoids, curcuminoids, carotenes, alkaloids, minerals, fiber, and water(Araújo & Leon, 2001; Duke, 2012). The primary compound of *C. longa* found in the rhizomes is curcumin (**Figure 6**). The compound is a polyphenol, responsible for the yellow color of turmeric and comprises approximately 2-5 percent of the spice(Aggarwal et al., 2007a). The phytochemical has hydrophobic properties and is soluble in several substances ranging from oils, ethanol,

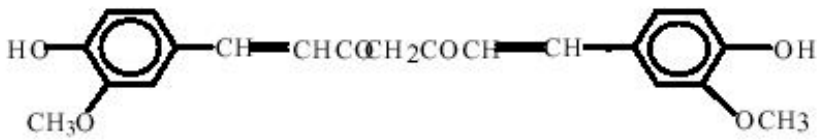

Figure 6. Chemical structure of curcumin (main compound in *C. longa*) (Image source: http://www.scielo.br/img/fbpe/mioc/v96n5/html/404001.html)

to acetone (R.A. Sharma, 2005). When curcumin is taken orally, it is broken apart into curcumin glucuronide and curcumin sulfonate. However, upon systematical administration it is metabolized into tetrayhudrocurcumin, hexahydrocurcumin, and hexahydrocurcuminol (Aggarwal et al., 2007a). Curcumin acts by down regulating iNOS enzymes and COX-2 and suppressing the NFKB pathway (Appleton, 2011). It also inhibits the scavenging free radicals and proliferation of inflammatory cytokines (Appleton, 2011).

Biological Activity

Antioxidant Effects

Turmeric is known for its high antioxidant effects. This is due to curcumin behaving as a scavenger of oxygen free radicals (Ishita Chattopadhyay, 2004). In various *in vitro* studies, curcumin was found to inhibit the generation of reactive oxygen species by stimulating macrophages. These species include ROS, H_20_2, and nitrate radicals. In transgenic mice with Alzheimer's disease, curcumin can decrease the oxidized proteins in amyloid pathology. In addition, it can reduce lipid peroxidation in rat brain homogenates, liver microsomes, and erythrocyte membranes (Ishita Chattopadhyay, 2004). Numerous studies have been conducted to examine

Plant Part	Active Constituents
Rhizomes	1-8 Cineole, 2-Hydroxy-Methyl-Anthrauinone, 4-Hydroxy-Cinnamoyl-Methane, Alpha-Atlantone, Alpha-Terpineol, Ar-Turmerone, Arabinose,Ascrobic Acid, Ash, Azulene, Beta-Carotene, Beta-Sesquiphellandrene, Bis Methane, Bis-Desmethoxycurcumin, Borneol, Caffeic-Acid, Carbohydrates, Chromium, Cinnamic-Acid, Cobalt, Copper, Cuminyl-Alcohol, Curcumin, Curlone, Curzerenone, Cyclo-Isoprenemyrcene, D-Alpha-Phellandrene, D-Camphene, D-Camphor, D-Sabinene, Dehydroturmerone, Desmethoxycurcumin, Di-Coumaroyl-Methane, Didesmethoxycurcumin, Diferuloyl-Methane, Dihydrocurcumin, Eo, Fat, Fiber, Fructose, Gamma-Atlantone, Glucose, Guicacol, Iron, L-Alpha-Curcumene, L-Beta-Curcumene, Manganese, Monodesmethoxycurcumin, Niacin, Nickel, P-coumaric acid, P-Cymene, P-Tolymethyl Carbinol, Phosphorous, Potassium, Resin, Riboflavin, Sodium, Thiamin, Turmerone, Ukonan, Water, Zinc, Zingiberene
Leaf	O-Coumaric Acid, Protocatechuic Acid, Syringic Acid, Vanillic Acid
Essential Oils	Alph-Pinene, Beta-Pinene, Caryophyllene, Curcumene, Curcumenol, Curdione, Curzerenone-C, Eugenol, Limonene, Linalol, Terpinene, Terpineol,
Plant	2-Bornanol
Roots	Bisabolene, Boron,

Table 1. **The chemical constituents of *C. longa* according to corresponding parts of the plant**

(Source: Information obtained from Dr. Duke's Phytochemical and Ethnobotanical Databases http://www.ars-grin.gov/cgi-bin/duke/farmacy2.pl)

curcumin's effect on lipid peroxidation (Araújo & Leon, 2001). It was consistently found that curcumin remains an effective antioxidant and restrains lipid peroxidation in rat livers (Pulla Reddy & Lokesh 1994). This lipid peroxidation plays an essential part in cardiovascular effects, inflammatory effects, and cancer (Araújo & Leon, 2001). Some extracts of *Curcuma longa* are fat and water soluble. These extracts along with curcumin exhibit strong antioxidant activities, comparable to vitamins C and E. One particular study of cardiac ischemia showed that a pretreatment with curcumin reduced ischemic injury (Thorne Research, 2002a). In addition, curcumin's effects on endothelial heme oxygenase-1 were examined in a *vitro* study using bovine aortic endothelial cells. As a result of a seemingly incessant incubation of approximately eighteen hours with curcumin, an increase in the cellular inhibition to oxidative damage was detected. (Thorne Research, 2002a).

Hepatoprotective Effects

Several animal studies have been conducted to determine turmeric's hepatoprotective effects. This ability to prevent

liver damage is due to the anti-hepatotoxic compounds in the plant, which include carbon tetrachloride, galactosamine, acetaminophen, and aspergillus aflatoxin (Thorne Research, 2002b). These compounds decrease the formation of inflammatory cytokines (Thorne Research, 2002a).

In addition, an interesting study done on ducklings infected with fungus *Asperigillus parasiticus,* showed that turmeric extract reduced the production of fungal aflatoxin by ninety percent (Thorne Research, 2002a). Moreover, research suggests that curcumin may also be used to treat cholelithiasis, commonly known as gallstones. (Thorne Research, 2002a).

Anti-inflammatory Effects

Curcumin acts by inhibiting the NF-kB activation and decreasing the TNF-a-induced expression of the gene in endothelial cells (Ishita Chattopadhyay, 2004). The anti-inflammatory effects of turmeric are stimulated by the down regulation of cyclooxygenase-2and inducible nitric oxide synthetase through repressing the NF-kB pathway (Ishita Chattopadhyay, 2004).

The volatile oils of turmeric along with curcumin produce anti-inflammatory effects. A study using rats with arthritis was performed in which curcumin was given orally. This caused a great reduction in the inflammatory swelling compared to the controls (Thorne Research, 2002a). Another study conducted on monkeys illustrated inhibition of neutrophil aggregation which is related to inflammation (Thorne Research, 2002a). In studies on mice with diabetes, curcumin has found to enhance the wound healing and the damage caused by H_20_2 in human fibroblasts(Ishita Chattopadhyay, 2004). Curcumin can also be administered topically to counter inflammation and irritation related to skin allergies (Thorne Research, 2002a).

Anti-carcinogenic/ Anti-tumor Effects

In vivo studies on rats and mice, have exhibited the ability of curcumin to inhibit carcinogenesis at three different stages: during tumor promotion, angiogenesis, and tumor growth (Thorne Research, 2002a). Studies examining colon and prostate cancer show that curcumin suppressed cell division and tumor growth (Thorne Research, 2002a). Several *in vitro* studies using human cell lines have indicated that turmeric has the capacity to restrain mutagenic and carcinogenic presence in various types of cells. This anti-carcinogenic effect of the plant is because of its antioxidant effects and the ability to help in hepatic detoxification of those carcinogens resulting in reduced nitrosamine production(Thorne Research, 2002a).

A study was conducted by Huang et al. (1988) examining the effects of ferulic acid, caffeic acid, chlorogenic acid, and curcumin on tumor growth in mice. It was discovered that curcumin was the most efficient in hindering the epidermal metabolism of arachidonic acid through the cyclooxygenase and lipoxygenase passages (Araújo & Leon, 2001).

Antimicrobial/ Anti-bacterial Effects

Essential oils of *C. longa* and its extracts are known to inhibit the proliferation of many types of bacteria, parasites, and fungi (Thorne Research, 2002a). An *in vivo* study conducted on chicks that had caecal parasite Eimera showed that the diets which were enhanced with one percent turmeric caused a decrease in small intestinal lesion scores and enhanced weight gain (Thorne Research, 2002a). In addition, a study performed on guinea pigs infected with pathogenic molds or

yeast showed that turmeric oil applied topically aided in the reduction of pathogenic fungi, however the curcumin did not have an effect on the yeast. The guinea pigs infected with fungi showed signs of improvements in the lesions. A week after the turmeric was applied to the animals, no lesions were detected (Thorne Research, 2002a).

In 1979, Murthy and Bhavani Shankar performed an *in vitro* study of turmeric fractions applied to intestinal bacteria. The results showed the inhibition of *Lactobacilli* growth when the bacterium was exposed to turmeric (Araújo & Leon, 2001). When examining the alcoholic extract, there were signs of inhibition but they were not as effective as the application of the whole fraction of turmeric (Araújo & Leon, 2001).

Cardiovascular Effects

Turmeric is also known to have positive effects on our cardiovascular system (Thorne Research, 2002a). These effects include lowering of triglyceride levels, cholesterol, and reducing vulnerability of low density lipoprotein to lipid peroxidation (Thorne Research, 2002a). It also aids in inhibiting platelet accumulation (Thorne Research, 2002b). An *in vivo* study performed on rabbits that were exposed to a low dose of turmeric extract showed a decrease in susceptibility of LDL to lipid peroxidation and reduced triglyceride levels. When the rabbits were exposed to a higher level of turmeric extract, the result indicated no decrease in lipid peroxidation of LDL, however there was a reduction in triglyceride and cholesterol levels. This occurred due to the decreased cholesterol uptake in the intestines and increase in exchange of cholesterol to bile acids found inside the liver (Thorne Research, 2002a). In addition, curcumin protects the harm caused by myocardial infarction by reducing the level of severity of pathological changes. It increases the chances of pharmacological interventions to repair the calcium $^{2+}$ homeostasis in the cardiac muscle by improving the Ca $^{2+}$ transport (Ishita Chattopadhyay, 2004).

Gastrointestinal Effects

Several compounds in turmeric serve as effective protective agents of the gastrointestinal tract (Thorne Research, 2002a). *C. longa* is demonstrated to restrain the presence of ulcers due to factors such as stress, alcohol, indo methacin, pyloric ligation, and reserprine (Thorne Research, 2002a). A study done on rats who suffered with these gastrointestinal problems were found to have increased gastric wall mucus production when exposed to the plant (Thorne Research, 2002a).

Clinical Studies

There have been clinical studies performed which apply turmeric power in patients with respiratory ailments (Ammon & Wahl 1991). It was discovered that the patients felt relief of symptoms such as cough, dyspea, and sputum production. Other studies accounted improvement in eighteen patients with rheumatoid arthritis upon administration of the drug orally on a daily bases (Araújo & Leon, 2001)

Studies examining curcumin have occurred over the last fifty years. The compound is known for its anti-carcinogenic properties as it suppresses the growth of tumor cells (Anushree Kumar, 2003). Human clinical trials performed showed no-dose limiting toxicity when given at doses up to ten grams per day (Anushree Kumar, 2003). These studies indicated that curcumin had the ability to prevent and treat various types of cancers such as breast and colon cancer (Anushree Kumar, 2003).

Another study was performed testing curcumin in cancer treatment at initial stages (Society, 2008). Fifteen patients with colorectal cancer were examined to observe their intake of curcumin safely. It was found that the patients could take 3.6 grams of curcumin without any side effects (Society, 2008). Some curcumin and its compounds were detected in the blood at this level of high dose. The lower doses may be beneficial for the intestine and stomach (Society, 2008). Although, it does not absorb effectively in the body, curcumin is able to absorb into the cancerous tissues of the colon. It was recommended from the study that a high dose of curcumin should be used when examining effects outside of the intestine. Further research indicated that individuals were able to ingest ten grams of curcumin per day for a few weeks without observing any side effects (Society, 2008). Currently, researchers are focusing on combining curcumin with other compounds in order to increase the absorption rate in the body (Society, 2008).

An interesting study was conducted on women in Southern India who use turmeric in order to prevent acne and reduce growth of hair (Jasmine H Shaffrathul, 2007). Seventy three individuals including female nurses, visitors, and workers of a hospital were surveyed concerning the period and reason for their turmeric use. The individuals were inspected for signs of acne. Thirty five of the 75 subjects used turmeric daily, while the remaining did not apply turmeric. The results of the study indicated no statistical significance in the *p* value between the individuals with acne who utilized turmeric and ones who did not employ the herb (Jasmine H Shaffrathul, 2007). In addition, the subjects who used turmeric did not indicate a reduction in the growth of hair when compared to the individuals who did not use turmeric.

A recent clinical study examined the use of a distinctive extract of *C. longa* on patients with osteoarthritis. In Italy, a team of scientists chose fifty patients with osteoarthritis in the knee in order to test a special formulation of turmeric known as Meriva ("Special Turmeric Extract Benefits Osteoarthritis Patients," 2010). After separating the individuals into two groups, the first group was exposed to the standard medical treatment by physicians, while the individuals in the second group were treated with curcumin extract in addition to the typical medical therapy. After a ninety day period, the individuals exposed to the extract had a 58 percent increase in their overall health and functionality when compared to the control group. Moreover, the Social and Emotional Index score for the Meriva patients increased by 300 percent, followed by a 16 fold decline of inflammation levels ("Special Turmeric Extract Benefits Osteoarthritis Patients," 2010). The most astonishing finding of the study was that patients treated with the turmeric extract reduced their use of NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) by 63 percent when compared to the patients with the standard medical treatment ("Special Turmeric Extract Benefits Osteoarthritis Patients," 2010).

Contraindications

Turmeric is generally considered to be a low toxicity plant, especially when used as a spice in foods. However, there needs to be more research conducted to examine the side effects of using turmeric as a herbal remedy. It is believed that ingesting large amounts of the spice orally can lead to stomach pains, indigestions, and nausea. Long term exposure to the plant can further be a cause of stomach ulcers and skin rashes. Individuals who have allergic reactions to ginger products or food colorings are likely be allergic to turmeric. Furthermore, women who are pregnant or breastfeeding and individuals

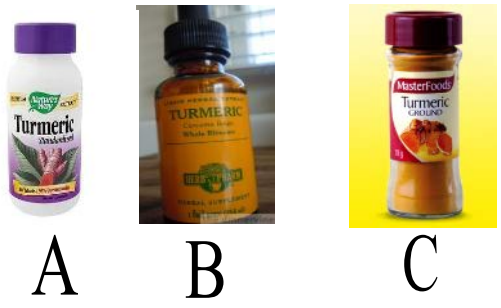

Figure 7. The different forms of turmeric which are marketed: Capsules (A), Tinctures (B), and ground powder (C) (Images Sources: http://www.nutritionexpress.com/natures+way/natures+way+turmeric+extract+500+mg+120+tablets.aspx **-A;** http://www.dinnervine.com/2012/01/turmeric-and-tinctures-and-rhizomes-oh-my/ **-B;** http://www.masterfoods.com.au/products/productdetail/tabid/83/productid/1574/turmeric-ground.aspx **-C**)

with bleeding disorders or a record of ulcers should avoid the use of turmeric (Society, 2008).

A study conducted in humans indicated that curcumin has the ability to make changes in the metabolism of oxalate. According to the results, the researchers cautioned individuals in using curcumin with conditions which can lead to kidney stones. In addition, people taking blood thinners should avoid the use of turmeric as a spice because it can cause detrimental herbal drug interactions (Society, 2008).

In various *in vivo* studies, it was found that turmeric can reduce the effectiveness of many anti-cancer drugs (Society, 2008). This is due to the antioxidant supplements which can intervene with the efficacy of cancer treatments such as radiation therapy and chemotherapy. It is often advised that people should not solely depend on turmeric to cure cancer as overuse of the plant can have side effects (Society, 2008). Therefore, it is vital to inform our health care practitioners about any herbal supplements we taking. According to the National Center for Complementary and Alternative Medicine, turmeric is a safe plant for most adults. However, long term exposure or high doses of the spice can be a cause of indigestion and diarrhea. Many studies in animals have shown that intake of high doses may lead to liver illnesses. Moreover, individuals who have gallbladder infections should avoid the use of turmeric as a dietary supplement (NCCAM, 2007).

Current Use in Allopathic and CAM therapies

Curcuma longa persists as an essential part of Ayurveda and Chinese Medicine today. Auyrvedic medicine remains one of the oldest medical systems worldwide. Ayurveda is comprised in the subcategory of CAM: whole medical system (NCCAM, 2005). This category includes practices that have evolved over time. Ayurvedic therapies consist of herbs, massages, and specialized diets (NCCAM, 2005). Studies have exhibited that practice involves using herbal therapies which include curcuminoids. This is believed to aid in cardiovascular diseases. In addition, botanicals such as ginger, turmeric, and boswellia are used in NCCAM as a treatment for inflammatory illnesses such as arthritis and asthma (NCCAM, 2005) Turmeric is also used to cure dental related issues (Chaturvedi, 2009). The process for treatment can include rinsing the mouth with turmeric water for relief, massaging the paining tooth with ground turmeric to eliminate any swelling, employing turmeric powder with weed seeds to clean the gums, and applying turmeric paste (½ teaspoon salt, ½ teaspoon mustard oil) to prevent gingivitis and periodontitis (Chaturvedi, 2009).

Currently, there are not any drugs derived from this plant. However, the roots of turmeric are on the approved list of

Form of turmeric applied	Dose recommendation for adults
Cut root	1.5-3 g per day
Dried, powdered root	1-3 g per day
Standardized powder (curcumin)	400-600 mg, 3 times per day
Fluid extract (1:1)	30-90 drops per day
Tincture (1:2)	15-30 drops, 4 times per day

Table 2. The amount of dose recommended for adults according to the form of turmeric used.
(Source: http://www.umm.edu/altmed/articles/turmeric-000277.htm)

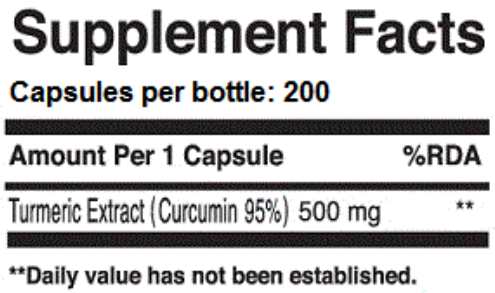

Figure 8. Turmeric supplement marketed as containing ninety five percent curcumin. (Image Source: http://www.turmeric-curcumin.com/)

herbs which are accessible as a spice in local grocery shops (Society, 2008). The average turmeric consumed commonly by Asians ranges from .5-1.5 g/day/person producing no toxic symptoms or side effects (Ishita Chattopadhyay, 2004). *C. longa* is available in the form of a capsule, tincture, and ground powder (**Figure 7**). Extracts of pineapple called bromalein are frequently combined with the products of turmeric as it has the ability to enhance the anti-inflammatory effects of curcumin ("Turmeric ", 2011). The supplements of turmeric have not been studied extensively in children; therefore there are no known recommended doses for children. However, there are suggestions of doses for adults (**Table 2**). Several sellers who market supplements of turmeric today argue that their products comprise of 95 percent curcumin compounds (**Figure 8**) (Society, 2008).

Discussion

The extensive use of *Curcuma longa* throughout history in Ayurvedic and Traditional Chinese Medicine is truly remarkable. Its wide array of uses as a spice, herb, medicine, dye, and various other applications enable the plant to cure and prevent numerous ailments. Moreover, the use of *C. longa* as a spice to give authentic foods its flavor and color remains an essential component of culinary practices today. Turmeric is beneficial for patients suffering from liver disorders, heart disease, diabetes, inflammations, osteoarthritis, and many other illnesses. Currently, turmeric is being studied to prevent aging, Alzheimer's disease, and most essentially cancer. There are numerous studies examining the ability of curcumin, the main compound in *C. longa*, in the treatment of cancer by inhibiting the proliferation of tumor cells. Despite widespread research on the possible medicinal applications of turmeric, there have not been any studies examining drug development of the plant. Today, curcumin is accessible in its pure form comprising of various biological activities. In the future, this could enable researchers to develop new drugs from curcumin by observing the pharmacological effects and mechanism of action of the compound. Curcumin may be employed as a

novel drug in the coming decades, which could prevent and treat a wide range of illnesses.

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Digitalis purpurea L., Plantaginaceae

Ian McCullough

Introduction

Commonly referred to as purple foxglove, *Digitalis purpurea* is a flowering plant in the Plantaginaceae family (USDA, 2012). *D. purpurea* is native to North Africa, Europe, as well as central and western Asia (Lin, Yang, Phua, Deng, & Lu, 2010). It was first mentioned in a Welsh Pharmacopeia published in the early 12th century AD, and was described as having blossoms which resembled a glove by Fuchsius in his 'De Historia Stirpium Commentarii Insignes,' published in 1542 (Rietbrock & Woodcock, 1985; Withering, 1785). Although *D. purpurea* has been used medically for over 700 years, the medicinal properties of *D. purpurea* were categorized by Withering in 1785 as a treatment for dropsy, which is now referred to as cardiac insufficiency or congestive heart failure resulting in edema (Whitfield, 1985; Withering, 1785). The medicinal value of *D. purpurea* results from high concentrations of potent cardiac glycosides it contains (Norn & Kruse, 2004). Two of these cardiac glycosides, digoxin, and digitoxin, have since become the most potent inotropic agents yet identified, as well as some of the most wildly prescribed inotropic agents in the world (Rietbrock & Woodcock, 1985).

Botanical Description

Digitalis purpurea, also known as common foxglove or purple foxglove, is a flowering plant that is native to North Africa, Europe, as well as Asia (Lin et al., 2010). However, purple foxglove was extensively imported to North America following the publication of Withering's book in the late 1700's, and it is

A)

B)

Figure 1. Habitat of Digitalis purpurea in western Europe (A), and North America (B).
Source A:
http://pbio209.pbworks.com/w/page/11342445/Snyder,%20K%20-%20Digitalis%20purpurea
Source B:
http://plants.usda.gov/java/profile?symbol=DIPU&photoID=dipu_008_ahp.tif

Figure 2. *Digitalis purpurea* (Purple foxglove).
Source:
http://plants.usda.gov/java/largeImage?imageID=dipu_007_avp.tif

now commonly found in the wild (**Figure 1**) throughout much of New England, the Mid-Atlantic States, the Pacific Northwest, as well as Canada (Rietbrock & Woodcock, 1985; USDA, 2012).

Withering describes purple foxglove (**Figure 2**) as a biennial common to dry gravely soil and flowering from late spring to early summer (Withering, 1785). It is a herbaceous plant with dark green foliage as well as a conspicuous purple flowers from which *D. purpurea* originally derived its name (USDA, 2012; Withering, 1785). The scientific name *Digitalis purpurea* directly translated to 'purple fingers' and was given to purple foxglove by Leonard Fuchs in 1542 (Rietbrock & Woodcock, 1985). These purple flowers grow in elongated clusters around the stem of *D. purpurea* and resemble fingers of a glove (Rietbrock & Woodcock, 1985). The plant tends to be between one and two meters tall at maturity and produces only foliage during its first growing season then typically flowers, produces seed, and dies in the subsequent growing season (Rietbrock & Woodcock, 1985; USDA, 2012; Withering, 1785).

Nearly all of the bioactive constituents derived from *D. purpurea* can be classified as cardiac glycosides (Doherty, 1973; Doherty & Kane, 1975). Although quantities of these glycosides can vary depending on growing conditions, the concentration typically found in as few as one or two leaves is nearly always toxic and potentially even lethal (Ramlakhan & Fletcher, 2007). That said, its bitter taste is generally sufficient to deter ingestion by herbivores (Rietbrock & Woodcock, 1985).

Traditional Uses

Historical accounts indicate that *Digitalis purpurea* has been used medically for over 700 years as part of traditional English folk medicine for treating dropsy, and written records of *D. purpurea* being prescribed medically date to as early as 1250 AD by Welsh physicians (Norn & Kruse, 2004). However, its medicinal use was inconsistent until the late 18th century

(Norn & Kruse, 2004). An English physician named William Withering became interested in *D. purpurea* in 1775 when he observed a patients recovery from dropsy, now referred to as congestive heart failure, following her consumption of herbal tea for several months (Rietbrock & Woodcock, 1985; Withering, 1785). Withering identified the active ingredient of this herbal tea as *D. purpurea*, and began conducting research into its effectiveness (Breckenridge, 2006). By 1785 he had treated 163 patients suffering from dropsy with *D. purpurea* and published his findings in 'An Account of the Foxglove and Some of its Medical Uses' (Whitfield, 1985). Withering drew upon the treatment outcomes of his own patients to objectively demonstrate the efficacy of using *D. purpurea* in treating dropsy, the edematous bodily swelling that typically accompanied heart failure (Withering, 1785). Withering, as well as medical practitioners in subsequent decades employed *D. purpurea* therapeutically by boiling its leaves into a herbal tea (Rietbrock & Woodcock, 1985). This process of boiling served to extract some of the more water soluble cardiac glycosides into the aqueous solution, including gitaloxin, which will be discussed later (Rietbrock & Woodcock, 1985).

Purple foxglove was widely cultivated throughout England and subsequently in North America following its introduction in the late 1700's (Rietbrock & Woodcock, 1985; USDA, 2012). Prior to identification of its medicinal properties *Digitalis purpurea* was predominantly grown for its ornamental flowers (Foster, 2000). *D. purpurea* proliferated widely in the United States following the discovery of its therapeutic properties. However, now that homemade *D. purpurea* preparations are no longer commonly made for the treatment of congestive heart failure *D. purpurea* is once again cultivated for its aesthetically pleasing flowers (Foster, 2000).

Chemistry & Pharmacology

The active components found in *Digitalis purpurea* include a number of cardiac glycosides, however, it is most widely known for containing the cardiac glycoside digitoxin (Rietbrock & Woodcock, 1985; Walter Lewis, 2003). The direct inotropic effects of the cardiac glycosides found in *D. purpurea* result from their inhibition of the activity of the sodium/potassium ATPase, also known as the sodium potassium exchanger, in the myocardium (Eric J. Eichhorn & Mihai Gheorghiade, 2002). The predominant method of expelling calcium from myocardial cell between contractions is the action of the membrane bound sodium potassium antiporter protein (Khatter, Agbanyo, Navaratnam, Nero, & Hoeschen, 1989). This protein uses the energy produced from moving sodium ions down their concentration gradient into the cell from the extracellular environment to pump calcium ions out of the cell against their concentration gradient (Khatter et al., 1989). By inhibiting the active transport of sodium ions out of the cell, *D. purpurea* derived cardiac glycosides bring the intracellular and extracellular sodium concentrations closer to equilibrium (Khatter et al., 1989). Because the sodium/calcium antiporter is powered by the movement of sodium down its concentration gradient into the cell, the reduction the sodium gradient decreases the activity of the sodium/calcium antiporter and results in increased levels of intracellular calcium ions (Eric J. Eichhorn & Mihai Gheorghiade, 2002). Increasing the concentration of intracellular calcium ions directly results in more interaction between the actin and myosin contractile proteins within the cells of the myocardium (Hauptman & Kelly, 1999). The increase in interaction between actin and myosin contractile proteins increase the contractile force generated by each cardiac myocyte (Doherty, 1973) . The increase in contractility results in a stronger heart beat as well as an increased ejection

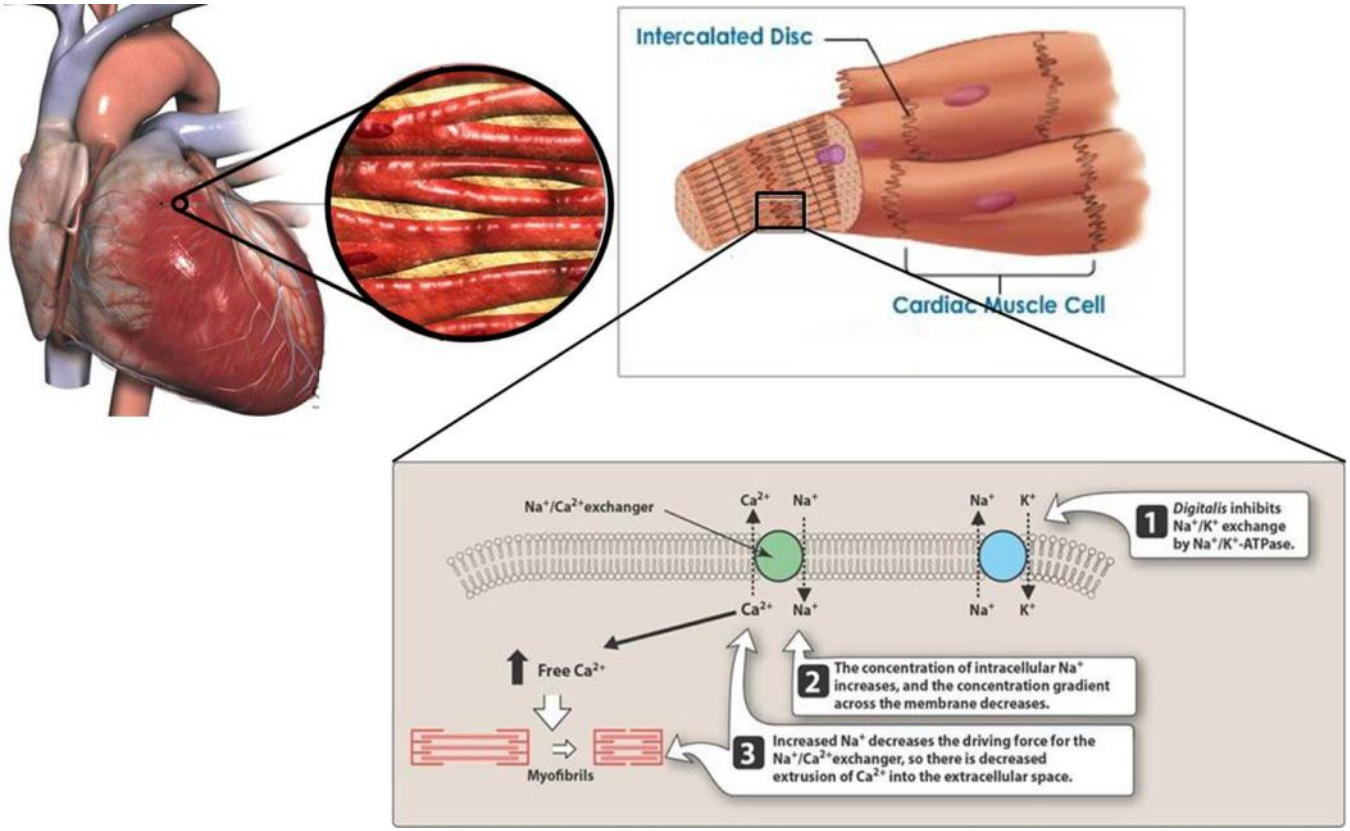

Figure 3. Mechanism of action of cardiac glycosides. Source http://e-learning.perubatan.org/index.php?topic=738.0

fraction (Eric J. Eichhorn & Mihai Gheorghiade, 2002). *D. purpurea* derived cardiac glycosides also exert an effect on the cardiac conduction system via inhibition of the sodium potassium exchanger (Doherty & Kane, 1975), **Figure 3**.

Multiple factors can and do influence the concentration of cardiac glycosides present in *D. purpurea* preparations such as the time of the year when the leaves are gathered, the climate and the soil conditions where the plant is cultivated (Goldman, 2001; Rietbrock & Woodcock, 1985). Withering noted that foxglove leaf preparations were efficacious in small, non-toxic doses and that their action varied according to the plant's stage of bloom (Rietbrock & Woodcock, 1985; Withering, 1785).

Major D. purpurea derived cardiac glycosides

Of the 38 cardiac glycosides found in *D. purpurea*, the two which have, or are currently used therapeutically are

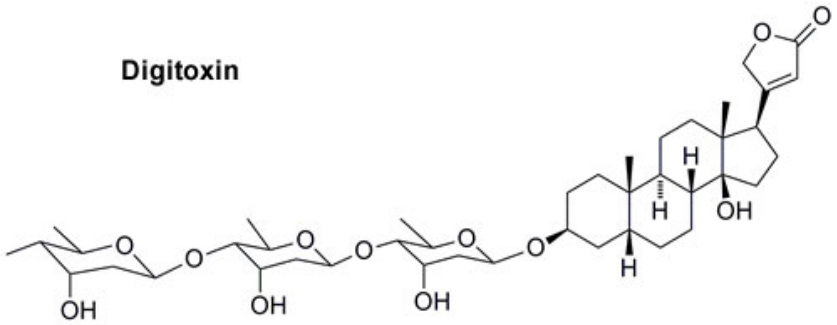

Figure 4. Chemical structure of Digitoxin
Source: http://www.soci.org/News/Hort-newsletter-jun-10

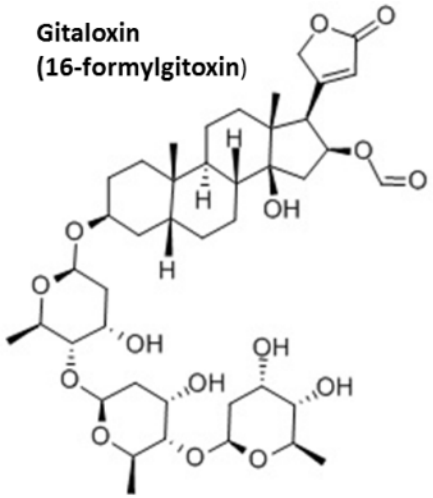

Figure 5. Chemical structure of Gitaloxin
Source:
http://www.chemicalbook.com/ProductChemicalPropertiesCB6877110_EN.htm

digitoxin (**Figure 4**), and gitaloxin (**Figure 5**) (Rietbrock & Woodcock, 1985). Gitaloxin is the only cardiac glycoside that can be collected from the leaves of *D. purpurea* in appreciable quantities by boiling (Rietbrock & Woodcock, 1985). Since

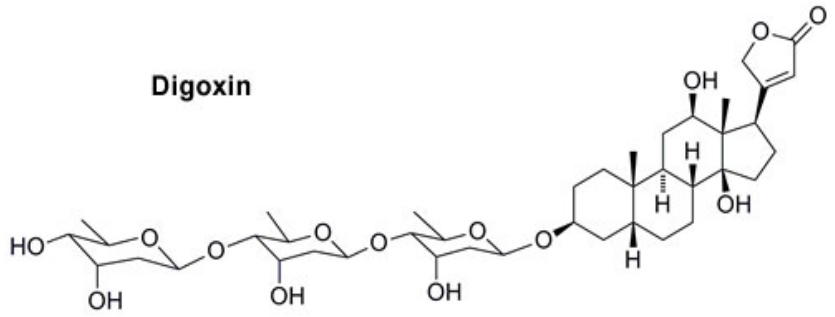

Figure 6. Chemical Structure of Digoxin
Source http://www.soci.org/News/Hort-newsletter-jun-10

traditional foxglove teas were made by boiling *D. purpurea* leaves in water, it is highly likely that gitaloxin was the cardiac glycoside responsible for the diuretic effects observed by Withering and other traditional practitioners of foxglove therapy (Rietbrock & Woodcock, 1985). The most commonly used cardiac glycosides employed pharmacologically today are digoxin (**Figure 6**) and digitoxin (Beermann, 1974; Doherty, 1973).

Dosing considerations

The cardiac glycosides derived from *Digitalis purpurea* have a relatively narrow therapeutic window with therapeutic dosages which can be as high as seventy percent of the dose required for toxicity (Walter Lewis, 2003). Moreover, the therapeutic dose varies widely from patient to patient. Withering, like physicians today, had to tailor the dose of *D. purpurea* preparations specifically for each patient individually as a dose which would be sub therapeutic on one patient may result in toxicity in another patient (Doherty, 1973; Doherty & Kane, 1975).

Causes, presentation, & management of overdose

The majority of cases involving *D. purpurea* toxicity result from an unintentional overdose on pharmacological agents derived from the plant (Antman, Wenger, Butler, Haber, & Smith, 1990). However, cases of toxicity resulting from intentional overdoses as well as from unintentional exposures to the plant occur occasionally as well (Church, Schamroth, Schwartz, & Marriott, 1962). Accidental toxicity can result from ingestion of water with *D. purpurea* growing in it or nearby (Ramlakhan & Fletcher, 2007). Toxicity may also result from inhalation of smoke produced by burning *D. purpurea* (Ramlakhan & Fletcher, 2007). Toxicity resulting from accidental ingestion by wild food gatherers who mistake *D. purpurea* for comfrey is one of the most common causes for toxicity not related to allopathic treatment with cardiac glycosides (Lin et al., 2010; Ramlakhan & Fletcher, 2007).

Symptoms of overdose from cardiac glycosides will vary from patient to patient, however, the most common indicators of toxicity include fatigue, anorexia, nausea, loss of visual acuity, as well as cardiac rhythm abnormalities which can be either tachycardic or bradycardic in nature (Doherty & Kane, 1975; Fisch & Knoebel, 1985; Ramlakhan & Fletcher, 2007). Symptomatic bradycardias induced by *D. purpurea* typically result from decreased conduction through the Atrioventricular node, and symptomatic tachycardias result from spontaneous depolarization of autorhythmic cells located in the ventricles (Fisch, 1962; Rodensky & Wasserman, 1962). First and second degree Atrioventricular blocks, as well as junctional rhythms also occur frequently following *D. purpurea* toxicity (Doherty & Kane, 1975; Fisch, 1962). However, the most reliable indicator of *D. purpurea* toxicity observed on an electrocardiogram prior to such symptomatic arrhythmias is depression of the ST segment with a concave upward slope (**Figure 7**) (Ramlakhan & Fletcher, 2007). If untreated, cardiac glycoside toxicity may result in death from ventricular fibrillation (Doherty, 1973; Walter Lewis, 2003).

The three primary objectives when treating a patient suffering from *D. purpurea* toxicity are supporting the heart and preventing the occurrence of arrhythmias, preventing further absorption of cardiac glycosides from the gastrointestinal tract, and reducing the concentration of serum glycosides as rapidly as possible (Antman et al., 1990). Various medications are used to protect the heart from arrhythmias. Atropine can be administered to counter the increased parasympathetic tone caused by the *D. purpurea* derived glycosides, short acting beta blockers may be administered to suppress tachycardias, and lidocaine may be administer in the case of persistent ventricular ectopy (Khatter et al., 1989; Ramlakhan & Fletcher, 2007). Synchronized cardioversion is indicated in cases of symptomatic tachycardias which are unresponsive to pharmacological interventions (Bourel & Gouffault, 1962; Ramlakhan & Fletcher, 2007). Symptomatic bradycardias should be electrically paced if they are unresponsive to pharmacological interventions (Ramlakhan & Fletcher, 2007). Activated charcoal is commonly administered orally to prevent further absorption of glycosides from the gastrointestinal tract (Rodensky & Wasserman, 1962). Most importantly, the concentration of circulating cardiac glycosides should be rapidly reduced via intravenous administration of anti-digoxin antibodies (Antman et al., 1990). Antibodies to digoxin were first created in 1967, but were only later employed as standard therapy for digoxin toxicity (Doherty, 1973; Ramlakhan & Fletcher, 2007). These antibodies to digoxin are currently marketed under the brand name Digibind and have also been effective in cases of digitoxin toxicity, although to lesser extent (Antman et al., 1990; Ramlakhan & Fletcher, 2007).

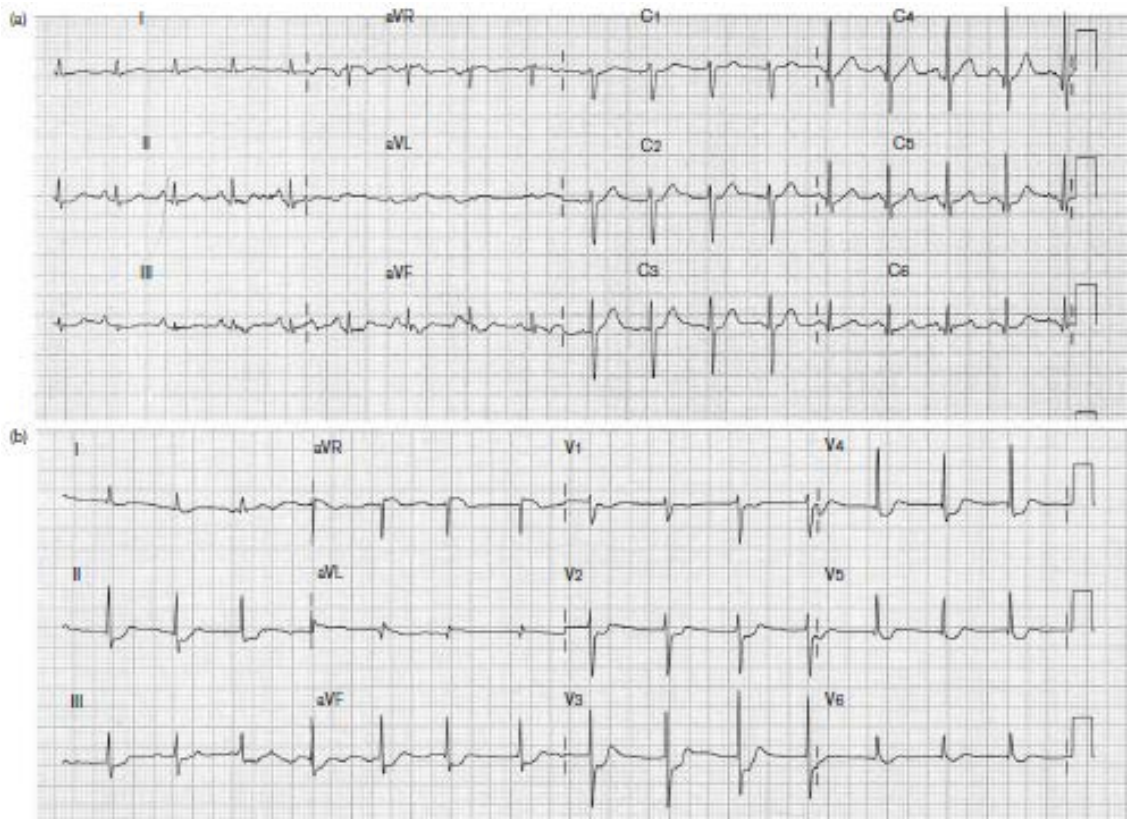

Figure 7. Electrocardiogram displaying the effects of *D. purpurea* toxicity. (a) depicts a normal ECG. Note the concave upwardly sloping ST segment depression in (b). Source (Ramlakhan & Fletcher, 2007)

Biological Activity

Withering and others describe the diuretic effect of *Digitalis purpurea* extracts; however, this effect is the indirect result of three direct effects of the cardiac glycosides found in *D. purpurea* on the heart (Beermann, 1974; Doherty, 1973; Doherty & Kane, 1975). The first of these three direct effects is an increase in myocardial contractility (Doherty & Kane, 1975). The second is an increase in parasympathetic tone which results in decreased heart rate which allows more time for the ventricles to fill between each beet (Eric J. Eichhorn & Mihai Gheorghiade, 2002). Finally, the third direct effect of *D. purpurea* derived glycosides is a slowing of the electrical impulses through the cardiac conduction system (Krantz & Ling, 1960). Such effects on the cardiac conduction system are confined to the atria, notably decreasing spontaneous depolarization in the Sinoatrial node and intermodal tracts, and slowing conduction through the Atrioventricular node

(Eric J. Eichhorn & Mihai Gheorghiade, 2002; Ramlakhan & Fletcher, 2007). Together these three effects increase contractility as well as decreased heart rate is in an increase in ejection fraction which increases the volume of blood pumped from the heart with each contraction (Doherty & Kane, 1975; Soffer, 1962). This increase in ejection fraction results in increased systemic perfusion. Specifically, renal perfusion is increased which enhancing the production and excretion of urine thereby eliminating excess fluid form the body (Doherty & Kane, 1975; Rietbrock & Woodcock, 1985).

Clinical Studies

Empirical evidence for the clinical effectiveness of *Digitalis purpurea* dates to the time of Withering, formal clinical trials of various *D. purpurea* preparations began at least as early as 1915 (Simson, 1962). Since then numerous studies of *D. purpurea* have demonstrated the positive inotropic effect of its cardiac glycosides on the myocardium (Cattell & Goodell, 1937). These studies have been conducted in animals such as frogs, cats and dogs to determine the mechanisms of action for the various cardiac glycosides extracted from *D. purpurea* (van Dyke & Li, 1935). Moreover, these animals were also to develop standardized concentrations for administration of digoxin and digitoxin which are two more recently identified cardiac glycosides (Doherty, 1973; van Dyke & Li, 1935)

Following the development of agreed upon standard for dosing were developed in the early to mid 1900's, studies into the effectiveness of the various glycosides were conducted (van Dyke & Li, 1935). In a 300 patient multicenter trial digoxin reduced hospitalization for congestive heart failure as well as increasing left ventricular ejection fraction (Eric J. Eichhorn & Mihai Gheorghiade, 2002). The Digitalis Investigation Group (DIG) trial was another example of the effect of digoxin verses alternative medicinal therapies for congestive heart failure. Over several months *D. purpurea* derived cardiac glycosides were shown to reduce hospitalization for congestive heart failure by twenty five percent (Gheorghiade & Pitt, 1997). Moreover, *D. purpurea* derived cardiac glycosides is the only currently marketed oral inotropic agents which do not result in increased mortality when administered to patients suffering from congestive heart failure (Eric J. Eichhorn & Mihai Gheorghiade, 2002).

Lastly, studies have indicated that concurrent use of aldosterone antagonists with the *D. purpurea* derived cardiac glycoside digitoxin may reduce the occurrence of toxicity associated with their use (Eric J. Eichhorn & Mihai Gheorghiade, 2002). Aldosterone is a compound secreted by the adrenal cortex in response to activation of the rennin-angiotensin-aldosterone-cascade which is not only up regulated in response to hypotension, but also in response to administration of digoxin (Eric J. Eichhorn & Mihai Gheorghiade, 2002). Because aldosterone has been observed to alter serum concentrations of potassium and potentially result in arrhythmias and potentiate the effects of digoxin toxicity, concurrently blocking the secretion aldosterone may increase the safety of *D. purpurea* based glycosides used for the management of congestive heart failure (Eric J. Eichhorn & Mihai Gheorghiade, 2002).

Contraindications

The two most commonly prescribe cardiac glycosides derived from *Digitalis purpurea* are digoxin and digitoxin. Digoxin is excreted from the body via the kidneys and extreme care should be taken when administering the drug to patients in renal failure their lack of renal clearance can result in the increase of serum cardiac glycoside concentration to toxic

levels (Hauptman & Kelly, 1999). However, digitoxin clearance is independent of renal function and is widely considered the safer of the two cardiac glycosides for administration to patients suffering from renal failure (Genazzani, 1977).

There are no medications which are completely incompatible with cardiac glycosides derived from *Digitalis purpurea*; however, diuretics which act by altering serum electrolyte concentrations, specifically potassium concentrations, such as furosemide, should be administered with care, as such medications can significantly increase the likelihood of developing arrhythmias (Ramirez-Ortega et al., 2007).

Current Use in Allopathic and CAM Therapies

The most widely prescribed *Digitalis purpurea* extract prescribed today is digoxin however, digitoxin is also occasionally prescribed as both are cardiac glycosides isolated in the early 1900's and both have relatively similar effects on the body (Rietbrock & Woodcock, 1985; Walter Lewis, 2003). These cardiac glycosides have become the most widely proscribed inotropic agents in the United States (Doherty, 1973). In fact, the *D. purpurea* derived cardiac glycosides are employed so widely in modern medicine that the term 'digitalis' is commonly used in place of 'cardiac glycosides' (Hauptman & Kelly, 1999).

No herbal supplements derived from *D. purpurea* are currently marketed however; it may still be grown and used by some practitioner's in England for the treatment of congestive heart failure (Rietbrock & Woodcock, 1985). It is worth noting that correct identification of plant species is essential for practitioners of CAM. As previously mentioned, several instances of severe toxicity have resulted from the misidentification of *D. purpurea* as *Symphytum offcinale*, commonly referred to as comfrey (Lin et al., 2010). Leaves of *S. offcinale* resemble those of *D. purpurea,* and during times of the year when *D. purpurea* lacks its distinctive purple flowers misidentification of the two plants becomes possible (Lin et al., 2010).

Discussion

The cardiac glycosides found within purple foxglove both increase contractility and reduces heart rate (Michael Heinrich, 2004). This increase in inotropy and reduction in chronotropy result in a slower but stronger heart beat and can increase cardiac output by as much as thirty percent (Walter Lewis, 2003).

As in the time of Withering, therapeutic administration of modern cardiac glycosides is often problematic for three reasons. First, its therapeutic window is very narrow, with the dosage required to achieve a physiological effect often approaching seventy percent of the dose necessary to produce toxicity (Breckenridge, 2006). In addition to this narrow therapeutic window, cardiac glycosides extracted from *D. purpurea* have relatively long half-lives, 36 to 48 hours in the case of digoxin, and approximately 10 days in the case of digitoxin (Doherty, de Soyza, Kane, Bissett, & Murphy, 1978). Repetitive dosing over time can, and often does progressively increase serum cardiac glycoside concentration eventually resulting in toxicity (E. J. Eichhorn & M. Gheorghiade, 2002; Pruitt, 1962). Finally, therapeutic doses of *D. purpurea* extracts vary widely from patient to patient (Pruitt, 1962). For example, the dosage required to achieve a therapeutic effect in one patient may be significantly greater than the dosage required for toxicity in another (Breckenridge, 2006; Pruitt, 1962). In fact, even the plasma concentrations of digoxin required to achieve a therapeutic effect can vary widely

between individuals (Breckenridge, 2006; Ramlakhan & Fletcher, 2007).

Interestingly, approximately twenty percent of patients receiving *D. purpurea* derived cardiac glycoside therapy today report experiencing adverse side effects, whereas the therapeutic success rate observed by Withering in the late 1700's was slightly higher, with only eighteen percent of his patients reporting side effects (Rietbrock & Woodcock, 1985) Moreover, Withering achieved this success rate while administering these glycosides in the form of teas brewed from foxglove, as opposed to modern practitioners who administer digoxin or digitoxin which are highly standardized and regulated *D. purpurea* derived cardiac glycoside, suggesting that herbal treatment administered by early practitioners such as Withering was no less successful, and no more likely to result in toxicity than modern synthetically derived *D. purpurea* therapy (Rietbrock & Woodcock, 1985).

Despite the potential for toxicity, *D. purpurea* derived cardiac glycosides have been a fixture in the treatment of congestive heart failure for over two centuries (Rietbrock & Woodcock, 1985). The use of *D. purpurea* derived cardiac glycosides will likely continue long into the future as they are the most potent inotropic agents yet identified and no suitable replacements have been identified (Rietbrock & Woodcock, 1985; Rodensky & Wasserman, 1962; Simson, 1962).

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Diospyros virginiana L., Ebenaceae

Ruby M. Lam

Introduction

Diospyros virginiana is a dicotyledon from the Ebenaceae family (Ebony). It may also appear as *Diospyros mosieri* S.F. Blake. In GRIN Taxonomy (Germplasm Resources Information Network from the USDA). *D. virginiana* has many common names including American Persimmon , Common Persimmon, Eastern Persimmon, Simmon, Possumwood, Sugar-plum, Date Plum, White Ebony, and Florida Persimmon. The genus name, *Diospyros,* is derived from the Greek words *διός (*Διος*)* meaning "god" and *pyros (πυρος)* meaning "wheat" or "grain"; which both allude to the life-giving properties of the edible fruit (Department of Agriculture, 1949). The origin of the name Persimmon is from the Algonquian tribes (Northern First Nations people) words for dried fruit—putchamin, pasiminan, or pessamin, which roughly translates to "choke fruit" (Curtis, Bausor, & Curtis, 1943). *D. virginiana* grows primarily in Southeastern United States but can be found as far north as Massachusetts and as far west as Oklahoma(Sargent, Faxon, & Gill, 1933). It was a very important source of food in the winter for the first settlers of Jamestown and confederate soldiers during the American Civil War. Besides providing consumable fruit, the plant also provides wood used for carving and bark used for color dying. The First Nation's people utilized its astringent properties for various medical ailments including thrush, diarrhea, gonorrhea, dropsy, dysentery, and bacterial infections (Briand, 2005).The bark , unripe and ripe fruit contain lignin, tannic acid, sugar, malic acid and pectin(Jiaofen, 2005). In the United States today, *D. virginiana* is often considered as just a minor fruit because its cousin the Japanese persimmon (*D. kaki*) cousin is cultivated, larger, and imported as an exotic fruit. Few Americans even know that that there are species of persimmon native to the Americas and consequently don't

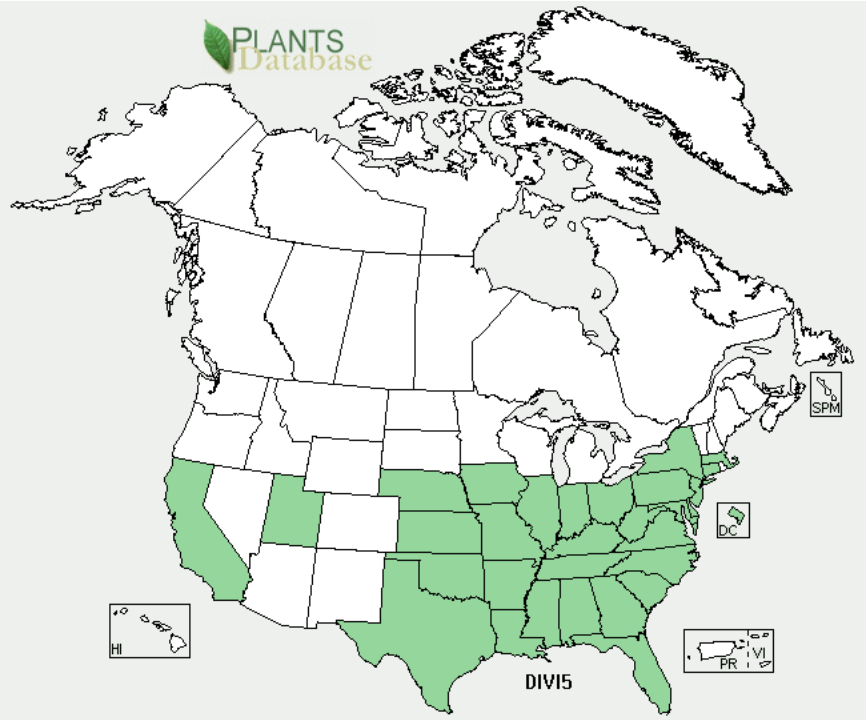

Figure1. A map showing the distribution of D. *virginiana* in 2010. Note that distribution in California and Utah are reflections of agricultural cultivations and not found in the wilderness. Source: http://plants.usda.gov/java/profile?symbol=DIVI5

Figure 2. Mature *D. virginiana* tree. Source: http://plants.usda.gov/java/profile?symbol=DIVI5&photoID=divi5_016_avp.tif

give the same attention to it as all the other "All American" foods such as the pumpkin, cornbread and turkey borrowed from the First Nations People (Native Americans). The American Persimmon's low profile existence may have caused many of us to overlook its cultural significance.

Figure 3. Close up of the square scale-like bark in the trunk of the *D. virginiana* tree. Source: http://forestry.sfasu.edu/faculty/jstovall/dendro/index.php/fact-sheets/photographs/82-diospyros-virginiana-common-persimmon

Botanical Description

Diospyros virginiana L. is one of two native North American Persimmon species and averages about 52-65 feet tall (16-20 meters). It grows predominantly in the Southeastern region of the United States (**Figure 1**) in light, sandy, well-drained soil and sometimes the deep, rich, bottomlands of river valleys. It is a deciduous woody perennial that is widely scattered and often covered with shrubby growth in abandoned fields and on the side of roads (Sargent et al., 1933). *D.virginiana* is a hardwood tree with a short, narrow trunk about 2-3 inches in diameter and grows to about tall with broadly spreading branches (**Figure 2**). It has a small,

Figure 4. A close up of the glossy, dark green color ,ovate-oblong leaves which are rounded at the base and arranged in an alternative fashion along the branch.
Source:
http://forestry.sfasu.edu/faculty/jstovall/dendro/index.php/fact-sheets/photographs/82-diospyros-virginiana-common-persimmon

dark, brown/black core and pale white wood with thick, dark, brown-gray square scale-like bark (Department of Agriculture, 1949) (**Figure 3**). The roots are thick, fleshy and is taproot in structure, reaching vertically downwards with smaller lateral roots (Sargent et al., 1933). They have ovate-oblong to oval leaves that are rounded at the base and have a glossy, dark green color which are arranged in an alternative fashion along the branches (**Figure 4**). *D. virginiana is a* dioecious species and therefore requires cross-pollination in

Figure 5: Unripe Fruit of *Diospyros virginiana* in the fall.
Source: http://davesgarden.com/guides/pf/showimage/34301/

order to bear fruit. Only female trees with solitary, flowers consisting of small stamens, smooth ovaries with eight mounted on four hair styles will become fruits(Curtis et al., 1943).The flowers are white and bloom in the spring; female trees produce yellow–green fruits in the fall (**Figure 5**);which ripens into an uneven dirty orange early in the spring after periods of frost (**Figure 6**). The unripe fruit is extremely astringent because of the high tannic acid content. As the fruit ripens, tannin levels decreases dramatically and the fruit softens, becoming increasingly sweeter. The inner bark is also astringent and bitter (Zim, Martin, Barlowe, & Barlowe, 1956).

There are several variations in the species that is not officially recognized by the USDA NRCS (United States Department of Agriculture Natural Resources Conservation Service). These categorizations are based on the different phenotypical expressions and include *D. pubescens* (Pursh) Dipp., *D. platycarpa* Sarg,*D.* mosieri(Small) Sarg and called Fuzzy Persimmon, Oklahoma Persimmon and Florida Persimmon respectively by pomologists (Fernald, 1950; Little Jr, 1983; Vines, 1960)

Figure 6: Ripe fruit and seeds in winter or early spring.
Source:
http://www.missouriplants.com/Whitealt/Diospyros_virginiana_page.html

Traditional Uses

First Nations people (Native American)

Folklore: In Native American folklore, the raccoon was a man who was told by the Great Spirit to go on a spiritual journey of the mind. He was not to stop to eat or drink until the task was completed. Unfortunately he was not ready for such a journey and indulged on the fruits of persimmon trees in a grove along the way. The furious Great Spirit turned him into a raccoon which is why the raccoon leaves footprints like a human, uses his hands like a man, and always knows when the persimmons are ripe (Dorsey, 1905). The persimmon tree and the fruit also appears in many other traditional stories, poems and songs.

Use: The main use of *D. virginiana* was sustenance. Native Americans incorporated it into bread, gruel, soup, stews, cornbread and pudding and preserved whole fruits by sun drying on the trees. The harvesting was usually in the late fall in preparation for the winter months. They also used dried seeds to brew beers and the leaves to brew teas.(Briand, 2005)

Medicine: Persimmon was made into poultices to treat burns given that the astringency tightens tissue, preventing burns from oozing liquids. Traditional American herbalists also use it to treat gastrointestinal bleeding. For the treatment of the fungal infection, thrush or "Oo-hah-lah-go-huh-skee", Cherokee Physicians made a mouth rinse from the inner bark and ashes, which were boiled and sweetened with honey(Chevallier, Compton, & Herrick, 2004; Foreman & Mahoney, 1975).

Western/ Current

History: The earliest reports of the Persimmon were in the 16-17th centuries by English and French explorers in the New World, who thought it looked like the medlar (*Mespilus germanicus*, Rosaceae)(Gravier, 1701). The first written description was made in A Brief and True Report of the New Found Land of Virginia by Roanoke colonist Thomas Harriot and John White in 1588. One of the first records of Persimmon consumption was made by Captain John Smith who aided in the settlement of Jamestown in Virginia in 1612. He wrote, "If it be not ripe it will drawe a mans mouth arwie with much torment; but when it is ripe, it is as delicious as an Apricock." It was quickly recognized as a vital part of the Native American's survival diets and adapted into the settlers' diets as well.

Use: During the Civil War, the Union soldiers blocked ports in the gulf, cutting off the Confederate soldiers from their food supply. Thus, they relied on Persimmon seeds as a substitute for coffee. The seeds were first boiled to remove the mucilaginous materials, roasted, grounded, and finally boiled in water with dried sweet potato to create the drink(Dodge, 1886). The seeds were also used to make buttons given that they are extremely strong and would not break when using the battling stick method to beat the laundry. (Dodge, 1886; Mathis, 1975; Porcher, 1863)

The fruit was used to make wine (Mehl-Madrona, 2003), brandy (Briand, 2005; Schery, 1954) white wine vinegar (Porcher, 1863) and beer (Edgeworth, 1860).The fruit was also used to make syrup and molasses by boiling its pulp until the saccharine material fully dissolves, straining the coagulations of fruit, and boiling off the juice until the desired viscosity is achieved (Shuster, Vigna, Sinha, & Tontonoz, 2011). Colonial settlers used the fruits to thicken and sweeten puddings. Persimmons were highly valued because they were easily accessible and thus could be turned into puddings without additional thickeners or sweeteners (Cutillas-Iturralde, Zarra, & Lorences, 1993; Elias & Dykeman, 2009). Today, the fruit is used to make traditional baked pudding , which has the consistency of custard and served during holidays like Thanksgiving and Christmas. In the state of Indiana, it is considered to be a legendary local dish (LASS, 1974).

The unripe fruit is also used to make ink by mashing the fruits and boiling it in water over a slow fire with small pieces of coppera(ferrous sulfate/green vitriol).This ink was called Gordon's Indelible Ink(Mason, 1871). Because the wood is very strong, hard, heavy, fine-grained, elastic, and resistant to wear, Persimmon wood was used to make gun stocks, golf clubs, drum sticks, billiard cues, wooden flutes, eating utensils, woodturning projects, screws, mallets and textile shuttles. It is also popular among bow craftsmen for the creation of traditional longbows (Chen, 1996; Kalm & Forster, 1972; Lim, 2012; Shukla & Bhatnagar, 1988).The ashes from burning the wood are also very high in alkali and therefore allowed for very good extractions of lye, which is needed for soap making (Candler, 1949).

Medicine

In the 18th century, *D. virginiana* was widely used as a medicinal plant. The inner bark, branches, roots and fruits were all harvested for medicinal use. The bark of the root was noted to have more medicinal value compared to the young twigs (Cook, 1869). The ripe persimmon fruit was reported to be an antiseptic and could be used in the same manner as Jesuit's bark, Cinchona (Cook, 1869)The fruit and bark were also used as an astringent for treating external ulcers, uterine hemorrhaging, and various other diseases such as diarrhea, dysentery, dropsy, diphtheria, syphilis, and thrush (Rafinesque, 1828). To treat diarrhea, a tonic made of the bark of *D. virginiana* and rhubarb (or unripe persimmon syrup with sugar with lozenges of green persimmon, red oak bark and blackberry preserve and gumarabic was commonly consumed(Butel-Dumont, 1753). To treat bacterial infections in the respiratory tract such as diphtheria, a tonic made from the inner bark of the *D. virginiana* tree and alum was gargled to washout the bacteria-infected areas of the throat(King & Felter, 1909; Rafinesque, 1828). For the treatment of dropsy, the seeds were often infused into the tonic with Digitalis to negate the side effect of diarrhea. To treat malaria, an infusion of the bark was used to subdue the fever (Lindley, 1836; Rafinesque, 1828).A decoction of Persimmon can also be used to treat sexually transmitted diseases (STIs) such as

gonorrhea and syphilis. The decoction for gonorrhea was made of sumac root, blackberry, and persimmon bark, boiled in 12 gallons of water until reduced to ½ gallon, and drunk until discharge symptoms were minimized. The decoction used to treat syphilis was made from white sumac, apple root, devils shoestring and persimmon root bark boiled in 4 gallons of water and drunken 1/8 pint, 3 times per day (Foreman & Mahoney, 1975)

A tonic made by boiling persimmon inner bark with ashes and infused with honey in "The Cherokee Physician" and "Indian Guide to Health" a was improved upon by the western settlers, who added Borax to the concoction to treat the fungal Thrush infections(Foreman & Mahoney, 1975).

Chemistry and Pharmacology

The main known chemical constituents isolated from *D. virginiana* are tannins (catechin, gallocatechin, betulinic acid), glucose, proteins, vitamins A and C, calcium, potassium, phosperous, iron, copper, manganese, carotenoids, and dietary fiber. They also contain the enzymes papain and bromelaine (**Table 1**). The astringent properties of the unripe fruit are often thought to be the result of the high tannin levels in the flesh.

Secondary metabolites like the flavonoid catechin and gallocatechin consists of benzene rings with many –OH groups which have a anti-oxidative, anti-infective, anti-inflammatory and anti-hemorrhagic properties(Rice-Evans, Miller, & Paganga, 1997). Betulinic acid is often correlated with antiretroviral, antimalarial, and anti-inflammatory properties(Tzakos et al., 2012). Persimmon also has beta-carotene, lycopene, lutein, zea-xanthin and cryptoxanthin, which are also very effective free radical scavengers, have shown to reduce the risk of lung cancer (Holick et al., 2002; Sies & Stahl, 1995) Papain and Bromelaine are protease enzymes that are present in the fruits which can help keep the inflammatory response in the body down and speed up healing processes(Maurer, 2001; Nayak, Pereira, & Maharaj, 2007).

The fruit is very high in glucose which is a dietary monosaccharide that can be easily digested and absorbed into the blood system. Because these carbohydrates provide energy for cells to function, the fruit is very important for sustenance. Persimmon fruit is also a very good source of vitamins A and C, which are important for normal retinal function and wound-healing and maintaining the integrity of capillaries respectively. Copper is a co-factor for vital enzymes like cytochrome c-oxidase which is also required for the production of red blood cells(Bertinato & L'Abbé, 2004; Soetan, Olaiya, & Oyewole, 2010). The manganese is a cofactor for the enzyme superoxide dismutase which acts as a powerful free radical scavenger (Martin et al., 1986; Van Remmen et al., 1999)

Biological Activity

Animal Studies

The tannins in the Persimmon are carcinogenic and can produce cancer in otherwise healthy rats. The tannin fractions from *Diospyros virginiana* and *Camellia sinensis* were very active and produced tumors at the subcutaneous injection site in 66% or more of the treated animals. (Kapadia et al., 1997) In addition to the carcinogenic effects in rats, larval mosquitoes' performance ie: survival rate decreased and days until pupation was increased significantly when treated with the leaves of the Persimmon plant as opposed to oak, maple or

Chemical Compound	Part of plant	ppm = parts per million tr = trace
(E)-2-HEXENAL	Fruit	
ALUMINUM	Stem	4 - 378 ppm
ASCORBIC-ACID	Fruit	660 - 1,855 ppm
ASH	Fruit	9,000 - 25,280 ppm
	Leaf	50,000 ppm
	Stem	22,000 - 54,000 ppm
BARIUM	Leaf	1 - 910 ppm
DUKE	Stem	3 - 1,080 ppm
BENZOTHIAZOLE	Fruit	
BORNEOL	Fruit	
BORNYL-ACETATE	Fruit	
BORON	Leaf	4 - 50 ppm
	Stem	1 - 38 ppm
CALCIUM	Fruit	270 - 758 ppm
	Leaf	1,150 - 12,500 ppm
	Stem	2,640 - 17,820 ppm
CARBOHYDRATES	Fruit	335,000 - 941,000 ppm
CHROMIUM	Leaf	1 ppm
	Stem	2.7 ppm;
COBALT	Leaf	100 ppm;
	Stem	54 ppm;
COPPER	Leaf	1 - 7.5 ppm
	Stem	0.2 - 108 ppm
DELPHINIDIN	Plant	
FAT	Fruit	4,000 - 11,230 ppm
	Seed	26,000 ppm;
FIBER	Fruit	15,000 - 42,135 ppm
GALLIUM	Leaf	0.25 ppm;
IRON	Fruit	25 - 70 ppm
	Leaf	15 - 500 ppm
	Stem	3 - 1,620 ppm
KAEMPFEROL	Plant	
KILOCALORIES	Fruit	1,270 - 3,567 /kg
LANTHANUM	Leaf	1.5 - 15 ppm
	Stem	0.7 - 16.2 ppm
LEAD	Leaf	0.5 - 35 ppm
	Stem	0.2 - 81 ppm
MAGNESIUM	Leaf	1,500 - 5,000 ppm
	Stem	660 - 5,400 ppm
MANGANESE	Leaf	25 - 1,500 ppm
	Stem	22 - 1,080 ppm
MOLBDENUM	Leaf	1 ppm
MOLYBDENUM	Stem	1.08 ppm;
MYRICETIN	Plant	
NEODYMIUM	Leaf	3.5 - 15 ppm
	Stem	1.5 - 37.8 ppm
NERYL-ACETATE	Fruit:	
NICKEL	Leaf	0.3 - 16 ppm
	Stem	0.1 - 8 ppm
PALMITIC-ACID	Fruit	
PHENYL-ACETALDEHYDE	Fruit	
PHENYLETHYL-ACETATE	Fruit	
PHOSPHORUS	Fruit	260 - 730 ppm
	Leaf	450 - 1,500 ppm
	Stem	132 - 2,592 ppm
POTASSIUM	Fruit	3,100 - 8,710 ppm
	Leaf	7,500 - 16,000 ppm
	Stem	1,716 - 16,200 ppm
PROTEIN	Fruit	8,000 - 22,470 ppm
	Seed	100,000 ppm
QUERCETIN	Plant	
SODIUM	Fruit	10 - 28 ppm

STRONTIUM	Leaf	7 - 1,000 ppm USG
	Stem	6.6 - 378 ppm
TITANIUM	Leaf	0.2 - 50 ppm
	Stem	162 ppm
VANADIUM	Leaf	0.25 - 1.5 ppm
	Stem	0.11 - 1.08 ppm
WATER	Fruit	644,000 ppm;
YTTERBIUM	Leaf	0.1 - 1 ppm
	Stem	0.04 - 0.38 ppm
YTTRIUM	Stem	0.1 - 16.2 ppm
ZINC	Leaf	5 - 25 ppm
	Stem	9 - 162 ppm
ZIRCONIUM	Leaf	1 - 3.5 ppm
	Stem	0.4 - 1.6 ppm

Table 1. Chemical components of *D. virginiana* (adapted mostly from Duke1992A). Source: (DUKE1992A: Duke, James A. 1992. *Handbook of phytochemical constituents of GRAS herbs and other economic plants*. Boca Raton, FL. CRC Press)

elm trees. This indicates that either the persimmon leaves have poor nutritional value or that there are active compounds inhibiting the maturation of the mosquito larvae. (Kesavaraju, Afify, & Gaugler, 2009)

There is also evidence that the naphthoquinones isolated from *D. virginiana*, juglone and analogs of juglone, are chemo-preventive agents. Chemo-preventative agents inhibit, delay, or reverse disease progression by antibacterial, antifungal, antiviral, insecticidal, anti-inflammatory, or antipyretic activity. In one study the naphthoquinones from *D. virginiana* showed potent anti-tumor promoting activity in vitro on the Epstein-Barr virus early antigen activation produced by the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and in vivo on stage 2 mouse skin carcinomas (Kapadia et al., 1997). In another study, Korean scientists found that persimmon leaf extracts administered to 180 in vivo sarcoma cells was cytotoxic (Park, Moon, & Kim, 1996).

Chemicals such as 7-methyljuglone (5-hydroxy-7-methyl-1,4-naphthoquinone or diospyrin) (**Figure 7**) and 8-6' dimer, Isodiospyrin were isolated from the wood of *Diospyros virginiana* and proved to be an effective termiticide (Carter, Garlo, & Stanley, 1978). 7-methyljuglone and its synthetic derivatives have also shown antibacterial activity against *Mycobacterium tuberculosis* both intracellularly and extracellularly in cell cultures (Mahapatra et al., 2007). While there is a high concentration of 7-methyljuglone isolates, the medicinally significant chemical activity was found but not discovered when investigating *D. virginiana*; they were actually discovered while studying the South American medical plant *Euclea natalensis*. Studies that isolated 7-methyljuglone and combined *Euclea natalensis* isolated 7-methyljuglone with the administration of anti tuberculosis drugs like Isoniazid and Rifampicin and reported a synergistic effect which increased the drug's ability to inhibit extracellular and intracellular activity of M. *tuberculosis* by 4-6 folds. Further research beyond recording the Fractional inhibitory concentration (FIC) indexes of 7-methyljuglone with different Tuberculosis drugs may lead to development of new drugs that work against multidrug-resistant (MDR) strains of *Mycobacterium tuberculosis. D. virginiana's* high 7-methyljuglone bioavailability may prove to be extremely important and lucrative when the demand for these drugs increase.(Bapela, Lall, Fourie, Franzblau, & Van Rensburg, 2006)

Many of the chemical constituents of *D. virginiana* show promising preliminary data for possible medicinal uses, however, much more research must be done starting with live

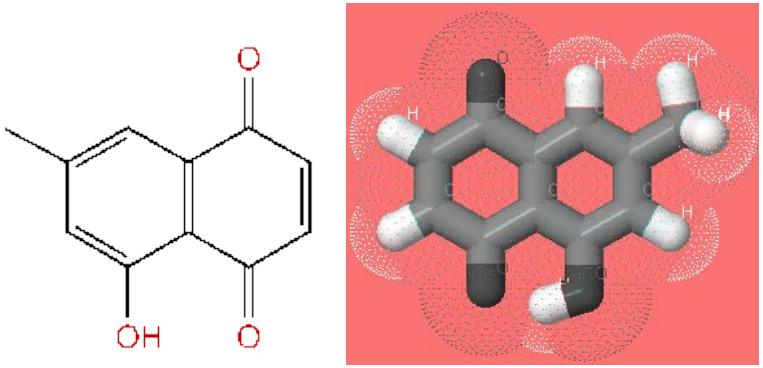

Figure 7. 7-Chemical structure of Methyl-5-hydroxy-1,4-naphthoquinone isolated from the wood of *Diospyros virginiana.* Source: http://www.pherobase.com/database/synthesis/synthesis-detail-7-methyljuglone.php

animal models. Until then, consumers should take advantage of the nutritional value of the .

Clinical Studies

There are currently no major clinical studies using this plant or its extracts but perhaps a survey that looks at the health differences between people and animals that eat persimmons yearly and those who don't could yield some interesting epidemiological patterns. Perhaps a closer look at the cultivation practices in each state can tell us more about how important this plant is to the ecological, social, cultural and physical health of America.

Contraindications

Excessive consumption of Persimmon fruits or consumption of unripe fruits may cause several gastrontestinal problems. Unripe persimmons have a significant amount of shibuol (a type of tannin) that, when mixed with the acidic environment of the stomach, polymerize to form a gluey precursor to a phytobezoar, a coagulum which entraps cellulose, hemicellulose, and protein in the stomach. Tannic acid also reacts with mucin, and decrease the cytoprotective mechanisms of the gastric mucosa. Today, in the United States of America, more than 85 percent of phytobezoars are caused by eating unripe Persimmon. Thus epidemics of phytobezoars occur most frequently in the Southeastern region of the country. Diospyrobezoars are a specific type of phytobezoar residing in the stomach made of a dense, wood-like trapped mass of fibrous plant material with a skin-like layer covering the surface. It presents symptoms such as a distended and tender stomach, dyspepsia (pain or discomfort in the area between the belly-button and the sternum), bloating, vomiting, weakness, weight loss , nausea, gastric outlet obstruction, perforation, abdominal pain, and bleeding(Sanders, 2004). One case in medical literature from 2004 documented a 51 year old patient who ate 2.2 pounds of unpeeled Persimmons everyday for 40 years came in with obstructed bowels which required retrograde double balloon enteroscopy (DBE) to find the bezoars blocking the distal ileum. Several days after having the bezoars surgically extracted, he was allowed to return home (Doo, Leung, & Lan, 2009).

Generally the recommendations for preventing the formation of Phytobezoars is to peel the fruit before consumption, not to consume more than several portions of the ripe fruit at one time, and to consume even smaller portions of the unripe, highly astringent fruits. The high potassium may also pose a risk if the consumer suffers from hypotension because it will decrease the fluid content in the blood and lower the blood pressure even more. The high sugar content of the ripe fruit

could also pose a risk for those who suffer from diabetes and cause an onset of hyperglycemia.

There are no known drug interactions and there are no know allergy reports, but there are several warnings about the preparation of the fruit passed down from traditional cooking practices. For example, persimmon is not encouraged to be paired with crab meat, goose, sweet potato, eggs, sauerkraut, black dates or with persimmon vinegar because it could encourage the formation of bezoars. The mechanisms of interaction is not known or studied very well, but these rules are generally followed by several different ethnic groups that consume a substantial amount of the persimmons produced around the world. The carcinogenic activity seen when extracts were injected into small rodents does not apply to orally consuming the fruit. It is generally regarded as safe to eat. The hand cream featured earlier in this article is also GRAS and is made with organically grown persimmons.

Larger grazing animals such as horses and cows should be kept away from persimmon trees in the late fall and winter because they will gorge themselves with these sweet fruits and promptly develop very large phytobezoars which often leads to bowel obstructions and infections that can kill the animal.

Current Uses in Allopathic and CAM Therapies

Though there are no current FDA approved uses for Persimmon, consumption and topical application of plant extracts can be beneficial in treatment of minor ailments. The fruit, which is low in sodium, has diuretic properties. Furthermore, patients with gout, arthritis, or hypertension can benefit from the high potassium content of the Persimmon because potassium negates some of the water retentioning

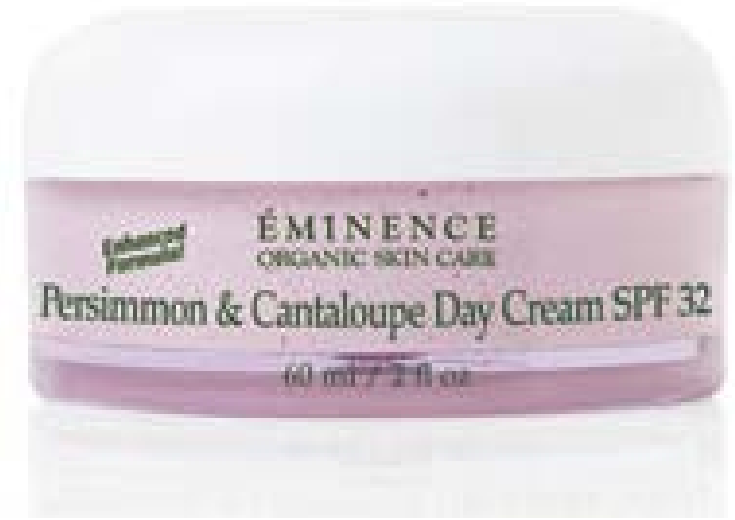

Figure 8: Example of an organic hand cream that is commercially available and marketed as rejuvenating by tighten facial pores and removing wrinkles.
Source: http://www.buynaturalskincare.com/persimmon-cantaloupe-cream-p-1004.html

effects of a high sodium diet. The highly astringent properties of unripe Persimmon fruits may help patients with diarrhea or sore throats. However, the abundance of pectin in the ripe Persimmon can treat constipation and increase the absorption of cholesterol from the intestines before it enters the bloodstream. Since choosing how ripe the fruit is before consumption depends on the state of the consumer, this is a complimentary diet based therapy. Currently, there are several hand creams that are commercially available that claim to be rejuvenating. This rejuvenation is accomplished by the tannins that tighten facial pores, allowing for a youthful glow (**Figure 8**).

Discussion

Diospyros virginiana is a plant with many historical and cultural ties to the United States but has been severely overlooked due to the importation of the larger fruited Japanese Persimmon trees and because of the odd qualities of the plant. It has a long history of use as food, material, and medicine and currently still provides hardwood for many unknowing users. It has played significant roles in feeding both the native people of this land, visiting explorers, and incoming settlers. Since the 18th century, the Persimmon plant has been well known to have astringent properties and therefore has been used for a broad spectrum of diseases from open wounds and fevers to gonorrhea and diarrhea.

In the US today, only a few Orchards in California and Utah grow the American Persimmon (**Figure 1**). Most of the trees in the southeast are growing in the wild and vary in flavor and size every season. These trees are generally regarded as nuisances because of the difficulty in uprooting their tap roots when they spring up in the middle of other crop fields. The persimmon tree actually has the potential to bear reliably high annual fruit yields with the careful care of a cultivator. The high seed count and astringency could be slowly bred out with careful control of pollination and may come back into favor in the American consumer public.

This plant has the potential to play a very important role in the future of human health as a beneficial dietary supplement to round out some of the nutrients that we are not getting from eating such a restricted modern diet. Furthermore, it is an excellent example of a food that is also medicine and can be consumed as needed depending on how one feels. Although this particular plant is not extensively cultivated because it takes at least ten years to mature enough to bear fruit, the tree is naturally fungi and insect resistant and should be preserved as part of the American heritage.

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Echinacea purpurea (L.) Moench, Asteraceae

Charity Coleman

Introduction

Echinacea purpurea (L.) Moench is 1 of the 477 genera in the Asteraceae family and 1 of 9 species in the *Echinacea* genus (USDA 2011). It is most commonly known as the purple coneflower. Other common names include eastern purple coneflower, snakeroot, Kansas snakeroot, broad-leaved purple coneflower, scurvy root, Indian head, comb flower, black susans, hedgehog and American coneflower (MDidea 2010). Echinacea is one of the most popular, top-selling, and most studied herbal treatments in today's society. Traditionally, Echinacea has been used to prevent and/or treat colds, flu and other upper respiratory infections. It is believed that Echinacea is capable of stimulating the immune system in order to help fight off infections. Also, Echinacea has been used to treat wounds and skin problems such as acne and boils, although these uses of Echinacea are less common (NCCAM 2005-2010). Echinacea is generally sold as a preparation derived from 3 of the 9 species including *Echinacea purpurea, Echinacea angustifolia,* and *Echinacea pallida*. The most commonly used preparation in the United States is a liquid extract made from the root of *Echinacea purpurea* (Kligler 2003). The active component of the plant has not been identified; however, the main components believed to be responsible for its immune-stimulating effects are the high-molecular-weight polysaccharides: heteroxylan and arabino-glactan (Kligler 2003).

Figure 1. Photograph of *Echinacea purpurea* and a female Pipevine Swallowtail butterfly (*Battus philenor).* (Source: Photograph by Rhonda Stewart. http://www.fs.fed.us/wildflowers/pollinators/butterflies.shtml)

Description

Echinacea purpurea is an herb that is native to the eastern and mid-western regions of North American and the southern regions of Canada. Although it does not grow naturally

anywhere else, it is cultivated worldwide because of its great use as an herbal medicine. Its habitat is mainly that of rocky areas, open woods, thickets, prairies, and roadsides. It is a single stem, erect, perennial with an active growth period in the spring and summer. Its mature height is approximately 1.2 feet but can reach heights of up to 4 feet (Upton 1997). The flower is purple in color, with elliptical petals radiating from the spiny seed head (cone), which is responsible for the plant's name (*echinos*: Greek word-root for "hedgehog") (Upton 1997). Foliage color is green and the fruit and seed color is black (FNA 2011). The seeds are produced in the cone and are small, 4 sided achenes. The stems are rough and have small hairs along their entire length with alternate, simple and ovate leads coming from them. The roots occur in a mass of thin, beige-brown rootlets that are approximately 12 inches in length. The taste of the stem has been described as a sweet but acrid flavor (Upton 1997). The growth rate is moderate, but regrowth rate after harvesting is quite slow (FNA 2011). Preferential growth requires full sun with low tolerance to drought. The optimum pH is between 6.5 and 7.2 for growth. Echinacea does not compete well with other plants (USDA 2011). When in bloom, the flowers of *Echinacea purpurea* attract pollinators such as insets and butterflies. Also, song birds such as Goldfinches are fond of the seeds during the fall. **Figure 1** is a photo of *Echinacea purpurea* along with one of its pollinators, the female Pipevine Swallowtail butterfly. There are no known pests of potential problems associated with *E. purpurea.* However, because it is such a popular herbal remedy, it is becoming increasingly rare in its natural habitat due to over harvesting.

Traditional Uses

Echinacea has been used for hundreds of years among Native Americans, North Americans and Europeans. Archaeological digs in the Lakota Sioux village sites uncovered evidence of Echinacea plants dating back to the 1600's. The use of Echinacea as an herbal medicine was introduced to Europe in the late 1800's by H.C.F. Meyer (MDidea 2010). It has been used in Europe extensively since the 1930s and is used by millions of Europeans today as their primary therapy for colds, flus and other upper respiratory infections (URIs) (MDidea 2010). As in European use, Echinacea is commonly used in the North America to treat/prevent colds, flus, upper respiratory infections, and also for general immune-boosting effects. It is also used, less commonly, for the treatment of urinary tract infections, treatment of wounds, and skin conditions such as acne, boils, psoriasis, and eczema (MDidea 2010). *E. purpurea* has also been used in Canada to treat abscesses in livestock (Lans, Turner, Khan, Brauer and Boepple 2007). Studies have shown activity against bacterial and viral infections and some of the chemical compounds extracted from Echinacea have shown inhibitory effects against certain forms of cancer (USDA 2011). Echinacea was introduced into the 1887 *Materia Medica* but was eventually removed due to lack of efficacy from clinical studies (MDidea 2010 and Upton 1997).

Traditional Medicinal Uses by Native Americans

Echinacea is said to have been the most commonly used medicinal plant by Native American tribes, being used for more illnesses than any other plant (Upton 1997). The most common uses of Echinacea by Native Americans were to treat toothaches, sore throats, coughs, infections, burns, venereal diseases, and even snake bites and other venomous bites, stings, and poisonings. The most common method for treating

coughs was to chew the root and swallow the juices (Moerman 2011). Use of Echinacea extended through many tribes including the Blackfoot, Cheyenne, Choctaw, Comanche, Crow, Dakota, Delaware, Kiowa, Lakota, Omaha, Pawnee, Ponca, Sioux, and more (Upton 1997). Use has also been reported by the Montana and Winnebago tribes (Borchers, Keen, Stern and Gershwin 2000). When using Echinacea to treat snakebites, most of the tribes employed the same preparations and applications. The root was used internally by chewing the fresh root, and externally by making a fresh poultice and applying it to the bite to draw out the poison (Upton 1997). Another common application throughout the tribes was to suck on the root to treat toothaches, sore throats, coughs, and infections. Other tribes applied juice, from the roots, to burns, wounds, ulcers and other skin conditions (MDidea 2010).

Specific Traditional Medicinal Uses by Tribe

The Blackfoot chewed the root to reduce pains from toothaches. The Sioux used freshly scraped root to make a poultice to treat hydrophobia caused by bites of rabid animals. The Dakotas also used freshly scraped root to treat hydrophobia, snakebites, and wounds that had putrefied (MDidea 2010). The Delaware tribe used *E. purpurea* to treat venereal disease by combining the roots with staghorn sumac roots. For more advanced cases of venereal disease, they would use an infusion of roots. The Delaware also reported high effectiveness with the use of *E. purpurea* in the treatment of gonorrhea (Moerman 2011). The Lakota tribe used Echinacea as a painkiller for toothache, tonsillitis, stomachache, pain in the bowels, and also when they were thirsty or perspiring by eating the roots and green fruits of the plant. The Omaha tribe used some parts of the plant for sore eyes. The Kiowa and Cheyenne chewed pieces of the root and let saliva run down their throats in order to treat colds and sore throats. The Cheyenne also made tea from the leaves of Echinacea to treat sore mouths and gums, toothaches due to large cavities, rheumatism, arthritis, mumps and measles. This same liquid was also applied to necks to relieve sore neck pain. Salves were also made by the Cheyenne to treat the same ailments externally. Echinacea root was also mixed with *Mentzelia laevicaulis* (blazing star) and boiled to make a tea for treating smallpox (MDidea 2010 and Upton 1997). The Choctaw tribe is reported to have used *E. purpurea* as a cough medicine by chewing the root and swallowing saliva containing the juice. They also prepared a tincture which they used for coughs as well. The Choctaw also used *E. purpurea* as a gastrointestinal aid to help with dyspepsia. They used the same methods of preparation as with the cough medicine (Moerman 2011). This information was also reported in the *American Journal of Clinical Nutrition* (Borchers, Keen, Stern and Gershwin 2000).

Non-medicinal Traditional Uses

Echinacea had traditions not only in medicinal uses, but decorative, ceremonial and other uses as well. Echinacea, due to its beautiful flowers, is grown as a decorative flower in many gardens and can be found in many homes. Native American tribes would use Echinacea in many religious ceremonies to show supernatural abilities. They would use the numbing qualities of Echinacea by mashing the fresh root and rubbing the juice on their hands with tallow to enable them to handle fire painlessly. Also, others would chew the root to numb the mouth in order to hold a hot coal in their mouths for religious ceremonies as well as for medicinal practices (Upton 1997). Winnebago medicine men would do this in order to create confidence in their ability to heal (MDidea 2010).

Children of the Pawnee tribe used Echinacea in a game in which the stems were twirled around each other. Many tribes have also been known to use the spiny seed cone as a hair comb (Upton 1997).

Plant Part	Active Constituents
Flower	Cichoric-Acid (12,000-31,000 ppm), Cyanadin-3-O-(6-O-Malonyl-Beta-D-Glycopyranoside), Cyanadin-3-O-(Beta-D-Glycopyranoside), EO (600-6,000 ppm)
Fruit	Alpha-Pinene, Beta-Farnesene, Beta-Pinene, Epishiobunol, Limonene, Myrcene
Leaf	2,3-O-Diferuloyltartaric-Acid, 2-O-Caffeoyl-3-O-Feruloyltartaric-Acid, 2-O-Caffeoyltartaric-Acid, 2-O-Feruloyltartaric-Acid, Apigenin, Ascorbic-Acid (2,140 ppm), EO (100-6,000 ppm), Flavanoids (3,800-4,800 ppm), Isorhamnetin-3-Rutinoside, Kaempferol, Kaempferol-3-0-Glucoside, Kaempferol-3-0-Rutinoside, Luteolin, Luteolin-7-Glucoside, Quercetagetin-7-Glucoside, Quercetin, Quercetin-3'-Glucoside, Quercetin-3-0-Galactoside, Quercetin-3-0-Xyloside, Quercetin-3-Robinoside, Quercetin-3-Xylosylgalactoside, Rutin, Rutoside, Selenium, Silicate (15,340 ppm), Silicon (301 ppm), Sodium (90 ppm)
Plant	(+)-Tartaric –Acid, (E)-10-Hydroxy-4,10-Dimethyl-4,11-Dodecadien-2-One, 2-Methyltetradeca-5,12-Diene, 2-Methyltetradeca-6,12-Diene, 2-O-Caffeoyl-3-(5-(Alpha-Carboxy-Beta-(3,4-Dihydroyphenyl…), 3,5-Dicaffeoyl-Quinic Acid, 4,5-O-Dicaffeoylquinic-Acid, Borneol, Bornyl-Acetate, Caffeic-Acid, Ferulic-Acid, Isotussilagine, Vanillin
Root	6-O-Caffeoylechinacoside, Alkylamides (40-1,510 ppm), Aluminum (786-12,900 ppm), Arabinogalactan, Ascorbic-Acid (843 ppm), Ash (80,000 ppm), Behenic-Acid-Ethyl-Ester, Beta-Carotene, Betaine, Calcium (3,290-7,760 ppm), Carbonate (7,100 ppm), Caryophyllene (42 ppm), Caryophyllene-Epoxide (26 ppm), Chloride (760 ppm), Chromium (19 ppm), Cichoric-Acid (6,000-21,000 ppm), Cichoric-Acid-Methyl-Ester (6,000-21,000 ppm), Cobalt (148 ppm), Cynarin, Deca-(2E,4E,6E)-Trienoic-Acid-Isobutylamide , Des-Rhamnosylverbascoside, Dodeca-(2E,4E)-Dienoic-Acid-Isobutylamide, Dodeca-(2E,6Z,8E,10E)-Tetraenoic-Acid-Isobutylamide, Dodeca-2,4-Dien-1-yl-Isovalerate, Echinacein (10-100 ppm), Echinacin, Echinacoside (3,000-17,000 ppm), Echinolone, EO (50-40,000 ppm), Fat (13,000 ppm), Fiber (111,000 ppm), Germacrene-Alcohol, Germacrene-D, Heptadeca-(8Z,11Z)-Diene-2-one, Heteroxylan (800 ppm), Humulene (8-12 ppm), Inulin (59,000-200,000 ppm), Iron (700-4,800 ppm), Kilocalories (2,800/kg), L-Pentadecene (400 ppm), Magnesium (1,170-1,860 ppm), Manganese (101 ppm), Niacin, Palmitic-Acid, Penta-(1,8Z)-Diene (400 ppm), Pentadeca-(8Z)-en-11,13-diyn-2-one, Pentadeca-(8Z)-en-2-one (4,000 ppm), Pentadeca-(8Z,11Z)-diene-2-one, Pentadeca-(8Z,13Z)-diene-11-yn-2-one, Pentadeca-8-en-2-one, Phosphorus (790 ppm), Polyacetylenes (20 ppm), Polysaccharides, Ponticaepoxide, Potassium (3,140-8,090 ppm), Protein (92,000 , ppm), Rhamnoarabinogalactan, Sulfate (2,450 ppm), Tetradeca-(8Z)-en-11,13-diyn-2-one, Thiamin (2.6 ppm), Tin (17 ppm), Trideca-1-en-3,5,7,9,10-Pentayne, Tussilagine, Verbascoside, Water (749,000 ppm), Zinc (51 ppm)
Shoot	12-Hydroxyoctadeca-(9Z,11E,15Z)-Trienoic Acid,2-O-Caffeoyl-3-O-Cumaroyltartaric-acid, Beta-Sitosterol, Chlorogenic-Acid, Isochlorogenic-Acid, N-Triacontanol, Sitosterol-3-Beta-O-Glucoside, Stigmasterol

Table 1. Chemical composition of *Echinacea purpurea*. (Source: Information obtained from Dr. Duke's Phytochemical and Ethnobotanical Databases. http://www.ars-grin.gov/cgi-bin/duke/farmacy2.pl)

Chemistry and Pharmacology

Due to the heterogeneity of the products used in the majority of studies of Echinacea, the main active component of *Echinacea purpurea* is not known. Most studies examine combinations of Echinacea species and others study Echinacea combined with other herbs (Kligler 2003). However, the chemical composition of *E. purpurea* has been well documented and is categorized based on plant parts in **Table 1.** The main constituents thought to be responsible for the immune-stimulating effects are heteroxylan and arabinoglactan (Kligler 2003). Other classes of compounds considered to contribute to the activity of Echinacea are alkaloids, polysaccharides, and polypropenoids, in particular, caffeic acid derivatives echinacoside and cichoric acid (MDidea 2010). Echinacoside is present in the roots at

approximately 7,000-13,000 ppm and cichoric acid is present in the flower at levels of 12,000-31,000 ppm and in the root at levels of 6,000-21,000 ppm (Duke 2011). Also, inulin is found at very high concentrations ranging from 59,000-200,000 ppm in the roots. **Figure 2** shows the chemical structures of some

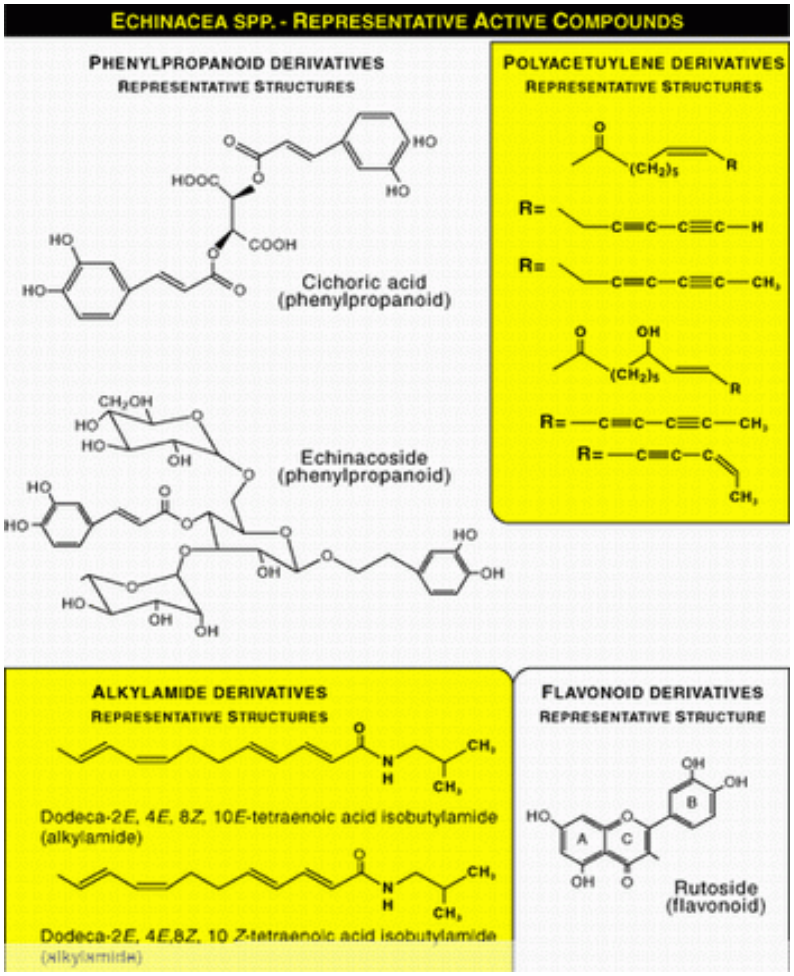

Figure 2. Chemical compounds found in *Echinacea purpurea*. (Source: Mdidea Exportind Division Extract Professionals.http://www.mdidea.com/products/herbextract/echinacea/data05.html)

of the main chemical compounds found in *E. purpurea.*

Biological Activity

There have been many *in vitro* and *in vivo* studies done to evaluate the efficacy and to try and determine the main active components of *Echinacea purpurea*. Due to the vastness of chemical constituents and the different compounds present in different parts of the plants and in different species, the main active ingredients are not known. Therefore, when sold, Echinacea extracts are generally a mixture of *E. angustifolia, E. pallida,* and *E. purpurea* as many herbalists believe that the effects of Echinacea are due to a complex interaction among the constituents (Fiebert and Kemper 1999).

Immunostimulant

One study incubated mouse serum with polysaccharides (thought to be one of the main active components for immune-stimulation) from *Echinacea purpurea* roots. They found that this stimulated proliferation of bone marrow cells and promoted phagocytosis by macrophages. Also, another study in rats showed that, in isolated, perfused rat livers, Echinacea extracts enhanced phagocytosis. A study performed in immunosuppressed mice showed a reduction in renal *Candida* load by 80% when the mice were injected with Echinacea polysaccharides prior to infection with *Candida albicans*. Bacterial counts in both the liver and spleen were also reduced by 95% in mice treated with Echinacea prior to infection with a lethal dose of *Listeria monocytogenes* (Fiebert and Kemper 1999). A study in undefined murine animals

(mice or related rodents) sought to define the effects of *E. purpurea* root and leaf extracts in dendritic cells. These cells are responsible for the generation of innate and adaptive immune responses. This study concluded that *E. purpurea* can function as an immunostimulant, immunosuppressive, and/or an anti-inflammatory agent. The achieved affects are determined by the portion of the plant used as well as the method of extraction applied (Benson, Pokorny, Rhule, Wenner, Kandhi, Cech and Shepherd 2010). Aly and Mohamed performed a study to investigate the immunostimulant effects of *E. purpurea* and garlic in Nile tilapia. They found that fish supplemented with *E. purpurea* and garlic showed an improvement in body weight gain, increased survival rates and increased resistance against challenged infections (Aly and Mohamed 2010).

Antimicrobial: Antiviral

In a study of cultured mouse cells, treatment with aqueous extracts of *Echinacea purpurea* caused a 50-80% resistance to infection of Influenza and Herpes viruses when treated four to six hours prior to exposure. The resistance lasted for 24 hours after exposure, but after 48 hours the cells were sensitive to infection (Fiebert and Kemper 1999).

Antifungal

An *in vitro* study of human granulocytes and monocytes treated with *Echinacea purpurea* extracts showed enhanced mobility. Also, there was an increase in phagocytosis of *Candida albicans* by 30-45%. Also *in vitro*, inhibition of *Candida albicans* growth was seen with use of purified polysaccharides from *E. purpurea* (Fiebert and Kemper 1999). A study on light-mediated antifungal activity of *E.* purpurea showed that extracts of *Echinacea* have phototoxic antimicrobial activity against fungi. Under near UV irradiation, growth inhibition was shown against yeast strains of *Saccharomyces cerevisiae, Candida shehata, C. kefyr, C. albicans, C. steatulytica* and *C. tropicalis.* These affects were contributed to the ketoalkenes and ketoalkynes found in roots of *E.* purpurea (Binns, Purgina, Bergeron, Smith, Ball, Baum and Arnason 2000).

Complementary immune support

In an *in vitro* study, a cytotoxic response of macrophages against tumor targets was seen with use of purified polysaccharides from *Echinacea purpurea.* One study with mice showed as increase in the number of white blood cells, compared to controls, after receiving intravenous *E. purpurea* polysaccharides prior to injection of cyclophosphamide. A study in rats that received dietary supplements of *E. purpurea* showed enhanced mobilization of vitamin E mediated oxidation/reduction pathways. This suggests that there is a protectant effect against radiation damage (Fiebert and Kemper 1999).

Mechanism of Action

Since the exact active components of *Echinacea purpurea* have not been identified, the exact mechanism of action is not known. However, the mechanisms are known for many of the constituents found in Echinacea. Several studies show that Echinacea provides an increase in immunological activity by increasing levels of interferon and increasing phagocytosis, cellular respiratory activity, and lymphocyte activation. This is achieved through the release of tumor necrosis factor, interleukin-1, and interferon beta-2 (Kligler 2003). Echinacea

promotes T-cell activation, natural killer-cell activity, lymphatic function and antibody binding. It also increases the reticulo-endothelial layer which increases antibodies by increasing the production of alpha, beta, and gamma globulin (Valentino and Yance 1999). Echinacea is thought in assist in wound healing, a cortisone-like activity, by inhibiting hyaluronidase enzyme that is associated with swelling and inflammation (Valentino and Yance 1999). This activity is accomplished by Echinacea's ability to maintain the structure and integrity of the collagen matrix in connective tissue (MDidea 2010). Echinacea is most properly termed an immunomodulator as it stimulates in the immune system in some conditions and suppresses it in others (MDidea 2010).

There has been much debate about the length of use of Echinacea and its long term effectiveness. Some sources say that it is only efficient for a seven to ten day period. However, one source indicates that this is a misconception and that it came from the mistranslation of a few German graphs from an oral double blind study with *E. purpurea* vs. a placebo (MDidea 2010).

Clinical Studies

Many clinical studies have been performed to determine the efficacy of Echinacea in treating human conditions with many mixed results reported. A double-blind, placebo-controlled randomized trial by Melchart, Walther, Linde, Brandmaier and Lersch on the prevention of upper respiratory tract infections found that no prophylactic effects of Echinacea extracts could be shown. The placebo group showed 36.7% infection while the group treated with *E. purpurea* only showed 29.3% infection, however these results were not conclusive enough to confirm efficacy. They did conclude however, based on their research and 2 other studies, that there could be speculation of a 10-20% risk reduction with treatment of Echinacea products (Melchart, Walther, Linde, Brandmaier, and Lersch 1998). Another randomized controlled trial on the effectiveness of *E. purpurea* in treatment of URIs found there was no difference in duration or severity of symptoms between patients treated with *E. purpurea* and those receiving a placebo (Taylor, Weber, Standish, Quinn, Goesling, McGann, and Calabrese 2003).

On the other hand, a study by Brinkeborn, Shah and Degenring (1999) concluded that preparations from *E. purpurea*, including Echinacea concentrate and Echinaforce, were low-risk, effective alternatives to standard symptomatic medicines in the treatment of the common cold. Also, according to the University of Maryland Medical Center, a study of 95 people with early symptoms of the flu who drank tea made from Echinacea felt better sooner than those who drank a placebo tea. They also reported that a review of 14 clinical trials revealed a 58% reduction in the odds of developing a cold when using Echinacea (UMMC 2011).

Contraindications

According to the National Center for Complementary and Alternative Medicine, when taken by mouth, Echinacea does not generally cause side effects. Generally uncommon and minor side effects include abdominal upset, nausea and dizziness (Kligler 2003). Although rare, some people have experienced allergic reactions such as rashes, increased asthma, and anaphylaxis (NCCAM 2010). People more likely to experience allergic reactions to Echinacea include those who are allergic to related plants in the daisy family such as ragweed, chrysanthemums, marigolds and daisies, as well as individuals with asthma (NCCAM 2010).

There have been no significant herb-drug interactions reported with the use of Echinacea. Although, theoretically the immune-stimulating properties of Echinacea could possibly interfere with immunosuppressive medications taken by individuals with autoimmune disease, such an effect has not been documented in humans or animals. Also, *in vitro* studies of Echinacea have shown that there may be a mild inhibition of the cytochrome P450 3A4 enzyme complex system which causes an increase in levels of drugs metabolized by the system. Drugs affected are itraconazole (Sporanox), fexofenadine (Allegra), and lovastatin (Mevacor). None of these interactions have been reported in humans but caution should be taken when using Echinacea along with these medications (Kligler 2003).

Current Use in Allopathic & CAM Therapies

There are literally hundreds of products containing Echinacea species currently on the market (Echinacea 2011). The most common uses of Echinacea today are for the treatment/prevention of colds, flus, upper respiratory tract infections, and general enhancement of the immune system. Echinacea is generally sold as a combination of *E. angustifolia, E. pallida,* and *E. purpurea* and can be purchased as extracts, tinctures, tablets, capsules, ointments, and in teas. It is also sold in capsule forms combined with other immune boosting herbs, vitamins, and minerals (UMMC 2011).

When purchasing Echinacea products, do take caution to make sure you are purchasing from a reputable, established company that distributes through trustworthy and knowledgeable establishments. According to the University of Maryland Medical Center, a study performed by ConsumerLab.com found that only 4 out of 11 brands of Echinacea purchased for testing contained what was stated on their labels. Moreover, approximately 10% of them had no Echinacea in them at all! Also, half of them were mislabeled as to which species of *Echinacea* they contained and the standardized preparations did not match the label (UMMC 2011).

The UMMC recommends the following dosages of Echinacea for general immune stimulating effects, during colds, flus, URIs or bladder infections: 1-2 grams of dried root or herb as a tea, 2-3 mL of standardized tincture extract, 6-9 mL of expressed juice (succus), 300 mg of standardized, powdered extract containing 4% phenolics, tincture (1:5): 1-3 mL (20-90 drops) or stabilized fresh extract: 0.75 mL (15-23 drops). It is recommended to choose one of these dosages and take it 3 times a day for 7-10 days (UMMC 2011).

Discussion

Echinacea purpurea has played an extremely important role in natural medicine for hundreds of years, dating back to the 1600s. It was the most widely used medicinal plant by the Native Americans in the past and is still one of the most widely used herbal medicines today. The vastness of use of this plant is extraordinary, ranging from treatment of the common cold to its tumor fighting properties and hundreds of preparations containing Echinacea are already on the market. Although the efficacy of Echinacea has not yet been truly confirmed, it is used by millions of people worldwide with personal opinions of success. With its use traversing hundreds of years, there are obviously some benefits. I believe that this plant will continue to be highly regarded as a beneficial herbal supplement and that future studies will find and confirm its efficacy.

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Ephedra sinica Stapf., Ephedraceae

Samuel C. Turner

Introduction

Ephedraceae is a family of herbs that have been exploited for their stimulating properties for nearly 5,000 years. Ephedraceae contains only one genus, *Ephedra*. Ephedra is composed of about 40 species. *Ephedra sinica* is the most potent in the genus *Ephedra*. *E. sinica*, known originally as Ma Huang, has had many epithets, including; Chinese Ephedra, Ephedra, upitonia, ephedrine (referring to its active ingredient), desert tea, desert herb (referring to its ecology), whorehouse tea and any number of other names (asiarecipe.com).

The Chinese have used *E. sinica* for thousands of years as a treatment of asthma, hay fevers, sweating and the common cold. Although Ephedra is one of the earliest recorded medicines; its active ingredients, ephedrine and pseudophedrine, have only been isolated in recent history (1887) (Frank, 2009). Ephedrine is an alkaloid that stimulates the central nervous system, constricts the blood vessels and opens lungs. Since its isolation it has used as a treatment for asthma, weight loss and athletic performance enhancer, as well as an antihistamine and decongestant (Thomas, 2010). Its abuses have led to increased scrutiny on its dosage, usage and availability.

Figure 1. *Ephedra sinica.* (Source: http://www.herbie sherbs .com/pages/herbpictures/ephedra2386L.jpg)

Description

Ephedra sinica is a small, shrub-like plant that grows to a maximum of three feet and is prevalent eastern China and Mongolia. Since its mass production, it has been planted in other, similar habitats in the Mediterranean, India, Persia and South America. Ephedra is dioecious and a gymnosperm. Its ideal habitat includes dry regions of sandy or rocky deserts. This habitat provides the well-drained soil needed for growth,

but may not be conducive to the copious amounts of water needed to provide the best conditions (Yang, 2004).

Ephedra is a green, erect species, with a plethora of branches, each individual branch contains roughly two opposing scale-like leaves, or a 'whorl' of three (**Figure 1**). During propagation, the dioecious (rarely, some are found to be monoecious) males produce egg-shaped seeds, which it stores. During autumn or spring, the smooth, mature male gametes are seeded into the female's mature, fleshy bract. Up to three seeds are inserted per bract. Ephedra can also propagate through root division, but this is a rare occurrence (Yang, 2004). Characteristics of *E. sinica* include; dark, reddish-brown seeds; bitter taste when brewed into coffee; and no particular smell (although decomposition can cause a distracting odor.)

Traditional Uses

Although *Ephedra sinica* has been used for many thousands of years, its history can be viewed as bleak. Its first use was noted around 3,000 B.C., where it was considered somewhat of a miracle drug. Physicians would prescribe Ephedra, or more commonly known at the time as, Ma Huang, to treat a number of conditions, including; the common cold, asthma, arthritis, hay fever, hypotension and other conditions.

Ma Huang was listed in the first chapter of a classical Chinese herbalism text, written by Sheng Nong in the first century A.D. Its many manipulations have allowed for a broad range of treatments. Although, *E. sinica* is limited to Asia, other *Ephedra* species have been used in the Middle East for asthma and other similar conditions (Frank, 2009).

Traditionally, Ma Huang has been prepared and administered as a tea, by adding two grams of the most concentrated portions of the Ma Huang into eight ounces of water, and boiling for ten minutes. This produces a 15 - 30 mg serving of Ephedrine (Crazyfortea.com, 2007). Presently, synthetic Ephedrine has been administered in several ways, most often as dietary pills, which range in concentrations of 10 - 80 mg (Herb Research Foundation, 1995).

Ephedra's traditional use has been limited to its physiological properties. Its physical properties as a shrub, do not lend it to more artistic uses, such as clothing or dye. But, its stimulant properties have led to more creative uses. Much like coffee, it has been brewed to make a recreational tea. Made by Mormon settlers in the 1800's to replace tea (considered taboo by their religion), Mormon settlers brewed to the herb to produce "Mormon tea" (Larsen, 2009). After the synthesis of ephedrine, use of *E. sinica* and its derivatives skyrocketed. Along with its prescribed use, ephedrine become synonymous with weight loss, athletic enhancement and substance abuse. In particular, weight loss products contained considerably higher doses than traditional use. These can be dangerous concentrations and prompted criticism over Ephedra's potential harm. Its abuse as an over-the-counter medication has resulted in several deaths, especially in teenagers (Nertheim, 2003).

Chemistry & Pharmacology

The chemical composition of *E. sinica* includes: Ephedrine alkaloids 0.481-2.47% (l-ephedrine, d-pseudoephedrine,l-norephedrine, d-norpseudoephedrine, lmethylephedrine, d-methylpseudoephedrine), 2,3,5,6-tetramethyl-pyrazine 2,3,4-trimethyl-5-phenyloxazolidine-benzylmethylamine, ephedroxane, essential oil 0.25% (l-α-terpineol) (Chen, 2004). Although the amount of constituents is rather abundant, the active ingredients comprise only a relatively small amount.

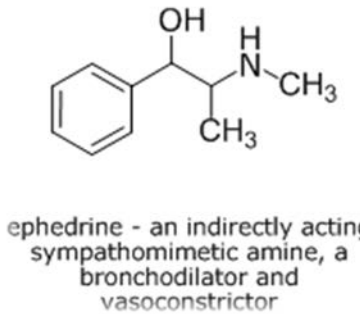

Figure 2. The structure of ephedrine. (Source: http://www.thekanjifoundrypress.com/sketch%20files/e%20GIF/ephedrine.gif)

The active components of *E. sinica* are alkaloids. Of the total makeup, alkaloids compose about .5 - 2.5% of the entire chemical composition. Of this .5 - 2.5%, ephedrine and pseudophedrine account for up to 90% (Haller, 2010).

Ephedrine (**Figure 2**) and pseudophedrine (**Figure 3**) work by increasing the amount of epinephrine and norepinephrine, which are sympathetic nervous system neurotransmitters. As well, ephedrine stimulates receptors found in the central nervous system and circulatory system (Edward, 2009).

Biological Activity

Ephedrine, the most abundant and potent alkaloid in *Ephedra sinica*, works by increasing the amount of norepinephrine reuptake. This is accomplished by increasing the amount of norepinephrine released from its terminal storage vesicles, into the synaptic cleft. More neurotransmitters allow for more activated terminal receptors. In turn, these receptors initiate a cascade of enzymatic reactions. Receptor stimulation increases production of adenylate cyclase, which prompts an

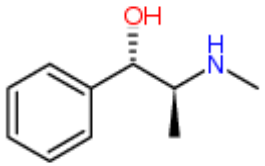

Figure 3. Structure of pseudoephedrine. (Source: http://isomer design.com /Cdsa/C/dl375.png)

increase in the enzyme kinase. This acts like a amino-acid based hormone and stimulates cyclic AMP levels, which increases lipid metabolism (Edward, 2009).

This mechanism (**Figure 4**) contains potential for medical miracles and recreational exploitation. The ability to manipulate the sympathetic nervous system allows for the systematic utilization of its benefits, such as increased metabolism. Conversely, this provides an amped or excited state popular among athletes. This explains the controversy and numerous conflicting studies over its potentially harmful effects.

Clinical Studies

In one study, the researchers investigated 37 cases of cardiovascular disease that were directly related to the use of Ma Huang. The results indicated that Ma Huang was temporarily related to stroke and death, even if there was no underlying medical problems and dosage requirements were followed (Samenuk, 2002). Another study was conducted over its effects as a dietary supplement. This study reported that its benefit/ detriment ratio that was well within acceptable limits. Stating that after an eight week trial, patients that used an *E. sinica*/caffeine combination lost over

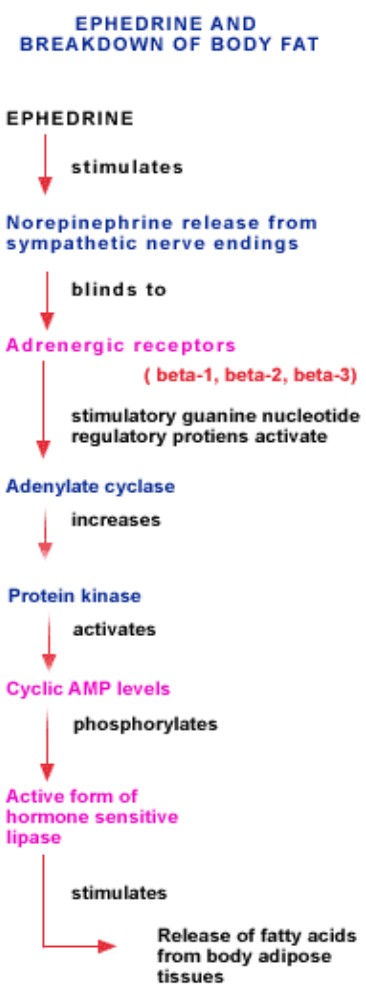

Figure 4. Catabolism of ephedrine in the body.

two pounds (lbs) more, per month, than the placebo-control group. However, the study also found that there was a definite increase in gastrointestinal and psychiatric symptoms, as well as an increased number of heart palpations (Pittler, 2004). There is a plethora of conflicting research over *E. sinica*. The results can be construed to fit your needs, but the overall consensus tends to be positive, if used under appropriate conditions.

Contraindications

The dangers associated with this plant stem from its stimulant activity. Patients should avoid *E. sinica* if they have a history of cardiovascular disease, angina, hypertension, thyroid disease, enlarged prostate, Insulin resistant diabetes, anxiety, history of substance abuse and other similar conditions. Adverse reactions can consist of nervousness, anxiety, insomnia, anorexia, hypertension, tachycardia, arrhythmias. Prolonged use can lead to addiction, eating disorders and its use has been linked to psychoses, strokes, heart attacks and abrupt deaths (Thomas, 2010).

Furthermore, care should be taken to avoid potentially harmful drug interactions. Combination of Ephedra with other stimulants can magnify the aforementioned adverse reactions. As well, Beta blockers and monoamine oxidase (MAO) inhibitors in conjunction with Ephedra can lead to lethal hypertension. Phenothiazines can counter the effects of Ephedra, causing an unsafe drop in blood pressure, known as hypotension. Theophylline can increase the risk of CNS and GI problems. Other examples of drug interactions that should be watch for, include; oxytocin, antidepressants, digoxin, guanadrel, guanethidine, mecamylamine, methyldop, thyroid therapies, diabetes medications, dexamethasone, diruetics, ACE inhibitors, ergot alkaloids, anethetic drugs, Morphine, gout medications and St. John's Wort. In short, any patient debating on using this medication regularly should seek a physician's advice (Thomas, 2010).

Current use in Allopathic and CAM therapies

Ephedra is a hybrid of sorts, graying the lines between Allopathic and CAM therapies. Because of its properties of a bronchodilator, it is used to treat asthma, bronchitis, nasal congestion, the common cold and sinusitis. These are both traditional practices and common allopathic uses (although, there are several more westernized medicines commonly used to treat these as well) (Thomas, 2010). Its effects as a stimulus have been used to treat hypotension, weight management and more notably among the alternative side, it has been used to treat attention deficient disorder in children (Myatt, 1990).

Discussion

Ephedra sinica is a unique and powerful medicine, that has transcended generations and its use today is no less significant than its use 5,000 years ago. The ability of a single medicine to treat an array of conditions is unparalleled. Through its sympathetic-stimulating mechanism, *E. sinica* has been able to treat conditions from hypotension to the common cold. This dynamic herb has had no humble beginning, earning a rare spot among the world's earliest medicines. Thus, Ma huang remains ageless and will only become more relevant in the next 5,000 years.

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Ficus insipida Willd., Moraceae

Jennifer C. Howell

Introduction

Ficus insipida Willd. also known by its other scientific names, *Ficus glabrata* H.B.K. and *Ficus anthelmintica* Martius and common names such as wild fig, ojé, posen, higueron, and glabrate fig belongs to the Moraceae family, also known as the fig family (Smithsonian; Tropicos; University of Hawaii; Hansson et al., 2005; de Armorin et al., 1999). It is a large buttressed tree that produces relatively small fruits (figs) and grows in the tropical regions of Central and South America, including the Amazon basin (Smithsonian; Tropicos). The latex of this tree has been copiously used throughout Amazonian villages, particularly in Peru, where it is applied traditionally as a vermifuge in both children and adults. The latex is used to treat helminthiasis, anemia, diarrhea, malnutrition, immune deficiency, and other such effects resulting from these physiological compromises (Quave 2000; de Amorin et al., 1999; Jernigan 2011; Phillips 1990).

Very few studies have specifically investigated the biological activity of latex derived from *F. insipida*. Of the studies conducted, the efficacy as an anthelmintic as well as the toxicity of the latex was proven, both at low and high doses (de Amorin et al., 1999; Hansson et al., 2005). The active constituent found to digest and get rid of the parasites, causing helminthiasis, was ficin. Ficin is a class of papain-like cysteine proteases (Gonzalez-Rabade et al., 2011). The use of this tree's latex is still in practice today as an alternative to allopathic medicine and/or as the primary source of treatment (Hansson et al., 1986; Natural Remedies Haven Ltd.).

Figure 1. *Ficus insipida* tree (Smithsonian).

Botanical Description

Ficus insipida can be found in forest areas in places such as Mexico, Central America (mainly in Panama, Costa Rica, and Peru), South America (stretching as far as Brazil), and in the Amazonian regions (Tropicos). This plant is a free-growing, non-flowering tree reaching approximately 40 m tall with a grayish-tinted smooth bark and large buttressed roots protruding from the ground (**Figure 1**). Additionally, a milky latex sap can be found in the tree, which gives the tree one of its defining characteristics. The leaves of the tree are characterized by their long stretched oval shape, ranging from 10-40 cm long and 5-16 cm wide depending on the maturity of

Figure 2. *Ficus insipida* leaves (Smithsonian).

the leaf; the veins occur in 15-30 pairs angled slightly up and out from the center and base (**Figure 2**). The fig, *F. insipda*'s fruit, at maturity displays a yellowish-green color with lighter yellowish-green spots. These figs only reach about 5-15 cm long when fully matured (**Figure 3**). In order for the tree to grow, the seedlings require a sufficient amount of light, meaning many of these trees are found in "younger" or newly formed areas of forests (Smithsonian). The sustainability of these trees includes guidelines that only allow for a particular tree to be tapped after the tree has reached at least seven years of maturity, leaving a gap of twelve months between each tapping, and not allowing a cut of more than 1 cm in the tree to ensure tree viability (Phillips 1990).

Because of its similarity to other species in Costa Rica and Panama, this particular species of fig tree has been confused

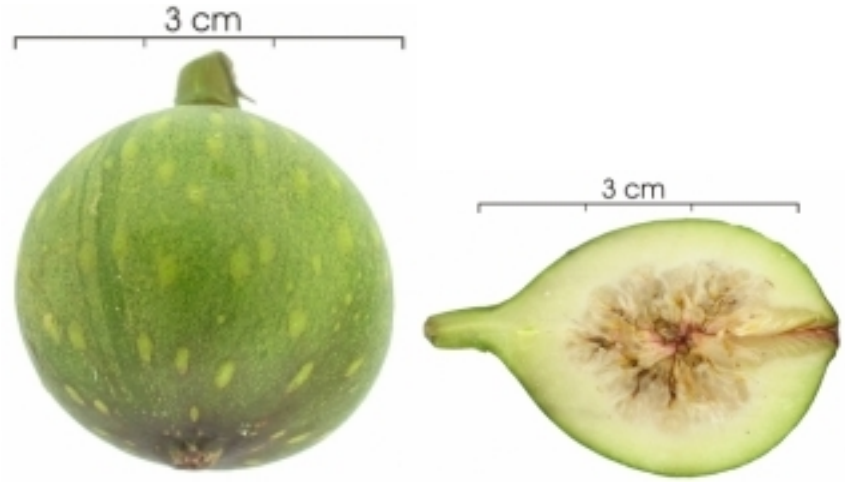

Figure 3. *Ficus insipida* fruit (Smithsonian).

(by untrained eyes) with species *Ficus crassiuscula* Warb. One of the main tell tale signs that these species are not *F. insipida* is that *F. insipida* only proliferates at elevations below 500 m whereas the other species occur at elevations above 1,000 m. Also, *F. insipida* has a different pollinator from the other two species (Smithsonian). Each *Ficus* species has a species-specific wasp that contributes to the pollination process. One of the known pollinators of *F. insipida* is the agaonid wasp. These wasps, like many of the other fig wasps, use the syconium, a fleshy hollow enclosure inside the surface of the fig fruit, as a mating receptacle. When these wasps retreat from the fruit, seeds are carried along and dropped along the wasps' path, thus spreading the seeds of the fig tree and prolonging the existence of F. Insipida (Smith and Bronstein, 1996). While studying *F. insipida* at the Le Selva Biological Station in Costa Rica, bats and fish were also found to be pollinators of the tree (Banack et al., 2002).

Traditional Uses

The latex from this species has been used traditionally as an anthelmintic in Central and South America (de Amorin et al., 1999). Based on current knowledge sought out by Hansson et al. (2005), indigenous populations living in the upper Napo River region of Peru that have used or currently use *Ficus insipida* to treat parasitic-related illnesses, mainly in children, did/do not relate the parasites to the illnesses (ex. anemia and diarrhea). Rather, they felt that since one was born with such parasites, they were merely treating the symptoms, such as diarrhea, respiratory complications, and pale complexion, and not the eradication of the parasites. Another reason for the disconnection between the parasites and illness is due to the fact that the transmission of these parasites to the child is accomplished via non-boiled or unsanitized drinking water and/or via the soil.

Preparation of the remedy involves collecting the latex in a jar the day before treatment, to ensure freshness. The latex is then mixed with the juice of oranges, cocona, or sugar cane and given orally in small doses (spoonfuls). The latex is sometimes mixed with sugarcane brandy to prevent decomposition and fungal growth, in order to prolong storage (Hansson et al., 2005). This is done in the early morning, before sunrise. Accompanying the latex mixture is a period of fasting in which only sanitized water is drunk throughout the day. At night a light meal of plantains and manioc is commonly eaten; foods such as peppers, salt, and meat are avoided several days before and after the use of this remedy. To treat anemia, diarrhea, and abdominal distension, this remedy is taken for 2 consecutive days once every 4 months, to avoid overdose. The remedy is considered successful when visible symptoms have disappeared (Quave 2000).

Among the peoples of the Peruvian Amazon that speak Iquito, the approach to healing involves not only dietary restrictions and limiting physical activity but also the use of plants associated with specific plant spirits, to avoid exacerbating illnesses. According to a study conducted by Jernigan (2011), the Iquito speaking people believe that diarrhea comes from temperature, dirty water, and bad food, parasites result from walking barefoot and eating bad, and anemia comes from having bad blood, lack of blood, and bad hygiene. Spirits associated with certain plants are integral into the type of diet and illness treated. Unlike the peoples living in the Napo River region, this group believes *F. insipida* has a plant spirit that helps in treating only parasites. *F. insipida* remedies are accompanied by strict diet guidelines because it is believed, by the Iquito, to be a "jealous" spirit. Another group that uses the latex mixture are the Ese-Eja Indians, who tap the tree for latex in the early morning when it is most viscous and distribute dosages according to the season (Phillips 1990).

Chemistry and Pharmacology

In a recent study, the chemical constituents of *Ficus insipida* were measured from the reserves at La Selva Biological Reserve in Costa Rica via the leaves collected from the tree. The leaves were dried and subsequently doused in 70% acetone to extract constituents. All constituents found can be seen in **Table 1** (Ardon and Pringle, 2009).

The active constituent that gives this tree its anthelminitc properties is the proteolytic enzyme ficin, also known as ficain (de Amorin et al., 1999). Ficin is a general term for the cysteine proteases, in the papain family, found within the *Ficus* genus (Azarkan et al., 2011). Ficin contains 2 L-cysteine thiol groups; these thiol groups act as catalytic sites (Malthouse and Brocklehurst, 1976). It is at these sites that

Known Constituents	% Mass
Condensed Tannins	0.5
Total Phenolics	11.8
Hydrolysable Tannins	2.7
Lignins	8.3
Cellulose	19.1
Hemicelluloses	14.7

Table 1. Known constituents of *Ficus insipida* expressed as a percent mass of the plant.

ficin is able to digest the parasitic invaders without harming the human host. Peak activity of ficin was found to be at pH 7.0 and found to be related to the solvents added, such as sucrose, trehalose, and xylitol (Devaraj et al., 2008). Cysteine proteases are used by the plant in insect resistance, protein degradation, maturation, to regulate the folding of proteins, and in some cases involved in proteolysis. They also are involved in the assembly of storage proteins for seed utilization. For insect resistance, it was found that the hydrolytic enzymes are involved in the inhibition of larvae growth (Gonzalez-Rabade et al., 2011).

Biological Activity

In a study conducted by de Amorin et al. (1999), mice infected with helminthes (*Syphacia obelvata*, *Aspiculuris tetraptera*, and *Vampirolepis nana*) and treated with *F. insipida*-derived latex at 3 ml/kg/day for 3 consecutive days showed significant elimination of *A. tetraptera* and *V. nana* but not *S. obvelata*. During necropsy of the animals they found hemorrhagic effects in the mucosa of the gastrointestinal tract. When a high dose of 10 ml/kg/day was applied for 3 consecutive days, the authors found a lethality of 60%, thus, demonstrating the toxicity of this latex at both high and low doses.

Clinical Studies

A clinical study consisting of 181 persons from three Amazonian villages sought to determine the safe and effective dosage of latex taken systemically. The persons involved held a 92% prevalence of helminthiasis: *Ascaris* 68%, *Strongyloides* 42%, *Trichuris* 41%, Ancylostoma/Necator 26%, and *Taenia* 1%. Biological activity was measured using a milk coagulation test. Pharmacological efficacy was determined using live *Ascaris, in vitro,* in which concentrations of latex (in physiological saline solution) as low as 0.05% was sufficient for the suppression and digestion of *Ascaris* (Hansson et al., 1986).

The anthelminitc activity of *Ficus insipida* was studied retrospectively over a 12-year period by Hansson et al. (2005) in Pucallpa, Peru. This area was of great interest due to the 39 reported cases, involving mostly children, of *F. insipida*-related symptoms due to toxicity. Of the 39 cases, the majority were due to overdoses (small>1.5 cm^3/kg, large>4 cm^3/kg). One fatality occurred among the hospitalized patients, while two fatalities were reported to have occurred outside the confines of the hospital. In severe cases of toxicity cerebral edema was reported, for which the treatment was mannitol.

Additionally, in less severe overdoses adults were reported as having primarily cardiovascular and respiratory complications and children were reported as having more musculoskeletal complications, while both adults and children were reported as having gastrointestinal issues. The treatment for the less severe cases involved various kinds of drugs including antacids, pyridoxine, atropine, and dexamethasone, depending on the symptoms present.

Contraindications

It has been reported in both adults and children that gastrointestinal complications, cerebral edema, and death have occurred. However, only large overdoses (>4 cm^3/kg) have been reported to cause the cerebral edema and fatalities (Hansson et al., 2005).

Current Use in Allopathic and CAM Therapies

There are no current uses of *Ficus insipida* latex in allopathic medicine; however, it is still used in today among Amazonian villagers and among those who live near those areas where *F. insipida* flourishes. In Peru, bottles of latex mixtures are sold by independent vendors in many of the main markets. Although the sale of the latex mixtures is still lucrative, it is not as prominent as it once was, due to the accessibility to allopathic medicine. According to the vendors, the recommended dose is approximately 2 cm^3/kg, which can be taken over the course of three days, and should be taken in the early morning on an empty stomach, following a conservative diet for the rest of the day. This regimen should be applied once or twice a year, as needed (Hansson et al., 2005). This slightly contradicts the recommended dose found to be safe through clinical trials: 1 cm^3 of latex mixture/kg for 3 days every 3 months (Hansson et al., 1986).

Natural Remedies Haven Ltd. currently markets, online, *F. insipida* latex as an alternative supplement, in the form of ojé latex sold in 25 ml bottles, to current pharmaceuticals (**Figure 4**). The ojé latex is mixed with either water or fermented with sugar. The company markets this to treat worms, bacteria, anemia, and rheumatic inflammations. As for the dosage, they recommend mixing one tablespoon of the latex mixture with one liter of water, and to drink this mixture every other day until the problems subside (Natural Remedies Haven Ltd.).

Figure 4. The 25 ml bottle of *F. insipida* latex marketed by Natural Remedies Haven Ltd. (Source: http://www.weight-care.com/herb_Ojelatex.htm)

Discussion

The latex derived from *Ficus insipida* Willd. has been used traditionally for generations among the peoples of the Amazonian region for the treatment of helminthiasis and its resulting symptoms. Because of this tree's distinct characteristics and the vast exposure Amazonians have experienced with this tree, it is not confused with other species from the *Ficus* genus. The traditional preparation of the latex is still in practice today. The maximum biological activity is achieved when the latex is mixed with sugar (sucrose), which reflects its traditional preparation. This latex mixture is sold locally in markets and internationally (on a very small scale) via the internet. Quality of the latex being

sold, at least among the local markets, is of little concern because of the abundance of the tree and the knowledge the vendors carry about the preparation and use of the latex. The toxicity experienced from those taking *F. insipida* mixtures is not due to taking the improper latex from other Ficus species, but rather taking an overdose of the *F. insipida* latex.

Furthermore, the use of this latex has yet to become a dominant remedy globally due to the limited availability of the tree worldwide and to the ability to access either allopathic or safer, alternative CAM therapies. Another reason these latex or pharmacologically-derived latex remedies are not found in allopathic medicine is that the associated risks and possible side effects far outweigh the benefits that come with the use of the latex compared allopathic remedies, which are often much safer and more readily available. Additionally, the active component, ficin, is currently not isolated from this tree, for reasons unknown, even though ficin has been found to be involved in several diseases and cell signaling pathways. However, because of the relatively easy access to this particular species and the growing importance of the cysteine proteases found within this plant, future pharmaceuticals will probably include some form the these cysteine proteases, whether it be synthetically derived forms or pure forms.

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Ginkgo biloba L., Ginkgoaceae

Benjamin Kramer-Roach

Introduction

Ginkgo biloba L. (**Figure 1**) is the sole surviving member of the Ginkgoaceae family, a family of "living fossils" that has its roots in the Mesozoic era (Taylor, 1993). In more recent times *G. biloba* is thought to only grow natively in a certain region of Southeast but it is now common to find them in many countries throughout the world. Botanist Engelbert Kaempfer introduced the ginkgo tree to Europe in 1690 and gave it the name "duck foot tree" due to the signature shape of its leaves (Bilia, 2002). The name "ginkgo" is derived from the Chinese word for silver apricot, and this name was given to the tree because of the size and coloring of the fruit (Mahadevan, 2008). Other common names of *G. biloba* include silver apricot, yajiao (duck foot), gong sun shu (grandfather tree), maidenhair tree, temple tree, noyer du Japon, Kew tree, and Ginkgobaum (Hori, 1997). The leaves and nuts of this tree have been used in traditional Chinese medicine (TCM) for millennia to treat a variety of ailments involving the brain, heart, and lungs. In modern medicine gingko extract is primarily known for cognitive enhancement and treatment of cardiovascular problems, but the standardized extract (EGb 761) has shown promise as a cancer-preventative as well. As with every plant-based medicine, it is challenging to attribute the medicinal effects of *G. biloba* to a single chemical or pathway because of the synergistic tendencies of the chemicals. Ginkgo leaves have been shown to contain terpene lactones, biflavones, and flavonoid glycosides, which act on a variety of pathway and receptors (Spinella, 2001). These chemicals provide it with ample resistance to infections and insect invasions, which has led to its proliferation in cities and gardens and has made it extremely popular in global markets for its perceived cerebral enhancing properties. In addition, it

Figure 1. *Ginkgo biloba* tree.
Source: David Stang,Tropicos.org

is annually a top-selling herbal supplement sold around the world for its effects in boosting memory (van Dongen, 2000).

Botanical Description

Approximately 180 million years ago, the Ginkgoaceae family extended to nearly every continent, from sub-polar regions like Canada to the tropics. *Ginkgo biloba,* though, the last remaining species, is now thought to only grow in the wild in the Zhejiang Province in China, specifically on the Tian Mu Mountain (Shen, 2005). However, mystery still shrouds the origins of this population, and there is debate over whether the trees are truly wild or are merely semi-wild descendants of trees cultivated in Buddhist temples (Del Tredici, 1992).

Ginkgo trees grows best in cool, wet climates and in deep soil with good water retention, but in recent decades it has been able to adapt to a variety of habitats around the world due to their high resistance to disease, insects, and air pollution (Andrews, 1947). Ginkgo trees are dioecious, meaning that each tree produces either all male or all female flowers. The trees themselves can grow to be around 100 feet (30 meters) high and have been known to live thousands of years. They do not start producing seeds for up to 25 years, but can remain fertile for as long as they live (Taylor, 1993). Flowers appear in spring from stubby appendages on the branches, called short roots, in small clusters as catkins. The plum-sized fruit ripens throughout the fall to a golden color and covers the seed in a plump outer covering and a woody shell. Fertilization occurs via motile, multi-flagellated sperm from the pollen (Dallimore, 1948). Due to the butyric acid content of the fruit, when the ripe fruit falls and splits open during autumn, it emits a powerful odor, akin to rancid butter, despite the edible nut inside (Andrews, 1947). Male trees, which do not bear fruit, tend to be more common in ornamental areas like cities and gardens to avoid the odoriferous fruit.

Figure 2. Leaves and fruit of *Ginkgo biloba*.
Source: W. H. Hodge from Tropicos.org

The leaves of *Ginkgo biloba* are flat and fan-shaped and exhibit two lobes divided by a cleft of varying sizes, a trait to which *G. biloba* owes its name **(Figure 2)**. Sprouting in clusters of 4 - 8 from the short roots, the leaves emerge light green in the spring and fade through dark green to a golden color in late autumn. The veins on the leaves are unique from other shade trees in that rather than having a mesh-like structure, ginkgo leaves have a branching venous pattern that protrudes from the stem and branches continuously to fill out the leaf (Andrews, 1947).

Traditional Uses

Ethnomedicinal uses

Ginkgo biloba has a long history of use in traditional Chinese medicine (TCM) with a host of uses. The seeds or nuts, removed from the outer fruit, were used to treat certain pulmonary disorders, like asthma, bronchitis, cough, and

enuresis, in addition to bladder inflammation, kidney ailments, and leucorrhea (Mahadevan, 2008; Bilia, 2002; Smith and Luo, 2004). The seeds have been used extensively in TCM for millennia. In contrast, leaves of *G. biloba* were only used marginally in the past and were first recorded to have medicinal properties in a book by Lan Mao, published in 1436 C.E., when they were recorded to have effects on treating skin disorders such as head sores. A little later, in 1505, C.E Liu Wan-Tai wrote about the first internal use of ginkgo leaves, which was for the treatment of diarrhoea (Bilia, 2002). The leaves, though, have grown to be seen as being beneficial to the heart, brain, and lungs (Mahadevan, 2008; Bilia, 2002).

Uses in cuisine

The seeds, often called Pak-Ko (meaning white fruit), are a popular snack and considered a delicacy in many East-Asian countries, including Japan, Korea, and China. They can be cooked into sweet desserts, like the dish Cheng Tengm, or roasted and eaten as an appetizer (Porterfield, 1951; Goh, 2002). Ginkgo seeds, shown in **Figure 3**, are in season during winter months after the fruit has matured (Goh, 2002). The extraction of the seed begins by harvesting the plum-sized fruits from the tree and allowing them to ferment in water for several days. (This process causes a highly unpleasant aroma.) If the fruit itself is eaten, it has a bitter taste and is therefore typically fermented or cooked (Mahadevan, 2008). Once soaked in water, the flesh of the fruit can be easily removed from the edible seed within. The seeds must be roasted or cooked because raw seeds are toxic and consumption of them often results in serious food poisoning (Bilia, 2002). Traditional Chinese foods like chawan-mushi, a custard-like dish, and nabe-ryori incorporate ginkgo nuts as a main ingredient (Bilia, 2002).

Figure 3. *Ginkgo biloba* seeds and nuts.
Source: Oregon State University, 1999-2012.

Cultural uses

Taoist beliefs incorporate the balances of yin and yang and are often represented by certain plants and animals. Ginkgo is used to exemplify this concept, with one temple serving a dish called ginkgo chicken, with ginkgo representing yin and rooster symbolizing yang. It is believed that when eaten as a whole, the dish replenishes vitality (Shermann, 1985).

Due to its beauty and hardiness against disease, insects, and air pollution, *G. biloba* has been a popular ornamental tree for thousands of years (Briskin, 2000). It appears in Buddhist temples in both China and Japan, but its popularity has grown and can now be found in many countries all across the world, particularly in urban centers (Bilia, 2002). The unique elegance of the leaf has also led to its incorporation in various forms of art and sculpture.

Chemistry and Pharmacology

Ginkgo biloba has a multitude of phytochemicals, including terpene trilactones, flavanol glycosides, biflavones,

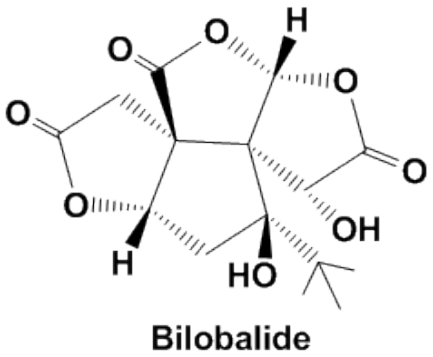

Figure 4. Structure of bilobalide. Source: http://www.pipitech.com/newproduct/bilobalide/bilobalideLS.htm

proanthocyanidins, alkylphenols, polyprenols, and 4-O-methylpyridoxine (van Beek, 2002). The unique, primary constituents found in *Ginkgo biloba* are terpene lactones, biflavones, and flavonoid glycosides. The terpene trilactones include bilobalide (technically a sesquiterpene trilactone), shown in **Figure 4**, and five types of ginkgolides (A, B, C, J, and M), which are twenty-carbon terpenes manufactured in the roots and leaves of the tree (Mahadevan, 2008; Smith, 2004; Spinella, 2001). The flavonol glycosides include compounds such as quercetin, rutin, kaempferol, and isorhamnetin (van Beek, 2002; Mahadevan, 2008). Other chemicals such as Isoginkgetin, ginkgetin, bilobetin and sciadopitysin are all biflavones (van Beek, 2002).

A standardized extract was developed by German and French pharmaceutical companies called EGb 761, which is the main extract used in experiments. The chemicals are extracted through either a water-ethanol or water-acetone process from green leaves harvested and dried in the summer (Mahadevan, 2008; Bilia, 2002). The chemical constituency of the leaves varies depending on the season, with greater flavanoid concentration in the fall than in the spring, and therefore the leaves must be analyzed during the multi-step extraction process for chemical levels and presence of heavy metals before being made into the extract (Mahadevan, 2008; Chan, 2007). The creation of this extract involves many procedures, some of which are undisclosed, and condenses leaves to extract in the ratio of 50 to 1. This concentration also removes unwanted components like biflavones, catechins, and polyprenols, which can have negative health effects (Chan, 2007; van Beek, 2002). Consuming ginkgo leaves, as in a tea for example, instead of extract has minimal health effects because of the extremely low concentration of active chemical compounds (Chan, 2007). The standardized *Ginkgo biloba* extract contains approximately 6% terpene lactones, 24% flavonoid glycosides and less than 5 ppm Ginkgolic acid (Van Beek, 2009; Goh, 2002). The chemical constituents of *G. biloba*

Class	Major Constituents	Approximate % in EGb 761
Flavanol glycosides	Quercetin, kaempferol and isorhamnetin	24
Carboxylic acids	Phenolic and non-phenolc acids	13
Proanthocyanidins	-	7
Terpene trilactones	Sesquiterpene trilactones (bilobalide) Ginkgolides A, B, C, J, and M	6
Alkylphenols	Ginkgolic acids, ginkgols and bilobols	5 - 10 ppm
Biflavones	Bilobetin, ginkgetin, isoginkgetin and sciadopitysin	0
Polyprenols	-	0
-	B6 4-O-methylpyridoxine	0

Table 1. Chemical constituents of *G. biloba*. (Information derived from van Beek, 2002)

are listed in **Table 1** with their associated concentrations in the extract.

Proanthocyanidins, also found in wine, are partly responsible for the antioxidant properties of the extract and contribute to ginkgo extract's medicinal effects (van Beek, 2002). B6 4-O-methylpyridoxine, a chemical found in primarily in the seeds, is toxic to humans and is removed in the creation of the extract (van Beek, 2002).

Biological Activity

In Vivo

Numerous studies have been conducted to test the benefits of standardized *Ginkgo biloba* extract (EGb 761), particularly for its memory enhancement capabilities and its efficacy in protecting against neurodegeneration.

A study by Winter (1997) tested the memory effects of EGb 761 extract on rats. The rats were first acclimated to a multi-armed maze and the number of false starts made by the rats was recorded. The research found that rats that had been orally administered EGb 761 via consumption of sweetened, condensed milk showed improved memory, as judged by fewer false starts. Moreover, rats that consumed EGb 761 lived significantly longer than those that had not (Winter, 1997). A study by Anna Walesiuk et al. (2005) found that rats subject to either stress or drug-induced memory loss regained memory function with the addition of EGb 761 into their diet In an experiment by Yohitake (2010), aged rats improved their spatial cognition after doses of *Ginkgo biloba* extract were administered. In addition, the extract was effective against Alzheimer's and Parkinson's disease in animals tested (Yohitake, 2010). Extracts have also been shown to improve memory function and spatial learning in young and old rats as well as short-term memory in mice (Perry, 2011).

By acting on the hippocampus in animals, which is involved with the creation of new memories, EGb 761 was shown to boost memory retention and acquisition in other vivo studies as well (Smith and Luo, 2004). EGb 761 was found to be beneficially involved with the following processes in rats: free radical scavenging, age-related brain losses, ischaemic neuronal death, hippocampal function, hippocampal uptake of high-affinity choline, regulation of hippocampal glucocorticoid receptors, plasticity of neurons, and stress-related cognitive deficits (Smith and Luo, 2004).

Smith and Luo (2004) found that *Caenorhabditis elegans*, a nematode, demonstrated increased resistance to both thermal and oxidative stress when fed EGb 761. Similarly, rats treated with moderate amounts of ginkgo extract through their drinking water showed reduced oxidative damage to mitochondrial DNA in the liver and brain (Chan, 2007). This emphasizes the importance of antioxidants as a component of ginkgo extract (Smith and Luo, 2004).

To highlight another pathway that ginkgo extract effects, mice fed EGb 761 for four weeks in one study showed a dramatic up-regulation in ten genes that have neuroprotective roles (Smith and Luo, 2004). Other studies along the same theme point towards the capability of ginkgo extract to regulate transcription of certain genes pertaining to adaptive response of organisms thereby increasing tolerance to oxidative stress (Smith and Luo, 2004). The stress-reducing activities of ginkgo extract suggest effects on receptors in the dopaminergic system (Yohitake, 2010).

Rats fed EGb 761, one study showed, had substantially decreased levels of stress, which researchers attributed to the effects of the ginkgolides on the expression of the peripheral

benzodiazepine receptor in the adrenal cortex (Smith, 2004; DeFeudis, 2000). This reduction helps to control levels of circulating glucocorticoids, which has extensive favorable consequences such as decreased immunosuppression, neurotoxicity, and neuroendangerment (Amri et al., 1996).

Certain flavanols, like kaempferol, have been tested for cancer prevention and some studies have found a positive effect on colon and prostate cancers (DeFeudis et al., 2003). Monte et al. (2004) found that in mice, EGb 761 inhibited lymphocyte-induced angiogenesis, a process essential to cancer growth. Similarly, DeFeudis et al. (2003) found that mice given ginkgo extract showed decreased expression of peripheral benzodiazepine receptor (PBR) and therefore suppressed proliferation of breast cancer cells. Feng, et al. (2009) found that the flavanoids in ginkgo extract exhibited anticancer properties. When the extract was administered to mice with leukemia, many of the treated mice showed signs of tumor suppression and reduction after a week and a half of treatment (Feng, 2009).

In Vitro

The neuroprotective qualities of EGb 761 are derived from its reaction with many cellular pathways simultaneously and synergistically (Smith and Luo, 2004). In vitro EGb 761 was observed to have multiple methods of action for its neuroprotective characteristics such as up-regulating certain proteins and maintaining membrane potential in mitochondria. (Smith and Luo, 2004). Likewise EGb 761 has been shown to improve cardiac conditions by altering ion concentrations across membranes, thus altering action potentials that results in vasorelaxation and vasodilation in aortic smooth muscles (Satoh, 2004).

Both the proanthrocyanidins and flavonol glycosides have antioxidant properties, such as free-radical scavenging, which can reduce oxidative stress (Chan, 2007). This is also responsible for some of the relaxation of blood vessels, and thus the increased blood-flow, effects of the extract (Kleijnen and Knipschild, 2002).

An altered version of EGb 761, called IPS200, was administered to human breast cells in an in vivo study conducted by DeFeudis et al. (2003). The study found that the altered version had regulatory effects on a number of genes involved with cell proliferation and apoptosis, suggesting anti-cancer properties.

Clinical Studies

There have been many clinical trials of *Ginkgo biloba* leaf extract, particularly EGb 761, for its cognitive enhancing abilities on both mentally impaired and healthy people, the treatment of other conditions involving cardiovascular deficiencies and neurodegenerative disorders, its antioxidant and anxiolytic effects, and its efficacy in treating antidepressant-induced sexual dysfunction. Many of these found ginkgo extract to have an overall beneficial effect.

There is a wealth of studies demonstrating the beneficial characteristics of EGb 761 on a number of ailments, but there are also many studies that found no substantial evidence supporting the use of EGb 761 for treating many of them. One study highlighted this schism in the literature by first positing that while they found mild cognitive improvement in patients treated with ginkgo extract, the trials with overwhelming success might have been due to other factors, and then in the same study, admitting that *G. biloba* is one of the plants most effective against dementia and other cognitive ailments like

premenstrual tension and tinnitus (Perry, 2010). The few studies showing no effect are lost among the flood of studies detailing the successes of ginkgo extract. In a series of clinical trials conducted by a research team, extract of *G. biloba* was found to be effective against a variety of conditions such as "early-stage Alzheimer's disease, vascular dementia, peripheral claudication, vertigo, heart disease, eye diseases, chronic cerebral insufficiency, accidents involving brain trauma, dementia, and tinnitus of vascular origin" (Cxavusxogˇ lu, 2011; Napryeyenko, 2007).

In two trials testing the effects of ginkgo extract on healthy volunteers aged 18-40 and 22-59, at the dosage of 120 mg/day for thirty days and fourteen days respectively, both found that exposure to extract from the leaves increased the working memory of volunteers, but did not effect long-term memory (Hartley, 2003). Numerous other studies echo similar results in finding that healthy volunteers who had ginkgo extract of a comparable dosage showed an improved working memory, particularly in older subjects, while not exhibiting any changes in other types of memory like reaction time, attention, or word recall (Hartley, 2003). From this information it can be deduced that certain sections of the brain, most likely the frontal lobe (responsible for working memory), and certain age groups (primarily the elderly) are most affected by *G. biloba* extract (Hartley, 2003).

Most studies agree that *Ginkgo biloba* is effective in the treatment of Alzheimer's disease and mental deterioration. This effectiveness can be attributed to several characteristics of chemicals found in the leaves. The extract inhibits the creation of the protein Aβ from its precursor β-amyloid, which is an essential step in Alzheimers disease pathogenesis. Aggregation of this proteolytic fragment exacerbates and promotes early onset and autosomal dominant versions of Alzheimer's disease, which can be suppressed by EGb 761. The reduction in Aβ also leads to decreased apoptosis of neurons: a counter to the cause of many neurodegenerative diseases (Mahadevan, 2008; Smith, 2004). Le Bars, et al. (1997) also found in a double-blind, placebo-controlled experiment that EGb 761 improved cognitive functioning in elderly patients with dementia or Alzheimer's disease. Kleijnen and Knipschild (1992) found that when ginkgo extract (120-160 mg daily for a period of more than four weeks) was given to forty patients with cerebral insufficiencies, most experienced beneficial outcomes. The cerebral insufficiencies encompassed a range of symptoms from tiredness to confusion.

The alkaloids in the extract were found to be powerful antagonists of platelet-activating factors (PAF) in several studies. Since PAF are inflammatory autacoids and are important to the pathologies of inflammatory conditions like ischemia, asthma, CNS disorders, and shock, ginkgolides are a valuable natural defense against these diseases (Braquet and Hosford, 1991). Along the same lines, ginkgolides reduce aggregation, increase blood circulation, and can be used to increase blood flow to the brain after an ischemic incident (Smith and Luo, 2004). The sesquiterpene trilactone bilobalide has shown promise in reducing volume of cortical infarct following a stroke or cerebral ischemia by amplifying flow of glucose and oxygen to the brain (Smith and Luo, 2004).

There have also been several studies that found there was statistically significant data to support the use of EGb 761 to increase standards of living and mental well being in terms of decreased depression, anger, and fatigue (Cieza A et al.; Kennedy, 2007). Ginkgo extract was also found to be anxiolytic in humans and to reduce depression (Smith, 2004; DeFeudis, 2000; Evans, 2009).

The antioxidant properties in EGb 761 have also been found to be useful in treating sexual dysfunction. The antioxidants have the effect of protecting nitric oxide from degradation, which in turn activates the enzyme guanylate cyclase. This effect leads to producing smooth muscle relaxation in the penis, thus allowing an inflow of blood much like Viagra (Chan, 2007). This explains the results of a similar study, in which ginkgo extract was found to be highly effective in increasing sexual potency and minimizing the effects of antidepressant-induced sexual dysfunction (Cohen, 1998).

Contraindications

Ginkgo biloba leaf extract has been shown to have vasodilatory properties, which has given rise to worries about possible interactions with drugs that have anti-platelet forming or blood-thinning effects. One study even found an increased risk of hemorrhage when ginkgo extract was used in combination with more potent anticoagulants and blood thinners due to its effects on the platelet-activating factor (Perry, 2010). However, similar to the debate over whether ginkgo extract has cerebral enhancing properties, there are conflicting studies involving the negative side effects of ginkgo extract consumption. Specifically, one study focused on testing case studies that suggested negative synergistic reactions between *G. biloba* extract and drugs such as warfarin, aspirin, and ibuprofen (Izzo, 2009). Despite past claims that patients suffered from spontaneous hyphaemas or intracerebral haemmorrhages due to drug reactions with ginkgo extract, this study found that there was no evidence for links between ginkgo extract and changes in blood coagulation parameters (Izzo, 2009).

On the other hand, there have been several instances of seizures, though not statistically significant, among people taking high doses of *G. biloba* extract or consuming large amounts of the seeds (Spinella, 2001). In Germany, where much of the supply of EGb 761 is manufactured, companies are required to include on the label symptoms such as "headaches, dizziness, palpitations, gastrointestinal disturbances and allergic skin reactions" that can result from overdoses (Chan, 2007). Though these side effects must be included on the labels, a study by Kleijnen and Knipschild (1992) showed that there were no negative side effects to taking EGb 761 over a period of three months (Chan, 2007).

If the seeds are consumed raw they can produce symptoms of food poisoning and gastrointestinal problems due to the presence of the toxic 4-O-methylpyridoxine (van Beek, 2002).

Current Use in Allopathic and CAM Therapies

Ginkgo biloba extract is one of the most popular drugs and herbal supplements in Europe and the rest of the world, and it is part of a billion-dollar industry, primarily for its perceived value in increasing cognitive function (van Dongen, 2000). In 2008 in the U.S., *Ginkgo biloba,* as a dietary supplement and prescription drug, was the top-selling herbal medicinal product with a retail value of over $150 million, even outselling popular herbal supplements such as echinacea, garlic, and ginseng (Smith and Luo, 2004). It is sold under a variety of trade names in America including "Ginkgobene, Ginkgold, Ginkoba, Vasan" and many others (Spinella 2001). Popular prescription and over-the-counter ginkgo-based drugs in Germany and France include brands like "Tebonin, Kaveri, rökan, and Tanakan" (van Dongen, 2000).

As a dietary supplement ginkgo extract is typically taken by people looking to increase mental alertness, memory, and

attention, which makes it especially popular among the elderly. In addition, ginkgo extract is a powerful antioxidant and has appeal for its ability to fight aging and age-related mental disorders (Goh, 2002; Bilia, 2002).

Discussion

Ginkgo biloba is exceptional in many characteristics that make it extremely valuable socially, medically, historically, and economically. Its unique chemical constituents have helped the tree remain largely unchanged for millions of years, and give it resistance to infection, insects, and air pollution. This tree is found in nearly every country around the globe in urban centers and in temples in Japan and China. Traditional Chinese medicine has used ginkgo seeds for thousands of years and incorporates them into many foods and delicacies. The standardized extract, EGb 761, has been shown to increase memory in rats as well as to help prevent Alzheimer's disease and dementia, and the antioxidants also show promise in having anti-cancer and anti-aging properties. In humans it has also proven effective in promoting cerebral enhancement in healthy volunteers, increasing brain function after ischemic events, and helping with cardiovascular disorders. There have been no conclusive studies proving any side effects of usage and most studies have found ginkgo extract to be safe. It is sold as an herbal supplement, in 120 mg tablets, for its memory enhancement effects including increased sharpness and energy. As an herbal supplement its popularity has skyrocketed and is now part of a billion-dollar industry, despite not being approved by the FDA in America. This beautiful and noble tree will no doubt continue to appear in gardens and cities all over the world and will provide many cures for cerebral insufficiencies and hopefully several forms of cancer as well.

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Glycyrrhiza glabra L., Fabaceae

Elsa Lake

Introduction

Glycyrrhiza glabra L. belongs to the Fabaceae family. The extract obtained from the roots is called licorice, and this is the part of the plant that people use in foods and medicine. The scientific name of the plant comes from the Greek word *glukurrhiza*, which means "sweet root" (Edwards, 2000, p. 119). Common names for *G. glabra* include European licorice, Spanish juice licorice root, liquorice, sweet wood, sweet licorice, lacrisse (German), reglisse (French), and regolizia (Italian) (Sena, 2010, p. 256; Lakshmi, 2011). The plant is native to the lands around the Mediterranean, and it is alleged that in the 1560's it was brought to Britain by Dominican monks (Edwards, 2000, p. 119). There are two closely related species which originate in different parts of the world: *G. uralensis*, which is known as Chinese licorice, and *G. lepidota*, known as American licorice (Sena, 2010, 256). The main active constituent of all three species of licorice is Glycyrrhizin, which has a sweet taste (Li et al, 2011). For this reason, licorice is commonly used to mask bitter tastes in medicinal preparations ("Glycyrrhiza glabra", 2005). The plant has also been used as a mild laxative, anti-arthritic, antiinflammatory, anti-biotic, anti-viral, anti-ulcer, memory stimulant (being an MAOinhibitor), anti-tussive, aphrodisiac, anti-mycotic, estrogenic, antioxidant, anti-caries agent, anti-neoplastic, anti-cholinergic, anti-diuretic, and hypolipidemic agent (Lakshmi, 2011). The many different chemicals in licorice continue to be studied for these and more medicinal properties.

Figure 1. Drawing of *Glycyrrhiza glabra.* (Source: Koehler's *Medicinal-Plants* http://upload.wikimedia.org/wikipedia/commons/a/a4/Glycyrrhiza_glabra_-_K%C3%B6hler%E2%80%93s_Medizinal-Pflanzen-207.jpg)

Botanical Description

Glycyrrhiza glabra is a halophytic perennial shrub that grows to a height of four or five feet (**Figure 1)** (Li et al, 2011; "Glycyrrhiza glabra", 2005). Every year, new stems are produced, which are sturdy, erect, rough at the top, and branched either from the base or from further up. The foliage leaves are 10 to 20 cm long, alternate, and odd pinnate. The leaflets are in three to eight pairs, and the stipules are very small and drooping (Lakshmi et al, 2011). The plant has pale, white to purplish flowers that resemble lilacs (Sena, 2010, 256). The axillary inflorescences resemble spikes, are 10-15 cm long, and are upright. Each flower is 1 to 1.5 cm long and short-pedicled. The petals are narrow. The carina petals are not fused, or beaked, but are pointed. The calyx is short, shaped like a bell, and glandular-haired. The tips of the calyx are pointed lanceolate and are longer than the tube. The fruit is a pod which is 1.5 to 2.5 cm long and 4 to 6 mm wide. It is flat with thick sutures, erect and splayed, glabrous, somewhat reticulate-pitted, and usually has three to five brown, reniform (kidney-shaped) seeds (Lakshmi et al, 2011).

G. glabra and *G. uralensis* look very similar in many respects, but they can be distinguished in that *G. glabra* has straight pods and linear-oblong shaped leaves, while *G. uralensis* has falcated pods and ovate shaped leaves (**Figure 2)** (Hayashi et al, 2003). There have also been plants documented in Kazakhstan that have fruit and leaves with an appearance in between that of the two species; these plants are thought to be hybrids (Hayashi et al, 2003).

There is an extensive root system that is subdivided into three to five subsidiary roots that are 1.25 m in length. The main taproot is 15 cm long. There are also several horizontal woody stolons that can reach eight meters (Lakshmi et al, 2011). The main taproot, which is harvested every third or fourth year for medicinal use, is soft, fibrous, and has a bright yellow interior (Loftin, 1953; "Glycyrrhiza glabra", 2005).

Figure 2. A Comparison of Fruits of *G. uralensis* (left), the Intermediate Plant (middle), and *G.* glabra (right) from Kazakhstan. (Source: Hayashi et al, 2003)

In symbiosis with rhizobia, *Glycyrrhiza* spp. fix nitrogen from the atmosphere. *G. glabra* provides nutrients and shelter to rhizobia (Li et al, 2011).

G. glabra thrives best in fertile, sandy or clay soil near a river or stream or under cultivation where it can be irrigated (Lakshmi et al, 2011). It tends to grow on the riverbanks and in the flooded fields of Mediterranean countries, which are subtropical (Loftin, 1953; "Glycyrrhiza glabra", 2005). *G. glabra* can currently be found in Spain, Italy, Turkey, the Caucasus, Central Asia and the western part of China (Hayashi et al, 2003).

Traditional Uses

Preparation of Licorice Extract

Dried roots of *Glycyrrhiza glabra* are collected in autumn, and the powdered and finely cut roots are used to prepare dry and fluid extracts (Barceloux, 2008, p. 538). A simple aqueous extract is prepared by boiling the root and then allowing most of the water to evaporate (Sena, 2010, p. 256). From this extract commercial forms of licorice can be made. The three main ones are licorice powder, licorice paste and ammoniated glycyrrhizin crystals.

Ethnomedical Uses

Stores of *G. glabra* roots were found in the tombs of Egyptian pharaohs such as the 3000-year-old tomb of King Tutankhamen (Loftin, 1953). These roots were placed there for the pharaohs to make a sweet beverage called *mai sus* in the afterlife. This ceremonial drink, still used today in modern Egypt, is used to honor the pharaohs (Sena, 2010, 259-60).

The Greek botanist Theophrastus, who lived in the fourth century BC, stated that licorice could be used to quench thirst and treat asthma, dry cough, and other respiratory diseases (Barceloux, 2008, p. 537). He also said it could be used to heal wounds when mixed with honey. The armies of Alexander the Great and later the Roman armies carried licorice root with them to quench soldiers' thirst during long marches (Sena, 2010, p. 260). In the first century BC, the Roman naturalist Pliny the Elder wrote in his work *Naturalis Historia* about the usage of licorice for reducing hunger and thirst and for treating asthma and sterility in women (Sena, 2010, p. 260; Barceloux, 2008, p. 537). He also wrote that licorice could be used in lozenges to clear the throat and as a remedy for dropsy, ulcerous sores of the mouth and genitals, and bladder and kidney ailments (Sena, 2010, p. 260).

During the Middle Ages, the knowledge of licorice was kept alive in southern European monasteries (Loftin, 1953). It was used at that time to treat hypotension (Barceloux, 2008, p. 537).

Licorice also plays a large part in traditional Chinese medicine; the *Shen Nong Ben Cao Jing* contains references to it (Barceloux, 2008, p. 537). The Chinese make frequent use of the species *G. uralensis*, but this species does share major active constituents with *G. glabra* (Kondo, 2007).

Usage in Food, Beverages, and Tobacco

Licorice candies consist of three main ingredients: licorice extract, sugar, and a binder (Sena, 2010, p. 258). The licorice extract is in the form of a spray dried powder or 'block juice'. The powder has a mild licorice aroma and bitter-sweet taste (Edwards, 2000, p. 119). Contrary to common beliefs, licorice powder is actually a light brown color. Artificial black coloring is frequently "added to licorice candy just to humor the consumers' preconceptions of what licorice should look like" (Loftin, 1953). Block juice is a solid block resembling coal and has an overpowering licorice flavor and bitter-sweet taste (Edwards, 2000, p. 119). Not all candies labeled "licorice" actually contain licorice extract; "red licorice" is actually made with cherry, strawberry, or raspberry flavorings, and many "black licorice" candies are actually flavored with anise seed oil, synthetic anisole, or anethole (Sena, 2010, p. 259).

Licorice is used to flavor and sweeten soft drinks, herbal teas and various liqueurs, among other beverages. For example, glycyrrhizin crystals are used as a sweetening and foaming

Root	**Plant**
18-ALPHA-GLYCYRRHETINIC-ACID	GLYCYRRHIZIC-ACID
18-BETA-GLYCYRRHETINIC-ACID	
ASPARAGINE	**Leaf**
GLABRIDIN	LICOFLAVANONE
GLYCYRRHETINIC-ACID	NICOTINIC-ACID
GLYCYRRHIZIN	
GLYCYRRHETINIC-ACID-MONOGLUCURONIDE	**Rhizome Essential Oil**
ISOLIQUIRITIGENIN	ALPHA-TERPINEOL
LIGNIN	BENZALDEHYDE
O-ACETYL-SALICYLIC-ACID	OCTANOIC-ACID
SUGAR	PAEONOL
Shoot	**Rhizome**
GLABRANIN	GLABRIDIN
PINOCEMBRIN	
URALENIC-ACID	
URALOZIDE-ACID	

Table 1. Non-ubiquitous chemicals in *G. glabra* that have a concentration over 1,000 ppm. (Source: Duke 2012).

agent for beverages like root beer and birch beer" (Loftin, 1953).

In Italy and Spain, the root of *G. glabra* is chewed for use as a mouth freshener. Licorice is also a conditioning and flavoring agent in many tobacco products (Sena, 2010, p. 259).

Miscellaneous Uses

G. glabra was cultivated in Uzbekistan for a period of four years in water-logged saline soils to keep the water table below the critical level. The plant decreased the soil and water salinity and increased the productivity of wheat and cotton crops (Li et al, 2011).

After licorice is extracted from the roots of the *G. glabra*, the root fiber itself has a variety of uses. It can be boiled with caustic soda to make a stabilizer used in fire-fighting foam, and it can be used to make a very effective insulation board. Pulp made from the leftover roots can be used to make a superior boxboard. The leftover roots can also be fortified with chemicals and composted for about a month, and the resulting fibers can be used as groundwork on which to grow mushrooms (Loftin, 1953).

Chemistry and Pharmacology

Some major chemical constituents in *Glycyrrhiza glabra* are listed in **Table 1** (Duke, 2012). Glycyrrhizin, also called glycyrrhizic acid, is the main compound in *G. glabra* root extract (**Figure 3**). It is a triterpenoid saponin glycoside, and licorice root contains around 2-15 % triterpenoid saponins (Sena, 2010, p. 256). Glycyrrhizin, when isolated as the pure compound, is an odorless, crystalline solid that is partially soluble in cold water and very soluble in both hot water and alcohol (Sena, 2010, p. 258). Glycyrrhizin tastes much sweeter than sucrose; Sena (2010, p. 258) reports that it is 30-50 times sweeter, while Barceloux (2008, p. 539) reports that it is around 100-200 times sweeter. The sweet flavor is recognized more slowly and lasts longer than sucrose (Sena, 2010, p. 258).

Glycyrrhizin is found in *G. glabra*, *G. uralensis*, and *G. inflate*. Other compounds common to all three species include liquiritin, liquiritin apioside, isoliquiritin, isoliquiritin apioside, and liquiritigenin (Kondo, 2007). Glabridin, a

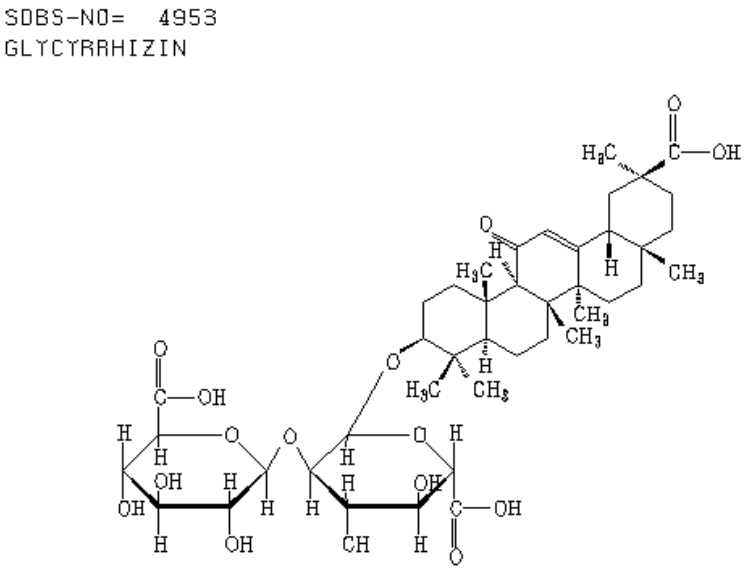

Figure 3. The Chemical Structure of Glycyrrhizin. (Source: http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/direct_frame_top.cgi).

polyphenic flavonoid, is a major compound specific to the species *G. glabra* (Hayashi et al, 2003). When licorice is used for medicinal purposes, the species does need to be taken into consideration because of the difference in constituents. Kondo (2007) advises that "in the assessment of the six kinds of main constituent contents, *G. glabra* and *G. inflate* can be used equally as medicine, but *G. uralensis* might be not able to use similarly with *G. glabra* or *G. inflate."*

Percentages of constituents also vary depending on the geographic location of the plant. Rutin was the major flavonol glycoside found in the leaves in Kazakhstan, but isoquercitrin was the major leaf flavonoid glycoside in specimens collected in Turkey, Italy, and Spain (Hayashi et al, 2003).

Biological Activity

Antiviral activity

Glycyrrhizin and glycyrrhizic acid have been shown to inhibit growth and cytopathology of numerous RNA and DNA viruses. *In vitro* research with a human hepatoma cell line has shown that glycyrrhizin completely suppresses the expression of the hepatitis A virus' antigen. Glycyrrhizin was proved to be 10 times more potent at reducing the infectivity of the virus. It was also more cell-selective by five-fold, meaning that it was less cytotoxic to the hepatoma cells. Glycyrrhizin has been shown to irreversibly inactivate the *Herpes simplex* virus, and it has been shown to inhibit viral replication and the infectivity of HIV, herpes zoster, *Varicella zoster*, and CMV ("Glycyrrhiza glabra", 2005).

Steroid-like anti-inflammatory activity

This activity is due to the inhibition of phospholipase A2 activity. *In vitro* research has also shown that glycyrrhizic acid inhibits cyclooxygenase activity and prostaglandin formation, as well as indirectly inhibiting platelet aggregation ("Glycyrrhiza glabra", 2005).

Antioxidant and hepatoprotective properties

Glycyrrhizin and glabridin inhibit the generation of reactive oxygen species by neutrophils at the site of an inflammation. *In vitro* studies have demonstrated that the licorice isoflavones hispaglabridin A and B inhibit Fe^{3+}-induced mitochondrial lipid peroxidation in rat liver cells ("Glycyrrhiza glabra", 2005).

Anticarcinogenic properties

Research has found that glycyrrhizin and other licorice components inhibit abnormal cell proliferation, tumor formation, and growth in breast, liver, and skin cancers ("Glycyrrhiza glabra", 2005). The licochalcones have been found to have radical scavenging properties, which may be due to the regulation of (per)oxidizing enzymes.

Antibacterial activity

Nand, Drabu, and Gupta (2011) found that methanolic extracts of licorice have broad-spectrum antibacterial activity. An *in vitro* study showed that licorice flavonoids show activity against clarithromycin and amoxicilin-resistant strains of *Helicobacter pylori*. Deglycyrrhizinated licorice (DGL) preparations may be effective against *H. pylori* infection since the flavonoids seem to be what is behind the antimicrobial property ("Glycyrrhiza glabra", 2005). Licorice root extract also showed significant antibacterial activity against the gram positive *Bacillus subtili* and *Stapphylococcus aureus* and the gram negative *Escherichia coli* and *Pseudomonas aeruginosa* (Lakshmi et al, 2011).

Antifungal activity

The compounds licochalcone A and glabridin are effective antifungal agents (Messier & Grenier, 2011). *G. glabra* extracts with 80% methanol (oil-based extract of licorice; OEL) were effective against filamentous fungi and some thermo-resistant bacilli. It also lessened contamination in polyethyleneterephthalate bottled tea based beverages, indicating potential application in the prevention of beverage and food spoilage due to microorganisms (Lakshmi et al, 2011).

Memory enhancement

Maze and passive avoidance testing in mice indicate that aqueous extracts of licorice may have memory-enhancing properties by reversing chemically-induced amnesia.

Clinical Studies

Herpes simplex virus

In one case report, a two-percent topical glycyrrhizic acid cream (carbenoxolone sodium) was applied six times daily to 12 patients with acute oral herpetic infections, and the result was that pain and dysphagia were resolved within 24-48 hours of the beginning of treatment. Ulceration and lymphadenopathy gradually healed within 24-72 hours (Lakshmi et al, 2011).

Stronger Neo Minophagen C (SNMC) is comprised of 0.2 % glycyrrhizin, 0.1% cysteine, and 2.0% glycine (Coon & Ernst, 2004). Oral and IV preparations of SNMC were given to infants with CMV (*Cytomegalovirus*, a genus of herpes virus). Liver dysfunction and weight gain improved in nearly all cases as compared to groups without treatment (Lakshmi et al, 2011).

HIV

A clinical study consisted of three HIV patients with hemophilia who were administered glycyrrhizin by IV at 400-1600 mg on six occasions over a one month period. The HIV p24 antigen, which was detected in all patients at the beginning of treatment, had either decreased significantly or become negative by the end of one month. P24 antigen levels immediately elevated when the glycyrrhizin dose was tapered off (Glycyrrhiza glabra", 2005).

Ulcers

In a double-blind study, 70 patients with endoscopically confirmed gastric or duodenal ulcers were given either 300 mg of carbenoxolone sodium or a placebo daily for seven days, then 150 mg daily for the next 3-5 weeks. In the experimental group, there was an increase in pH of the stomach antrum from 1.1 to 6.0 and a reduction in basal and histamine-induced gastric acid secretion at pH 3 and 5. 70 percent of ulcers in the experimental group healed within 3-5 weeks, while 36 percent of ulcers in the placebo group healed within that time frame (Glycyrrhiza glabra", 2005).

In one study on aphthous ulcers, twenty patients used a DGL mouthwash four times a day, and in fifteen there was 50-75 percent clinical improvement after just one day, and after three days, the canker sores completely healed. A double-blind trial was completed where 24 patients with recurrent aphthous ulcers were randomly allocated to consume either oral licorice mouthwash or a placebo three times a day after meals for four weeks. The mouthwash consisted of 2 g glycyrrhizin (carbenoxolone sodium) in 30 mL of warm water. The use of the mouthwash significantly reduced the average number of ulcers per day, pain sores, and the development of new ulcers compared to the placebo (Glycyrrhiza glabra", 2005).

Clinical trials using DGL formulations, however, do not all show clear evidence of effectiveness in treatment of ulcers. In a placebo-controlled, randomized clinical trial of 96 patients with gastric ulcers, the group given DGL showed no differences in healing than the placebo group over a period of 4 weeks (Barceloux, 2008, p. 538).

Weight Loss

Fifteen normal-weight subjects (seven males, eight females, ages 22-26) were given 3.5 mg of a commercial licorice preparation every day for two months. Plasma rennin activity and aldosterone were suppressed and there was a decrease in body fat mass, but there were no changes in body mass index. The results indicate that licorice and its components may be able to reduce body fat by inhibiting 11-R-hydroxysteroid dehydrogenase in fat cells ("Glycyrrhiza glabra", 2005).

Contraindications

Side Effects and Toxicity

In the United States, the US Food and Drug Administration (FDA) regulated licorice as a food supplement. As a flavoring agent in food products, the FDA regards licorice as GRAS (generally recognized as safe) (Barceloux, 2008, p. 538). When taken in higher concentrations as a supplement, however, a common side effect of licorice consumption is a condition known as pseudoaldosteronism. Glycyrrhizin's structure is very similar to the hormones secreted by the adrenal cortex, so glycyrrhizin is capable of potentiating aldosterone action while binding to mineralocorticoid receptors in the kidneys. This can cause high blood pressure, hypokalemia (potassium loss), high blood pressure, and sodium retention, which results in edema ("Glycyrrhiza glabra", 2005).

The high concentration of glycyrrhizin in the root of *G. glabra* is also evidenced to be toxic when chronically ingested in moderate to excessive amounts. An example of an "excessive" amount would be the ingestion of two to four licorice twists daily for a month, which can increase glycyrrhizin levels to over 400 ng/mL. Symptoms are usually related to abnormalities in cortisol metabolism and include edema,

weight loss, weakness, hypertension, confusion, and hypokalemia. The toxic effects of high levels of glycyrrhizin in the body usually fade away by themselves once the consumption of licorice is halted (McLawhon, 2010, p. 438)

Drug Interactions

There are reported licorice-drug interactions with hydrocortisone, prednisolone, and oral contraceptives (Sena, 2010, p. 268). Use of diuretics and licorice simultaneously may cause hypokalemia in patients with essential benign hypertension. When licorice is used in conjunction with digoxin, there is an increased likelihood of cardiac arrhythmias, especially in people with ischemic heart disease ("Glycyrrhiza glabra", 2005).

Current Use in Allopathic and CAM Therapies

Powdered preparations of licorice root usually contain 4-9 percent glycyrrhizin, and oral doses of 1-4 g per day divided into three to four doses have been used. Licorice extract is also available in liquid form, which contains 10-20 percent glycyrrhizin, and oral doses of 2 – 4 mL per day are used (Sena, 2010, p. 259). Individuals vary widely in how much licorice is safe to consume, but at daily oral intake, the 1-10 mg of glycyrrhizin (1-5 g of licorice, which contains 2% glycyrrhizin) is an estimated safe dose for most healthy adults. In studies of DGL for peptic ulcers, the dosages ranged from 760 to 2,280 mg daily ("Glycyrrhiza glabra", 2005).

The German Commission E approved daily doses of 200 – 600 mg glycyrrhizic acid for the treatment of peptic ulcers. However, treatment is limited to five weeks, and patients with cardiovascular or renal disease are discouraged from using licorice unless closely supervised by a physician (Barceloux, 2008, p. 538). Licorice extract is also available as the above mentioned deglycyrrhizinated licorice (DGL) for treatment of ulcers ("Glycyrrhiza glabra", 2005). Glycyrrhizin has been removed from these extracts, and thus the metabolic side effects caused by glycyrrhizin do not appear to occur with use. DGL comes in tablet form and in studies on peptic ulcer treatment, DGL was used in dosages ranging from 760- 2,280 mg daily (Sena, 2010, p. 259; Glycyrrhiza glabra", 2005). DGL formulations use a different mechanism than many other medications for ulcers. Others act by suppressing gastric acid release, but DGL formulations increase mucous production and blood supply to the damaged stomach mucosa, which aids in mucosal healing ("Glycyrrhiza glabra", 2005).

Several preparations specific to other countries include constituents from *G. glabra*. Stronger Neo Minophagen C (SNMC), mentioned above, has been used by the Japanese for over 40 years in the treatment of Hepatitis C (Kumada, 2002). It is an intravenous drug containing glycyrrhizin, glycine, and L-cysteine (Miyake et al., 2002). Ankaferd Blood Stopper (**Figure 4**) is used in Turkey for the management of external hemorrhage and dental surgery bleedings. It is a standardized mixture of *G. glabra, Thymus vulgaris, Vitis vinifera, Alpinia officinarum* and *Urtica dioica* (Goker et al., 2008).

Licorice is also used in hand, skin, and body lotions, and as an ingredient in anti-aging creams (Sena, 2010, p. 259). Glycyrrhizin crystals are also used to mask disagreeable flavors and as a base for many drugs (Loftin, 1953)

Figure 4. Ankaferd Blood Stopper (Source: http://www.pakimarket.com/wp-content/uploads/classipress/ankaferd-blood-stopp-592027747.jpg).

Discussion

Glycyrrhiza glabra has been used, documented, and recommended by a variety of prominent figures throughout history for many different ailments such as ulcers, bacterial and viral infections, and inflammation. Constituents of the plant, especially glycrrhizin, have been and continue to be extensively studied for medicinal properties. However, conclusive evidence regarding the effectiveness of standard licorice preparations for many conditions still has not been established. The extent to which licorice has been used in traditional medicine, however, does suggest strong medicinal properties, and more research will likely lead to development of powerful new drugs in the future. Overall, *G. glabra* has very useful components that will certainly continue to benefit human health. One must keep in mind, however, that just like many other medicinal plants, licorice is a flavoring in small amounts, a medicine in average amounts, and in large amounts, it can be a deadly poison.

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Hamamelis virginiana L. Hamamelidaceae

Sarah Howard

Introduction

Hamamelis virginiana L. is of the Hamamelidaceae family and the Hamamelidales order. Most commonly known as American witch hazel, *H. virginiana* is also known as spotted alder, snapping hazel, tobacco wood, pistachio, and winter bloom (Erichsen-Brown, 1979). Found in the Eastern region of the United States of America, the leaves, bark, and twigs have been used to treat a wide variety of illnesses, including skin irritation, burns, insect bites, sore throats, colds, fevers, etc. Its uses were first recorded by Native Americans who used the plant in a wide variety of medical treatments. *H. virginiana* is most commonly used today for its astringent qualities in cosmetics and relief from skin inflammation or irritation. Its main active chemical constituents are hamamelitannins and proanthocyanidins, which have been well established for their astringent properties.

Figure 1. *Hamamelis* Tree
Notice how the tree is developed from several small trunks clumped and rooted together, opening up into a leafy, bushy canopy. Source: http://www.bestplants.org

Botanical Description

Growing up to six meters tall, *Hamamelis virginiana* is a deciduous shrub, often with several trunks growing together in one cluster (**Figure 1**), with an average trunk diameter of 0.3 meters. Often, several smaller trunks grow together in clusters, forming what appears to be a single tree. The bark of the trunk is light brown and slightly scaled. The thin twigs are orange-brown to red-brown in color and transition from pubescent[1] to glabrous[2] over the course of their lifetime. The shape of the leaf varies but is most frequently obovate or subcordate with crenated edges and can be up to 6 cm broad and 2 cm long. The top side of the leaf is dark green while the bottom side is a pale green. The entire leaf is glabrous with the exception of pubescent veins. Flowers are found in small, axillary clusters with four bright yellow, linear petals (**Figure 2**) (Mohlenbrock & Thomson, 2009). The delicate flowers bloom after the foliage drops in October or November and have a light, spicy fragrance (Brand, 2001).

[1] Pubescent: with hair

[2] Glabrous: smooth surface, no hair present

Figure 2. Petals of *Hamamelis virginiana*
Each flower has four bright yellow thin petals radiating from the center. Each stem has multiple flowers. Source: http://www.ausable.org

H. virginiana is bisexual but remains dependent on insect pollination, most frequently flies of genus *Bradysia* and small bees (Anderson & Hill, 2002). The capsular fruits are orange-brown, pubescent, and obovoid in character. Within each capsule is two hard, elongated black seeds up to 5 mm long (Mohlenbrock & Thomson, 2009). The fruits mature in late August and remain on the plant until the capsule dries and erupts, expelling the seeds (**Figure 3**). The freshly expelled seeds travel an average distance of 3.45 meters. The only species of genus *Hamamelis* to blossom in the fall (most flower from late winter to early summer), it is suspected that *H. virginiana* evolved the early blossom to avoid competition for pollination with *H. vernalis* (Anderson & Hill, 2002). The preferred habitat for *H. virginiana* is a moist forest with rich

Figure 3. Opened *H. virginiana* capsules
As visible by the far left capsule, each capsule has two pods, which each contains one black seed. Upon opening, the seeds are expelled from the plant. Source: USDA-NRCS PLANTS Database

soil. *H. virginiana* grows in the eastern half of North America (**Figure 4**).

H. virginiana is largely unaffected by pests but may host the saddled prominent caterpillar and the witch hazel leaf gall aphid (Gilman & Watson, 1993). The saddled prominent is a

Figure 4. Distribution across North America
The green shading indicates the presence of *Hamamelis virginiana* in at least one area of the region. Based on the map, *H. virginiana* is primarily located in the eastern half of North America. Source: USDA-NRCS PLANTS Database

yellow-green to brown caterpillar named for the red marking on its back resembling a saddle (**Figure 5**). Though saddled prominent caterpillars will occasionally feed on *H. virginiana,* they prefer American beech, sugar maple, yellow birch, and paper birch (Rush & Allen, 1987). The primary pest of *H. virginiana* is the witch hazel leaf gall aphid, which can cause

Figure 5. Saddled prominent caterpillars
Source: US Department of Agriculture, Forest Service

green galls[3] up to ½ inch long to form on upper leaves. The galls cause no permanent damage to the plant and the aphids are usually controlled by natural predators (Gilman & Watson, 1993).

Traditional Uses

Native American tribes, including the Cherokee, Chippewa, Iroquois, Monhegan, Menomini, Potawatomi, Mahican, and Mohawk Indians, used *Hamamelis virginiana* extensively, but primarily for medicinal use (Spruce & Thrasher, 2008).

[3] Gall: abnormal growth formed on plants and trees in response to presence of insect activity.

Magical Properties

The most common name of the plant, witch hazel, was initially assigned to *H. virginiana* due to the many magical qualities the plant was believed to possess. Menomini and Monhegan Indians both used forked branches of witch hazel as a diviner of water and buried treasure, specifically silver and gold (Smith, 1970; Tantaquidgeon, 1972). Forked branches are held in each hand and the location of the water or treasure is indicated by where the point of the fork drops to the ground. The Menomini also used the dried, blackened seeds of the "pisewa'^{x}tîk" or "pisä'-kiwûs" to test the potential recovery of an ill individual in a traditional medical ceremony known as "mê'gîsê" (Densmore, 1932; Smith, 1970). Other noted non-medicinal uses of *H. virginiana* by Native American tribes are the use of its twigs and branches to fashion brooms, brushes, and even bows (Tantaquidgeon, 1972). The hard wood, weighted at approximately 43 pounds per foot would be a sturdy option for the creation of such products (Erichsen-Brown, 1979).

Muscle Relaxer

Known to the Forest Potawatomi tribe as "bwaote'ît" or "paga'nîmîg," *H. virginiana* twigs were placed in water with hot rocks to create a steaming bath said to bathe sore muscles (Smith, 1933). Similarly, the Menomini tribe steeped twigs to cure lame backs, adapted as a known use by neighboring Stockbridge Indians. There were also reports of a sudorific bath prepared with seven different herbs, including the twigs of *H. virginiana*. Also in the Menomini tribe, a decoction of the plant applied topically to the legs was believed to be useful during games to keep the athletes "limbered up" (Smith, 1970).

Poultice

Native Americans, particularly Cherokee and Monhegan tribes, used *H. virginiana* to treat a wide variety of topical conditions including cuts, bruises, insect bites, burns, varicose veins, tumors, and hemorrhoids, etc. (Hamel & Chiltoskey, 2002; Tantaquidgeon, 1972). In one account, a visitor to a tribe of Mohawk Indians described the healing of a man who had reached almost total blindness due to blunt force trauma to his head (treating a large bruise). Ultimately, he was healed by a warm stream of water that had soaked the bark of *H. virginiana* was poured over the location of injury. Of course, the preparation of the plant depends on the specific use. When treating a small cut or insect bite, the leaves are often ground or chewed and applied topically as a paste (Hamel & Chiltoskey, 2002). In treatment of varicose veins, a condition of enlarged and painful veins, a white cotton cloth is soaked in the extract and applied to the site. Tumors are dressed with a combination of *H. virginiana* and flax seed. Painful, inflamed eyes are treated with a poultice of inner bark rind from the plant.

Anti-hemorrhagic

Due to the astringent properties of the plant, anti-hemorrhagic properties of *H. virginiana* have been used extensively by Native Americans to treat everything from heavy menstrual bleeding, ulcers, bleeding in the mouth and throat, dysentery, and any internal bleeding. The dried powdered capsules of the flower can also stop bleeding of small cuts (Erichsen-Brown, 1979). These inflictions are most commonly treated with a decoction of the witch hazel, which is taken in the form of tea, 3-4 times per day. Alternatively, if experiencing bloody discharge, the extract, prepared by simmering one ounce of bark or leaves in one pint of water for ten minutes, is injected

directly into the rectum after each bloody discharge. The Iroquois also had an interesting pattern of using a decoction of the leaves and twigs to regulate blood in the kidneys.

Upper Throat and Lungs

Iroquois used a decoction specifically from young twigs as a respiratory aid for chest colds as asthma (Herrick, 1997). Cherokee tribes also used teas from the leaves and twigs of *H. virginiana* to treat a wide variety of ailments including colds, sore throats, general pain, tuberculosis, and fevers (Hamel & Chiltoskey, 2002; Moerman, 2010). The taste of the raw plant is described as initially bitter, noticeably astringent, and ultimately leaves a sweet after taste.

Chemistry and Pharmacology

Many of the chemical constituents of *Hamamelis virginiana* have been identified by both structure and function. Given that they serve notably different purposes to the plant, the leaves and bark of *H. virginiana* also vary in the chemical constituents they possess. The most well established constituents of the plant are its tannins, flavonoids, and volatile oil.

The over-arching group of phenolic compounds is vital to the function of the plant in treating various illnesses. A phenolic compound is a benzene ring with at least one alcohol group attached. An analysis of an extract of *H. virginiana* leaves resulted in the identification of 27 different phenolic constituents. It was found using a high-performance liquid chromatography diode-array detection that gallotannins with 6-11 galloyl units constitute the majority of phenolics (Duckstein & Stintzing, 2011).

The notable astringent qualities of *H. virginiana* are attributable to the tannins present in the leaves and bark. Tannins are polymeric phenolic compounds, meaning they consist of repeating phenolic groups. The astringent qualities of tannins are a result of the fact that they cause proteins to complex, which ultimately decreases the permeability, and therefore the secretions, of the dermis (Brown & Dattner, 1998). The leaves boast 9.6% tannins including oligomeric proanthocyanidins and gallotannins, which are composed of glucose and gallic acid. The tannin content corresponds to nearly 39% of the leaf's dry matter. Hamamelitannin, the object of many scientific studies, is largely found in the bark, which consists of 6–9% tannins.

The volatile oil, known to have anti-inflammatory effects, is present anywhere from 0.1-0.5% in the bark and 0.01%-0.5% in the leaves. The oil consists of 40% aliphatic alcohols, 15% aliphatic esters, 25% generic carbonyls (groups with a double bonded carbon and oxygen), and up to 0.2% safrole. Specifically the ketones and esters of the volatile oil reduce inflammation.

Flavonoids are another form of a phenolic structure and are thus hydroxylated (containing an alcohol or –OH group) but they also include a carbonyl group. There is a wide range of flavonoids active in *H. virginiana* including kaempferol, quercetin, quercitrin, and isoquercitrin in the leaves. Other catechins, a subgroup of flavonoids, are found in the bark: ellagitannin, gallocatechin, epicatechingallate, and epigallocatechingallate(Zeylstra, 1998). Finally, the bark also includes procyanidins and proanthocyanidins, yet another subgroup of flavonoids, which have been identified for their efficacy in tumor treatment.

Other constituents include gallic acid, caffeic acid, resin, and fat. See **Table 1** for a table of the above chemical constituents and their structures.

Biological Activity

There have been numerous in vivo and in vitro studies conducted regarding the efficiency of extracts of *H. virginiana* in treatment of tumors, dermatitis, and aging. Though general functions of the main constituents have been assigned, the research outlined below extends the knowledge of specific treatments and uses.

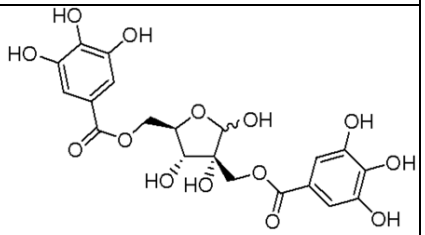
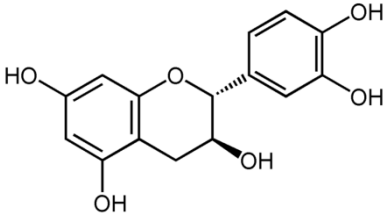
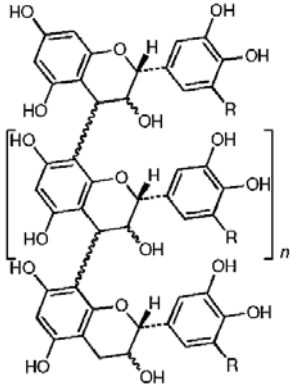
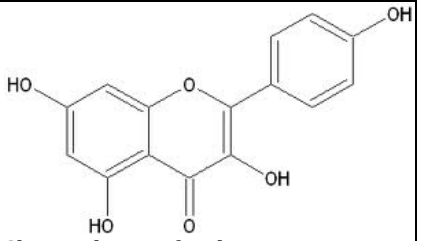
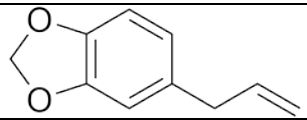
Chemical name	**Location and Percentages**	**Chemical structures**
Tannins – Gallotannins and hamamelitannins	Leaf, 9.6% (39% of dry matter) Bark, 6-9%	 Source: American Society for Pharmacology and Experimental Therapeutics
Phenol - Catechin (+)-catechin, (+)-gallocatechin, (-)-epicatechingallate (-)-epigallocatechingallate	Leaf and bark	
Flavonol - Proanthocyanidins Oligomeric cyanidin and delphinidin	Leaf and bark	 Source: WHO Monographs
Flavonoids Kaempferol Quercetin Quercitrin Isoquercitrin	Leaf	 Shown: kaempferol
Aliphatic alcohol (volatile oil)	Leaf, 40%	
Aliphatic esters (volatile oil)	Leaf, 15%	
Carbonyl substances (volatile oil)	Leaf, 25%	
Safrole	0.2%	

Table 1. Chemical Constituents of *Hamamelis virginiana* and their structures Source: *Hamamelis virginiana,* British Journal of Phytotherapy

Tumor Treatment

One study in 2012 indicated that hamamelitannins and pentagalloylculcose with proanthocyanidin from the bark of *H.*

virginiana were successful in inducing apoptosis, necrosis, and the prevention of DNA replication in the S-phase specifically in colon cancer growth. While hamamelitannin had no harmful effects on the processes of normal colon cells, pentagalloylglucose inhibited both cancer and normal cell growth (Sanchez-Tena et al., 2012).

In another *in vitro* study using human cells, hamamelitannins inhibited the apoptosis and endothelial cell death caused by the tumor necrosis factor-α (TNF). TNF was investigated not only for its effect on endothelial cell death but also its adhesion to monocytes, which are the white blood cells involved in immune system responses. Treatment of cells with TNF led to DNA fragmentation while treatment with concentrations of hamamelitannin, the major tannin found in *H. virginiana,* inhibits the apoptosis and fragmentation caused by TNF. This is consistent with the observation that the hamamelitannins in the prior study did not cause damage to the non-cancerous cells. There was still a TNF-induced increase in the number of receptors on the surface of target cells indicating that there are different pathways in the expression of cell adhesion molecules and endothelial cell death (Habtemariam, 2002).

Anti-Cancer

An *in vivo* study with human derived hepatoma (liver cancer) cells demonstrated that *H. virginiana* extracts are not only tumor suppressors but can also inhibit the reproduction of the cancerous cells and also reduce the DNA damage the cells cause. The study tested three main chemical constituents of *H. virginiana*: hamamelitannin and two proanthocyanidins. The effects of proanthocyanidin became evident at a minimum dose of 2 µg/mL and were significantly stronger than the catechins and hamamelitannin. Further tests revealed that the effects were due to the scavenging of the mutagen by the plant constituents (Dauer, Hensel, Lhoste, Knasmuller, & Mersch-Sundermann, 2003).

Erythema[4] Treatment

In two *in vivo* studies by Hughes-Formella et al, *H. virginiana* extracts were tested for their ability to treat erythema. In 1998, 30 volunteers were exposed to ultraviolet B (shortwave) rays to induce erythema and then treated with one of three ointments – pH5 aftersun lotion with 10% hamamelis distillate, pH5 aftersun lotion without hamamelis distillate, and a former pH5 lotion of different composition. Erythema was suppressed by 27% at 48 hours after exposure compared to about 15% suppression of the other lotions. Not only did the hamamelis extract reduce the erythema more effectively than the other lotions, but it also was demonstrated to have anti-inflammatory action at just 10% potency (Hughes-Formella et al., 1998).

In 2002, 40 volunteers were exposed to three different UV doses and given one of three different lotions. The lotions contained 10% hamamelis distillates in three different vehicles: dimethindene maleate 0.1% gel, hydrocortisone 1% cream, or hydrocortisone 0.25% lotion. Anti-inflammatory effects were observed for each lotion in the 48 hours after exposure. Though the discrepancy between the lotions was minimal, it was noted that the hydrocortisone formulations were most effective in treating erythema (Hughes-Formella, Filbry, Gassmueller, & Rippke, 2002).

The effect of hydrocortisone was also investigated in a study regarding the anti-inflammatory properties of hamamelis distillate. Twenty-four healthy subjects were exposed to

[4] Erythema: superficial redness of skin

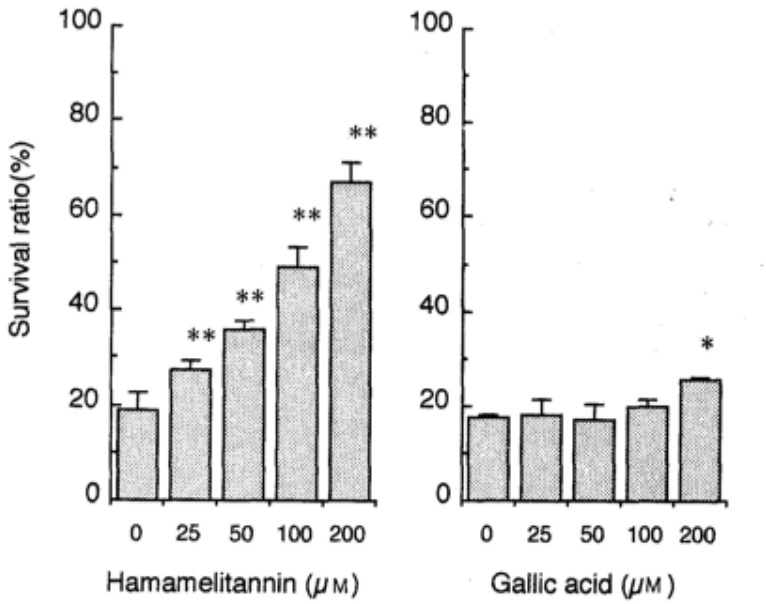


Figure 6. Graph of Cell Survival After Treatment of Hamamelitannin and Gallic Acid
Treatment with increasingly higher doses of hamamelitannin increases the survival rate of cells under attack by superoxide anion radicals. Gallic acid does not have a significant impact on the survival but increases slightly at a dose of 200 μM.
Source: Biological and Pharmaceutical Bulletin, 1995

ultraviolet irradiation and cellophane tape to induce erythema and subsequently treated with hydrocortisone cream, hamamelis phosphatidylcholine cream, hamamelis cream (without PC), or chamomile cream. The results demonstrated that inflammation was reduced when treated with hamamelis PC cream but that the hydrocortisone treatment was more effective. Furthermore, an increase in dosage of the hamamelis PC cream by four times did not improve activity of the treatment. Regardless, anti-inflammatory properties were confirmed (Korting, Schafer-Korting, Hart, Laux, & Schmid, 1993).

Anti-oxidative Properties

In addition to anti-inflammatory and anti-tumor properties, *H. virginiana* extracts were also found to have strong antioxidant properties. An in vitro study by Masaki et al. in 1995 found that hamamelitannin has superoxide-anion scavenging activity. Furthermore, hamamelitannin is more effective than gallic acid in preventing cell damage by superoxide anions, another constituent of *H. virginiana* extracts. The minimum concentration of efficacy for hamamelitannin, 50 μM, was half of that of the necessary concentration of gallic acid, which was 100 μM (**Figure 6**). Thus, pre-treatment of fibroblasts, cells that synthesizes the extracellular matrix and collagen, with *H. virginiana* extracts increases their survival rate (Masaki, Atsumi, & Sakurai, 1995).

General Anti-inflammatory Properties

In an *in vivo* study of human keratinocytes[5] by Deters et al, polysaccharides and proanthocyanidins were tested for their impact on keratinocyte proliferation and differentiation. While the polysaccharides did not impact the keratinocytes, the proanthocyanidins increased the proliferation of cells. Proanthocyanidins were also shown to reduce transepidermal water loss and erythema formation. This study confirmed the efficacy of chemical constituents found in the bark of *H.*

[5] Keratinocyte: epidermal cell producing keratin, a fibrous protein with important structural properties.

virginiana. Furthermore, it established that not only does an extract of the plant reduce erythema, but also specifically the proanthocyanidins cause these effects (Deters, Dauer, Schnetz, Fartasch, & Hensel, 2001).

In an *in vivo* study with rats, the anti-inflammatory properties of *H. virginiana*, *Polygonum bistorta*, and *Guaiacum officinale* were used to treat arthritis. *H. virginiana* and *G. officinale* were only active during chronic phases of swelling while *P. bistorta* was inhibited both phases of the arthritis (Duwiejua, Zeitlin, Waterman, & Gray, 1994).

Anti-viral

In an *in vivo* study with the Herpes simplex virus type 1, an extract of *H. virginiana* bark was shown to have significant anti-viral activity. The same extract was also shown to have anti-oxidative properties that led to the inhibition of human leukocytes (Erdelmeier et al., 1996).

Clinical Studies

Wolff and Kieser conducted one of the first clinical studies with hamamelis ointment on children. There is little data regarding potential effects on children or pregnant women; thus, its use is often not recommended. In the study, 309 children from the age of 27 days to 11 years with minor skin injuries, diaper dermatitis, or a localized inflammation of skin were prescribed either hamamelis ointment or dexpanthenol. Dexpanthenol is a well-established option to heal topical wounds by stimulating epithelization and granulation. It has also been proven useful in the reduction of itching and the treatment of diaper dermatitis. Although the two ointments are very similar in composition and the results were ultimately similar, hamamelis treatment was significantly more effective than the dexpanthenol ($p < 0.001$). Specifically, there were clear advantages in infants with diaper dermatitis and in 1 – 5 year olds with skin inflammation (Wolff & Kieser, 2007).

	Reference: vehicle Age: 32 (13) years Weight: 64 (12) kg Sex: 3 m, 33 f		Reference: hydrocortisone Age: 32 (10) years Weight: 67 (14) kg Sex: 9 m, 27 f	
Parameter	Hamamelis	Vehicle	Hamamelis	Hydrocortisone
Itching Baseline	2.0	2.0	2.1	2.1
Itching 1 week	1.5	1.6	1.5	1.3
Itching 2 weeks	1.4	1.4	1.2	0.8
Erythema Baseline	1.9	1.9	1.7	1.8
Erythema 1 week	1.6	1.6	1.5	1.3
Erythema 2 weeks	1.4	1.2	1.4	1.0

Table 2. Effect of *Hamamelis* ointment on atopic eczema
The table below displays that over the course of two weeks, the hamamelis ointment reduced both itching and erythema. However, the hydrocortisone was more effective, dropping itching at erythema by 1.3 and 0.8, respectively compared to 0.9 and 0.3. Source: European Journal of Clinical Pharmacology (17)

Despite the evident anti-inflammatory and protective properties of *H. virginiana* extracts, a study pertaining to atopic eczema found that hamamelis cream was not more effective than the results obtained from base preparation (**Table 2**). Although no significant benefits were observed, it was still noted that the hamamelis cream was very well

tolerated and can be considered for higher doses than other treatments. Given that this was a clinical study, the treatment course was adhered to for 14 days. Patients made note that the treatment was considerably slower to take effect than the glucocorticoids they were used to (Korting et al., 1995).

To date, there are no prominent clinical trials regarding the effectiveness of *H. virginiana* extracts in treatment of bruises, hemorrhoids, hemostasis, or of use as an antioxidant.

Contraindications

Hamamelis virginiana is generally recognized as safe for topical application. In fact, in an in vivo study with mice and rats, oral doses of 10-20 mg showed no adverse effects. Furthermore, daily oral doses of 100 mg per kg body weight also showed no toxic effects. However, regular ingestion, particularly by pregnant women, is not recommended due to the high tannin content of the plant. Hypersensitivity or allergies to the active substances may lead to allergic contact dermatitis, or severe irritation of the skin (Zeylstra, 1998). There are no known drug interactions with *H. virginiana* but patients should use caution and inform their doctors if they use the product regularly.

Individuals with allergies to the Asteraceae or Compasitae family should exercise caution when using products of *H. virginiana.* In an *in vitro* study of patients with the allergy, they were exposed to chamomile-containing preparations, arnica-based preparations, avocado oil, and *H. virginiana.* Though neither avocado nor witch hazel is in the Asteraceae family, they were consistently detected as sensitizers and may cause an allergic reaction (Paulsen, Chistensen, & Andersen, 2008).

Current Use in Allopathic and CAM Therapies

Today, the astringent properties of *Hamamelis virginiana*, largely attributable to the tannins present in the extract, are used to tighten pores after washing or shaving. Thus, the extract is frequently used in commercial shaving creams and toners. The extract of witch hazel is also often commonly found in mouthwashes.

H. virginiana, sold commercially as Witch Hazel, is found in numerous natural products but has not yet been used in the development of a new pharmaceutical drug. Thus, all forms of witch hazel are sold over the counter. Stand-alone witch hazel products include distilled witch hazel, which is available in pharmacies and grocery stores and is advertised as an "All Natural Astringent for Face and Body." The CVS brand contains 86% witch hazel and 14% alcohol. The alcohol is added to prolong the product's shelf life and prevent the loss of efficacy. This product can be used for homemade toners (used cosmetically to tighten facial pores) or to remove make-up. *H. virginiana* leaves are also sold in capsules, marketed to support health circulation and veins with a daily dosage of 1-2 capsules, three times per day.

Witch hazel has been incorporated into elective medicine in America since the 1850s in the form of Pond's Extract, used by many Homeopathic and Allopathic doctors as a cure-all for any skin complaint, ranging from burns to bites to "female complaints". Theron Pond took an interest in the plant that many Native Americans believed to have magical properties. He formed a partnership with the Indian Medicine Man he had been learning form and began to sell the product. A small production at first, it eventually expanded from the "Golden Treasure" to Pond's Extract, which is still sold today in a variety of creams with small amounts of witch hazel included (Lloyd & Lloyd, 1935).

Arnica Plus, sold by Peaceful Mountain, Inc, as an unapproved homeopathic product, is marketed for relief from sore muscles (similarly to how the Potowatomi and Menominee Indians used the extract). The product is sold with a labeled warning that the FDA is "not aware of scientific evidence to support homeopathy as effective." Interestingly, *Hamamelis virginiana* root bark and stem bark is listed as an active ingredient at 6X and 12X dilutions and witch hazel bark is listed as an inactive ingredient. The product is recommended for topical use, to be applied daily over the affected area (NLM, 2013).

Due to its astringent properties, witch hazel is found in varying concentrations countless other health and cosmetic products such as toners, mouthwashes, aftershaves, rectal creams, and antiseptic wipes. Witch hazel is also found as an inactive ingredient in cosmetic products such as Clinique Acne Solutions, anti-aging, anesthetic, body wash, and antiseptic products (NLM, 2013). Additionally, witch hazel cooling pads are common for the treatment of hemorrhoids. One such product, AER Pre-Moistened Witch Hazel Pads, contains 50% witch hazel and is marketed specifically as an astringent for temporary relief of anal itching and burning (NLM, 2013).

Discussion

The use of *Hamamelis virginiana* by Native Americans has been extensively documented; its uses range far and wide. Although clinical, *in vivo*, and *in vitro* studies have focused on and validated the value of the plant extracts use as an anti-inflammatory, tumor suppressor, and antioxidant, little research has been conducted regarding the other uses Native Americans identified. For example, the over the counter hemorrhoidal drugs that include witch hazel are marketed without approval by the FDA. Multiple studies have also found that the most active compounds of *H. virginiana* are the hamamelitannins such as gallotannins, proanthocyanins, and catechins. Currently, there is a great deal of research that can be done to further our knowledge of medicinal uses of *H. virginiana.* For example, Native Americans used the plant in the form of tea, paste, and distillate. Given the wide availability of *Hamamelis virginiana* in the United States, further research is a logical investment given that it is highly coveted and has been repeatedly documented by indigenous cultures.

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Humulus lupulus L., Cannabaceae

David Kyle Choe

Introduction

Humulus lupulus L, most commonly known as hops, is not only a major ingredient in the production of the alcoholic beverage, beer but also for its practical and traditional medical uses. It was first used to brew beer in England in the 1500s where it was immediately characterized as a "'wicked weed' that would 'endanger the people'" (Chevallier, 102). *H. lupulus* is commonly found in Europe and Asia where it grows on the sides of roads (Chevallier, 102). Brewing the strobiles or the female flowers (**Figure 1**), of the bines is the most common preparation of hops, however, there have also been other uses such as aromatherapy. The bitterness of hops is a distinct mark of not only its famous taste but also its chemical constituents that are essential to its medicinal uses. *H. lupulus* is comprised mainly of bitter compounds, flavonoids, tannins, and other constituents. The bitter compounds including lupulin, lipulon, and valerianic acid, also a sedative, are digestive stimulants (Chevallier, 102). There have been many recent studies undertaken in which the antibiotic and antiseptic qualities of *H. lupulus* have been tested, with varying results.

Botanical Description

The only other member and perhaps an equally well-known member of the cannabaceae family is *Cannabis sativa*, more commonly known as marijuana, a popular recreational drug. Within the genus *Humulus*, there are other species based mostly on their geographic localities. *H. lupulus*, is the common hop found widely in the northern hemisphere in

Figure 1. Picture of a *H. lupulus strobile* (Chevallier, A. (1996). The encyclopedia of medicinal plants. New York, NY: DK Publishing.)

areas such as Europe and North America. *H. japonicus* is found throughout China and Japan and is not found elsewhere. *H. yunnanensis* is found mostly in Asia, however very little is known about this particular species because there are very few observable specimen and no cultivated plants (Neve, 1).

H. lupulus is a dioecious species, meaning that this species has distinct male and female reproductive bodies, most visible in their difference in physical morphology (Neve, 2). *H. lupulus* is a climbing plant yet it does not use tendrils to bind to climbing surfaces, however, its bines or stems climb up anything on which they are able to entwine (**Figure 2**). The bines round up in a clockwise direction and use hooked hairs, to ascend their climbing surface. (Neve, 2). The leaves of the plant are most commonly found in pairs, but there have been instances in which three-leaf arrangements have also been found (Neve, 2). The flowers of the plant differ by the sex of the plant. Its loose panicles, or a cluster of flowers with a grouping of five sepals and five anthers, characterizes the male plant. The anthers have a small cavity in which the valuable resins are located. It is in these small cavities that the pollen is produced in large quantities. Hops are majorly pollinated by the wind (Neve, 2). The female flowers are found in similar clusters of flowers as the male but differ in that they are found on a central nave (Neve, 2). Each section of the flower has two bracts; these are the leaves, which surround the small flower of the female plant. The flower houses the ovary together with two stigmas (Neve, 2). As these clusters of flowers grow and age, the bracts become larger and eventually form the strobiles. The strobiles are the cones containing the main commercial product of the plant (Neve, 2). Pollinated plants are easily distinguished from the non-pollinated plants because of the differences in coloration.

Although aforementioned resin is found in both the male and female flowers it is comes in much smaller quantities in the male flower. The resins, which are produced by the lupulin glands, are most active in the seeded hops wherein which one third of the total resins are produced (Neve, 2).

Figure 2. Breakdown of *H. lupulus*
(http://www.pfaf.org/user/Plant.aspx?LatinName=H.+lupulus)

Also previously mentioned, these plants are perennial where the supra terram part of the plant or the aboveground part, dies each winter but the roots are able to survive (Neve, 5).

A variety of geographical conditions leads to key differences in the morphology of the plant. Noteworthy examples include the

H. japonicus and the H. yunnanensis. The *H. japonicus* has been identified as an annual species. Similar to *H. lupulus,* it is a dioecious but the cones look very different morphologically. *H. japonicus* does not have many lupulin glands and are consequently not commonly used to brew beer with. *H. yunnanensis* is mostly found in the higher altitudes of China, particularly the Yunnan Province (from which the name is derived). There is little known about this species, as alluded to previously, yet there is limited evidence suggesting that it may have been the origins of the commercially bred variety (Nave, 9-10).

Traditional Uses

In Europe, where *H. lupulus* has been used since the mid-ninth century, the plant was traditionally harvested from the wild. It was favored mostly as a substitute for the more widely consumed beer additive, *Myrica gale*. It was not until the eighteenth century when *H. lupulus* became more popular than *Myrica gale* because of its preservative advantage (Zanoli, 384). The beer brewing industry currently utilizes 98% of all hops worldwide, which is drastically different from its original use as a preservative (Zanoli, 384). The antimicrobial activity of hops that is currently under investigation was identified early on, but only later were hops used for their distinct flavor. Medicinal values of *H. lupulus* were noted by the renowned ancient healer, Pliny the Elder. He first used the plant's younger shoots as an edible vegetable, common among the Romans (Zanoli, 384). The leaves and the heads of the flowers give brown dye extracts. The flowers were also used as food-additives that would give unique flavorings to "cereals, spices, sauces, tobacco and alcoholic beverages other than beer" (Zanoli, 385). The fabric-generating stems played an important role in the paper manufacture industry, which is similar to the value of hemp in terms of fabric-making (Zanoli, 385). The plant also contains active ingredients found in perfumes and for skin creams and lotions (Zanoli, 385).

Traditional Medicinal Uses

Famously, *H. lupulus* has been used to treat a wide range of maladies. Its volatile oil is mildly sedative, so hops alleviate insomnia and anxiety (Zanoli, 385). *H. lupulus* remits excitability and restlessness, attributed to tension headaches. It aids appetite and digestion. Additionally, it also relieves tooth pain as well as ear pain and neuralgia (Zanoli, 385). Hops also allegedly works as a "diuretic, antispasmodic, and anaphrodisiac" agent (Zanoli, 385).

In Native American tribes it has been used similarly as a sedative, to treat rheumatoid arthritis, as a pain reliever and as an urination aid (Zanoli, 385). The Native Americans also utilized *H. lupulus* as a pneumonia treatment. They would heat up hops and use it as a poultice. Decocted hops were used for intestinal pain and fevers as well (Zanoli, 385).

H. lupulus has also been known in Indian Ayurvedic medicine as a treatment for restlessness caused by tension, headache and indigestion (Zanoli, 385). It is also found in traditional Chinese medicine (TCM) where it used similarly to treat "insomnia, restlessness, dyspepsia, and lack of appetite" (Zanoli, 385). The Chinese have also used alcoholic forms of hops to successfully treat conditions such as leprosy, pulmonary tuberculosis, acute bacterial dysentery, silicosis and asbestosis" (Zanoli, 385). Hops have been used in treating skin conditions such as crual ulcers and other skin injuries. It has been applied to areas where muscle spasms and nerve

pain occur. They were also used aromatically for the skin, for breathing aid, restlessness and other conditions (Zanoli, 385).

Chemistry and Pharmacology

Though *H. lupulus* is filled with many different and potentially useful chemical constituents, its main chemical compounds are terpenes, bitter acids and chalcones. Flavonol glycosides and catechins are also present in abundance. In fact, dozens of terpenoic compounds were found in the volatile oils making up around 0.3-1.0% of its chemical composition. These terpenoid compounds included B-caryophyllen, farnesene, and humulene, and myrcene. The bitter acids, mainly α-acids and β-acids, constitute the bulk of the chemical composition of the plants with 5-20% of the strobile weight (Zanoli, 385), **Figure 3**. These bitter acids exist in hops in various and complex forms and concentrations, serving different purposes for the well being of the plant. The α-acids are composed of mainly humulone (35-70% of total α-acids), cohumulone (20-65% of all α-acids), adhumulone (10-15% of all α-acids). The β-acids mostly consist of lupulone (30-55% of all β-acids), colupulone and adlupulone (Zanoli, 386). Between these two acids are the α-acids that are responsible for the high-quality hops used for the brewing of beer. These α-acids are not only crucial for the flavor of the beer, but they are also essential for the foam stability and for the antibacterial activity exhibited by hops-based beverages (Zanoli, 385). There are also crucial prenylflavonoids in the plant. The most important is chalcone xanthohumol, which can be converted to isoxanthohumol in the presence of heat, therefore, making it the main flavonoid in beer (Zanoli, 386). The presence of these bitter acids and XH differ based on geography, species and sex of the flower. Male plants have been shown to have similar levels of XH and bitter acids as young flowering cones (Zanoli, 386).

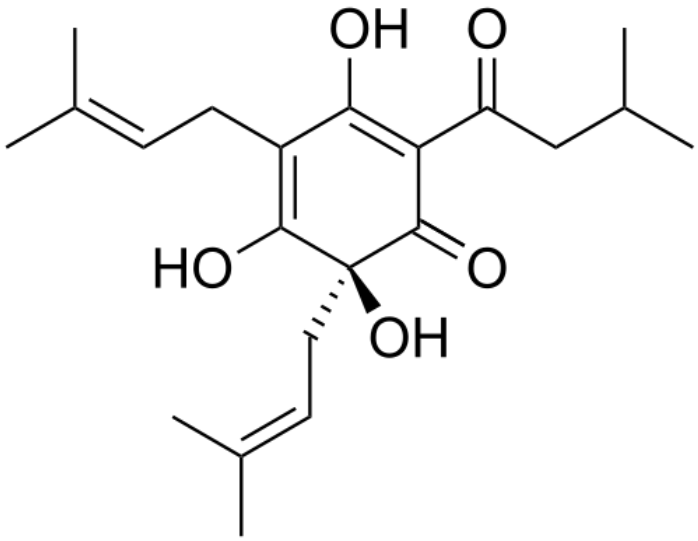

Figure 3. Molecular structure of an alpha acid.

In terms of extraction itself, the hop cones must be dried by artificial heat, wherein the water inside the cones must be significantly reduced (to 8-10% water). The method of extraction has changed from water and ethanol to more advanced methods utilizing steam and carbon disulfide.

Biological Activity

Sedative properties

The mildly sedative activity of hops was first observed when hop-pickers were reported to suffer from sleepiness and fatigue, which was surmised due to unconscious digestion during harvesting (Zanoli, 387). Germany was among the first countries to approve hops as an acceptable treatment for "mood disturbances, such as restlessness and anxiety and sleep disturbances", however it was not closely studied until

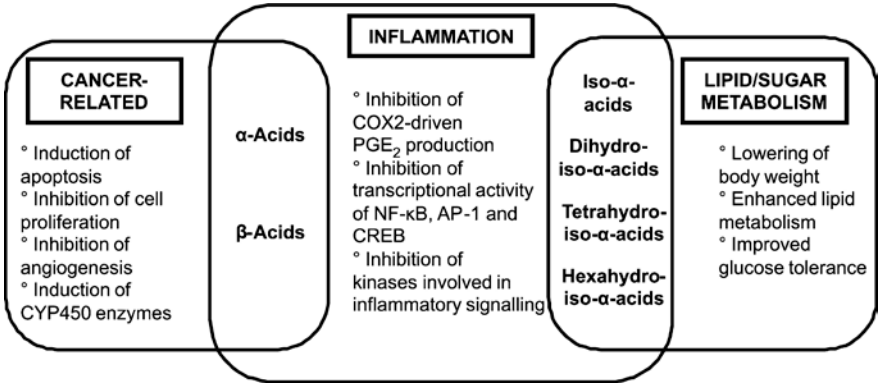

Figure 4. Biomedical properties of hops. (Source: http://pubs.acs.org/doi/full/10.1021/np800740m)

more recently (Zanoli, 388). Numerous studies on its sedative properties yielded mixed results. In the first *in vivo* study done by Hansel and Wagener in 1967, the researchers treated the mice with three different types of hop extracts prepared from two different solvents: ethanol and methylisobutyl-ketone; however, they did not find any conclusive results indicating any muscle-relaxant effects (Zanoli 388). In another study where the neuropharmacological effects of hops were tested, hop extracts were injected into mice in order to evaluate the hypothermic, analgesic and anticonvulsant activities, in which positive results were found that they did indeed serve as hypothermics, analgesics and anticonvulsants (Zanoli, 388).

A particularly significant study done by Zanoli focused on the use of hops with a CO_2 extract and "single fractions containing α-acids and β-acids". This CO_2 extract displayed remarkable "pentobarbital" sleep-enhancing effect. However, the test showed a striking result in which the hops extract produced an antidepressant-like effect. They further studied and concluded that the α-acids were producing these pentobarbital effects coupled with the antidepressant activity found after the administration of the drug.

Antimicrobial and antibacterial properties

Perhaps one of the most interesting and potentially beneficial studies done on *H. lupulus* is that of its effect on the *Mycobacterium tuberculosis*. This study, done by Serkani et al, examined the possibility of in the treatment of *M. tuberculosis,* which currently affects one third of the world's population. Thirty-seven *M. tuberculosis* strands from varying regions of Iran were tested using the proportion method. Hops extracts were also tested on a wide array of bacteria including *Bacillus subtilis, E. coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* The tests showed that there were statistically significant inhibitory effects against *M. tuberculosis* strains, with the female plants having the strongest effects. This result is interesting in comparison to its lack of effects against other bacteria such as *E. coli* and *Pseudomonas aeruginosa*. The efficacy of *H. lupulus* was close to that of the drug rifampin (Serkani et al, 235-242).

Another study evaluated the effectiveness of the chemical constituents in hops against acne vulgaris and different bacteria associated with the proliferation of acne (Yamaguchi et al, 369). They targeted this disease because it is one of the most prevalent skin diseases, affecting both children and adolescents. Acne vulgaris is caused mainly at the onset of puberty with secondary infections occurring because of various bacteria present on the skin. Yamaguchi conducted the research by comparing the effectiveness of the hops' main chemical constituents to well-known antimicrobials. The efficacy was measured by the minimum inhibitory concentration (MIC) and the minimum bactericidal concentrations (MBC), where the MIC stands for the "lowest

concentration that resulted in no visible growth after two days for *P. acnes* or 1 day for the others" and the MBC being defined as the "lowest concentration at which the microorganisms failed to grow in each medium" (Yamaguchi et al, 371). They found that the lowest MIC values, which indicate the most potent of the chemical constituents, were the naturally derived lupulones. The researchers have claimed that there has not been such a strong inhibitory ability of any natural product from an edible plant. This research has significant implications on the future of skincare products made from these naturally derived matter.

Estrogenic activity

Rich evidence shows that *H. lupulus* demonstrates significant estrogenic activity, first identified when female hops pickers experienced menstrual abnormalities (Milligan et al, 4912). Hop baths were used in Germany to treat certain gynecological disorders and have also been used to reduce hot flushes in menopausal women (Milligan et al, 4912). It is currently being used in commercial products and being marketed and breast enlargement products. The chemical compound that is highlighted in this study is 8-prenylnaringenin, which has been widely accepted as a powerful phytoestrogen. In fact, 8-prenylnaringenin, has been shown to have the one of the greatest phytoestrogenic activities among all the plant estrogens (Milligan et al, 4912). This study concludes that the endocrine activity of hops is because of the chemical compound 8-prenylnaringenin. Because of this recognition of its potency as a phytoestrogen, concerns have been raised as to possible threats and dangers in over-exposure to this chemical, but also suggest potential benefits in menopausal therapy.

Clinical Studies

In another study, tests were done on human subjects where subjects were treated with 250mg/day of hops extract for five days to treat epilepsy. In this test researchers found that there was indeed a significant decrease in the spontaneous motor activity. However, this decrease was related to the type of solvent used to extract the .

A major and novel study done by Heyerick et al was the first "prospective, randomized, double-blind, placebo-controlled study on the use of a standardized hop extract to alleviate menopausal discomforts" (Heyerick et al, 164). The introduction of the hops-based dietary supplement began to pique interest in its medicinal potential specifically its estrogenic potential. It was found that it was the closest plant compound to the 17β-estradiol, which is naturally found in the female body (Heyerick et al, 164). This study focused on the daily intake of a hops based dietary supplements, which are supposed to have beneficial effects on the experience of uncomfortable menopausal conditions. This study stands out in that it was done using three groups, a control receiving a placebo, and two active groups receiving 100 and 250 μg of 8-PN, respectively (Heyerick et al, 164). The study was done using a qualitative measure of the Kupperman index used to measure the level of discomfort caused by menopausal side effects. The results showed that there was a significant reduction of menopausal discomfort after six weeks of use. However, there was neither a dose-response nor a change in discomfort after the twelve-week period. This study is significant in that is one of the only clinical trials done using hop extracts that definitively show the efficacy of the 8-PN for treatment of a disorder (Heyerick et al, 173). Because of the thorough nature of the study, it might be said that there is greater potential for this drug to be developed for future use for menopausal symptoms.

In another study done by Wei-Jen Chen and Jen-Kun Lin, they found that the major constituents of hops, namely β-acids presented strong inhibitory effects on specific leukemia cells. This shows that bitter acids in hops may be a potential source of future cancer therapies.

Focusing on the strong barbiturate properties of hops, this study by *Zanoli et al* (2005), showed interesting and promising results concerning sleep-enhancing and antidepressant activities of *H. lupulus*. The researchers found that the age old practice of using hops as a sleeping aid to be supported by the dose-dependent responses to hops. In addition to this finding, the researchers observed there to be a reduction of immobility after use of the drug that they connected to anti-depressant effects by comparing the results to that of synthesized antidepressants.

Current Use in Allopathic and CAM Therapies

As mentioned previously one of the most widely studied uses of *H. lupulus* is found in its antimicrobial properties. This study done by Natarajan et al, reviews the efficacy of derived compounds lupulone and xanthohumol in their antibiotic qualities. The researchers then proceeded to create an antibiotic cream in which the active ingredients were added into the mix. They tested the antibacterial mixes on bacterial lawns and measured the size of the inhibitory zone in the dish. They found that that the greatest coaction found was between the hop constituents and the sulfa or polymyxin drug that was dropped into the dish. These researchers support other studies' method of action that suggests the mechanism of action being that hop constituents cause a change in the permeability of the membrane. The study concludes that the use of hop constituents might be expanded to include co-action with existing anti-bacterials (Natarajan et al, 194-201).

Perhaps one of the most important chemical compounds that are derived from *H. lupulus* is xanthohumol (XN), which was mentioned in the study above. In a review done by Clarissa Gerhauser, she goes through the effects of XN on bacteria, viruses, fungi and malaria. One study by Bhattacharya et al that Gerhauser reviews is that of the efficacy of XN verses a major compound found in a popular mouthwash, thymol (Gerhauser, 829). This study showed that XN stopped the growth of *S. mutans* significantly more than the most potent version of the common additive. Another study that she reviews is by Buckwold et al, in which hop chemical constituents were tested against a line of different DNA and RNA viruses. This study was particularly exciting because of XN's inhibitory effects against bovine viral diarrhea virus, cytomegalovirus, herpes simplex 1 and herpes simplex 2. However, the most potent example of the utility of XN was found in its effectiveness against HIV-1. In this study by Wang et al, it was found that XN "was able to inhibit HIV-1 induced CPE" which essentially suggests that XN inhibits key replication activity of the HIV-1 virus. This study has larger implications on the potential for the XN compound for future research and development (Gerhauser, 830). XN's antifungal properties were also reviewed in this article. The study done by Mizobuchi and Sato tested the constituents XN, naringenin, 6-prenylnaringenin, 8-prenylnaringenin and IXN against the human fungi *Trichophyton mentagrophytes, Trichophyton rubrum, Candida albicans, Fusarium oxysporum and Mucor rouxianus.* The results showed that XN and 6-prenylnaringenin were the most effective in fighting off the fungi. However, it is also significant to point out that IXN was more potent when it was subject to methylation. The last and perhaps one of the more exciting developments is XN's activity against malaria. Infamously known as the disease that was failed to be eradicated, malaria continues to take a major toll on this earth, combatted with an outdated and resisted drug, choloroquine.

However, using XN, researchers such as Herath et al and Frolich et al have been able to support the findings that XN has antiplasmodial effects that inhibited the activities of the malaria strains (Gerhauser, 830).

Discussion

It seems to be the case that *H. lupulus* has played a larger role in history of the earth than one might initially think. As a key ingredient to the fine beverage that beer is, *H. lupulus* has affected and will continue to affect such a large number of people across every culture and every geography. Many of its traditional uses: as a sedative, antimicrobial and antibacterial, seem to be shaping the ways in which the drug is being studied today. The very fact that *H. lupulus was* first used as a preservative in beer is a clear indication to scientists that there exists potential in this plant for future study. It was discovered that this plant has the potential not only to flavor alcoholic drinks but also to preserve them. Though alcohol often has a deeply negative connotation, it seems as if the key chemical constituents within hops, and therefore in beer, have unique and vital roles to play in the future of botanical medicine.

Although they have been studied for many years now, compounds such as XN and 8-PN are only just being studied in-depth and their powerful medicinal properties only barely beginning to be understood. To think that the future of malarial drugs, HIV drugs, cancer drugs, and estrogenic drugs might be found in *H. lupulus* is an incredible idea to grasp. Though it may not seem a researcher's first choice of subject, hops is an ideal plant to invest more research into because of its availability and relatively low variety within its genus. And in our current medical world, wrought with drug-resistant strands it seems imperative that we begin to look for alternatives, and *H. lupulus* seems like a great place to start. Plants like *H. lupulus* serve as an impetus of hope and a well of potential for future cures and vaccinations, and ultimately a healthier world.

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Hypericum perforatum L., Hypericaceae

Yinan Yang

Introduction

The genus of Hypericum contains about 370 species; within this large group, *Hypericum perforatum* or St. John's wort is known to be a highly variable tetraploid that propagates asexually. Hypericum is derived from its Greek name, hyperikon, meaning above (hyper) and image (eikon) (Radun 2007). The common name, St. John's wort, is named after John the Baptist in Christian tradition. The herb *H. perforatum* is usually harvested on June 23rd, the eve of the festival that celebrates St. John the Baptist, and it was used to ward off evil spirits, purify the surroundings and promote the harvest. Wort also comes from the Anglo-Saxon origin meaning medicine (Kim et al. 1999).

The use of *H. perforatum* can be traced back to ancient times and is currently widely cultivated around certain continents of the world like Asia and Europe. It is traditionally used as an herbal tea and antiseptic or as a topical medicine for burns and wounds but recently most commonly for its antidepressant activities. St. John's wort can also be used for treating skin irritation, asthma, inflammation and ulcers. Although the main components of St. John's wort extract are hyperforin, hypericin and pseudohypericin, the 2 latter ones are not yet linked with mechanisms for reducing depression. Other compounds in St. John's wort include tannins, flavonoids, proanthocyanidins and xanthones. St. John's wort is one of the top 15 best selling herbs in the world (Radun 2007). *H. perforatum* is a perennial herb but is also invasive; it was first introduced to the US in 1700s for ornamental purposes but has been overtaken some the native species (De

Figure 1. Illustration of *Hypericum perforatum*.

Smet et al. 1996). In terms of medicine, St. John's wort is one of the top 15 best selling herbs in the world (Radun 2007).

Description

Until recently, the origin of St. John's wort was thought to be in Africa that later spread to Asia, Australia and America. However, recent evidence tells us that it actually originated in the Mediterranean in Europe and later grew all over Europe, except in the coldest of places (Radun 2007). Today, St. John's wort can be located in most parts of Europe, North America, Australia and Asia, in the temperate climates and also in the tropics. St. John's wort grows in the shrub habit and also in tree-like habit in the tropics. Its habitat also includes the prairie, woodland, forest and rangeland (Radun 2007). While St. John's wort doesn't have very strict conditions for growth, its growth is optimally in the full sunlight with plenty of moisture. Lean or gravel soil is also a good condition to grow in as it reduces growths of other plants (Radun 2007).

St. John's wort grows upright and ranges from 30-100cm in height (Radun 2007). Its stems have 2 distinct ridges and branches off in bundles at the upper part of the plant (**Figure 1**). The leaves of St. John's wort are narrow and oval, ranging from 1.5-3cm in length and 1.5-5mm in width; they also have perforated holes spread throughout the leaves that produces essential oil (Radun 2007). In the summer, St. John's wort produces bundles of small yellow flowers with black dots; they are very abundant, often produced in clusters of 25-100 per stem. The fruits of St. John's wort look like capsules that store around 50 seeds per plant. Each plant produces up to 34,000 seeds per season and the seeds are around 5mm in length and black in color (Radun 2007).

H. perforatum is an invasive species that is capable of self-pollination and therefore, does not depend on pollination by insects. In many instances, *H. perforatum* is introduced to a new habitat along with an alien insect species that feeds on it. Alien insect species are more likely to feed on the plant they are familiar with than those that are not and *H. perforatum* is an example of an invasive weed species that has been biologically controlled by specialist insect herbivores (De Smet et al. 1996).

Traditional Uses

Uses of St. John's wort have been thought to exist from prehistory. St. John's wort is documented to be used mostly as medicine as other uses such decoration and food has not been found. Uses of St. John's wort as a medicine have had a long history whether as a topical medicine or an oral medicine. The uses of St. John's wort are many and are described as follow, however, in current times, St. John's wort is mainly used as an antidepressant. For example, in historical Britain, doctors used St. John's wort as a tranquilizer and to ward off evil spirits (Radun 2007). In Herzegovina (Southern region of Bosnia), the documented use of St. John's wort has been traced back to the 13th century (Radun 2007). The perforation of the leaves of St. John's wort was thought to be useful in covering deep wounds as it had antimicrobial properties (De Smet and Nolen 1996). Many notable Greek figures such as Hippocrates, Dioscorides, Theophrastus and Galen have been documented in using St. John's wort for treating menstrual problems, neurological disorders, treating wounds and as a diuretic. The Greeks also used it to ward off evil spirits in religious rituals and valued the plant greatly; this practice was carried on in Britain until recently (Zobayed, Murch, Rupasinghe and Saxena 2003). Paracelsus is also documented to use St. John's

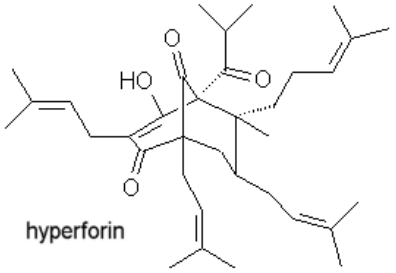

Figure 2. Structure of hyperforin (Source: biologie.uni-hamburg.de).

wort for treating wounds in the 1500s (Radun 2007). Traditionally among the ordinary citizens, St. John's wort has been used in Europe for treating anxiety, depression, wounds, inflammation and burns (Radun 2007). St. Johns wort is also used as herbal tea and it is widespread in many countries such as Turkey, Bosnia, Pakistan, Britain, Germany and China as well as by Native Americans. The tea is used to treat ulcers, asthma, and bronchitis. An oil extract can also be used for skin irritation and bruises (Radun 2007). St. John's wort has also be used as liquid extracts, powdered herbs and tinctures, and is applied orally or topically in historical Europe (De Smet et al. 1996). Many of these methods to using St. John's wort is still being used today like oral capsules.

Chemistry and Pharmacology

Many kinds of chemicals are naturally found in St. John's wort: they are tannins and proanthocyanidins (catechin, epicatechin, leucocyanidin) (6.5-15%), flavonoids (hyperoside, rutin, quercitrin, isoquercitrin, quercetin and kaempferol) (2-5%), biflavonoids, phloroglucinol derivatives (including 4% hyperiforin), phenolic acids, volatiles oil (up to 1%), higher alkanes, sterols, naphthadian-thrones (including

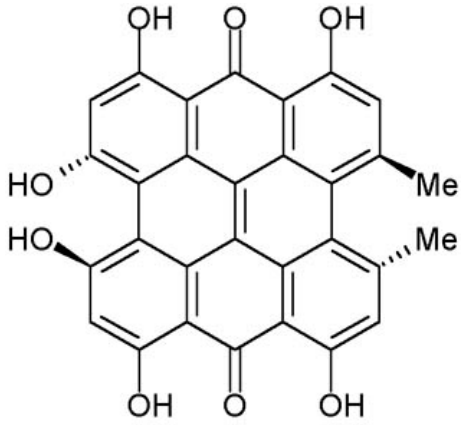

Figure 3. Structure of hypericin (Source: biopsychiatry.com).

hypericin and pseudo-hypericin), vitamin C and A and xanthoes (10ppm) (Radun 2007).

The most notable compounds are hyperforin (**Figure 2**), hypericins (**Figure 3**) and pseudohypericin (**Figure 4**). Capsules of St. John's wort have a standard dose around 300mg (0.3% hypericin) standardized around hypericin and pseudohypericin (Kopleman, Augsburger, NguyenPho, Zito and Muller 2001). Hyperforin is a compound that is naturally present in humans, and this is the compound responsible for inducing metabolism of St. John's wort. Among some popular brands that are sold in stores, the content of hyperforin ranged from 0.01-1.89%. Normally 1-6% of hyperforin is required in treating mild depression (De Los Reyes and Koda 2002). The content of hypericin and pseudohypericin in commonly sold drugs can be highly variable and in a study by De Los Reyes and Koda (2002), these compounds ranged from 0.03-1.29% in weight: this is approximately 56.6-130% of what all of the labels claimed to have in the amount of hypericin and pseudohypericin (0.15, 0.2 or 0.3%).

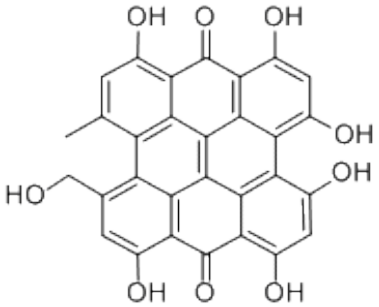

Figure 4. Structure of psuedohypericin (Source: chemicalbook.com).

Biological Activity

Flavonoids (rutin, hyperoside, isoquercitrin, quercitrin and quercetin) are thought to be responsible for the antidepressant activity of St. John's wort as well as some antioxidant activities (which are thought to increase the amount of extraction by preventing degradation of other chemicals). The napthodianthrones (hypericin and pseudohypericin) are used as standards in pharmacy, and was until recently also thought to contribute to antidepressant activity (Lewis and Elvin-Lewis 2003).

St. John's wort is an inhibitor of the reuptake of serotonin at the presynaptic axons and it also seem to alter neurotransmitters at the postsynaptic axons. Norepinephrine is also inhibited during reuptake but to a lesser extent. *In vitro* studies revealed that the ß-adrenoceptor is down-regulated in a dose-dependent manner in similar fashion to other anti-depressants, however, the activity could not be traced back to hypericin nor hyperforin and therefore, the identity of this active chemical is still currently unknown. Still in other studies like by Muller et al**.** (2001) it was found that St. John's wort extract was able to inhibit not only serotonin and norepinepherine, but also dopamine, GABA and L-glutamate. It is hypothesized that another mechanism using sodium ion channels is responsible for the mechanism. So far, hypericin is the only compound known to be a preclinical antidepressant with a completely new mechanism (Lewis and Elvin-Lewis 2003).

Clinical Studies

In clinical studies, extract of St. John's wort has proven to be significantly more effective than placebo in treating mild to moderate depression. The efficacy is not significantly different as that of tricyclic antidepressants, but it seems that St. John's wort causes fewer side effects than those other antidepressants. These conclusions are based on a meta-analysis of 23 randomized trials with over 1700 patients (Lewis and Elvin-Lewis 2003). Several clinical trials have been used in testing St. John's wort: one study compared the efficacy of 800mg of St. John's wort with 20mg of Prozac, and found that these had equal efficacy.

Other recent clinical studies and meta-analysis have found that the use of St. John's wort is twice as less likely in causing side effects as the tricyclic antidepressants. There were also less drop outs observed in using St. John's wort than tricyclic antidepressants in those trials: both of these are importnat factors for the patients since many treatments are abandoned due to onset of side effects causing patients to drop out (Kim, Streltzer and Goebert 1999).

Many of the clinical trials, however, have design problems with methods and sample sizes so a definitive conclusion that St. John's wort is effective cannot be drawn (Kim, Streltzer and Goebert 1999). For example, some of these studies used only subjective standards or older diagnostic criteria to measure the presence of depression (Kim, Streltzer and Goebert 1999).

Certain studies also lack certain information that are critical to the study like the patient's history of depression, family history of depression, and the duration of depression which all cause the studies to lack strong support and structured diagnosis (Kim, Streltzer and Goebert 1999).

Another questionable point in many of the clinical studies involving St. John's wort and depression is that many of these trials last only 4-6 weeks often with no follow up and so do not address any long term effect of St. John's wort. Currently there haven't been any study involved in using St. John's wort over the long-term efficacy, side effects, relapse…etc. for depression and other ailments.

Finally, many of these clinical trials and diagnosis were done by generalized primary care physicians instead of specialized psychiatrics. This raises the question of the accuracy in treating and diagnosing patients as primary physician have been known to mistreat and misdiagnose depression 50% of time (Kim, Streltzer and Goebert 1999), and so this further weakens the conclusions gotten by these clinical studies (Kim, Streltzer and Goebert 1999).

Contraindications

So far, St. John's wort has had no indication of any significant severe adverse effect that has been observed (Jobst, McIntyre, St. George and Whitelegg 2000). St. John's wort is currently used in clinical setting in Germany and has so far produced no record of any severe implications, overdose or toxicity (De Smet and Nolen 1996). St. John's wort is currently used in Germany for mild to moderate depression, anxiety, psychogenic disturbances and nervousness (De Smet and Nolen 1996). Some common mild side effects have been reported which include gastrointestinal problems, allergic reactions and fatigue, but all of these complaints combined constituted less than 2% of all clinical uses. On the other hand, St. John's wort is poisonous to livestock especially in the tropical regions or Southern regions of the US due to photosensitization of the chemicals (Lewis and Elvin-Lewis 2003).

Another point to consider is the amount of variation in the active ingredients found in different brands of St. John's wort that are sold. As mentioned before, hypericin and pseudohypericin are used to measure the standard dose, but research done by Liu *et al.* found that there were significant variations in other compounds within the different products that are sold (De Los Reyes and Koda 2002). Since hyperforin is considered to be the main ingredient in treating depression, and that it is not standardized (since it is difficult to standardize), different brands of St. John's wort may cause different degrees of effectiveness.

Research has not yet shown that the effect of St. John's wort is linked to hypericin and having variable amounts of other ingredients may cause deleterious effects, especially when combined with other drugs. It is still currently generally unknown that combination with any other drug may lead to dangerous effects and more research is needed.

Current Use in Allopathic & CAM Therapies

Currently, the only nation in the world that allows its doctors to prescribe St. John's wort as anti-depressant is Germany. In fact, in 1994, German doctors prescribed 66 million doses of medicine containing hypericum each day (De Smet and Nolen 1996). There are currently 20 times more St. John's wort prescriptions in Germany than Prozac that are used to treat depression (Kim, Streltzer and Goebert 1999). The German

healthcare system favors individualized and subjective standards for evaluating depression as opposed to a more objective system, and this may be another reason why they use St. John's wort in mainstream medicine (Kim, Streltzer and Goebert 1999).

Despite these promising data, the use of St. John's wort toward major depression, especially long-term trials, lack sufficient number of study and therefore, is not proven to be yet effective in treating them. Furthermore, relapse of depression and late side effects haven't been adequately studied (De Smet and Nolen 1996). In order for the extracts to be used for major depression, further research is needed and it must also meet the same regulations that govern other antidepressants. The extracts of St. John's wort also need 2 to 4 weeks to affect the mood.

There are currently many herbal supplements in the US and can often be seen sold in bottles over the counter as oral capsules. In the US, they are simply marked as capsules containing St. John's wort branded under certain names like Nature's Way, Sundown and Now Foods. The standard dose of hypericin and pseudohypericin in these capsules are around 300mg.

The use of St. John's wort is also growing in North America and it has also been used in aromatherapy and is currently under investigation for using it against HIV. The traditional uses of St. John's wort for curing wounds, bruises, ulcers and inflammation still remain (Radun 2007).

Discussion

St. John's wort is a commonly known herb that almost everyone in North America has heard of its existence. The popularity of this well known herb can be partly due to its widespread use across the world that dates back to ancient times. Although allopathic medicine continues to grow, the reality is that a large portion of the population in the world depends on traditional methods like medicinal plants to support their well being and cure diseases. This is especially true for rural areas around the world where there is no access to allopathic medicine. Plants like St. John's wort are also used to for food, flavoring, liquors, perfumes, cleaning products and essential oil.

Many clinical trials have obtained significant result for St. John's wort versus placebo, however, lack of an organized and standardized procedure and diagnosis for clinical trials confounds the promising results obtained: further studies are seriously needed in long-term trials that objectively assess the follow up of patients to definitely conclude that St. John's wort is effective in treating depression.

The use of St. John's wort is not recommended as a stand-alone treatment for depression and any use should be consulted by a doctor. The fact that St. John's wort produces fewer side effects than conventional anti-depressants opens a door for doctors to consider using it for patients that have side effects with conventional antidepressants (Kim, Streltzer and Goebert 1999). However, this can only be said for mild to moderate depression, and major depression and other severe psychiatric disorders require more attention.

It is interesting to note that St. John's wort is widely prescribed in Germany by doctors even though the effects of St. John's wort are not totally conclusive. As mentioned before, this is probably due to the more subjective treatments practices utilized.

One point that needs to be researched is whether hypericin and pseudohypericin have any effect against treating depression through an alternative mechanism yet to be

discovered. The difficulty in storing hyperforin (the main ingredient acting against depression) prevents it from being used as the standard in conventional drugs. There is also the possibility that some other compounds may be responsible.

The traditional use of St. John's wort around Europe for treating wounds and inflammation still exists today after thousands of years. Due to its multitude of uses, *Hypericum perforatum* will probably never cease to be used by people around the world, at least not in the foreseeable future.

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Ilex paraguariensis A. St.-Hil., Aquifoliaceae

Avery Berlin

Introduction

Ilex paraguariensis A. St.-Hil is a member of Aquifoliaceae or the holly family (Tropicos 2012). Classified by Auguste Francois César Prouvencal de Saint-Hilare, its common names include "té del Paraguay" in Paraguay, "erva mate" and "chimarrão" in Brazil, and "maté" in Uruguay and Argentina (**Figure 1**). These refer to its hot tea form. But when it is prepared as a cold beverage, it is called "tereré" (Cordoba et al. 2011). However, it is also universally known throughout most of the world as yerba maté. Yerba comes from the word 'herb' in the Quichua language, and maté is the name of the gourd from which it is traditionally consumed (Stern 2000).

Grown naturally as well as cultivated throughout South America, this plant has remained an integral part of the culture of Argentina, Brazil, Paraguay, and Uruguay since the time of the Guaraní Indians, who employed this tea as a form of social ritual (Joyce 1934). Now, it is universally consumed throughout South America as well as around the world within everything from candy to beer to soft drinks to creams and even energy drinks (Andrade et al. 2012). However, the most common method of consumption of *I. paraguariensis* is in the form of tea (**Figure 2**) also known as "maté cebado" (Conforti et al. 2012).

Recently, *I. paraguariensis* has become available in the form of supplements and pills. Mainly consumed as a tonic and a stimulant, it is used in the treatment of cardiovascular, hepatic, digestive, and inflammatory disorders. People commonly ingest yerba maté for its elevated caffeine content to combat fatigue and to promote weight loss. Important

Figure 1. ***Ilex paraguariensis*** **plant (leaves, flowers, and berries).**
Source: http://www.nhm.ac.uk/nature-online/species-of-the-day/biodiversity/economic-impact/ilex-paraguariensis/index.html

purine alkaloids, phenolic compounds, saponins, tannins, and flavonoids provide *I. paraguariensis* with anti-oxidant, anti-viral as well as anti-inflammatory effects, which in turn give

Figure 2. **Traditional yerba maté tea contained in a gourd with a 'bombilla'.** Source: http://guayaki.com/product/61/Pre-Columbian-Gourd-Gift-Pack.html

this plant much of its medicinal properties (Bastos et al. 2007).

Botanical Description

Native to South America, *Ilex paraguariensis* mainly occupies the subtropical region of the continent (Rakocevic et al. 2011). While this shrub tree grows in Bolivia, Colombia, and Ecuador, it flourishes specifically in northeastern Argentina, southern Brazil, eastern Paraguay, as well as throughout Uruguay (Service, U. N. R. C.; Gorzalczany et al. 2001). *I. paraguariensis* prefers the full, direct sunlight and extreme humid conditions of this subtropical region, enjoying temperatures averaging around 21 degrees Celsius (Giberti 1998). As a member of Aquifoliaceae, otherwise known as the holly family, yerba maté expresses "monopodial and rhythmic" proliferation, occurring in biannual growth spurts in the spring and autumn seasons (Giberti 1998; Rakocevic et al. 2011). It can even live up to one hundred years. The pollination that it undergoes is entomophilous or via insects such as bees and flies; birds then scatter the seeds within the fruit of *I. paraguariensis* (Gauer & Cavalli-Molina 1999).

The characteristics of this perennial, dioecious plant depend on how it is grown—whether it is grown naturally or cultivated. Naturally, wild yerba maté resides in the South American forests as an understory evergreen tree, preferring the sandy and clay soils present on the banks of local waterways. Here, this small to medium sized tree grows upwards of eighteen meters tall. It is, however, cultivated throughout South America as a part of their agroforestry programs, seeking to combine the benefits of both agriculture and forestry in order to produce a flourishing crop population (Giberti 1998).

In addition to agroforestry, yerba maté farmers cultivate this plant as the sole crop on plantations due to the increasing demand for the leaves of this plant (Rakocevic et al. 2011). Compared to the wild versions, cultivated *I. paraguariensis* remains smaller, about two to three meters total in height (Shua & Prada 2003). By comparing the picture of cultivated yerba maté in **Figure 3** to the wild version of the plant in **Figure 4**, the height differences remain quite noticeable.

There are also distinct leaf differences in addition to the asymmetry in stature between the wild and cultivated yerba maté. The leaves of *I. paraguariensis*, which are the part of the plant most commonly used, average twenty centimeters in size in the wild (Giberti 1998; Stern 2000; Shua & Prada 2003). The cultivated leaves are much smaller, only about five

Figure 3. ***Ilex paraguariensis*** **cultivated through monoculture on plantations.**
Source: http://www.nhm.ac.uk/nature-online/species-of-the-day/biodiversity/economic-impact/ilex-paraguariensis/biology/index.html

centimeters. (Shua & Prada 2003). The tea from cultivated leaves also tastes less bitter (Rakocevic et al. 2008).

Despite how they are grown, the leaves are eye-shaped with tooth-like edges. These shiny, deep green colored leaves have a leathery, fleshy quality. The leaves are attached via deep red petioles. **Figure 1** displays the specific leaf, fruit and flower structures. The tree's flat and somewhat smooth bark alternates in color between brown, gray, and green. The white colored flowers of *I. paraguariensis* give rise to spherical shaped fruits—magenta tinted berries—in the autumn months (Giberti 1998).

Due to *I. paraguariensis*' significant and ever present role in South American society as well as their economy, the plant population has suffered. No longer can the naturally grown tree stripped of its leaves produce enough supply for its

Figure 4. Wild ***Ilex paraguariensis*** **grown in the forests of South America.**
Source: http://www.nativayerbamate.com/12.jpg

increasing demand. This abuse directly influenced the *I. paraguariensis* population, causing a significant decrease in numbers. Even through cultivation, specifically the use of plantations to grow this crop, the population continues to deteriorate until a balance is created between supply and demand (Giberti 1998).

Traditional Uses

Since ancient times, *Ilex paraguariensis* has been richly steeped in South American culture. Recently, this plant's cultural role remains severely intertwined with its economical importance. However, for hundreds of years, *I. paraguariensis* has continued to occupy an integral part of South American society. Not only did the ancient South Americans recognize the important medicinal implications held by this plant, but they also utilized this tea within social settings. Dating back hundreds of years, the Guaraní Indians, dwelling in the regions of Brazil and Paraguay, consumed this plant known to them as 'caamini' or 'caaguazú' (Gorzalczany et al. 2001; Joyce 1934). Its tea was deemed a social and ritual drink, one that promoted "trust and communion" between people and facilitated meaningful conversations. It was also involved in relaxation rituals (Gorzalczany et al. 2001).

Legend has is that yerba maté was given to the Guaraní Indians by the Goddess of the Moon. Yasí, the moon goddess, and her sister Araí, the cloud goddess, left Heaven one night to travel to Earth. Unable to defend themselves against a jaguar attack, they were rescued by an elderly Guaraní Indian who shot the jaguar with a bow and arrow and saved the goddesses lives. Out of thanks, the Goddess of the Moon gave this Guaraní yerba maté, along with directions on its preparation. This drink was then shared with the rest of the Guaraní tribe (Shua & Prada 2003).

The traditional method of drinking the yerba maté tea can be seen in **Figure 2**. The leaves are gathered, dried over the fire, pulverized, and then steeped in water to produce a brewed tea. The gourd that the tea is commonly contained in is known as a maté, and a metal 'bombilla' strains the dried leaf particles as it is being consumed (Conforti et al. 2012). Amazed at the tea's caffeinated effects, the Spanish Jesuits who were missionaries to the Guaraní then proceeded to cultivate the plant for the first time on plantations. The Guaraní Indians also chewed the leaves to extract the caffeine (Butler 1900).

In addition, this tea was used historically for its medicinal properties, which have provided relief from a wide variety of ailments for hundreds of years. These include, but are not limited to, digestive disorders, inflammatory processes, weakness of the body and mind, cardiovascular problems, as well as joint issues (Andrade et al. 2012). The plant is mainly ingested via tonics. However, cataplasms, moist compresses with pulverized plant parts, were also placed on inflamed and ulcerated skin lesions in order to soothe as well as treat. The plant was first documented in South America for its remedial effects within *Examen de los Simples Medicinales* in 1617 (Joyce 1934). Due to these yerba maté botanical remedies, studies have resulted in order to not only isolate the active compounds, but also to potentially find treatments or cures for specific illnesses.

Chemistry and Pharmacology

The active constituents within *Ilex paraguariensis* are responsible for not only increased plant survival, but also the plant's medicinal properties. Amongst the plant's most prominent constituents are alkaloids, phenols, flavonoids, saponins, tannins, as well as a host of vitamins and minerals (Bastos et al. 2007). **Table 1** lists these major constituents within *I. paraguariensis*.

The three most active alkaloids, specifically purine alkaloids, within yerba maté are caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), and theophylline (1,3-dimethylxanthine). **Figure 5** shows the structural differences

Compound	Class of Compound
Caffeine	Purine alkaloid
Theobromine	Purine alkaloid
Theophylline	Purine alkaloid
Caffeic acid	Phenol
Ferulic acid	Phenol
Sinapic acid	Phenol
p-coumaric acid	Phenol
5-O-caffeoylquinic acid	Phenol
caffeoylshikimic acid	Phenol
dicaffeoylshikimic acid	Phenol
caffeoyl glucose	Phenol
Matesaponin	Saponin
Ursolic acid	Saponin
Oleanolic acid	Saponin
Quercetin	Flavonoid
Rutin	Flavonoid
A	Vitamin
B complex	Vitamin
C	Vitamin
E	Vitamin

Table 1: Major Constituents within *Ilex paraguariensis* A. St.-Hil.
Source: Bastos et al. 2007

between these three types of xanthines. While some scientists still debate the presence of theophylline within the plant, caffeine and theobromine are present in high doses, with caffeine being the highest. This provides the plant with a means of preventing predation as well as fighting off competing plants in its surrounding area. The differences in the caffeine concentrations among plants of *I. paraguariensis*

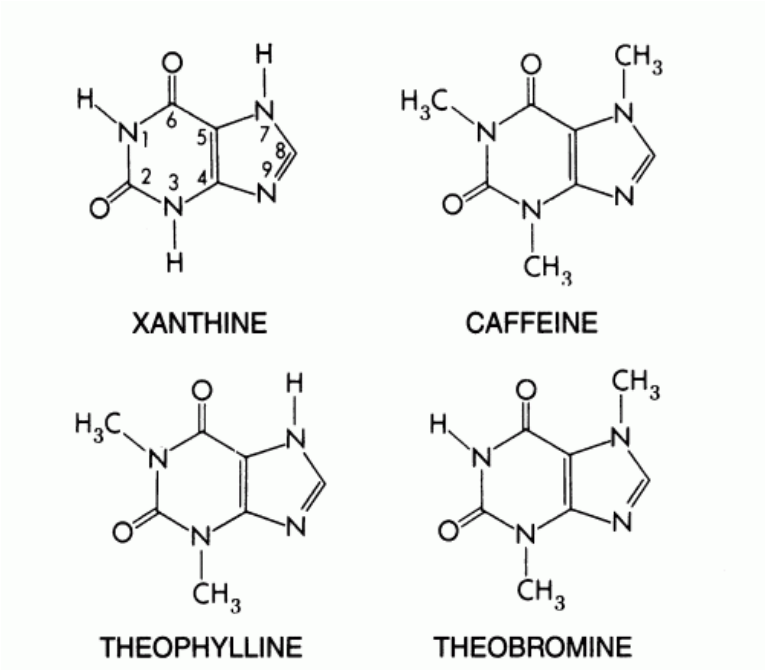

Figure 5. The chemical structures of the major alkaloid constituents within *Ilex paraguariensis*, which are caffeine, theophylline, and theobromine (the three most prevalent xanthines).
Source: http://www.benbest.com/health/xanthines.gif

may be due to the time of year as well as the location that the plant is growing. The winter and autumn months result in a decreased concentration of caffeine. "Light intensity and temperature, stress conditions, presence of predators, kind and frequency of trimming" may also influence the amounts present. Also, studies differ on whether or not the concentrations of these purine alkaloids are elevated post-drying of the leaves (Bastos et al. 2007).

In addition to alkaloids, phenolic compounds are present within the leaves of *I. paraguariensis*, making up roughly thirty percent of the plant constituents (Matsumoto et al. 2009). These mainly occur in the form of esters as chlorogenic acids or CGAs and include caffeic (3,4-dihydroxicinnamic), ferulic (3-methoxy, 4-hydroxy), sinapic (3,5-dimethoxy, 4-hydroxy) and p-coumaric (4-hydroxy) acids. Amongst these acids, the characteristic chlorogenic acid within yerba maté is 5-O-caffeoylquinic acid or 5-CQA. Other extracts of the dried leaves revealed caffeoylshikimic acid and dicaffeoylshikimic acid. Caffeoyl glucose remains another present polyphenol within the plant. The anti-oxidant properties owe themselves mainly to the phenolic compounds present in yerba maté. However, there is debate over the methodology responsible for the anti-oxidant properties. This oxidation prevention occurs either due to the donation of excess electrons to "free radical scavengers" or the "chelating [action] of transition metal ions" (Bastos et al. 2007).

Similarly, saponins are prevalent throughout the *I. paraguariensis* plant. The majority of these glycosides are triterpenoid, but there are "monodesmosidic and bidesmosidic saponins found in the aerial parts of the plants" (Bastos et al. 2007). In 1989, research of yerba maté yielded the "isolation and elucidation of a new saponin" known as matesaponin, a "three sugar residue bidesmoside" (Bastos et al. 2007). Since then, research observed matesaponin 1 through 5 within the plant. Ursolic acid and its isomer oleanolic acid are minor saponins occupying the leaves of the plant. In all, 29 different saponins have been identified within *I. paraguariensis* (Bastos et al. 2007).

The rest of the important constituents are flavonoids and tannins, as well as some vitamins. Quercetin and rutin are two common flavonoids present in yerba maté. The tannin content is partly responsible for the bitter nature of the tea. Vitamins A, B complex, C, and E are present; however, their amounts are highly influenced by how the tea is produced as well as where it is grown (Bracesco et al. 2011).

Biological Activity

Over the past few decades, many researchers have studied the effects and mechanisms of action of *Ilex paraguariensis* in hopes of gaining new and more in depth medical insight. Because this plant has been used for years in medical treatments, researchers are determined to uncover the medicinal basis of yerba maté. In order to do so, biological assays are performed *in vitro* as well as *in vivo* in reference to a variety of different health issues and diseases.

Herpes

Due to the rapid increase in drug resistance, researchers are desperately searching for new modes of action in the treatment of infections due to the herpes simplex virus. Because certain constituents of the plant have anti-viral effects, yerba maté was examined for its potential anti-herpes properties. Extracts from *I. paraguariensis* were tested against HSV-1, KOS strain, and HSV-2. Through the laboratory study, all plant samples used were positive for anti-herpes effects. Furthermore, it showed "reduced HSV-1 and HSV-2 infectivity to concentrations ranging from one to three times below their original infectivity concentration value." This information may propose microbicidal properties, which may "inactivate the herpes virus and interfere with virion envelope structures or mask viral structures necessary for absorption or entry into host cells." The studied fraction decreased the spread of the virus into adjacent cells and the number of virions transferred to unaffected cells. The saponins found in yerba maté were

specifically believed to be responsible for its anti-herpes effects (Lückemeyer et al. 2011).

Weight Loss

I. paraguariensis has long been associated with weight reduction. Therefore, studies were performed to assess the accuracy behind this association. One particular *in vivo* study sought to examine the effects of yerba maté on mice obesity. These mice were fed high fat diets and then were randomly assigned either water or yerba maté extracts. The results proved that the extracts in fact led to a considerable reduction in body mass of those mice, which consumed the extract. The evaluation of the mice showed a minimization in "serum levels of cholesterol, triglycerides, LDL cholesterol, and glucose." In addition, the expression of many genes and proteins that were increased due to the high fat diet experienced a reduction. Chlorogenic acid, a phenolic compound, is believed to be associated with the metabolism of glucose via the enzymatic activity of glucose-6-phosphatase. It also plays a role in the prevention of cardiovascular disease by the anti-oxidation of cholesterol. The purine alkaloids and saponins potentially work with the phenolic compounds to produce these effects visible in the mice (Arcari et al. 2009).

Bile Flow

Because *I. paraguariensis* was historically utilized to treat digestive and hepatic disorders, this *in vivo* study examines the effects of yerba maté on bile flow and peristalsis of the digestive tract. While under anesthesia, the rats underwent surgery to measure their bile output in order to calculate bile flow. They then received either saline or *I. paraguariensis* extract intravenously or intraduodenally. To measure the intestinal propulsion via peristalsis, mice were fed charcoal and killed approximately twenty minutes later. The distance that the charcoal traveled down the small intestine was measured. A considerable increase of bile flow resulted from the 250 milligram per kilogram dose. *I. paraguariensis* failed to show any effects on peristalsis. However, it does increase the output of bile from the liver, thereby restating the hepatoprotective effects (Gorzalczany et al. 2001).

Clinical Studies

Bone Mineral Density

Ilex paraguariensis has been involved in a multitude of clinical studies to further test the notions of health benefits associated with this plant. One such study sought to investigate bone mineral density associated with osteoporosis in postmenopausal women. This study examined 146 women who drank an average of one liter per day of yerba maté tea and 146 women who never ingested the tea as the control group. Bone mineral density or BMD was measured for each woman's lumbar spinal region and hip joint via a dual-energy X-ray absorptiometry. Women who regularly consumed yerba maté tea showed considerably higher bone mineral density in both of the regions. This amounted to an increase of 9.7% for the lumbar spine and 6.2% for the femoral neck of the hip joint. Therefore, *I. paraguariensis* shows protective activity against osteoporosis in postmenopausal women (Conforti et al. 2012).

Diabetes

Another clinical study set to investigate the correlation between *I. paraguariensis* and diabetes, specifically patients who are type II diabetic and pre-diabetic. Because research

has proved that this plant decreases blood sugar in some animals as well as decreases cholesterol levels in humans, this study wanted to test the notion that yerba maté may be of benefit to these people. There were three treatment groups—yerba maté tea, dietary counseling, and yerba maté tea in conjunction with dietary counseling. 29 individuals with diabetes mellitus and 29 pre-diabetic individuals were utilized in this study. The yerba maté tea reduced "levels of fasting glucose, glycated hemoglobin, and low density lipoprotein cholesterol." The clinical study found that yerba maté regulated blood sugar levels and decreased cholesterol in these diabetic and pre-diabetic individuals. This may point to yerba maté containing preventative means against coronary artery disease (Klein et al. 2011).

UADT Cancers

Because the incidence of upper aerodigestive tract cancers is significantly greater in South America, a clinical study was performed to test the hypothesis that the increased consumption of yerba maté may influence these rates. Upper aerodigestive tract cancers include cancers of the oral cavity, oropharynx, hypopharynx, larynx, and esophagus. 1,168 individuals as well as 1,026 control individuals were involved in this clinical study. This study was indicative of a positive correlation between esophageal cancer and excessive consumption of yerba maté tea. If an individual drank the tea consistently for roughly twenty years, he/she was 3.5 times more likely to acquire this type of cancer than someone who had never drank the tea. For forty years of consumption, the likelihood increases to five times. There has been controversy regarding previous studies, which believe that this association is due to the temperature of the tea. A high temperature could induce thermal damage to the head and neck regions. However, this particular study found that the increased risk was greater for those who drank the tea either cold or warm than those who drank it hot or very hot. This proposes that yerba maté may in fact have carcinogenic effects especially in the UADT (Szymańska et al. 2010).

Contraindications

Ilex paraguariensis ingested in any form is considered GRAS or generally recognized as safe. However, despite the fact that it is described as nontoxic whether in the form of tea, pills, supplements, etc., there remain contraindications as well as positive associations with certain diseases and cancers. Due to these dangerous interactions, *I. paraguariensis* should potentially be avoided by certain people whose health issues may be exacerbated by the active constituents within the plant.

An *in vivo* study with rats and rabbits tested toxicity levels immediately following consumption as well as after continued intake for up to three months. Not only did the study show no forms of toxicity for a single dosage, it was also found to be safe for chronic consumption. Other studies have confirmed that there is no toxicity associated with the liver, the kidneys, or the bladder. *I. paraguariensis* even shows a potential protection again DNA strand damage in an *ex vivo* study. The constituents prevented the DNA strands from damage due to peroxide content, and they enhanced the mending of DNA within liver cells (Andrade et al. 2012).

Because *I. paraguariensis* contains caffeine and other xanthines, people that consume any form of this plant should avoid it if they have allergies to either compound. In addition, women who are either pregnant or breastfeeding should refrain from ingesting yerba maté. The caffeine within the

plant reduces fatigue and increases alertness; therefore, it may cause problems with sleep if ingested around bedtime. It also should be avoided when taken in conjunction with sleeping medications due to drug interference. Yerba maté also has diuretic effects and should not be consumed if diuretic effects are to be avoided especially with urinary tract obstruction. People suffering from cardiovascular diseases such as hypertension and arrhythmia should avoid yerba maté. Those who show signs of hyperthyroidism and gastric and duodenal ulcers should refrain from consumption. In addition, use with monoamine oxidase inhibitor drugs or MAO-inhibitors should not occur. These drugs are used in the treatment of depression. *I. paraguariensis* decreases the sedative and anti-anxiety processes within these drugs and therefore, exacerbate the side effects associated with sympathomimetic drugs (Products, C. o. H. M. 2010). Unfortunately, there has been a positive correlation with head and neck cancers, especially oropharyngeal and esophageal cancers (Szymańska et al. 2010). However, due to the anti-oxidant effects of yerba maté, some of the constituents have anti-carcinogenic effects. While people with specific health conditions should avoid *I. paraguariensis*, the plant remains GRAS and is established as nontoxic.

Current Use in Allopathic and CAM Therapies

Today, *Ilex paraguariensis* still remains a leading medicinal plant. Based on the knowledge that native South American Indians possessed of its curative properties, yerba maté continues to be used to treat a multitude of ailments. Use of this plant within traditional medical practices propagated its use in the current world of complementary and alternative medical therapies. This has instigated the creation, production, and use of *I. paraguariensis* in many forms, including capsules, tablets, and pills. The majority of these medicinal remedies remain in the form of supplements. Mainly, yerba maté is utilized as a stimulant to increase alertness and decrease fatigue (Gorzalczany et al. 2001). These supplements on many occasions are used in conjunction with guarana, kola nut, and green tea to enhance the effects (Bastos et al. 2007). 29 different dietary supplements of yerba maté from the years 1963 to 2006 gained international patents with a significant influx in supplements after the turn of the century. These included but were not limited to supplements to facilitate weight loss, promote sexual arousal, enhance hair thickness, and decrease levels of cholesterol. Other uses for yerba maté aim to treat the underlying issues of "arthritis, headache, constipation, rheumatism, hemorrhoids, obesity, fatigue, fluid retention, hypertension, slow digestion, and hepatic disorders" (Gorzalczany et al. 2001).

Albeit no prescription or over the counter drugs are either on the market or have been approved by the FDA, many studies are attempting to gather more information regarding *I. paraguariensis* in the hopes of isolating the mechanisms of action for its active constituents. While studies have produced positive results concerning the treatment for hypercholesterolemia as well as osteoporosis, more research needs to be done in order for a FDA-approved drug. Until more information is known, yerba maté will continue to be a commonly used supplement and CAM therapy.

Discussion

Hundreds of years ago, the Guaraní Indians of South America recognized the importance as well as the medicinal potential of *Ilex paraguariensis*. Today, researchers are beginning to recognize this same thing. For the Guaraní, this highly

regarded tea remained an integral part of their culture, from social rituals to traditional medicinal practices. Over the years, it has been associated with the treatment of a variety of different ailments. This yerba maté decoction treats everything from weakness of the body and mind, digestive disorders, inflammatory processes, cardiovascular problems, as well as joint issues.

Recently, researchers have gone further to investigate these treatments, looking at the constituents of *I. paraguariensis* as well as their mechanisms of action. These constituents include purine alkaloids, phenolic compounds, saponins, tannins, and flavonoids, providing anti-inflammatory, anti-viral, and anti-oxidant effects. Within *in vitro* and *in vivo* studies, *I. paraguaniensis* showed anti-herpes activity, weight loss as well as a reduction in LDL cholesterol and an increase in bile flow. In regards to clinical studies, while it increased BMD and lowered LDL in diabetic patients, it did show a carcinogenic effect. Knowing which problems yerba maté has been known to treat through traditional medicine, researchers may be able to learn more about *I. paraguariensis* and its potential for the future. However, to be able to utilize this plant for generations to come, we must protect it from environmental abuse and overharvesting.

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Lavandula angustifolia Mill., Lamiaceae

Cimma Sefat

Introduction

Lavandula angustifolia, also known as true lavender, is a member of the Lamiaceae family (**Figure 1**). It is commonly known as English lavender or xun yi cao in Chinese ("Lavandula angustifolia Mill. ," 2012). *L. angustifolia* is the most common species of lavender, and other species include *L. burnamii, L. dentate, L. dhofarensis, L. latifolia, and L. stoechas* (Basch et al., 2004). This plant is native to the western Mediterranean region, and its hybrid lavandin is widely cultivated in Australia, Europe, and the United States. *L. angustifolia* has been known for its cosmetic and medicinal uses throughout history (Basch et al., 2004). Advertised as a calming agent, *L. angustifolia* is said to ease restless minds. Its aromatic essential oils are highly prized and used for aromatherapy, candles, cooking, laundry detergent, massage oils, perfume, and soap (Basch et al., 2004). Being a member of the Lamiaceae or mint family, lavender leaves also serve a culinary purpose and are commonly used in salads, stews, tea, honey, jam, and jelly (Craig, 2006).

Figure 1. Lavender field in Provence, France. (Source: http://gourmetcyclingtravel.com/wp-content/uploads/Lavender-Field-800X2752.jpg).

Botanical Description

Lavender is an evergreen subshrub plan that grows approximately two to three feet tall and produces small, slender blue-violet flower spikes on the stalks and narrow silver green foliage. This detail can be noted in **Figure 2**. The *angustifolia* species is a stockier plant with fuller flowers unlike other the lavender species ("Lavender," 2007). Native to low mountains and altitudes of 1,970-3,940 feet, *L. angustifolia* originated in the Mediterranean basin and is cultivated in Albania, Argentina, Australia, Bulgaria, China, France, Hungary, Italy, Moldova, Montenegro, the Netherlands, Russia, Serbia, Spain, the United Kingdom, and the United States (Craig, 2006). *L. angustifolia* is capable of surviving in temperatures as low as -15°C (Bourne, 2004). This plant prefers full sun and can also survive in a wide range of soils but these soils must be well drained since the plant cannot tolerate sitting in very wet soils (Fowler, 2004). *L. angustifolia* flourishes on hard pruning, and if pruned every year, it can survive for approximately 20 years (Bourne, 2004). Lavender is a popular and reliable drought-tolerant perennial for gardens and hedges, requires little fertilizer, and is bothered by few pests (Anonymous, 2011).

The hybrid lavandin is much more widely cultivated and exceeds *L. angustifolia* in essential oil production; however, the finest oil used in cosmetics, perfume, and aromatherapy

Figure 2. Close up of *L. angustifolia* (Source: http://scienceray.com/biology/lavender-a-fragrant-plant-of-the-family-lamiaceae/).

come from *L. angustifolia* (Bourne, 2004). Since lavender has been cultivated for so long, most lavender found in gardens are hybrids and identification can be difficult (Craig, 2006). Dried lavender flowers contain less than 13 ml/kg of essential oil that is obtained by steam distillation from the flowering parts (Basch et al., 2004).

Traditional Uses

Lavender is derived from the Latin word *lavare*, which means to wash. The Grecians, Persians, and Romans used lavender flowers as a perfume in the baths and for laundry (Craig, 2006). As a part of mummification, ancient Egyptians soaked wrapping linen in lavender oil containing asphalt as a method of casting. The bodies would be wrapped in these linens and dried in the sun until the casts hardened (Craig, 2006). Lavender is known as a "healing agent" in India and Tibet, and can also be used medicinally to treat insanity and psychoses in Tibetan Buddhism (Basch et al., 2004). It has been reported that in fourteenth century France, Charles VI's court used cushions stuffed with lavender which provided not only a pleasant aroma but also helped repel insects (Craig, 2006). During the twelfth century in Grasse, located in southeast France, lavender oil was the main component of commercial perfumes. Many thought the scent of lavender surpassed all other scents due to its delicate and unique aroma (Craig, 2006). In Iranian folk medicine, lavender leaves are used to treat various inflammatory diseases (Hajhashemi, Ghannadi, & Sharif, 2003). Infusions of the plant have been used as a carminative, diuretic, anti-epileptic, anti-rheumatic, and pain reliever especially for headache and migraine pain. Associated with cleanliness, lavender maintains great popularity as fresh or dried flowers, potpourri, gifts, crafts, and more (Anonymous, 2011).

Chemistry and Pharmacology

Lavender is known for its rich content of volatile oils, and for centuries it has been used for both fragrance and medicine. Aromatherapy is thought to be effective due to the effect of odor and the physiological effect of inhaling volatile compounds like the oil of *L. angustifolia*. Lavender is composed of several hundred constituents which include flavonoids, cineole, coumarins, triterpenes, tannins, limonene, camphor, linalyl acetate (**Figure 3**), perillyl alcohol, and linalool (**Figure 4**) (Basch et al., 2004). The highest concentration of naturally occurring compounds in lavender oil include linalool, linalyl acetate, 1,8-cineole, beta-ocimene,

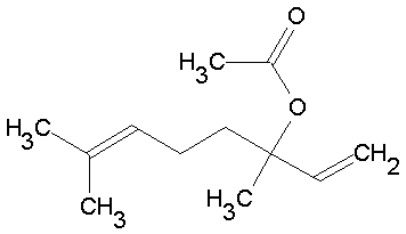

Figure 3. Linalyl acetate (Source: http://www.rdchemicals.com/chemicals.php?mode=details&mol_id=8000).

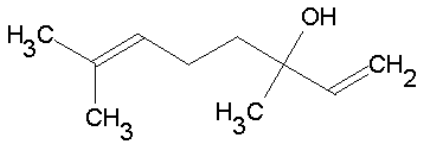

Figure 4. Linalool (Source: http://www.rdchemicals.com/chemicals.php?mode=details&mol_id=7998).

terpinen-4-ol, and camphor (Oliff, 2003). Linalool and linalyl acetate are rapidly drawn into the skin when massaged and believed to cause central nervous system depression. Linalyl acetate has narcotic actions while linalool acts as a sedative (Basch et al., 2004). Due to the calming actions of these constituents, they may be the origin of the use of lavender pillows to induce sleep (Oliff, 2003). Lavender leaves yield a pale-yellowish essential oil with a fresh and pleasant odor (Hajhashemi et al., 2003). Twenty-one components were characterized which represent 99.1% of the total oil components detected, listed in **Table 1**.

Table 1. Gas chromatographic composition of the essential oil (%; uncorrected values)

Constituents	*Lavandula angustifolia*
α-Pinene	0.14
Camphene	0.10
β-Pinene	0.11
Myrcene	0.64
Limonene	0.20
1,8-Cineol	1.06
γ-Terpinene	traces
p-Cimene	9.05
Camphor	1.19
Linalool	27.34
Linalyl acetate	32.10
Terpinen-4-ol	3.63
Lavandulyl acetate	1.05
Bornyl acetate	traces
β-Caryophyllene	2.68
Lavandulool	0.86
Borneol	0.30
α-Terpineol	0.29
Neryl acetate	0.18
Geranyl acetate	0.40
Geraniol	0.38

Table 1. List of essential oils in *L. angustifolia* (Source: Hajhashemi et al., 2003).

Biological Activity

In Vitro

Oil of *L. angustifolia* is active against several bacterial and fungal species, and it has been suggested that lavender oil may be useful in treating bacterial infections resistant of antibiotics (Lodhia, Bhatt, & Thaker, 2009). In a study by Hui et al., *L. angustifolia* oil was studied for antimicrobial activity for

	8.5 mg/ml	17 mg/ml	34 g/ml	51 mg/ml
Staphylococcus aureus	+	+	+	-
Micrococcus ascoformans	+	+	+	-
Proteus vulgaris	+	+	+	-
Escherichia coli	+	+	+	-

Table 2. Anti-bacterial activities of lavender essential oil (Source: Lu Hui, 2010).

potential treatment for rhinitis patients. It was found to be active against all tested strains of bacteria seen in **Table 2**. The oil's activity is dependent on concentration. At 51 mg/ml, none of the tested bacteria survived. The study believes that the *L. angustifolia*'s essential oil is capable of disrupting the cell membrane, and the cell looses chemiosmotic control (Lu Hui, 2010). Antimicrobial activity of lavender oil may not be related to the main constituents in the oil and there is little known about the possible relationship. Many of the lavender antimicrobial studies do not have standardization so directly comparing studies is impossible (Oliff, 2003).

Another study published in 2007 studies essential oils in plants like *L. angustifolia* as an acaricide, or a substance poisonous to mites or ticks. There is a long tradition that aromatic herbs such as lavender are used to repel insects. In folklore, essential oils are held in high regard because of their use as an insecticide and repellent. It is known that different essential oil components render different species of insect susceptible. The study performed by Perruci et. al has shown that when the mites *Psoroptes cuniculi* (mange mite of the rabbit) is exposed to linalool, the parasite looses mobility (5). It is suggested that inhalation and direct contact to linalool causes lose of mobility. The experiment placed the mites (10-15/sample) on a 6 cm petri dish covered with a filter. Then that petri dish is placed in a 9 cm petri dish containing the *L. angustifolia* oil. The experiement was done at room temperature (22 °C). The mites were exposed to a series of doses. To verify that linalool was the only effective constituent responsible for acaricidal activity, an artificial mixture of 27% linalool (the percentage found in the essential oil) and 73% saline (1mL/plate) with 2% vaseline oil (1mL/plate) was tested the same way. The controls used were plates containing just the physiological saline and plates containing solely vaseline oil. Motionless mites were observed with a stereoscopic microscope after a 24-hour period, and then transferred to a fresh petri dish and observed again for another 24 hours. The mites were stimulated with a needle, and any lack of reaction or continued immobility indicated death. However, further studies on the toxicity and mode of action and in vivo activity are necessary to confirm whether lavender essential oil and linalool can be used as an alternative acaricide.

In Vivo

There also was a study on anti-inflammatory and analgesic properties of leaf extracts and essential oil of *L. angustifolia* published in 2003. The study evalulates the analgesic and anti-inflammatory activities of the hydroalcoholic extract, polyphenolic fraction and essential oil of plant leaves in mice and rats. The leaves of *L. angustifolia* were collected near Isfahan, Iran. The leaves were then air dried and powdered, and the essential oil was isolated by hydro distillation. The hydroalcoholic extract was made by taking the powdered *L. angustifolia* leaves (100 g) and macerating with EtOH-H_2O (500 g) for 48 hours. The extract was shaken, filtered, and evaporated until dry. The polyphenolic fraction of the plant (100 g) was made in 2 steps. The fraction was extracted with EtOH:H_2O (9:1) then again with a 1:1 ration of the same solution. The mixture was left for 12 hours. Then the two

extracts were combined and evaporated to 1/3 its original volume. The remaining aqueous solution was extracted with chloroform and evaporated until dry, which left semi-solid masses of both the hydroalcoholic extract and polyphenolic fraction (yield 42.0% and 12.2%, respectively). Analgesic activity was tested with two tests: a formalin test and an acetic acid-induced writhing test. For the first test, mice were injected with 20 µl of 2.5% formalin (in 0.9% saline) in their right hind paw. The hydroalcoholic extract, polyphenolic fraction and essential oil were given orally prior to the formalin injection.

For the acetic acid-induced writhing test, 6 mice received different oral doses of hydroalcoholic extract, polyphenolic fraction, and essential oil one hour prior to the intraperitoneal injection of 1% acetic acid (volume 10 ml/kg). Anti-inflammatory activity was evaluated by carrageenan-induced paw edema test in the rat. The rats were anaesthetized with either and injected with 0.1 ml of 1% suspension of carrageenan in isotonic saline. The left hind paw was injected with 0.1 ml saline as a control. The extract and essential oil mixture was diluted and administered 1 hour before the carrageenan injection.

While the hydroalcoholic extract failed to produce any significant analgesic effects in the first phase, when given in does of 400, 800, and 1600 mg/kg, paw licking time was reduced 69, 72, and 93%, respectively. The polyphenolic fraction and essential oil suppressed paw licking in both phases of formalin test. For the acetic-induced writhing test, hydroalcoholic extract did not apply a significant decline of abdominal twitches, while the polyphenolic fractions (doses of 400 and 800 mg/kg) and essential oil (100 and 200 mg/kg) significantly reduced writhing. The results were not dose-dependent since the increased dose did not alter the response. For the carrageenan test, the neither the hydroalcoholic nor the polyphenolic fraction in high doses (such as 4000 mg/kg) did not exhibit a considerable anti-inflammatory effect. At a dose of 200 mg/kg, the essential oil showed a 48% inhibition of carrageenan-induce paw edema (S. Perrucci, 1996).

A study by the Cline group investigates the anxiolytic effects of the compound linalool and the $GABA_A$ receptors in rats. Forty-four rats were given a 1 mL total volume intraperitoneal injection of positive control, midazalom, linalool, or a linalool and flumazenil mixture. Then, they were put on an elevated maze and had their corticosterone levels measured. The data suggests that linalool does not produce anxiolytic effects by regulating $GABA_A$ receptors but it may modulate motor movements in the rats. The rats were placed on an open arm of the elevated plus maze (EPM) for a 5-minute evaluation of behavioral response to anxiety. The EPM was networked with MotorMonitor software to track the number of times the rats would enter either type of arm (open or closed), time spent in the arms, and basic motor movement. After the 5-minute test on the EPM, the rats were humanely killed by guillotine and their blood was collected for corticosterone and catecholamine (epinephrine and noepinephrine) analysis. There was no significant analysis of the ratio of open arm time versus total time in the EPM between the linalool and the flumazenil plus linalool group. There was significant difference in the open arm, a measurement of behavioral changes with anxiety, between the midazolam and the control groups. As for fine motor movements, analysis showed a significant decrease in movement in both the midazolam group and flumazenil plus linalool group compared to the control. The behavioral measurements comparing time spend in different arms on the EPM does not suggest that linalool produces anxiolysis. There was no difference between the mixture group therefore suggesting that linalool does not regulate the benzodiazepine receptor site. The results do not

support that linalool modulates the $GABA_A$ receptor. The neurohormonal data did not demonstrate a significant difference amongst the groups. Motor movement exhibited that midazolam and the flumazenil and linalool combination significantly decreased both basic and fine motor movements. The study is unsure how to interpret these results but it is speculated that another neurotransmitter site is being modulated. It is suggested that linalool modulates receptors at the neuromuscular junction thus decreasing acetylcholine release and limiting motor activity. Although the data does not support anxiolytic effects of linalool in rats, there is evidence to suggest linalool regulates the central nervous system by producing unconsciousness and deterioration of motor movements (Cline, Taylor, Flores, & Bracken, 2006).

Clinical Studies

The chemical composition and pharmacological study and evaluation of *L. angustifolia* have been the subject of several studies for years. Most of these studies focus on extracts and essential oils of lavender. In pharmacological studies, the extracts and essential oils are believed to have CNS-depressant, anti-convulsive, sedative, spasmolytic, local anesthetic, antioxidant, anti-bacterial, and mast cell degrandulation effects (Hajhashemi et al., 2003).

A cross-over randomized study conducted at nursing homes in Hong Kong studied pharmacological intervention of *L. angustifolia* for agitated behaviors among people with dementia. Seventy participants diagnosed with dementia with significant agitation were recruited, and participants taking psychotropic medication to control their aggression (51.4%) continued to take their medication. Participants were assigned to group A or B but both groups received both treatments. Either treatment A or treatment B was administered for 3 weeks with a wash out period (2 weeks) in between treatments. Then, the other treatment was given. Treatment A was *L. angustifolia* inhalation, and treatment B was sunflower inhalation (placebo). Aroma diffusers were placed at each side of the participants' bed during sleep at night for 1 hour. No adverse effects were reported from either staff or patients during the study period. Caregivers noted a significant decrease in agitated behavior in patients recieving Treatment A. Agitation scores decreased from 6.49 to 5.63. Irritability decreased from 4.81 to 4.53. The aromatherapy treatment was well tolerated and resulted in a significant decrease in agitation of patients with dementia (Wan-ki Lin, Wai-chi, Fung-leung Ng, & Chiu-wa Lam, 2007).

Support of lavender as an anxiolytic is still weak but some evidence does suggest an effect in the plant's ability to relieve anxiety (Basch et al., 2004). In one study, nurses used aromatherapy on 20 hospitalized children with HIV to relieve them of physical pain. They used a variety of essential oils known for their "analgesic and nervine" properties including *L. angustifolia* oil. Nurses noted that the children responded well to the essential oils, which helped reduce the need for analgesic drugs. Chronic chest pain, intermittent muscle spasm, and peripheral neuropathy were alleviated almost completely. This is a descriptive study and no statistical data was analyzed (Buckle, 1999, p. 44).

Another study suggests that there is some scientific evidence regarding the use of lavender oil baths for relieving postpartum perineal pain. The effect of lavender oil baths was examined on 635 postpartum women in a randomized trial. The women were divided into three groups. One group added a natural lavender oil extract, the second group added synthetic lavender oil, and the third group added a GRAS substance. Neither the midwives nor the mothers know what kind of oil was administered. Daily baths were routine for the

mother's care after delivery, and each day the midwife added six drops of the oil from which the mother's name was placed on the bottle. Thirty minutes after the bath, the mothers were asked to record the degree of discomfort experienced, and the midwives assessed the mother's perineum as part of the normal daily examination of the mother. Daily visits helped reinforce the procedure for the mother, and assessment continued until the 10th day. Mood scores were analyzed over a 10-day period. The mean discomfort scores showed a steady reduction. There was missing data from this study possibly due to the fact that those whose pain improved were more likely to stop recording the amount of discomfort experienced. The women in the pure lavender oil group showed the lowest mean discomfort scores after the first 5 days of childbirth. No side effects by the mothers or midwives were reported during the trial (Dale & Cornwell, 1994).

Contraindications

There have been few reports of sensitization after topical use of lavender. The lavender species is considered "likely safe" or GRAS (generally recognized as safe) for internal and external use. Generally, lavender is well tolerated with mild side effects. People with known allergy or hypersensitivity to lavender should exercise caution when using this plant. People with lavender allergy may experience mild local skin reactions after topical use of lavender oil. Lavender is believed to the potential of heightening effects of barbiturates and other depressants to the central nervous system. It is said to exhibit narcotic like effects on both humans and animals. In theory, lavender may intensify the effects of other sedative or hypnotic agents. Since lavender contains varying amounts of coumarins, it theoretically can increase the effect of anticoagulant medications. There is no reported lethal dose of *L. angustiofolia* but nausea, vomiting, and anorexia were reported after consuming large doses of lavender (>0.5 g/day). Lavender has still not been proven safe for consumption for pregnant women or children, and more research is necessary to understand the medicinal action of lavender (Basch et al., 2004). Those should avoid the plant if they allergies to members of the Lamiaceae family. People with sensitivity to plants with volatile oils or sensitivity to strong smells are advised to avoid contact with *L. angustifolia*.

Current Use in Allopathic and CAM Therapies

Throughout the United States and Europe, lavender oil has been used in aromatherapy, balneotherapy (treatment of disease by bath), and massage therapy. In Germany, lavender tea is used to promote sleep and ease stomach illness and nervous conditions. Lavender is used as an anti-depressant in Ayurvedic medicine in India while Tibet includes the plant in psychiatric treatment. Also, lavender oil is used externally to medicate burns, sunburn, wounds, insect bites, and muscle pain (Craig, 2006).

Aromatherapy is acknowledged as complementary therapy for "increased comfort, relief of pain, relaxation, improved coping, reduction or moderation of stress, and an increased sense of well-being" (Buckle, 1998, p. 56). Because many essential oils like lavender have calming and soothing properties aromatherapy has gained popularity in the general population and specifically among nurses who want to incorporate a holistic approach to their care (Buckle, 1998, p. 54). This kind of aromatherapy is known as clinical aromatherapy. Unlike recreational aromatherapy, clinical aromatherapy heavily relies on understanding the different species of the plant, not just their common name. For example, *L. angustifolia* is a relaxant, *L. latifolia* is a stimulant, and *L. stoechas* may exhibit

neurtoxic properties but is effective in treating pseudomonads or gram-negative bacteria (Buckle, 1998, p. 59).

In nursing, aromatherapy often times is used with topical applications such as compresses, therapeutic baths, or gentle massage. For each method, 1 to 5 drops of essential oil is diluted; essential oils are highly concentrated and should not be directly applied to skin if undiluted. The essential oil can be diluted in cold-pressed vegetable oil, cream, gel, or water. For baths, the essential oils should be diluted in a small amount of milk before being added to the tub since these oils are insoluble in water and will merely float on the surface of the water and provide uneven treatment (Buckle, 1998, p. 57). One example of clinical aromatherapy that utilizes lavender is to mimic the effect of diazepam (valium). Diazepam reduces the effect of external emotional stimuli by increasing inhibitory neurons in the amygdala, which governs emotional response in the brain. *L. angustifolia* is thought to have a similar effect and produce a sedative effect on patients (Buckle, 1998, p. 56).

Discussion

There is a lack of consistent data and reporting on the true effects of lavender and its therapeutic importance (Oliff, 2003). Although research is still needed, the potential contribution lavender has on human health can be noteworthy. Its use in clinical aromatherapy offers a more sensitive, holistic care especially for critical care patients. Also, there can be future research opportunities to explore the efficacy of linalool as a complement with anesthesia or the interactions with anesthesia medication. Many of the studies, especially concerning the research done by Buckle, show that *L. angustifolia* oil can help with pain management. According to Buckle, the United States spends over $70 million on chronic pain therefore utilizing *L. angustifolia* oil can be a holistic and cost-effective approach to pain management. Lavender is an extremely diverse plant in terms of its uses from cosmetic to culinary to medicinal. One cannot deny the historical significance of *L. angustifolia* as well as its fantastic and pleasant scent.

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Linum usitatissimum L., Linaceae

Alexa Kahn

Introduction

Linum usitatissimum L., commonly referred to as Flax, is a member of the Linaceae family. Another common name is linseed (Linnaeus 1857). Flaxseeds are known to be rich in α-linolenic acid and when used in the diet, it can be a therapy for reducing serum cholesterol and triglyceride levels, and ultimately used as a supplement to reduce platelet aggregation. Some data on antiplatelet effects are inconclusive, and more research needs to be conducted to better define flax as a functional food in reducing diseases such as cardiovascular disease. Secoisolariciresinol diglucoside (SDG) is another main constituent of flax and is the primary lignan found in the crop that is responsible for its therapeutic benefits. Flaxseeds have been studied for their benefits in treating conditions such as arteriosclerosis and high cholesterol (Lewis and Elvin-Lewis 2003). Flax is available in multiple forms such as, raw seeds, powder and oil. All forms are beneficial for both industry as well as in the human and animal diet and nutrition.

Figure 1. *Linum usitatissimum* (Flax capsules*)*
Source: http://en.wikipedia.org/wiki/Flax

Botanical Description

Linum usitatissimum is among the oldest crop plant that is cultivated for obtaining fiber and oil. In Linnaeus' book "Species Plantarum" he gave the botanical name for flaxseed: *Linum usitatissimum* (Linnaeus 1857). There are currently 200 species of flaxseed known. It has a shallow root system and is known to be an annual herbaceous plant. The shallow system allows the plant to better survive on sandy or rocky terrain.

The plant is short in height and has secondary branches and seed balls (**Figure 1**) (Jhala & Hall, 2010). Flax is a blue flowering crop that can produce small seeds that are flat, ranging in golden yellow to a reddish brown in color (**Figure 2**). In stores, flaxseeds sold in stores are normally available in ground seed (powder-like), whole seeds, and also in an oil form. The varieties of flax used for fiber come from plants with

Figure 2. ***Linum usitatissimum*** **(flower and seeds).**
Source. http://www.colourbox.com/preview/2288281-427644-flax-seeds-with-flower.jpg

longer stems, 80-120 cm tall, containing fewer branches and seeds. The oil forms come from shorter plants approximately 60-80 cm tall, and has stems dense with branches. The flax used to extract oil, also generally has a greater number of seed capsules and larger seeds itself (Shahidi 2005). Due to the versatility of flax, many universities and laboratories around the United States are showing an increased interest in studying the plant to solidify and learn more about its medicinal properties.

The flaxseed is home to warm and cool climates, and has been cultivated for many centuries across the world, primarily for its stem fiber, seed and linen cloth. Another common name used is linseed (Carter 1993). In India and the United Kingdom, the crop is called linseed, whereas throughout the United States and Canada the crop is termed flaxseed. In Canada, flax has a dark brown seed coat. In the United States, flaxseeds are available with yellow seed coats. The differences

Figure 3. Golden Flax
Source: http://en.wikipedia.org/wiki/Flax

Figure 4. Brown Flax
Source: http://en.wikipedia.org/wiki/Flax

in color are generally aesthetic (**Figures 3 and 4**) (Shahidi 2005) and not significant medicinally.

During a ten-year period, the total average yearly production of flax was 2.52 million metric tons. This ranked higher than

Figure 5. Graph of the major world producers of flaxseed during a ten year period (Shahidi, 2005).

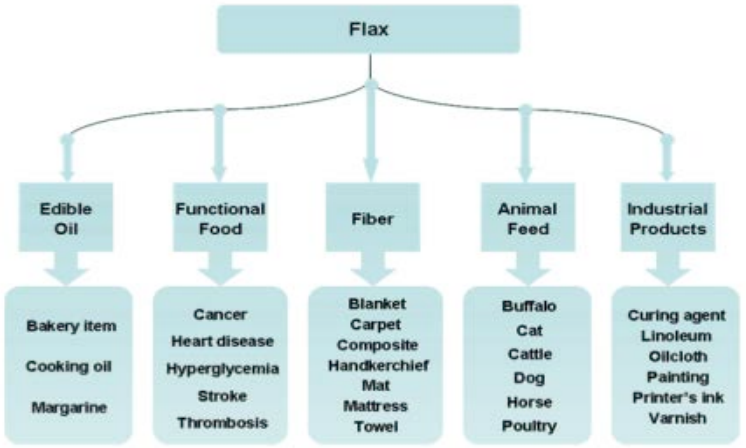

Figure 6. Schematic diagram of flax uses (Jhala & Hall, 2010).

sunflower, yet slightly lower than the soybean. Canada is shown to be the highest growing region for flax (**Figure 5**). In the northern latitudes, higher production of oil from the seeds is seen with high levels of polyunsaturated fatty acids contained in the oil. In the United States, North Dakota is the flaxseed production leader with over 95% of the region growing with flax (Shahidi 2005).

Traditional Uses

Flax has been used in food cooking throughout Europe and Asia since 5000-8000 BCE. Specially, the fiber contained in the flax stem has been utilized for linen cloth (Bloedon & Szapary, 2004). Originally, the stem fiber was brought to North America for its use in making linen and parchment paper. Over the past 100 years, flaxseed has been used throughout the Northern Great Plains region of the United States and Canada, as a commercial oilseed crop sold in many "health foods" stores as a dietary supplement (Carter 1993). Flax is grown in a wide range of countries and is known for its adaptability and product diversity (Jhala & Hall 2010)

Flax has many uses (**Figure 6**) and has stemmed back to ancient cultures in China, Greece, Egypt and India. In the flax growing region of the People's Republic of China, many of the villages used flaxseed oil for low temperature stir-frying in many of their home cooked food dishes. (Carter 1993). In ancient Greek and Egyptian cultures, flax was utilized as a natural laxative and food source by many of the villagers.

In India, flax was mainly grown for its oil, as a baking and cooking item. However, flax was additionally used as a fiber source to make blankets, mattresses, linen clothes, and parchment paper (Vajpeyi, Abhishek, Shukla & Srivastava, 2008). As a result, the fiber obtained from flax was a main contributor to the textile industry of India. In Egypt, the art of

Linum usitatissimum, the whole flaxseed	
% Fat	41
% Protein	21
% Dietary Fiber	28

Table 1. **The percentage components of one whole flaxseed** (Bloedon & Szapary, 2004).

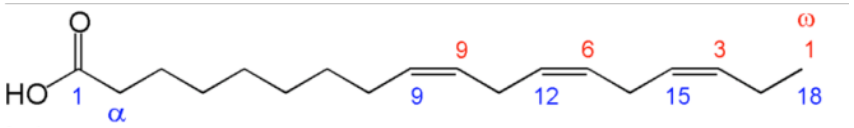

Figure 7. alpha-Linolenic acid molecule
Source: http://en.wikipedia.org/wiki/Alpha-Linolenic_acid

woven flax fiber to linen became important for the making of clothes for the upper class figures, such as Pharaohs in their society (Jhala & Hall 2010). Additionally, they used linseed oil as the main ingredient of the "finishing oil" for their woven and wooden furniture. The linseed oil is added to paints to create a more fluid and transparent liquid that can then be applied to hard surfaces, specifically wood. This leaves a nice, shiny appearance before it is then sold in markets in their villages.

It was measured that Europe generally has taller flax compared to the United States, which can be a reason why Europeans still use flax for linen cloth. The United States uses the fiber from flax less for linen and clothing since the discovery of cotton has been made (Carter 1993). While today flax is less used for its properties useful for parchment making or clothing for a community of people, we still do use flax as a cooking item. Today many people add flax in the form of seeds or even powder to their cooking to meet their daily fiber requirements.

Chemistry and Pharmacology

Linum usitatissimum, the whole flaxseed, is composed of 41% fat, 21% protein, and 28% dietary fiber, along with other minerals and vitamins (**Table 1**). The oil in flaxseed on the other hand, isunique in that it is concentration in 73% polyunsaturated fatty acids, 18% monounsaturated fatty acids, and only 9% saturated fatty acids. This lends itself to be considered a low-saturated fat food. The richest source of omega-3 fatty acid comes from flaxseed oil, which comprises approximately 55% of the total fatty acids (Bloedon & Szapary, 2004).

Flax is known for its higher fiber content, both an insoluble and a soluble source of fiber. They are rich in α-Linolenic acids (**Figure 7**) as well as phytoestrogens, or lignans. Lignans are phenolic compounds. The richest source lignans are, seciosolariciresino diglucosides (SDG) (**Figure 8**) (Bloedon & Szapary, 2004).

Flaxseed is known to contain approximately 35-45% oil, 25% protein with 10% moisture. The flaxseed has high constituents of oil, but extracting this oil is difficult to do and often requires double pressing to do so. Prior to crushing the seeds, clean ones are needed in order to ensure moisture levels of 9.5-10%. Ultimately, this will minimize the formation of fine particles when the seeds are cracked and will then maximize the removal of oil from them. Next, the moisturized seeds pass through a set of corrugated and smooth rolls to be cracked and then flaked. Consequently, the production of flax oil is differentiated from that of Solin or Linola oil. In order for flaxseed to be consumed by humans, it is necessary for it to be cold-pressed. The process of cold-pressing entrails the temperature of oil coming from extruder to not exceed 35 °C,

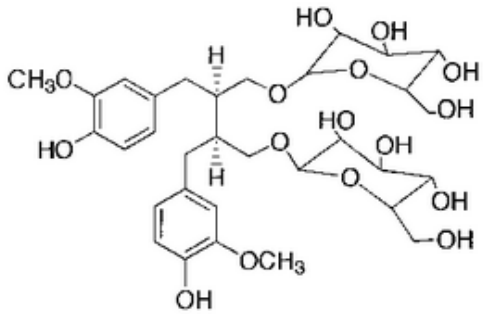

Figure 8. Seciosolariciresino diglucosides (SDG)
Source:
http://www.biopurify.com/admin/editor/UploadFile/20071018141427125.gif

(Carter 1993) and the pressing is done under a blanket of nitrogen in order to protect it from oxygen. Expellers can be used to cool parts of the press that are in contact with the seeds and the oil in hopes of controlling the temperature during the extensive processing technique (Vassel & Nesbitt 1945).

Flax may be used as a protective agent against cardiovascular disease through a number of different mechanisms of action. Flax is capable of reducing serum cholesterol, inflammatory marks, and platelet aggregations. Furthermore it acts as an antioxidant. The high soluble fiber content of flax allows for enhanced gastric emptying, which interferes with the bulk phase diffusion of fat, while also increasing the excretion of bile acids. The viscous fiber component of flax has been associated with increase glucose tolerance (Bloedon & Szapary, 2004).

Some evidence suggests that SDG is correlated with lower serum cholesterol levels. Lignans are potentially capable of lowering serum cholesterol through the modeling of enzymes, specifically, 7α-hydroxylase and acyl CoA cholesterol transferase, which are both involved in cholesterol metabolism. The lignans in flax have been shown to reduce oxidative stress, and SDG, has been shown to act as an antioxidant and further as an antagonist of platelet activating factor (Lewis and Elvin-Lewis 2003).

Biological Activity

Many studies have been conducted to assess the biological activity of flax. Both *in vivo* and *in vitro* studies have been conducted to determine the mechanism of action of flaxseeds to ultimately better understand the biological activity in animals as well as humans

In vivo

Animal studies have been conducted to question the capability of flaxseed products improving serum lipids. The rats fed 20% and 40% flaxseed for 90 days has substantially lower serum total cholesterol and LDL level than those rats that had a diet absent of flax. The results found that SDG found in flax reduced hypercholesterolemic atherosclerosis by 73% suggesting the effect was associated with lower serum levels (Prasad 1997). Additionally, the antioxidant activity of flaxseed lignans in rabbits was evaluated *in vivo*. Results showed that a 7.5 g/kg regular flaxseed added to an antioxidant free diet, along with the addition of 1% cholesterol, decreased atherosclerosis in the aorta by nearly 46% and lowered the number of inflammatory polymorphonuclear leukocytes as compared to the same diet in the absence of flax. The study projects that dietary flaxseed supplementation could be used to minimized heart attacks and strokes (Prasad 1999).

Overall, purified SDG from flax seed have been evaluated in mammary, colon, prostate, and thyroid cancers as well as diabetes, liver and heart disease in animal rat models. On the whole, results suggest beneficial effects of lignans. Some caution should be taken in relation to the dose of lignan and time of administration during reproductive stages in females (Westcott & Muir, 2003). Although the mechanism of action is not completely understood, researchers suggest that it may involve influence of hormonal or anti-oxidant activity.

Skin and hair conditions were studied in animals that consumed flaxseed or flaxseed oil. In Capuchin monkeys, it was reported that severe skin lesions were healed after consumption of flaxseed. Although the scientists have not yet confirmed and documented the effects of flax in human skin conditions, current research suggests possible improvements of a variety of skin disorders after assessing the improvements of psoriasis in humans who had sufficient consumption of flaxseed (Sinclair, Fiennes, & Crawford, 1973). Additionally, a study with Swiss mice showed that α-linolenic acid inhibited progression of skin cancer development (Ramesh & Das, 1993).

In vitro

The outcome of linseed oil fed to New Zealand white rabbits were compared to the outcomes of a diet that consisted of soy oil and safflower oil. An *in vitro* study looked at peripheral blood lymphocytes that were cultured with T-cells mitigens. The blood lymphocytes were noticeably higher in the group of rabbits that were fed linseed oil as compared to the group that was given either soy or safflower oil. Lymphocytes are a type of white blood cell that is in the immune system of vertebrates. The immune system of the rats that were fed linseed oil appeared more responsive compared to the rabbits that were given the other two types of oil. The results suggests that the increase in immunoresponsiveness in the linseed oil fed rabbits may be useful in fighting infections in the body (Kelley, Nelson, Senator, Schmidt & Branch, 1988). Based on the results of this study, researchers hope to investigate the effects of a linseed oil based diet in immunocompromised humans as well as healthy individuals for comparison.

Clinical Studies

In a North Dakota State University clinical study, the effects of flaxseed supplementation were examined in overweight patients who had hypertension and a family history of diabetes. The study determined that flaxseed consumption decreased insulin resistance, in other words, decreased glucose concentration in patients after use of flaxseed supplementation. The bioactive component of flaxseed, lignan, is responsible for do decreasing insulin resistance as well as inflammation (Rhee & Brunt 2011). Another study looked at the relationship between flax consumption and treatment of diabetes. 6 patients were monitered as they fasted overnight, and in the morning were randomly given a meal that consisted of 50g carbohydrate bread that was either made from white flour (control) or flax flour (experimental). The researchers concluded that the blood glucose levels were 28% in those subjects that were given the flax bread (Shahidi 2005).

In Toronto, flaxseed was used as alternative to hormone replacement therapy, since it is rich in the phytoestrogen precursor, secoisolaricriesinol diglucoside (SDG) and α-linolenic acid. It was hypothesized that these factors may be protective against vascular disease. The study analyzed 35 women with vascular disease ranging from ages 54 to 70. These women were administered different strains of flax: Flanders (high in α-Linolenic acid and low in lignan), AC

Linors (a mixture of intermediate levels of lignan and α-Linolenic acid) as well as Linola 989 (low α-Linolenic acid and high lignan). Results showed that all three types of flax lowered blood pressuring during mental stress. The Flax phytoestrogens ameliorate specific responses to stress, which in turn can protect patients against atherosclerosis, thickening of the arteries (Spence, Thornton, Muir & Westcott, 1973).

A two year study was done with subjects having multiple cardiovascular risk factors, correlating the risk of ischemia heart disease and nutritional diets. Two groups were randomized, giving one group margarine righ in α-linolenic acid while the other group obtained margarine that was rich in linoleic acid. After the two year period, results suggest that individuals who had a rich source of α-linolenic acid, showed a higher total cholesterol to HDL cholesterol ratio than when the experiment first began. This improvement of cholesterol levels decreased estimated ischemia heart disease (Bemelmans, Broer, Feskens & Smit, 2002).

In another study in Toronto, seven eldery patients were give two flax muffins per day. The results of the study reported increased bowel movements and frequency of bowel movements per day (Cunnane, Ganguli, Liede, Hamadeh, Chen, Wolever & Jenkins, 1993). This increase of bowel movements suggests the laxative properties of flax.

A clinical trial in China examined the SDG-rich flaxseed extract to determine the possible effects of SDG on total cholesterol, HDL-cholesterol, LDL-cholesterol as well as glucose concentrations in a group of 35 men and 20 women over the course of 8 weeks. In order to be eligible, subjects needed to meet the requirements of LDL-cholesterol levels no lower than 1400 mg/l. Additionally, they could not be taking any drugs, herbal supplements, products containing flax, or any products used to lower cholesterol. Subjects were randomly assignment to the 300mg SDG, 600mg SDG or the placebo group. Results showed that the dietary flaxseed lignan extract (SDG) did decrease plasma cholesterol and glucose concentrations significantly in hypercholesterolaemic subjects (Zhang, Wang, Lui & Tian, 2007).

Contraindications

There is minimal risk with the consumption of flaxseeds in humans. Food allergies to flax seem to be quite rare. Those who question a possible allergy to Flaxseed should consult their physician for permission of use prior to incorporating it into their daily diet. Those individuals with allergies to nuts should be cautious. It's also important to realize that flaxseed is rich in α-linolenic acid, which is has been a major component of the Mediterranean diet for many years. In a 5-year prevention trial, a high content α-linolenic acid Mediterranean proved to be more efficient than present day diets in preventing coronary diagnosis and deaths (Bemelmans, Broer, Feskens & Smit, 2002). Its wide use for many years with no major side effects allows it to be viewed as safe.

However, one concern lies in the consumption of uncooked flaxseed due to cyanogenic glycosides (HCN) content, which in high quantities can be toxic to animals as well as humans. No cases have been reported of acute or chronic cyanide toxicity from flaxseed consumption. It is important to note that baking flaxseed is capable of removing any potential risks from HCN (Bloedon & Szapary, 2004). By cooking the raw flaxseeds (in baking products or hot cereals; some oatmeal's contain flaxseed) this seems to eliminate the potentially toxic cyanogenic glycosides (Cunnane et al, 1993). Furthermore, those who experience harmful side effects from cyanogenic glycosides generally have a poor quality diet as well. For the

most part, populations in North America have well-balanced diets and receive proper nutrients that allow the body to eliminate the potentially harmful, HCN, which is found flax (Shahidi, 2005).

Current Uses in Allopathic and CAM Therapies

The flaxseed has only recently been introduced in North America. Some known benefits of flaxseed in human and animal nutrition include, its high component of alpha linolenic acid (also known as omega-3 fatty acid) content pertinent to humans, its high percentage of insoluble and soluble dietary fiber, and lastly, it has the highest content of plant "lignans". Lignans are important because they are known for their anti-carcinogenic properties (Shahidi, 2005).

The use of flax in industry and food is continuing to grow and develop. Around 200 new food and health care products were introduced and marketed in the United States in 2005 (Jhala & Hall, 2010). Today, ground or whole flaxseed can be easily added to almost any baking recipe. Ground flax in a powder form is often used in liquid foods such as yogurt and soup to hide the taste, whereas whole flaxseed can be used in bread, pancakes, waffles to add a nutty flavor. Whole flaxseeds are a great food additive in recipes to add a crunch (Shahidi, 2005). Many U.S. companies are manufacturing fiber bars for those who don't receive adequate amounts of daily fiber that contain high quantities of flax. Additionally, there are few side effects of flaxseed making it a more widely used product among many Americans.

The National Cancer Institute is now considering flax a "designer" food. The term "designer" means the product consists of more than one ingredient that contributes to the overall beneficial qualities. Flax contains nutrients for good health but also is known to be protective against cancer and cardiovascular disease (Carter, 1993).

Another fairly new and current use of flax is in the form of flaxseed gum, or mucilage (Mazza, 1989). The gum is reported to be extracted using hot and cold water. Then it can be mixed with ethanol, eventually dehydrated and then freeze-dried. The acid hydrolysis of gum then produces polysaccharides. In experimental study, it was found that flaxseed gum is a similar alternative to gum arabic, which is a mixture of complex polysaccharides, which is used as a food stabilizer. In fact, it was noted that flaxseed mucilage has great emulsifying properties compared to gum arabic, enabling it to be more efficient. It was estimated about 7-10 lbs of gum could be yielded from about 100 lbs of flaxseed. Today, the usefulness of flaxseed gum is in improving loaf volume of bread while also keeping the quality fresh (Mazza, 1992).

Today, the Food and Drug Administration and several universities are conducting extensive research in feeding flaxseed to animals as well as humans to further discover health benefits of flax. Although flaxseed and cold-pressed linseed oil don't already have the Generally Recognized as Sage status, there is a "no objection" status, that seems to be adequate while flax undergoes more testing in the laboratory (Carter,1993).

Discussion

Linum usitatissimum is a crop grown in various countries and climates for different purposes, and has been around for over 6,000 years. Flax is known for its adaptability and diversity as a product traditionally, as well as in current allopathic and CAM therapies. Its prime use today is in dietary supplements and cooking. North Dakota is currently the leading state for

flax production. With the high content of α-linolenic acid and SDG in flax, both *in vivo* and *in vitro* studies as well as numerous clinical trials have examined its effects in controlling diabetes, as a laxative, managing cardiovascular disease as well as a tool to enhance the immune system. Some in vivo studies also looked at flax in monkeys and its effects on fur condition. From this research in monkeys, scientists predict that flax can be a source of improving skin conditions such as psoriasis. Since the flax is available in the form of oil, the scientist suggests topical use of flaxseed oil can be beneficial to numerous skin diseases in individuals. However, more clinical trials are underway to confirm theses hypotheses.

Many Americans are first becoming aware of flax and its health benefits. Flax is an easy food supplement to any current diet and has a high nutritional component with a great source of fiber. The high fiber contents are not only beneficial for food intake but also in the linen and parchment paper industry. Although flax has been used for many years now, further clinical studies can be done to better understand the mechanism of action of flax itself as well its main components such as omega-3 fatty acids, α-Linolenic acid, and SDG.

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Matricaria recutita L., Asteraceae

Carolyn Wiegert

Introduction

Matricaria recutita, most commonly known as German chamomile*,* (synonymous with *Matricaria chamomilla* L.) is a flowering plant of the daisy family, Asteraceae. It is also known as Hungarian Chamomile, mayweed, sweet false chamomile or wild chamomile (McKay & Blumberg, 2006). With a history dating back to ancient foundation societies like Rome, Egypt, and Greece German chamomile has been an ever-present herb in world history. Prized for its essential oil known as "blue oil" and flower heads, *M. recutita* appears in traditional and modern cultures—both western and eastern—as a common herbal medicine. Its main chemical constituents are of the flavonoid and sesquiterpene families. Examples are apigenin, chamazulene, and α-bisabolol. Although scientific studies of the plant are all fairly recent—within the last two decades—German chamomile possess great medicinal potential.

Figure 4. Wild growth of *Matricaria recutita;* plant in its uncultivated form. Image source: http://www.flickr.com/photos/hinkelstone/2811407254/.

Botanical Description

Herbaceous *Matricaria recutita* is an indigenous plant of Southern and Eastern Europe (Guimaraes et al., 2013), but additional countries across the world cultivate it. Cultivators include India, Germany, France, Russia, Italy, South America, and the United States. Hungary, who exports the flowers and essential oil to other European countries, produces the largest quantity of the plant in the world. The plant flourishes in nearly any soil type and propagates via seeds, making it a versatile cultivar. *M. recutita* is the fifth most traded herb in the world. On the international market, traders refer to its essential oil as "blue oil" and with the rising popularity of CAM (Complementary and Alternative Medicine) and allopathic therapies, an increase in demand should be expected (Barnes, Powell-Griner, McFann, & Nahin, 2004; Singh, Khanam, Misra,

& Srivastava, 2011). As shown in **Figure 1**, the plant resembles other daisy species from the Asteraceae family with similar petal shape and size. Singh et al. (2011) describes the flowering annual plant as having spindle shaped-roots and an erect stem that can grow to more than 80 cm high (**Figure 2**). It produces golden yellow florets with small, white petals and yellowish-brown fruit. Its odor is described as "sweet, grassy, and lightly fruity" ("Matricaria chamomilla (German chamomile)," 2008).

Traditional Uses

The most common traditional preparations of *Matricaria recutita* are teas and tisanes, used by various cultures both in ethnobotanical and ethnomedical contexts. Most of the alleged health claims from traditional uses have little or no scientific evidence to back them simply because all research on the plant is fairly recent and few studies have been replicated. In addition to teas, dried flower heads are also utilized for multiple flavoring, aromatic, and dyeing uses (McKay & Blumberg, 2006). Although German chamomile teas are present in all areas of the globe, several particular populations in Southern Italy, the Italian Archipelago, Mexico, Central Serbia and Syria give the herb to a special cultural status.

Basilicata, Southern Italy

The Albanian minority ethnic group known as the Arbëreshë uses *Matricaria recutita* in two key ways. This small group lives in the Basilicata province, also referred to as Lucania, located in southern Italy. Today, this group utilizes western medicine as their primary form of health care, but plant remedies are still used to supplement western treatment. Referred to as *Kamohill* in the Arbëreshë language, *M. recutita*

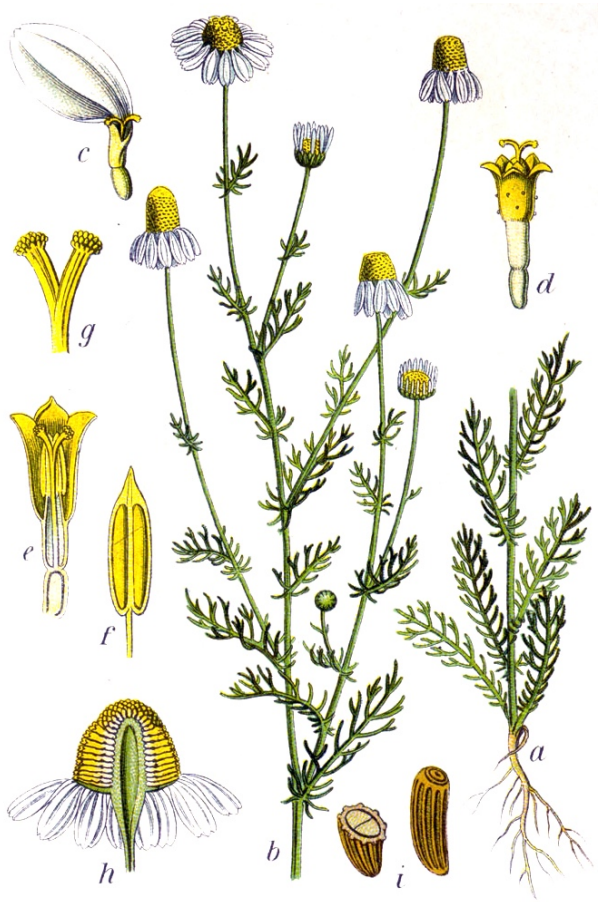

Figure 2. Botanical illustration of *Matricaria recutita;* notice the small white petals and yellow stamen. Image source: http://commons.wikimedia.org/wiki/File:Matricaria_recutita_Sturm13045.jpg.

is used for its flowers, leaves, and other aerial parts. Most traditional knowledge of the plant has been preserved by the elderly women of this cultural group and therefore, these women prescribe and administer most ethnomedical

treatments. Tea decoctions of the plant's parts are used as digestives and sedatives. Parts of the plants are also used to treat the Arbëreshë wind illness known as *mal vjnt*. To treat *mal vjnt*, which appears as a skin rash on the body, the whole form of the plant is bundled with other gathered plants and waved over the rash while simultaneously reciting a special prayer. The herb bundle ceremony is repeated for 9 nights. Both of these uses are documented in the research of Pieroni, Quave, Nebel, and Heinrich (2002).

Tuscan Archipelago, Northern Italy

To a lesser extent, *Matricaria recutita* is also used as an herbal remedy on island of Elba. Elba is a member of a small chain of islands off the coast of northern Italy known as the Tuscan Archipelago. Locally referred to as *Camomilla di Campo* or *Camomilla masseta,* the plant is used in both its decoction and infusion form. Decoctions are used for external treatments while infusions are ingested to treat internal ailments. The island's population uses the plant as an analgesic, anti-inflammatory, digestive, eye compress, and a topical treatment for reddened skin (Uncini Manganelli & Tomei, 1999).

Mexico

Commonly referred to as *Manzanilla té* in the country, *Matricaria recutita* is the second most cited plant used in combinational herbal remedies by Mexicans. Utilized primarily in its tea decoction form to treat conditions of gastrointestinal distress, special *Chamanes* healers in rural areas or a female relative in urban areas are often the main prescribers. The plant is also prescribed for conditions of fever, high pressure, conjunctivitis, flu, sore throat, and diseases of the "evil eye" (Josabad Alonso-Castro et al., 2012).

In addition, even after emigration, Mexican Americans also rely on *Manzanilla* as a key home herbal remedy. Continuing to prepare the plant as a decoction tea in the United States, adult Mexican American women prepare the drink by adding an unmeasured handful of dried flower heads to boiling water and allowing them to steep for 5-10 minutes (Waldstein, 2006). Waldstein (2006) explains that the plant can be purchased fresh in its whole form at many Mexican American grocery stores. In accordance with ancient medicinal humoral theory, *Manzanilla* is administered as a "hot" therapy for "cold" ailments such as menstrual cramps, stomachaches, infant colic, sinus cleanses, and evening tonics or eyewashes for anxiety. The "hot" humoral properties are believed to be purifying to the stomach by relieving bloating, pain and prompting burping, which is believed to be the release of anxiety by the body. After administering its curative properties to the body, *Manzanilla* is believed to exit the body through urine (Waldstein, 2006). For Mexican Americans, *M. recutita* is just one of many herbal remedies used to supplement an adoption of conventional medicine.

Central Serbia

In a survey ethnobotanical study of Kopaonik Mountain in Central Serbia, *Matricaria recutita* was found to be one of the most consumed plants for medicinal and functional food purposes (Jaric et al., 2007). Only collected and consumed in its wild form, the plant is locally known as *Kamilica*. Jaric et al. (2007) explains that the mountain's population of Serbians is mostly elderly with livelihoods dependent on cattle herding or agriculture. *Kamilica* is referred to as a panacea (remedy for all illnesses) by the local population and can be found in most

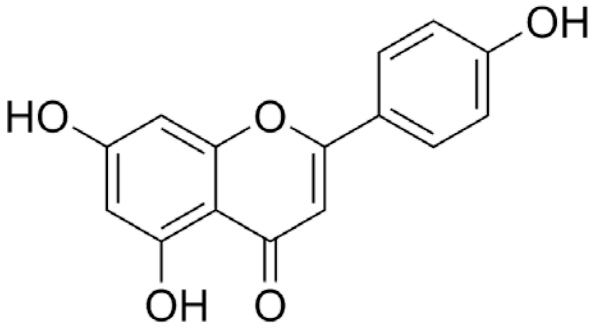

Figure 3 Chemical structure of apigenin. Image source: http://commons.wikimedia.org/wiki/File:Apigenin.svg.

homes on reserve. Locals scald the plant parts before storing them with the belief that this better preserves their active ingredients. *M. recutita,* only used in tea form, is ingested and applied externally for antiseptic, anti-inflammatory, and sedative purposes. Serbians ingest the tea as a stomach painkiller and a treatment for dyspepsia and constipation. The tea form is applied externally to treat skin wounds, burns, and galls or as a vaginal douche. The steam of the tea is inhaled to clear the sinuses of those suffering from sinusitis. Serbians prefer phytotherapy uses like these to conventional medicine and very few members ever visit an actual doctor (Jaric et al., 2007).

Syria

In Syria, *Matricaria* recutita exists as a functional food and post-meal digestive. It is a single herbal component of a multi-species herbal tea commonly known as *Zahraa. Zahraa is* sold from market stands and dispensaries across the country, but is particularly common in Damascus marketplaces. Bedouin herders and rural populations do much of the collection of *M. recutita* for sale. *Zahraa* tea mixtures can contain up to 14 species depending on which dealer they are purchased from, and are a key component to Middle Eastern Unani medicine. The tea is served hot after meals in Damascus restaurants, homes, and cafes and is thought to have digestive properties (Carmona, Llorach, Obon, & Rivera, 2005). Syrian people believe found that the plant contains medicinal properties for the following body systems: digestive system, infectious ailments, circulatory system, respiratory system, nervous system, and skin ailments (Carmona et al., 2005).

Chemistry and Pharmacology

Over 120 chemical constituents have been identified over the years in *Matricaria recutita* (McKay & Blumberg, 2006). The compounds of primary importance are mainly of the sesquiterpene and flavonoid families and their derivatives. Many of these compounds are found within the plant's flowers' essential oil, which is approximately 0.24 to 2.0 percent of the flower ("Matricaria chamomilla (German chamomile)," 2008). The research of McKay and Blumberg (2006) shows that the flower heads contain the following approximate percentages of phenolic compounds: 16.8 percent of the notable flavone glycoside apigenin (chemical structure shown in **Figure 3**), 9.9 percent of the flavonoid quercetin, 6.5 percent of the flavonal patuletin, 1.9 percent of the flavone Luteolin, both coumarins herniarin and umbelliferone, and ferulic and caffeic acid derivatives. Important essential oil compounds are α-bisabolol, a sesquiterpene alcohol, and chamazulene, a sesquiterpene derivative. Other notable chemical constituents are tannins, choline (0.3 percent in plant) ("Matricaria chamomilla (German chamomile)," 2008), polysaccharides, and amino and fatty acids (McKay & Blumberg, 2006). It is important to note that these concentrations decrease when the plant is prepared

in decoction or infusion form. Decoctions possess higher concentrations of phenolic compounds relative to infusions (Guimaraes et al., 2013). McKay and Blumberg (2006) states that teas brewed of *M. recutita* only contain 10-15 percent of the plant's original volatile oil content.

Biological Activity

Matricaria recutita has been studied for biological activity with encouraging preliminary results. In various studies, *M. recutita* has shown antioxidant, anti-inflammatory, anti-tumor, antimicrobial, anti-platelet, anti-cancer, anti-genotoxic, and hypocholesterolemic activity. Much of the anti-cancer potential of the plant is attributed to the chemical constituent apigenin. With more research, German chamomile has the potential to benefit the heart, liver, gastrointestinal and central nervous systems of the body. Some examples of the mechanisms of action of this plant and its chemical constituents are explained in more detail below.

Irritable Bowel Syndrome

Because inflammatory markers and oxidative stress have been linked to the expression of irritable bowel syndrome (IBS), Asadi-Shahmirzadi et al. (2012) conducted a study on male albino rats to test the combined effect of *Matricaria recutita* and *Aloe vera,* and individual effect of *M. recutita* on the diarrhea-predominant form of IBS. Both *A. vera and M. recutita* are known to possess inflammation-inhibiting qualities. *A. vera* has been shown to inhibit inflammation biomarkers TNF-α, IL-1 β and IL-6. Although not thoroughly tested, *M. recutita's* compounds chamazulene, α-bisabolol, and apigenin have been shown to possess anti-inflammatory properties (Asadi-Shahmirzadi et al., 2012).

Rats were divided into six groups for the study: control, normal (sham), *M. recutita* (German chamomile), mix group 1, mix group 2, mix group 3, and a loperamide group for comparison. Loperamide is an opiod drug commonly used to treat diarrhea. For seven days, animal groups were administered either saline (control and normal), 300 mg/(kg [illegible] (Mix-150), 300 (Mix-300), or 450 (Mix-450) mg/(kg [illegible] of German cham *A. vera* and German chamomile, or 10 mg/(kg [illegible] d) of a 5 loperamide. Whole leaves of *A. vera* and the flower heads of *M. recutita* were used to produce aqueous extracts of German chamomile and extracts of combined *A. vera and* German chamomile. After day 2, experimenters induced IBS in rats using plastic tube retainers for forced restraint. After day 7, gastric emptying and small bowel movement were measured via phenol red, colonic transit evaluation was measured via fecal pellet count, and biochemical assays were used to measure effects on inflammatory markers TNF-α, MPO, and LPO via TBARS as well as the total protein of colon homogenate content.

German chamomile exhibited significant treatment potential on its own by reducing gastric emptying and small bowel transit, TNF-α, MPO, and increasing colonic antioxidant capacity in comparison to the control group. However, the synergistic effect of combined *A. vera* and German chamomile seemed to be more powerful as an anti-inflammatory and treatment for IBS overall. Results were not be dose dependent (Asadi-Shahmirzadi et al., 2012). However, mix-150 did demonstrate more potential than higher dose mixes, but not in a statistically significant manner. A combination of *A. vera* and *M. recutita* could be used to reduce diarrheal movements of the bowels from IBS and the oxidative stress associated with it. On it's own, *M. recutita* shows promise for reducing inflammatory biomarkers and small bowel transit, but more

research is necessary to determine its exact method of action.

Heliobacter pylori Infections

Heliobacter pylori bacteria are one of the main causes of general gastrointestinal distress and gastrointestinal disorders and diseases. *H. pylori* infections have been linked to the development of chronic gastritis, ulcer disease, gastric neoplasms, and peptic ulcers. Combinational therapies of antibiotics, proton pump inhibitors, bismuth subsalicylate, and H-2 blockers are the current conventional treatment for *H. pylori.* These treatments can be and are effective, however they often produce unwanted side effects and are not effective against antibiotic resistant strains in patients (Shikov, Pozharitskaya, Makarov, & Kvetnaya, 2008). Shikov et al. (2008) details an *in vitro* investigation of the potential inhibitory effects of an olive oil *Matricaria recutita* extract on *H. pylori.*

For the experiment, dried *M. recutita* flowers were used to create an olive oil extract. A *Heliobacter pylori* strain, *Heliobacter pylori* ATCC 43504, was inoculated into liquid media and cultured in a micro aerobic atmosphere. Researchers obtained a single colony from the culture and placed it on Colombia agar plates containing 10 percent sheep's blood. The *H. pylori* strain was tested both with German Chamomile extract and olive oil alone as a control (Shikov et al., 2008).

German chamomile olive oil extract prevented the growth of *H. pylori* into full, opaque colonies. The colony treated with the olive oil extract produced colonies smaller in size with rough edges. German chamomile seems to inhibit growth by preventing the bacteria from producing urease, a substance necessary for the bacteria's survival in the stomach. *M. recutita*'s coumarins, terpenes, and spiroethers, are thought to be responsible for this method of action. Shikov et al. (2008) concluded that German chamomile could be a viable treatment option for suffers of *H. pylori* who are allergic or possess resistant strains to antibiotics.

Allergic Reactions

Mast cells, present in many tissues and organs of the body, are known to be important regulators of the body's allergic reaction response and histamine release. Chandrashekhar et al. (2011) conducted an *in vivo* study in male albino rats to examine the potential anti-allergic activity of *Matricaria recutita. Chandrashekhar et al. (2011)* noted that German Chamomile possesses significant tannin and flavonoid content that may help inhibit anaphylaxis, pruritus (itching of the skin), and histamine release during allergic reactions.

For the experiment, methanol extracts made from the dried flower heads of *M. recutita* in dosage quantities of 100, 200, and 300 mg were administered to three experimental groups of ten rats each. A separate group of ten rats was administered 10 mg of the mast-cell stabilizing drug disodium cromoglycate. Control and normal groups of ten rats each were administered saline. To induce pruritus and anaphylaxis, the compound 48/80 in dosage quantities of 3 mg/kg and 8 mg/kg was administered to rats respectively in all groups except normal. Scratching behavior and death from anaphylactic shock was observed. Mast-cell stabilizing potential, blood histamine release, and nitric oxide levels in the rats were also measured (Chandrashekhar et al., 2011).

M. recutita showed dose-dependent protection and inhibition from pruritus and anaphylaxis, with the 300 mg dose showing the greatest positive effect. *M. recutita* also dose-dependently

lowered histamine release in the blood and nitric oxide levels throughout the body. Nitric oxide is thought to increase free radical production in the body by inducing inflammatory responses. Tannins and flavonoids have been shown to suppress histamine release and therefore, the experimenters hypothesize *M. recutita'*s tannin and apigenin and luteolin flavonoid content may be responsible for its anti-allergic activity. Further research is necessary to determine German chamomile's exact method of action.

Wound Healing

According to Martins et al. (2009), corticosteroids are the most common form of therapy used by medical practitioners to aid wound healing. Corticosteroids, unfortunately, can cause adrenal suppression and subsequent kidney malfunction when they are used over a long period of time. Because *Matricaria recutita*'s chemical constituents are known to possess antibacterial, anti-inflammatory, and antifungal capabilities, Martins et al. (2009) decided to test, via *in vitro* and *in vivo* studies, the plant's potential as a wound-healing drug. The cytotoxicity of the plant was also examined.

For both the *in vitro* and *in vivo* studies, five test groups were created: control, German chamomile, and three different forms of corticosteroids. For the *in vitro* study, cells were cultured under nutritionally deficient conditions to simulate the environmental stress on cells during wound healing. Cell mitochondrial activity and cell viability were measured in the presence of no drugs, chamomile, or the corticosteroids. For the *in vivo* study, 125 male albino rats were evenly divided amongst the five test groups. After mild anesthetization, experimenters inflicted traumatic ulcers on the rats' tongues. No drugs, chamomile, or corticosteroids were administered at time intervals (every 12 hours) during the 14-day experiment period depending on the rats' test group designation. On days 1, 3, 5, 7, and 14 experimenters examined the tongues for wound healing progress in five sacrificed rats from each group. Wounds were classified either "in repaired" or "no repaired" (Martins et al., 2009) based on a scale of grades 1 to 5. Grade 1 represented the greatest wound healing progress and 5 the least.

Study results revealed *M. recutita* to be non-toxic after cytotoxicity tests. Surprisingly, *M. recutita* demonstrated the least wound healing power out of all groups *in vitro*, but the greatest power *in vivo*. Rats who were in the German chamomile group had almost all their lacerations healed completely by day five, while the control and corticosteroid groups required the full 14-day experiment period to reach grade 1 healing status. The German chamomile group also exhibited low rates of abscess formation *in vivo* during healing, demonstrating the antibacterial activity of the plant. Martins et al. (2009) inferred these results were due to chemical constituents of German chamomile such as chamazulene, flavonoids, etc. as documented by other studies. Although German chamomile demonstrated the lowest cell viability during the *in vitro* study, researchers believe results could have been influenced by said antibacterial activity. Therefore, German chamomile could serve as an alternative wound treatment that is safer and faster with fewer side effects than corticosteroids.

Clinical Studies

Most recent clinical studies have focused on testing *Matricaria recutita* as an alternative therapy for the treatment of depression and/or anxiety. Other clinical trials, as examined by McKay and Blumberg (2006), have demonstrated positive

effects of topical applications and inhalation treatments. McKay and Blumberg (2006) reports on a study that demonstrated German chamomile's topical application effect was equal to that of .25 percent hydrocortisone cream in the treatment of atopic dermatitis, eczema, radiation therapy, and erythema. McKay and Blumberg (2006) also examined two studies that showed German chamomile's essential oil potential to alleviate pain and create a "comfortable feeling" when inhaled.

Anxiety and Depression

Amsterdam et al. (2009) and Amsterdam et al. (2012) are two studies that examine the potential anxiolytic and anti-depressant activity in German chamomile. The search for an herbal therapy for anxiety and/or depression is relevant because many patients decide not to seek treatment for various reasons such as cultural (many societies have a stigma towards these two mental disorders), financial (psychiatric treatment is often costly), or personal reasons. In addition, modern conventional therapies such as selective serotonin reuptake inhibitors and benzodiazepines can cause undesirable side effects (Amsterdam et al., 2009).

Although the researchers responsible for these two studies mention various caveats present in their methods, they do believe their results still show potential for *Matricaria recutita* as a possible treatment. Both clinical trials were randomized, double blind, and placebo-controlled. All patients included had a standardized diagnosis of Generalized Anxiety Disorder or Depression. Those patients who had major mental co-morbid disorders, unstable medical conditions, or allergies to the Asteraceae family or chamomile were excluded. Researchers examined the mean scores of HAM-A and HAM-D scoring tests and determined that administration of oral chamomile extract resulted in lowered HAM-A and HAM-D scores over time.

German chamomile's method of action for these anxiolytic and antidepressant results is unknown, however researchers have several hypotheses. The anxiolytic effect may be a result of *M. recutita'*s flavonoid content acting on γ-amino butyric acid, noradrenalin, dopamine, and serotonin neurotransmissions or modulating hypothalamic-pituitary-adrenocortical axis function. Another hypothesis is that apigenin may bind to benzodiazepine receptors and reduce γ-amino butyric acid-activated activity. Researchers believe the anti-depressant mechanism of action could be a result of flavonoid content modulating central noradrenalin, dopamine, serotonin, and γ-amino butyric acid neurotransmission (Amsterdam et al., 2009; Amsterdam et al., 2012).

Contraindications

The FDA (Food and Drug Administration) categorizes *Matricaria* recutita, German chamomile, as GRAS (Generally Recognized as Safe) and ADRs (Adverse Drug Reaction) to the plant are rare. However, a small amount of ADR cases have been recorded in the form of allergic reactions. Most reactions to *M. recutita* occurred in patients with preexisting allergies to other members, such as mugwort or ragweed, of the Asteraceae family (McKay & Blumberg, 2006).Therefore, those with said allergies should avoid interaction with *M. recutita*. Oral ingestion, topical application, and use as an eye rinse of *M. recutita* can cause severe hypersensitivity and anaphylaxis, allergic conjunctivitis, and contact dermatitis (Rodriguez-Fragoso, Reyes-Esparza, Burchiel, Herrera-Ruiz, & Torres, 2008). Cases of anaphylaxis are extremely rare and of the few-recorded ADRs most were associated with conditions of the skin and its deeper subcutaneous layers (Jeschke et al., 2009).

Cases in which *Matricaria recutita* was prescribed: Conditions, Diseases, Infections
Respiratory: • Acute upper respiratory infections • Acute lower respiratory infections • Chronic lower respiratory diseases • Other diseases of the respiratory system • Other diseases of the upper respiratory tract
Digestive distress
Other infectious diseases
Diseases of oral cavity, jaw, and salivary glands
Non-infective enteritis and colitis
Diseases of middle ear and mastoid

Table 1. Cases in which German allopathic physicians prescribe *Matricaria recutita.* Adapted from Jeschke et al. (2009).

An additional potential cause of ADRs among patients is contamination of chamomile supplements and products with a similar species known commonly as "dog chamomile" (Srivastava, Shankar, & Gupta, 2010).

There are no scientifically proven drug interactions for *M. recutita* however several theories for potential interactions exist. It is thought that *M. recutita*'s coumarin content may increase the power of the drug Warfarin by interfering with the blood coagulation process. In addition, *M. recutita* may add blood thinning effects to the existing anti-platelet effects of aspirin, NSAIDs (Non-steroidal Anti-Inflammatory Drug), and acetaminophen and increase the effects of several central nervous system depressant sedative drugs (McKay & Blumberg, 2006).

Current Use in Allopathic and CAM Therapies

Modern day uses of German chamomile are diverse and far-reaching across the globe. German chamomile is one of the most consumed herbs worldwide, with over a million cups of its herbal tea consumed on the daily (Srivastava et al., 2010). Because most clinical studies on the plant are fairly recent and have not been replicated, there are no conventional chamomile-derived or synthetically modified chamomile drugs on the market today. In fact, *Matricaria recutita* is still typically consumed/utilized in its traditional preparation forms: teas, tisanes, tinctures, eye rinses, and topical skin applications. German chamomile for tea brewing is sold in dried flower heads or tea bags made from powdered *M. recutita* alone or in combination with other popular medicinal herbs. Drinking of the tea or inhalation of the essential oil is common for German chamomile's reported overall calming effect and alleviation of depression, anxiety, insomnia (Srivastava et al., 2010). In Germany, *M. recutita* is the most prescribed herb of the Asteraceae family by allopathic physicians (Jeschke et al., 2009). See **Table 1** for a list of cases in which *M. recutita* was prescribed for treatment. According to "Matricaria chamomilla (German chamomile)" 2008) courtesy of the Alternative Medicine Review, the recommended dosage is 1 dried flower head prepared via infusion and consumed orally. Chamomile is also a common ingredient in aromatherapy, cosmetic, and hair products (Srivastava et al., 2010).

Discussion

German chamomile, *Matricaria recutita,* is a plant with a detailed ancient history in cultures across the world, but is a relatively new plant in the world of western medicinal research. Patients using both eastern and western traditional

forms of medicine (Unani, Aberëshë, etc.) have reported ethnomedical and ethnobotanical benefits for centuries. In particular, German chamomile seems to be one of the most frequently used traditional herbal remedies for overall gastrointestinal distress and anxiety relief. These herbal uses of the plant appear across cultures that developed an ocean apart. It's surprising to find that more older scientific studies are not available on the plant, but logical as well. With western medicinal struggles such as antibiotic resistance, conventional drug side affects, and the costliness of healthcare emerging only in the past few decades, it makes sense that most research on alternative therapies is fairly recent. German chamomile's biological activity presents the exciting potential for safer and less expensive therapies for conditions involving oxidative stress, bacterial infections, cancer cell and tumor proliferation, and much more. It has also presented itself at the forefront of research on new therapies for mental distress (depression, generalized anxiety disorder, and insomnia). These exciting findings and claims of both past and present should encourage more research on the plant's phytochemical constituents for potential new drugs and whole herb treatments.

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Melaleuca alternifolia Cheel, Myrtaceae

Elizabeth M. Cappello

Introduction

Melaleuca alternifolia Cheel is an angiosperm in the Myrtaceae family. The common names of Melaleuca alternifolia are tea tree and paperbark tree. *M. alternifolia* has many different uses, both traditional and modern, including flavoring, antiseptic, antibiotic, antiviral, anti-inflammatory, antifungal and topically for skin abrasions (Brophy et al., 1989; Carson et al. 2002; Hammer et al., 2004). *M. alternifolia* has been used as a natural preservative in both pharmaceutical and cosmetic products (Cox et al., 2000). Halberstein noted that *M. alternifolia* has been prescribed as an antibiotic and is similar to Elecampane flower (2005). The main constituents are terpinen-4-ol, 1,8-cineole and α-terpineol (Carson et al., 2002). The essential oil extracted from *M. alternifolia* has become popular within the last century and is an economically significant essential oil (Homer et al., 2000).

Figure 1. ***Melaleuca alternifolia*** (Source: http://toptropicals.com/catalog/uid/melaleuca_alternifolia.htm)

Botanical Description

The Myrtaceae family is known for its popular genus, Eucalyptus, and also includes *Psidium guajava* L. (common guava) and *Syzygium aromaticum* L. (clove). *M. alternifolia* (**Figure 1**) is a small evergreen tree found in New South Wales and Queensland and is native to Australia, found in the low and swampy areas near the Clarence and Richmond rivers (Butcher et al., 1994; Carson and Riley., 1993). A correlation has been observed between the composition of tea tree oil (TTO), the essential oil of *M. alternifolia*, and the geographic location of the tree in Australia; high levels of terpinen-4-ol were seen mostly in northern New South Wales, low levels of terpinen-4-ol (high levels of 1,8-cineole) were seen near Grafton and terpinolene was seen in abundance in M. alternifolia populations in Queensland (Homer et al., 2000). It has been hypothesized that this chemical distribution

according to geographic location is because of genetic rather than environmental pressures; the close proximity of these trees may have caused a type of "inbreeding" effect allowing for a more "pure breed" within each geographic area (Homer et al., 2000). *M. alternifolia* has very small white flowers and a papery, black bark with narrow leaves, growing up to 22 meters in length. The evergreen grows to about 5 meters in height (Carson and Riley, 1993). The essential oil of *M. alternifolia*, famous for its biological activity, is pale, yellow, viscous oil with a pungent odor (Carson and Riley, 1993).

Traditional Uses

The use of *M. alternifolia* has been a part of the Australian Aboriginal tradition, particularly the Bundjalung Aborigines (Budhiraja et al., 1999; Hammer et al., 2003). When traditionally used by Aborigines, the leaves and branches were crushed or decayed leaves were placed in water (Carson and Riley, 1993). The collection of *M. alternifolia* was originally done by coppicing process, a method of cutting down the plant to its tree stump and allowing it to grow back (this make take up to 10 years) (Carson et al., 2006). The use of the essential oil, tea tree oil, became popular within the 20th century. Prior to the scientific discoveries of the benefits of TTO from *M. alternifolia* in the 1920s and 1930s, the raw materials of *M. alternifolia* were used (Carson et al., 2006). A steam distillation is preformed of the leaves to extract the essential oil, which is now in demand today (Carson and Riley, 1993). In a more crude process of extracting the essential oil, people would simply break off the leaves and terminal branches and do a very simple steam distillation over a temporary wood fire (Carson et al., 2006).

The Aborigines used *M. alternifolia* for skin infections, bruises, insect bites and cuts by sprinkling the crushed leaves over wounds (Carson et al., 2006). *M. alternifolia* has also been used for cleansing and dressing wounds (Carson and Riley, 1993). Medicinally in Aboriginal culture, the crushed leaves were inhaled for coughs and colds while an infusion of the leaves were used for treatments of sore throats (Carson et al., 1993; 2006). The Aborigines also noted sacred healing rivers in which leaves of the *M. alternifolia* fell in the river, creating a simple infusion; these rivers were used for healing (Carson et al., 1993; 2006). It is unlikely that the Aborigines used extractions of *M. alternifolia* for its essential oil, but they were aware of the plant's medicinal benefits (Carson et al., 1993).

There was no documentation of medicinal uses of *M. alternifolia* prior to colonization of Australia as the Aborigines had an oral tradition (Carson and Riley, 1993). Due to this oral tradition, there are no ancient texts documenting the medicinal uses of *M. alternifolia*. The oral tradition of the Aborigines influenced modern physicians to look more closely at *M. alternifolia* that led to the increase of scientific knowledge of the tea tree in the past century.

The use of *M. alternifolia* has changed over time. The raw materials were originally used, but now the focus is on the essential oil that is extracted through steam distillation. It is now harvested for its oil (Butcher et al., 1994). The traditional uses of *M. alternifolia* are still relevant today. These uses provide scientists and physicians with an idea of what it could be used to treat. Since the Aboriginal culture has relied on *M. alternifolia* for so long, this indicates that there must have been some efficacy associated with its medicinal use.

Chemistry and Pharmacology

The essential oil of *M. alternifolia*, tea tree oil (TTO), is the focus of research and is extracted by steam distillation from

the evergreen's leaves (Brophy et al., 1989). This essential oil is similar to nutmeg oil and contains terpinen-4-ol (**Figure 2A**), 1,8-cineole (**Figure 2B**) and α-terpineol (**Figure 2C**) (Borphy et al., 1989; Carson et al., 2002). Tea tree oil from M. alternifolia is insoluble in water (Carson and Riley, 1993).

There are actually three different varieties of *M. alternifolia* according to the concentration of cineole; low, medium and high, with low being the most sought after for medication (Brophy et al., 1989). The essential oil of *M. alternifolia* is composed of monoterpenes, terpinen-4-ol, cineole and various hydrocarbons (Carson and Riley, 1993). Of the monoterpenes, 50% are oxygenated and 50% are hydrocarbons (Cox et al., 2000). The International Standard of *M. alternifolia* oil is a minimum of 30% terpinen-4-ol and a maximum of 15% 1,8-cineole (Homer et al., 2000). The composition of the oil of *M. alternifolia* may change if not stored properly (the proper storing requires a cool, dry, dark setting in a container with as little air as possible) (Carson et al., 2006).

Biological Activity

The essential oil of *M. alternifolia* known as tea tree oil (TTO) is the main constituent that contributes to the biological activity of *M. alternifolia*. While there is still speculation regarding how TTO is able to disrupt the cell membrane functions, it is hypothesized that there is not one single mechanism of action due to its heterogeneous composition and structure (Carson et al., 2002). The essential oil of *M. alternifolia* is able to disrupt cell membrane function by increasing permeability, disrupting homeostasis and inhibiting cell respiration (Carson et al., 2006). TTO and other terpenes have been shown to affect cell respiration, leading to the hypothesis that it has an adverse effect on the mitochondria organelles within cells (Hammer et al., 2004).

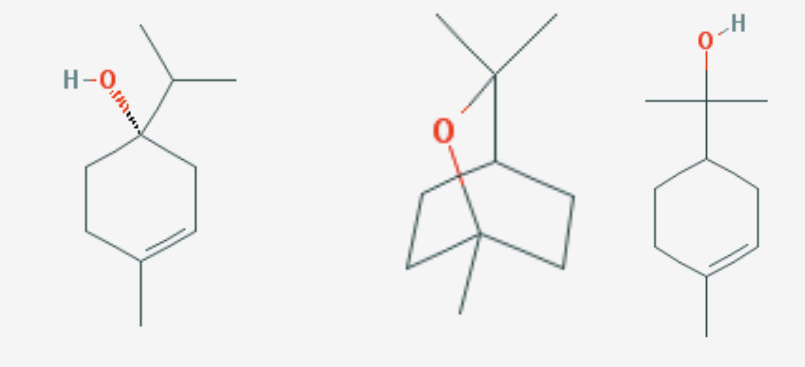

Figure 2. Chemical structures of (A) Terpinen-4-ol, (B)1,8-cineole, and (C) α-terpineol – all main constituents of *Melaleuca alternifolia* oil. (Source: http://pubchem.ncbi.nlm.nih.gov.proxy.library.emory.edu/summary/summary.cgi?cid=2724161&loc=ec_rcs)

These effects from TTO lead to cell death (Cox et al., 2000). TTO has also been observed to increase potassium ion leakage from cells (Cox et al., 2000). TTO can permeate skin very easily, but this also dependent on how it is applied to the skin (an ointment will permeate the skin more than a cream for example) (Reichling et al., 2006).

TTO has demonstrated a variety of uses ranging from antibacterial and antifungal to antiviral and anti-inflammatory (Carson et al., 2002; 2006). *M. alternifolia* is even used for flavoring and an antiseptic for mouthwashes (Brophy et al., 1989). *M. alternifolia* has been shown to be great for treating oral infections, such as oral candidiasis, but is not very useful as a preventative measure (Hammer et al., 2003). While TTO has demonstrated a variety of uses, there have not been many investigations displaying these uses *in vitro* (Carson et al., 2006).

M. alternifolia has demonstrated to be effective against furunculosis, wood-destroying fungi, vaginitis, oral vaginitis,

dermal infections, ulcers and cuts (Brophy et al., 1989; Budhiraja et al., 1999). TTO can penetrate body tissues and shows high biological activity with low irritation effects (Budhiraja et al., 1999). White blood cells were activated after interacting with TTO (Budhiraja et al., 1999).

As an antibacterial, TTO was shown to kill *Staphylococcus aureus* during its stationary phase within the host (Carson et al., 2002). During one investigation of antiviral uses of the essential oil of *M. alternifolia*, TTO was tested on tobacco plants with tobacco mosaic virus (Carson et al., 2006). Tobacco plants treated with TTO did show a decrease in lesions in comparison to controls (Carson et al., 2006). Tea tree oil was also studied for antiprotozoal activity, showing a 50% decrease in growth primarily due to terpinen-4-ol found in TTO (Carson et al., 2006). *M. alternifolia* is best when attacking "free" viruses that have not yet infected a cell (Carson et al., 2006). *In vitro* studies of TTO have shown both antimicrobial and anti-inflammatory activity (Carson et al., 2006). TTO has been difficult to evaluate for antimicrobial activity because it is not soluble in water (Carson et al., 2006).

While investigating the antibacterial use of TTO on *Escherichia coli, Staphylococcus aureus* and *Candida albicans*, it was concluded that TTO was able to inhibit cell respiration and increased the permeability of the bacterial cytoplasm and yeast cell membrane through the increased uptake of propidium iodide, which is normally impermeable to the cell (Cox et al., 2000). Potassium ion leakage of *E. coli* and *S. aureus* cells was observed when exposed to TTO (Cox et al., 2000).

One *in vitro* study of TTO investigated the effect on bacterial vaginosis, which is a growth of fungus within the vagina, not to be confused with a yeast infection (Kessel et al., 2003). A capsule composed of a few drops of TTO and gelatin inserted into the vagina overnight for six days was able to control the infection by inhibiting both aerobic and anaerobic respiration (Kessel et al., 2003). This treatment was also estimated to be of low cost to the patient ($2.00 - $20.00 per day) (Kessel et al., 2003).

M. alternifolia is an effective treatment of fungal infections such as dandruff or oral candidiasis, a yeast infection that allows for fungal growth on the mucous membranes of the mouth typically the tongue (Hammer et al., 2004). In an *in vivo* study of *M. alternifolia* against *Candida albicans*, terpinen-4-ol of TTO was showed to be the main contributor to treatment of the fungal infection and eliminating the fungus within three weeks (Mondello et al., 2006). The use of *M. alternifolia* for treatment of *C. albicans* would be cost effective because the only active compound is terpinen-4-ol, allowing for faster production and lower costs to patients (Mondello et al., 2006).

TTO showed antioxidant activity during an investigation by Kim et al. (2004) using two assays to determine the activity: 2,2-diphenyl-1-picrylhydrazyl and hexanal/hexanoic acid. The assays confirmed antioxidant activity due to α-terpinene, α-terpinolene and γ-terpinene, rather than the expected terpinen-4-ol (Kim et al., 2004). The antioxidant activity of *M. alternifolia* oil is similar to butylated hydroxytolene (BHT), a synthetic antioxidant used as an additive to food and cosmetic products; this suggests that TTO may be a good, natural alternative to butylated hydroxytolene (Kim et al., 2004).

M. alternifolia is used topically for infections of the skin (Carson et al., 2006). Several investigations have been conducted to observe the effect of tea tree oil on mild acne. Oil was extracted from *M. alternifolia* and made into a gel (Magin et al., 2006). This was compared to 5% benzoyl peroxide, a typical compound used in skin care products (Magin et al., 2006). While 5% benzoyl peroxide was better at controlling inflammatory lesions, *M. alternifolia* oil was able to reduce

non-inflammatory lesions and provides less side effects such as itching, redness and stinging, etc. (Carson and Riley, 1993; Magin et al., 2006).

Hammer et al. investigated resistance of *Staphylococcus aureus, S. epidermidis* and *Enterococcus faecalis* (all Gram-positives) to TTO and rifampicin (an antibiotic) (2008). Low resistance of *S. aureus, S. epidermidis* and *E. faecalis* to TTO was observed ($<10^{-9}$), indicating that single-step mutants of these bacteria have low resistance to TTO (Hammer et al., 2008). Recently, Hammer et al. investigated resistance of *S. aureus* and *Escherichia coli* to TTO (2011). In this study, multi-step mutants were evaluated and Hammer et al. concluded that TTO has little effect on the development of resistance of *S. aureus* and *E. coli* (2011).

TTO has been seen to be effective against cancerous melanoma cells (Buzzoto et al., 2011). One reason that melanoma is so deadly is because it is resistant to chemotherapy and radiation treatment (Buzzot et al., 2011). TTO has been observed to inhibit the growth of melanoma cells by overcoming their resistance of apoptosis (Buzzoto et al. 2011). This data shows how TTO is a possible alternative to chemotherapy and radiation to treatment of melanoma.

Clinical Studies

Barker and Altman investigated the efficacy of TTO, lavender oil, suffocation product and pyrethrin products against head lice (2010). Lavender oil and TTO was almost four times more effective than products containing pyrethrin in killing lice after one day of treatment (Barker and Altman, 2010). These results show that not only could TTO be used in place of pyrethrin-containing products for individuals that would have an adverse effect to pyrethrin, but also that TTO is more effective (Barker and Altman, 2010).

TTO has also been used in a clinical study to evaluate double-blind testing (clinical trials where both the investigator and research volunteers are unaware of receiving the control or treatment) (Carson et al., 2008). TTO is difficult to use in clinical trials due to its distinctive odor (Carson et al., 2008). Carson et al. investigated the use of deception by looking at TTO as an effective treatment of recurrent herpes labialis (cold sores) (2008). Carson et al. concluded that double-blinding is not ineffective (only 50% of volunteers in each test group were able to correctly identify if they received a placebo product or TTO product) (2008).

Contraindications

The long history of use of *M. alternifolia* and its essential oil has led to a general consensus that it is safe and because of this, there is very little scientific documentation of its safety with the exception of case studies (Carson et al., 2006). While the essential oil of *M. alternifolia* was shown not to be mutagenic by the Ames test, there are still cases of toxicity (Carson and Riley, 1993). That being said, ingestion of the essential oil, especially orally, can result in toxicity but there have yet to be any human deaths due to the oil of *M. alternifolia* as of yet (Carson et al., 2006). There are dangers of toxicity when TTO is ingested orally as it has been shown that even a modest intake of *M. alternifolia* has resulted in toxicity symptoms (Budhiraja et al., 1999; Carson and Riley, 1993; Jacobs and Hornfeldt, 1994). Toxicity due to TTO ingestion was observed in a 23-month-old after found with an empty bottle of T36-C7, which is made up of entirely tea tree oil (Jacobs and Hornfeldt, 1994). The child was described to have decrease motor coordination and was disoriented. Physicians were able to treat the patient and release him from the

hospital relatively quickly, but this case did show that a small amount (the bottle was 10ml) of oil extracted from *M. alternifolia* could be toxic when ingested orally (Jacobs and Hornfeldt, 1994). This has raised concern because of the prevalence of tea tree oil in soaps, lotions, creams, cleaning products and toothpaste (Jacobs and Hornfeldt, 1994).

Any skin irritation due to using TTO topically is mostly due to its improper storage and production rather than the constituents themselves (Carson et al., 2006). It is difficult to store TTO because plastics absorb the oil quite readily (Carson et al., 2006).

Current Use in Allopathic and CAM Therapies

You can currently purchase an array of products with TTO as a constituent. You can also find tea tree oil in soap, shampoos, skin care products, etc. (Jacobs and Hornfeldt, 1994). One commercial product of tea tree oil is Melasol (Carson and Riley, 1993). The production of Melasol was so important during World War II that those employed to collect *M. alternifolia* were not required to enter the service (Caron and Riley, 1993).

M. alternifolia is marketed as "tea tree oil." It may be purchased as just the oil, but is also part of many skin care and hair care products. The Body Shop has a complete "Tea Tree" line including facial washes, concealers and exfoliants. The tea tree oil used in these products is *M. alternifolia* that is cultivated in Africa. Typically tea tree oil is marketed as 100% pure essential oil and is advertised to be part of first aid kit to be used on cuts and abrasions. Other companies, including Desert Essence market tea tree oil skin ointments and creams, but the products are not entirely composed of the essential oil from *M. alternifolia* and are instead a mixture of oils from several plants.

Some more obscure current uses of *M. alternifolia* include its use for ceasing cannibalism among chickens (Carson and Riley, 1993). Not only did *M. alternifolia* heal the wounds of chickens, but also the constituents within the plant created an irritant to chickens causing them to stop eating other chickens because of taste (Carson and Riley, 1993).

Discussion

Melaleuca alternifolia is an economically important plant that has a variety of medicinal uses. *M. alternifolia* has been a central part of Australian Aborigine medicine and is now a sought after plant for its essential oil. TTO has shown to be beneficial for a variety of treatments and as more clinical investigations are conducted, we will have a greater understanding of TTO's power to be used medicinally.

TTO is used as an antibacterial, antimicrobial, antifungal and antiviral. It also is useful for skin abrasions, mild acne and cuts. Further research should be done of how the Aboriginal culture used *M. alternifolia* and see how those traditions and methods can still be applied to medicine today. The many studies discussed here show how *M. alternifolia* and more specifically, TTO, can be beneficial for human health, providing more cost effective treatments than those currently used.

From its use as an antibiotic to another therapy in battling melanoma, TTO has great potential. Tea tree oil is more than just an essential oil to be purchased at a health food store, but instead an oil that has a wealth of opportunities to help human health.

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Momordica charantia L., Cucurbitaceae

Soraya Chanyasubkit

Introduction

Many plants serve a dual role as food and medicine. With a majority of the world's healthcare system based in traditional medicine, up to 80% dependency in some regions according to the World Health Organization, plants are critical in health promotion and disease prevention. The term "functional food" refers to a modified food or specific food ingredient that can provide a health benefit beyond basic nutrition (Hasler, 2002) and can be applied to the many herbs and plants used in traditional healing. *Momordica charantia* is such an exemplary plant that is widely consumed in the tropics of Asia, the Caribbean, Africa, and the Amazon (**Figure 1**). It is in the Cucurbitaceae family, which also includes melons, squashes, and gourds. The plant's defining characteristic is its bitter taste, hence its common names like bitter melon, bitter gourd, balsam apple, balsam pear, sorosi, karela (Hindi), and ampalaya (Tagalog) (Kumar *et al.*, 2010; Grover and Yadav, 2004).

Aside from its culinary use, *M. charantia* is also employed in traditional medicine due to its hypoglycemic, antibacterial, antioxidant, wound-healing, and other therapeutic properties. It is typically prepared as a tea or bath by soaking the leaves, stem, and fruit in water or directly eating the juice or the fruit itself (Behera *et al.*, 2010). The key anti-diabetic compounds are charantin and momordicin, but bitter melon is known as a health-promoting vegetable due to the presence of many kinds of chemical compounds such as phenolic acids, saponins, triterpenes, and proteins (Kumar *et al.*, 2010). It has been incorporated into many cultures for over 3,000 years and continues to be an integral part of diet, ceremonies, and health worldwide (Behera *et al.*, 2010; Taylor, 2002).

Figure 1. Momordica charantia as the fruit and the plant.
(Image source: http://kookiecurious03.files.wordpress.com /2010/10/ampalaya.jpg)

Botanical Description

The most well-known part of *Momordica charantia* is its edible fruit which has an oblong shape, similar to a large cucumber, but with a rough surface full of ridges and warts. Unripe, it has varying shades of green but matures into a brilliant yellow-orange fruit that bursts to reveal the vibrant red seeds within. In a cross section of the fruit, the flesh is crisp and full of water and has a mostly hollow cavity similar to a bell pepper. In this cavity, a mesh, white pith suspends its flat seeds (**Figure 2**).

The fruit of *M. charantia* can be categorized into three groups which are described in **Table 1**. Depending on the phenotype, the fruit can range from a smoother, pale green (Chinese) to an incredibly warty and spiky surface of deep green (Indian). The size of the fruit can range from 9-12cm to 30-60cm, again depending on variety. However, a more recent categorization for Indian and Southeast Asian bitter melons is by diameter: *M. charantia* var. *minima* (< 5cm) and *M. charantia* var. *maxima* (> 5cm) (Behera *et al.*, 2010).

This herbaceous, tendril-bearing vine has leaves with jagged edges hence the genus name *Momordica* which means "to bite" in Latin. The plant itself can grow to a height of 2-4 meters. *M. charantia* is monoecious and its flowers have five petals and are typically yellow. Bees are the primary pollinators. All parts of *Momordica charantia* are bitter (Taylor, 2002).

M. charantia can tolerate a variety of environments from tropical to subtropical climates but it grows best in hot, humid areas. The cultivation of bitter melon is similar across countries except for the spacing between plants. Frost can kill the plants, and cooler weather can slow development. Ideally, it is grown in a sunny, warm area with well-drained sandy soil at pH 6.0 – 6.7 (Behera *et al.*, 2010).

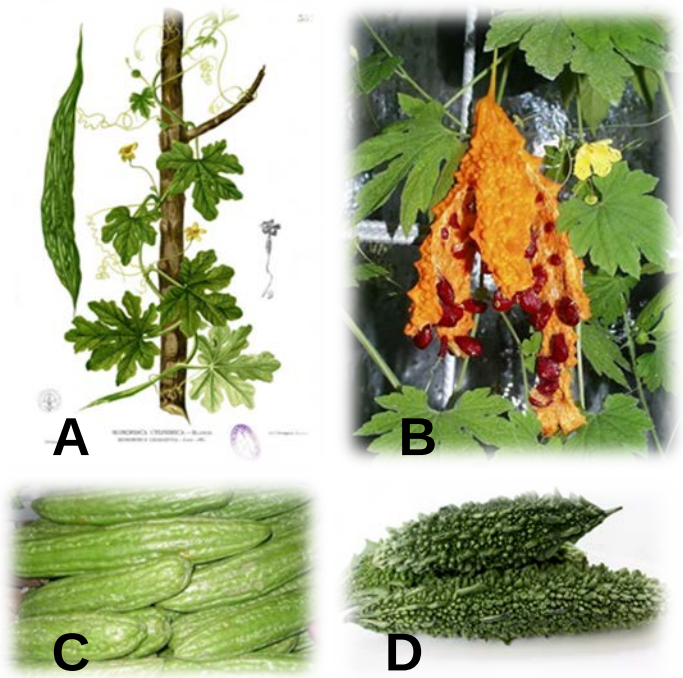

Figure 2. The growth and phenotypes of *M. charantia*. Bitter melon is a tendril-bearing vine with jagged leaves and yellow flowers (**A**). At maturation, the fruit is a brilliant yellow-orange and bursts to expose the seeds (**B**). The phenotypes of Chinese and Indian bitter melons are easily distinguishable (**C and D**).

(Image sources: **A** - http://www.herbal-supplement-resource.com/images/bitter_melon_herb_img.jpg; **B** - http://www.whitetigernaturalmedicine.com/wp-content/uploads/2010/10/Herb_0109.jpg; **C** - http://yourhomegardenblog.com/wordpress/wp-content/uploads/2009/03/chinese-bitter-melon.jpg; **D** - http://farm4.static.flickr.com/3318/3475604705 _27b918fed6.jpg)

	Small fruit type (var. *charantia* - Indian)	Long fruit type (var. *charantia* - Chinese)	Triangular fruit type (var. *muricata*)
Size	10-20 cm in length/ 5-8 cm in width	30-60 cm in length/ 3.5-6.0 cm in width	15-25 cm in length/ 9-12 cm in width
Weight	0.1-0.3 kg	0.2-0.6 kg	0.3-0.6 kg
Color	Dark green	Light green	Light-dark green
Bitterness	Extremely bitter	Slightly bitter	Medium-strongly bitter

Table 1. Categories of *Momordica charantia*. The phenotypes of M. charantia can be classified due to the distinct appearances of each type. The most commercial is the Chinese bitter melon due to its more suave bitter flavor.

Traditional Uses

The origin of *M. charantia* is unknown, though the domestication of the plant is thought to be in Asia, namely China or India. The first mention of the bitter melon is in Ayurvedic texts dating back 2000-200 BCE by Indo-Aryans in India. The ancient Chinese medicinal book Pen Ts'ao (or Ben Cao in pin-yin) referenced the bitter vegetable later in 1370 AD (Walter and Decker-Walters, 1988; Yang and Walter, 1992). The use of *M. charantia* moved westward through the African continent toward the New World into the Americas via the slave trade as one of many domesticates introduced from the Old World as early as the 16th century (**Figure 3**). The melon is a versatile vegetable used in soups and curries, stir-fries, or is stuffed or pickled. Before culinary use, the bitter melon is typically boiled, blanched, or soaked in salt water to attenuate the bitter flavor (Behera *et al.*, 2010).

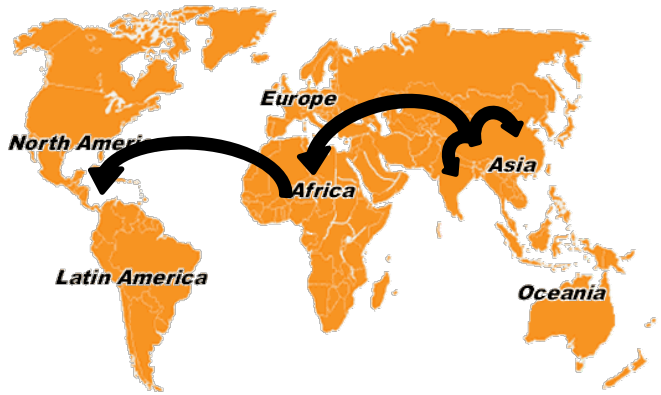

Figure 3. Origin and movement of *M. charantia*. Bitter melon is believed to originate in Asia near China and India, and its domestication spread to Africa and later the New World via the transatlantic slave trade.

Historically, the plant has been used all over the world as an edible plant product to treating a variety of ailments from gastrointestinal to veterinary to, most famously and universally, diabetes. Though its use has been primarily medicinal, the exact applications differ from region to region. Preparation across cultures typically involves extracting juice from the unripe fruit and leaves or soaking and boiling the plant in water to create tea or a bath (Basch, 2003).

Africa

M. charantia has a variety of uses to both traditional healers and the general population. According to Beloin *et al.* (2004), in Togo, bitter melon is used primarily not only to combat

gastrointestinal diseases and childhood viral infections like chicken pox, but also to treat infections and skin afflictions. The groups soaked the vine in water for several hours and the resulting liquid was drunk or bathed in, sometimes using vines as a sponge if necessary. The seed is applied locally in gynecological circumstances. It is notable that traditional healers and the villagers used the plant to treat the same diseases, except for diabetes where none of the general population uses the plant as a treatment and 33% of healers do. Healers use all parts of the plant except for seeds which 47% of healers agree that they are toxic. It is also notable that many healers hold university degrees and a few even have medical degrees and still rely heavily on plant-derived remedies and therapy.

Bitter melon also has strong spiritual and ritual properties in Togo. It is used to provide protection against evil spirits, diseases, malignant, intentions, and madness. It is viewed as a purifying plant and used in ceremonies involving sacred objects. The vine itself is worn as jewelry or a crown during traditional rituals. This custom is likely derived from the mid-1600s when the Guin tribe moved to current Togo due to warfare and the slave trade and wore the vines to protect themselves from enemies and ensure a safe journey (Beloin *et al.*, 2004).

In the Democratic Republic of Congo, whole plant decoction is used to treat many gastrointestinal afflictions such as intestinal worms, colic, diarrhea, dysentery, constipation and for general ailments like, diabetes, cold, hypertension, malaria, fever, anemia, psoriasis, and jaundice. It can also be used as a laxative and an aphrodisiac (Mesia *et al.*, 2008).

M. charantia is also one of the more frequently used plants in the treatment of measles in Nigeria and Cucurbitaceae is the most widely mentioned family according to interviews conducted by Sonibare *et al.* (2009). The primary methods of extraction are juicing the leaves or boiling the leaves and ingesting or bathing in the juive. According to recipes gathered from Nigerian herbalists, fresh leaves of M. charantia (local name: ejinrin) are washed and squeezed to extract juice. Two tablespoonfuls of this liquid is taken orally three times a day and in bathing morning and evening. In another recipe the leaves of ejinrin are boiled with the leaves of *Newbouldia laevis* Seem. (Bignoniaceae), *Vernonia amygdalina* Chevallier (Asteraceae) and *Ocimum gratissimum* (Lamiaceae) and the resulting concoction is drunk three times daily as well.

The Americas

Generally, across the Americas and the Amazon, locals and indigenous peoples grow bitter melon in their gardens as a source of food and medicine. Some applications are similar in use and purpose and other remedies are more highly emphasized depending on the region. In Mexico, the most common afflictions are diabetes and dysentery. Peruvian shamans use the plant mainly to treat different types of inflammation (Taylor, 2002).

However in Brazilian herbal medicine, the whole plant or various parts are used to combat many skin infirmities such as scabies, eczema, and leprosy, in addition to tumors, inflammation, allergies helminthiasis, rheumatism, diarrhea, dandruff, and wounds (de Albuquerque *et al.*, 2007; Taylor, 2002). In Colombia, it is taken as an oral infusion to treat intestinal parasites, fever, and pruritic ailments (Gomez-Estrada *et al.*, 2011). Various Amazonian Bolivian indigenous groups also use the mashed leaves for skin infirmities and considers *M. charantia* a valuable medicinal plant (Boudary *et al.*, 2000; Thomas *et al.*, 2010).

In eastern Nicaragua, bitter melon is used extensively in fertility health and childbirth among the Rama midwives. Taken orally or applied topically, *M. charantia* can relieve abdominal and back pain, post-partum abdominal pain, menstrual hemorrhage and pain, fever, vaginal infections, weakness and anemia and promote contraception and induce abortions (Coe, 2008).

The Caribbean

Trinidadian hunters use botanical and zoological methods to stimulate their hunting dogs, typically foxhounds, or to enhance their sense of smell to ensure more successful hunting trips. Lans et al (2001) interviewed a hunter who uses *M. charantia* to help his dogs acclimate to hunting for larger game, specifically wild hogs. First, he bathes the dog in congo lala (*Eclipta prostrate* L., Asteraceae) and the dog will begin hunting the small matte lizard. Next, the dogs are bathed in M. charantia (caraaili in Trinidadian Creole) and barbadine leaf (*Passiflora quadrangularis* L., Passifloraceae) and they will hunt for bigger game. Finally, the dog is bathed with unspecified plants that promotes hunt for wild hogs (Lans, *et al.*, 2001).

M. charantia usage is also based on the doctrine of signatures which dictates that a specific plant trait resembles the desired outcome; thus after application of this plant, the foxhound would want to hunt for the particular animal the plant corresponds to (**Figure 4**). In this case, there is an association between caraaili and agouti, a type of rodent similar to the guinea pig (Lans, *et al.*, 2001).

Another use of caraaili by Trinidadian hunters for dogs is for medical treatment rather than improved hunting. Crushed leaves and vines of *M. charantia* are steeped in bathwater for

Figure 4. Doctrine of Signature. Comparison of the agouti rodent and *M. charantia* which Trinidadians use in hunting (Image source: http://plantecology.files.wordpress.com/2010/05/agouti.jpg; http://upload.wikimedia.org/wikipedia/commons/5/54/Momordica_charantia_003.JPG)

dogs infected with mange, or parasitic mites, and then a poultice of other plant species can be applied to the affected areas. Aside from veterinary use, the vine is steeped in water to create a "cooling" tea for individuals who have too much "heat" or impurities in their system e.g. blood. In the Caribbean as a whole, *M. charantia* is used as an abortifacient, to treat diabetes, and to lower high-blood pressure (Lans, 2006).

China

Traditional Chinese Medicine is one of the oldest whole medical systems beneath the umbrella of complementary and alternative medicine founded on the principle of balance between *yin* and *yang*. If they are out of balance, this leads to disease. In the case of diabetes, a *yin* deficiency results in dryness in the body that can be remedied by eliminating the heat and promoting fluid (Li *et al.*, 2004). This can be achieved by the incorporation of a common traditional Chinese plant into the diet of the patient. *M. charantia* is one of 13 plants listed in Liu *et al.* (2004) study and has been used to treat diabetes in China for thousands of years through oral administration of the extract or fruit juice.

In addition, many plants in the Cucurbitaceae family are mentioned in ancient Chinese texts including *M. charantia*. Even in modern-day China, use of bitter melon is still highly prevalent to treat rheumatism, gout, dysentery, and toothaches (Yang and Walters, 1992). In tea form, it is also used to stimulate appetite and treat gastrointestinal infections (Kumar *et al.*, 2010).

India

As one of the countries with the longest tradition of *M. charantia* use, India has extensive applications for the plant. This exhaustive list includes but is not limited to "antidiabetic, abortifacient, anthelmintic, contraceptive, antimalarial and laxative and is used for treatment of dysmenorrheal, eczema, emmenagogue, galactagogue, gout, jaundice, kidney (stone), leprosy, leucorrhea, piles, pneumonia, psoriasis, rheumatism, and scabies" (Grover and Yadav, 2004).

In specific regions of India, use of the M. charantia (local name: karela) is more specialized. For example, in the Moradabad district of northern India, it serves an ethnoveterinary role (Ali, 1999). The fruit is mixed with onion (*Allium cepa* L., Amaryllidaceae) and vinegar and the resulting paste is applied to the forehead, horns, and legs of cattle with ephemeral fever. Boiled in equal quantities with *Boerhavia diffusa* L. (Nyctaginaceae), *Luffa acutangula* L. (Cucurbitaceae), onion, and sugarcane, it is an oral remedy for stomach disorders and fever.

In the northwestern state of Rajasthan, karela treats a variety of afflictions. The whole plant is combined with cinnamon, long pepper, rice and *Hydnocarpus wightiana* Blume, (Achariaceae) oil for topical application of scabies and other skin disorders. The leaf juice is externally applied around the eyes for night blindness. The paste of its roots is also used topically over piles. Of course, ingestion of one spoonful of fruit paste in water can reduce blood sugar (Namsa *et al.*, 2011).

Other miscellaneous applications of karela across India include a topical snakebite antidote when mixed with olive oil (Samy *et al.*, 2008), topical paste when mixed with lime for skin diseases like dhobis itch and ringworm (Mahishi *et al.*, 2005), and as a blood purifier for diabetes and eye infections (Jain *et al.*, 2005)

Chemistry and Pharmacology

Momordica charantia has a variety of phytochemicals dispersed throughout the plant anatomy, totaling to 228 known compounds. The leaves are rich in alkaloids, flavonoids, hecogenins, steroidalglyscoside, saponins, and tannins. Many minerals like iron, calcium, copper, and zinc are present in the roots (Bakare, 2006). The essential oil from the

seeds is comprised mostly of trans-nerolidol (61.6%) and apiole (8.9%) and many trace compounds (Braca *et al.*, 2007).

The fruit, the most used part of the plant, contains glycosides, saponins, alkaloids, triterpenes, proteins, and phenolic compounds and steroids, which contribute to the range of medicinal uses. Namely, charantin (a steriodal saponin), momordicin (an alkaloid), and protein P-insulin demonstrate anti-diabetic activity (**Figure 5**). Phenolic compounds have an antioxidant, anticarcinogenic, and anti-inflammatory effects. The protein MAP-30 shows potential anti-HIV properties. The plant in general is rich in vitamin A, vitamin B, and vitamin C (Kumar *et al.*, 2010, Singh *et al.*, 2011). A more exhaustive list of known chemical constituents in *M. charantia* can be found in **Table 2**.

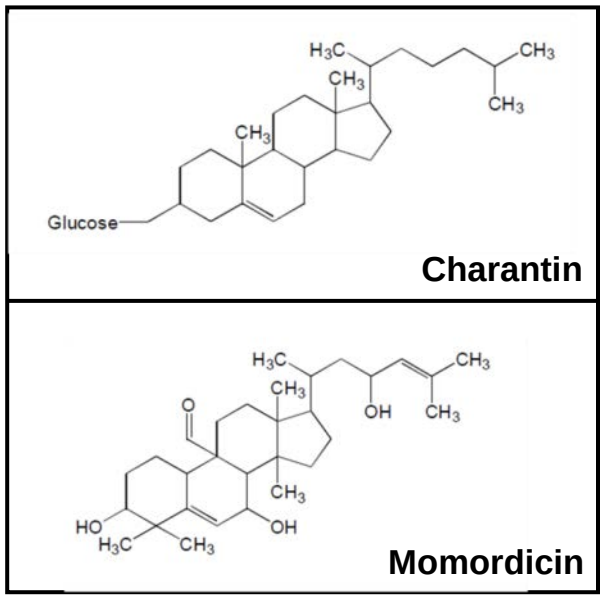

Figure 5. Structures of Charantin and Momordicin.

Plant Part	Chemical Constituents
Fruits	Momordicoside K, L, two acylglycosylsterols-3-O-[6%-O-palmitoyl-b-D-glucosyl] stigmast-5,25(27)-diene and 3-O-[6%-O-stearyl-b-D-glucosyl]stigmasta-5,25(27)-diene, benzyl alcohol, myrtenol, *cis*-3-hexenol, *trans*-2-hexenal, 1-penten-3-ol, *cis*-2-penten-1-ol, charantin, stigmast-5,25-diene-3b-O-glucoside
Leaves	Octacosane, 1-triacontanol, 7-stigmasten-3b-ol, 7,25-stigmastadien-3b-ol, 5,25-stigmastadien-3b-ol glucoside, phytosphingosine, momordicine I, II, III
Tendrils	Benzyl alcohol, myrtenol, *cis*-3-hexenol, *trans*-2-hexenal, 1-penten-3-ol, *cis*-2-penten-1-ol
Seeds	b-sitosterol-b-D-glucoside, stearic acid, two lectins, two new triterpene glycosides – momordicosides A and B, characterized as 3-O-b-gentiobioside and 3-O-b-D-xylopyranosyl (1.4)-[b-D-glucopyranosyl (1.6)]-b-D-glucopyranoside, respectively, of cucurbit-5-en-3b,22(*S*),23(*R*),24(*R*),25pentaol; momordicosides C, D and E characterized as 3-O-b-gentiobiosides of cucurbit-5-en-3b,23,24,25-tetraol, cucurbit-5,24-dien-3b,22,23-triol and 3b-hydroxy-23.24,25,26,27-pentanor-20(j) cucurbit-5-en-22-al, respectively; two cytokinins – zeatin and zeatin riboside –; two proteins a and b momorcharins, p-cymene, hexadecanol, menthol, nerolidol, pentadecanol, and squalene, 10a-cucurbit-5,24-dien-3b-ol, 24-methylencycloartanol, taraxerol, b-amyrin, campesterol, cycloeucalenol, 24b-ethyl- 5a-cholesta-7-trans-22-dien-3b-ol, 24b-ethyl-5acholesta-7-trans-22,25(27)-trien-3b-ol, lophenol, 4a-methylzymosterol, obtusifoliol, spinasterol, stigmasterol, stigmasta-7,25-dienol and stigmasta- 7,22,25-trienol, momordica anti-protein (MAP 30), ribosome-inactivating-proteins (RIPs)
Unripe fruits	Momordicosides G, F1, characterized as 3-O-b-D-allopyranoside and 3-O-b-D-glucopyranoside, respectively, of 5,19-epoxy-25-methoxy-5b-cucurbita-6,23-dien-3b-ol, momordicosides F2, I characterized as 3-O-b-D-allopyranoside and 3-O-b-D-glucopyranoside of 5,19-epoxy-5b-cucurbita-6,23-dien-3b,25-diol, 3-O-[6%-O-palmitoyl-b-Dglucosyl]-stigmasta-5,25(27)-diene and stearil derivative

Table 2. Chemical constituents of Momordica charantia by plant part. *Courtesy of Scartezzini and Speroni, J of Ethnopharmacology 71 (2000).*

Biological Activity

Hypoglycemic activity

Since its initial documented use thousands of years ago, *Momordica charantia* has been associated with reducing blood sugar levels. The compounds involved in this process have come to light, though the exact mechanisms of action are still under debate. Charantin, a steroidal saponin, momordicin, an alkaloid and protein P-insulin all have structures similar to animal insulin, leading to the hypothesis that their makeup plays a hypoglycemic role. Unfortunately, there is a dramatic lack of standardization among the studies, and concrete conclusions are difficult to glean. There are discrepancies among concentrations, extraction procedures, experiment duration, and model organisms used; though for the most part, there is a consensus that *M. charantia* does reduce plasma glucose levels (Basch *et al.* 2003).

Some proposed methods include stimulation of pancreatic insulin secretion, attenuation of hepatic gluconeogenesis, or rise in hepatic glucagon synthesis. Application of *M. charantia* fruit juice increased the number of functional beta cells which produce insulin in the pancreas though not to the normal amount. Other literature suggests an extra-pancreatic mechanism of action, resulting in increased glucose breakdown in the liver, inhibition of glucose-6-phosphatase, or enhanced GLUT4 transporter protein in muscles (Kumar *et al.*, 2010, Singh *et al.*, 2011).

Fernandes *et al.* (2007) attempts to provide experimental evidence for hypoglycemic activity by using a well-standardized *M. charantia* extract in alloxanic diabetic rats compared to glibenclamide, a current anti-diabetic drug. The sample was obtained through an ethanolic extract and received as 10% processed powder. Their results showed an increase in insulin serum, a decrease in plasma glucose, and a less damaged pancreas compared to control untreated rats. Thus, the authors concluded "MCE [Momordica charantia extract] exhibited promising anti-diabetic activity in alloxan diabetic rats" (p 7). In this study, the researchers suggested that the extract increased pancreatic beta cells insulin stimulation.

New literature proposes that *M. charantia* extract possesses 11β-hydroxysteroid dehydrogenase type 1 inhibitor (11β-HSD1) which is involved in the etiology of obesity and thus type 2 diabetes. 11β-HSD1 has been implicated in regulating obesity: 11β-HSD1-deficient mice have better lipid and glucose profiles and overexpressed-11β-HSD1mice are obese and have diabetes. Blum *et al.* (2011) suggests that the presence of this inhibitor contributes to *M. charantia*'s anti-diabetic activity and opens another avenue for future research.

Antioxidant/Anticarcinogenic activity

Aside from its strong hypoglycemic activity, *Momordica charantia* has also been implicated in having antioxidant and antitumor properties as well. Though there is no evidence to suggest that bitter melon can treat cancers, research suggests that it can retard or suppress tumor growth. Pitchakarn *et al.* (2010) observed bitter melon leaf extract reduced cell growth, invasion, and migration in rat prostate cancer cell line PLS10 in vitro. The authors also observed reduced metastatic lesions in lung cancer, though not in incidence in rats. This study significantly showed the anti-metastatic activity of *M. charantia in vitro* and *in vivo*.

Crude extracts have also shown anticancer effects in leukemia, lymphoma, melanoma, breast cancer, and many other cancers. Methanol extract has demonstrated cytotoxicity in human

cancer cell lines. The mechanisms of action for this property include inhibition of guanylate cyclase, disruption of G2 and M phases of cell cycle, activation of NK cells, or instigation of apoptosis (Kumar *et al.*, 2010).

Antimicrobial/Antiviral activity

Another property of *M. charantia* that makes it a widely-used and beneficial plant is its ability to fight off nearly any type of infection. The leaf extracts of bitter melon have demonstrated a wide-range of antimicrobial effects including activity against *Escherichia coli, Salmonella paratyphi, Helicobacter plyori* and *Myobacterium tuberculosis*. It also has demonstrated antihelminitic properties against *Ascaridia galli* worms, antiprotazoal properties against *Trypanosoma brucei brucei*, and antifungal properties against *Candida albicans* and *Cryptococcus neoformans* (Grover and Yadav, 2004). Such infections are common in the developing world and the value and benefit of bitter melon is immediately obvious in these circumstances.

Notably, protein MAP 30 has potential as antiviral drug, specifically in HIV treatment. This activity can be credited to inhibition of HIV-1 integrase and viral reverse transcriptase. *M. charantia* extract has also been shown to combat herpes and the polio virus, likely by inhibiting protein synthesis (Grover and Yadav, 2004).

Clinical Studies

Few clinical studies have been conducted to verify, quantify and explore the mechanism of action of glucose tolerance due to *Momordica charantia.* Leatherdale *et al.* (1981) conducted a study with nine non-insulin-dependent diabetic Asian subjects. The human subjects underwent glucose tolerance tests (GTT) as a standard test and after consumption of 50 ml of bitter melon juice and eight to 11 weeks of consuming fried bitter melon. In both instances where the subjects ingested *M. charantia* regardless of modality, the blood sugar level declined. There appears to be a significant decrease in plasma glucose level between the standard and the consumption of the juice. However, it is not apparent if the authors conducted a statistical analysis between consumption of the fried vegetable and the standard or the juice. They conclude their study demonstrated *M. charantia*'s hypoglycemic properties.

Basch *et al.* (2003) compiled the literature of previous clinical studies in a review article including Leatherdale *et al.* (1981)'s study and noted the many shortcomings that were common in all the previous research conducted: not randomized and double-blind, lack of standardization and controls, poor experimental set-up, and establishment of patient history and proper baselines. The authors suggested that though the research has shown *M. charantias*'s antidiabetic activity, these are only preliminary studies.

A more recent study, however, provides more conclusive data and results. In Thailand, Fuangchan *et al.* (2011) conducted the first multi-week, randomized, large sample size, double-blind, active-control trial in four paralleled treatment groups to determine the minimum effective dose of M. charantia in newly diagnosed type 2 diabetes patients. Headache, dizziness, and increased appetite in the highest concentration group were noted as low-incident side effects and bitter melon was generally well tolerated. This study concluded that 2000 mg/day of bitter melon significantly lowered fructosamine levels and had a modest hypoglycemic effect.

Contraindications

Though *Momordica charantia* is widely eaten and incorporated into many diets around the world, it should be avoided in certain circumstances. Bitter melon has historically been used as an abortifacient and should not be ingested during pregnancy as it might result in premature birth or accidental abortion. In general, it has been shown to reduce fertility in females and sperm production in males. Thus, couples trying to get pregnant should avoid eating this vegetable (Basch *et al.*, 2003).

A large-scale study has not been conducted investigating the potential harmful effects of bitter melon, but rather isolated cases of strong side effects have been reported. Of course, any individuals with known allergies to the Cucurbitaceae family such as melon, pumpkin, or squash should avoid consumption of bitter melon (Basch *et al.*, 2003). Fruits in this family have been reported to be one of the most allergy-causing fruits in the United States (Figueredo *et al.*, 2000). Similarly, people of Mediterranean or Middle Eastern descent or that have a glucose-6-phosphate dehydrogenase deficiency should take caution in the ingestion of bitter melon seeds which has the compound vicine that can cause favism, or hemolytic anemia. The red arils enveloping the seeds are also toxic to children and can cause headaches (Basch *et al.*, 2003). All parts of the plant register low toxicity, but extracts administered intravenously or intraperioneally in high doses can cause death in laboratory animals (Gover and Yadev, 2004).

Due to its hypoglycemic effects, it has had great success as a possible treatment for diabetes. However, this property can be exacerbated when used in conjunction with other anti-diabetic medication. Two incidences of hypoglycemic coma and convulsions have been reported in children after consumption of bitter melon tea. Also, additive-glucose lowering effects have been reported when bitter melon was consumed while the patient was taking sulfonylureas, a class of antidiabetic drugs that stimulate insulin production (Basch *et al.*, 2003).

Current Use in Allopathic and CAM Therapies

Generally, the current market for *Momordica charantia* is directed to promoting healthy blood sugar levels, reducing hyperglycemia and stimulating the immune system **(Figure 6)**. According to Birdee and Yeh (2010) in *Clinical Diabetes*, approximately a third of adults with diabetes use some type of

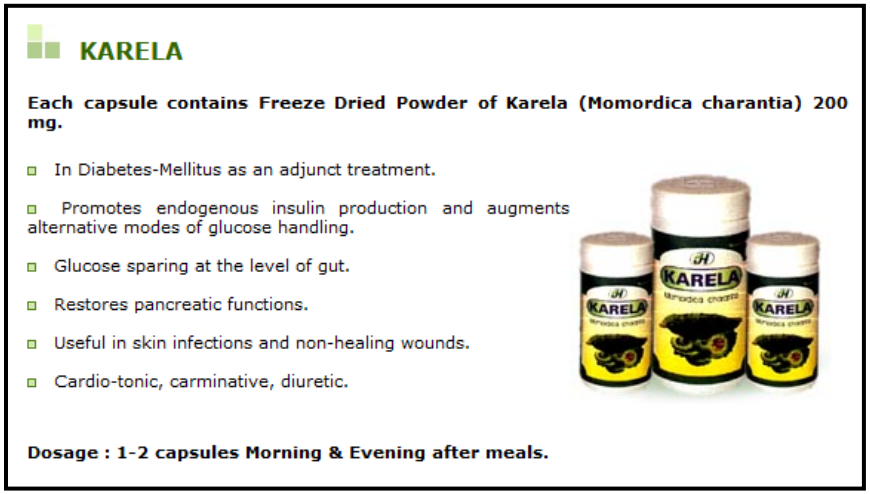

Figure 6. Example of how *M. charantia* is marketed today as an herbal supplement. Rather than the traditional method of eating the whole vegetable, pharmaceuticals have condensed the plant and marketed it as convenient, easy-to-swallow capsules. However, there is no mention of potential side effects or a consultation with a certified physician. Courtesy of http://www.ayurvedatoday.org/karela.htm

CAM therapy, which can include botanical sources, dietary supplements, and mind-body medicine. Many diabetes journals suggest *M. charantia* reduces blood glucose but also cautions its use due to lack of conclusive clinical studies (Birdie and Yeh, 2010; Yeh *et al.*, 2003; Krawinkel and Keding, 2006). Bitter melon can come in many forms. The encapsulated extract dosage is usually 100-200 mg 2-3 times daily or liquid form is 50-100 mL/d and the dry powder can range from 3-15g daily (Najm and Lie, 2010).

In Traditional Chinese Medicine, *M. charantia* is dried, powdered, and placed into capsules to make pills. This is the most common form that is available in the herbal remedy market (Covington, 2001). In local communities across the globe that have a long history with bitter melon, it is incorporated as a functional food rather than any direct treatment to an illness, and this is considered the safest method of ingestion because extract dosage has not been standardized nor extensively studied (Geil and Shane-McWhorter, 2008).

Discussion

The breadth of activity and illnesses that *Momordica charantia* encompasses and treats is truly far-reaching. History can witness the medicinal and therapeutic value this plant has had over many continents and throughout millennia. Though its current and traditional primary use is treatment of diabetes mellitus, many other applications have arose that are integrated to everyday life such as gastrointestinal affliction, skin infirmities, human fertility, infections and many more.

This wide spectrum of treatment is due to the hundreds of chemical constituents that comprise the bitter melon. Research continues today to analyze and better identify the components of this versatile plant. The one grave deficit in this vegetable's history is the lack of reliable, conclusive clinical trials in humans. The studies thus far have been poorly executed and thus have not effectively showed that the research conducted in mice, rats, and rabbits can be translated to human subjects.

Such conclusive research would be invaluable to current human health with the global increase in obesity, type 2 diabetes, and cancers. Incorporation of this vegetable into the daily diet, with certain restrictions of course, and purification of the active compounds into medication could produce more potent and efficacious resources in the future.

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Nepeta cataria L., Lamiaceae

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Introduction

Nepeta cataria L. of the Lamiaceae family (also known as the Labiatae family) is colloquially known as "catnip," "catmint," "catnep," "catwort," or "field balm." The genus name is derived from the city of Nepi (historically referred to as Nepete) in Italy and the species name from the late Latin word for cat, "cattus" (Coffey, 1993). Catnip is generally thought of in relation to its feline-attracting properties and the euphoric effect that the plant induces on cats. However, the herb has a long history of medical use, most prominently in treating fevers, anxiety, and gastrointestinal ailments (Grognet, 1990). The biological activities of catnip are generally attributed to the chemical nepetalactone, a monoterpenoid, and related stereoisomers (D'Amelio, 1999; Frohne & Pfander, 2005). Although neither the herb itself nor its active compounds are medically researched today, the compounds in *N. cataria* could have potential uses in insect repellants, including one for the malaria mosquito (Birkett, Hassanali, Hoglund, Pettersson & Pickett, 2011). These applications of the plant can still relate, however indirectly, to human health prevention and treatment.

Botanical Description

Geography & Related Plants

Nepeta cataria L. (**Figure 1**) is a perennial herb that grows up to three feet in height. It is indigenous to most of Europe, as well as to Southwestern and Central Asia. It has been reported that the plant was once much more common in some areas,

Figure 1. A flowering *Nepeta cataria* plant.
(Source:http://companionplants.com/catalog/product_info.php?products_id=981

especially in the United Kingdom, and may now even be extinct in Ireland (Stace, 2010). Catnip has been historically cultivated in North America and is thought to have been introduced to the continent by European settlement (Grognet, 1990). Today, it is particularly prominent in the United States and Canada, located nearly everywhere in the States and the lower Canadian provinces, especially in the northeast and north central states (**Figure 2**) (Sih & Baltus, 1987; Swerdlow, 2000). The plant grows in open fields and meadows and is adept at growing in dry, drained areas such as along roadsides, in waste lands, and in calcareous, rough soils

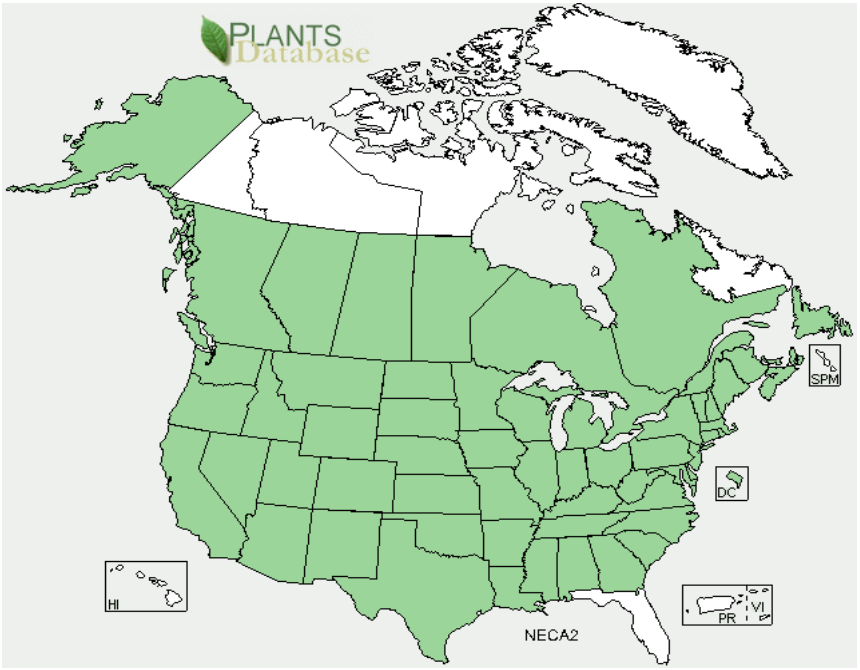

Figure 2. Growth range of *Nepeta cataria* in the United States and Canada. (Source: http://plants.usda.gov/java/profile?symbol=NECA2)

(Meuninick, 2008; Stace, 2010). One variation of the basic catnip plant is *Nepeta cataria* var. *citriodora*, or lemon catnip, which contains a higher level of the compound citronellal, giving this variety its distinct citrus scent (Baranauskiene, Venskutonis, & Demyttenaere, 2003). The term "catmint" can be used to describe the *Nepeta* genus as a whole, which includes several other cat-attracting herbs related to *N. cataria* such as dwarf catmint (*Nepeta racemosa*) and giant catmint (*Nepeta grandiflora*). The different species of the *Nepeta* genus are similar in leaf and flower structure and most are aromatic. The genus is also extremely diverse; over 150 of them are indigenous to temperate regions of Central Asia and

Figure 3. *Nepeta cataria* leaves. (Source: http://www.missouriplants.com/Whiteopp/Nepeta_cataria_page.html)

over 67 species are endemic to Iran (Domokos, Peredi, & Halasz-Zelink, 1994; Zomorodian, et al., 2012). Given that N. cataria is part of the Lamiaceae family, it is also more distantly related to many commonly used, aromatic culinary herbs such as basil (*Ocimum basilicum*), mint (*Mentha spicata*), and thyme (*Thymus vulgaris*) (Duke, 1985).

Herbal Description

Nepeta cataria L. has grey, quadrangular, erect stems with many branches and a downy texture (D'Amelio, 1999; Grognet, 1990; Meuninick, 2008). The heart-shaped leaves have a velvet-like quality to them; the blades have serrated edges and are green-grey in color with a grey underside,

which often gives them a white appearance (**Figure 3**) (Meuninick, 2008; Stace, 2010; Swerdlow, 2000). The small flowers of the plant grow in large clusters (Meuninick, 2008). The petals are usually white with purple spots and can also have violet or purple hues (**Figure 4**) (Crellin & Philpott, 1990; Stace, 2010). The flowers have four stamens with red anthers, which are shorter than the surrounding corolla (Stace, 2010; Swerdlow, 2000). *N. cataria* blooms starting in summer, from late June to September (Foster & Duke, 2000). All parts of the plant have a mint-like odor. The leaves have a sharp, bitter flavor that is also reminiscent of mint (D'Amelio, 1999; Grognet, 1990). A typical catnip plant will remain alive for four years after planting; a plant dies after its seeds ripen (Domokos, et al., 1994). The herb is typically pollinated by bees including honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) (Sih & Baltus, 1987).

Traditional Uses

Non-medicinal Uses

Nepeta cataria L. has a long history of both medicinal and non-medicinal use. The herb is best known for its euphoria-inducing effect in cats, producing a state resembling sexual excitement. Domestic cat owners have often grown the plant as a stimulant treat for their pets or for use in cat toys. In ancient Egypt, the plant was sacred to the cat goddess, Bast. Because the "catnip response" is also shown in larger, wild cats, the essential oil of catnip has historically been used as bait for trapping bobcats and mountain lions (Duke, 1985; Swerdlow, 2000). The taste and smell of catnip has appealed to humans as well as cats, however, due to its close relation to other aromatic, culinary herbs. The aerial parts of the plant have been used as a spice or flavoring, especially for sauces,

Figure 4. A *Nepeta cataria* flower cluster (Source: http://www.missouriplants.com/Whiteopp/Nepeta_cataria_page.html)

cheeses, and soups in Iranian cuisine (Duke, 1985; Zomorodian, 2012).

In the 1960s, catnip began to be experimented with as a hallucinogen, though the outcomes are not well-documented (Swerdlow, 2000). *N. cataria* was sometimes used in conjunction with or as a replacement for marijuana (Cannabis sativa). It could be mixed with tobacco or an alcohol tincture of the herb could be sprayed on the tobacco and smoked, which supposedly gives a stronger effect than smoking the leaf

alone. It is claimed that the effects of smoking catnip are similar to the relaxing, euphoric "high" induced by smoking marijuana; visual or auditory hallucinations also might occur (Grognet, 1990). However, catnip is reported to be less potent and quicker-burning than marijuana, meaning more catnip would have to be consumed in order to conjure an effect equivalent to that of cannabis. Little mention of any hallucinogenic effects were reported before the 1960s and the findings are mostly based on case studies. Most individuals report uplifted moods after smoking *N. cataria*, as well as an appreciation for music. Some researchers have suggested that the effects of catnip as a recreational drug are more similar to LSD than to marijuana (Jackson & Reed, 1969).

Medicinal Uses

Aside from the herb's obvious feline-entrancing qualities, *Nepeta cataria* L. has been mentioned in medical texts since the 16th century. The herb has been and still remains common in peasant gardens across Europe (Domokos, et al., 1994). Many uses were especially listed in early English literature; John Gerard, a British herbalist, mentioned "nep, catmint, and small catmint" in his 1597 herbal, claiming the plant had deobstruent properties (Crellin & Philpott, 1990). The frequent mentions of catnip were likely related to the fact that teas made out of the *N. cataria* plant were popular in England until black tea was introduced from China in the late seventeenth century. Nicholas Culpeper, an English scientist, prescribed catnip as a remedy for bruises, hemorrhoids, and head scabs (Swerdlow, 2000). The herb was also used as an English folk remedy for inflammatory conditions (Prescott, Veitch, & Simmonds, 2011). However, around the time of the eighteenth century in Europe, few writers mentioned catnip as an herbal remedy and in the several accounts given, few uses were mentioned. The treatments mentioned in relation to *N. cataria* around this time included those for gastrointestinal ailments, uterine disorders, and nervousness (Crellin & Philpott, 1990). Before it temporarily fell out of use, catnip was thought to induce fierceness in users; hangmen reportedly chewed the root of the herb before being led to their executions (Swerdlow, 2000). The supposed stimulating effect of the plant's root was opposite the sedative effects traditionally associated with catnip's leaves and flowers (Grognet, 1990). By 1847, catnip was administered in the form of a tea in Europe, parts of Asia, and the Americas, where it was cultivated for its ability to calm infant colic (Swerdlow, 2000). The herb was also traditionally thought to help with digestive issues, insomnia, headaches, and as a refrigerant (Grognet, 1990).

By the 1800s, catnip had become naturalized in America and was commonly mentioned in medical books (Crellin & Philpott, 1990). Among Americans living in the Ozarks, leaves were chewed to soothe toothache, and catnip tea was used as a nerve tonic and to cure colds, hives, fevers, and stomach ailments. Dried leaves were often also smoked as respiratory treatments (Duke, 1985). Besides its use by early colonists, the herb was also used widely among different Native American tribes across the States for a variety of ailments. **Table 1** shows a range of uses and preparations of *N. cataria* traditional to several of these tribes; the use of the herb was spread across all parts of the United States throughout different tribal regions. Among Native American groups, catnip is commonly cited for its use as a febrifuge, a medicine for children, and a general cold and cough remedy. The leaves and flowers are often prepared into a tea or other infusion, but other ways of preparation such as smoking the dried leaves or topically using the leaves have also been reported (Moerman, 2009). The plant's common use as a febrifuge

correlates to the plant's use in the Menominee tribe as a diaphoretic. In Europe as well as the Americas, catnip was mentioned for its ability to induce sweating without raising the body temperature; this helps to disperse the body heat associated with a fever (Swerdlow, 2000). Throughout its use worldwide, catnip has been frequently used in the treatment of infant colic and gastrointestinal issues (including diarrhea, flatulence, and stomach cramps), often in the form of infusion, as well as for bronchial and pulmonary conditions, fevers, and insomnia (D'Amelio, 1999; Duke, 1985). The herb is commonly mentioned for its supposed sedative effects in humans (unlike the stimulant effect induced in felines) and therefore has been used in easing headaches, menstrual cramps, muscle spasms, anxiety, stress, and sleep disorders (Meuninick, 2008). Catnip tea was once recommended as a treatment for scarlet fever and smallpox (Duke, 1985).

Tribe (Cardinal location in US)	Uses for *N. cataria*	Traditional Preparations (if known)
Cherokee (S, centr.)	Abortifascient Antihelmintic Anticonvulsive Cold remedy Cough medicine Dermatological aid (hives) Dermatological aid (boils, swelling) Febrifuge Gastrointestinal aid (colic, stomachaches) Pediatric aid Sedative Stimulant Tonic	Used in infusion Mixed into a syrup with honey Used in infusion Leaves applied to skin Used in infusion
Chippewa (N, NW)	Febrifuge	Concoction of leaves
Delaware (N, centr.)	Pediatric aid	Used with peach seeds
Keres (SW)	Stimulant	Used in baths for tiredness
Hoh (NW)	Pediatric aid	
Iroquois (N, NE, centr.)	Analgesic (headaches) Antiemetic (from unknown cause) Cold remedy Cough medicine Febrifuge Gastrointestinal aid Laxative Oral aid (excess salivation) Pediatric aid Sedative Throad aid	
Shinnecock (NE)	Antirheumatic	Dried leaves are smoked
Menominee (N)	Diaphoretic Pulmonary aid Sedative	Applied to chest for pneumonia
Ojibwa (N)	Blood purifier Raises body temperature	Used in baths

Table 1. Traditional uses and preparations of *Nepeta cataria* in various Native American tribes (Moerman, 2009)

Cosmetically, the herb can be mixed with other plants such as wintergreen and chaparral in a concoction that is used to soothe puffy eyes. The cooling effects of the herb have also contributed to its use as an antidandruff treatment (D'Amelio, 1999).

Though *N. cataria* has been cited for use in the treatment of a wide variety of conditions and ailments and became widespread in America after colonization, the herb began to be dismissed in the nineteenth century as a simple folk remedy. The Dispensatory of the United States in 1836 noted that catnip was used most often in domestic practices for colic, amenorrhea, and anemia, but was not used in formal medicine (Crellin & Philpott, 1990; Jackson & Reed, 1969). One source quotes that, in 1847 Pennsylvania, the herb was a popular medicine among "good ladies who deal in simples," suggesting that the herbal remedy was used more often by uneducated homemakers rather than physicians or other healers (Coffey, 1993).

Figure 5. The molecule structure of nepetalactone, the primary active compound in *Nepeta cataria.* (Source: http://www.creaturecomfortsinc.com/ThePetZone/CatnipInfo.htm)

Chemistry and Pharmacology

The main constituents in *Nepeta cataria* L. are a non-glycosidic iridoid, nepetalactone, and several related compounds, including nepetalic acid, nepetariaside, and nepetol rosemarenic acid (D'Amelio, 1999; Frohne & Pfander, 2005). Nepetalactone (**Figure 5**) is a methylcyclopentane monoterpenoid found in the essential oil of catnip, which can constitute up to one percent of the fresh, flowering tops of the plant (Downing & Mitchell,1974; Duke, 1985). The essential oil consists mostly of sesquiterpines and monoterpines (Heuskin, et al., 2008). Of the essential oil, up to 99% can be nepetalactone, though many studies using gas chromatography have shown the nepetalactone content to fall around 75% (Crellin & Philpott, 1990; Regnier, Waller & Eisenbraun, 1967). Nepetalactone is found in various amounts in all Nepeta species; some species also contain epinepetalactone, a related compound with a structural difference in one hydrogen group (Regnier, et al., 1967). Nepetalactone is believed to be the main cat-attracting compound in N. cataria and is thought to induce the mild cooling and sedating effects in humans. It is synthesized from mevalonic acid and related compounds such as mevalonic acid phosphate; these precursors are also involved in the biopathways that synthesize terpenes and steroids (Downing & Mitchell, 1974). The plant contains other terpenoids often found in the Lamiaceae family, including carvacrol (characteristic of oregano) and thymol (found in thyme) (Duke, 1985). These monoterpenoids contribute to the distinct aromas of the herbs. A monoterpenoid found

especially in *N. cataria* var. *citriodora*, or lemon catnip, is citronellal, which contributes to the lemony odor of the plant (Baranauskiene, et al., 2003). Several other compounds recently isolated from the catnip plant have showed to play a role in regulating inflammation. The caffeoyl phenylethanoid glycosides known as verbascoside and lamiuside A inhibit calcineurin, a protein phosphatase which regulates the gene expression related to T cells in the human immune system. This process leads to decreased immune response and could account for some of the traditional plant uses related to decreasing inflammation (Prescott, et al., 2011). The seed oils are also high in linoleic and linolenic acids, fatty acids that have antioxidant properties (Domokos, et al., 1994).

Biological Activity

Nepeta cataria L. displays biological activity in organisms other than humans and cats. The essential oil of the plant has been studied for its antimicrobial, antifungal, and antibacterial properties. This effect is believed to be related to the high concentrations of nepetalactone in the herb and its essential oil. The essential oil has bacteriostatic and bactericidal effects against *Salmonella* and *Shigella* species, *Staphylococcus aureus*, and *Escheria coli*. (Zomorodian, 2012). The oil inhibits gram-positive bacteria in lower concentrations than gram-negative bacteria. Ants, cockroaches, Afro-tropical mosquitoes (*Anopheles gambiae* and *Culex quinquefasciatus*, vectors of malaria and West Nile virus, respectively), the brown ear tick (*Rhipicephalus appendiculatus*), the red poultry mite (*Dermanyssus gallinae*), the stable fly (*Stomoxys calcitrans*) and the house fly (*Musca domestica*) have all been shown to be repelled by the essential oil in N. cataria (Birkett, et al., 2011; Regnier, et al., 1967; Zhu, et al., 2009). Nepetalactone and related compounds are released by certain species of female aphids as a pheromone during the sexual mating phase. Because catnip is a common target for aphid attacks, it is hypothesized that the plant produces this compound in order to attract aphid predators in order to reduce the levels of infestation (Kelly, 1996).

The mechanism of action that results in the feline euphoria related to *N. cataria* is relatively unknown. While cats sometimes ingest the leaves and stems of the plant, the "catnip effect" can be induced simply by smell. The response to the herb's aroma is characterized by actions similar to those shown in female cats in estrous; these displays include rolling on the ground, head shaking, rubbing the face and head on the ground and plant (**Figure 6**), chewing, and scratching (Kelly, 1996). Although these actions resemble females in heat, male cats also show these behaviors after being exposed to *N.*

Figure 6. A housecat exhibiting the "catnip response" to *Nepeta cataria*. (Source: http://www.vaswim.org/wp/wp-content/uploads/2011/04/cat_catnip2.jpg)

cataria (Palen & Goddard, 1966). The tendency for this reaction to catnip is governed genetically; the response is a dominant autosomal trait found in about two-thirds of all domestic cats (Foster & Duke, 2000). This effect is said to be similar to the effect of cannabis on cats. Similar activity is found for female cats in response to the urine of tomcats, which contains feline pheromones that may induce sexual receptivity in females. However, because the catnip response is also characteristic of male cats, it cannot be assumed that nepetalactone and related compounds are mimicking these pheromones (Mabberley, 2008). The effects of N. cataria in male cats do not result in characteristic sexual excitement; male cats show no mounting or aggression when presented with a cat-sized object after exposure to catnip (Palen & Goddard, 1966).These catnip-induced behaviors are also evident in larger members of the cat family. Lions and jaguars have shown high excitability in response to catnip, yet bobcats, tigers, and cougars showed little characteristic response. This same study also showed that the catnip response is higher in these larger felines around reproductive age than older or immature cats. Therefore, the catnip response differs not only with species of feline, but also with relative age of an individual (Hill, et al., 1976).

In addition to the cat family, *N. cataria* has shown to induce excitatory effects in male rats (Frohne & Pfander, 2005). The penile erections of rats have been shown to increase after the animals are fed catnip leaves. However, the overall activity of the rats reduces after ingesting *N. cataria*, except for a slight increase in sexual activity (Bernardi, Kirsten, Lago, Giovani, &, Massoco, 2011). This differs from the feline response in that there is a direct effect on the sexual organs of the animal, yet little effect on overall euphoric behaviors. It is hypothesized that sexual arousal is increased due to *N. cataria's* action on the dopaminergic system of the rat (Bernardi, et al., 2011). Catnip oil, which mainly consists of nepetalactone, has shown to increase sleeping time in rats as well. This may be related to the sedating effects of the compound which is evident in humans (Harney, Barofsky, & Leary, 1978).

Clinical Studies

Few clinical studies have been performed with *Nepeta cataria* L. or its active compounds, most likely due to the herb's declared status as a home remedy rather than a medical treatment. Rather than testing the biological effects of catnip in humans themselves, one study investigated the insect-repellent effects of two isomers isolated from the essential oil of catnip, E,Z- and Z,E- nepetalactone, along with whole catnip oil. In this study, using human volunteers, the biting activity of Aedes aegypti, the red eye Liverpool mosquito, was measured. The effects of nepetalactone were compared to effects from N,N-diethyl-3-methylbenzamide (the common, yet reportedly toxic, insect repellent, deet) and chiral (1S,2S)-2-methylpiperidinyl-3-cyclohexene-1-carboxamide (a new repellent isolated by the same lab, known as SS220). Repellant activity was tested both in vitro, using human blood, and *in vivo* using skin treatments with each chemical on six human volunteers. Catnip oil and both forms of nepetalactone were shown to repel over half of the mosquitos (measured by the proportion of mosquitoes not biting) and were equally as effective as deet. The new compound SS220, however, repelled more mosquitoes than any catnip extract or deet (Chauhan, Klun, Debboun, & Kramer, 2005). These data suggest catnip oil and its extracts as a safe and effective alternative to deet as a topically-applied insect repellent. Aside from these possible uses as a mosquito deterrent, however, the United States Pharmacopeial Convention has declared that the plant has no therapeutic value other than

that of an aromatic (Coffey, 1993). Overall, *N. cataria* has received little attention from the medical community, despite evidence for its antimicrobial and antibacterial activities (Crellin & Philpott, 1990).

Contraindications

It is recommended that pregnant women do not use *Nepeta cataria* L. treatments (Meuninick, 2008). Case studies have reported headache and a distorted sense of reality after smoking catnip; however, this was in relation to catnip as a hallucinogenic drug and not as a medical treatment (Jackson & Reed, 1969). Few side effects to using catnip have been reported, likely due to the fact that the plant only has a mild effect on humans in regular doses. The LD-50 of catnip oil is reported to be around 1300 mg/kg, meaning it would require ingestion of over 90 grams of pure essential oil to kill the average human (Harney, et al., 1978). The FDA has classified *N. cataria* as an "herb of undefined safety" (Duke, 1985).

Current Use in Allopathic and CAM Therapies

Nepeta cataria L. is still used in alternative medicine communities as a natural remedy for many of its traditional uses. Catnip is sold in many countries worldwide in its whole plant form, as dried leaves and flowers, and in extracts of its essential oil. The plant is still used as an allopathic treatment for fevers and headaches because of its cooling, sedating, and mild analgesic effects (D'Amelio, 1999). The tea has been used to stimulate the gallbladder and digestion. Catnip is mixed with elderberry to treat infections and used in tincture for arthritic joins when applied topically (Meuninick, 2008). The leaves are still chewed as a mild painkiller for toothache, especially in Appalachia where it grows abundantly in the wild. In these mountainous regions, the plant is also still used as a remedy for colds, skin infections, anxiety, and digestive issues (Duke, 1985). However, because of its lack of medical attention, the herb is mostly used and researched for its non-medical properties, such as the insect repellent compounds found in its essential oil and its popularity as a treat and toy for domestic cats.

Discussion

Most modern research on *Nepeta cataria* L. has focused on studying the effects of compounds in the plant on non-human animals and insects. Although the herb has been long-abandoned in way of its medical legitimacy, catnip still has potential to make an impact on human health. The repellant effect of catnip's essential oil towards several species of mosquitos and flies could help slow the spread of diseases carried by these insects, including malaria, West Nile virus, and filariasis. Using the plant as a lure for wild cats may not only save human lives by relocating these big cats from dangerous areas, but may also assist in the conservation and protection of these great felines. The herb has also shown promise in stopping the growth of or killing certain bacteria, fungi, and microbes. This may be applicable in future research for use as a topical or ingestible treatment, although the drug-resistant capabilities of catnip essential oil have not been researched. Even though *N. cataria* may not currently have much of a future in medical research, the plant can still impact human health as it has done historically, whether through its effects on other organisms or through its use as a mild home remedy.

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Panax ginseng C.A. Mey., Araliaceae

Mengshi Dai

Introduction

Panax ginseng C.A. Mey, otherwise known as Asian ginseng or Chinese or Korean ginseng, is an important medicinal plant in the Araliaceae family (Duke, 2012). Asian ginseng is called 'ren shen' (man plant) in Chinese, due to the root's forked shape which resembles a man's body. The genus *Panax*, derived from the Greek word 'panakos' (panacea) meaning 'all healing', refers to the many inclusive curative properties attributed to this plant (Swerdlow, 2000). Not to be mistaken for its American counterpart (*Panax quinquefolius* L.) or Siberian ginseng, (*Eleutherococcus senticosus* (Rupr. ex Maxim.) Maxim.), which belong in the same family, Asian ginseng is solely native to China and Korea. However it is also cultivated in Japan, Russia, and parts of North America. Asian ginseng has been used for centuries in Asia, but was introduced to the West in the 1700s by Father Jartroux who advocated its use in the treatment of fatigue, pleurisy, and convalescence. Ginseng's primary constituents are ginsenosides and panaxans. Ginsenosides stimulate the cardiovascular and central nervous systems to enhance physical and mental performance. Panaxans are polysaccharides derived from ginseng extract which lower blood sugar, strengthen the immune system, and treat diabetes (Swerdlow, 2000). Ginseng also has antioxidant, antiaging, and appetite-stimulating properties. In addition, the Chinese and Koreans use this plant for almost every ailment ranging from sexual potency to colds and even to cancers in the forms of teas, tonics made from decoctions, and infusions of the roots or powdered extracts (Harriman, 1973).

Figure 1. *Panax ginseng.* (Source: http://foodspeople.com/wp-content/uploads/2011/05/ginseng1.jpg)

Botanical Description

Panax ginseng (**Figure 1**) grows on mountainous or hilly forest slopes in the Manchurian region of China, and North Korea. It thrives in small patches in climates with cold and dry air, especially in wooded regions where the shady forest

Figure 2. Stem, Leaves, and Flowers of *Panax ginseng*. (Source: http://i01.i.aliimg.com/photo/v0/269775813/plant_extract_panax_ginseng_P_E_ginsenoside.jpg)

canopy offers protection from wind, rain, and sun. Asian ginseng grows among hardwoods rather than conifers, and often grows together with basswood (*Tilia tomentosa)* and royal paulownia (*Paulownia tomentosa*) (Harriman, 1973). In addition, Asian ginseng requires rich, moist soil and around five to seven years to mature fully (Swerdlow, 2000). These difficult growing requirements cause ginseng to remain quite

Figure 3. Fruit of *Panax ginseng*. (Source: http://www.herbs.org/greenpapers/panaxq.jpg)

rare and elusive, contributing to extremely high prices for roots of good quality. The plant is a perennial shrub, growing up to two and a half feet tall with an erect stem and a single cluster of greenish yellow or pink flowers in groups of 15 to 30 shooting up each spring. The leaves are thin and finely serrated, clustered in groups of three to five, which grow up to eight inches long (**Figure 2**). The fruits are scarlet, smooth, and glossy (**Figure 3**). The root is cream-colored or white, resembling a parsnip with rootlets branching in the form of a human body shown in **Figure 4** (hence the indigenous name 'ren shen', or man plant). Ginseng root extracts and tea have a distinctive sweet taste mixed with underlying bitter tones. There is little or no odor and the root is starchy with small amounts of resin, volatile oils, and the medicinally active compound, *panaquilon* (Harriman, 1973).

Figure 4. Root of *Panax ginseng*. (Sources: http://www.bouncingbearbotanicals.com/images/ginseng_root.jpg, http://www.buzzle.com/img/articleImages/489358-262329-53.jpg)

Traditional Uses

Increased sexual potency

Panax ginseng has been used in China for thousands of years. It was documented for its life-prolonging effects in one of the ancient medical texts, *Shen Nong Ben Cao Jing* (Shen Nong's Materia Medica) as early as 502-557 in the Liang Dynasty (Yun, 1996). Aside from its inherent medicinal properties, ginseng was used to increase potency. In Chinese medicine, health is holistic. The balance of yin (feminine attributes) and yang (masculine attributes) and humans with nature was essential to good physical, mental, and spiritual health. Ginseng was considered the most "yang" of all the herbs. As such, true believers wore a well-shaped ginseng root as a sexual amulet to increase sexual potency. Folklore has it that an emperor once paid $10,000 to buy a particularly shapely root. To be able to afford fine ginseng was sign of wealth and commanded respect because it was used by wealthy Oriental aristocrats to keep concubines satisfied. Even now, men take ginseng before and after intercourse to enhance performance and to replenish his sapped energy and sexual potency after a taxing encounter (Harriman, 1973). According to Jartroux, the man who introduced *Panax ginseng* to the West, ginseng was a necessity in all the prescriptions set by the Chinese physicians, and only the Emperor had the right to collect its roots (Swerdlow, 2000). Ginseng was, and still is, a scarce, expensive, and highly prized possession.

Religious Uses

During the 18th and 19th centuries, several reports of ginseng found their way back to America from missionaries and officials serving in Asia. They discovered that the Chinese and Koreans attributed stimulating and restorative properties to Asian ginseng, and that this plant has been held in high esteem for centuries. A report in 1900 from a president of one of the largest trading companies in the Orient said that the Chinese used ginseng for thousands of years for medicinal and religious purposes. He also elaborated that its use has been firmly established in their religion for generations. Due to its seemingly supernatural powers, it was also worshipped (Harriman, 1973). Owing to its long history of use, several folklores exist about ginseng. One tale from the 6th century attributes ginseng with human qualities. One day, a general coming back from war stopped to stay at a stranger's house to rest. That night, he heard the cries of a man coming from a close distance. The strange cries continued for several nights with the entire house searching for the source of the voice. At

last, the general followed the voice to a distance of about half a kilometer from behind the house, and there he found a single ginseng plant. He dug until the root was exposed, and was startled by its human-like appearance. In fact, it had been this root that had been calling out in a man's voice over the previous nights (Harriman, 1973). Stories where dreams or voices led to the discovery of wild patches of ginseng are common in China. The people believe that if someone finds wild ginseng, they will be blessed and luck and fortune will come their way (Harriman, 1973).

Medicine

Panax ginseng remains famous in the East and West for its numerous and ubiquitous health benefits, ranging from colds to cancers and everything in between. Even now, Chinese people believe in corresponding ginseng body parts to the actual limbs that are hurting. For example, taking medication derived from part of the root that looks like an arm will be beneficial to that individual's ailing arm (Harrison, 1973). The root was, and still is, often used as a tonic. One can chew a bit of root or take a spoonful of extract each morning on an empty stomach to help the body gain vigor and resilience. Preparation of ginseng tonic involves ginseng extract, which is produced by soaking the root in water and evaporating the solution. The extract is then kept in a tightly sealed container, and no metal except silver may come into contact with it (Harriman, 1973). Ginseng extract can be used alone or in conjunction with several other ingredients, which will enhance the individual healing properties of each drug. Most Chinese medicines are decoctions or infusions of various ingredients, and certain plants are frequently used with ginseng. These herbs are ginger, licorice, fou ling, cassia, and huang ch'i. Taken together, these decoctions are powerful tools against stomach and bowel pains as well as gas, coughs, and chest and lung problems (Harriman, 1973). Aside from *Shen Nong Ben Cao Jing*, ginseng medicinal recipes are found in additional ancient texts in China and Korea, such as *Ben Cao Kang Mu* and *Pang Yak Hap Pyung* (Harriman, 1973). These formulas serve as guides and healers adapted them to fit the specific cases of their patients. Some examples of these medicinal recipes include a tonic decoction using equal parts of several herbs, including ginseng; a tincture of ginseng using powdered ginseng root fermented with rice and yeast; date and ginseng pills made by pounding together fresh ginseng root with dates and others plants. One Cantonese ginseng tonic uses ginseng in combination with several other ingredients to treat loss of vitality, loss of appetite, fever, weakness of legs, jaundiced appearance, and laborious breathing. It is also used to strengthen the spleen and stomach and to increase yang forces. Since ancient times, ginseng has been used as a panacea, treating many serious illnesses. A list of conditions and diseases involving the use of ginseng in traditionally prescribed remedies in China is shown in **Table 1**, and in Korea is shown in **Table 2** (Harriman, 1973).

Tuberculosis	Dysentery
Fitful coughs	Enuresis
Nausea	Gout
Diabetes	Suppurating sores
Thirst accompagnied by dry skin and flushed throat	Degeneration of the kidneys, characterized by continual thirst, polynuria, and pain in renal area
Indigestion	Rheumatism in lower limbs
Diarrhea	Sexual potency

Table 1. Conditions and diseases involving the use of Ginseng in traditionally prescribed remedies in China (Harriman, 1973).

Acute gastritis	Endometritis
Infant diarrhea	Nipple infection resulting from nursing
Excessive thirst accompanying fever	Suppuration
Chronic cough	Furuncle
Anemia	Toothache
Alcoholism	Malaria
Abnormality of the blood	Hysteria

Table 2. Conditions and diseases involving the use of Ginseng in traditionally prescribed remedies in Korea (Harriman, 1973).

Chemistry and Pharmacology

Panax ginseng has many constituents. The primary ones are ginsenosides (**Figure 5**), which stimulate the cardiovascular and central nervous systems to enhance physical and mental performance. Panaxans, polysaccharides from ginseng extracts, are also important compounds. They lower blood sugar, strengthen the immune system, and treat diabetes (**Figure 6**) (Swerdlow, 2000). Ginsenoside-RB-1 is present in 5000 ppm in the ginseng root, offering antiamnesic, antistress, and antipsychotic activities. Ginsenoside-RB-2 is present in 2000 ppm in the root and has calcium-antagonist and hypocholesterolemic properties. Ginsenoside-RC is present in 3000 ppm in the roots and is also a calcium-antagonist. Other constituents in the root with biological activity include ascorbic acid, beta-carotene, beta-sitosterol, calcium, magnesium oleonolic-acid, pathothenic acid, and selenium. These compounds contain analgesic, antiaging, anticancer, and antibacterial properties. They are also anticarcinomic, antiarrythmic, antiarthritic, antiaggregant, and antiangial. Many more chemical compounds exist in the plant in lower concentrations, and a full list of the constituents is shown in **Table 3** (Duke 2012).

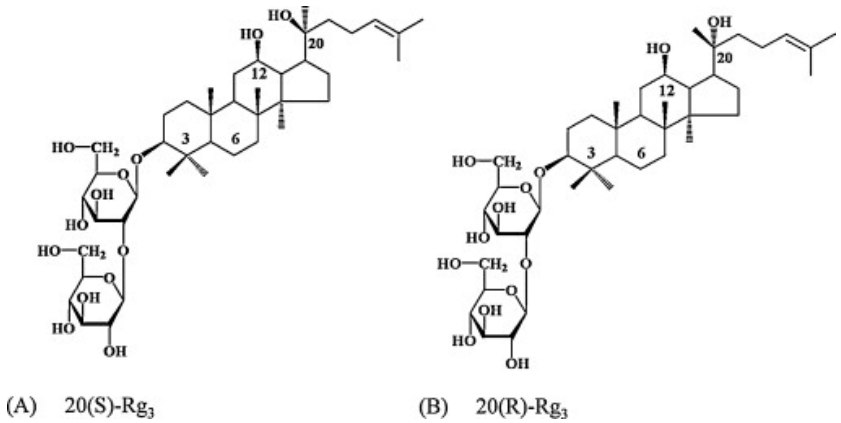

Figure 5. Chemical Structures of Ginsenoside (A) 20(S)-Rg3 and (B) 20(R)-Rg3. (Source: http://ars.sciencedirect.com/content/image/1-s2.0-S0006295211009555-gr1.jpg)

Biological Activity

In Vitro

Many studies have been conducted evaluating the potency of *Panax* ginseng extracts on cancer. One study evaluated the cytotoxic potency against a variety of cancer cells by using ginseng ethanol extracts (RSE), protopanaxadiol (PPD/PPT)-type ginsenosides fractions, and their hydrolysates. Cytotoxic potency of the hydrolysates of the extracts and ginsenoside fractions was much stronger than the original control. Using two synthesized derivatives of the fractions and the original ginsenosides, a comparative study was performed on various cancer cell lines. The results suggest that compounds with less polar chemical structures have more cytotoxic activity towards cancer cells, and are involved in suppressing tumors and mediating cell growth (Dong et al., 2011).

Chemicals/Molecules	ppm	Plant Part	Chemicals/Molecules	ppm	Plant Part	Chemicals/Molecules	ppm	Plant Part
Carbohydrates	834000	Root	Ginsenoside-RG-1	2000	Root	Chromium	11	Root
Fiber	72000	Root		15000	Leaf	Panthothenic-acid	6.6	Root
ASH	50000	Root		2000	Flower	Riboflavin	1.8	Root
Fat	17700	Root		200	Fruit	Thiamin	1.7	Root
Calcium	2880-4140	Root	Ginsenoside F-1	4000	Leaf	Biotin	0.9	Root
Ginsenoside-RB-1	5000	Root	Ginsenoside-F2	2000	Leaf	Ascorbic Acid		Root
	2000	Bud	Ginsenoside-F3	2000	Leaf	Beta-Carotene		Root
	1000	Leaf	Carbon-disulfide	1500	Root	Beta-Flemene		Root
Ginsenoside-RB-2	2000	Root	Choline	1000-2000	Root	Beta-Sitosterol		Root
	4000	Leaf	Panaxynol		Root	Beta-Sitosterol Glucoside		Plant
	2000	Flower	Water	788000	Root	Camptesterol-6'-linolenylglucoside		Root
	200	Fruit	Protein	109000	Root	Camptesterol-6'-linolenylolglucoside	Root	
Ginsenoside-RB-3	50	Root	Disaccharides	33000	Root	Camptesterol-6'-oleylglucoside		Root
Ginsenoside-RC	3000	Root	EO	500	Root	Camptesterol-6'-palmitylglucoside		Root
	2000	Flower	Potassium	2430-10700	Root	Camptesterol-6'-stearylglucoside		Root
	2000	Leaf	Magnesium	481-1950	Root	Citric Acid		Root
	1000	Fruit	Iron	180	Root	D-Fructose		Root
Ginsenoside-RD	2000	Root	Heptadeca-1-EN-4,6-Dien-3,9-Diol	150	Root	D-Glucose		Root
	15000	Leaf	2-Glucoginoside-RF	50	Root	Fumaric Acid		Root
	2000	Flower	Zinc	27		Panaxene		Root
	1000	Fruit	Aluminum	22	Root	Panaxic Acid		Root
Ginsenoside-RE	2000	Root	Manganese	19-180	Root	Panaxin		Root
	15000	Leaf	Tin	16	Root			
	25000-60000	Fruit						
	28000	Flower						

Table 3. List of active chemicals and biological molecules in ppm in *Panax ginseng* (Duke 2012).

Another study was conducted on the immunological effects of ginseng on cells. Water-soluble ginseng oligosaccharides (WSGO) were obtained from extracts of ginseng roots and separated into fractions. Preliminary immunological tests suggest that WSGO were potent T and B-cell stimulators and

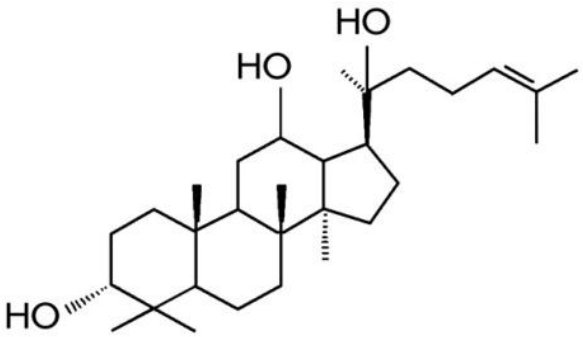

Figure 6. A Panaxoside in *Panax ginseng.* (Source: http://actanaturae.ru/article.aspx?id=190)

were effective towards lymphocyte proliferation (Wan et al., 2011).

In Vivo

Research has shown *Panax ginseng* extracts, specifically ginsenosides, have a positive effect on lowering hypertension in rats. The extract stimulated nongenomic Akt-mediated endothelial NO synthase (a regulator of systematic blood pressure) activation. It also enhanced NO production and vessel wall thickening, and alleviated hypertension in hypertensive rats (Hong et al., 2012). Ginseng is also proven to protect against influenza. One study shows that mice infected with the H1N1 virus that were treated with ginseng polysaccharides (GP) had higher survival rates than the control mice. Mice exposed to GP had lower levels of lung viral titers and inflammation (Yoo et al., 2012). Ginseng can also suppress asthma through the inhibition of lung inflammation by the ginsenoside RG-II (Jung et al., 2012).

One traditional use of ginseng was for the purpose of antiaging. In ancient China, people used to believe ginseng could be taken to achieve immortality (Harriman, 1973). In a modern experiment, the effect of fermented *Panax ginseng* extract (GINST) on oxidative stress and antioxidant activities in major organs of aged rats was studied. Oxidative stress throughout life is the major cause of aging. The study found that administration GINST to aged rats resulted in increased activities of superoxide dismutase, catalase, and glutathione peroxidase as well as levels of ascorbic acid and α-tocopherol. Levels of malondialdehyde, aspartate aminotransferase, and urea were lowered, on the other hand. These findings indicate that ginseng extract can decrease oxidative stress and age-related disorders due to free radicals (Ramesh, 2012). Another research study also showed ginseng to possess antioxidant effects. The ginsenoside RG3 inhibited Cy-induced oxidative stress by increasing levels of catalase and superoxidase dismutase while lowering activities of xanthine oxidase and nitric oxide (Wei et al., 2012).

So far, there has been no research supporting issues of the development of drug resistance. Ginseng is mainly taken as a 'bu yao' and 'bu yin,' which are herbs or plants used to enhance and nourish the body (Guo, 1961). Even when ginseng extracts are used to target biological receptors in the body, they are not used to fight microbiological agents, which is why there have been no instances documenting drug resistance to ginseng.

Clinical Studies

Although *Panax ginseng* has been used for thousands of years for its obvious health benefits, not many controlled clinical studies have been carried out. One study, however, investigates experimental and epidemiological evidence of cancer-preventive effects of ginseng on humans. The relationship between ginseng consumption and cancer was

evaluated by interviewing 905 human pairs of case and control subjects matched by various factors, such as age, sex, and date of admission to the hospital. Ginseng extract and powder were more effective at fighting and reducing cancer than fresh sliced ginseng, ginseng juice, or ginseng tea (Yun et al., 1996).

A later experiment was carried out to further study the effects of types of ginseng products that have the most cancer-prevention. The study also investigated the duration of ginseng consumption, types of preventable cancers, and effect of ginseng on cancers related to smoking. Ginseng intake was found to decrease risk of cancer and there was an inverse relationship between cancer risk and frequency and duration of ginseng intake.The longer and more often an individual ingests ginseng, the lower risk of developing cancer. There was an effect on lung, lip, oral, and liver cancer, but no effect on breast, uterine cervix, or thyroid gland cancers. With regards to smokers, cancer of the lung, lip, and oral cavity decreased as opposed to smokers who did not intake ginseng extract. These findings show that ginseng intake can decrease cancer at various sites (Yun et al., 1996)

The mechanism for this anticancer effect is still not understood. However, it is hypothesized that saponins are the active components of ginseng, giving this plant its anticarcinogenic effects. Saponins have been shown to have antimutagenic activity, growth inhibition activity against several mouse tumor cells lines, growth inhibition of human ovarian cells, and immunomodulating activity in mice. Polysaccharides, another main active constituent in ginseng, have anticomplementary activity, reticuloendothelial system-potentiating activity, and alkaline phosphatase-inducing activity. Also, polyacetylenes extracted from ginseng have been shown to have cytotoxic activity. It is reasoned that the synergy of the combination of these different constituents is what is reducing cancer risks. However, further studies need to be carried out to support this theory and to identify unknown constituents that may have anticarcinogenic effects (Yun et al., 1996).

Aside from reducing cancer, ginseng has also been shown to lower hypertension in humans. One study compared blood pressure from two groups of adults. One group took 200 mg daily extract of ginseng and the other group took 200 mg daily of the placebo. The study showed that taking ginseng extract reduced blood pressure by 5 mm Hg in two hours after ingestion (Caron et al., 2002). There are also studies of ginseng on diabetes. Although used traditionally to treat this disease, there are mixed results from experiments regarding its effect on diabetes. Some studies have found ginseng extract to reduce diabetes while others did not produce conclusive evidence. A study investigating the effect of red ginseng (heated *Panax ginseng*) on type 2 diabetes did not yield supporting data for the effectiveness of red ginseng on glucose control (Kim et al., 2011). Other studies investigating ginseng and cardiovascular illness also produced mixed conclusions. One experiment showed taking ginseng extracts did not lower deaths related to cardiovascular disease, but did decrease mortality overall (Yi et al., 2009). Although the results were not definite, this study supports the holistic practice of taking ginseng to enhance and nourish the body rather than to treat specific diseases (Guo 1961).

Like most medicinal plants, clinical evidence of ginseng efficacy in treating various diseases are contraindicating and sometimes produce no conclusive evidence in the benefits of ginseng intake. However, this problem is mostly due to the scarcity of well-designed, randomized, and controlled clinical trials rather than to any problems related to the plant (Karmazyn et al., 2011).

Contraindications

Harriman states in 1973 that no studies so far have shown any toxicity related to *Panax ginseng* intake. In fact, of all the research done on ginseng, not one has shown even the slightest deleterious side effect of this gentle "Queen of the herbs" (Harriman, 1973). Toxicology and carcinogenesis studies of ginseng were made in rats, mice, and other microbes. Genetic toxicology studies were performed in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes, as well as on live mice and rat subjects. The mice and rats all survived in 2-week, 3-month, and 2-year studies. No increases in the incidences of neoplasms or nonneoplastic lesions were attributed to the administration of ginseng. Almost 30 years later, in modern studies contradictions exist in data regarding ginseng toxicity. In a genetic toxicity study, ginseng was not mutagenic in either of the two independent bacterial mutagenicity assays. Under the conditions of these 2-year studies, there was no evidence that ginseng was carcinogenic in male or female rats (National Toxicology Program, 2011).

However, another study found that Rg3, a ginsenoside, suppresses normal physiological responses *in vitro* and leads to vascular remodeling in rats, which is surprising concerning ginseng's proven protective effects on vascular function (Karmazyn et al., 2011). Potential embryotoxic effects exist for mice embryos that were directly exposed to the Re ginsenoside, but no side effects were found in embryos exposed to the Rc ginsenoside (Chan et al., 2004). In general, ginseng has relatively few adverse effects although this could be due to low reporting instead of low incidences (Karmazyn et al., 2011). The other recorded side effects were provided mostly through case reports and were not conclusive data taken from controlled experiments. A female without cardiovascular risk factors reported developing long QT syndrome (which can lead to arrhythmias) during periods when she was consuming large amounts of ginseng (Torbey et al., 2011). Another report of an elder man developing bradyarrhythmia after prolonged exposure to ginseng (Liao et al, 2011) also calls into question the potential toxicity of ginseng.

Current Use in Allopathic and CAM Therapies

The current uses of *Panax ginseng* have not changed much from the thousand-years-old traditions of the ancient people. Ginseng is one of the most important herbs used in traditional Chinese medicine. According to a study asking elderly Chinese patients in America to list their medications, ginseng was listed as a home remedy benefitting the lungs and spleen, used against weakness with chronic illnesses, vaginal bleeding, diabetes, and palpitations (Guo, 1961). Ginseng is still used by modern people to fight colds and chronic illnesses, amongst many other traditional uses. The preparations of decoctions and tonics have not changed since earlier times. The roots are still soaked in water then kept in an airtight container for later use. Ginseng roots are still being sought after, and prices on the market vary according to the quality. Fine ginseng costs many times more than rougher forms. Eastern people regard the root shape (how closely it resembles a man) as the defining factor in quality while Westerners look for ginsenoside content. **Table 4** shows the difference in prices of various grades of ginseng. Asian ginseng costs more than American or cultivated ginseng. Ginseng is a highly priced and popular root that has spread from Asia to the Western hemisphere. Even in the United States, all oriental drugstores carry the root or extracts of it. The harvesting and processing of Korean and Chinese ginseng is done entirely under supervision and the seal on packaged products ensures the

Grade	Price (per Catty)
Chinese Wild Imperial	$5000.00
Korean and Chinese Red	$26.50
Korean White	$196.50
American Wild	$133.30
Japanese and American Cultivated	$36.60

Table 4. 1971 Hongkong wholesale prices of ginseng according to grades (Harriman, 1973).

identity of this herb. Whole roots are shipped to the US in boxes weighed in cattys, with one catty equal to 1.33 lbs. There have been many attempts (now successful ones) to cultivate this precious herb outside of its native Chinese and Korean forests, in America, Japan, and Russia. The popularity of this herb maintains the use of tonics and decoctions found in traditional Chinese medicine and herbal teas, and powdered forms can be taken as drugs in the form of capsules (Harriman, 1973).

Discussion

Panax ginseng has been an important plant in the treatment of a broad range off diseases and illnesses since ancient times. Today, it continues to be one of the leading herbs used in traditional Chinese medicine. Its active biological constituents include various ginsenosides and polysaccharides. Research has been done on this herb to classify its active constituents on biological pathways and diseases, with results showing antihypertensive, anti-inflammatory, and anti-carcinogenic effects. As with the use of all plants and herbs, well-controlled and effective studies are few in number. Further research still needs to be made in clinical trials, as well as continued in vitro and *in vivo* studies. The preventive and curative effects of ginseng extract on cancer and tumor cells are especially exciting, with studies documenting favorable results supporting the anticancer properties of this herb. Research data is favorable in the use of ginseng as a possible herb against cancer, and future anticancer drugs containing ginseng is possible. However, the pathways in which ginseng suppress cancer still remain to be elucidated. The main theory involves the synergy created by the various components acting together although this hypothesis has not been proven yet.

Although new studies are emerging in the potential adverse effects of ginseng, these cases are few and far between. Ginseng has been used for over two thousand years as a medicinal plant, and traditional techniques in preparing ginseng decoctions remain almost unchanged to this day. Ginseng acts as a panacea, restores energy, and nourishes the body. In addition, its antihypertensive and anticarcinogenic properties are most exciting, as these can be utilized to treat chronic diseases for which Western medicine still lacks a cure. The potential for the active constituents to be made into drugs is high and favorable, as *in vivo, in vitro*, and clinical studies continue to support ginseng's various positive effects on health.

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Papaver somniferum L., Papaveraceae

Ben Jarvis

Introduction

Papaver somniferum L.*,* commonly known as the opium poppy or the poppy plant, belongs to the family Papaveraceae. The opium poppy is one of the most well known plants because of its many uses. It has a long history throughout the world as being one of the most useful as well as one of the most abused plants. In fact, opium is perhaps one of the oldest narcotics known to man with evidence of its use found in limestone caves in Spain from about 55,000 years ago (Schultes 1995). The earliest writings of the opium poppy date back to about 4000 B.C., where the Sumerians refer to it as the "joy plant" (Lewis 2003). The opium poppy has a range of uses from the commonly used food ingredient, the poppy seed, to one of the most effective pain relievers, morphine. Other common drugs derived from the opium poppy include codeine, noscapine, thebaine, and papaverine. Because of the addictiveness and abuse caused by this plant's effect on the central nervous system, there has been a lot of conflict and controversy over its growth and cultivation. In fact, the Opium Wars that took place between England and China in the nineteenth century were over this very plant. Even today, with the United States and other countries battling a war front in Afghanistan, Opium farming and its funding of the Taliban remains a key issue in the war. The opium poppy has many therapeutic uses in humans, but its addictive nature also brings about a great deal of controversy and concern.

The two most common food ingredients of the opium poppy are poppy seeds and poppy seed oil. They provide a unique flavor that can be found in many cuisines from cultures around the globe. The seeds and their oil contain very low levels of opiates and are considered to be safe ingredients for all ages.

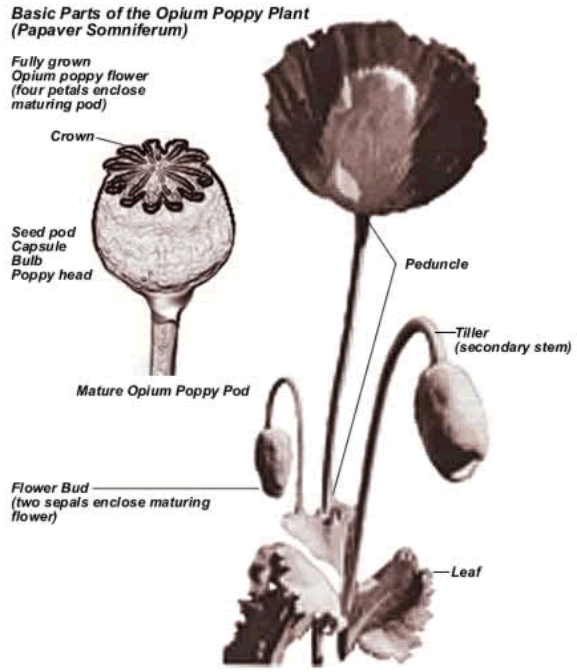

Figure 1. The basic parts of the opium poppy are shown above. The opium poppy consists mainly of a stem, leaves, flower and flower buds, peduncle, tiller, and typically three seed pods with a crown. (Image Source: poppies.org)

P. somniferum also has many medicinal uses that have had a great impact on anesthesia and healthcare as a whole. There are over 25 alkaloids that come from the opium poppy, the

most common of which is morphine. Morphine, a benzylisoquinoline alkaloid with two additional rings, is derived from the poppy's latex, or milky fluid commonly found in angiosperms, and is commonly sold under 125 different brand names worldwide.

Description

The plant species, *Papaver somniferum*, can be found in fields and flower gardens throughout the world. The opium poppy consists mainly of a stem, leaves, flower and flower buds, peduncle, tiller, and typically three seed pods with a crown (**Figures 1 and 2**). It is these seed pods which contain the valuable latex that is so commonly used in codeine, morphine salts, and other drugs derived from the plant. The plant secretes this thin latex as a response to mechanical damage, but it is still unclear why the opium poppy produces the latex in the first place. Most recent evidence shows that the metabolism of morphine to bismorphine serves as a defense mechanism in the opium poppy (Morimoto 2001). Morphine comes from two molecules of the amino acid L-tyrosine through a series of at least 17 enzymatic steps (Grothe 2001).

Perhaps the most commonly seen poppies are in the movie "The Wizard of Oz," where Dorothy and her friends travel through the poppy fields (**Figure 3**). It is in that movie that many people learn about the sedative effects of the opium poppy. The opium poppy is not only grown in fields. Many growers include the opium poppy as an ornamental flower in their gardens. The flowers of the opium poppy can be found in many different colors, but are most frequently seen as red, white or pink flowers. The flowers have two sepals that fall off as the flower blooms. The exact number varies, but the poppy flower can usually be seen with four to six petals. The seeds of the poppy plant are most commonly planted in the fall under a thin moist layer of soil. The opium poppy grows very quickly and a seedling can be seen within a week or two. The pods of the poppy plant contain the seeds and the latex that are commonly sought after.

Figure 2. The image above shows another view of the major parts of the opium poppy. Notice the different color flowers. (Image Source: botanical.com)

P. somniferum also has over 75 close relatives which are grown as weeds or flowers. A few common relatives are the corn poppy, *Papaver rhoeas*, the Western poppy, *Papaver californicum*, and the Oriental poppy, *Papaver orientale*.

Figure 3. In this popular scene from the "Wizard of Oz," Dorothy and her friends pass through the poppy fields on their journey. (Image Source: joewmccord.com)

Although less common, there have been medicinal uses found in these other species as well.

In *P. somniferum,* most of the flowers are planted by humans in controlled environments where they can keep a close watch on the plant. Because of the value obtained from the latex in the opium poppy, growers pay close attention to the growth and harvesting of the crop.

Traditional Uses

Papaver somniferum is used not only as one of the most common analgesics and narcotics, but it is also commonly used in food, gardening and recreational drug use. The majority of its influence, however, has been because of its drug effects.

Some of the earliest writings focused on the opium poppy show that it was used in anesthesia. In the Roman Empire, a sponge was soaked with the latex from the opium poppy and placed under the nose of the patient. The vapors that were released when the sponge was moistened would induce sleep. Mandrake wine and other concoctions containing morphine were also used to induce sleep, but it was quickly noted that high dosage could cause negative side effects and often lead to death (Carter 1996). William Shakespeare referred to the poppy's sedative effects in two of his most common plays, in *Othello* and in *Romeo and Juliet.* It was from the opium poppy that the potion was made that put Juliet to sleep.

Throughout recorded history, there is evidence of the use of the opium poppy for anesthesia and for its sedative effects. The opium poppy has also been used to treat many other ailments and as an aphrodisiac. Papaverine, one of the alkaloids from the opium poppy, has been used as an antiviral to combat cytomegalovirus (CMV), measlesvirus, and human immunodeficiency virus (HIV) and has potential to help AIDS patients who also have CMV disease. Papaverine has also been used to treat conditions of the nervous system, peptic ulcers, painful menstruations, gastrointestinal complications and spasms among many other things. Cryptopine, a colorless alkaloid obtained from the opium poppy, has shown to control arrhythmias and slow myocardium tissues. Protopine, another alkaloid found in the opium poppy, has been shown to inhibit histamine inhibitors and show bradycardial action. A powder from the poppy seed has also been shown to relieve toothache (Lewis 2003). The most common use of alkaloids from *Papaver somniferum* has been to relieve pain. Codiene has been used in cough syrups as an antitussive. Amorphine, an emetic derived from morphine, has been used in the removal of poisons from the stomach and in aversion therapy.

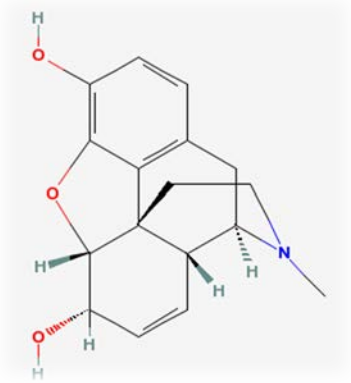

Figure 4. The chemical structure for morphine ($C_{17}H_{19}NO_3$). Notice the ring structure bearing nitrogen, which is a common characteristic of alkaloids. (Image Source: pubchem.ncbi.nlm. nih.gov)

Some of the common analgesics previously mentioned including morphine, codeine, noscapine, thebaine, and papaverine also have addictive tendencies associated with their use. Both mental and physical addictions are common with the abuse of these drugs. Overcoming the addiction, however, is not easy and can often require professional help. Withdrawal symptoms can be quite severe and include nausea, vomiting, and diarrhea among many other things (McClung 2006).

Perhaps one of the most addictive drugs, heroin, comes from the opium poppy. Heroin, or diacetylmorphine, is a semi synthetic drug created from morphine. In some countries, heroin is used to treat severe acute pain (van den Brink 2003). Unfortunately, the recreational use of heroin is much more popular than the medical uses. Heroin is a commonly sought after drug due to the induced euphoria effects. Tolerance forms quickly in heroin users and creates a need for more.

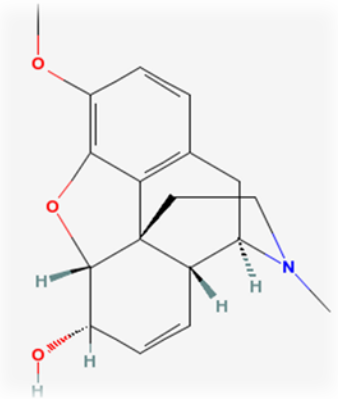

Figure 5. The chemical structure for codeine ($C_{18}H_{21}NO_3$). Like morphine, codeine is an alkaloid. (Image Source: pubchem. ncbi.nlm.nih.gov)

Many famous drug related deaths have been attributed to heroin.

A safer and very common use of the opium poppy includes the use of poppy seeds and poppy seed oil. Poppy seed and their oil are used to add flavor and texture to many foods. The seeds are commonly used in many baked goods, casserole dishes, and in several types of Indian curry.

Chemistry and Pharmacology

Alkaloids are commonly identified by their ring structure bearing nitrogen. Morphine (**Figure 4**) and codeine (**Figure 5**) are both alkaloids. Morphine has the chemical formula $C_{17}H_{19}NO_3$ and a molecular weight of 285.338 g/mol. The crystal structure of an anti-morphine antibody and its complex with morphine are shown in **Figure 6**. Codeine has the chemical formula $C_{18}H_{21}NO_3$ and a molecular weight of

Alkaloid	Chemical Formula	Molecular Weight
Noscapine	$C_{22}H_{23}NO_7$	413.421 g/mol
Thebaine	$C_{19}H_{21}NO_3$	311.375 g/mol
Papaverine	$C_{20}H_{21}NO_4$	339.385 g/mol

Table 1. Chemical formulas and molecular weights of some common drugs derived from the opium poppy.

299.364 g/mol. The chemical formula and molecular weight of thebaine, noscapine, and papaverine are shown in **Table 1**.

Biological Activity

There has been a great deal of research surrounding the opium poppy and it uses. In 1988, an *in vitro* study showed how thebaine, an intermediate of morphine and codeine biosynthesis in the opium poppy, could be transformed to oripavine, codeine, and morphine by rat liver, kidney, and brain microsomes. (Kodaira 1988). In 1995, Lenz and Zenk showed the properties of codeinone reductase (NADPH), which catalyzes the production of codeine from codeinone, another step in the biosynthetic pathway shown in **Figure 7** (Lenz 1995).

Morphine and other opiate drugs greatly affect the central nervous system (CNS). There are four major receptive sites found in the brain and spinal cord of most vertebrates, known as the opioid receptors. The four opioid receptors are identified as MOP (μ), KOP(k), DOP(δ) and the nociceptin receptor (NOP). Each of the four receptors has a different location in the brain and spinal cord and has a different function affecting the reception of pain (Dreborg 2008). A stimulation can activate these receptors, which will then

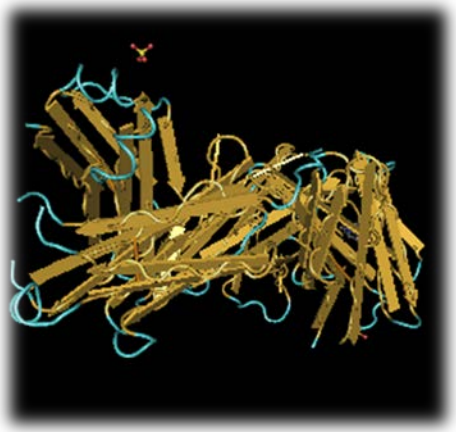

Figure 6. The crystal structure of an anti-morphine antibody and its complex with morphine are shown. (Image Source: pubchem.ncbi.nlm.nih.gov)

initiate a series of intracellular signals that affect many hormones and neurotransmitters throughout the body and can often cause respiration depression (Pattinson 2008). While the effects of many of the opiate drugs are well studied, the mechanisms of actions for many of them are still unclear. Although the mechanism of action for papaverine remains unclear, some researchers have found evidence that it is a strong inhibitor of cyclic-3',5'-nucleotide-phosphodiesterase (Kukovetz 1970).

Clinical Studies

New drugs, such as Kadian, are in clinical trials to determine the effectiveness of low dosage or sustained release morphine capsules. There are also clinical studies to determine more effective means of overcoming addictions to morphine and other narcotics. A current, ongoing study sponsored by the Norwegian University of Science and Technology is trying to

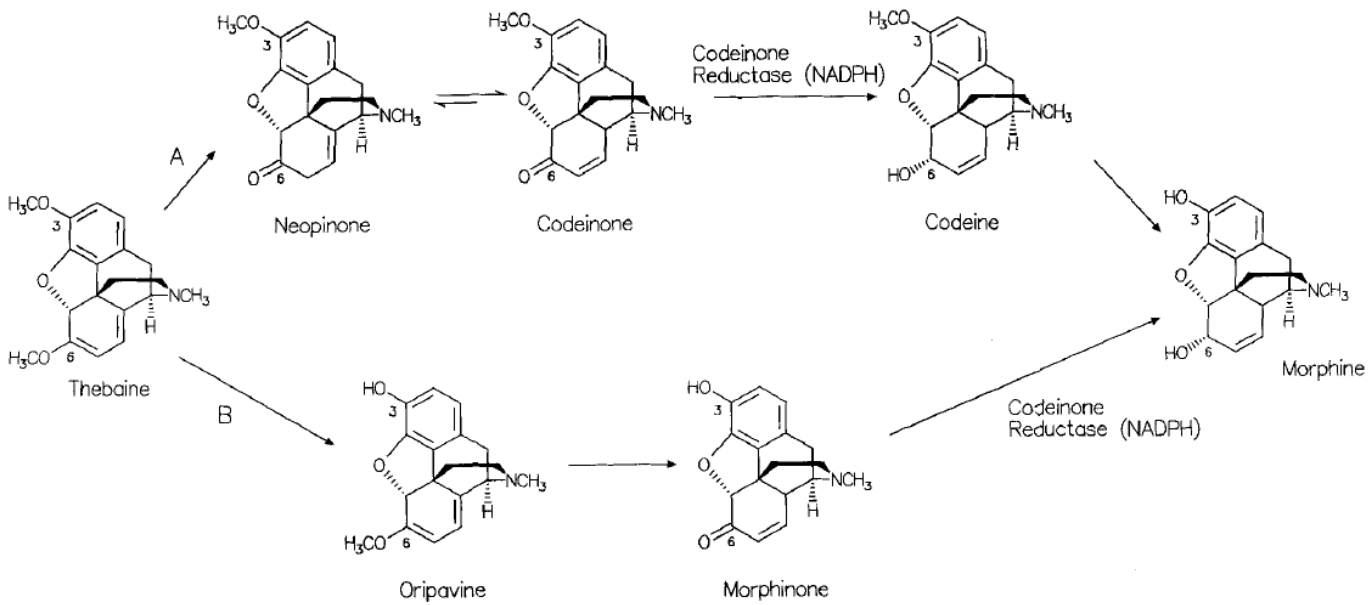

Figure 7. Codeinone reductase (NADPH), which catalyzes the production of codeine from codeinone is shown above. (Image Source: Lenz 1995)

determine if it is possible to switch from morphine treatments to methadone in patients with advanced cancer or short life expectancy.

Contraindications

There are many interactions to watch out for with the use of drugs from the opium poppy. Some of the more common interactions include alcohol, barbiturates, antidepressants, muscle relaxants, antihistamines, sleep medications, antipsychotics, and other narcotics. Morphine itself does not interact with grapefruit juice, but codeine, on the other hand, will cause an interaction. The two most common dangers associated with alkaloids from the opium poppy include interactions and the addictive nature of the drugs. Pregnant women should be especially careful to avoid the abuse of morphine. Babies born from mothers addicted to morphine often have the physical addiction themselves (Becker 2005).

Current Use in Allopathic & CAM Therapies

Because of the addictive nature of the drugs and the widespread abuse, opiates are not commonly used in complementary and alternative medicine therapies. Codeine can be found in some combination preparations such as Tylenol with codeine and antitussives. There are many drugs derived from morphine, but in nearly every country, they are closely regulated by the government to prevent such abuse.

Morphine and other opiate drugs are often administered in hospitals and clinics to manage post-operative pain. Many doctors will choose to use NSAIDs instead of opiate analgesics because there is less risk involved, both in addiction and respiratory depression. NSAIDs are a good for mild to moderate pain, but it is recommended that practitioners "use opioid therapy for the short-term management of breakthrough pain that is not responsive to NSAIDs or acetaminophen" (Becker 2005).

Discussion

Papaver somniferum has generated many medicinal uses that have greatly impacted human health over the years. The controversy surrounding the opium poppy, however, has often overshadowed its positive effects. The highly addictive nature of heroin and other opiates can even be passed on through pregnancy. Over-dosage of opiates, like most narcotics, can often lead to death. Many lives have been improved and many lives have been lost over the opium poppy plant. With proper use and direction, morphine and codeine can help alleviate pain, spasms, coughs and many other conditions. Even when used correctly, one must be very careful to avoid addiction. While many drugs have been derived from the opium poppy, there is still a lot of research surrounding this plant.

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Passiflora incarnata L., Passifloraceae

Ronni Shalem

Introduction

Passiflora incarnata (**Figure 1**) is from the Passifloraceae family. It is most commonly known as the purple passionflower but alternate names include: apricot, ground ivy, Holy Trinity flower, mayapple, maypop, or mollie cockle (McGuire, 1999). *P. incarnata* is indigenous to warm temperate and tropical North America (Dhawan, Dhawan, & Sharma, 2004). It has a perennial vine (present at all seasons of the year) with white and blue or purple flowers, and an edible fruit. The fruits are berries and oval in shape (**Figure 2**). The main constituents include flavonoids, flavonoid glycosides, alkaloids, cyanogenic glycosides, carbohydrates, amino acids, benzopyrone derivatives and volatile constituents (Elsas, Rossi, Raber, White, Seelay, Gregory, Mohr, Pfankuch, & Soumyanath, 2001).

Passiflora has been used traditionally as a sedative, anxiolytic, antispasmodic, analgesic, anticonvulsant, and for the treatment of whooping cough, bronchitis, and asthma (Dhawan, Kumar, & Sharma, 2002). Topically, it is has been used for hemorrhoids, burns, bruises, and inflammation. More recently, *P. incarnata* has been recommended for insomnia, neuralgia, seizures, menopause, and as an anti-diabetic (Tovar & Petzel, 2009; Tenney, 2007).

The pharmacological effects of *P. incarnata* include anticonvulsant, anti-asthmatic, antitussive, and anxiolytic activities (Grundmann, Wang, McGregor, & Butterweck, 2008). Although there is a vast use of *P. incarnata*, the pharmacological work on this plant has been insufficient and inconclusive in determining the mode of action of the plant as

Figure 1. *Passiflora incarnata* L. (Image source: http://www.wildflower.org/plants/result.php?id_plant=PAIN6)

Figure 2. Fruit of *Passiflora incarnata* L.
(Image source: http://www.duke.edu/~jspippen/plants/passiflora.htm)

Figure 3. Juice with Passionflower
(Image source: http://tastesfromaroundnz.co.nz/4-x-325ml-teza-peach-passionflower-juiced-teas.html)

well as the phyto-constituents responsible for the anxiolytic and sedative effects of the plant (Soulimani, Younos, Jarmouni, Bousta, Misslin, & Mortier, 1997).

The passionflower fruit is widely eaten today and is used in juice drinks (**Figure 3**), beverages, baked goods, cereals, yogurt, ice cream, candy, jams, and jellies (Ngan & Conduit, 2011). It is currently used in complementary and/or alternative medicine for its treatment of anxiety, asthma, insomnia, nervousness, neuralgia and more (Tenney, 2007).

Botanical Description

Passiflora incarnata is an herbaceous perennial vine that climbs with the support of tendrils. Individual shoots are unclassified, grow up to ten meters long, and are usually branched. The plants often develop additional shoots from the underground roots and rhizomes. The shoots die in autumn, and new shoots grow each spring from buds on rhizomes and roots that live over from one season to the next (McGuire 1999).

P. incarnata's wild growth, branching, and underground spreading can make it very difficult to manage in cultivation. The stems are not self-supporting, so plants must be bolstered in cultivation to protect the foliage and developing fruits from pests. The petioles, up to eight centimeters long, have two nectaries (gland that secretes nectar) attached at the base of the laminae (expanded part of a foliage leaf). Laminae of adult leaves are moderately to deeply three-lobed, and are 6-15 cm long along the mid vein. Two meristems (plant tissue that allow cells to differentiate and produce tissues and organs) in the axil of each leaf allows for the possible upper development into a branch, and the lower into a tendril, or a tendril and a flower (McGuire 1999).

Flowers are born individually on the stalks up to ten centimeters long. Three leaves, each bearing two nectaries, circle the base of the floral bud. Floral buds grow two to three centimeters long before opening. Open flowers are up to nine centimeters wide and have the complex floral structure typical of the *Passiflora* genus. The sepals and petals are white to pale lavender on their displayed surface. The prominent corona (crown) consists of five or six series of thin and flexible appendages. The outer two filaments are 10-20 mm long, colored white, pink, lavender, and/or purple, and variously banded. The remaining series of corona filaments are much shorter (two to four millimeters long) (McGuire 1999).

At maturity, fruits separate at the region of articulation on the peduncle, and the floral parts and a portion of the peduncle remain at the base of the separated fruit. The fruits are berries, oval in shape, and up to seven centimeters long. Immature fruits have smooth, green rings that at maturity

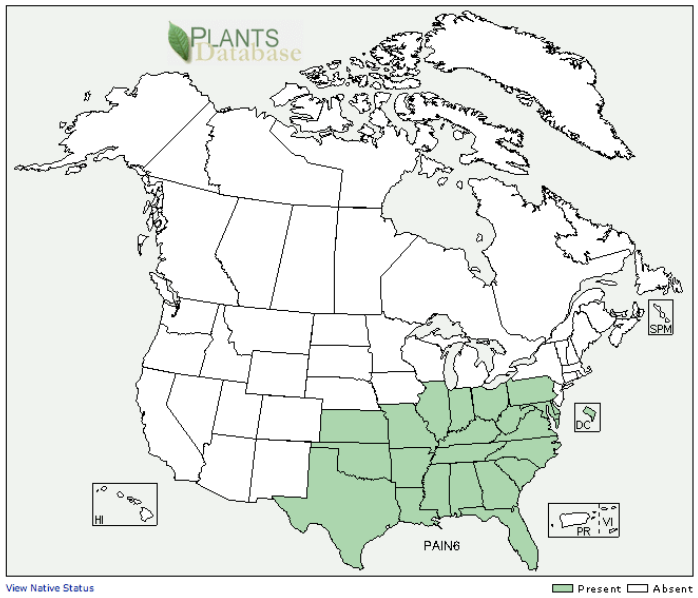

Figure 4. *Passiflora incarnata* is native to southeastern North America.
(Image source: http://plants.usda.gov/java/profile?symbol=pain6)

sometimes become wrinkled, yellow, and eventually brown. Some plants bear fruits with three longitudinal maroon stripes originating at the juncture of the peduncle and fruit and continuing down partially to the end of the fruit. Within the thin skin are three longitudinal parietal placentae, and the fruit interior contains up to 120 seeds, dark brown at maturity, four to five nun long, three to four mm wide, and individually enclosed. The arils (exterior covering) vary in size among fruits and contain an edible, aromatic, creamy yellow juice (McGuire, 1999).

P. incarnata can reproduce asexually from stem cuttings and root or rhizome fragments. Root or rhizome fragments four to eight cm long have nearly 100 percent germination when planted one cm deep, but shorter fragments have lower germination percentages. Root or rhizome fragments lose their capability to grow, develop and even live very easily if they are not kept moist (McGuire, 1999).

There are four important aspects of *P. incarnata*'s reproductive biology that could affect fruit and seed set in cultivation. Firstly, *P. incarnata* is typically self-incompatible. Secondly, insect pollinators (generally Xylocopa spp.) are required to deposit compatible pollen on stigmas. Thirdly, flowers must have both male and female reproductive organs (hermaphrodites) to receive pollen, and plants vary hermaphroditic flower production in response to resource status. Finally, when pollinator visits are not frequent, seed set may cause a relatively long delay between flower opening and effect the way the plant bends (McGuire, 1999).

P. incarnata is a native of temperate and tropical North America (**Figure 4**). *P. incarnata* seeds occur in many human archaeological sites from the Late Archaic (3500- 800 B.C.E.) to Historic (1550-1800 C.E.) periods in the southeastern U.S., indicating that Native Americans were consuming the fruit. Moreover, seed abundance increases over time in the archaeological record. This suggests that human fruit consumption rose at this time. During the same time period, agriculture expanded in southeastern North America, and increased human consumption of *P. incarnata* fruits may indicate an increased cultivation of this plan. It is also possible that since *P. incarnata* prefers disturbed habitats, fruit consumption may have risen simply because the plant became more common, as it invaded the disturbed habitats associated with an expanding agriculture (McGuire, 1999).

Early European travelers in North America noted that Algonkian Indians in Virginia and Creek people in Florida ate *P. incarnata* fruit gathered from cultivated, semi-domesticated, or wild plants. The European settlers

themselves also consumed the fruit and praised its flavor (Dhawan, Dhawan, & Sharma, 2004). Since then, inhabitants of the U.S, as well as people globally, have continued to eat wild and cultivated *P. incarnata* fruits.

Traditional Uses

The discovery of seeds dating back to thousands of years from archaeological sites in North America provides evidence of the historic use of the fruit by the ancient Red Indians. Early European travels in North America (particularly Southeastern parts) documented the consumption of the fruit of *Passiflora* by the Algonkian Indians in Virginia and the Creek peoples in Florida. These fruits were eaten from cultivated as well as wild sources (Dhawan, Dhawan, & Sharma, 2004). The Native Americans also used passionflower as a tonic for bruises and injuries. The Aztecs used it as a sedative and for pain relief (Tenney, 2007).

The use of *Passiflora* as a medicine was documented for the first time in 1569 by Spanish researcher Monardus in Peru. The Matrica Medica Americana, published in Germany in 1787, mentions the use of *P. incarnata* to treat epilepsy for older people. An ancient report also describes its use in spasmodic disorders and insomnia in infants and the elderly (Dhawan, Dhawan, & Sharma, 2004).

P. incarnata is a popular traditional European remedy and a homoeopathic medicine for insomnia and anxiety. In North America, it has a history of use as a sedative tea. In America, it is used to treat diarrhea, dysmenorrheal, neuralgia, burns, hemorrhoids, and insomnia. In Brazil, *Passiflora* has been used as an analgesic, anti-spasmodic, anti-asthmatic, wormicidal, and sedative. In Iraq, it has been used as a sedative as well as a narcotic. In Turkey, *Passiflora* has been used to treat dysmenorrhea, epilepsy, insomnia, neurosis, and neuralgia. It has been used to cure hysteria and neurasthenia in Poland. *P. incarnata* has also been used to treat morphine addiction in India (Dhawan, Dhawan, & Sharma, 2004).

The Houma, Cherokee and other Native American tribes used *P. incarnata* for food and medicinal purposes. In 1612, Captain Smith reported that Native Americans in Virginia planted the vines of *Passiflora* in order to produce the fruit of the plant. They were eaten raw, boiled to make syrup, and squeezed to make a drink from the juice. The roots were used in a tea infusion to treat boils, inflammation, and to cure liver problems. The plant was also used as a sedative to treat nervousness and hysteria (USDA).

The above ground (aerial) parts have been used as a sedative, anxiety reliever, pain reliever, as well as a convulsion preventative and reliever for epilepsy. Aqueous extract (extract prepared by evaporating a watery solution of the soluble properties to a semisolid or solid consistency) of *P. incarnata* roots has been used topically on ulcers and hemorrhoids. The whole plant has also been used in the treatment of insomnia, anxiety, and other central nervous system disorders (Dhawan, Kumar, & Sharma, 2001; Dhawan & Sharma, 2001). However, to date, there is little scientific validated evidence for the constituents of *P.incarnata* that are responsible for the sedative and anxiolytic effects (Ngan & Conduit, 2011).

P. incarnata is currently listed in the pharmacopoeias of Great Britain, United States, India, France, Germany, Switzerland and others. The active ingredients have not been conclusively defined. Most available data suggests flavonoids and indole alkaloids as possible active components (Elsas et al., 2010).

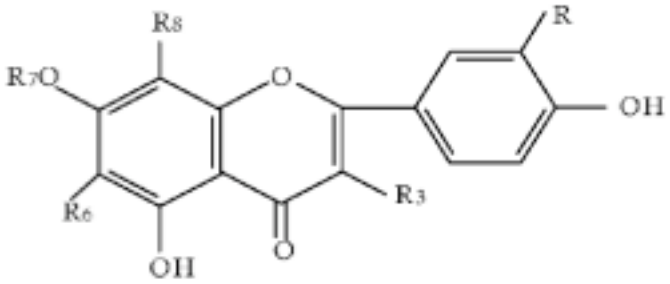

Figure 5. Flavonoid compound chemistry structure (Dhawan et al, 2004).

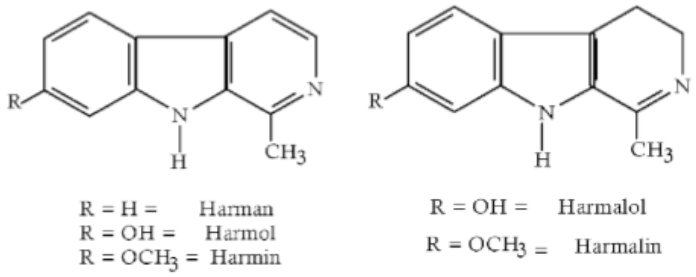

Figure 6. Indole alkaloids chemistry structure (Dhawan, 2004).

Passionflower fruit is widely eaten today and is used in juice drinks, beverages, baked goods, cereals, yogurt, ice cream, candy, jams, and jellies (Ngan & Conduit, 2011).

Chemistry and Pharmacology

The constituents of *Passiflora incarnata* include flavonoids, flavonoid glycosides, alkaloids, cyanogenic glycosides, carbohydrates, amino acids, benzopyrone derivatives and volatile constituents, as well as coumarins from the roots (Soulimani et al., 2007).

The main chemical constituents of *Passiflora incarnata* are flavonoids (0.25%). These include vitexin, isovitexin, orientin, isoorientin, apigenin, and kampferol, luteolin, quercetin, schaftoside, isoschaftoside (**Figure 5**). The greatest accumulation of flavonoids is found in the leaves (Dhawan, Dhawan, & Sharma, 2004). The simple indole alkaloids (0.1%) such as harman, harmin, harmalin, harmol, and harmalol are also active compounds (**Figure 6**). The medicinal extra of the plant has 10-20 ug/100 ml of harman and harmine. *P. incarnata* also contains the active compounds y-benzo-pyrone derivative maltol and ethyl-maltol (0.05%) (**Table 1**).

Other constituents include carbohydrates such as raffinose, sucrose, d-glucose, and d-fructose; essential oil with hexanol (1.4%), benzyl alcohol (4.1%), linalool (3.2%), 2-phenylethyl alcohol (1.2%), 2-hydroxy benzoic acid methyl ester (1.3%), carvone (8.1%), trans-anethol (2.6%), eugenol (1.8%), isoeugenol (1.6%), β-ionone (2.6%), α-bergamotol (1.7%) and phytol (1.9%). The constituents responsible for the odor are limomene, cumene, prezizaene, zizaene, amino acids; and cyanogenic glycoside gyanocardin (Dhawan, Dhawan, & Sharma, 2004).

The pharmacological effects of *P. incarnata* include anticonvulsant, anti-asthmatic, antitussive, and anxiolytic activities (Grundmann et al., 2008). Although there is a vast use of *P. incarnata*, the pharmacological work on this plant had been insufficient and inconclusive in determining the mode of action of the plant as well as the phyto-constituents responsible for the anxiolytic and sedative effects of the plant (Soulimani et al., 1997).

Researchers have proposed different theories on the bioactive chemicals of *P. incarnata*. However, there has been no

Active Compounds
Flavonoids (0.25%)
Vitexin
Isovitexin
Orientin
Isoorientin
Apigenin
Kampforel
Luteolin
Quercetin
Schaftoside
Isoschaftoside
Indole Alkaloids (0.1%)
Harman
Harmin
Harmalin
Harmol
Harmalol
Maltol and ethyl-maltol (0.05%)

Table 1. Constituents of *Passiflora incarnata.*

consensus regarding the exact mode of its pharmacology. Some researchers have highlighted the role of the flavonoid chrysin and even the b-pyrone derivative maltol to be responsible for the CNS effects of the plant (Movafegh, Alizadeh, Hajimohamadi, Esfehani, & Nejatfar, 2008). The indole alkaloids and flavonoids have been speculated to be the main bioactive constituents of the plant. They are believed to be so due to their MAO enzyme inhibiting properties (Dhawan, Dhawan, & Sharma, 2004). Some findings have attributed the sedative and anxiolytic activities in *P. incarnata* to benzodiazepine and GABA receptors in the body. By increasing levels of GABA in the brain, lowering the activity of some brain cells, one feels more relaxed (Dhawan, Kumar, & Sharma 2001). Yet contrary to many of these reports, the inclusive pharmacological studies on *P. incarnata* by Soulimani et al. (1997) ruled out the main phyto-constituents of flavonoids and indole alkaloids as the sources of psychotropic activity. Although the sedative and anxiolytic properties of the plant were shown in their studies, the mode of action of both the aqueous and hydroalcoholic extracts were not found.

Biological Activity

In vitro

Passionflower fruit, which is used to produce mark, jams, and juices, has shown to contain a considerable amount of lycopene. This finding shows that passionflower fruit products may serve as a good alternative for those people who do not or cannot eat tomatoes and tomato products (which is known to have a high amount of lycopene). Lycopene, a carotenoid antioxidant, has been shown to protect against oxidative damage in many epidemiological and experimental studies. In addition to its antioxidant activity, other metabolic effects of lycopene have also been demonstrated (Mourvaki, Gizzi, Rossi, & Rufini, 2005).

Dhawan, Kumar, and Sharma (2001) show that roots of *P. incarnata* do not have anxiolytic effects, but act as natural adulterants and should be separated from the aerial parts prior to any pharmacological, phytochemical and standardization studies on *P. incarnata*. The presence of flowers along with leaves and stems is also undesirable. Using the whole plant for pharmacological studies (both commercial and medicinal purposes) is not optimal for finding the CNS effects of *P. incarnata*. Although the separated leaves afford the best possible results, the selection of the entire aerial parts (except for the flowers) may prove to be the best approach for

identifying the bioactive parts of the *P. incarnata* plant.

In vivo

Studies in animal models show efficacy of *Passiflora incarnata* extracts and flavonoid fractions against pentylenetetrazol (PTZ) induced seizures. It is possible that *Passiflora* inhibits the benzodiazepine site antagonist Ro 15-1788, suggesting the involvement of GABAA receptors. Flavonoids bind to the benzodiazepine site of the GABAA receptor, but appear to alter GABAA and also GABAC receptor currents by a different mechanism than benzodiazepines (Elsas et al., 2010). This finding suggests that the anticonvulsant activity of the plant is attributed to the benzodiazepine and GABA receptors mediated biochemical processes in the body (Dhawa, Dhawa, & Sharma, 2004).

Soulimani et al. (1997) examined the potential anxiolytic and sedative effects of aqueous and hydrocoholic extracts of *Passiflora* on mice. The aqueous extract appeared to induce sedative effects at 400 and 800 mg/kg. It reduced activity in the staircase and free exploratory tests. The hdyroalcoholic extract did not have such sedative effects, rather, it appeared to enhance activity and have an anxiolytic effect at 400 mg/kg.

Singh, Singh, and Goel (2001) used hydroethanolic extracts of *Passiflora* to explore the anticonvulsant effects of the plant. Treatment with the extract significantly decreased seizure severity at 300 and 600 mg/kg. This finding suggests that the presence of flavonoids, which act by agonizing the GABA-benzodiazepine receptor may be responsible for the anticonvulsant activity. Since depression in the mice was induced by seizures, the anti-depressive effect can be due to the suppression of seizures.

In Grundmann et al. (2008), oral administration of an extract prepared from the herb of *Passiflora incarnata* induced an anxiolytic-like effect in mice, since it increased the number of entries and the time spent in open arms in the EPM test at a dose of 375 mg/kg. Although the anxiolytic effects of various extracts from *Passiflora* have been demonstrated in animals and in humans, the mode of action and the identity of the active constituents of the different *Passiflora* species remained unclear.

In Dhawan and Sharma (2001), the methanol extract of the leaves of *P. incarnata* was shown to be significantly active in suppressing the SO2 -induced cough in mice. These results provide evidence for the folklore claims on the effectiveness of the plant in managing “tough” cough conditions. Moreover, this finding showed that *P. incarnata*, having no reports to possess an addictive quality, could be an optimal alternative to cough-suppressants opiates, which have several negative effects including CNS depression, dryness of the mouth, blurred vision, and severe gastrointestinal effects.

The ethanolic extract of *P. incarnata* showed anti-inflammatory properties at a dose of 125 to 500 mg/kg in rats when inflammation was induced by cotton pallets, dextran and carrageenin (Borrelli, Pinto, Izzo, Mascolo, Cappaso, Mercati, Toja, & Autore, 1996). This provides evidence for the plant’s anti-inflammatory properties.

Clinical Studies

Although the effects of *Passiflora incarnata* have been studied less in humans, Akhondzadeh, Naghavi, Vazirian, Shayeganpour, Rashidi, and Khani (2001) and Movafegh, Alizadeh, Hajimohamadi, Esfehani, and Nejatfar (2008), are two clinical trials that have demonstrated *Passiflora*’s efficacy in the treatment of anxiety. The findings of the studies

attribute the effectiveness of the treatment to the anxiolytic and sedative actions of the passionflower extract (Ngan & Conduit, 2011). Akhondzadeh, Naghavi, Vazirian, Shayeganpour, Rashidi, and Khani (2001) also explored the use of the plant in the treatment of opiate withdrawal and attention-deficit hyperactivity disorder (ADHD).

Akhondzadeh et al. (2001) study on anxiety used the Hamilton anxiety rating scale to assess the anxiety levels of 36 outpatients diagnosed with generalized anxiety disorder. The anxiety scores indicated that passionflower (45 drops of *P. incarnata* extract per day) was just as effective as the positive control condition (30 mg of oxazepam per day) in reducing anxiety levels at the end of the 28-day treatment. In another study, Movafegh et al. (2008) used a numerical rating scale to assess the anxiety levels of 60 ambulatory surgery patients during the 90 minute period between premedication (placebo or 500 mg of passionflower tablets) and surgery. They found that the group that received passionflower had significantly lower anxiety ratings than the placebo group after premedication. These clinical findings indicated that *P. incarnata* may be effective in reducing short term as well as long term, heightened anxiety (Ngan & Conduit 2011).

Akhondzadeh et al. (2001) study on opiate withdrawal randomly assigned 65 opiate addicts to treatment of *Passiflora* extract and clonidine tablet or a placebo and clonidine tablet. Although both were effective in treating the physical symptoms of withdrawal, *Passiflora* in addition to clonidine had a significant increase in managing mental symptoms than clonidine on its own. These results suggest that *Passiflora* extract may be effective for the management of opiate withdrawal.

Contraindications

The FDA lists *Passiflora incarnata* as a safe herbal sedative. Since the mode of CNS depressant activity has not been conclusive, it is advised to take caution when taken with other CNS depressants or stimulants. The neuromuscular relaxing effects of the extract have synergetic effects with aminoglycoside antiobiotics such as clindamycin (Dhawan, Dhawan, & Sharma, 2004).

The use of the plant up to one month is considered safe. However, negative affects associated with the plant include dizziness, confusion, sedation, and a lack of coordination (Tovar & Petzel, 2009).

There has been a report about a woman who developed severe nausea, vomiting, weakness, drowsiness, slower heart rate, and ventricular arrhythmia, after two days self-administration of an herbal remedy containing *P. incarnata* (Fisher, Purcell, & LeCouteur, 2000). A case of hypersensitivity with skin vasculitis (inflammation of blood vessels) and hives after ingestion of tablets containing *Passiflora* extract has been reported (Movafegh et al., 2008). Five patients have been reported to suffer altered consciousness after taking an herbal preparation from *P. incarnata* called Relaxir (Dhawan, Dhawan, & Sharma, 2004).

In an *in vivo* study, no acute toxicity was observed after an injection into the peritoneum of mice in doses larger than 900 mg/kg (Movafegh et al., 2008).

Passiflora may induce uterine contraction so its use is contraindicated during pregnancy (Movafegh et al., 2008). The use of *Passifora* is also highly discouraged in lactating mothers. There is also a severe contraindication of the plant with the synthetic MAO inhibitor drug Phenelzine (NARDIL) (Dhawan, Dhawan, & Sharma, 2004).

Current Use in Allopathic and CAM Therapies

Passiflora incarnata is currently used primarily to treat alcoholism, asthma, insomnia, nervousness, anxiety, eye tension, headaches, menopausal symptoms, and neuralgia (acute pain). It's secondary applications include bronchitis, depression, muscle spasms, seizures, convulsions, diarrhea, epilepsy, pain (for example, menstrual) and restlessness (Tenney, 2007). It is available as a liquid extract, capsule, tea, and tincture (**Figure 7**). The recommended dose for anxiety is 45 drops (90mg) daily (Tovar & Petzel, 2009).

Gupta, Kumar, Chaudhary, Maithani, and Singh (2011) showed the anti-diabetic properties of the *Passiflora incarnata*. This study explored the hypolygemic and hypolipidemic effects of the methanolic extract of *P. incarnata* on induced diabetic mice. At a dose of 200 mg/kg, the mice had significantly increased tolerance in glucose. This depicts that the leaf can be a good alernative medicine for diabetes as it has significant anti-hyperglycemic activity in streptozocin-induced diabetic mice.

Frontier markets the herbal supplement to naturally promote relaxation. To make the tea, you boil 1-2 teaspoons of the herb, cover it, and let it seep for 5 to 10 minutes. Gaiia markets the passionflower to support the nervous system, to promote calm and relaxation, and to support healthy sleep. Its recommended dose is 1 capsule, 2 times daily between meals. Gaia also makes an extract of the passionflower, and its suggested use is 40-60 drops in a small amount of water, three times daily between meals.

The Passionflower fruit itself is eaten globally and is widely used in juice drinks, beverages, baked goods, cereals, yogurt, ice cream, candy, jams, and jellies (Ngan & Conduit, 2011).

Figure 7. Herb, extract, and capsule form of *Passiflora incarnata.* (Photo by Ronni Shalem).

Discussion

Passiflora incarnata was an important plant for the Native Americans in traditional medicine. It served many purposes including food and beverage as well as having medicinal properties. After being introduced to the Europeans, *P. incarnata* became a popular traditional European remedy and a homoeopathic medicine for insomnia and anxiety. It is now used all over the world. This is evident by the fact that it is listed as an official plant drug in the British Herbal Pharmacopoeia (1983), Homeopathis Pharmacopoeia of India (1974), United States Homoeopathic Pharmacopoeia (1981), Pharmacopoeia Helvetica (1987), as well as the pharmacopoeias of Egypt, France, Germany, and Switzerland (Dhawa, Dhawa, & Sharma, 2004).

The importance of the plant is exemplified in its use all over the globe. In Brazil, *Passiflora* has been used as an analgesic, anti-spasmodic, anti-asthmatic, wormicidal, and sedative. In Iraq, it has been used as a sedative as well as a narcotic. In Turkey, *Passiflora* has been used to treat dysmenorrhea, epilepsy, insomnia, neurosis, and neuralgia. It has been used to cure hysteria and neurasthenia in Poland. *P. incarnata* has also been used to treat morphine addiction in India (Dhawan, Dhawan, & Sharma, 2004).

Almost every component of the plant has shown to have some form of pharmacological activity. There have been many studies on the plant both in vivo and in vitro, although mostly *in vivo*, to explain the chemical activities of *P. incarnata*. Studies in animal models show efficacy of *Passiflora* extracts and flavonoid fractions against pentylenetetrazol (PTZ) induced seizures. In Grundmann et al. (2008), *P. incarnata* extract induced an anxiolytic-like effect in mice when administered orally. In Dhawan and Sharma (2001), the methanol extract of the leaves of *P. incarnata* was shown to be significantly active in suppressing the SO2 -induced cough in mice. Although the effects of *P. incarnata* have rarely been studied in humans, two clinical trials have demonstrated its efficacy in the treatment of anxiety. It has also been shown to be effective in opiate withdrawal. *P. incarnata*, serving so many medicinal purposes, has much to contribute to our world of medicine.

In the future of our medical sphere, as people continue to consider holistic lifestyles of alternative medicine, *P. incarnata* will be an important plant to turn to for its variety of medicinal applications. Its various uses to treat conditions from fevers to neuralgia and seizures to diarrhea, the multiplex applications of the plant are pretty remarkable. For someone who is not comfortable taking synthetic drugs or synthetically derived drugs to treat medical ailments such as anxiety and insomnia, they can turn to *P. Incarnata* as a natural remedy. In addition, people can incorporate the passionflower fruit in their diets for its lycopene content, which is extremely notable for its antioxidant activity. To do so, the fruit can simply be blended into a juice.

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Persea americana Mill., Lauraceae

Corinne Grady

Introduction

Persea americana Mill. of the Lauraceae family is commonly known as the avocado tree. This edible fruit is also called the alligator pear, el aguacate in Spanish, and e li in Chinese ("Persea americana (Mill),"). The English name avocado is derived from the Spanish aguacate, which in turn comes from the Nahuatl, or Aztec, name ahuacatl (Popenoe, Zentmyer, & Schieber, 1997). As depicted in **Figure 1**, *P. americana* is a tall, green leafy tree with single seeded fruit. The plant originates from the tropical and subtropical climate regions of Central and South America, where indigenous people first cultivated it. For thousands of years, the fruit, leaves, and seeds of the avocado tree have been used as a food source and for medicinal purposes. The uses of *P. americana* range from emollients for the skin to treatments for hypertension. The main chemical constituents that lend to the activity of avocado are aliphatic acetogenins, flavonoids, terpenoid glycosides, and furan-ring derivatives (Yasir, Das, & Kharya, 2010). Today, the flesh of the fruit is cultivated and consumed worldwide and is generally recognized as safe as a food source (Morton, 1987).

Figure 1 *Persea americana* Mill (Source: http://www.public.asu.edu/~camartin/plants/Plant%20html%20files/perseaspecies.html)

Botanical Description

The genus *Persea* contains 8 species, which are all trees or shrubs ("Persea americana Mill, avocado,"). A polymorphic tree native to Central America, *Persea americana* has a variety of subspecies, which include the commercial varieties of avocado. The varieties of *P. americana* are *americana, drymifolia, floccosa, guatemalensis, nubigena, steyermarkii, tolimanensis,* and *zentmyerii*. Each subspecies has distinct botanical features as described in **Table 1**. Three of these varieties (the West Indian, Mexican, and Guatemalan avocado) have been domesticated and are used commercially for their fruits, which all slightly differ in size, shape, and color. The necessary climate and soil type for each subspecies varies. For example, the Mexican variety is the hardiest and can survive in the coldest weather (25°F). The Guatemalan variety

Subspecies	Common name	Distinctive botanical features		
		Tree	Leaf	Fruit
P. americana var. *americana*	West Indian avocado	Small to large, low elevation	N/A	10-15cm long green mature fruit with flesh more than 1 cm thick
P. americana var. *drymifolia* (rare in Guatemala, common in Mexico)	Mexican avocado	Small to large	N/A	<5cm long purple-black mature fruit with little flesh, anise flavored
P. americana var. *guatemalensis*	Guatemalan avocado	N/A	N/A	Nearly round fruit >4cm diameter, 2-3 cm of flesh, rough to smooth exocarp, green to black
P. americana var. *floccosa*	N/A	Native to eastern Mexico	Young growth of floccose pubescent	Thick green to black exocarp, not much longer than broad
P. americana var. *nubigena*	N/A	N/A	Secondary veins 30-50° divergent, glaucous	Thick green to black excocarp, little flesh <4cm diameter
P. americana var. *steyermarkii* (rare)	N/A	N/A	Secondary veins 45-75° divergent, glabrous	Thick green to black excocarp, little flesh <4cm diameter
P. americana var. *tolimanensis*	N/A	Branching begins high on trunk	Dull foliage	Round to oblong fruit 6-8cm diameter, bitter taste, irregular rough green exocarp
P. americana var. *zentmyerii* (very rare)	N/A	N/A	N/A	3.5-5cm diameter, little flesh, thick green to black exocarp covered with corky ridges

Table 1: Key features of the subspecies of *P. americana* (Whiley et al., 2002)

originated in the subtropical highlands of Central America and while not as hardy as the Mexican variety, it is tougher than the West Indian race, which only grows in tropical or near tropical climates like southern Florida and Puerto Rico.

Although the avocado tree needs a particularly humid and non-windy climate, it can grow in an assortment of different soil types. Some soil types include red clay, volcanic soil, sand, limestone, and slightly alkaline soils. The pH level preferred by *P. americana* is between 6 and 7, however avocados in southern Florida can grow on land with pH levels between 7.2 and 8.3. Good drainage is also essential for tree growth. The trees can grow up to 30 meters tall with leaves up to 20 centimeters long and 24 centimeters wide. The trees are almost evergreens, only losing their leaves briefly during dry seasons. The leaves are glossy and dark green on top with a paler bottom surface. They vary from oval to oblong and rounded to obtuse in shape. The flowers of *P. americana* are small in comparison; only reaching from a few millimeters to a couple of centimeters long. They are small, yellow or pale green in color, and do not have petals. Instead, the flowers have sepals, whorls of perianth lobes and nine stamens (**Figure 2**). The female and male organs of the flower do not operate simultaneously. Unlike all other flowering plants, the avocado's flowers first function as only female – accepting

Figure 2. Avocado Flower (Source: http://www.doyletics.com/images/avocflwr.jpg)

pollen but not producing any from their stamen. This stage only lasts for two to three hours and is the only time in which sperm from another flower can fertilize the egg. Bees and sometimes other large insects are the pollinators for the avocado flower in addition to its self-pollination capabilities. Once the two to three hours have passed, the flower's sepals close for about twenty-four hours. When the flower reopens, it sheds pollen rather than receiving it until the flower closes again. This second closing is permanent and if fertilization is successful, an avocado will begin to grow. Cross pollination is able to occur because every plant has two different varieties of flower – the A type and the B type. Usually, the A type flowers open as female in the morning and are male the following afternoon while the B type do the opposite so that there is

Figure 3. A single fruit on a *P. americana* tree
(Source:("Persea americana Mill, avocado,")

pollen to fertilize the female flowers during both parts of the day. When temperatures fall below 70°F, however, the flowers' cycles become irregular and the plant produces less fruit.

In general, *P. americana* produce fruit, called avocados (**Figure 3**). These single seeded berries range in size from 3.5 cm to 20 cm long, can have smooth to rough skin, and vary in color from green to purple-black. The fruit does not ripen until removed from the tree due to inhibition from the stem. Some related species include *Persea schiedeana* (Lauraceae) and *Beilschmiedia anay* (Lauraceae). These are both trees native to Central America. They can grow up to 20 meters high, which is slightly shorter than *P. americana.* Both produce fruit similar to varieties of the avocado, however, their flowers are very different. *P. schiedeana* produces yellow-green, downy flowers with stamens that redden over time. *B. anay* produces 5-inch long green flowers. The fruit of *P. schiedeana* is marketed locally while the fruit of *B. anay* is not (Bergh, 1973; Morton, 1987; Whiley, Schaffer, & Wolstenholme, 2002).

Traditional Uses

Food Uses

Avocados have been used as a food source in the Americas, particularly in Central America, for approximately 10,000 years. The three types of edible avocados consumed by the indigenous people are *Persea americana* var. *drymifolia*, *P. americana* var. *americana*, and *P. americana* var. *guatemalensis* (Williams, 1977*).* These three subspecies were selected and cultivated by indigenous populations of Mexico, the Pacific coast of Central America, and Guatemala respectively (Whiley et al., 2002). In addition, avocados were intentionally taken from their centers of origin to other places throughout Central and South America for cultivation purposes even before Europeans landed in the Americas. Evidence for the cultivation of avocado trees was discovered in excavations in Peru which revealed avocado remains dating back to 4000BC (Whiley et al., 2002). The Aztec people in Central America used avocado, or ahuacatl, in the dish ahuacamulli, which is now known as guacamole. This term "mulli" means sauce or soft dish in the Nhuatl language. So the Aztec compound word for guacamole is a description of the dish, which is made by mashing up the avocado fruit into a soft paste and adding other ingredients. Ahuacachiaualloti, meaning avocado oil, was also used by Aztec people thousands of years ago (Popenoe et al., 1997). Indigenous American peoples also eat avocados whole with salt and tortillas. Today, avocados are served with salads, in sandwiches, and are added to hot dishes just before serving. The avocado becomes very bitter when cooked due to a significant amount of tannins in the flesh so the fruit is normally served cold. In Brazil, New Zealand, Hawaii, and Java the avocado is treated more like a fruit than a vegetable like in the Americas. The fruits are used in milkshakes, ice creams, and fruit salads sweetend with sugar (Morton, 1987).

Ethnomedicinal Uses

P. americana has been, and continues to be, used for a variety of ailments as a traditional medicine. The leaves of *P. americana* are used in traditional medicine to treat hypertension in Trinidad and Tobago (Lans, 2006). In Togo, a decoction of *P. americana* leaves and *Theobroma cacao* leaves are taken orally as an antihypertensive agent. In addition, a combination of *Lippia multiflora* leaves, *Stachytarpheta angustifolia* leaves, *Allium sativum* bulbs and *P. americana* fruit are made into a powder and taken orally for the treatment of hypertension (Karou et al., 2011). In the Philippines, the seeds or a decoction of *P. americana* are used to relieve toothaches by applying them to tooth decay (Lewis & Elvin-Lewis, 2003). In the Dominican Republic, the seeds are fried and mashed along with several other plants to treat

lice (Lewis & Elvin-Lewis, 2003). Avocado are also used to treat snakebites through neutralizing the hemorrhagic effect of snake venom (Lewis & Elvin-Lewis, 2003).

Other Uses

Different parts of *P. americana* are utilized for nonfood and non-medicinal purposes. For example, the bark of *P. americana* is used to set the color of dyes in Guatemala. Wood from avocado trees is used in construction. During the time of the Spanish conquistadores, documents were written with the red-brown ink expelled from avocado seeds (Morton, 1987). In cosmetics, avocado oil is used an emollient and sunscreen (Swisher, 1988).

Chemistry and Pharmacology

The main chemical components that have been identified in *Persea americana* include alkanols (or aliphatic acetogenins), flavonoids, terpenoid glycosides, furan-ring derivatives and a coumarin. These chemical groups all contain different functionality, which enable diverse biological activity (Yasir et al., 2010). There are over six hundred biological activities known for the compounds identified in *P. americana* (Duke, 2012). As described in **Table 2**, most of the known compounds in avocados have multiple activities ranging from antibacterial and antiviral to hypotensive and antidiabetic effects (Duke, 2012). Persin, **Figure 4**, is an alkanol constituent of avocado leaves that has been studied rather extensively. Persin was first studied due to its observed toxic effects on animals, especially among lactating mammals (Oelrichs et al., 1995). Later research found that persin has *in vitro* activity against breast cancer, as later described (Butt et al., 2006). Other major components of *P. americana* fruit are lipids. With up to 864,000 ppm in the fruit, fat makes up a significant portion of avocado chemistry (Duke, 2012). The main form of glycolipids and phospholipids in *P. americana* are oleic and linolenic acids. There is a different composition of the lipids in the fruit and in the seed; the most significant difference being a higher oleic acid content in the fruit compared to the seed (Pacetti, Boselli, Lucci, & Frega, 2007). While many of the chemicals in *P. americana* are well understood and defined in terms of their biological activity, there are even more compounds with unknown activity and more studies are needed to elucidate a fuller picture of the avocado's chemical and biological effects within the human body.

Biological Activity

There is extensive biological activity among the constituents of *Persea americana*. Several studies have examined the effects of various extracts of different parts of *P. americana,* both *in vivo* and *in vitro.* These studies can be classified into several categories based on the type of activity found, including analgesic, anti-inflammatory, anticonvulsant, anticancer, antioxidant, and hypotensive activity, among others.

Analgesic and anti-inflammatory

Adeyemi et. al found in an *in vivo* study with mice, that an aqueous extraction of dried avocado leaves, which were processed through boiling, filtration, and drying, had significant analgesic and anti-inflammatory effects. For example, a 1600mg/kg dose of the extract inhibited acetic acid-induced pain (evaluated based on the writhes of the mice) equivalent to a 100mg/kg dose of acetylsalicylic acid. An 800mg/kg dose inhibited pain response to heat equivalent to

Plant Part	Antibacterial	Anticancer	Antioxidant	Colorant	Multiple activities[1]	Unknown
	Ascorbic-acid[2] Proanthocyanidins[2]	Alpha-carotene[2] Alpha-tocopherol[2] Folic acid [2] Linoleic acid[2] Lutein[2] Oleic acid[2] Serine	Alanine[2] Histidine[2] Lecithin[2] Manganese[2] Methionine[2] P-coumaric acid[2] Palmitic acid[2] Riboflavin[2] Tartaric acid[2] Threonine[2] Tryptophan[2]	Alpha-cryptoxanthin[2] Violaxanthin[2]	Alpha-tocopherol, arginine, aspartic-acid, beta-carotene, biotin, boron, caffeic acid, calcium, carnitine, chlorogenic acid, copper, fiber, folacin, glutamic acid, glycine, glycerol, iron, isoleucine, lysine, magnesium, mufa, niacin, nonacosane, pantothenic acid, phylloquinone, phytosterols, potassium, propionic acid, pufa, pyridoxine, salicylates, serotonin, stearic acid, thiamin, tyramine, tyrosine, valeric acid, valine, zinc	1-acetoxy-2,4-dihydroxyheltadeca-16-ene, abscisic acid, ash, carbohydrates, d-erythro-d-galacto-octitold-erythro-l-gluco-nonulose, d-glycero-d-galacto-heptitol, d-glycero-d-galacto-heptose, d-glycero-d-galacto-octulose, d-glycero-d-manno-octulose, d-mannoketoheptose, d-taloheptulose, dopamine, fat, hentriacosane, heptacosane, isolutein, leucine, linolenic acid, myo-inositol, p-coumaryl-quinic acid, pentacosane, phenylalanine, phytate, proanthocyanidin-a-2, proline, sfa, triacosane
Fruit[3]	N/A	4,8-biscatechin	N/A	N/A	N/A	avocadene, avocadenofuran, avocadenone-acetat, avocadineofuran, avocadyne, avocadynofuran, avocadynone-acetate, avocatins, d-arabitol, galactitol, isoavocadienofuran, perseitol
Seed[4]	Pinene[2]	N/A	Tannin[2]	N/A	anethole, estragole, beta-sistosterol, methyl-chavicol, quercetin,	N/A
Leaf[5]	N/A	N/A	N/A	N/A	N/A	n-caffeoylputrescine
Flower	N/A	N/A	N/A	N/A	Anethole Estragole	N/A
Bark	1,2,4-trihydroxyheptadeca-16-ene Cycloartenol	Catechol[2]	Campesterol[2] Cholesterol	Epsilon-carotene	Phosphorus Subaphyllin	24-methylene-cycloartenol Feruloyl-putresine

Table 2: Chemical composition of *Persea americana* Source: (Duke, 2012)

1. Compounds with 3 or more biochemical activities are listed here. The activities for an individual compound may include those listed in the table or any of the 630 known activities for *P. americana* compounds.
2. Compounds also have multiple biological activities, however they are listed under one of their significant activities.
3. Also contains: citrostadienol which is an antirheumatic; /cryptoxanthin which is antimutagenic; cystine which is antihomocystinuric; palmitoleic acid which makes soap; sodium; vit-B6; and vit-D.
4. Also contains volemitol, which is a sweetener.
5. Also contains paraffin, an emollient.

a 2mg/kg does of morphine. Swelling and inflammation induced by carrageenan, a compound extracted from seaweed, was also inhibited by a previously administered dose of the extract (Adeyemi, Okpo, & Ogunti, 2002). In another study, polyhydroxylated fatty alcohols from avocados were found to suppress inflammatory response (Rosenblat et al., 2011).

Anticonvulsant

P. americana aqueous leaf extracts were tested on mice for the extract's ability to delay and reduce seizures induced by pentylenetetrazole, picrotoxin and bicuculline. In each type of induced seizure, the aqueous leaf extracts yielded positive results indicating that the extract has some effect on GAGAergic action and/or neurotransmission involved with seizures (Ojewole & Amabeoku, 2006).

Anticancer

Extracts of avocado have been found to inhibit cancer growth and have some chemo-protective effects. In one study, a chloroform soluble extract of the avocado fruit flesh was found to have activity inhibiting oral cancer cell growth. The mechanism of action of two aliphatic acetogenin compounds isolated from this extract was found to involve the blocking of phosphorylation of protein kinases and growth factor receptors in the EGFR/RAS/RAF/MED/ERK1/2 cancer pathway. The two compounds work together to produce this anticancer effect (D'Ambrosio, Han, Pan, Kinghorn, & Ding, 2011). Another study investigated the cartenoid content of avocado fruit and found that an acetone extract of the fruit inhibited prostate cancer cell growth *in vitro.* The main carotenoid in the extract was lutein, at 70%, but alone it did not have the anticancer effects that the extract possessed. A mechanism of action involving the monounsaturated fat content of avocado and the relationship with absorption of the phytochemicals into the bloodstream was proposed but has yet to be verified (Lu et al., 2005). Another chemo-protective effect was discovered with avocado fruit extracted using methanol. This effect works with regard to the anticancer drug cyclophosphamide, which may have genotoxic effects on healthy cells (Paul, Kulkarni, & Ganesh, 2011). A study on the effects of persin found that both *in vivo* and *in vitro* the compound is cytotoxic for mammary epithelium for mice and human breast cancer cells, respectively. Applications of persin to human breast cancer cells *in vitro* result in a halt of the cell cycle, preventing further division, and apoptosis due to interference with microtubules (Butt et al., 2006).

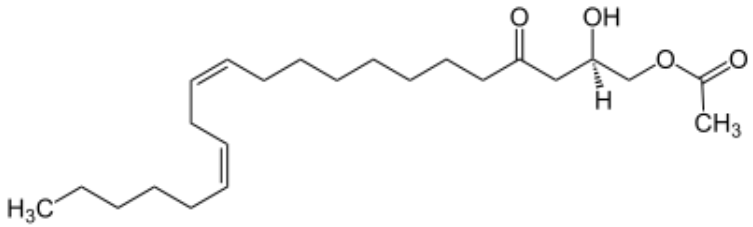

Figure 4 Chemical Structure of Persin. (Source: http://upload.wikimedia.org/wikipedia/commons/thumb/f/f6/Persin_Structural_Formulae_V.1.svg/400px-Persin_Structural_Formulae_V.1.svg.png)

Antioxidant

P. americana contains many chemical compounds that have antioxidant capabilities. Wang et al. investigated the antioxidant capacity of avocado along with its phenolic content and proyanidins. The seeds had the highest antioxidant capacity and proyanidins and phenolic content while the fruit flesh had the lowest antioxidant capacity (Wang, Bostic, & Gu, 2010). Another study found that the catechins, proyanidins and hydroxycinnamic acids in the peels and seeds were present in larger amounts and were more antioxidant *in vitro* compared to those constituents in the pulp (Rodríguez-Carpena, Morcuende, Andrade, Kylli, & Estévez, 2011). An *in vivo* study on rats found that a methanol extract of avocado probably has an antioxidant effect on intracellular defense mechanisms used to cope with increased oxidant stress. This mechanism supports the finding that the extract could protect against oxidative stress and paracetamol toxicity by increasing the activity of the particular defense enzymes during hepatic damage (Yasir et al., 2010).

Antiulcer

An *in vivo* study of albino rats, it was found that the rats treated with an aqueous leaf extract of *P. americana* experienced a significant and does- dependant antiulcer effect when ulcers were induced (Yasir et al., 2010).

Antiviral

An infusion and an ethanol extraction of *P. americana* were compared regarding their inhibitory effects on viral replication. The infusion had activity against the three viruses AD3, HSV-1, and ADV whereas the ethanol extract was only active against HSV-1 and ADV (Yasir et al., 2010).

Hypoglycemic

In one study, areduction in blood glucose level was found to occur when aqueous leaf extract of avocado was administered to normal rats (Yasir et al., 2010).

Hypotensive

Dose-dependent hypotensive activity was discovered for intravenous doses of *P. americana* aqueous and methanol leaf extracts in normotensive rats. Doses used ranged from 6.25mg/kg to 50mg/kg with those above 12.5mg/kg having hypotensive activity when compared to the control group (Adeboye, Fajonyomi, Makinde, & Taiwo, 1999).

Vasorelaxant

There are several different mechanisms of action proposed for the vasorelaxant effects of aqueous *P. americana* extracts. In an *in vivo* study examining the aortas of rats, it was found that the vasorelaxation response occurs due to the administration of the extract and is concentration related (Yasir et al., 2010).

Wound healing

Normally, 17 days are required for an excision wound to heal in rats. However, in a study by Nayak et al. complete healing of an excision wound occurred in 14 days after oral or topical treatment with *P. americana* fruit extract at 300mg/kg/day (Nayak, Raju, & Chalapathi Rao, 2008).

Clinical Studies

Although there has been extensive research into the biological activity of *Persea americana*, few clinical studies have been done examining the avocado's effects in humans. There have been four clinical studies investigating the use of the non-soap-forming, or unsaponifiable, extract of *P. americana* combined with the unsaponifiable extract from soybeans, known as avocado/soybean unsaponifiables (ASUs), to treat osteoarthritis. These studies focused on osteoarthritis of the hip and/or knee. In two of the studies, 300mg of the extracts taken each day for three months resulted in lower use of non-steroidal anti-inflammatory drugs for pain and inflammation. There was no significant difference found between 300 and 600mg/day doses. In a 6-month study, a two month delayed onset of effects was observed and effects continued for two months past the end of treatment. No long-term effects on the prevention of joint narrowing were observed. The fourth study investigated effects of ASUs on hip osteoarthritis with two years of treatment. With a 300mg/day treatment, no statistically significant difference was found between the ASUs and placebo concerning levels of pain and NSAID usage after one year. Despite these negative results, ASUs may have symptom-relieving effects for the most severe hip osteoarthritis. All of these findings require further study for confirmation, whether positive or negative, concerning ASUs ability to modify osteoarthritis symptoms (Ameye & Chee, 2006).

Research investigating the antidiabetic and cholesterol-lowering capabilities of *P. americana* has found positive results in clinical trials. For example, a study by Lerman-Garber et al found that a diet high in monounsaturated fatty acids from avocados and olive oil for patients with non-insulin-dependent diabetes patients resulted in minor decrease in cholesterol and a 20% decrease in plasma triglycerides (Lerman-Garber, Ichazo-Cerro, Zamora-González, Cardoso-Saldaña, & Posadas-Romero, 1994). Another study comparing an avocado enriched diet high in monounsaturated fatty acids and a high carbohydrate diet also found that the avocado enriched diet was more effective in lowering total cholesterol levels and low-density-lipoprotein (Colquhoun, Moores, Somerset, & Humphries, 1992). These clinical trial results coincide with the hypocholesterolemic activity of some of the chemical constituents in *P. americana* (Duke, 2012).

Contraindications

Although the fruit of *Persea americana* is widely cultivated and consumed safely as a food source, there are contraindications for the avocado. Avocado hypersensitivity, while rare, is one contraindication. Possible symptoms include anaphylaxis, angioedema, bronchial asthma, rhinoconjunctivitis, urticaria and vomiting (Blanco, Carrillo, Castillo, Quiralte, & Cuevas, 1994). In a study of avocado hypersensitivity, an allergic reaction usually occurred when the patient had a preexisting allergy to latex, chestnut, and/or other fruit, such as banana (Blanco et al., 1994). Interactions with Warafin, an anticoagulant, have been documented although the

mechanism through which avocado prevents the anticoagulant effects of Warafin is unknown (Blickstein, Shaklai, & Inbal, 1991). In addition, the leaves of *P. americana* contain a toxic compound, persin, which has been found to cause necrosis of lactating mammary glands and the myocardium in mice (Oelrichs et al., 1995). Instances of poisoning have been reported in rabbits, horse, cattle, goats, fish and canaries (Lewis & Elvin-Lewis, 2003). These poisoning are most likely due to the persin content present in the avocado plant (Yasir et al., 2010).

Current Use in Allopathic and CAM Therapies

The only major current use of *Persea americana* in allopathic medicine is as an osteoarthritis treatment in which the unsaponifiable portions of the fruit are added to soy bean unsaponifiables (Ameye & Chee, 2006). These ASUs (avocado-soybean unsaponifiables) are also used as dietary supplements in complimentary and alternative medicine therapies for the treatment of arthritis and have support for effectiveness from positive results in clinical trials (Soeken, 2004). Another current use of avocado is the preparation of skin products with the oil due to the strong emollient properties of avocado oil. The oil is also a component of hair products. Some CAM treatments for skin disorders, like sclerosis, include the use of avocado oil (Khan & Abourashed, 2010). Although there are not many targeted medicinal uses of avocado being marketed at this time, the avocado is advertized as a health promoting food and is consumed internationally.

Discussion

Persea americana has been used for thousands of years as both food and medicine. The beneficial activity of avocado occurs due its composition of bioactive compounds, which affect a myriad of pathways in the human body. Further studies are needed, especially clinical studies, to verify the efficacy of traditional uses of *P. americana.* The many biological activities found in laboratory studies also need to be investigated with clinical studies to determine the effectiveness of specific activities in humans. Since instances of cancer and diabetes have become more widespread threats to human health worldwide, the avocado's activity in preventing and treating these diseases needs to be further researched. In particular, the development of cancer treatments should be studied since *P. americana* has shown activity against so many different types of cancer. In addition, the many chemical components with unknown biological effects must be studied for complete understanding of the effects of *P. americana* in the human body (**Table 2**). As a widely consumed food, whose cultivation has spread globally in more recent years, the avocado will continue to remain an important food source that provides nutrition along with flavor (Whiley et al., 2002). With proper investigation and drug development, the avocado has the potential to provide much needed medicines for a wide range of ailments in the future.

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Petroselinum crispum (Mill.), Apiaceae

Ellen Chiang

Introduction

Petroselinum crispum Mill. commonly referred to as parsley, belongs to the Apiaceae (Umbelliferae) family, which includes other popular plants such as carrot, coriander, and celery (Anthony & William, 1977). Parsley has a unique aroma that distinguishes it from other leafy, green herbs (Stace, 1991). The aromatic herb is used worldwide to garnish and flavor dishes. The perfume industry also uses the plant's essential oil, which is responsible for the aroma, in fragrances (Teuscher, 2005). The essential oil contains the most medically significant compounds, mainly terpenes and phenolic compounds (Taiz & Zeiger, 1998). These compounds contribute to the efficacy of the herb as a diuretic, immunomodulator, antioxidant, abortifacient, and hypoglycemic agent. *P. crispum* is one of the most widely known herbs, especially in the U.S. where it is found fresh in most supermarkets or in supplements, dietary pills, and essential oil extracts (Peter, 2004).

Botanical Description

Dioscorides named *P.crispum* "rock celery" because the undomesticated plant grew amongst rocks (Peter, 2004). *P. crispum* is native to the Mediterranean but naturalized throughout Europe. Parsley is now heavily cultivated, especially in the U.S., Europe, and western Asia (Navazio, 2012). Two of the most popular parsley varieties are *Petroselinum crispum* var. *crispum,* also known as curly-leafed parsley, and *Petroselinum crispum* var. *neapolitanum*, referred to as flat-leaf parsley or Italian parsley (Teuscher, 2005).

Figure 1. Flower umbels of flat-leaved parsley
Image source:
http://www.dispatch.com/content/stories/home_and_garden/2012/06/03/1-plant03-gm4hh52o-1.html

Parsley is a biennial herbaceous plant; the herb foliage grows in the first year and sets seed in the second year (Navazio, 2012). If parsley is being cultivated for its leaves, stems, or

Figure 2. Flat-leaved parsley
A close up of the morphological traits of Italian parsley leaves. Image source: http://www.sunlandherbs.com/wp-content/uploads/2010/07/parsley.flatleaf.HZell2009.WikiCom.jpg

Figure 3. Fool's Parsley
Fool's parsley leaves are structurally similar to flat-leaved parsley.
http://www.biolib.cz/en/taxonimage/id133998/?taxonid=40297

roots then these are collected the first year. However, seed collection occurs in the second year after the flowering period. *P. crispum* forms yellow-green flowers in 10-20 compound umbels (Anthony & William, 1977) (**Figure 1**). Wasp and hoverfly species cross-pollinate the parsley florets, each of which have five stamens, two styles, and a two-celled ovary. This type of ovary produces two seeds, one from each cell (Navazio, 2012). The ideal temperature for pollination and subsequent seed production is 65-85 degrees Fahrenheit; therefore, the flowering period typically occurs during the warmer months of June and July (Teuscher, 2005). Parsley seeds take about 3 weeks to germinate. This process can be accelerated by soaking the seeds in hot water a day prior to sowing in order to soften the seed coat (Peter, 2004).

P. crispum grows up to 60 to 120 cm tall in sunny or slightly shaded areas that have humid soil with a pH level of 5.3 to 7.3 (Navazio, 2012). *P.crispum* var. *neapolitanum* has dark green triangular leaves, which are flat and divided into 2-3 leaflets. The leaflet edges are pinnately separated (Polunin, 1969) (**Figure 2**). Fool's parsley, *Aethusa cynapium,* also has flat, triangular leaves with similar pinnation. These similarities often result in misidentifying *A. cynapium* as *P. crispum* var. *neapolitanum* (**Figure 3**). *A. cynapium* also belongs to the Apiaceae family but is poisonous (Stace, 1991). The unpleasant odor of fool's parsley helps distinguish it from

Figure 4. Curly-leaved parsley
Curly-leaved parsley displays a voluminous and ruffled appearance**.**
Image source:http://www.sunlandherbs.com/wp-content/uploads/2010/07/parsley.leaf_.WikiCom.jpg

Figure 5. Hamburg Root
Hamburg parsley variety has significantly larger roots.
Image source:
http://www.mariquita.com/images/photogallery/umbelliferfamily.jpg

parsley, which has a sharp, grassy aroma (Tisserand, 1995). *P. crispum* var. *cripsum* is similar to the *neapolitanum* variety except that there are more leaflets per stem and the leaves have a ruffled appearance (**Figure 4**). Parsley roots grow up to 20 cm in length and 5cm in width. The roots are a faint yellow color and carrot-shaped. However, *P. crispum* var. *radicosum*, or Hamburg root parsley, has much larger roots (**Figure 5**) and is commonly used in European cuisine (Teuscher, 2005).

P. crispum does not have many predators. The most common pest is the larva of *Papilio polyxenes asterius*, the black swallowtail butterfly. The larvae are often referred to as parsley worms. They feed on both celery and parsley foliage for several weeks before pupating. Parsley worms do not cause any major damage to the plant and can easily be dealt with by manually picking them off the plant (Schrock, 2004).

Traditional Uses

Symbolic

The ancient Greeks believed that parsley originated from the blood of Archemorus, the "Forerunner of Death" (Scoble & Field, 2001). The herb was a symbol of imminent death and was used in funeral rites. For example, parsley was given to

the terminally ill and parsley garlands were placed on corpses. Parsley also eventually became associated with Satan and was dedicated to the queen of the underworld, Persephone (Peter, 2004). Virgins were warned not to plant parsley, for doing so would result in losing their virginity to Satan. The Greeks believed that the parsley seed traveled to and from the underworld several times before germinating (Scoble & Field, 2001). Due to these negative associations, parsley was not consumed or incorporated into dishes. Interestingly, the Greeks began to crown athletic victors with parsley garlands in memory of important predecessors. Over time, parsley shifted from a symbol of death and Satan to a symbol of strength (Castleman, 2009).

Deodorant

The Romans had a less superstitious view of parsley, but they also did not widely consume the herb. However they often chewed on parsley to hide the lingering smell of garlic from their meals (Castleman, 2009). This may have contributed to the use of parsley to garnish plates of food. The Romans also believed that wearing parsley garlands would prevent intoxication and were thus worn at large banquets and parties (Peter, 2004).

Medicinal

P. crispum has a long history of medicinal use. Hippocrates classified parsley as a diuretic. During the Medieval period, parsley compresses were used to treat arthritis and chest pain was addressed by drinking wine boiled with parsley. In the 17th century, an English herbalist expanded on the medicinal relevance of parsley and recommended using *P. crispum* to alleviate menstrual symptoms. Parsley was administered both topically and internally (Lis-Balchin, 2006). In 1850 the U.S. pharmacopeia documented parsley as a laxative, diuretic, and quinine substitute (Castleman, 2009). Many communities continue to implement traditional medicinal practices. For example, Colombian immigrants of London continue to use parsley as a condiment for its digestive and cleansing properties. They also use parsley infusions to treat and regulate menstrual pain (Ceuterick, Vanderbroek, Tony & Pieroni, 2008). The powder of parsley seeds has also been massaged into the scalp in order to stimulate hair growth. Additionally, parsley juice was used to prevent and treat insect bites (Peter, 2004).

Compounds	Parsley Leaf Oil (% composition)	Parsley Seed Oil (% composition)
α-pinene	26.42	15.73
Sabinene	1.1	0.64
β-pinene	18.04	10.01
Myrcene	4.24	0.22
α-phellandrene	0.51	0.12
β-phellandrene	6.48	2.14
Terpinolene	2.52	0.01
p-mentha-1,3,8-triene	16.41	0.12
Myristicin	11.92	39.65
Elemicin	2.71	4.84
2,3,4,5-tetramethoxy-allybenzene	0.72	7.82
Apiol	0.27	18.32

Table 1. Essential oil content of parsley leaves and seeds
The table shows the percentages of each compound found in the essential oils of parsley leaves and seeds.

Culinary

The first uses of parsley as seasoning trace back to Europe during the Middle Ages. Charlemagne, who grew the herb on his property, is often credited for increasing the popularity of incorporating parsley in food (Peter, 2004). The plant's distinct aroma and flavor explains its many culinary applications (Polunin, 1969). The raw leaf stalks and roots can flavor food or can be eaten as a side dish (Teuscher, 2005). Because parsley originated in the Mediterranean region, the herb is commonly associated with Italian cuisine, hence the name "Italian parsley" (Stace, 1991). In the U.S., the curled leaves of *P.crispum var. crispum* are used as a garnish or plate decoration. In Germany, parsley is the most commonly used herb in cooking. Tabbouleh, a very popular Middle Eastern dish, uses parsley as the main ingredient (Teuscher, 2005). The Jewish Passover Seder has parsley in the dishes to symbolize a new beginning (Peter, 2004).

Chemistry and Pharmacology

Secondary products function as plant defense mechanisms or pollinator attractants (Sumner, 2000). These compounds have various medicinal applications. In parsley, the essential oil (**Table 1**) and seed coating primarily contain terpenes and phenolic compounds, which are responsible for parsley's anti-oxidant, anti-inflammatory, anti-microbial, diuretic, and hypoglycemic properties (Taiz & Zeiger, 1998).

Leaves

The leaves of *P. crispum* are made up of 0.02-0.9% volatile oil. The main components of the oil are α-pinene, β -pinene, *p*-mentha-1,3,8-triene, and myristicin (Peter, 2004). The monoterpenes: α-pinene ,β –pinene, and *p*-mentha-1,3,8-triene act as pest control. The terpenes are responsible for the characteristic parsley aroma. This aroma is meant signal the presence of compounds that are toxic to several insects. However, some aphids are able to feed on parsley in a way that specifically avoids ingestion of the toxins. Due to this adaptive mechanism, the parsley aroma now serves as a food signal to these pests (Rosenthal & Berenbaum, 1991). Myristicin is a toxic phenolic compound that has hallucinogenic properties (**Figure 6**). Nutmeg contains higher levels of myristicin and, at high intake levels, can act as a psychoactive (Hallström & Thuvander, 1997). Vitamin C, vitamin A, vitamin K, and folate are also found within the parsley leaves (Teuscher, 2005).

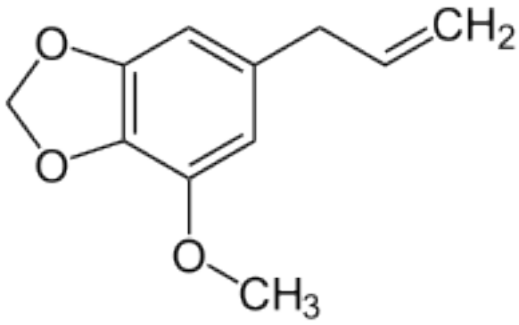

Figure 6. The chemical structure of myristicin.
Myristicin's structure is directly related to its significant functions.
Image source: http://en.wikipedia.org/wiki/Myristicin

Seeds

Parsley's seeds contain 2-8% volatile oil and 13-22% fixed oil. The major compounds are α-pinene, β-pinene, myristicin, and apiol (Peter, 2004). Apiol is a phenolic compound that is responsible for parsley's abortifacient properties (**Figure 7**).

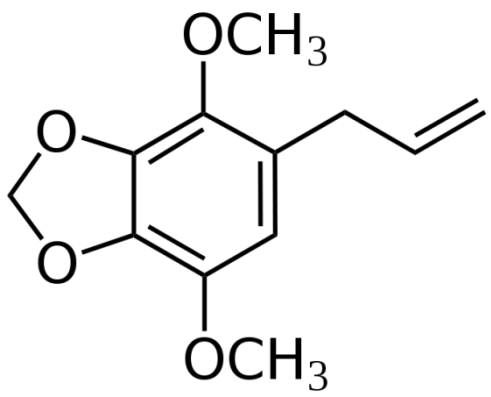

Figure 7. The chemical structure of apiol.
Apiol is similar to myristicin but has an additional methoxyl group.
Image source: http://en.wikipedia.org/wiki/File:Apiol_acsv.svg

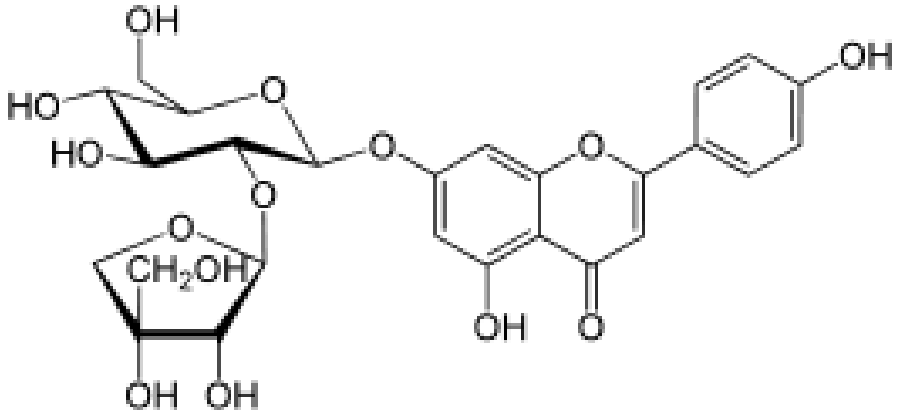

Figure 8. Apiin Structure
The chemical structure of apiin, an important compound in parsley.
Image source: http://en.wikipedia.org/wiki/File:Apiin.svg

These compounds stimulate the uterus and can also be used to induce the beginning of a menstrual cycle (Castleman, 2004). Furanocoumarins, such as oxypeucedanin, account for a large part of the seed coat (Maderfeld et al, 1997). Furanocoumarins are simple phenolic compounds that inhibit DNA transcription after exposure to UV-A light causes the compounds to enter a high-energy state. These compounds are therefore believed to have allelopathic effects via the regulation of weed and other plant germination (Roenthal & Berenbaum, 1991). In fact, these compounds may inhibit parsley itself, thus explaining the tough seed and long germination period (Kato et al., 1978).

Roots

Essential oil makes up 0.2-0.75% of parsley's roots. The oil mainly consists of terpinolene, β-pinene, apiol, and myristicin (Peter, 2004). Additionally, apiin makes up 0.2-1.6% of the roots and is from a large subset of phenolic compounds known as flavonoids (Taiz & Zeiger, 1998) (**Figure 8**). Apiin is a glycosidic form of apigenin, which has been shown to help prevent chemoresistance (Gupta & Seshadri, 1952). Phthalides including: ligustilide, senkyunolide, and butylphthalide as well as polylines such as falcarinol and falcarindiol also make up parsley's roots (Teuscher, 2005).

Biological Activity

In vitro

Antioxidant

Free radicals lead to oxidation of biomolecules which then lead to cell damage or death. Antioxidants mitigate the effects of oxidative stress by scavenging these free radicals and

donating electrons to them in order to keep biomolecules intact and undisturbed (Wong & Kitts, 2006). Myristicin and apiol are believed to be the major contributors to the observed antioxidant properties of parsley, which are characteristic of phenolic compounds (Taiz & Zeiger, 1998).

The research of Zhang et al. supported this hypothesis by testing antioxidant capabilities through three in vitro assays: β-carotene bleaching assay, 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay and Fe^{2+}-metal chelating assay. While the metal chelating properties where insignificant, parsley's essential oil showed activity in β-carotene bleaching and free radical hunting. Apiol showed more free radical scavenging activity than myristicin despite being present in lower concentrations. The presence of an additional methoxyl group, a prominent electron donor group, most likely contributes to this observed difference (Zhang, Chen, Xi & Yao, 2006).

Anti-microbial

Parsley has evolved defense mechanisms against microbial predators such as bacteria and fungi. The secondary plant phenolic compounds such as apiol, myristicin, and furanocoumarins play important roles in these functions (Manderfeld, Schafer, Davidson & Zottola, 1997). One study extracted parsley leaves in a 70% alcohol solution after they were washed, dried, and ground up. The solution then underwent filtration and evaporation to obtain a semi-solid residue. This parsley extraction showed significant inhibitory activity against *Staphylococcus aureus*. Additionally, the addition of fresh parsley leaves to Kareish cheese showed reduced levels of yeast after two hours. However, parsley was less effective in reducing the proliferation of mold (Wahba, Ahmed, & Ebraheim, 2010). Similarly, parsley is susceptible to *Erysiphe heraclei*, which causes growth of powdery mildew on the leaves (Koike, Gladders, & Paulus, 2007). *P. crispum* has also been shown to reduce the growth of *Listeria monocytogenes*, *L. innocua*, *Escherichia coli* O157:H7, *E. coli* Bs-1 and *E. carotovora* (Wong & Kitts, 2006).

Anti-inflammatory

The immune system protects humans from foreign antigens, wards off microbes, etc. However, chronic activation of the immune system results in inflammation and is linked to tissue damage, neuropathological diseases, and autoimmune disorders. Myristicin found in parsley has been shown to reduce inflammation, by inhibiting the production of nitric oxide and cytokines, inflammatory proteins released by the immune system, through the calcium pathway (Lee & Park, 2011).

Yousofi et al. treated mice splenocytes, white blood cells from the spleen, with phytohemagglutinin (PHA) or lipopolysaccharide (LPS) in order to stimulate an immune response from the T and B cells respectively. In the experimental group treated with PHA, parsley essential oil suppressed T-cells proliferation and therefore inhibited the growth of the splenocytes. Parsley's essential oil displayed similar suppression of B-cells but only at higher concentrations. Therefore parsley seems to be more efficient at targeting T-cells. These researchers also found that parsley suppressed nitric oxide concentrations without toxic effects (Yousofi, Daneshmandi, Soleimani, Bagheri, & Karimi, 2012).

In vivo

Diuretic

In traditional medicine, parsley is most used as a diuretic. One study examined the effect of parsley seed extract on the urine output of rats. The rats alternated between drinking aqueous parsley seed extract and water without any additives. The results show that when the rats drank the *P. crispum* extract, they had significantly higher levels of urine output every 24 hours compared to when they only drank water. Parsley increases urine output by inhibiting the activity of the Na+- K+ ATPase. This inhibition increases the K+ concentration in the lumen, therefore leading to increased water flow into the lumen via osmosis. This process results in increased urine flow and output (Kreydiyyeh & Usta, 2001).

Hypoglycemic Agent

As traditionally used in Turkey, parsley displays hypoglycemic effects (Patel, Kumar, Laloo & Hemalatha, 2012). One *in vivo* study compared the effectiveness of parsley extract versus gilbornuride, an anti-diabetic treatment, in diabetic rats. One group of diabetic rats received parsley extract for 28 days. The second group of diabetic rats took gilbornuride for 28 days. All of the rats had lower body weight, which may indicate the loss of tissue due to oxidative damage from diabetes. However the gilbornuride treatment group had higher body weight compared to the parsley treatment group. This difference may be attributed to the herb's diuretic effects. The diabetic rats treated with parsley showed decreased blood glucose levels and decreased lipid periodization in the liver. Liver glutathione (GSH) functions as an antioxidant to diminish the effects of high levels of free radicals caused by oxidative stress from diabetes. Parsley treatment in rats showed increases in GSH levels, therefore protecting and reducing damage to the liver tissue (Ozsoy-Sacan et al., 2006).

Clinical Studies

Parsley has been shown to have abortifacient effects when taken in high doses (Lis-Balchin, 2006). A retrospective study was conducted in Montevideo, Uruguay where calls to the poison control center were made after ingesting an herb with the intention to abort a fetus. Of the 86 reported cases, 13 involved the use parsley either in a single herb infusion or in a homemade mixture with other herbs. Three cases of taking parsley with *Ruta graveolens* lead to multiple organ failure in the women. In 7 cases, where parsley was either taken by itself, with prescription drugs, or in addition to self-mutilation, the end result was abortion. Although causation between ingestion of parsley and abortion cannot be conclusively determined, this study addresses the need for greater awareness when using herbal medicines at home without the supervision or guidance of someone that is knowledgeable in the field (Carmen & Laborde, 2003).

The antioxidant properties of parsley have been studied in humans. One clinical trial focused on the potential antioxidant properties of apiin, a flavonoid glycoside found in *P.crispum*. The 14 subjects were both males and females. Those in both the control group and the experimental ate the same meals with the exception that fresh and microwaved parsley was added to the experimental group's meals. The subjects' urine outputs were measured for apiin concentration. Low concentrations of apiin in the experimental group indicated that the intestine readily absorbs apiin. However, the high variability of apiin concentrations between subjects suggests that there are different aptitudes for apiin absorption. Two antioxidant enzymes, GR and SOD were monitored In order to

determine whether apiin ingestion results in antioxidant activity. The subjects that consumed parsley in their diets showed increased activity levels of these two enzymes (Nielsen et al., 1998).

Contraindications

General Safety Guidelines

There is a common misconception, especially among Westerners, that plant based therapies have negligible or no side effects. However, herbal remedies also carry dangers related to dosage, application method, and drug interactions. Traditionally, the society's healers carried the knowledge of these safety and efficacy parameters. However, botanical medicine is now a global industry. In the U.S. and many other countries, there is a need for further regulation on the sale and distribution of herbs. Universal parameters are necessary in order to ensure the quality, safety, and efficacy of these natural products (Lewis & Lewis, 2003).

Most people purchase the fresh herbal form of parsley but supplements, teas, and oils are also for sale. Parsley seed oil has high levels of myristicin and apiol; therefore, the seeds and oil should not be used in cooking. At high doses, the oil content may lead to gastroenteritis, headache, kidney and liver damage, shock and coma. Additionally, high quantities of myristicin and apiol may stimulate the uterus and should therefore be avoided by pregnant women. Babies and young children have higher susceptibility levels to the toxic compounds so exposure to parsley essential oil should be limited. Parsley leaves, stalks, and roots have low levels of these compounds and are safe to consume. Those with sensitive skin may experience skin rashes aggravated by the plant's furanocoumarin content (Lis-Balchin, 2006). All parsley components should be avoided by those with allergies to plants in the Apiaceae family (Castleman, 2009).

Drug Interactions

Those who are taking Warfarin, a blood thinner, should closely monitor parsley intake, which may reduce the effectiveness of the drug (Heck, DeWitt, & Lukes, 2000). Additionally, those taking diuretic drugs may want to refrain from taking parsley because both treatments work to increase water excretion. Too much water loss can lead to dehydration, dizziness, and low blood pressure. Those with parsley allergies should avoid taking aspirin with parsley because the aspirin will increase the body's sensitivity to the herb and may result in increased bleeding risk (Grossberg & Fox, 2008).

Current Use in Allopathic and CAM Therapies

U.S. physicians do not prescribe *P. crispum* as a medicinal treatment. However, many people use parsley in place of water pills because the herb is a cheaper diuretic. Parsley's ability to increase urine output makes it a popular herb to recommend for treating congestive heart failure and high blood pressure (Kreydiyyeh & Usta, 2001). In Germany, parsley seed tea is a very popularly proscribed high blood pressure treatment. For optimal results, it's recommended to drink 3 cups of this infusion per day. However, the therapeutic levels can easily be surpassed and reach toxic levels, so the dosage must be carefully monitored. Parsley is officially recognized as a diuretic by the German equivalent of the FDA and the German Commission E recognizes parsley as a kidney stone treatment (Peter, 2004). The diuretic properties of parsley contribute to its detoxifying uses in juice cleansers and health tonics.

Parsley tinctures, the herb dissolved into alcohol, can also be made or bought for around $16 per 2 ounces of tincture. The recommended dose is 1-3 ml, three times per day (Johnson, 2012). Parsley supplements are very common in the U.S. and can be bought over the counter at drug stores such as Walgreens. The supplements usually come in capsule form. Fresh parsley can be bought at almost all supermarkets for around $2 /bunch. Most CAM therapies utilize nutrition as disease prevention, therapy, and health promotion. Parsley is a perfect example of this type of treatment. Many cultures have and continue to use parsley as a condiment or main ingredient in food preparation and consumption. Although it may seem cheaper or more convenient to buy supplements, the dried form of parsley may have lost most of its biological activity and effectiveness and there is risk of product adulteration due to the weak regulations on herbal products (Lewis & Lewis, 2003). Accidental adulteration of parsley products with plants of greater toxicity, such as Fool's parsley, poses a very serious risk.

Discussion

Despite the prominence of allopathic healthcare systems in many countries, patients often refer back to traditional remedies when conventional medicine has proved to be ineffective or too expensive. In urban areas, where there are large immigrant populations, healthcare professionals with knowledge of these cultural traditions are able to better serve their patients. Additionally, some immigrants rely heavily on traditional treatments because their illegal status limits their access to national healthcare systems (Ceuterick, Vanderbroek, Tony & Pieroni, 2008).

P. crispum has a long history of symbolic, medicinal, and culinary uses and continues to be one of the world's most popular herbs. Data from in vitro, in vivo, and clinical studies of parsley provide empirical support for the traditional remedial applications of the herb as an antioxidant, anti-inflammatory, anti-microbial, diuretic, abortifacient, and hypoglycemic agent. Myristicin and apiol are the main compounds responsible for this array of medicinal properties. Both chemicals are most concentrated in parsley seeds, which contain the highest amount of volatile oils. Apiin has very interesting properties but has been much less studied. However, the research that has been conducted on apiin in the form of apigenin shows great potential for its biological significance in cancer therapy (Gao et al., 2013). Myristicin may also have anticancer effects (Zheng et al., 1992).

Incorporating fresh parsley into one's diet can serve as an effective, economic, and relatively safe way to boost overall health. However, there is a need for more clinical studies in order to assess the efficacy and safety of the therapeutic applications of parsley in humans, especially in regards to pregnant women and children. Safe dosage levels allow humans to receive therapeutic effects and avoid the ramifications of excess toxin intake. In the past, parsley was reserved for momentous events such as victory and death, but nowadays the herb often goes unnoticed on the corner of our plates. Further research on parsley and its components could lead to important discoveries that impact both botanical and human health.

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Rosmarinus officinalis L., Lamiaceae

Kedra Woodard

Introduction

Rosmarinus officinalis, commonly known as rosemary, is a perennial plant that belongs to the Lamiaceae family. It is also known in Chinese Pinyin as mi die xiang. The Lamiaceae family is a large group, rich in aromatic species and is used in many ways such as culinary herbs, part of folk medicine, and fragrance. Many of the species of this family are rich in essential oils. Studies have shown that species of the Lamiaceae family have antimicrobial, anticarcinogenic, antioxidant, cognitive-improving, and glucose level lowering properties due to the essential oils that are typically secreted by glandular trichomes (Okoh, Sadimenko, & Afolayan, 2011). Trichomes are present in most plants and are specialized epidermal cells. Glandular trichomes, found in most species in the Lamiaceae family, contain secretions produced by plants including volatile oils. There are typically two types of trichomes in the leaves and stems of plants, peltate and capitate. An illustration of both can be found in **Figure 1**. *R. officinalis* was first cultivated in the Mediterranean and was then transplanted in China. Today, *R. officinalis* is cultivated worldwide (Waggas & Balawi, 2008).

Botanical Description

Rosmarinus officinalis is an herb with ever-green needle-like leaves. It is indigenous to the Mediterranean where it grows wild. It can especially be found near the Mediterranean seashore in Spain, Portugal, Morocco and Tunisia where it can grow up to 6 feet high. Rosemary thrives best in warm, sunny

Figure 1. Picture on the left is of (A)peltate trichome and the picture on the right is of (B)capitate trichome. Source: http://www.knottybits.com/jftrich/trichome.htm

areas near seashores, which makes the Mediterranean seashore a perfect growing location. *R. officinalis* received its common name from where it can be found in abundance, near the sea. The term rosemary means “dew of the sea” (González-Trujano et al., 2007). Rosemary has been recorded in an Italian herbal, circa 1500 (Foley 1974). The picture from this document of *R. officinalis* can be seen in **Figure 2**. Rosemary can be identified by its dark green leaves, small blue, violet, or white flower, and minty odor.

The primary pollinators of rosemary are honeybees because rosemary is rich in highly concentrated nectar. Studies have shown that when compared to nine other plants of the Lamiaceae family, rosemary is one of the most preferred by honeybees (Zer & Fahn, 1992). One study discusses the

Figure 2. Rosemary illustration from an Italian herbal, circa 1500
Image Source: http://en.wikipedia.org/wiki/Rosemary

percentage of dry matter in the nectar of rosemary in varying locations in the summer. The amount found in Israel may be as high as 62-65% and about 44% in Jerusalem. The sucrose: glucose: fructose ration is about 2-83:1:1-85 however amino acid levels are pretty low (Dafni et al., 1988). Because honeybees pollinate based on the amount of nectar that can be found, rosemary in these areas are preferred.

There are several common cultivations of rosemary. Many of these cultivations, such as Albus, Arp, Salem, and Wilma's Gold, are frequently sold in grocery stores and nurseries (Mulas, Brigaglia, & Cani 1997). **Table 1** is a chart illustrating more frequently sold cultivars and their varying descriptions.

Cultivars	Description
Albus	White flower
Arp	Leaves light green, lemon-scented
Aureus	Leaves speckled yellow
Benenden Blue	Leaves narrow, dark green
Blue Boy	Dwarf, small leaves
Golden Rain	Dark green leaves, with golden streaks
Gold Dust	Low and lax, trailing, intense blue flower
Irene	Procumbent selection from Tuscan Blue
Lockwood de Forest	Shrubby
Ken Taylor	Pink flowers
Majorica Pink	Distinctive tall fastigate form with wider leaves
Miss Jessop's Upright	Pink flower
Pinkie	Lower groundcover
Prostratus	Fastigate form, pale blue powers
Pyramidalis	Pink flowers
Roseus	Pale blue flowers, cold hardy similar to Arp
Salem	Spreading, low-growing, with arching branches; flowers deep violet
Severn Sea	Traditional robust upright form
Wilma's Gold	Yellow leaves

Table 1. Rosemary cultivars and descriptions (Mulas, Brigaglia, & Cani 1997).

Traditional Uses

Rosmarinus officinalis is used topically in Mexico to relieve rheumatic pain. The rosemary is prepared by maceration, which is the process of softening using an alcohol, in ethanol. This mixture is applied externally and directly to the skin

Chemical	Plant Part Present	Percent per million (ppm)
(+) Limonene	Plant	16-76
Acetic Acid	Esin, Exudate, Sap	
Alpha Terpineol	Plant	24-1555
Ascorbic Acid	Plant	612-673
Betulinic Acid	Leaf	
Cadalene	Plant	
Caffeic Acid	Plant	
Calcium	Plant	10919 - 16150
Camphor	Plant	60 - 5800
Carbohydrates	Plant	640,600 - 704660
Carnosic acid	Leaf, Plant	448.4- 5000
Chlorogenic Acid	Plant	
Copper	Plant	5 – 6
Ethanol	Plant	
Fenchone	Plant	250
Fiber	Plant	165420 - 206338
Glycolic Acid	Plant	
Iron	Plant	220 – 400
Lavandulol	Plant	7 – 34
Limonene	Plant	1,950
Methyl Ether	Plant	
Rosmaric Acid	Plant	3,000 - 3,500
Rosmarinic Acid	Leaf	3,500
	Plant	25,000
	Shoot	13,500
	Tissue Culture	38,957
Rosmarinol	Plant	
Safrole	Plant	32 – 95
Salicylates	Leaf	70 – 680
Sodium	Plant	462 – 592
Tannin	Plant	
Zinc	Plant	30 – 38
Zingiberene	Resin, Exudate, Sap	

Table 2. A few important constituents found in *Rosmarinus officinalis.*

(Ventura-Martínez, Rivero-Osorno et al., 2011). Rosemary is also used in ethnomedicine as a general stimulant, for improvement of circulation, hyperglycemia and skin care (Hamedo, 2009). Rosemary tea, as a folk remedy, can be used for nervous headaches and healing colds. Some studies have reported its use as an effective diuretic and mood stabilizer (Haloui et al., 2000).

Rosemary was believed to strengthen memory in ancient Greece. Rosemary was consumed often in hopes to avoid forgetting. It was also very popular to see Greek students wearing rosemary leaves in their hair as they studied so they could remember reviewed information. Rosemary became a symbol of remembrance (Foley 1974).

Rosemary was cultivated in many of the ancient European countries, including France, Germany, Denmark, England, and parts of Scandinavia. It was too cultivated in ancient Central America, Venezuela, and the Phillippines. This plant has been associated with love and marriage as well as birth and death in many of these cultures. In Shakespeare's *Hamlet,* it is used as a symbol of love (al-Sereiti, Abu-Amer, & Sen, 1999).

Cosmetic Uses

Rosmarinus officinalis is commonly used as a fragrant additive in soaps and other cosmetic products. Rosemary is used in these products for treating cellulite, wrinkles, and normalizing excessive oil secretion from the skin (Hamedo & Abdelmigid, 2009). Rosemary can also be found in hair dyes and rinses to darken and retain color and in oils and shampoos to prevent premature balding.

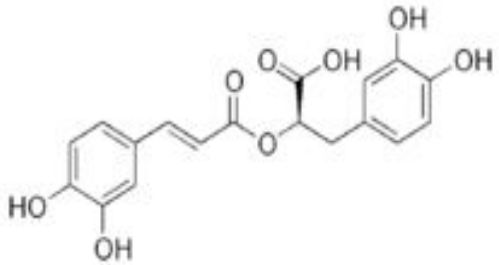

Figure 3. Chemical structure of rosmarinic acid.

Culinary Uses

Rosemary is an ancient spice. Because it is native to the Mediterranean, it is extremely common in Mediterranean cuisines. Extracts produced from the leaves of rosemary can also be used in food production to keep materials from spoiling due to its antioxidant properties. Its effectiveness in food preservation can be witnessed in many different types of foods including refrigerated beef and frozen pork patties (Lara, Gitierrez, Timón & Andrés, 2011).

Other Uses

When combined with other herbs, the chemical activity of *Rosmarinus officinale* can be increased. For example, a mixture of its leaves and juniper berries were burned in hospitals around France to kill germs during World War II (Foley 1974). Also, using rosemary leaves mixed with myrrh can be effective in treating bleeding gums.

Chemistry and Pharmacology

Rosmarinus officinalis has many important constituents with important biological activity. Some studies reported 1,8–

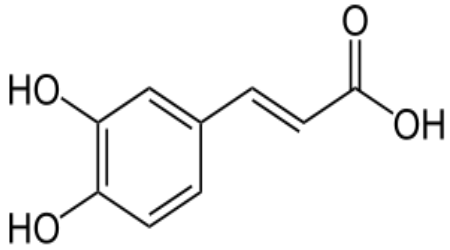

Figure 4: Chemical structure of caffeic acid.

cineole as a main component (Stojanović-Radić, Nešić, Čomić & Radulović, 2010) though, there are not many studies that discuss the role of this compound. **Table 2** displays several of the constituents found in rosemary, as well as its plant location, and abundance. The synergy of the constituents helps to produce the odor, appearance, and nutrients of rosemary. Rosemary extracts are effective in relaxing the smooth muscles of the trachea and intertine. It has choleretic, hepatoprotective, and anticarcinogenic properties. Recently, scientists seem to be particularly interested in three of the acids found in rosemary: carsonic acid, caffeic acid and one of its derivatives, rosmarinic acid. All of these compounds have antioxidant effects (al-Sereiti, Abu-Amer, & Sen, 1999). **Figures 3** and **4** illustrate the structure of rosmarinic acid and caffeic acid, respectively.

Carnosic acid is a carbon dioxide solution found in many plant molecules. It can be found in abundance in rosemary. Carnosic acid has been found to promote weight loss. Ibarra et al. discovered this phenomenon while observing its effects in mice. In this study, standardized 20% carnosic acid rosemary leaf extract was used to identify its effects on weight gain, glucose level, and lipid homeostasis in mice (2011). Three groups were observed: mice given a high-fat diet, mice given a low-fat diet, and mice given a high-fat diet supplemented with 500mg of the standardized rosemary leaf extract since

juvenile. The mice were monitored for sixteen weeks. The rosemary extract reduced cholesterol level. This was visibly noted when the group given a high-fat diet and the extract were compared to those only given a high-fat diet. There was too, a direct correlation between fecal fat excretion and rosemary extract. This, however was not witnessed with decreased food intake (Ibarra et al. 2011). Though further studies are needed for this one to be conclusive, this study shows that perhaps rosemary rich in carnosic acid can be used for weight loss.

Caffeic acid is a naturally occurring compound that can be found in most plants. This compound may contribute to the prevention of cardiovascular disease (Olthof, Hollman, & Katan 2001). Margreet Olthof, Peter C. H. Hollman, and Martjin B. Katan conducted an experiment to determine the amount of caffeic acid absorbed in humans (2001). This was determined through a cross-over study using four healthy female and three healthy male subjects. The calculation used to determine the amount absorbed was the amount ingested minus the amount excreted. Eleven percent of the caffeic acid absorbed was excreted in urine (Olthof, Hollman, & Katan 2001). Accounting for human error, almost all of the caffeic acid intake will be absorbed by humans.

Rosmarinic acid, the ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, is a compound commonly found in the Boraginaceae family but unique to rosemary in the Lamiaceae. In the plant, rosmarinic acid behaves as a defense compound (Petersen & Simmonds 2003). Rosmarinic acid is a phenolic compound; it has one phenolic ring from phenylalanine via caffeic acid and one phenolic ring from tyrosine via dihydroxyphenyl-lactic acid. Rosmarinic acid can be well absorbed through membranes such as the gastrointestinal tract and the skin. It is capable of increasing the PGE2, prostaglandin E2, and reducing the production of leukrotriene B4, a fatty signaling molecule, and inhibits the complement system (al-Sereiti, Abu-Amer, & Sen, 1999). PGE2 is important in childbirth labor because it softens the cervix and causes the uterine to contract; however if activated early can be an abortifacient. Many of the activities rosmarinic acid is involved with have therapeutic potential. For example, relaxing the bronchial and intestinal smooth muscles can treat bronchial asthma and be antispasmodic. Reduction of leukotrienes and the increase of PGE2 production can also treat bronchial asthma, peptic ulcers, and inflammatory diseases.

The three constituents of rosemary discussed, caffeic acid, carnosic acid, and rosmarinic acid, are also found to have therapeutic potential in treatment. These constituents are able t inhibit lipid peroxidation which can then treat hepatotoxicity atherosclerosis and inflammatory diseases. They also prevent the formation of the carcinogen-DNA adduct which gives rosemary its anticarcinogenic property (al-Sereiti, Abu-Amer, & Sen, 1999).

Biological Activity

In vitro

Rosmarinus officinalis was tested in a study conducted by Vina Yang and Carol Clausen for its effectiveness in controlling mold growth on wood (2007). Moisture is the most critical component to consider when controlling mold growth, however when moisture cannot be controlled, one must consider what to do to control mold growth. In this experiment, seven plants, rich in essential oils were test for their antifungal effects including rosemary. Petri dish tests and tank test chamber gave similar results. Rosemary essential oil extract was among the group of essential oils

shown to have promising antifungal effects in this experiment (Yang and Clausen 2007).

In vivo

Fawzia A. Fahim and Amr Y. Esmat conducted an experiment to study the antimutagenic and hepatoprotective effects of the essential oil of *Rosmarinus officinalis* and ethanolic extract (1999). They used carbon tetrachloride as hepatotoxic and cyclophosphamide as mutagenic compounds. For three weeks, rats were administered rosemary ethanolic extract (0.15 g/100 g BW). This produced the better hepatoprotective results than when compared to silymarin. Mice were pretreated for seven days with rosemary essential oil (1.1mg/g BW) and later injected with cyclophosphamide. This significantly reduced the induced mitodepression of bone marrow cells in the mice. The presence of high percentages of phenolic compounds within the rosemary essential oil extract contributed to its antioxidant effects (Fahim & Esmat, 1999).

Hossein Hosseinzah and Mahnaz Nourbakhsh investigated the effects of aqueous and ethanol extracts of *R. officinalis* on morphine withdrawal syndrome in mice (2003). The aqueous and ethanol extraction of rosemary induced significant activity in the writhing test that showed a decline in sensitivity to painful stimuli and this activity was then inhibited by naloxone, am opioid antagonist drug, pretreatment. The mice developed dependence to morphine by being induced daily for three days usimg subcutaneous injections of morphine. Day four, the mice were injected two hours prior to the intraperitoneal injection of naloxone with morphine. The effects of the withdrawal syndrome was observed in the mice and were then measured by the number of jumps within the 30 minute period after the naloxone injection was administered. It was found that the number of jumps reduced due to the aqueous and ethanol extracts of rosemary (Hosseinzah & Nourbakhsh 2003). This study introduced the possibility of using rosemary extractions to treat morphine withdrawal syndrome.

Clinical Studies

Moss and Cook conducted an experiment using human subjects to test the cognitive effects of *Rosmarinus officinalis* and lavender (Moss & Cook 2003). Many nontoxic herbal spices from Europe are traditionally used to treat cognitive deficiency. This experiment, however only tests two. Moss and Cook assessed the olfactory impact of the two plants on cognitive performance and mood. Rosemary was found to produce enhancement in secondary memory factors and terms of overall quality of memory. However, it was found to impair the speed component of memory (Moss & Cook 2003). This could be due to rosemary's essential oils; they produce a soothing effect.

Rosemary essential oils were found to moderately inhibit acetyl-cholinesterase (Orhan et al., 2008). The inhibition of acetyl-cholinesterase by rosemary essential oil can be explained by the synergy produced by the interaction between 1,8 cineole and 2-pinene. Inhibition of acetyl-cholinesterase is currently one of the treatment strategies against several neurologic disorders such as Alzeimer's and other dementias. Because rosemary was found to have this activity, it is now being further investigated to see how it can potentially treat these diseases (Orhan et al., 2008).

Contraindictions

Rosmarinus officinalis is not known to be toxic. However, a large intake of the plant or its oils could pose a threat to

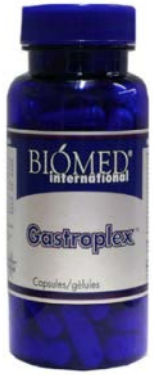

Figure 5. Gastroplex
Image source:
http://www.google.com/imgres?q=Gastroplex&um=1&hl=en&sa=N&biw=1441&bih=747&tbm=isch&tbnid=kRZczBPNBrnZrM:&imgrefurl=http://biomedicine.com/products-page/biomed-supplements/gastroplex%25E2%2584%25A2&docid=yr_hRbG4MT6qfM&imgurl=http://biomedicine.com/wp-content/uploads/2011/07/11080.jpg&w=600&h=480&ei=c91aT7baBsq9tweB_OSEDA&zoom=1

pregnant women (al-Sereiti, Abu-Amer, & Sen, 1999). A study was conducted on mice to see the dietary effects of rosemary on the fertility of adult rats. It was reported that sperm motility and density decreased. In addition, the number of aborted fetuses was increased. It was concluded that the increased amount of rosemary given caused infertility (Nusier et al. 2007). Because humans are much larger, it would take a lot more rosemary to cause this effect. The amount to cause such effects, however are not known due to lack of studies. There is no evidence that the intake of a moderate amount of rosemary could be harmful to breast-feeding women or their children. There is potential for the oil to act as an abortifacient, which is an agent that may induce abortion (al-Sereiti, Abu-Amer, & Sen, 1999). This is only due to its effects on PGE2. Also, a frequent intake of rosemary can cause iron deficiency in susceptible individuals.

Current Uses in Allopathic and CAM Therapies

Extracts from *Rosmarinus officinalis* are presently used in herbal medicine today, such as in the drug Gastroplex. Gastroplex is used for the treatment of dysbiosis, the rapid overgrowth of yeast, parasites, and bacteria. Gastroplex is used to regain the proper function of the gastrointestinal tract and reclaim its health. This drug cannot be taken during pregnancy or lactation. A photograph of the drug can be found in **Figure 5**.

Because of the high concentration of volatile oils, rosemary is used in aromatherapy. It has also been effectively in use in massage therapy. Its scent has the effect of producing a relaxing sensation. Other uses of rosemary include its use as a diuretic. Teas with rosemary extract are used by the general public and are widely marketed and can be purchased with convenience.

Discussion

Rosmarinus officinalis is an amazing plant with many health benefits. It is anti-inflammatory, anticarcinogenic, antibacterial (Jarr et al., 2010), and an antioxidant. It is rich in phenols and tannins. It can be used medicinally and used in cuisines. Though originating in the Mediterranean, today this plant can be found in all parts of the world. This plant can even be grown in indoor pots inside the home. By simply incorporating *R. officinalis* into the diet, many bacterial infections can be avoided while gaining nutritious benefits.

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Salix alba L., Salicaceae

Kinsey McMurtry

Introduction

The plant being investigated in this paper has the scientific name *Salix alba* L., which is in the plant family Salicaceae. *Salix alba* L. is commonly called white willow, catkins willow, European willow, salicin willow, and withe withy (Highfield & Kemper, 1999). The white willow contains salicin, which is metabolized into salicylic acid in the body. Synthetic salicylic acid is used to make Aspirin by acetylating the synthetic salicylic acid. The discovery of Aspirin can be attributed to the knowledge of the medical uses of willow bark. Willow bark is used as an analgesic and anti-inflammatory due to its main constituents such as flavonoids, polyphenols, tannins, and salicin (Vlachojannis, 2011). While it is mostly known for its use in Aspirin, the use of the white willow tree dates back to the ancient Egyptians who used it to treat pain and inflammation. Its use has been well documented by many healers including Hippocrates, Dioscorides, and Pliny the Elder. White willow bark, roots, and leaves are also well documented in Native American medical rituals for a variety of ailments including hair loss to healing skin lesions (Moerman, 1998).

Figure 1. White willow tree (http://www.herbal-supplement-resource.com/white-willow-bark.html)

Botanical Description

The willow family, Salicaceae, has four genera containing trees and shrubs. These 350 species are found in northern temperate climates. *Salix alba* originated from Europe and contains tannins and salicin (Pfister, 1992). The white willow is a tree that grows 6-18 meters high. It blooms with yellow male flowers and green female flowers. The white willow tree is native to North America, Asia, and Europe. It can survive in cold temperatures and can grow in a variety of climates. The bark of young branches from the tree is usually harvested during spring (Highfield & Kemper, 1999). As far as the willow tree's habitat, typically the white willow must grow in an environment near water for its seeds to germinate. Moisture is critical during seed dispersal because the seeds of the willow tree are only viable for a few weeks. However,

after the plant has taken root in the soil constant moisture is not necessary. The seeds of the willow tree can travel great distances because they are lightweight and have hair-like attachments (Kuzovkina, 2004). See **Figure 1** for a picture of the tree in its typical habitat.

Traditional Uses

The modern use of white willow dates back to 1763 when its antipyretic effects from the dried and powdered bark was first reported (Vlachojannis, 2011). However, the use of willow bark dates much further back.

Traditional Analgesic Uses

The Ancient Egyptians used the white willow tree bark to treat pain and inflammation. Willow bark was also used as a remedy for gout and rheumatic joint diseases during the eras of Hippocrates and Dioscorides (Highfield & Kemper, 1999). Hippocrates recommended chewing the leaves of the willow tree during childbirth because of its analgesic effects. He also recommended using the juices of the tree for eye diseases. Dioscorides also reported the analgesic effects of willow leaves and he similarly suggested its use to treat gout. Pliny the Elder, a Roman encyclopedia writer, described the analgesic effects and other uses of the gum, leaves, and bark of the willow tree (Mueller & Scheidt, 1994). Native Americans also used willow for its analgesic properties. Willow bark's traditional uses include rheumatic pain, back pain, toothache, headache, menstrual cramps, relieving sore throats, fever, and headaches associated with flu and upper respiratory infections (Highfield & Kemper, 1999).

Traditional Antipyretic and Anti-inflammatory Uses

Galen, a Roman physician, first described the antipyretic and anti-inflammatory properties of willow leaves (Mueller & Scheidt, 1994). *Salix alba* has been recorded as a traditional use for treating fever and malaria in Epirus, Greece as well (Vokou, Katradi & Kokkini, 1993). Similarly, Cherokee Indians in North America used white willow to create infusions to treat fevers (Moerman, 2009). In England, before disease and infection were fully understood it was common to think that illnesses were related to the geographic locations in which they occurred. Therefore, it was argued that remedies could be found where the diseases were. This is the case for white willow. Because white willow is found near water the bark was used as a cure for agues (chills and fevers) because agues typically occurred in people living near water (Porter, 2006).

In 1757 Edward Stone began to search for a new antipyretic drug to replace quinine. Upon tasting the bark of the willow tree he noticed the similarity in the extreme bitter taste between the cinchona bark and the willow bark. Stone performed studies to test the anti-inflammatory effects and reported his positive findings in a letter to the Royal Society in 1763. In addition, Stone's findings gave scientific evidence to the already well-known use of willow bark for the treatment of fevers and inflammatory illnesses. His work allowed for science to take the next step in analysis by isolating and purifying the active constituents of the white willow bark for clinical study and production (Mueller & Scheidt, 1994). In 1829 a French pharmacist, H. Leroux, identified willow's active chemical constituent salicin. Salicylic acid was prepared in pure form in 1838. Both Salicylic acid and salicin were a popular treatment for rheumatic fever and gout in the 19th century. The benefits and therapies of willow bark were also known in Italy. Bartolomeo Rigatelli used willow bark extract as a therapeutic agent in the early 1800s. In 1838

Raffaele Piria, an Italian chemist, extracted the salicylic acid compound from the white willow bark. In 1855 Cesare Berganini published a detailed report about the adverse events that are caused by a salicylate overdose (Marson & Pasero, 2006).

Traditional Antidiarrheal Uses

Cherokee Indians used white willow as an antidiarrheal by creating an infusion with the bark. The infusion was taken to check the bowels (Moerman, 2009).

Traditional Respiratory Uses

Cherokee Indians chewed the roots of white willow as a respiratory aid while playing sports. White willow is also used as a throat aid. Cherokee Indians also made infusions with the inner bark of the willow tree to treat a lost voice and the root was chewed to treat hoarseness (Moerman, 2009).

Traditional Cosmetic Uses

Cherokee Indians used the white willow as a dermatological aid. A decoction or infusion of the bark was used as a wash to make hair grow. The Blackfoot tribe created tonics for dandruff and to straighten the hair by soaking the dried, crushed willow roots in water and adding grease. The Costanoan tribe created a hair rinse by making an infusion of the willow leaves. The leaves were also used to treat balding by creating a paste that was rubbed on the scalp. Creek Indians treated swellings by creating an infusion with willow roots that was then used as a wash. The Isleta tribe used a decoction of willow leaves as a skin bath (Moerman, 1998).

Active Chemical Constituents	Percentage
Glycosides	1.5-11%
Tannins	8-20%
Aromatic aldehydes and acids	
Salycil alcohol	
Flavonoids	

Table 1. Active chemical compounds in *Salix alba* (Highfield & Kemper, 1999).

Traditional uses in teas

In west Bosnia and Herzegovina *Salix alba* is used in herbal teas. One tea is used for the treatment of renal ailments. Another tea is used for the treatment of restlessness (Saric-Kundalic, Dobes, Klatte-Asselmeyer & Saukel, 2010).

Chemistry and Pharmacology

The active chemical constituents of the white willow bark include glycosides (1.5-11%), tannins (8-20%), aromatic aldehydes and acids, salicyl alcohol, and flavonoids. The glycosides include salicylates such as salicin, salicortin, populin, fragilin, and tremulacin. The aromatic aldehydes and acids include salidroside, vanillin, syringin, salicylic acid, caffeic, and ferulic acids (Highfield & Kemper, 1999). See **Table 1** for an organized representation of the chemical constituents.

Using decoctions of herbs and plants, like willow bark and leaves, containing salicylates, as anti-inflammatory therapies have been well documented and used throughout history. The creation of synthetic salicylate led to the acetylated form of salicylic acid. This drug, known as Aspirin, has anti-inflammatory, analgesic and antipyretic actions. Aspirin

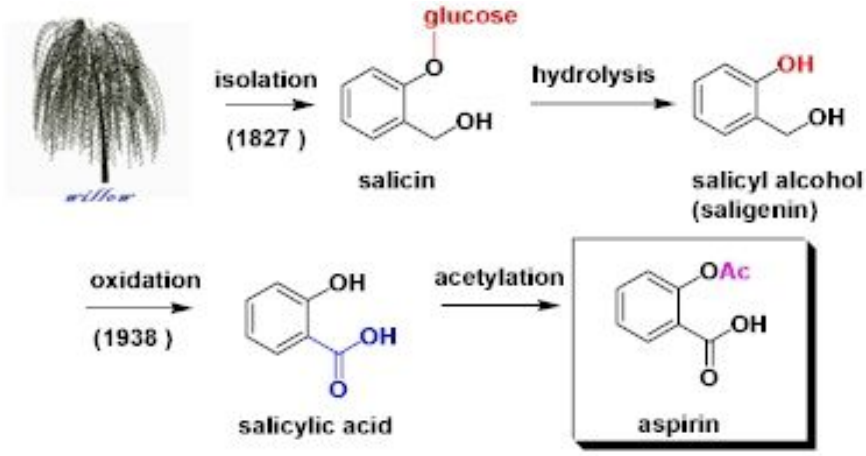

Figure 2. The Creation of Aspirin
http://www.mdidea.net/products/herbextract/salicin/data02.html

inhibits the activity of the cyclooxygenase (COX) enzyme. This enzyme leads to the creation of prostaglandins (PGs), which cause inflammation, swelling, pain and fever (Vane & Botting, 2003). See **Figure 2** for a graph of the molecular structures.

Biological Activity

In vitro

Anti-inflammatory Effects

A study of the inhibition of pro-inflammatory biomarkers in THP1 macrophages by polyphenols in willow, chamomile, and meadowsweet showed that willow bark had the greatest anti-inflammatory activity. This was achieved by using aqueous herbal extracts and isolated polyphenolic compounds. These were incubated with THP1 macrophages. Interleukin (IL)-1β, IL-6 and tumor necrosis factor-alpha (TNF-α) were measured. The willow bark reduced IL-6 and TNF-α production more than the meadowsweet and chamomile but all three showed positive results (Drummond, Harbourne, Marete, Martyn, Jacquier, O'Riordan & Gibney, 2012).

Another study investigated the anti-inflammatory mode of action in rosehip, willow bark, and nettle leaf. The study was performed as an *in vitro* model of primary canine chondrocytes. The extracts from the rosehip, willow bark, and nettle leaf all showed anti-inflammatory and anabolic effectiveness on chondrocytes (Shakibaei, Allaway, Nebrich & Mobasheri, 2012).

Since the discovery of Aspirin, studies on the white willow have declined greatly due to Aspirin's magic bullet characteristics. However, in one study the final objective was to determine if white willow and meadowsweet could be used to create a beverage with anti-inflammatory properties. The first part of the study investigated which type of herb drying allowed for the phenolic compounds to remain effective. The herbs were air-dried, freeze-dried, and oven-dried. The results showed that freeze-drying and oven drying had no significant effect on the phenolic constituents for both meadowsweet and white willow. The temperature used was 30 degrees Celsius but when the temperature was increased to 70 degrees Celsius the herbs dried faster but there was a loss of some phenolic compounds (Harbourne, Marete, Jacquier & O'Riordan, 2009). This study is important because an anti-inflammatory beverage that contained plant constituents could be very beneficial and could be used to circumvent the ill effects of current popular NSAIDs.

Anti-proliferative and Proapoptotic Effects

Salicin is known for its anti-inflammatory and analgesic effects in willow bark. It was used to create acetylsalicylic acid, but

this synthetic non-steroidal anti-inflammatory drug (NSAID) and other NSAIDs have shown to have anti-proliferative effects on cells. A study was done to investigate the possible anti-proliferative and proapoptotic effects of willow bark on the cells of the lining of the colon. A water extract (STW 33-I) and a polyphenolic rich fraction of willow bark were tested by using the colon-carcinoma cell line (HT-29). The willow bark extract (STW 33-I) contained 23-26% total salicin derivatives and therefore was an effective analgesic and anti-inflammatory drug. It also contained flavonoids, condensed tannins and polyphenols. The in vitro experiment demonstrated that the willow bark extract had pro-apoptotic effects on the HT-29 cancer cells. This experiment showed that willow bark extract is similar to other NSAIDs in its anti-proliferative and pro-apoptotic effects. This is important for the prevention of side effects such as gastric erosion that is common in NSAID use (Bonaterra, Kelber, Weiser, Metz & Kinscherf, 2010).

In vivo

The acetylation of salicylate, a naturally occurring anti-inflammatory found in *Salix alba*, is how Aspirin is produced. There have been many studies to investigate the effects of Aspirin on cancer prevention (Burn, Chapman, Bishop & Mathers, 1998). One such study investigated the effect of aspirin on colonic carcinogenesis in rats. Groups of rats were given daily doses of aspirin for 18 weeks. The dosages given were 0, 5, 30, or 60 mg/kg. Half of each of the four groups was given 18 x 30 mg/kg/wk injections of dimethyl hydrazine (DMH) colonic cancer. The results showed that the doses of 5, 30, or 60 mg/kg/dy had a progressive effect on the reduction of the number of tumors. The dosages also reduced the size of the tumors. Aspirin doses of 30 and 60 mg/kg/dy significantly reduced the occurrence of tumors in the rats (Davis & Patterson, 1994).

Clinical Studies

Headaches

In a clinical study to investigate the effects of salicin cream on headaches, it was found that the salicin worked better than the placebo. In the study, 54 patients were given anticephalgic photo protective premedicated masks to treat migraines. They were also given bottles of topical medication that either contained salicin or a placebo. They were instructed to apply the medication to their foreheads, put the mask on, and lie down. They were then told to rank the relief using a scale of 0 to 10. Seven of the 20 patients who had placebo reported the medication and mask helped. 28 of the 34 patients who used salicin reported that the treatment was effective in relieving the pain (Hyson, 1998).

Blood Thinner

In another study, the effects of salicylates on the hemostatic properties of platelets in males were investigated. According to the results, aspirin has a more significant effect on the bleeding time in six normal males in comparison to sodium salicylate. This is due to the finding that aspirin prolongs bleeding time because it inhibits the release of platelet ADP while sodium salicylate does not (Weiss, Aledort & Kochwa, 1968). This information is important for those who take aspirin daily and therefore have much thinner blood, which can cause problems when combined with other drugs or when an injury occurs.

Therapies	Lesion Improvement by percentage	
	4 weeks	6 weeks
Conventional therapy	62.60%	89.90%
Herbal therapy with acid	42.90%	61.30%
Herbal therapy without acid	55.10%	87.80%

Table 2. Results from The Effect of Clove Bud, Nigella and *Salix alba* on Wart and Comparison with Conventional Therapy (Rezaei, Jebraeili, Delfan, Noorytajer, Meshkat & Maturianpour, 2008).

Warts

Salicylic acid is commonly used to treat warts. *Clove bud, Olive, Nigel*, and *Salix alba* are all disinfectants, anesthetics, analgesics, and have wound healing properties. Because of these properties, one study investigated the effects of these plants on wart treatment in comparison to conventional treatments. The investigation was performed as a randomized double blind study on 291 female students. The students were organized into three groups. One group was treated with the conventional treatment of Salicylic acid (16.7%), lactic acid (16.7%) in a collodione body. The second group was treated with herbal medicine in olive oil that contained no acid. The third group was treated with herbal medicine along with salicylic acid (1%) and lactic acid (1%) in olive oil. The results showed that after four weeks the improvement of the wart was 62.6% for conventional therapy, 42.9% for herbal therapy with acid, and 55.1% for herbal therapy without acid. At six weeks the improvement of the wart was 89.9% for conventional therapy, 61.3% for herbal therapy with acid, and 87.8% for herbal therapy without acid (**Table 2**). This study showed that the use of herbal therapy with or without acid is significantly efficient (Rezaei, Jebraeili, Delfan, Noorytajer, Meshkat & Maturianpour, 2008).

Dosage	Percentage of patients who didn't need Tramadol
120 mg Salicin 393 mg of dry willow bark extract	21%
240 mg Salicin 2 pills of 393 mg of dry willow bark extract	39%

Table 3. Results from chronic lower back pain trial (Setty & Sigal, 2005).

Back Pain

A randomized double blind study investigated the efficacy of willow bark extract for the treatment of chronic lower back pain in 210 patients. These patients either received a twice-daily dose of 120 or 240 mg of salicin per day or a placebo. Patients in the low dose group also received a pill of 393 mg of dry willow bark extract and the high dose group received 2 pills of 393 mg of dry willow bark extract. A rescue medication of tramadol was available. The end goal of the study was to see how many patients were pain free and did not need tramadol for at least 5 days in the last week of the study. The results showed that 39% of the high dose group and 21% of the low dose group were able to go five days without tramadol by the end of the study. More tramadol was required for the patients in the placebo group (Setty & Sigal, 2005). See **Table 3** for a visual representation of these results.

Osteoarthritis

Another study was done to investigate the efficacy of willow bark for the treatment of osteoarthritis of the hip or knee. There were 78 patients in this double blind, placebo controlled study. Patients were given two doses of willow bark tablets that were equivalent to 240 mg salicin/day or a placebo. Additional NSAIDs were not allowed in the study. The Western Ontario and McMasters University Osteoarthritis Index was used to score the pain. The patient's pain index was lowered by 14% after 2 weeks of treatment. There was a pain index increase of 2% in the placebo group. The results showed that there was a moderate analgesic effect (Setty & Sigal, 2005).

Oral Administration

One study evaluated the effectiveness of salicin when it is given orally as a willow bark extract. Ten volunteers ingested 240 mg of salicin twice in a period of 24 hours. Throughout the 24 hours, urine and serum levels of salicylic acid and its metabolites were measured. Renal excretion rate, elimination half-life and total bioavailability of salicylates were also measured. The results showed that salicylic acid was the main metabolite of salicin that was detected in the serum at 86%. The peak levels of salicylic acid occurred less than two hours after ingested. Renal elimination occurred mainly as salicyluric acid. The peak serum levels of salicylic acid averaged at about 1.2 mg/l. This study concluded that the current therapeutic dose of willow bark extract leads to much lower serum salicylate levels than analgesic doses of synthetic salicylates. According to this study, the formation of salicylic acid alone cannot explain the analgesic or anti-rheumatic effects of willow bark (Schmid, Kotter & Heide, 2001).

Contraindications

Willow bark has a high concentration of tannins, and because of this, it usually leads to gastrointestinal toxicity before a therapeutic concentration of salicylates is reached. However, it is safe as an analgesic and wart remover when applied topically. Along with tannins, salicin and salicortin are also potentially toxic compounds. Allergies to willow bark are possible. Toxicity from Aspirin is more common than with willow bark because willow bark has much lower levels of salicylates. High doses can cause gastric and renal irritation, nausea, vomiting, and gastrointestinal bleeding. There are no reported interactions with other herbs or pharmaceuticals (Highfield & Kemper, 1999).

While Aspirin inhibits the formation of prostaglandins, which cause inflammation, swelling, pain, and fever, Aspirin also inhibits the formation of physiologically important PGs. Some of these PGs are responsible for the protection of the stomach mucosa from hydrochloric acid damage. They also maintain kidney function and amass platelets when needed (Vane & Botting, 2003).

Current Use in Allopathic and CAM Therapies

Acetylsalicylic acid was first synthesized in 1853 and was rediscovered by the Bayer Company in Germany. See **Figure 3** for a visual representation of the key plants used in the making of Aspirin, the chemical compounds, and who discovered these elements. Today, Bayer Aspirin is one of the most popular drugs sold worldwide. Synthetic acetylsalicylic acid is used as an analgesic and antipyretic. Aspirin is also

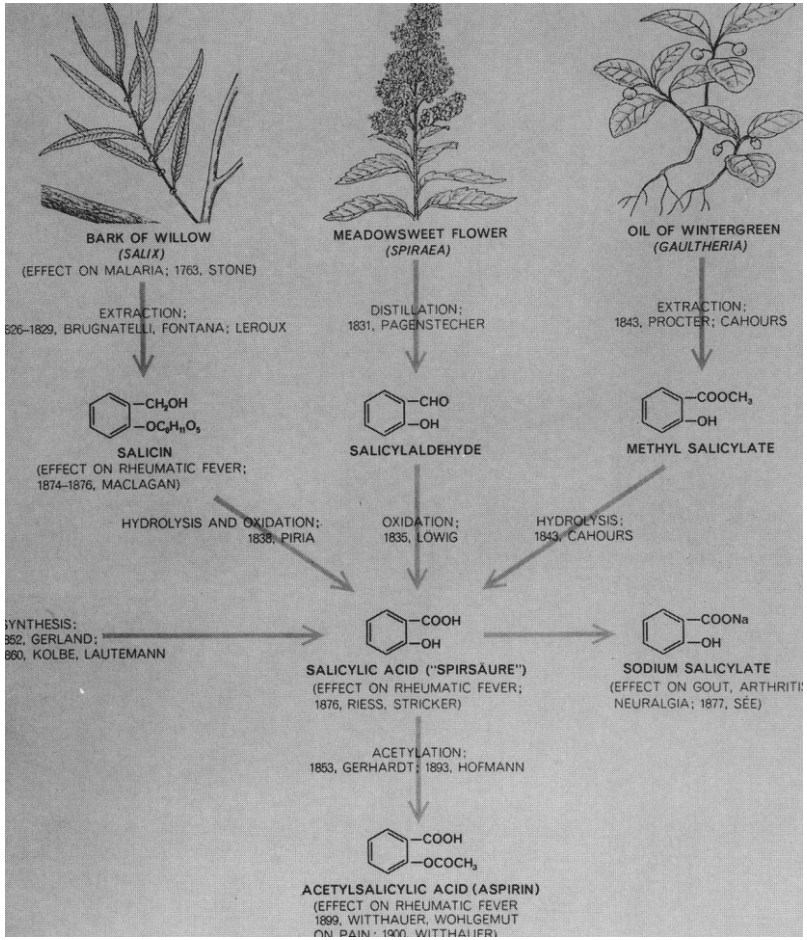

Figure 3. Chart of the salicylates and how they make Aspirin (Mueller, 437)

used to prevent myocardial infarctions, strokes, and colorectal cancer. Some herbalists believe willow bark extract can be used as a natural substitute for aspirin. This belief is used in Germany where willow bark is typically taken with aspirin in order to minimize side effects and enhance therapeutic effects (Highfield & Kemper, 1999). In a study done where informants in Jordan were asked about the typical plants used for certain illnesses, *Salix alba* was mentioned. According to these informants, *Salix alba* is most commonly used for intestinal colic, gastric disturbances, and renal calculi (Al-Qura'n, 2007). White willow bark is usually dried and prepared in creams, ointments, tablets, liquids, and capsules (Highfield & Kemper, 1999).

Discussion

Salix alba has a long history of use throughout Europe, North America, and Asia. Its use was widely known and documented by healers like Dioscordes, Hippocrates, y Pliny the Elder. Its early use as a treatment for pain, fever, and an anti-inflammatory led to further research by scientists in the early 1800s. *Salix alba* is one of the first herbal remedies that lead to a synthetic drug. Salicin had long been known for its curative powers and with advancements in science; chemists began to experiment with the chemical structure of Salicin. Salicylic acid was first isolated from meadowsweet, which led to the creation of acetyl-salicylic acid. Bayer "rediscovered" the acetyl-salicylic acid and created aspirin. After the creation of Aspirin there was a decline in research on the white willow because Aspirin treated so many illnesses.

There are a couple studies such as the study by Rezaei (2008) and Hyson (1998) that show the efficacy of the almost forgotten herbal form of salicin. Salicin is a strong anti-inflammatory and anesthetic but medical uses in its natural form are not very common because of the invention of Aspirin. More studies need to be done to further investigate the potential uses of salicin. There also needs to be more studies on its use as a cancer treatment. Because of Salicin's apoptotic effect on the cell lining of some organs, it could be used as a potential drug for cancer treatment. In the study done by

Bonaterra et al., the dangers of overuse of NSAIDs are shown but it would be interesting to see what further investigation could indicate (2010). As much of a wonder drug the white willow has proved to be it still has potential to be even more beneficial. Further study should also be done on how the Native Americans use white willow. There are very distinct differences in how Europeans historically used salicin and how the Native Americans used the white willow. Many tribes use the willow tree as more than just a pain reliever but few of these methods have been investigated scientifically (Moerman, 1998).

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Sambucus nigra L., Adoxaceae

Katie Nelson

Introduction

Sambucus nigra L., commonly known as Elderberry, belongs to the Adoxaceae family. Other common names include European Elder, Black Elderberry, Elder Flower, Elder, Flor de Novia (Peru) (Tropicos, 2001), Cinta de Novia (Peru) (Tropicos, 2001), Savuchə or Shtog (Arbëreshë) (Quave, 2009; Quave and Pieroni, 2005), Saúco (Spain) (Cavero et al., 2011), Savucu (Calabria) (Tagarelli et al., 2010), Sambuco (Italy), Sureau (France) and Sabugueiro (Brazil) (Charlebois, 2011). The origin of *Sambucus* is believed to be from the Greek word sambuca, which was a triangle-shaped instrument made from the bark. (Austin, 2004; Watts, 2007).

Elderberry is most commonly used today in Europe to treat colds and influenza (Anderson, 2007; Watts, 2007; Zakay-Rones et al, 2004). Other suggested uses include use as a laxative, for dropsy or for topical skin treatments of wounds, burns or insect bites (Picon et al, 2010; Quave and Pieroni, 2005; Uncini-Manganelli et al, 2005; Watts, 2007). Modern-day products of Elderberry extract include Sambucol and phytotherapic Brazilian tea. Studies have shown Elderberry extract to have antioxidant, antiviral and antibacterial activity and insulin-like properties (Gray et al, 2000; Heinrich et al, 2004; Roxas and Jurenka, 2007; Swanston-Flatt et al, 1991; Uncini-Manganelli et al, 2005; Wu et al, 2004). Its main constituents are comprised of cyanogenic glycoside, flavonoids, triterpenoids and anthocyanidins (Atkinson and Atkinson, 2002; Duke, 1992; Roxas and Jurenka, 2007). The Elderberry tree is referred to extensively in European folk-lore. (Anderson, 2007; Burne, 1914; Grimassi et al, 2011;

Figure 5: *Sambucus nigra*. (Image source: http://irapl.altervista.org/nit/viewpics.php?title=Sambucus+nigra)

Keightley, 1850; Morgenstern, 2000; *Notes and Queries*, 1907; Watts, 2007).

Botanical Description

Elderberry is a deciduous shrub that can range from 4m to 10m in height. The shrub initially grows straight and vertical from the base and has arching branches (Atkinson et al, 2002; Kabuce et al, 2006). The bark of the shrub is moderately acidic and is highly capable of holding water (Atkinson et al, 2002). The branches contain porous white pith. Individual leaves

grow up to 20cm in length and are oval shaped with a pointy tip. The flowers are a creamy white color and 5mm in diameter. The flowers commonly have 5 petals and have cream colored anthers (**Figure 1**). The fruits of this shrub are spherical black berries that grow in clusters and contain 3-5 seeds (Atkinson et al, 2002; Kabuce et al, 2006). They have a lifespan of up to 25 years (Atkinson et al, 2002).

Elderberries are believed to be native to continental Europe but are cultivated as far north as the Scandinavian countries, as far south as northern Morocco, Algeria and Tunisia, but it is also grown in North America, areas of Australia, New Zealand and east Asia (Atkinson et al, 2002). American elder (*Sambucus canadensis* or *S. nigra* subsp. *canadensis*), a close relative of *S. nigra*, is more commonly cultivated in North America and is sometimes confused with *S. nigra* (Charlebois et al, 2007).

Elderberry shrubs are commonly found in either naturally or unnaturally disturbed sites. These sites tend to have eutrophic soils or nutrient-rich soil (Atkinson et al, 2002). Disturbances can refer to woodland margins, hedgerows, neglected gardens, floodplain terraces, roadsides and building sites. The shrubs show stunted growth under shade, which suggest that they prefer sun-lit habitats (Atkinson et al, 2002; Kabuce et al, 2006).

The only pollinators known to impact elderberry are birds. They spread the seeds either by regurgitation or defecation after eating the berries (Atkinson et al, 2002; Charlebois et al, 2007; Kabuce et al, 2006).

Subspecies

In this monograph, *Sambucus nigra* subsp. *nigra* is being addressed, but another subspecies is *canadensis*. This subspecies *canadensis* is known as American Black Elderberry and is predominately found in eastern United States (Austin, 2004; Charlebois et al, 2007; Tropicos, 2001). Another subspecies is *cerulea*, which is known as the Blue Elderberry in primarily found in western United States (Austin, 2004; Tropicos, 2001).

Traditional Uses

Medicinal/Ritual

Elderberry is mentioned in ancient texts and appreciated for its medicinal properties. Hippocrates is said to have called the elderberry a "medicine chest" in 400 BC. Additionally, Pedanius Dioscorides, author of *De Materia Medica* (50-70 AD), and Theophrastus used and wrote about elderberry as a medicinal plant (Krawitz et al, 2011).

The medicinal uses of elderberry are numerous and span across Europe. In general the flowers were used to heal sore or red eyes and gastrointestinal tract problems, and the fruit was used as a laxative (Uncini-Manganelli et al, 2005).

Primarily in the United Kingdom, tea was made from the leaves of *S. nigra* to treat diabetes (Gray et al, 2000; Swanston-Flatt et al, 1991). Elderberry tea was also used for the treatment of colds, coughs, fevers and influenza (Anderson, 2007; Watts, 2007). In medieval England, berry juice or tea was used for dropsy. In England, elderberry leaves were also used on wounds and lacerations, and were especially effective if picked in April (Uncini-Manganelli et al, 2005). Also, the leaves were used to heal insect bites (Watts, 2007). The inner bark was used to treat burns (Quave and Pieroni, 2005; Watts, 2007). It was common in England to use elder flower water, the inner bark or flower lotions to clear freckles and blemishes, whiten skin and heal sunburn (Watts, 2007).

Elderberry was also used in treating witchcraft that came in the form of wounds from animals or plants. One such remedy ritual involved placing the pus of the wound into a hole in the side of the elderberry tree and covering it with elderberry material (*Notes and Queries*, 1907).

In the Arbëreshë communities in Italy, there are numerous uses of elderberry to treat foot sores and blisters, toothache, sore throat, abscess and headache, and worked as an anti-inflammatory, anti-rheumatism and anti-pruritic. Flowers are used for sore throat, foot sores and blisters. For insect bites, wounds, inflammations, rheumatisms and abscesses the leaves on are topically. The community also smokes the dried stems to relieve toothache (Quave, 2009). A cream made from elderberry bark, olive oil and bees-wax was used to treat '*cigli alla testa'*, which is characterized by a headache on the top of the head. Part of the healing ritual for '*cigli alla testa'* includes a prayer being said under the elderberry (Quave, 2009; Quave and Pieroni, 2005). Another Arbëreshë ritual using Elderberry in conjunction with other plants (*Malva sylvestris, Matricaria recutita* and *Parietaria diffusa*) is used to treat '*mal vjnt', or* 'wind illness'. In this community '*mal vjnt'* is identified as a skin rash and he four plants are bundled together and brushed over the rash while a specific prayer is said (Pieroni et al., 2002).

In the region of Calabria (southern Italy), elderberry was traditionally used as an anti-rheumatic and purgative, to treat swollen breasts and legs, insect bites, toothache, colic and conjunctivitis. The plant materials used include the inflorescence, fruit, leaves, bark and dry flower heads (Tagarelli et al., 2010). Additionally, fresh leaf bunches were used to attract flies so could them kill them (Passalacqua et al., 2006).

In Spain, communities in the Middle Navarra had traditionally used elderberry for multiple purposes. Fourteen such uses were documented by Cavero et al. (2011). The inner bark and the inflorescence were used together or separately for the topical treatment of wounds, burns, blisters and animal bites. The plant material was roasted then mixed in either oil or wax, forming an ointment which was applied topically. The inflorescence had additional uses for toothaches, catarrhs, sore throat and as an expectorant. For the previously mentioned purposes the inflorescence was boiled and the smoke inhaled with the exception that a tea was made for sore throats. The researchers also noted that this community was aware that some medicinal usage knowledge about elderberry has already been lost (Cavero et al., 2011). Adjacent to the Navarra region is the Pyrenees area which separates Spain and France. In this area, elderberry was used for medicinal purposes as well (Akerreta et al., 2007). Another nearby region where the medical use of elderberry was prevalent was that of Catalonia. Here, elderberry was used to reduce blood thickness and as an anti-pneumonic (Agelet and Vallés, 2003).

In North America, the subspecies *canadensis* was used by the Native American tribes Cherokee, Menominee, Iroquois, Creek, Choctaw and Houma. The Cherokee and Seminoles prepared tea from the bark for stomach complaints. The Houma however, used bark tea for difficult childbirths, as analgesic and anti-inflammatory. The Iroquois also used elderberry as an anti-inflammatory. Among the Iroquois bark was used to treat headaches, cuts and measles. They used a mixture of roots and bark as poultice for newborn infant navels. Branches or bark could be used as a laxative and the pith from stems treated heart disease and gonorrhea within Iroquois culture Berry tea was used for rheumatism among the Cherokee and for fevers in Menominee and Cherokee. The Creeks treated swollen breasts with water soaked roots, the

Figure 6. Example of one type of elderberry wine. (Image Source: http://heatherhillfarms.com/proddetail.php?prod=manischewitz-elderberry-wine)

Choctaw mixed leaves with salt for headaches (Austin, 2004; Banks, 2004; Moerman, 1998).

The subspecies *cerulea* was commonly utilized by western Native American tribes including the Thompson and Kawaiisu and selectively used by eastern tribes Choctaw and Houma. The Kawaiisu used a mixed of leaves and flowers in a steam bath for headaches and colds. Additionally, leaf tea is used to wash blood poisoned appendage among Kawaiisu. The Thompson tribe treated arthritis with boiled bark, from a nonmetal pot otherwise will be poisonous. Bark was also used for toothaches and dried flowers treated syphilis in Thompson tribe. The Choctaw used leaves topically for swollen hands and internally as a purgative. The bark is used by the Choctaw to treat diarrhea and the Houma as an analgesic (Austin, 2004; Moerman, 1998).

Figure 7. Elder flower fritters. (Image source: http://utmarketgarden.files.wordpress.com/2011/03/elder-flower.jpg)

Culinary

Culturally, elderberry was most commonly used in the culinary arts. During the nineteenth and twentieth century elderberry was used to make soup, jam, wine and juice in the Polish countryside (Luczaj, 2010; Luczaj and Szymański, 2007). In traditional Europe and United States, elderflowers were used to make elderberry beer and the berries to make elderberry ale or Ebulon. The recipe for elderberry beer calls for fresh or dried elderflowers, water, sugar and yeast. Elderberry ale is made with elderberries, water, malt extract and yeast (Buhner, 1998). Elderberry wine was also a popular in England, possibly due to the belief that it was beneficial for sore throat, asthma, sciatica and longevity (**Figure 2**) (Watts, 2007). In Germany, a recipe called Hollerküchln, encompassed elderflowers deep fried and the leftover oil was used further for the treatment of ear infections (**Figure 3**) (Buhner, 2004).

This same recipe is used in the Calabria area of southern Italy too; there the flower fritters are called '*pitte cu majiu*' (Passalacqua et al., 2006).

The berries of both subspecies *cerulea and canadensis* were used in many tribes to make wine, pie, jelly and sauce and in one instance as a fish marinade (Moerman, 1998).

Folk-Lore

There is much folk-lore associated with the elderberry tree throughout Europe and America in traditional beliefs of Christianity, Wicca and others. In England, Germany, Scotland and Denmark it was considered bad luck to burn or cut an elderberry tree without asking permission (Burne, 1914; Keightley, 1850; Morgenstern, 2000; *Notes and Queries*, 1907; Watts, 2007). It was bad luck to burn the tree because by doing so the tree would release evil. Each culture had a different specific prayer or saying for the proper way to ask permission of the Elderberry, but all cultures had expressed a bargain to return wood to the elderberry (Keightley, 1850; *Notes and Queries*, 1907). In Germany and Denmark, if you did not ask permission before using the wood to make a cradle, the entity within the tree would torment the baby (Keightley, 1850; *Notes and Queries*, 1907; Watts, 2007). Additionally, in the areas near the Rollright Stones and Wales, people believed that the elderberry would bleed when cut (Blaine and Wallis, 2007; Burne, 1914; *Notes and Queries*, 1907; Watts, 2007). This stemmed from the belief that the entity within the tree was injured. The name of the entity was different in various countries: in England it was Old Lady, Old Girl or witch and in Denmark it was Hyldemoer, which translates to Elder-Mother (**Figure 4**) (Anderson, 2007; Blaine and Wallis, 2007; Burne, 1914; Grimassi, 2000; Keightley, 1850; Morgenstern, 2000; *Notes and Queries*, 1907; Watts, 2007).

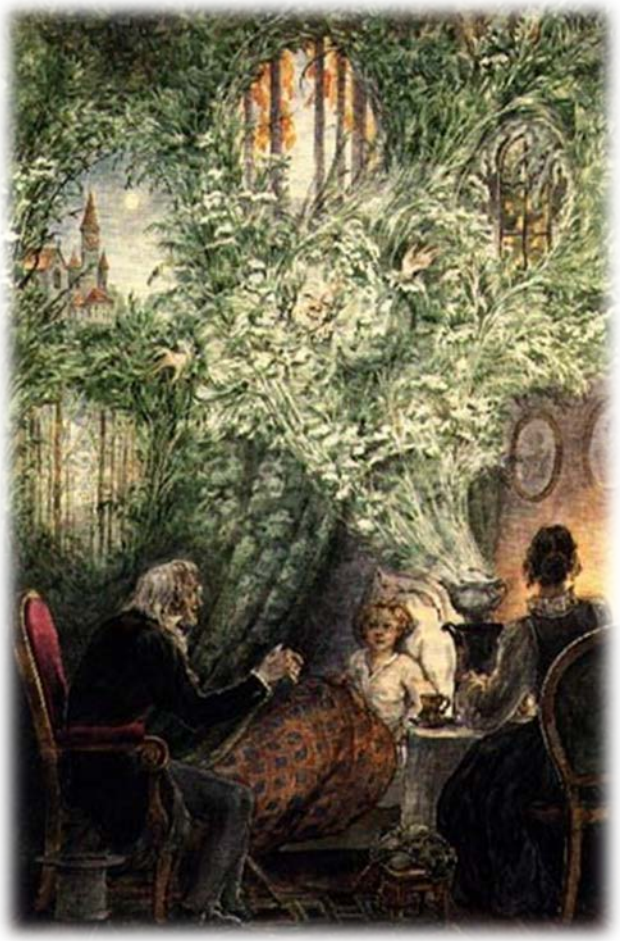

Figure 4. Illustration of Hyldemoer (Elder-Mother Tree) from a Hans Christian Anderson story. (Image Source: http://polarbearstale.blogspot.com/2010/06/sct-hans-and-elderflowers.html)

One of the protective activities associated with the Elderberry was as a safeguard against lightning because it was never struck. In some Christianity sects, this was believed because Judas Iscariot hung himself from an Elderberry tree and the

Compound Class	
Flavonoids (<3%)	quercetin, rutin, isoquercitrin, kaempferol, astragalin, nicotiflorin and hyperoside
Triterpenes	Ca 1% α- and β- amyrin, occurring mainly as fatty acid esters; Triterpene acids: Ca 0.85% oleanolic and ursolic acids, 20β-hydroxyursolic acid
Volatile Oil (.03-.14%)	65% of free fatty acids including palmitic acid and linoleic acid. 7% alkanes. Others include ethers, oxides, ketones, aldehydes, alcohols and esters.
Caffeic acid derivatives (3%)	chlorogenic acid
Sterols	β-sitosterol, stigmasterol, campesterol and cholesterol
Minerals	potassium
Other constituents	tanin, muclilage, plastocynin (protein), pectin and sugar

Table 1. Compounds and their percentages found in Elder flowers. (Image Source: http://www.ema.europa.eu/docs/en_GB/document_library/Herbal_HMPC_assessment_report/2009/12/WC500018238.pdf)

cross used during Jesus Christ's crucifixion was an elderberry tree (Morgenstern, 2000; *Notes and Queries*, 1907; Watts, 2007). Additionally, it became a common practice to place a stick of elderberry over a grave, because if it grew into a tree then the soul of the deceased was happy (Morgenstern, 2000; Watts, 2007). In Wicca, standing beneath the elderberry tree and inhaling the scent of its flowers was believed to reveal the fairies that live there (Grimassi, 2000; Watts, 2007). Elsewhere, a pregnant woman standing under the elderberry tree would have an abortion (*Notes and Queries*, 1907). In England, boys beaten with wood from elderberry were believed to have stunted growth (*Notes and Queries*, 1907; Watts, 2007).

Other

The flowers of *S. nigra* were used as a dark yellow dye for white doilies in the Latíum area of Italy. The fruit juice was used as blackish ink and as a blackish dyeing agent for must (result of first step in wine making) in Latíum, Abruzzo and Marche areas. These three areas are in the central portion of Italy adjacent to one another (Guarrera, 2006). The berries have been used as a dye to color wine since Greek and Roman times and their dying properties were utilized in other materials as well (Austin, 2004; Morgenstern, 2000; Watts, 2007).

The subspecies *canadensis* and *cerulea* were used by the North American Indian tribe Houma, to make blowguns. *Canadensis* specifically was used by the Menominee to make toy guns. The Kawaiisu used the subspecies *cerulea* to make a flute after removing the pith and making six holes in the wood (Moerman, 1998).

Chemistry and Pharmacology

The main constituents of elderberry are cyanogenic glycoside, flavonoids, triterpenoids and anthocyanidins (**Table 1**). Sambunigrin is the main cyanogenic glycoside and is found in the leaves (**Figure 5**). The common flavonoids found are rutin, which is found in the flower and berry, and quercetin, which is found in the berry and leaves (Atkinson and Atkinson, 2002; Duke, 1992; Roxas and Jurenka, 2007). There are numerous triterpenoids found in elderberry: ursolic acid, oleanolic acid, α-amyrin and β-sitosterin (Atkinson and Atkinson, 2002; Christensen et al, 2007; Duke, 1992). The latter is classified as a subtype called steroid alcohols (Heinrich et al, 2004). The two anthocyanidins present in the berries are cyanidin-3-sambubioside and cyanidin-3-sambubioside-5-glucoside

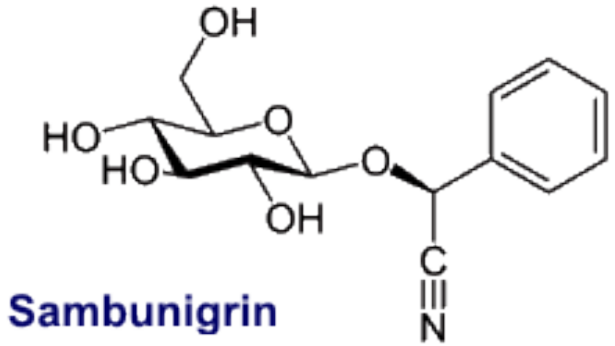

Figure 5. Sambunigrin is one of the chemical constituents of *Sambucus nigra.* (Image Source: http://www.awl.ch/heilpflanzen/sambucus_nigra/index.htm)

(Charlebois, 2007; Duke, 1992; Roxas and Jurenka, 2007). The berries also contain the sweetener compounds dextrose and levulose (Duke, 1992). The noteworthy fatty acids present in the flower are linoleic acid and α-linolenic for their activity on peroxisome proliferator-activated receptor (PPARγ) (Christensen et al, 2007; Duke, 1992).

The elderberry's antioxidant capacity is due to the flavonoids and anthocyandins, which are responsible for the color of the flower (Heinrich et al, 2004; Roxas and Jurenka, 2007; Wu et al, 2004). Cyanogenic glycosides are toxic and thus, thought to be a defense against herbivore. Triterpenoids contain steroid type compounds and are useful in mammals (Heinrich et al, 2004).

Biological Activity

There have been many studies addressing the biological activity of elderberry. In bacterial liquid culture standardized elderberry extract (Rubini) was effective in decreasing growth in Gram-positive bacteria such as, group C and G *Streptococci* and *Streptococcus pyogenes*, and in Gram-negative bacteria including *Branhamella catarrhalis* and *Haemophilus influenza*. However, in the study using Rubini, the extract did not inhibit bacterial growth of *Staphylococcus au*reus, *Streptococcus mutans* and *Haemophilus parainfluenzae*. Rubini was also used in cell cultures where it decreased grow of avian type-A influenza virus (H5N1) and type B influenza virus (Krawitz et al, 2011).

Elderberry fruit extract was used in an *in vitro* study to determine the flavonoids effect on type-A influenza virus (H1N1). The extract was more effective in inhibiting H1N1 infection compared to when each flavonoid was used individually. The flavonoids used were a type of quercetin and dihydromyricetin because they were bound to the virus. It was suggested that either of these flavonoids bound to the domain of the virus responsible for binding to a host cell (Roschek et al, 2010). A different study used dried elderberry flower boiled in water in an *in vitro* for insulin-like properties. The compounds lectin, rutin and β-sitosterol were identified in the extract. The extract significantly increased glucose uptake, glucose metabolism and insulin secretion from pancreatic β-cells. The suggested mechanism of action was sulfonylureas receptor activity on β-cells, which depolarizes membrane and causes an influx of calcium ions. Diazoxide, which inhibits sulfonylureas activity, decreased insulin secretion by the extract (Gray et al, 2000). The effectiveness of elderberry extracts used for inhibition of H1N1 and insulin-like properties were dose-dependent (Gray et al, 2000; Roschek et al, 2010).

Additional antiviral properties were studied of elderberry bark with the feline immunodeficiency virus (FIV) *in vitro*. The boiled bark extract significantly inhibited syncytia formation (formed by virus) (Uncini-Manganelli et al, 2005).

The insecticidal activity of Elderberry is due to the plant protein type-2 ribosome-inactivating protein (RIP) of *Sambucus nigra* agglutinin I' (type-2 RIP SNA-I') found in the bark. In bioassays, tobacco plants (*Nicotiana tabacum*) were transformed with type-2 RIP SNA-I' and were found to be toxic for tobacco aphids (*Myzus nicotianae*) and pea aphids (*Acyrthosiphon pisum*) when ingested (Shahidi-Noghabi et al., 2008). In an additional study, transgenic tobacco plants (*Nicotiana tabacum*) with type-2 RIP SNA-I were harmful when ingested by tobacco aphids (*Myzus nicotianae*) and beet armyworm (*Spodoptera exigua*). The mechanism of action of RIP is proposed to inactivate eukaryotic ribosomes. The B-chain RIP binds to the cell surface receptors which allows the A-chain of the RIP to enter the cell where it inactivates ribosomes (Shahidi-Noghabi et al., 2009).

Clinical Studies

In a Norwegian clinical study, patients who took the elderberry product Sambucol syrup showed a faster recovery from influenza type A and B compared to placebo (**Figure 6**). The patients also reported no adverse effects after taking the syrup (Zakay-Rones et al, 2004).

In Brazil, a mixture of *Pimpinella anisum*, *Foeniculum vulgare*, *Cassia augustifolia* and *S. nigra* prepared as a tea is used to treat constipation. The efficacy of this mixture was supported. The patients had significant increases in evacuations per day (after day 2), and self-reported improvement of bowel functions. There were no reported adverse effects, but it was not recommended to be used with diuretics (Picon et al, 2010).

A clinical study addressed the before and after differences when obese people ingested a combination of psyllium, *S.*

Figure 6. A variety of Sambucol products. (Image Source: http://www.nutranutra.com/nutraport/?cat=15&paged=3)

*nig*ra juice and berries and two sets of tablets, one made from *S. nigra* and the other *Asparagus officinalis*. This diet regiment consisted of 370mg flavonol, 150mg hydroxycinnamates and 1mg anthocyanin from *S. nigra* and 19mg saponins from *A. officinalis*. The participants had significantly decreased body weight and improvement of blood pressure, emotional and physical well-being (Chrubasik et al., 2008).

Contraindications

There are very few dangers associated with elderberry plant material use. The unripe berries can be toxic and might cause nausea, vomiting and severe diarrhea in the worst cases (Morgenstern, 2000). It is also recommended that elderberry extracts not be taken in conjunction with diuretics (Picon et al, 2010).

Figure 7. Example of laxative tea made with elderberry, not necessarily the same one from the study. (Image Source: http://oilnatural.com/product_info.php?cPath=42&products_id=31)

Current Use in Allopathic and CAM Therapies

Today there are some elderberry extract products used in CAM therapies but they are primarily used in Europe. One such product is Sambucol, a syrup used to alleviate and shorten duration of colds and influenza. In a clinical trial there were no side effects and it is advertised for both adults and children (Zakay-Rones et al, 2004). Another cold and influenza medicine is Rubini, but it has not been clinically supported for efficacy of treatment.

A phytotherapeutic tea of elderberry is used in Brazil to treat constipation (**Figure 7**). The tea contains one-third elderberry extract and the other two-thirds are comprised of Aniseed, Fennel and Senna. This tea also has no adverse side effects (Picon et al, 2010).

OptiBerry contains elderberry along with other berries including bilberry, blueberry, cranberry, raspberry and strawberry (Zafra-Stone et al, 2007).

Discussion

Elderberry has widespread traditional and modern-day uses and great treatment potential for colds, influenza and constipation. The modern-day uses are based on some of the traditional uses, but there are many more traditions that have yet to be explored. There are few clinical trials supporting the efficacy of extracts for colds and influenza and no clinical trials for laxative use. Additionally, a majority of the mechanisms of action of the elderberry extract's compounds have yet to be identified or replicated. While there is support for elderberry's antiviral and antibacterial activity, the same cannot be said for its laxative and insulin-like properties. Furthermore, its traditional use as a topical skin healing treatment has not been addressed. The alternative medicinal products mentioned previously are only available in Europe and Brazil, and are largely unknown to the America public. For the most part, Americans are unaware that its health benefits rival those of other berries, which is a shame because of all its wonderful medicinal properties and culinary uses.

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Silybum marianum (L.) Gaertn., Asteraceae

Annelies Carl

Introduction

Silybum marianum (L.) Gaertn, commonly known as Milk thistle, St. Mary's thistle, and Blessed Thistle, is a member of the Asteraceae family, commonly known as the daisy family (Abenavoli et al., 2010). Historically, milk thistle was administered to treat liver aliments, hemorrhoids, and used as a diuretic. Today this plant is still used in the same manner, and the isolated active compounds have a myriad of applications including anti-toxin properties and liver cleansing. This plant is also suggested as a panacea on many herbal Internet sources, reflecting milk thistle's popularity and frequency of usage. The main active ingredient in milk thistle is silymarin, which is found in the highest concentrations in the seeds of the plant but other parts of the plant like the leaves and fruit are also used. Milk thistle seeds or the isolated compound silymarin are sold in many products from biomedical pharmaceuticals to herbal supplements, which range in price. Although many clinical trials have been conducted that showed numerous positive effects of milk thistle components, many of the studies provide contradictory results because of unstandardized concentrations and preparations of milk thistle.

Botanical Description

Milk thistle is native to the Mediterranean basin but has spread throughout the world, and in some areas this plant is now considered to be a noxious and invasive weed that can often be found growing along roadsides, in waste ground, and among cereal crops. Removing milk thistle can be a costly effort for those farmers who consider milk thistle to be a prickly annoyance, since the milk thistle is a very hardy plant. Milk thistles with well-developed root systems can be grown in light soils with periods of water deficiency. Milk thistle will also grow in heavier clay soils and will tolerate a wide variety of pH levels ranging from 5.5 to 7.6 (Karkanis et al., 2011). Although milk thistle is obvious highly adaptable, it prefers fertile soils. Also reflective of its adaptability, milk thistle can grow as an annual or biennial.

Figure 1. Milk thistle. Note the milky white veination patterns. (Source: http://altmed.creighton.edu/MilkThistle/What%20Is%20It.htm)

The stem of milk thistle can grow from 40cm to 200cm tall. The stem itself grows vertically and is slightly downy. Towards the upper part of the plant the stem becomes branched. When the stem branches, the lowermost leaves are large and alternate with spiny edges. These large leaves grow from 50cm to 60cm long and from 20cm to 30 cm wide (Karkanis et al., 2011). Milky white veins, a distinguishable characteristic, found on milk thistle leaves are what give the plant part of its name. Due to the white veins, tradition says that milk thistle was given the name 'marianum' based on the legend that the milk from the Virgin Mary landed on the plant when she was sheltering under a bower formed from milk thistle leaves, and this milk is what turned the veins of the milk thistle white (**Figure 1**).

The leaves on the stem are smaller than the leaves on the base. At the end of each stem, a flower head will form that is around 5cm in diameter with reddish–purple coloration. The seeds have a white pappus, are about 5 to 8 cm long, and range in color from brown to black. On average, each flower head produces about 190 seeds. While in the soil, the milk thistle seed can remain viable for nine years. It has not been observed that the seed needs dormancy (Karkanis et al., 2011) (**Figure 2**).

Many pests and diseases can threaten milk thistle. There have been reports of rust fungus (*Puccinia punctiformis* (F.Strauss) Rohl.) in the U.S, smut fungus *(Microbotryum silybum* Vanky & Berner) in Greece, and cucumber mosaic virus (CMV) in Spain. Milk thistle is also a host for the tomato spotted wilt virus. In addition, there are many insects that feed on milk thistle including the seed-head weevil in Egypt and aphids (*Dysaphis lappae cynarae and Aphis fabae cirsiiacanthoidis*) in Iran and Greece (Karkanis et al., 2011).

Figure 2. Botanical drawing of *Silybum marianum*. (Source: Prof. Dr. Otto Wilhelm Thomé *Flora von Deutschland, Österreich und der Schweiz* 1885, Gera, Germany. Permission granted to use under GFDL by Kurt Stueber. www.biolib.de)

Traditional Use

The seeds of the milk thistle have been used for over 2,000 years in the treatment of liver aliments and gallbladder disorders including hepatitis, cirrhosis, and jaundice. Although other parts of the plant like the flowers are occasionally used, medicinally the seeds are most commonly used. Milk thistle was also used to protect the liver from damaging poisons from sources like snakebites, insect stings, mushroom poisoning, and alcohol (Abenavoli et al., 2010).

In the 4th century B.C. Theophrastus described milk thistle under the name *Pternix*. Milk thistle was later included in Dioscorides *De Materia Medica* in the 1st century A.D. All parts of this plant have been historically eaten as vegetables with no toxic side effects (Karkanis et al., 2011) and the root was also eaten as a nutritional supplement. Milk thistle was used in the Mediterranean and across Europe. Milk thistle was also used in Indian and Chinese medicine. In Traditional Chinese Medicine (TCM), milk thistle is thought to restore balance by fortifying *yin*, which is helpful in treating illness since illnesses that drain *yin*, the dark force that opposes yang (Bensky, 1986).

In Dr. Finley Elingwood's 1919 classic, *American Materia Medica, Therapeutics and Pharamacognosy*, milk thistle was used to improve the general bilious conditions like jaundice, hepatic pain, and swelling. In a study conducted in Mujib region of Jordon on the medicinal plants of the area, milk thistle was found to be an extremely important plant used for the treatment of liver diseases. The flowers and seeds were made into either decoctions or infusions (Hudaib et al., 2008). A different study conducted in the Calabria region (Southern Italy) found that milk thistle leaves are made into a decoction to treat hemorrhoids or make into an infusion as a diuretic (Passalacqua, Guarrera, and Fine, 2007).

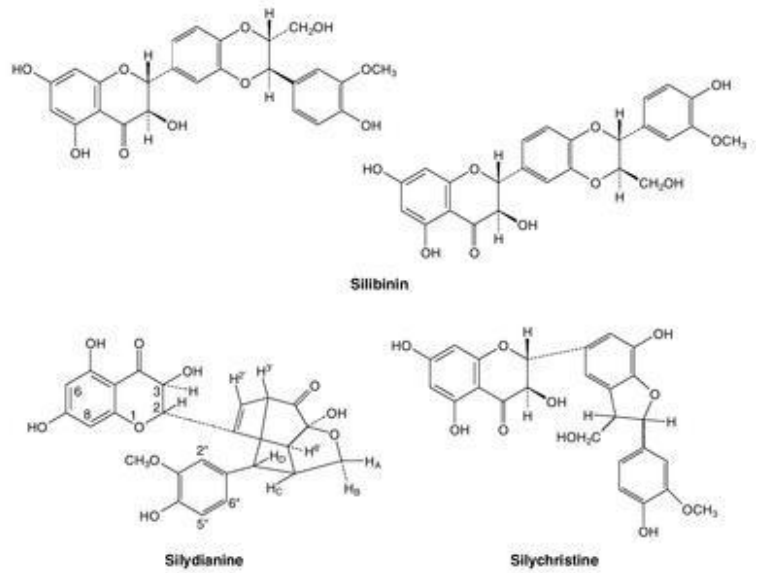

Figure 3. The components of silymarin: silibinin, silydianine, and silychristine. (Source: http://www.medscape.com)

Chemistry & Pharmacology

Most of the medicinal compounds found in milk thistle are present in high concentrations in the seeds. These compounds include silymarin, which is composed of three isomer flavonolignans: silybin, silydianin, and silychristin (Karkanis et al., 2011). Silymarin is 50 to 70% silybin. Silymarin has been shown to have the most biological activity and is a strong antioxidant. The seeds are about 20 to 35 % fatty acids and other polyphenolic compounds (Ramasamy and Agarwal, 2008). Seeds of milk thistle have also been discovered to contain other flavonolignans including isosilybin, dehydrosilybin, desoxysilycristin, silandrin, silybinome,

silyhermin, and neosilyhermin (Karkanis et al., 2011). Silymarin has very low toxicity, and silymarin is not water-soluble, so it is usually given in capsules that contain about 70 to 80% silymarin (Karkanis et al., 2011) (**Figure 3**).

Biological Activity

There are many hypotheses regarding the mechanism of action; the multitude of explanations concerning the mechanism of actions causes confusion and debate in the literature. Silymarin is absorbed orally and from there is distributed into the alimentary tract, which includes the liver, stomach, intestine, and pancreas (Karkanis et al., 2011). One theory is that silymarin's hepatoprotective action is due to its anti-free radical and anticarcinogenic ability, but its heptoproductive action may also be due to other actions like its antioxidant, anti-lipid, peroxidative, antifibrotic, anti-inflammatory, immunomodulatory, and liver regenerating activity (Karkanis et al., 2011). Silymarin is a toxin-blocking agent, which inhibits how toxins bind to hepatocyte cell membrane receptors (Abenavoli et al., 2010). In addition, silymarin has shown anti-inflammatory and immune-modulation activity in various structures and pathways in the cell.

The anti-inflammatory properties of silymarin may be due to the way it regulates inflammatory mediators like the tumor necrosis factor (TNF), tumor necrosis factor-alpha, nitrous oxide, interleukin-6, and interleukin-1 receptors (Manna et al., 1999, Zi X et al., 1997, and Tager et al., 2001). This information suggests that silymarin may help to prevent and treat infectious disease.

With regards to the cytoprotective activity of milk thistle, several mechanisms have been observed. Milk thistle has been shown to promote neuronal differentiation *in vitro* (Kittur et al., 2002). Another study suggested that silymarin inhibits leukotriene formation by Kupffer cells *in vitro* (Dehmlow et al., 1996). In addition, silymarin has been shown to increase the expression of growth factor beta-1 and c-myc in mice (He et al., 2002). Other *in vivo* studies have shown that silymarin may protect the pancreas from damage due to cyclosporine (von Schonfeld et al., 1997), protect the kidney from acetaminophen, cisplatin (Platinol), and vincristine (Oncovin) (Sonnenbichler et al.,1999), and protect the liver from damage due to carbon tetrachloride by reducing lipid peroxidation (Kravchenko et al., 2001). In baboons, silymarin was shown to slow the progression of alcohol-induced liver fibrosis (Lieber et al., 2003). Many other *in vitro* and *in vivo* studies suggest that milk thistle might have anti-cancer properties against cancers of the prostate, breast, skin, colon, tongue, and bladder (Tyagi et al., 2002, Zi et al., 1998, Kohno et al., 2002).

Clinical Studies

Most clinical trials have been done in patients with either hepatitis or cirrhosis, although other studies have been conducted concerning hyperlipidemia, diabetes, and *Amanita phalloides* (death-cap mushroom) poisoning. Overall, clinical trials have found lower bilirubin serum levels, which are a sign of an improved liver because high bilirubin levels are an indicator of liver inflammation, damage, and disease. One study on patients with hepatitis A and B found that silymarin helped lower levels of AST, ALT, and bilirubin in five days (Magliulo, Gagliardi, and Fiori, 1978). On the other hand, many clinical trials have shown that silymarin has no effect on liver disease or liver enzyme levels (Flisiak and Prokopowicz, 1997)

There is debate regarding whether or not silymarin has a direct effect on the hepatitis C virus, with contradictory evidence from various clinical trials. In a trial conducted by the National Institute of Diabetes and Digestive and Kidney Diseases, users who were given silymarin reported fewer symptoms from their chronic liver disease and had a better quality of life than those who did not use the products containing silymarin. One report found that silymarin helped inhibit a tumor necrosis factor and increased peripheral blood mononuclear cells (Polyak et al., 2007). In addition, silymarin has also shown prophylactic and therapeutic effects against hepatitis C virus infection and helped increased the inhibitory ability of interferon-alpha against hepatitis C virus replication. This evidence shows that silymarin has both anti-inflammatory and antiviral properties for patients who suffer from hepatitis C.

Trials conducted on patients with chronic liver disease have also been promising. In a large study of over 2,500 patients with chronic liver disease, an eight week treatment of 560 mg/day of silymarin produced reductions of serum AST, ALT, and gamma-glutamyltranspeptidase (a sign of bile duct disease) and decreased the occurrence of palpable hepatomegaly (Albrecht et al., 1992).

Trials have discovered that silymarin may be useful in the treatment of cancer. In two case reports silymarin was used in individuals with cancer as a treatment and additional therapy. In one case a 34-year old woman with leukemia was given 800 mg of silymarin during maintenance therapy, which helped reduce the number of breaks in therapy due to abnormal liver enzyme levels (Invernizzi et al., 1993). In another case, a 52-year-old man with hepatocellular carcinoma was given 450 mg of silymarin per day and his tumor regressed although he had not started anticancer therapy (Grossmann, Hoermann, Weiss, et al., 1995). A report conducted on the use of silybin claims that it is the only effective antidote for patients suffering from liver damage due to *Amanita phalloides* related poisoning (Enjalbert et al., 2002). In addition, a meta-analysis of herbal supplements on glycemic control, silymarin was shown to increase insulin resistance when used for eight weeks (Suksomboon et al., 2011).

Although milk thistle is arguably one of the most researched plants in the treatment of liver disease (Karkanis et al., 2011), studies done on silymarin and/or silybin are often controversial and are constantly reassessed (Kren and Walterova, 2005). Many studies have shown that constituents in various parts of milk thistle, most often the seeds, help the function of the liver but not all studies agree (Kren and Walterova, 2005). Studies concerning milk thistle also suffer from undefined mechanisms of action, undefined disease populations, different formulations of active components, and varying length of therapy (Kren and Walterova, 2005). Other studies are poorly designed. Overall, the undefined composition of silymarin preparations that are used in the studies is the most common cause of controversy (Kren and Walterova, 2005). This lack of standardization is because there is variation in the proportions of components in silymarin that depends on the source of the milk thistle seeds including the specific plant under cultivation and the conditions of cultivation (Kren and Walterova, 2005). In addition, silymarin extract and the pure compound of silymarin are often confused in the literature (**Table 1**).

Contraindications

In general, silymarin and silybin are thought to be safe with rare adverse effects. One study, Jacobs et al., 2002, reported three serious side effects. After being given a combination herbal formula which contained milk thistle one of the

Efficacy	Acute and chronic viral hepatitis, alcoholic liver disease: conflicting evidence. Cytoprotection: rigorous randomized controlled trials ongoing; limited evidence suggests benefit. Anticarcinogen: clinical trials ongoing Amanita phalloides poisoning: insufficient data
Adverse effects	Generally well tolerated; infrequent reports of gastrointestinal disturbances; rare reports of pruritus, eczema, rash, and anaphylaxis*CAUTION: do not use in patients with allergies to members of the aster family.
Interactions	No significant drug interactions
Dosage	Milk thistle seed extract, 150- to 175-mg capsule, standardized to 80 percent silymarin, three times daily. Ultrathistle (seed extract bound to phosphatidylcholine), 360-mg capsule, three times daily
Cost	$15 to $30 per month at 150 to 175 mg three times daily$42 per month at 360 mg three times daily
Bottom line	Safe, no known drug interactions; insufficient data to recommend for treatment of liver disease; under investigation for anticarcinogenic and chemoprotective effects

*—*Three nonfatal case reports, only one of which is sufficiently attributed to the herb*

Table 1. Key points about milk thistle (Rainone, 2005).

patients in the study suffered from gastroenteritis symptoms. Jacobs et al. also reported anaphylactic reactions after drinking milk thistle tea. Other smaller reactions have been reported, the most common of which being gastrointestinal symptoms, dermatological effects, and headaches, but these symptoms were also reported in the placebo groups. Due to the low solubility of silybin, it is very difficult to reach toxic concentrations *in vivo*. Although silybin and its related flavonolignans showed inhibition of the catalytic activities of cytochrome P450(CYP) isoenzymes *in vitro*, the concentrations required to achieve this are almost physiologically impossible (Kren and Walterova, 2005). These findings suggest that the likelihood of drug interactions involving silymarin and silybin are lower, although further studies are required to better determine the mechanisms of actions in order to better understand the adverse side effects (Kren and Walterova, 2005). On the other hand, other studies suggest that interactions between silymarin and other prescriptions may lead to serious clinical reactions. Milk thistle constituents may have negative reactions when taken with nifedipine, iriontecan, metronidazole, indinavir, pyrazinamide, digoxin, rosuvastatin, and ranitidine (Wu et al., 2009).

Current Use in Allopathic & CAM

Milk thistle is sold in many herbal products for the treatment of various aliments but mainly for liver problems. These products can be found online and in many stores from herbal specialty stores to supermarkets. Commonly, these products contain either the dried milk thistle seeds or extracted silymarin. Silymarin is sold under a wide variety of trade names including Legalon, Silipide, and Siliphos (**Figure 4**). These products can cost anywhere from three dollars to seventy dollars, but theses costs can change dramatically depending on the supply of milk thistle that year.

In allopathic medicine, milk thistle, and the associated chemical silymarin, is mainly used in clinical settings for the treatment of hepatitis and cirrhosis (Post-White et al., 2007). The main constituent, silymarin, is given as a milk thistle seed extract, 150 mg to 175 mg per capsule with a standardization

Figure 4. Legalon – one of the tradenames that silymarin is marketed under. (Source: http://www.poisoncentre.be/_article.php?id_article=223)

of 80 percent silymarin three times daily (Rainone, 2005). Milk thistle is also taken in other forms like liquid extracts and tinctures. This is taken to treat liver disease and used in conjunction with other treatments that may have liver damaging effects. Milk thistle may also be given to reduce damage from a variety of poisons.

Discussion

Milk thistle has been an important botanical throughout the world, especially in Europe and Asia, but also in the U.S in the growing herbal market for people interested in more natural alternatives to biomedicine. It is incorporated in many treatments, both allopathic and CAM, for liver aliments and other various health problems like diabetes, hemorrhoids, and liver damage from alcohol abuse. Like many other botanicals, there are many online sources that suggest milk thistle is a panacea, but these claims should used with care until more research can be completed. Although milk thistle may be as effective as herbal supplies describe, milk thistle is a relatively safe plant with low toxicity.

Currently, information on how milk thistle was traditionally prepared is rare in the scientific literature, perhaps because knowledge on the use of this botanical was spread by word of mouth. Studies that do include traditional uses of milk thistle lack detail, listing the part of the plant and the type of preparation made but not how that preparation is made. Historically, milk thistle was also reputed to help nursing mothers produce milk and eaten as a nutritional supplement, but these claims are not supported by the scientific literature although one study has suggested that milk thistle spouts may be a good source of nutrients and antioxidants (Vaknin, 2008). Milk thistle is used to improve liver function and often administered when certain diseases result in decreased liver function. There has been promising research that milk thistle might have many anti-cancer properties and also help relieve the liver damaging effects of many cancer treatments. Negative side effects of milk thistle are rare although there may be significant drug reactions, so consultation with a physician is important when combing milk thistle with biomedical drugs.

Future studies on milk thistle might clear up confusion regarding the effectiveness of milk thistle constituents. Milk thistle's most biologically active set of compounds found in silymarin may be found to have greater effects in the treatment of various liver diseases, cancers, and anti-toxin abilities. New treatments for these problems will become increasingly important as toxins build up in the environment, which will lead to increased cancer rates. As history has shown, cancer and chronic disease are on the rise due to many factors, some determined while others are unknown. The rise

in rates of liver disease and diabetes may be due to increased alcoholism and obesity, and there is potential for milk thistle to be used to combat the side effects from both diseases. Since milk thistle has a long historical use and numerous current applications, it should be tested further. Such studies may lead to new treatments for these health problems and many others.

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Solidago virgaurea L., Asteraceae

Alisha Morris

Introduction

Solidago virgaurea (European goldenrod) belongs to the Asteraceae family. The herbal substance consists of the dried, flowering parts of the plant. It has been used for treatment of different diseases such as arthritis and diabetes. Today, it is mostly used as an aquaretic agent. Studies have found that the active constituents help reduce inflammation, relieve muscle spasms, fight infections and cancer, lower blood pressure, and have antioxidant effects (UM Medical Center).

Description

European goldenrod is a perennial found along roadsides and in open fields. It has a single woody stem that grows to heights of 3 to 7 feet. It has yellow flowers, about 1/4 of a inch wide and come in large clusters. They appear mainly in August and September (**Figure 1**). The leaves alternate between toothed and smooth edges. The plant is native to Europe and the Asia mainland. Different species of the goldenrod can be found in many locations outside of Europe because of its ability to crossbreed with other plants. Its pollen grains are meant to be carried by insects because of their heaviness in comparison to plants with airborne pollens (UM Medical Center).

Traditional Uses

The goldenrod has been used to treat tuberculosis, diabetes, enlargement of the liver, gout, hemorrhoids, internal bleeding, asthma, and arthritis. Topically, it is used to treat inflammation of the mouth and throat as well as slow-healing

Figure 1. Picture of Golden rod, Solidago virgaurea (Source: Naturephoto-cz.eu).

wounds. It may be taken in a variety of forms, including the dried herb; for teas and capsules, tincture (a medicine consisting of an extract in an alcohol solution), or fluid extract (UM Medical Center). It also used frequently in Europe as a treatment for urinary tract infection and to prevent or treat kidney stones. It has been approved by the German Commission E as a diuretic, anti-inflammatory, and antispasmodic for the treatment of urinary tract disorders (Vitamin for life 2002). In the Appalachian Mountain region of the U.S., its leaves have been used to prepare tea, which is taken as a treatment to combat fatigue and physical exhaustion (Annussek 2001).

Chemistry & Pharmacology

European goldenrod contains about 1.5% flavonoids (quercetin, kaempferol, astragalin, and rutoside) and antocyanidins. Others constituents include about 2% of triterpene saponins, between 0.08% and 0.48% bisdesmosidic phenol glycosides leiocarposide and virgaureoside A, diterpenoid lactones of cis-clerodane type phenolic acids including caffeic acid and chlorogenic acid (0.2-0.4%), and small amounts of essential oils (**Figures 2**). The pharmacological effects of goldenrod have been described in several researches and reviews. A synergistic action of several components of the *S. virgaurea* are proposed. Therefore, the herbal substance or herbal preparations from goldenrod must be considered as the active ingredient (European Medicines Agency, 2008).

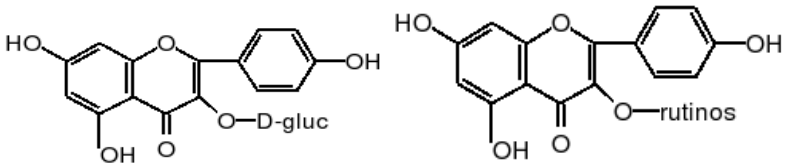

Figure 2. European goldenrod flavonoids (Dzyubak).

Biological Activity

Anti-inflammatory activity

Anti-inflammatory influence of goldenrod extracts from Phytodolor N on the activity of myeloperoxidase liberated by the activated granulocytes was estimated in *in vitro* experiments. Goldenrod extract did not inhibit myeloperoxidase activity at concentrations up to about 1%. It proved that its anti-inflammartory and anti-rheumatic properties were comparable to non-steroidal anti-inflammatories, but with little or no side effects (Von Kruedener et al. 1995). Extracts of *S. virgaurea* (aqueous/alcoholic) were tested individually for anti-inflammatory activity using carrageenan induced edema and adjuvant induced arthritis of the rat paw as well as Extracts of Populus tremula and Fraxinus excelsior were also tested. All of the extracts showed significantly reduction of the carrageen edema and the volume of the arthritic paw (El-Ghazaly et al. 1992). Phytodolor (composition of *S. virgaurea, Fraxinus excelsior* and *Populus tremula*) was tested for an anti-inflammatory, analgesic, and antipyretic activity in rats. Activity was similar to that of the reference substances salicylic alcohol and indomethacin. All of the extracts displayed considerable efficacy (Okpanyi et al. 1989).

Antibacterial activity

Nine clerodone diterpenes were isolated and characterised from ethanol-ethyl acetate extract of *S. virgaurea*. Many of these showed moderate antibacterial activity against Staphylococcus aureus (Starks et al. 2010). Antimicrobial activity of the extracts from *S. virgaurea* were tested *in vitro* by use of free-dried biomass from 4 week old callus cultures of the plant. The minimal bactericidal concentrations of the extracts estimated with the agar diffusion assay method showed moderate activity (Thiem and Goslinska 2002).

Antifungal activity

Triterpenoid glycosides obtained from *S. virgaurea* and *Beallis perennis* inhibited the growth of human pathogenic yeasts. The intensity of growth inhibition is influenced particularly by the carbohydrate chains of the glycosides. Monodesmosidic and bisdesmosidic glycosides of polygalacic acid exert

fungicidic effects (Bader 1990). Glycosides of polygalacic acid are isolated from the aerial parts of S. virgaurea. The glycosides were tested against humanpathogenic strains of *Candida albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* using a micro-dilution assay. The antifungal action can influence the variation of the etherglycosidically bonded carbohydrate nits at C-3 and the acylglycosidically bonded oligosaccharide at C-28 of the aglycone (Bader et al 2000).

Antioxidant activity

Ethanolic extracts of *S. virgaurea, Potentilla anserina*, *Radix Rubiae tinctorum, Equisetum arvense*, *Oleum juniperi* and *Petroselini sativi fructus* mixtures were used *in vitro* to estimate glucose consumption by rabbit brain slices. With an increase of glucose consumption and the aerobic formation of lactic acid, the swelling of the brain slices was significantly diminished (European Medicines Agency, 2008; Dittmann 1973). Extracts (aqueous/ethanolic) from *Fraxinus excelsior, Populus tremula* and *S. virgaureaI,* all carriers of phytodolor, were tested *in vitro* for antioxidant activity. The activity of xanthine oxidase, diaphorase, lipoxygenase, riboflavin and rose bengal were studied. The results showed inhibition of production of reactive oxygen species that were mentioned above (Meyer et al. 1995).

Analgesic activity

The analgesic activity of *S. virgaurea* was tested *in vitro* for affinity to three neuropeptide receptors involved in the mediation of acute pain in mammals. The receptors were bradykinin (expressed in Chinese hamster ovary cells), neurokinin 1 (expressed in astrocytoma cells), and calcitonin gene related peptides. The results showed there was significant inhibition of radioligand binding for bradykinin receptors (Sampson et al. 2000).

Spasmolytic activity

Extract of leaves from *S. virgaurea* inhibited muscarinic M2 and M3 receptor-mediated contraction of rat and human bladder muscle strips. 0.01% concentrations appeared to result from non-competitive muscarine receptor antagonism. 0.1% concentrations might have a non-specific inhibitor effect. However, the relationship between in vitro concentrations and therapeutic doses remained unclear due to unknown bioavailability of the active ingredients of the extract (Borchert et al. 2004). According to Westendorf and Vahlenieck (1981), *S. virgaurea* ethanolic extract induced spasmolytic activity in the range of 14.7% of papaverin in an in vitro experiment on isolated smooth muscles of intestines of guinea pig (European Medicines Agency, 2008). According to Racz et al. (1980) and Duarte et al. (1993), the presence of flavonoids in goldenrod preparations may contribute to explain vascular smooth muscle relaxation. The vasodilatory action depends on the inhibition of protein kinase C, inhibition of cyclic nucleotide phosphodiesterase, or decrease of calcium uptake (European Medicines Agency, 2008). The phytotherapeutic product (Extr Rad. Rubiae tinct. Spir, Extr. Sem Ammeos visnagae spir., Extr. Herb. *S. virgaurea* spir., Extr. Rad. Taraxaci) and aescin exhibited spasmolytic activity in vitro in acetylcholine pretreated urinary bladder of the rat (European Medicines Agency, 2008, Westendorf and Wahlensieck 1983).

Anticancer activity

In a study using mice with an allogenic sarcoma-180model and syngenic sacroma model, significant tumor inhibitory action against Virgaurea saponin E was found (European Medicines Agency, 2008; Bader et al. 1996; 1998b). In another series of experiments the antitumor effects of polysaccharides were demonstrated. In an SCID mouse model antineoplastic activity of *S. virgaurea* on prostatic tumor cells was texted and cytotoxic activity on various tumor cell lines were demonstrated. The active fraction of the extract corresponding to a molecular weight of about 40,000 grams per mole, was administered intraperitoneal or subcutaneous every 3 days for 25 days in an experimental tumor model in mice. Tumor growth was inhibited at 5mg/kg (European Medicines Agency, 2008; Gross et al 2002).

Immunobiological activity

According to European Medicines Agency, Plohman *et. al* (1997,1999) stated that the immunomodulatory and antitumor activity of triterpene saponins were shown in *in vitro* experiments (Plohman et al. 1997, 1999). Choi et. al (2005) used *in vitro* mouse peritoneal macrophages to showed that two compounds from *S. virgaurea* exhibited stimulation macrophage function that suggested potential use in the treatment of infectious diseases and tumors (European Medicines Agency, 2008).

Diuretic activity

The diuretic properties are prevalence in studies performed on the European goldenrod. Leiocarposide was isolated from *S. virgaurea* var. *leiocarpa* (European Medicines Agency, 2008; Hiller et al. 1979) and was found in *Solidago virgaurea* L (European Medicines Agency, 2008; Chodera et al. 1985a, 1985b; Budzianowski 1999). The compound exhibited diuretic activity in rats: only 75% lower than furosemide (European Medicines Agency, 2008; Chodera et al. 1985a). The diuretic action was delayed but, began 5 hours after administration and lasted up to 24 hours (European Medicines Agency, 2008; Chodera et al. 1985b). The flavonoid fraction of *S. virgaurea* was administered to rats and showed an increase of diuresis. Decrease of an overnight excretion of potassium and sodium and an increase of excretion of calcium ions was observed (European Medicines Agency, 2008; Chodera et al. 1991).

According to the European Medicines Agency (2008), Schilcher et. al (1989) demonstrated that leiocarposide diuretic activity was reduced by the presence of flavonoids and saponins. In contrary, some researchers suggest that diuretic activity of goldenrod is exerted by the mixture of flavonoids and saponins. Others demonstrated in animal studies that relative inactivity of the flavonoid mixture present. After 6 weeks of administration of leiocarposide, a significantly decreased of growth of the renal calculi was observed in experimentally induced renal calculi models in rats (European Medicines Agency, 2008; Chodera et al. 1988). After oral administration of an infusion of *S. virgaurea*, significant increase of diuresis in rats together with an increased elimination of sodium, potassium and chloride ions was observed. The lower dose was more efficient though (European Medicines Agency, 2008; Schilcher and Rau 1988). Active flavonoides of S. virgaurea inhibit NEP and the converting enzyme activity of angiotensin (European Medicines Agency, 2008; Schilcher and Rau 1988; Melzig et al. 2001a; Melzig and Major 2000; Major 2001). According to Melzig and Major (2000), the mechanism of beneficial renal and cardiovascular activity of S. virgaurea can depend on

modulation of neutral endopeptidase activity. By increasing water and sodium excretion and arterial and venous vasodilatation, Solidago treatment can regulate water and sodium balance and cardiovascular homeostasis by blocking the hydrolysis of the vasoactive peptides (European Medicines Agency, 2008).

Clinical Studies

An ethanolic extract made from fresh *S. virgaurea* was tested in an open post marketing crossover study with placebo in 22 healthy patients between the ages 17 and 61. The patients received 100 drops/day of the ethanolic extract for 2 days. In Solidago treated groups a significant increase of daily volume of urine was observed (European Medicines Agency, 2008; Klinisch-Experimentelle Studie Nr 23223. P 1. 1992). The ethanolic extract of *S. virgaurea* L., made from fresh plant, was tested in 53 patients in a year long open multicenter postmarketing study. The patients consisted of 45 females and 8 males between the ages 6 and 83 with symptoms of urinary tract inflammation, dysuria, pollakisuria, and tenesmus. Patients with renal stones, renal carcinoma, gonorrhoea, syphilis, AIDS and marked prostate hyperplasia were excluded, as well as patients with bacterial counts in urine over 104. After treatment, 65.4% of treated patients showed significant clinical improvement with significant reduction of dysuria, pollakisuria and tenesmus (European Medicines Agency, 2008; Klinisch-Experimentelle Studie Nr. 23223.P2. 1992). In an open multicenter study, the efficacy of the dry extract of *S. virgaurea* was tested. It was performed by 289 physicians in 745 female patients between 12 and 94 years of age with dysuria of different origins. After 14 days of treatment with Solidago extract, 69.2% of patients' micturition frequency was decreased as the other symptoms of cystitis (European Medicines Agency, 2008; Schmitt 1996). The efficacy of the *S. virgaurea* extract was estimated in a postmarketing study performed on 1487 patients with several urinary tract diseases. Patients were treated in average for 4 weeks. 79% of patients reached significance in global improvement when evaluated by physicans (European Medicines Agency, 2008; Laszig et al. 1999).

An open multicenter study was performed on 1,487 patients with chronic recurrent irritable bladder conditions. They were treated for five weeks. The patients received *S. virgaurea* dry extract. In result, 96% of the patients treated showed improvement registered in clinical global impressions scale, and in 80.1% of patient´s estimation of effectiveness was good or very good. Side effects were not registered (European Medicines Agency, 2008; Melzig et al. 2001b; Pfannkuch and Stammwitz 2002). According to Laszig et al. (1999), there was a case report of patients treated with *S. virgaurea* dry extract for 4 weeks after extracorporeal shock wave lithotripsy resulted with spasmolytic effects, and lack of additional spasmolytic treatment needed (European Medicines Agency, 2008).

Contraindications

Solidago virgaurea is contraindicated for patients with the following conditions: hypertension, hypotension, and osteoporosis. This should not be taken if pregnant, breast feeding, or have heart or kidney disease. Goldenrod may increase the effects of diuretics, increasing the risk of dehydration. It could cause of lithium to build up in the blood, if taking lithium medication (UM Medical Center). Acute toxicity of leiocarposide in rats was reported (European Medicines Agency, 2008; Chodera et al. 1985b).

Current Use in Allopathic & CAM

Solidago virgaurea is used in Pauri Garhawl and Uttrakhand for kidney trouble, asthma, and throat infection. It serves as an alternative to modern medical treatments available to the local inhabitants who have poor economic conditions (Pala et al. 2010). Phytodolor, an active constituent, is a reasonable alternative to NSAIDs and to cyclooxygenase-2-inhibitors such as recoxib (Gundermann and Muller 2007). In Europe, it is used as a supportive treatment for bladder infections, irritation of the urinary tract and for bladder/kidney stones. It increases the flow of urine, helping to wash out bacteria and kidney stones and it may also soothe inflamed tissues and calm muscle spasms in the urinary tract. Goldenrod is useful for the treatment of upper respiratory catarrh and it helps to thin mucus secretions and relieve congested mucus. This herb has been used in combination with other herbs in the treatment of influenza. It is beneficial when used for flatulent dyspepsia. It also promotes the healing of wounds. When gargled, it can relieve sore, throat, laryngitis and pharyngitis. When used as a wash or compress, it aids in wound healing, headaches, and rheumatism. As a douche, it can treat yeast infections. It can also be used as a poultice for bee stings, as a hair rinse for blond hair, and as a bath herb for facial steams (Viable Herbal Solutions).

Discussion

Research studies have shown diuretic, anti-inflammatory, antioxidant, analgesic, spasmolytic, antibacterial, antifungal, anticancer, and immunodulatory activity of *S. virgaurea*. There is no single ingredient responsible for these activities but result from many of the constituents working together. However, the whole herbal preparation of goldenrod's flowering part must be considered as the area containing the medicinal constituents. Acute toxicity of the leiocarposide in rats was reported (European Medicines Agency, 2008; Chodera et al. 1985b). European goldenrod is a reasonable alternative for treatment of diseases mentioned or can be used as supportive treatment in conjunction with antibiotics.

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Taraxacum officinale F.H. Wigg., Asteraceae

Dhondup Tso King

Introduction

Taraxacum officinale, commonly known as the common dandelion, is a member of the Asteraceae family. There are nine species of the *Taraxacum* genus. *T. officinale* has been used as a folkloric medicine for a diverse range of diseases such as hepatic disorders, breast and uterus cancers. It was also used as a diuretic and an anti-inflammatory remedy (Jeon et al., 2008). The genus name *Taraxacum* is derived from the Greek words "taraxis" for inflammation and "akeomai" for curative (Schütz, Carle, & Schieber, 2006). The leaves, flowers and the roots of the plant are either collected or bought from the market and have been used for medicinal and culinary purposes (Yaldiz, 2012). The main constituents of *T. officinale* are sesquiterpene lactones, triterpene/phytosterols, phenolic acids, coumarins, inulin, and flavonoids **Table 1** (González-Castejón, Visioli, & Rodriguez-Casado, 2012). *T. officinale* is especially wide spread in temporal regions of the Northern Hemisphere. Most of the known traditional uses for *T. officinale* as an herbal remedy are based on empirical findings and not scientific studies.

Dandelion Root	Phytochemical Group & Biological Activities	Phytochemicals
Terpenes	Sesquiterpene lactones *Anti-inflammatory and antimicrobial properties* Triterpene/phytosterols *Promote reduced cholesterol absorption*	Taraxinic acid beta-glucopyanoside 11, 13 dihydro-taraxinic acid beta glucopyanoside Traxasterol Beta-sitosterol beta-D-glucopyanoside Stigmasterol
Phenolic compounds	Phenolic acid *Immunostimulatory properties* Coumarins *Act on cardiovascular*	Chicoric acid Monocaffeoyltartaric acid Caffeic acid Chlorogenic acid p-hydroxyphenylacetic acid
Storage carbohydrate	Inulin *Prebiotic activity*	
Aerial dandelion parts (leaves and stems)		
Terpenes	Sesquiterpene lactones Triterpenes/phytosterols	Taraxinic acid beta-glucopyanoside 11, 13 dihydro-taraxinic acid beta glucopyanoside
Phenolic compounds	Phenolic acid *Immunostimulatory properties* Coumarins *Act on cardiovascular*	Chicoric acid Monocaffeoyltartaric acid Caffeic acid Chlorogenic acid p-hydroxyphenylacetic acid
Phenolic compounds (dandelion flowers)	Phenolic acid *Immunostimulatory properties* Flavonoids *Antioxidant properties*	Monocaffeoyltartaric acid Caffeic acid Chlorogenic acid Luteolin 7-O-glucoside Luteolin 7-diglucoside Free luterolin Free chrysoeriol

Table 1. Phytochemical composition of dandelion. Main purported pharmacological properties of phytochemical components shown in italics. Source: adapted and modified from (González-Castejón et al., 2012)

Botanical Description

Taraxacum officinale (**Figures 1 and 2**) in most of the western countries is seen as a perennial weed that grows up to 15-30 cm in length with large, serrated leaves clustered in a rosette around the base of the plant (Wright, Van-Buren, Kroner, & Koning, 2007). *T. officinale* is native to Europe and Asia and begins to grow at the beginning of the summer. It is widely distributed in warm temperate zones of the Northern Hemisphere, especially in fields, gardens, wild, semi-arid land, and along roadsides (Steven & Foster, 2008; Wright et al., 2007). Dandelions prosper in nitrogen rich pasture, gardens and lawns and are cultivated in Germany and France (Steven & Foster, 2008). The leaves sprout at ground level with a stem and are arranged in a rose-like, serrated pattern. Another common name is lion's tooth, from the French word "dent-de-lion" (Schütz et al., 2006). In general, dandelion leaves lack fuzz, which is another way to distinguish it from other similar plants with serrated leaves. It blooms bright yellow blossoms that are about 10 to 30 cm long, sprouting from the middle of the plant (Escudero, De Arellano, Fernandez, Albarracin, & Mucciarelli, 2004). The fruit of the dandelion is the spherical, fluffy seeded heads that helps self-pollinate with the assistance of wind. Its spherical, fluffy, seeded heads give it another common name: blowball. The dandelion's root and the leaves secrete bitter, milky latex.

Figure 1. A complete depiction of Taraxacum officinale with its aerial parts (serrated leaves, stem, fluffy blowball seedlings atop, and root). (Source: http://www.survivalschool.us/wp-content/uploads/dandelion.jpg)

Traditional Uses

Taraxacum officinale has played a significant role in traditional herbal medicine. *T. officinale* has also been reported to being used in folk medicine as a "blood purifier" and to maintain healthy liver. Studies on animals (mice) revealed that dandelion's polysaccharides have hepatoprotective effects by decreasing hepatic lesions. Traditionally, dandelion was used as anti-fibrotic agent in hepatic disorders and recent evidence supports this traditional practice of treatment (González-Castejón et al., 2012).

Figure 2. *Taraxacum officinale*-plant leaves with flowers.
Source: (Šaćiragić, 2011)

T. officinale has been known since ancient times for its curative properties and treatment of a wide range of ailments. *T. officinale* has traditional uses in Germany, North America, Turkey and China (Wright et al., 2007). In Germany, it has a history of treatment for gout, diarrhea, blisters, and spleen and liver complaints. In North America, it has been used as a treatment for kidney diseases, dyspepsia, and heartburn. In Turkey, the herb is utilized as a laxative, diuretic, and anti-diabetic medicine. *T.officinale* has also been reported to be a medicinal treatment for arthritis, rheumatoid arthritis, diuretic and skin conditions such as eczema.

According to Schutz et al., (2006), Arabian physicians first referred to the therapeutic use of T. officinale during the 10th and 11th centuries to treat liver and spleen ailments. *Taraxacum* genus plants have long been employed as a diuretic for over 2000 years in Traditional Chinese Medicine (TCM) and Ayurveda medicine (Clare, Conroy, & Spelman, 2009). Dandelion's medicinal values were recognized by the ancient Chinese prior to Arab physicians' introduction as a strong herbal medicine to the Western herbal medicine (Steven & Foster, 2008). In addition, German physician and botanist Leonhard Fuchs (1543) has provided extensive records of the medicinal application of *T. officinale* and described its uses to treat gout, diarrhea, blisters, and spleen and liver complaints (Schütz et al., 2006). The French have several terms for the common dandelion including *dent-de-lion*, which refers to the deep jagged shape of the leaves, and *pissenlit* which is literally translated as "piss in bed," a reference to dandelion's diuretic effect (Steven & Foster, 2008).

According to Steven & Foster (2008), the first recorded reference to *T. officinale* in TCM was in 659 A.D. This detoxifying herb was used to treat ailments ranging from digestive disorders, appendicitis, breast inflammation, to stimulate milk flow, and uterine and lung tumors (Chatterjee, Ovadje, Mousa, Hamm, & Pandey, 2011; Steven & Foster, 2008). After its introduction to Europe, European herbalists have used dandelion as remedies for common fever, eye problems, diarrhea, diabetes, and liver complaints. The East Indians in the 16th century used *T. officinale* as a hepatic stimulant, diuretic, and for liver disorders and chronic skin diseases (Chatterjee et al., 2011) . The Native Americans used dandelion decoctions to treat kidney disease, indigestion, swelling, skin problems, heartburn, and dyspepsia (Chatterjee et al., 2011; Steven & Foster, 2008).

Today, TCM specialists prescribe dandelion for lung and breast tumors, jaundice, hepatitis, mastitis, abscesses, and urinary tract infection (Steven & Foster, 2008). In Western medicine, dandelion is used in a wide range of conditions. Fresh dandelion and dried preparations are used to stimulate

appetite and ease stomach distress. The root of dandelion is used as mild laxative to improve digestion, jaundice, and liver related conditions, skin conditions like eczema and psoriasis. In addition, the sap from the root is used to treat diabetes.

Nonmedical Uses

Taraxacum officinale's serrated leaves are often eaten as vegetables in salads, or boiled and flavored as a side dish or in soups. The roots can be roasted and utilized as coffee substitutes or fermented into beer, while the flowers have been used to make wine and other soft drinks (Steven & Foster, 2008). Although there is a limited amount of sources supporting the use of dandelion parts as food, a research done in the Province of San Luis, Argentina by Escudero et al. (2004) has shown that even though dandelion is not a protein source, it is still considered a food source due to its high content of minerals, fiber, vitamins, laxative effects, and low toxicity **Tables 2 and 3**. The use of dandelion for weight loss with experimental diet is due to the *T. officinale's* diuretic and effects of *T. officinale* in combination with high quantity of fiber content resulting in increased fecal volume (Escudero et al., 2004).

Chemistry and Pharmacology

The constituents and pharmacological activities in the nine different species of the genus *Taraxacum* remain unclear. However, recent research revealed that ethanol extract of *Taraxacum* officinale contains anti-angiogenic, anti-inflammatory and anti-nociceptive activities (Jeon et al., 2008). The main constituents in *T. officinale* are sesquiterpene lactones, triterpenes/phytosterols, phenolic acids, coumarins, inulin and flavonoids (González-Castejón et al., 2012). Dandelion's bitterness is due to its sesquiterpene lactones found only in the aerial dandelion parts (leaves and stems) and the root of dandelion. Many of the components of dandelion have been isolated and identified, but the pharmacological activities from these components are still under research or have not yet been studied. **Figures 3 and 4** provide the chemical structures of the most representative phytochemicals in dandelion. Dandelion contains important mineral salts such as calcium, potassium, iron, magnesium, phosphorus, silicon and sodium (Šaćiragić, 2011).

Determination	(g/100 g)
Moisture (MF)[a]	91.53 ± 0.83
Residual moisture	8.23 ± 0.15
Protein (N × 6.25)	15.48 ± 0.47
Ash	14.55 ± 0.64
Ether extract (petroleum ether)	3.39 ± 0.04
Total carbohydrates [b]	58.35 ± 0.32
Soluble dietary fiber	6.69 ± 0.36
Insoluble dietary fiber	41.11 ± 0.85
Total dietary fiber	47.80 ± 0.63

Mean ± standard deviation of triplicate determinations.
[a] Fresh basis.
[b] Calculated as 100 – (% residual moisture +% protein +% ether extract +% ash).

Table 2. Proximate chemical composition of flour from *Taraxacum officinale* leaves. (Source: Escudero et al, 2004)

Determination	(g/100 g)
Calcium	695.00 ± 4.00
Total phosphorus	700.00 ± 3.00
Potassium	2520.00 ± 4.00
Magnesium	470.00 ± 2.00
β carotene (vitamin A)	13.80 ± 0.20
Ascorbic acid (vitamin C)	53.00 ± 0.10

Mean ± standard deviation of triplicate determinations.

Table 3. Mineral and vitamin contents in flour from *Taraxacum officinale* leaves. (Source: Escudero et al, 2004)

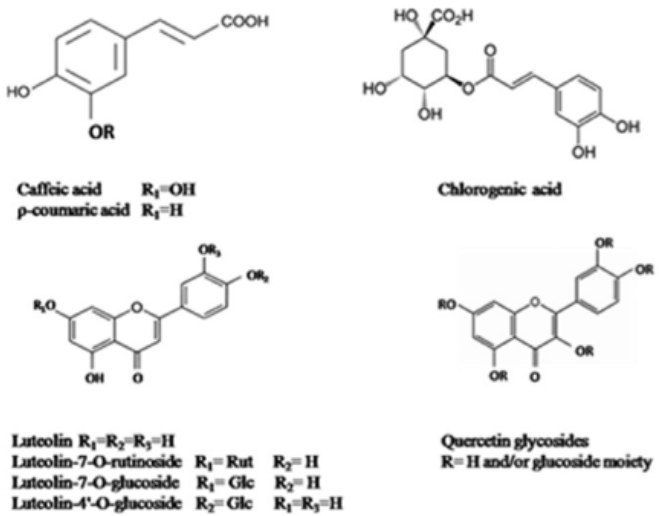

Figure 3. A general structure and substitution patterns of Phenolic Acids and Flavonoids from *Taraxacum officinale*. Sources: (González-Castejón et al., 2012)

Dandelion root

The roots of *T. officinale* contain sesquiterpene lactones (Taraxicin acid beta-glucopyanoside, 11,13-dihydro-taraxinic acid beta-glucopyanoside) which biological activities include anti-inflammatory and antimicrobial properties (González-Castejón et al., 2012; Yaldiz, 2012). Triterpenes and phytosterols in the roots include teraxasterol, taraxerol, homotaxasterol, beta-amyrine, beta-sitosterol beta-D glucopyanoside, and stigmasterol to promote reduced cholesterol absorption. Other chemical ingredients of dandelion are sterine, nicotine acid, choline, various resins and waxes (Šaćiragić, 2011). The root also encompasses phenolic acid such as chicoric acid for its immunostimuulatory properties and coumarins that act on cardiovascular system.

In vitro studies were done on mice to examine the efficacy of *T. officinale* on fatigue and immunological parameters by giving the mice a force-swimming test. The results showed that *T. offcinale* decreased the immobility time in the forced swim test, giving a potential future treatment with immune-enhancing effects. However, more studies are needed to further support this finding (Bo-Ra, Jong-Hyun, & Hyo-Jin, 2012). Furthermore, dandelion roots also contain 15.35% free carbohydrates, carotenoids, fatty acids, minerals and pectin (González-Castejón et al., 2012; Olennikov, Tankhaeva, & Rokhin, 2009). Dandelion root extract is thick and is the main component for obtaining the appropriate pharmaceutical pills (Šaćiragić, 2011).

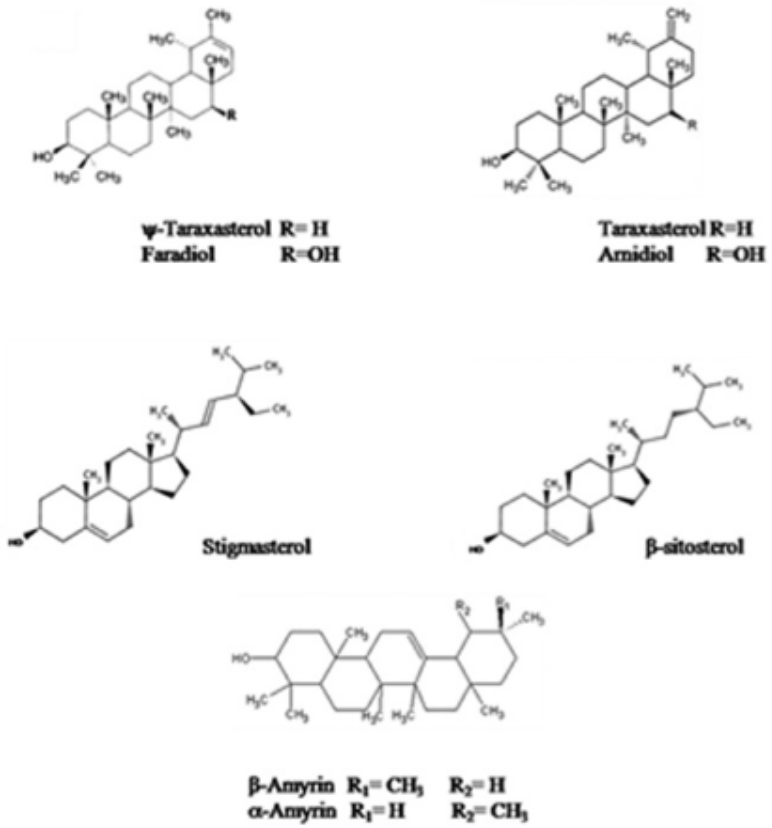

Figure 4. **Chemical structure of some Triterpenes and Phytosterols of *Taraxacum officinale***. Source: (González-Castejón et al., 2012)

Dandelion Leaves and Flowers

T. officinale's aerial parts including the flowers, all contain polyphenol compounds content. However, the content is much higher in the flowers and the leaves of dandelion than in the roots. Most of the phenolic compounds found in the leaves and the flowers of the dandelions are derivatives of hydroxycinnamic acid. A main carotenoid pigment of the flowers is a diester of taraxanthin for which the name "taraxien" was suggested. The major constituents of dandelion flowers, roots and leaves are chicoric acid and monoaffeoyltartaric acid caffeic acid and luteolin 7-diglucoside, and current research studies have focused on chicoric acid for its potential immunostimulatory activities (González-Castejón et al., 2012).

The biological component of sesquiterpene lactones suggested dandelion's anti-inflammatory and anticancer effects (Chatterjee et al., 2011; González-Castejón et al., 2012). *T. officinale* also contain several phenylpropanoids, terpenoids, polysaccharides playing their role in immune regulation, hepatoprotective effects and antitumoral activity (González-Castejón et al., 2012). Dandelion is also a rich source of vitamins and contains a high level of potassium. According to, the phytochemical composition of dandelion strongly depends on the season, time of harvesting, ecological factors and varies among the parts of the plant **Table 2**. For example, sesquiterpene lactones that contribute to the bitter taste of the plant are more prominent in the leaves compared to roots. However, it is noticeable in the roots when it is harvested in the spring. Overall, sitosterol is the most abundant sterol in the leaves.

Biological Activity

Although it is not very strong in caffeine level, many studies have mentioned the use of *Taraxacum officinale* roots as coffee substitute. Emerging evidence suggests that dandelion and its constituents (flavonoids and sesquiterpene lactones) have antioxidant and anti-inflammatory activities that result in diverse biological effects. Many consider it to be an annoying weed and spray it down with herbicide without knowing the diverse biological activity of this herb. With more research,

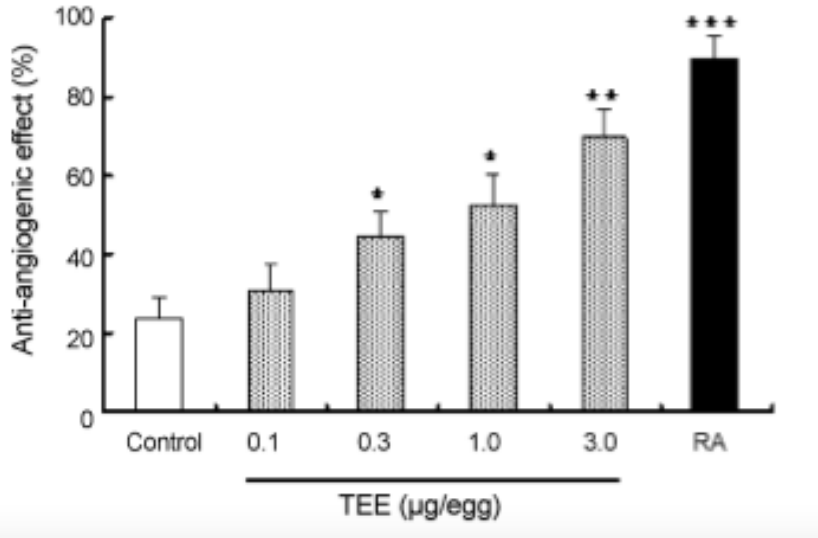

Figure 5. The inhibitory effect of the 70% ethanol extract (TEE) from *Taraxacum officinale*. Sources: (Jeon et al, 2008)

the benefits of dandelion as an choleretic, diuretic, anti-rheumatic and anti-inflammatory will be better understood. Recent studies have paid more attention to dandelion's antioxidant activity and its possible beneficial effects against the development of obesity, cancer, and a number of cardiovascular risks (Gonzalez-Castejon, Visioli, & Rodriguez-Casado, 2012; González-Castejón et al., 2012).

In vivo Laboratory Studies

In vivo experiments was conducted by (Jeon et al., 2008) using male imprinted controlled region mice with a room temperature of 23 ± 2°C, a 12 hour light/dark cycle, and food supply. Dried parts of *T. officinale* were grounded under liquid nitrogen and extracted for an entire month. Anti-angiogenic activity was determined from chorioallantoic membrane assay from chicken egg (Jeon et al., 2008). Current anti-angiogenic strategies are the interference of rapid mitosis of endothelial cell, obstruction of endothelial cell migration and adhesion. The retinoic acid plays a significant role in inhibiting angiogenesis by interfering the expression of factors that promote the formation of new blood vessels (Jeon et al., 2008). **Figure 5** displays that anti-angiogenic activity from ethanol extract (TEE) increases with respect to increase in dosage. Luteolin, a flavonoid compound from *T. officinale* is considered as one of anti-angiogenic principles in dandelion after it increased inhibition in the CAM assay from,(Jeon et al., 2008)'s research. Therefore, the anti-angiogenic activity of *T. officinale* from this study provides a pharmacological basis on its traditional use in treating various acute anti-inflammatory diseases and cancer (Jeon et al., 2008). Anti-nociceptive activity was determined through writhing response after stimulated with acetic acid and there was also a relative inhibition pattern revealed from writhing response induced by acetic acid (Jeon et al., 2008).

Anti-inflammatory Activity

T. officinale was found to contain anti-inflammatory activity in cholecystokinin-induced acute pancreatitis in rats (Jeon et al., 2008). There is an indication of an anti-inflammatory activity from *T. officinale* in the central nervous system from cultures of rats astrocytes and that it hinders the production of tumor necrosis factor alpha by inhibiting the production of interleukin-1 (Jeon et al., 2008).

Antioxidant activities

In both *in vivo* and *in vitro* laboratory studies on rats,

dandelion exhibit antioxidant activities when dandelion root and leaf extracts are administered. Due to dandelion's high phenolic compounds such as flavonoids and coumaric acids found in its flowers, the flowers have great potential of being a source of natural antioxidant (González-Castejón et al., 2012). On the other hand, leaf extract from dandelion contain a greater content of both polyphenol and flavonoid compared to the roots and has the prospective protection from free-radical formations (González-Castejón et al., 2012). Dandelion tea was studied for the ability of its flavonoids to protect V79-4 cells from free-radical induced toxicity (González-Castejón et al., 2012). *In vitro* studies on mice provide scientific evidence that dandelion's antioxidant and antiproliferative activities on hepatic cells and gallbladder disorders give grounds for the use of dandelion in traditional medicinal use (González-Castejón et al., 2012). Dandelion also interferes with the expression of certain proinflammatory mediators. *In vitro* studies show that dandelion root methanol extracts and leaf extract influence inflammatory mediators, while *in vivo* studies show that ethanol, aqueous, and methanol extracts inhibits the production of inflammatory cytokines in rats. Furthermore, *in vivo* studies in humans with seven herb combination including dandelion found significantly less rectal bleeding and anti-inflammatory activity in inflammatory bowel disease **Table 1**. A recent study reveals that dandelion seem to demonstrate protective anti-inflammatory effects on an acute lung injury in mice induced by lipopolysaccharide (González-Castejón et al., 2012).

Pharmacological effects

Animal studies have reported the effects of different dandelion extracts on a range of risk factors of cardiovascular disease such as obesity, hyperlipidemia, hypertriglyceridemia and hypercholesterolemia (González-Castejón et al., 2012). Potent inhibitory activity of dandelion on pancreatic lipase has been evaluated in *vitro* and in *vivo* studies for its potential as an anti-obesity agent (González-Castejón et al., 2012). Flavonoids such as luteolin from dandelion leaves might exert pancreatic lipase inhibitory activity (González-Castejón et al., 2012). Pancreatic cancer is deadly and research studies have been underway to find alternative treatments for this disease. The efficacy of dandelion root extract in inducing apoptosis and autophagy and resistance to pancreatic cancer cells was evaluated and the research data reveals that dandelion's root extract led to the collapse of the mitochondrial membrane, consequently leading to degradation of cancerous cells with no significant effect on the healthy, noncancerous cells (Ovadje et al., 2012).

Hypoglycemic effects

The mechanism of action of its hypoglycemic properties gives controversial results. However, certain dandelion extracts stimulate the release of insulin by pancreatic beta-cells, resulting in reduced blood sugar level (González-Castejón et al., 2012). More thorough research study is needed to confirm which of the dandelion extract initiates the insulin release.

Chemotherapeutic potential

Aqueous extracts of *T. officinale* have been long used in traditional medicine throughout Asia, Europe and North America as a treatment for various types of cancer including breast cancer and leukemia. However, the mechanism of action is unknown. *In vitro* laboratory studies have been done to investigate the anti-carcinogenic effects of dandelion on cell proliferation and metastasis formation. The results conclude

that dandelion extracts kill human hepatitis cells (HepG2) and exhibit toxicity to the uncontrolled cancerous cell growth in humans (Chatterjee et al., 2011; González-Castejón et al., 2012). Crude extract of dandelion leaves have the benefit of decreasing the growth of breast cancer cells by 40 percent. Meanwhile, root extract was found to block the invasion of MCF-7/AZ breast cancer cells while leaf extract block the invasion of LNCaP prostate cancer cells (Chatterjee et al., 2011; González-Castejón et al., 2012). Preventing rapid tumor cell growth from *Taraxacum* extracts attributed to triterpenoids (especially taraxasterol and taraxerol) and sesquiterpenes suggesting chemopreventive agents (González-Castejón et al., 2012).

In test tube studies, dandelion extracts have shown strong anti-tumor activities against liver, colon, and melanoma cancer cells (Steven & Foster, 2008). This is important, as melanoma is one of the leading cancers targeting adolescents and young adults in North America and its aggressive and chemotherapy resistant nature have lead many researchers to try to find alternative treatments. Chatterjee et.al (2011) have investigated the efficacy of dandelion root extraction on human melanoma cell lines in *in vitro* laboratory studies and discovered that dandelion root extract is effective in inducing apoptosis. (González-Castejón et al., 2012) and (Chatterjee et al., 2011) state that the possible mechanism of action is through the activation of caspase-8 by the dandelion root extracts.

Detoxifying and hepatoprotective effects

Traditional folk medicines have been using *Taraxacum officinale* for hepatic disorders as a detoxifying agent. In one research study, extract from dandelion leaves was investigated for its hepatoprotective effects by inducing mice with methionine and choline deficient diet. Non-alcoholic steatohepatis (NASH) is a form of metabolic liver disease, which is becoming a serious global health problem. NASH is caused by the increasing accumulation of fatty acids triggering oxidative stress and inflammation (Munkhtugs et al., 2013). Therefore, a developed treatment for NASH includes inhibiting fatty acid build up, oxidative stress, and inflammation. Dandelion extract has shown efficiency in reducing oxidative stress and fibrosis that cause liver injuries. Dandelion leaf extract with its high content of polyphenol and luteolin inhibit lippolysaccharide induced oxidative stress and protect against liver injury (Munkhtugs et al., 2013). Conversely, the mechanisms by which the dandelion leaf extract exert hepatoprotective effects are unclear. Another study done recently also shows that the combination formula (HV-P411) of seeds from *Vitis vinifera*, *Schisandra chinesis* and *T. officinale* was used to treat D-Galactosamine (D-Ga1N), hepatotoxicant, showed that the formula did have preventive effect on liver fibrosis (Kang et al., 2012).

Renal effects

In traditional folk medicine*, T. officinale* has been employed as a diuretic throughout Europe, Asian and Americas, and is still utilized in modern phytotherapy. Dandelion leaves have been shown to possess diuretic effects in rats and account for 100% weight loss in the testing animals **Table 4**. Studies done on human subjects given fresh hydroethanolic extracts to assess urine volume and frequency of urination indicate diuretic effects but additional studies are needed (Clare et al., 2009).

Antimicrobial / antiviral effects

In vitro laboratory studies data demonstrate that there are

Compounds	Other activities of diuretic compounds
Ascorbic acid Caffeic acid	Nutrient Antiaggregant, anti-inflammatory, antioxidant, anxiolytic
Calcium Chlorogenic acid	Nutrient Anti-inflammatory, antioxidant, cardio protective
Isoquercitrin	Anti-inflammatory, antioxidant, hypotensive
Luteolin	Anti-inflammatory, antioxidant, hypocholesterolemic, vasodilator
Magnesium	Nutrient
Mannitol	Anti-inflammatory, antioxidant
Potassium	Nutrient

Table 4. Diuretic compounds in *T. officinale* and other properties. Source: (González-Castejón et al., 2012)

antiviral effects against the human herpes virus type 1 and antimicrobial effects attributed to the flavones from the dandelion (González-Castejón et al., 2012). Dandelion was used in traditional medicine in Rize region in Turkey for its antimicrobial activity. The milky sap from the roots and leaves of dandelion has shown efficacy in removing verruca and warts on the skin in traditional medicine (Yaldiz, 2012) and melting gallbladder stones and reducing inflammation.

Other activities

Unfortunate events, like the Deepwater Horizon oil spill, have led to an intense search for hyper accumulating plant species that have the potential to be utilized in absorbing a great concentration of heavy metals. A field research was conducted during the month of May 2008 in several different locations on the territory of Bosnia and Herzegovina to investigate the levels of plant contamination with heavy metals (Šaćiragić, 2011). *T. officinale* has been investigated in this field research due to its bio indicator of the degree of contamination of the environment with heavy metals according to (Šaćiragić, 2011). Throughout the research, the content of heavy metals varied depending on the parts of the plant, the site where the survey was done, the level of contamination of the environment with heavy metals, and other ecological factors. As a result of the research, dandelion was classified as a hyper accumulator plant and as bio indicator of heavy metals, particularly lead (Šaćiragić, 2011).

Clinical Studies

One study was done in which fresh leave hydroethanolic extract of *Taraxacum officinale* was given to human volunteers to assess their urine volume and frequency of urination in the subjects (González-Castejón et al., 2012). The results indicated that the dandelion hydroethanolic extract did exert diuretic activities in humans. However due to the small sample size of only seventeen subjects, further experimentation/data collection is advised.

Contraindications

Taraxacum officinale has been found to have low toxicity, and suggested for dietary supplement and as a food source. However, since studies indicate that *T. officinale* is a heavy metal absorber in phytoremediation of agricultural land, it would probably be highly recommended not to pick dandelion grown in recreational parks, off the side of roads, or in backyards due to people's habit of spreading herbicide in these areas and pollution in the environment. According to

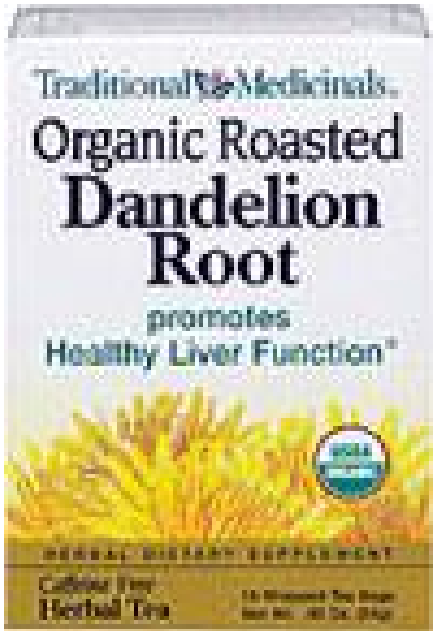

Figure 6. Dandelion tea from the market. (Source: http://www.dandeliontea.org)

(Šaćiragić, 2011)'s, research support dandelion as a bio-indicator of heavy metals, especially lead, which further cautions collecting dandelion where there is high pollution area (i.e. roadside with heavy flow of traffic).

Current Use in Allopathic and CAM Therapies

Taraxacum officinale has a long history of use as an alternative medicine in a variety of cultures in Europe, Asia, and the Americans as a diuretic effect. The extracts have displayed significant potential to induce apoptosis in human melanoma and pancreatic cancer (Chatterjee et al., 2011; Ovadje et al., 2012). In the current market, dandelion tea and supplements are available as dietary supplements **Figure 6**.

Discussion

Despite the fact *Taraxacum officinale* and its related species are commonly considered as a pesky weed, recent research findings have discovered significant medicinal and dietary contribute of dandelions. Emerging evidence and studies suggest that *T. officinal*e and its constituents have a wide-range of biological activities such as antioxidant and anti-inflammatory activities attributing to diverse biological effects. *T. officinale* is steadily gaining credibility with the support of growing scientific research on the plant.

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Taxus brevifolia Nutt., Taxaceae

Wallace Wyeth

Introduction

The Pacific yew tree, *Taxus brevifolia*, is the source of a very important drug used in the treatment of cancer. The Pacific yew, also known as the western yew, is a member of the Taxaceae family (Lewis and Elvin-Lewis 2003). The discovery of *T. brevifolia* as a source of a biologically active phytochemical resulted from its collection in 1962 by botanists from the US Department of Agriculture (Kingston 2007). This collection was part of a large scale screening program then being conducted by the National Cancer Institute. The tree was among a group of 35,000 plants screened for bio-active metabolites (Vongpaseuth and Roberts 2007). The biologically active phytochemical found in this slow growing tree is paclitaxel, a diterpenoid that is extracted from the bark. The Pacific yew is so slow growing that it takes approximately 100 years for a tree to be large enough to harvest. Even then, the yield of anticancer diterpene is very low, in the range of about 0.01 to 0.02%. With such a low yield, it takes three 100 year old trees to produce 1 gram of paclitaxel. Each course of treatment requires 2 grams of the drug (Heinrich, et al. 2004), resulting in the need for a tremendous number of trees. Renneberg described the needed amount of paclitaxel in terms of how many trees would be required each year to treat the patients with ovarian cancer. 360,000 trees per year would be needed just to treat the US patients (Renneberg 2007). In order to protect *T. brevifolia* from being over utilized, a renewable source of paclitaxel would have to be found.

Figure 1. *Taxus brevifolia* in a forest in Canada. The brush in the foreground and the conifers in the background provide a sense of scale (Tigner 2008).

Figure 2. This photograph of the trunk displays the scaly nature of the bark and the multiple hues of purple described in the text (McDougall 2003).

Description

Taxus brevifolia Nutt. (Taxaceae) is described as a small tree, or a large evergreen shrub (**Figure 1**). The bark is thin, scaly, and is purplish in color (**Figure 2**). The tree may reach a height of 15 meters in areas conducive to maximum growth such as coastal areas or some low lying inland areas. Trees of this size are approximately 60 cm (about 2 feet) in diameter at chest height. When conditions are less favorable and at higher elevations, the Pacific yew will only reach a height of a few meters and will be proportionally smaller in diameter as well (Francis n.d.). Leaves are narrow and vary from 8 to 35 millimeters in length (up to 1 1/3 inch). The Pacific yew produces an ovoid seed 5 to 6 millimeters long that is enclosed in a fleshy cup shaped red aril (**Figure 3**). The range

Figure 3. Leaves and fruit of *Taxus brevifolia.* The leaves and seeds are toxic, but the red aril surrounding the seed is not (Pacific Yew Taxus brevifolia n.d.).

of the species is from southern Alaska to northern California. It is sometimes found as far inland as just east of the Oregon or Washington eastern borders. The largest examples of the species are found along the coast with those further inland generally diminishing in size. It is found from sea level to an elevation of 2,200 meters (Francis n.d.). *T. brevifolia* is usually the dominant understory plant, most commonly in coniferous forests. It sometimes grows exposed at higher elevations and also when exposed by logging of the overstory species. It is generally considered a hardy species (Francis n.d.), but is very vulnerable to fire, and controlled burns are not recommended in forests where the Pacific yew is found (Tirmenstein 1990). The same report stated that due to its thin bark, Pacific yew is always killed by even small ground fires. If the species is abundant in a given area its presence indicates a significant period with an absence of fire. It was observed that in one part of Idaho where the species was eliminated by fire, a

period of 6 years transpired before seedlings began to germinate (Tirmenstein 1990).

The Pacific yew can propagate both by seed and by vegetative means. Layering is the primary method of propagation in some areas (Tirmenstein 1990). Layering is a process where lower limbs that come into contact with the ground form roots and establish a new plant. Pacific yew is dioecious and male plants produce abundant 3 mm staminate cones. Ovulate cones are green and appear individually on the underside of branches. The fruit is red and berrylike as described above and is regularly consumed by several bird species (Tirmenstein 1990). It is common for birds to disperse the seeds of the Pacific yew. Seeds are known to exhibit dormant behavior. Passage through the avian gut may affect this dormancy in some way. It takes 250 to 350 years for a Pacific yew to reach maturity and individuals often survive for several centuries (Tirmenstein 1990).

Traditional Uses

Ethnomedical Uses

People of the Pacific Northwest made a tonic from the Pacific yew that was used medicinally (Tirmenstein 1990). Moerman (2004) lists a number of ethnomedical uses by the various tribes in that region. It was used either topically or taken internally as an infusion or decoction. The Chehalis people used *Taxus brevifolia* as a diaphoretic (Moerman 2004). An infusion was made from the crushed leaves that was then used as a wash to cause perspiration. These same people also used it as a panacea, a cure all, by making a similar infusion and using it as a wash to improve general health (Moerman 2004). The Bella Coola boiled the branches complete with leaves to make a decoction used as a pulmonary aid. The Cowlitz people made a poultice from the ground leaves for use as a topical dermatological aid that was applied to wounds (Moerman 2004). Another group made a decoction of the wood and bark that was used both as a gastrointestinal aid to relieve stomach ache and also for other internal ailments. Several other tribes used products from the Pacific yew for similar treatments as well as using it as an analgesic to relieve pain, for the treatment of bloody urine, to treat internal injuries, to treat sunburn, and as a blood medicine to purify the blood (Moerman 2004). The Tsimshian people used it for internal ailments and to treat cancer, though the method of preparation for the cancer medication is not described (Moerman 2004).

Other Ethnobotanical Uses

In his book, Native American Ethnobotany, Moerman (2004) provides a list of the uses made by Native Americans of various plants. On the list of non food, non medical uses, *Taxus brevifolia* is second on the list with 84 uses, behind only *Thuja plicata* (Cupressaceae), the Western Red Cedar, with 121 uses (Moerman 2004).

The wood from *T. brevifolia* is fine grained and hard as well as being very strong and decay resistant. The sapwood is light yellow whereas the heartwood is orange or a rosy red. The wood finishes well and is sometimes used in cabinet making or to make tool handles or boat paddles. Despite this, the wood is not considered to be commercially important (Tirmenstein 1990). Native Americans also used the wood of the Pacific yew to make boat paddles, but additionally used it for making harpoons, spear handles, clubs and, most importantly, bows. Pacific yew is considered to be an excellent source of material for making

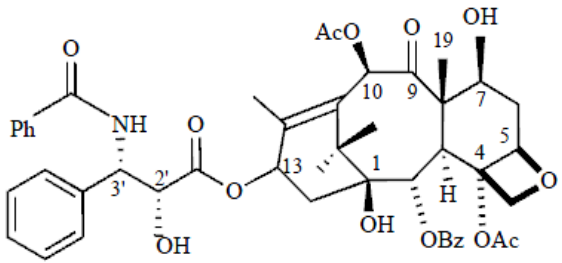

Figure 4. Structure of paclitaxel. Note the side molecule attached at carbon number 13 (Kingston 2007).

bows. The Salish people called it the bow plant (Tirmenstein 1990). Pacific yew is still used to make bows. These bows are very expensive because the wood from which they are made is usually cured for decades (Tirmenstein 1990). Though the Pacific yew is supposedly toxic to domestic livestock, it provides important browse for wildlife species including deer, elk and particularly moose. It is reported that moose depend on Pacific yew over the winter months and from it obtain approximately 40% of their nutritional requirements during this period. The claim that Pacific yew is toxic to livestock has not been substantiated. Some livestock owners claim it is not toxic when eaten as browse, but becomes dangerous if cut and allowed to decay. There are also claims that the fruit is toxic to some species, but songbirds and some mammals are known to feed on the fruit (Tirmenstein 1990). Native Americans also ate the fleshy portions of the fruit, but not the seeds which are poisonous. Native Americans used the foliage as a deodorant due to its fragrance and also used it as a cleaning agent. Some Native Americans associated the Pacific yew with death and bereavement (Tirmenstein 1990).

Chemistry and Pharmacology

The principle active molecule in *Taxus brevifolia* is paclitaxel. Originally known as taxol, paclitaxel is a very complex plant secondary metabolite (**Figure 4**). Because of this complexity, it took years to determine the molecules structure and that it was the active constituent in *T. brevifolia.*

After its initial collection in 1962, plant extracts from the Pacific yew underwent testing in 1963. These tests confirmed the cytotoxicity of these extracts against human KB cells (Kingston 2007). This eventually led to a second bark collection in 1965 that was sent to the Research Triangle Institute (RTI) for further testing. Activity against mouse leukemia was confirmed in 1966, but nevertheless, the active constituent molecule was not isolated until 1969 (Kingston 2007). Taxol was identified as the isolated active constituent (Kingston 2007) but not until 1971 was it confirmed that taxol was the anti-tumor component of the *T. brevifolia* extracts (Vongpaseuth and Roberts 2007). This pattern of discovery and long lapses of time with little progress became the signature of the investigation of the extracts obtained from *T. brevifolia*.

Despite its activity against mouse leukemia that was discovered in 1966, and activity against other leukemia's and carcinosarcoma demonstrated later, other available compounds worked as well or better in these tests, so interest in the products from the Pacific yew remained low (Kingston 2007). There remained just enough interest in taxol, as it was still called then, that more testing was conducted in the early 1970's. These results led to even further testing and the eventual selection of paclitaxel as a development candidate in 1977. The end to the start and stop process of investigation did not come until the mechanism of action was discovered (Kingston 2007). It was the discovery of the unique tubulin

Year	Events
1963	Anti-tumor activity in the extract of *T. brevifolia* bark tissue was discovered during large-scale screening of 35,000 plants.
1971	Paclitaxel was identified as the active component of anti-tumor activity.
1974	First callus culture of *T. baccata* induced.
1979	Paclitaxel's unique mode of action, i.e., tubulin stabilization, was identified.
1983	NCI begins conducting clinical trials of paclitaxel's safety and its effectiveness against various types of cancer.
1989	Paclitaxel produced partial or complete responses in 30% of previously treated patients with advanced ovarian cancer.
1989	First reports of paclitaxel production from callus and cell suspension cultures.
1991	Bristol-Myers Squibb is selected by the NCI to be its commercial partner in developing TAXOL injection and signs a CRADA with the NCI.
1992	FDA approved the use of paclitaxel for refractory ovarian cancer. Clinical trials using paclitaxel demonstrated that the drug is effective against advanced breast cancer.
1993	Bristol-Myers Squibb ends *T. brevifolia* bark harvesting for the manufacture of TAXOL and begins to develop other renewable sources for the drug.
1994	FDA approved the use of paclitaxel for breast cancer that has recurred within 6 months of completion of initial chemotherapy and for metastatic breast cancer that is not responding to combination chemotherapy.
1994	Two separate groups independently report the total synthesis of paclitaxel. Unfortunately, efficiency is not high enough for industrial production.
1995	The semisynthetic form of paclitaxel receives clearance for marketing from the FDA.
1995	Phyton, Inc. announces that it has licensed its *Taxus* spp. cell culture technology to Bristol-Myers Squibb.
1997	FDA-approved semisynthetic TAXOL for the second-line treatment of AIDS-related Kaposi's sarcoma. Paclitaxel produced by plant cell culture was approved in Korea and was marketed as Genexol by Samyang Genex.
1998	FDA-approved semisynthetic TAXOL in combination with cisplatin for first-line treatment of advanced carcinoma of the ovary.
1999	FDA-approved semisynthetic TAXOL in combination with cisplatin for first-line treatment of non-small cell lung cancer in patients who are not candidates for potentially curative surgery and/or radiation therapy.
2001	Export of cell culture-based Genexol by Samyang Genex.
2004	Bristol-Myers Squibb receives a Presidential Green Chemistry Challenge Award for development of a green synthesis for TAXOL manufacture via plant cell fermentation and extraction.
2005	FDA approved abraxane, a drug that is composed of paclitaxel conjugated to albumin to increase efficiency of delivery. It is approved for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within six months of adjuvant chemotherapy.
2006	Studies examing the use of paclitaxel in Alzhiemer's and heart disease treatments begin.

Table 1. History of paclitaxel (Vongpaseuth and Roberts 2007).

stabilization mode of action in 1979 (Vongpaseuth and Roberts 2007) that solidified interest in the molecule (**Table 1**).

Despite its halting nature, research of the chemical properties of the *T. brevifolia* extracts continued through the 1960's and 1970's. As earlier stated, Taxol was isolated in 1969. However, the yield of Taxol from the bark of the Pacific yew was only 0.01% and the concentration in other parts of the plant, such as the wood and needles, was even lower so little Taxol was available for experimentation. It was not until 1971 that the structure of paclitaxel was finally determined after the molecule was degraded by the cleavage of a side chain from the main molecule (Figure 5). The cleavage resulted in two molecules, 10-acetylbaccatin and β-phenylisoserine ester (Kingston 2007). There were a number of reasons why it was so difficult to determine the structure of paclitaxel. Magnetic imaging was not nearly as advanced at that time as it is now. The complex nature of the molecule was a contributing factor as was the extremely limited quantity of paclitaxel that was available for analysis (Wall and Wani 1995). Although a number of crystalline compounds were obtained prior to degradation of the main molecule, none of them were suitable for x-ray analysis which was necessary to help determine the structure of the molecule. After the cleavage of the side chain, the resulting smaller molecules were then crystallized and were suitable for x-ray analysis (Heinrich, et al. 2004). Determining the structure of the constituent molecules allowed the structure of the parent molecule to be determined (Wall and Wani 1995).

Biological Activity

Paclitaxel does exhibit biological activity. The extracts from the bark of *Taxus brevifolia* were first studied because of anti-

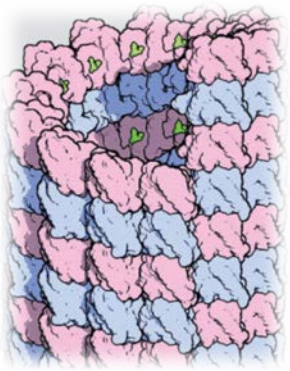

Figure 6. **Paclitaxel (green) binding tubulin and stabilizing the microtubules** (Renneberg 2007).

tumor activity discovered during screening using KB cytotoxicity assay (Kingston 2007). *In vivo* activity testing was done in 1966 that confirmed the bark extract was active against mouse leukemia. Other *in vivo* testing was done against other leukemia's and Walker 256 carcinosarcoma that showed only modest activity. Additional *in vivo* bio assays were developed in the early 1970's. The paclitaxel results in the B16 mouse melanoma assay led to growing support within the National Cancer Institute to pursue development. New tests were developed for testing mammary and colon xenografts in mice. Paclitaxel demonstrated good activity in these tests and was selected for development in 1977 (Kingston 2007).

Susan Horwitz discovered the mechanism of action of paclitaxel in 1979. Paclitaxel works by inhibiting mitosis. Other products discovered before paclitaxel such as vincristine and colchicine also inhibit mitosis. However, paclitaxel works completely opposite those other products. While vincristine for example inhibits mitosis by binding tubulin and preventing the formation of the microtubules required for mitosis, paclitaxel stabilizes microtubules and

prevents depolymerization (Heinrich, et al. 2004). Paclitaxel and its analogue docetaxel have their greatest effect on cells that divide rapidly such as cancer cells. It binds to the tubulin subunits of microtubules (**Figure 6**) and prevents disassembly. The interruption of the cell cycle leads to programmed cell death (Renneberg 2007). The novel method by which paclitaxel inhibited mitosis and initiated cell death contributed to the growing interest in the compound and led to clinical trials (Heinrich, et al. 2004).

Many patients do develop resistance to paclitaxel (Renneberg 2007). The mechanisms that result in resistance are complex and included many potentially contributing processes. Factors listed include rates of expression for cell membrane transporters with increased expression of efflux and decreased expression of influx transporters. Increased expression of resistance associated proteins was also reported. Other factors include over expression of enzymes that metabolize paclitaxel, tubulin mutations that reduce paclitaxel binding, and changes in the signaling pathways leading to microtubule formation (Kumar, et al. 2010).

Clinical Studies

Preclinical toxicology testing of paclitaxel was completed in 1982. Phase I clinical testing was started in 1984 and phase II trials followed in 1985. Serious side effects in the form of hypersensitivity reactions were encountered. Because of its low solubility, paclitaxel formulations include a surfactant (Cremophor EL). The side effects encountered during clinical trials were believed to be caused by Cremophor. Two deaths resulted from these unpredictable hypersensitivity reactions and clinical trials were almost ended (Kingston 2007). A 24 hour infusion protocol was developed that avoided the hypersensitivity reactions. Through these clinical trials, melanoma response to paclitaxel was reported in 1987. Responses in ovarian cancer and breast cancer were reported in 1989 and 1991 respectively (Kingston 2007). Other clinical trials found paclitaxel also effective against advanced breast cancer (Vongpaseuth and Roberts 2007). Paclitaxel was approved for use in 1993 under the trade name Taxol (Heinrich, et al. 2004). The initial FDA approval was for use against refractory ovarian cancer. Additional FDA approvals follow over the next several years. Paclitaxel was approved for use against breast cancer in 1994, Kaposi's sarcoma in 1997, and for use against non-small cell lung cancer in 1999. The FDA approved the marketing of semi-synthetic paclitaxel in 1995 (Vongpaseuth and Roberts 2007).

Other more recent papers have reported clinical results as well. Many of these clinical trials compared the use of paclitaxel in combination with other medications. Platinum compounds are known to be active against ovarian cancer, so testing was done using paclitaxel in combination with cisplatin. Phase I trials provided information that the two drugs could be used safely in combination (Kumar, et al. 2010). Subsequent trials have compared cisplatin-paclitaxel combinations against combinations of other agents. For instance, one trial (GOG 111) compared the effectiveness of a combination of cyclophosphamide-cisplatin against the cisplatin-paclitaxel combination. The progression free survival period (PFS) was increased from 13 to 18 months by using the paclitaxel containing combination (Kumar, et al. 2010). This same trial reported a median survival increase from 24 to 38 months. I similar trial (OV-10) reported similar findings with the cisplatin-paclitaxel combination. These studies established this combination treatment as the first line therapy for ovarian cancer (Kumar, et al. 2010). Subsequent trials such as ICON3, reported in 2002, have not demonstrated the same clear superiority of this combination treatment as

reported by GOG 111. However, though not demonstrating a superior PFS and overall survival rate, the toxicity profile was superior and the cisplatin-paclitaxel combination remained the recommended first line therapy for ovarian cancer (Kumar, et al. 2010). Since these trials concluded, other work has resulted in the replacement of cisplatin by carboplatin. This is a better tolerated platinum compound that is proven equally effective. Most patients will respond to first line therapy, but unfortunately the majority of ovarian cancer patients also suffer recurrence (Kumar, et al. 2010). Work continues to better understand the complex interactions involved. New therapies continue to be tested for both platinum sensitive and platinum resistant cancer.

The combination of carboplatin-paclitaxel remains important for the treatment of ovarian cancer. Recently, clinical trials have been initiated to evaluate adding a third agent along with carboplatin-paclitaxel (Kumar, et al. 2010).

Contraindications

There are a number of toxic side effects related to use of paclitaxel. Neutropenia, an abnormally low number of neutrophils in the blood, is the most common toxic effect. Inflammation of the lining of the mouth and the lining of the gastrointestinal tract are also possible. Muscle pain is a common side effect. Cardiac rhythm disturbances have also been encountered (Georgatos and Theodoropoulos 2001).

Drug interactions with paclitaxel must also be considered. It is important that the dosing level of paclitaxel be relative to the metabolic capacity of the elimination pathway. If the dose exceeds this level, toxicity is increased. If the dose is below this level, efficacy is decreased. Any interactions that alter the rate at which paclitaxel is metabolized and eliminated can lead to increased toxicity or a reduction in the cytotoxic antitumor activity (Kumar, et al. 2010). Paclitaxel is known to have interactions with several other cytotoxic drugs as well as some non-cytotoxic drugs. Even though cisplatin and paclitaxel have been demonstrated to be safe to use together, dosages have to been altered when doing so. Cisplatin administered prior to paclitaxel is known to increase neutropenia and reduce the antitumor activity of paclitaxel (Kumar, et al. 2010). Other cytotoxic drugs with known interactions include doxorubicin, etoposide, topotecan and trastuzumab. Interactions of trastuzumab with paclitaxel have resulted in increased incidence of congestive heart failure (Kumar, et al. 2010).

Known interactions with anticonvulsant drugs such as phenytoin and phenobarbital result in reduced cytotoxic and antitumor activity of paclitaxel. Cytochrome P450 induction is involved in this interaction (Kumar, et al. 2010).

Current Use in Allopathic & CAM Therapies

Paclitaxel is currently marketed under the trade name Taxol by BristolMyersSquibb. Docetaxel is a semi-synthetically produced variant of paclitaxel that is also a taxane diterpene. Both paclitaxel and docetaxel were produced semi-synthetically from 10-acetylbaccatin III. Docetaxel has a modified side chain that is different than the side chain of paclitaxel (Heinrich, et al. 2004). Docetaxel is used for the treatment of breast and ovarian cancer and is marketed under the name Taxotere. Both of these medications are administered intravenously (Heinrich, et al. 2004). Paclitaxel is approved by the FDA for use against "…breast cancer, ovarian cancer, non-small cell lung cancer…" and is also used against Kaposi's sarcoma (Vongpaseuth and Roberts 2007, 219). It is also being studied for use against Alzheimer's and

as a follow up to angioplasty. It is hoped that paclitaxel can limit scar formation to prevent artery narrowing.

Discussion

The discovery of taxol in the Pacific yew was a very important discovery for the treatment of several forms of cancer. The discovery is also indicative of the potential importance of natural products. It is an often cited example of the random sampling method of discovery. The process by which this compound was researched and brought to market is a true example of persistence and tenacity. At several stages of the process, seemingly insurmountable obstacles were encountered. These included the complex nature of the molecule, the molecules weak activity in early assays and most importantly the incredibly low yield of the host plant, the Pacific yew. Research scientists and pharmaceutical companies have derived a number of methods of producing the medication without overexploiting the original source, *Taxus brevifolia*. To prevent extinction of the species, a method was derived where the drug was produced semi-synthetically from 10-acetylbaccatin III harvested from the needles of *T. baccata*. Because this does not kill the tree, this is a sustainable process. Still not satisfied, as the process is sustainable but still not environmentally friendly, research continues to try and find better ways of producing this important medicine. Currently, Taxol is produced by plant cell fermentation (Vongpaseuth and Roberts 2007), a method implemented in 2004. Recently, a new process was announced based on an improved cell culture using *T. brevifolia* stem cells that will be even more environmentally friendly. This process involves culturing cambial meristematic cells and promises higher growth rates and paclitaxel production (Roberts and Kolewe 2010).

Despite this progress, more research is still needed to derive more analogues that can avoid the problems of dangerous side effects and acquired resistance.

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Theobroma cacao L., Malvaceae

Kira Ohmart

Introduction

Theobroma cacao, commonly known as cacao or cocoa, belongs to the Malvaceae family, or the mallow family, which includes marshmallow plants, kola nuts, okra, and cotton. *T. cacao* is one of the most significant perennial crops due to the massive manufacture of chocolate, which is how the plant is almost exclusively used (Almeida and Valle 2007). In 2006, the estimated world output of the cacao plant was 3.5 million tons. The plant has been cultivated and exploited for the seeds, which are used for the manufacture of chocolate (Almeida and Valle 2007). The main constituents of *T. cacao* include the methylxanthines theobromine, a similar compound to caffeine, and caffeine itself, both of which have stimulant properties. Other compounds in *T. cacao* include flavonoids, which exhibit antioxidative effects among other effects, lipids, sterols and, interestingly, trans-resveratrol, which may have significant effects on cancer (Colombo, Pinorini-Godly et al. 2012). The plant is native to tropical regions and can be found in neotropical rainforests, particularly the Amazon basin and Guyana Plateau (Colombo, Pinorini-Godly et al. 2012).

Figure 1. Typical flower found on cacao tree. These grow in clusters directly on the trunk of the tree. Each flower has five fertile stamen and five infertile stamen. (Image source: panoramio.com)

Botanical Description

Theobroma cacao is a small evergreen tree that is native to tropical and subtropical areas, and grows at elevations of 20 to 400 meters. The cacao tree requires a humid climate, with regular rainfall, and healthy soil to survive and produce fruit, tending to grow in areas with 1000-3000 millimeters of rainfall each year and only within 10 degrees north or south of the equator (Colombo, Pinorini-Godly et al. 2012). The cacao tree is an understory tree, reaching 20 to 25 meters in height in its natural habitat, growing beneath the canopy of taller plants in the rainforest in shady areas indicating that it does not require lots of sunlight to survive (Almeida and Valle 2007). There are three different subvarieties of the species that are identified by their physical and genetic characteristics, and the geographical regions they are located in. These three subtypes are Criollo, Forastero, and Trinitario

(Almeida and Valle 2007). The cacao tree originates in the river valleys of Middle and South America. It was originally used by Central Native American groups including Maya, Aztecs, and Toltecs. Now that the *T. cacao* species is cultivated, however, it has been known to be grown not only in Central and South America, but also Asia and Africa (Almeida and Valle 2007).

The cacao plant has two different types of branches: those that grow vertically (orthotropic) and those that grow laterally (plagiotropic). In the *T. cacao* species the vertically growing branches are known as chupons, and the laterally growing ones are known as fan branches. The cacao tree is cauliflorous indicating that it produces flowers on older branches and the main trunk of the plant (Colombo, Pinorini-Godly et al. 2012), **Figure 1**. The leaves of the cacao tree are dark green and shiny with a leathery texture. The leaves are elliptically shaped and undivided blades. They grow to be about approximately 20 to 35 centimeters long and 7 to 8 centimeters wide (Colombo, Pinorini-Godly et al. 2012).

The flowers are tiny, usually just 1 to 2 centimeters in diameter, and contain a pink calyx. The flowers' color typically ranges from white with a yellowish tinge to a very pale pink. Unusual for the plants in the Malvaceae family, the flowers of the *T. cacao* plant grow throughout the entire year, producing, on average, between 50 thousand and 100 thousand flowers per year, and have no nectar or perceptible scent to them (Colombo, Pinorini-Godly et al. 2012). The flowers are produced in little clusters directly on the trunk and older branches (cauliflorous), and each has five petals, five fertile stamens, and five infertile stamens. The plant is pollinated by the *Forcipomyia midges* species, a tiny fly. Interestingly, when these flowers are pollinated, their morphology makes a drastic change from tiny to quite massive, becoming the large pods

Figure 2. Pod of *Theobroma cacao* tree. The husk or outer part is characterized by a yellow color. On the inside, the sweet white mucilage surrounds the seeds of the tree that give chocolate its typical flavor. (Source: EcoLibrary.org)

from where cocoa beans originate, the main ingredient of chocolate (Colombo, Pinorini-Godly et al. 2012).

The pods or berries, as they are sometimes referred to, are an ovular shape, ranging from about 15 to 30 centimeters long and 8 to 10 centimeters wide. These weigh around 500 grams, or a little over 1 pound. The color of the pods ranges between a yellowish to an orange color. Each pod typically contains 20 to 60 seeds, or what are commonly considered cocoa beans. These seeds are enveloped by sweet, white mucilage that is said to have a lemony taste or a taste similar to that of mango (**Figure 2**). Monkeys and other mammals break the pods open to eat this sweet mucilage, which is how the seeds of the plant

are dispersed. The seeds or beans have an extremely bitter taste to them (Colombo, Pinorini-Godly et al. 2012).

Present within the *T. cacao* species, there are fungal endophytes, particularly *Fusarium* species that grow within the plant tissues. These fungal endophytes do not necessarily have a harmful effect on the host unless the host is under environmental stress. Although they are not harmful, there have been no conclusive studies on the benefit of these organisms in the cacao tree (Colombo, Pinorini-Godly et al. 2012)

Traditional Uses

The *Theobroma cacao* species had several different uses in indigenous cultures, including culinary, medicinal, and even monetary uses. It had cultural significance within the Maya and Aztec cultures, among others. The first European encounter with the plant was in 1502, during one of Christopher Columbus's returns to the Americas. By this time, the cacao plant had already been cultivated for up to 2000 years. The indigenous peoples gave him the concoction that they usually create from the cocoa beans and his response was immediate and momentous. He claimed it a "divine drink which builds up resistance and fights fatigue. A cup of this precious drink permits a man to walk for a whole day without food" (Colombo, Pinorini-Godly et al. 2012). This reaction may have been due just to the very strong taste that accompanies the consumption of the bitter beans, or due to the small presence of the methylxanthines theobromine and caffeine.

The concoction that Columbus was given was a beverage drunk by the Mayan peoples. The beverage has an intricate preparation in these cultures. The fruits, or pods, are allowed to ripen throughout the course of a year, picked when ripe, and the beans are removed and laid out on mats to dry. To create the beverage, the beans are roasted over a fire, and then ground between stones on a mortar, creating a paste. The paste is put into cups made from calabashes, similar to a gourd, produced from *Lagenaria seceraria,* mixed with water, sometimes spices were added, and then finally drunk (Colombo, Pinorini-Godly et al. 2012). The Maya groups consider *T. cacao* a significant ritual food due to the stimulant effect of the plant (Kufer, Grube et al. 2006).

Another traditional use of the *T. cacao* species is by the Choco Indians. These people originate in the Amazonian forest. They typically do not make chocolate beverages or even use the beans. This group is more interested in consuming the white pulp surrounding the beans as a snack between meals (Duke 1970).

A different indigenous group, the Cuna of Panama and Colombia, also consume *T. cacao,* in the form of a paste made from the beans. The Cuna people eat 25 grams of this paste per day as a snack. The Cuna also used *T. cacao* for medicinal purposes to treat malaria and fever (Duke 1975). The preparation for these treatments included burning the beans ceremonially with hot peppers. Additionally, the fruit pulp will be turned into a decoction, and taken by pregnant women to relieve their pregnancy symptoms. The Cuna people also use the leaves of the cacao tree to make an infusion for weary children. In addition, adolescent leaves are applied as antiseptics, while flowers are used to treat eye parasites (Duke 1975).

The Aztecs actually used cacao as a form of currency, and when consumed, it was as if one was "eating money". This made the cacao plant a luxury only ever drunk or eaten by the Aztec nobility because commoners simply could not afford to eat their money (Colombo, Pinorini-Godly et al. 2012).

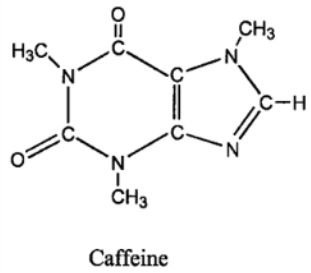

Figure 3. Chemical structure of caffeine, one of the purine alkaloids present in *Theobroma cacao*. Both caffeine and theobromine are considered methylxanthines. (Image source: med-chemist.com)

Filipinos have been known to use *T. cacao,* for specific ailments. For example, a decoction of the roots of the plant has been taken orally when menstruation has resulted in excessive and unceasing bleeding. Filipino people have also used scrapings of the skins of the pod placed around boils for two or three days to excrete and remove pus (Langenberger, Prigge et al. 2009).

In comparison to all of these seemingly advantageous uses for different parts of the cacao plant, the modern medicinal application for the plant is somewhat wanting. Because of the huge chocolate industry, the plant is primarily cultivated for chocolate production. However, chocolate has been marketed for its antioxidant properties, resulting in increased production value of the plant.

Chemistry and Pharmacology

Over the past fifteen years, there has been piqued interest in the potential of the products of the cacao plant, including the cocoa removed from the seeds and dark chocolate. There has been evidence to support the antioxidative properties and benefits of phytochemical-rich components of chocolate.

Component	Nib %	Shell %
Water	3.2	6.6
Fat (cocoa butter)	57	5.9
Cocoa powder	4.2	20.7
Nitrogen	2.5	3.2
Theobromine	1.3	0.9
Caffeine	0.7	0.3
Starch	9	5.2
Crude fiber	3.2	19.2

Table 2. Table of component percentages found in certain parts of the cacao plant. Nib refers to internal part of the plant, while shell refers to the external husk (Colombo, Pinorini-Godly et al. 2012).

The constituents include a large variety of components that interact with each other. These are varieties of catechins, flavonoids, tannins, essential oils, alkaloids, polyphenols, proanthocyanidin glycosides, carboxylic acids, purine alkaloids, and fatty acids (Giorgetti, Negri et al. 2007).

Cacao is rich in methylxanthines, which are an alkaloid, particularly theobromine, theophylline, and caffeine (**Figure 3**). These can tend to have both negative and positive health effects, but primarily serve as stimulants. There are also flavonols and flavonoids present in the cacao plant, including flavan-3-ols, which exhibit antioxidative effects, protection from cardiovascular disease, and anticancer effects (Colombo, Pinorini-Godly et al. 2012). There are also a large variety of lipids present, especially in the seeds or beans of the pod. When ground into a paste, a cocoa butter results along with fine brown particles that are the main constituents of chocolate (**Table 1**). The cocoa butter makes up 50 to 57 percent of the dry weight of cocoa and contains a significant

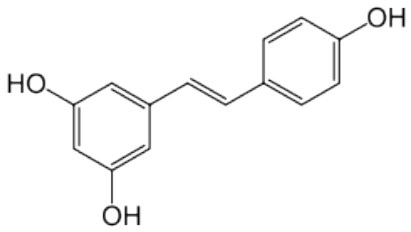

Figure 4. Chemical structure of trans-resveratrol, one of the most promising medicinal components found in *Theobroma cacao*. (Image source: mhhe.com)

amount of fat and fatty acids, including saturated stearic acids (35%), saturated palmitic acids (25%), monosaturated oleic acids (35%), and polyunsaturated linoleic acids (3%) (Colombo, Pinorini-Godly et al. 2012). There are also a number of sterols, and possibly most significantly, trans-resveratrol (**Figure 4**). Trans-resveratrols have exhibited a significant amount of anti-inflammatory effects, anticancer effects, and cardioprotective and estrogenic activities (Colombo, Pinorini-Godly et al. 2012).

Biological Activity

Several studies have been done involving the compounds found in *Theobroma cacao. In vitro* studies have provided evidence that suggest that the flavonols and procyanidins present in cocoa may possess immunoregulatory effects that may help modulate immune responses. Additionally, other studies have suggested that the removal of theobromine and caffeine from the cacao plant has actually enhanced the antioxidant effects of cocoa. This result provides evidence that these components may inhibit antioxidative activity thereby reducing the effectiveness of the plant as an antioxidant (Colombo, Pinorini-Godly et al. 2012). However, this is not conclusive and needs more research.

Another *in vitro* study done has resulted in the discovery that flavonoids (including epicatechins and isoquercitrin) in cacao have affected transcription, resulting in enhanced expression in peripheral blood human mononuclear cells. There was also a decreased nitric oxide production along with cytokine secretion modulation reported when cocoa extracts were used rather than using the isolated chemical components suggesting that the synergism of all of the constituents present in cacao makes it more effective than isolating the compounds (Ramiro, Franch et al. 2005).

Some *in vivo* studies have indicated the antioxidant effects of epicatechin as well, enhancing oxidation scavenging activity in plasma. Antioxidant activities of this constituent have resulted in reduction of the concentration of free radicals, diminishing of oxidation of lipids, proteins, and nucleic acids. Additionally, other *in vivo* studies have provided support that caffeine and theobromine have resulted in reproductive toxicities in animals, particularly males, among other toxicities from the isolated compounds. However, the cacao plant does not have a high percentage of these methylxanthines, and, in fact, is known to have less caffeine than a cup of decaffeinated coffee (Colombo, Pinorini-Godly et al. 2012).

The mechanism of action involved in *T. cacao* constituents is still in debate, but a range of potential mechanisms of the flavanols in cocoa has been shown to improve cardiovascular health. Some of these possible mechanisms of action include nitric oxide activation, as seen in the above *in vivo* study. Additionally, there have been some anti-platelet effects that tend to improve endothelial function, blood pressure, and other heart conditions (Colombo, Pinorini-Godly et al. 2012). Flavonoids have also shown some bioactivity in the

modulation of cell signaling pathways that regulate several cell functions including survival and inflammatory responses (Giorgetti, Negri et al. 2007). In addition, the presence of

sterols also contributes to heart health because they contribute to an improvement of blood lipid profiles through the mechanism of competitive inhibition of cholesterol absorption through the gastrointestinal tract (Colombo, Pinorini-Godly et al. 2012). There is no known threat of drug resistance against the constituents in *T. cacao.*

In another *in vivo* study, the biological activity of cacao proteins were studied and identified. This supported the traditional uses of the plants against tumors and free radical scavenging, particularly inhibiting murine lymphoma L5178Y, a model cancer cell found in mice. It was found that the cacao albumin fraction (a certain protein isolated from the cacao plant) had a significant effect on tumor growth that differed from the other tested protein fractions, which tended not to be dose-dependent. The results suggested that antitumor activity was due to the amino acid profile that was present in the albumin fraction, which was rich in cysteine, leucine, arginine, and lysine. The presence and high percentage of these amino acids relates this protein to the trypsin inhibitor potential. There was also a significant correlation between radical scavenging capacity assays in cacao, and evidence shows that the cacao protein fractions can be considered a chain breaking antioxidant. This indicates that it halts the transfer of hydrogen atoms, creating a stable radical, rather than one that continues the chain reaction (Preza, Jaramillo et al. 2010).

Clinical studies

For the development of drugs using the constituents of the cacao plant, few clinical studies have been done, merely because the primary use of cacao is for chocolate use. However, the few that have been done have suggested that the polyphenolic compounds present in the cacao have been useful in increasing blood flow and perfusion of the brain. Some clinical studies have discussed the use of flavanols to treat neurodegenerative diseases like Alzheimer's disease. Evidence has supported that flavanols may delay the onset of this type of disease (Colombo, Pinorini-Godly et al. 2012).

Current use in allopathic and CAM therapies

The current use of *Theobroma cacao* is commercial exploitation of the crop. The cacao plant has had a huge success in the marketing industry. *T. cacao* has not only been used for the manufacture of chocolate, but also derivatives and byproducts of the plant have been used for cosmetics, fine beverages, jellies, and juices (Almeida and Valle 2007).

The modern process of changing the cacao seeds into cocoa or chocolate has similarities to the ancient Mayan process. The cacao plant is allowed to ripen throughout the year, and once ripe, the pods are harvested. After this process, the cacao pods are cracked open and the seeds, or beans, are extracted from the pulp and pod. These are then fermented, dried, and finally roasted. These steps are what contribute to the typical chocolate flavor and color that most people are familiar with. The roasted seeds are then ground into a chocolate liquor, which contains 55 percent cocoa butter, and cocoa powder, which consists of nonfat, fine, brown particles of the beans. The cocoa powder is where the flavor of the chocolate primarily comes from and where the polyphenol antioxidants are located. This nonfat part of the cocoa liquor is what is actually used in the production of chocolate or the cocoa powder that is used for culinary and beverage purposes (Colombo, Pinorini-Godly et al. 2012).

It is important to note that chocolate and cacao are not interchangeable terms. Chocolate is actually a combination of the cocoa powder, cocoa butter, additional sugars, and other added chemicals that are made into a food. Different chocolates have different percentages of cacao, but usually range from 50 to 80 percent cacao.

Medicinally, one of the most important constituents in cacao is the trans-resveratrol. This is currently one of the phytochemicals in cacao with the greatest potential for use as a pharmacological drug. These are intended to prevent or reduce the risk of certain diseases (Colombo, Pinorini-Godly et al. 2012). Resveratrol is of significant interest because of its antioxidatve, anti-platelet, anti-inflammatory, estrogenic, anticancer, anti-tumor, and antiviral activities. In one study, trans-resveratrol and its glycoside, trans-piceid, were found in dark chocolate extracts. Additionally, the same study discovered that presence of procyanidins increase the antioxidant activity of chocolate (Counet, Callemien et al. 2006).

Another study provides strong evidence to support that cacao husk lignin fractions prepared by acid precipitation and ethanol precipitation yielded an unexpected amount of anti-human immunodeficiency virus activity. These activities amount to almost as much anti-HIV effects as conventional treatments and popular anti-HIV chemical compounds. The husk lignin fractions have showed anti-flu virus activity as well. However, there was no reported antibacterial activity. This study also supports that there is stimulation in the generation of nitric oxide by macrophage-like cells, suggesting the possibility of an effective complementary alternative medicine (Sakagami, Satoh et al. 2008).

Discussion

Theobroma cacao is now primarily used for the economical benefit for the countries that cultivate and produce chocolate. The chocolate industry provides a powerful incentive for Mesoamerican and South American countries to distribute this cash crop widely. The commercial benefits of chocolate have a profound effect on these tropical and subtropical countries.

The cacao plant's traditional uses have guided researchers and ethnobotanists to compose research on the possible medicnal uses for cacao. These studies have resulted in a multitude of results that provide support for many different potential medicinal therapies. It can be used to effectively reduce oxidation, diminish tumor activity, and improve cardiovascular health. Some of the constituents found in *T. cacao* are also effective anti-inflammatory and estrogenic constituents including the trans-resveratrol. Stimulants, including but not limited to theobromine and caffeine, also contribute to the chemical compounds present in cacao plants.

As discussed previously, the economic value of the cacao tree has influenced the countries that grow and farm the plant to increase production and actually destroy native forests for the cultivation of cacao. This is problematic for the maintenance of forests and completely hinders the efforts to conserve native rainforests, which are already diminishing quickly with deforestation due to human expansion and other such reasons (Almeida and Valle 2007).

In the next ten years, it has been projected that the consumption of cacao will increase by nearly one million tons of cacao seeds. It is expected that there will be an increase in cacao production in West African countries. However, this would have an impact on the economy and resources of these countries. The chocolate industry actually foresees a challenge in the cacao supply in the future (Almeida and Valle 2007). In

fact, if cacao is not supplemented by another crop, this could actually be problematic for the chocolate industry and for individuals of certain indigenous groups (Hazlett 1986).

Besides this, there is also an ethical debate over the cacao plant and chocolate industry. In the United States, successful chocolate manufacturers, including Hershey's and Mars, were reported for using child labor on West African cocoa farms. Subsequent investigations revealed the conditions that children worked under as some of the most atrocious forms of child labor. Child labor probably produced a massive percentage of the chocolate products that Americans consider affordable luxuries (Mustapha 2010). The result of this discovery bears the presence of ethical concerns that were not previously considered or even detected by the huge chocolate industry. These threats make the cultivation of cacao into an entirely other issue, where the moral indications have to be considered.

Although there are some ethical concerns regarding the cultivation of the *T. cacao,* the plant is still a delectable treat, with a variety of different medicinal effects in humans. The traditional uses of cacao have permeated through today, in the social aspect of drinking the fine beverages. In addition, the stimulant effects of the plant make it a recharging treat.

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Thymus vulgaris L., Lamiaceae

Carly McCabe

Introduction

Thymus vulgaris is commonly known as Thyme, however, it goes by many other names such as common thyme, garden thyme, tomillo, thym, 0v'tos (by the Greeks) (Andrews, 1958) and many others (Basch, Ulbricht, Hammerness, Bevins, & Sollars, 2004). It is a member of the Lamiaceae family, which is the mint family, and so it is related to common plants like peppermint, rosemary, and oregano. It is native to the Mediterranean, however, it can be found in many regions of the world—these regions include Egypt, Iran, Israel, and the United States among others (Arraiza, Andres, Arrabal, & Lopez, 2009). Thyme has many applications and it is highly valued for its antibiotic, antispasmodic, antioxidant, antimicrobial and antifungal effects. Thyme has also been purported to possess analgesic, diuretic, carminative and stimulant properties. Additionally, it has been used in flavoring applications within the processed foods industry, as a soap, detergent and preservative (Arraiza, et al., 2009). Its medicinal activity comes from the diverse essential oil content—two main compounds within the extract are thymol and carvacrol (Lee, et al., 2005).

Botanical Description

Thyme is a small, shrubby, aromatic plant native to the Mediterranean (Robineau, 1997). It is a branched, woody plant with numerous stems and fibrous roots that usually grows to be eight to ten inches in height (Culpeper, 1955). At the base, the stems grow erect or along the ground without setting roots (Darlington, 1847). The leaves are small, quite short, broad, pointed, and dusky-green (Culpeper, 1955). They can either be an oblong oval shape or a narrow oval shape (**Figures 1 & 2**), curling on the edges, and bunching at their axils (Darlington, 1847). Its flower is commonly small and light blue or light purple. The calyx is hirsute, strongly ribbed, and studded with what appears to be tiny dots or holes, while parts of the lower lip subulate (Darlington, 1847). *T. vulgaris* is considered the garden thyme; wild thyme (*Thymus serpyllum*) and lemon thyme are others commonly encountered among the 100-plus varieties (**Figure 3**). The most suitable environment for thyme growth is one with poor, light and dry soil. When found in moist or rich environments,

Figure 1. Oblong-oval leaves of *T. vulgaris* (Modzelevich, 2008).

Figure 2. Long-oval leaves of *T. vulgaris* (Landing, 2012).

the plant thrives or becomes prolific. However, this causes the plant to lose many of its characteristic aromatic properties; nor is it then able to survive the winter (Johnson & Emerson, 1858). The plant also enjoys an environment comfortably surrounded by other plants, although not crowded. Thyme can be grown from either seed or rooted slips; if using seeds, sowing should be done around the middle of March until the beginning of May. Additionally, it should not be sown thin nor should it be more than half of an inch from the surface. During growth, the seedlings must be kept clear of weeds and should be lightly watered two times a week, which should continue until the plants take root. Thyme is a perennial, however, after three or four years the plant no longer grows productively (Johnson & Emerson, 1858).

Figure 3. Botanical drawing of *Thymus vulgaris* (Future, 2010).

Traditional Uses

The growth of various species and the use of thyme in ancient Egypt, Greece and Rome was primarily to perfume unguents (a viscous substance uses as an ointment), for embalming and of course for its medicinal properties (Stahl-Biskup & Sáez,

2002). Bardeau proclaimed thyme as "an indispensable plant, which should be consumed to conserve health. Furthermore, if one could replace ones' morning cup of coffee with an infusion of thyme, you would quickly appreciate its positive effects: animation of spirit, sensation, lightness in the stomach, absence of morning cough, and its euphoriant and tonic effect (Stahl-Biskup & Sáez, 2002)." Thyme earned itself a place in many ancient cultures, and it is referenced in many ancient texts for a variety of uses.

Medicinal Applications

The medicinal application of thyme and its essential oil are quite concordant when it comes to the many cultures that could take advantage of and had access to the plant. Nicholas Culpeper detailed the use of thyme in his herbal, originally published in 1652, for a sundry of applications, which find homology in other traditions. He listed it as a strengthener of the lungs, a remedy for the chin-cough (whooping cough) in children, it purges the body of phlegm, is a remedy for shortness of breath, and as cure for helminthiasis. He references it as 'the notable herb of Venus' such that it provokes the terms, gives a safe and speedy delivery to women in labor, and stimulates the afterbirth. When used as an ointment, it eases swelling and warts, helps sciatica and dullness of sight, and eases the pain and hardness of the spleen. It can be useful for gout and pains in the loins and hips. Additionally, when ingested it has beneficial effects on the stomach and expels wind (Culpeper, 1955). The essential oils have also been recognized for the treatment of respiratory conditions like bronchitis, whooping cough, as well as tooth ache (Porte & Godoy, 2008). In México, Thyme is commonly known as Tomillo and is popularly used for bronchial affectations, cough, cold, angina, inflammation, stomachache, diarrhea, and parasitism (Navarro, Villarreal, Rojas, & Lozoya, 1996). Each of these prescriptions (especially ones for respiratory ailments) finds similarity in other cultures and other ancient texts that reference it.

Historical Texts

Cairo Genizah

A millennium ago, Old Cairo (Fustat) was one of the most important centers of social, economic and religious activity (Amar, 2008). The Perfumers' Square in Fustat as well as in Alexandria were centers of the drug and perfume business (Amar, 2008). The Ben Ezra synagogue was a place of worship for the Palestinian Jews of Fustat. In this synagogue existed a "Genizah" or depository, in which sacred books were stored. Until recently, this treasure trove of historical and cultural knowledge was kept from the hands of researchers and scholars. However, the manuscripts were revealed to the world and later purchased by many institutions. These institutions have installed research efforts aimed at studying and understanding the texts and the information retained within them. The Cairo Genizah has its own *materia medica* in which it details the medicinal use of Mediterranean plants in and around Egypt (Amar, 2008). Thyme is mentioned in recipes for stomach ailments, colic, excessive lachrymation, eyelids growth, and as a tonic (Amar, 2008).

The Canon of Medicine

Avicenna recommends the use of thyme as a treatment for fresh wounds, abscesses, spleen swellings, chronic fevers, and scorpion stings, and as a diuretic, a purgative and menstrual stimulant (Amar, 2008).

al-Ghafiqi

An important scholar and physician in Andalusia, al-Ghafiqi describes thyme as a diuretic, menstrual stimulant, and as a cure for wounds, ulcers, bruises, and stings of poisonous insects (Amar, 2008).

Cooking

As mentioned, 0v'tos is the Greek name for thyme. However, the name was commonly applied to the species *Thymbra capitata*; many centuries ago this name was carried out of Greece by colonists and into southern Italy. From there, the Romans adopted the name in the form *thymum* and applied it to reference *Thymus vulgaris*, which grew all along the west side of the peninsula (Andrews, 1958). The use of thyme in ancient Greece is referenced with less frequency, however it is mentioned for its use in flavoring salt and grating it to flavor drinks (Andrews, 1958). Apicius cataloged the use of thyme by the Romans; he mentions thyme as an ingredient in sauces, whether that is boiled foods or meats, as well as with fish and vegetables. Today in Italy thyme is called *timo* and its dried or fresh versions are used to flavor soups, gravies, stews, sauces sausages and dressings (Andrews, 1958). These sources that reference thyme as a culinary supplement provide the appearance of thyme as a fine condiment used, quite secretively, by chefs.

Today

Thyme alone or in combination with other herbs such as sundew is one of the most commonly recommended herbs in use in Europe today; it is used for dry, spasmodic coughs, as well as whooping cough. It is prepared as a topical salve that consists of thyme, myrrh, and goldenseal to treat oral herpes.

Compound	**Concentration (mg/g)**
Monoterpene hydrocarbons	
α-Terpinene	0.005
γ-Tepinene	0.007
p-Cymene	0.013
Oxygenated monoterpenes	
1,8-Cineole	0.245
Camphor	0.148
3,7-Dimethyl-1,6-octadien-3-ol (linalool)	0.479
α-Terpineol	0.291
Borneol	0.244
Sesquiterpene hydrocarbons	
δ-Selinene	0.010
Oxygenated sesquiterpenes	
β-Eudesmol	0.014
Aliphatic alcohols	
(*Z*)-3-hexenol	0.017
Aliphatic aldehydes	
(*E,Z*)-2,4-heptadienal	0.004
Aliphatic esters	
Methyl 2-methylbutyrate	0.001
Aliphatic ketones	
Methyl jasmone	0.012
Aliphatic acids	
Octanoic acid	0.004
Aromatic compounds	
2-Isopropyl-5-methylphenol (thymol)*	8.554
2-Isopropyl-2-methylphenol (carvacrol)*	0.681

Table 1. Sampling of the compound in *Thymus vulgaris* essential oil. Notice the bolded compounds and their concentrations (Lee, Umano, Shibamoto, & Lee, 2005).

It is also used to treat chronic candidiasis and halitosis (Taheri, Azimi, Rafieian, & Zanjani, 2011). As a result of its antibiotic, anti-fungal, and antimicrobial properties, the

essential oil of thyme is a very useful preservative in the food industry for stored food items and even for the preservation of stored fresh meat, specifically chicken (Kumar, et al., 2008), (Fratianni et al., 2010).

Chemistry and Pharmacology

When inspecting the volatile oil profile and the antifungal, antibiotic, antimicrobial, and antioxidant activities of thyme (which exist a result of these oils), one will encounter many factors that influence results. Specifically, the level of volatile oil content differs depending on when the plant is harvested (month, season), from what part of the region it is collected, and the country from which it is being surveyed (Torras, Grau, López, & de las Heras, 2007). Another factor to note is the method in which the volatile oil is extracted; depending on which method is chosen, the quality and yield of the essential oil can be affected. Steam distillation is a common method used in the laboratory and in addition to being very efficient it typically yields high quality oil. The use of organic solvents for extraction, on the other hand, has a tendency to result in residual solvent in the extracts as well as insufficient solvent selectivity that allows other compounds to be dissolved in addition to the active substances (Grigore et al., 2010). Learning about the volatile oil in thyme is very important to understanding its various medicinal activities, for it is within the oil that the compounds responsible for these effects exist.

Volatile Oils

T. vulgaris possess many volatile compounds ranging from aromatics to monoterpenes (**Table 1**). Within this list of volatile compounds, there exists another list of compounds responsible for the characteristic aroma of thyme. A study

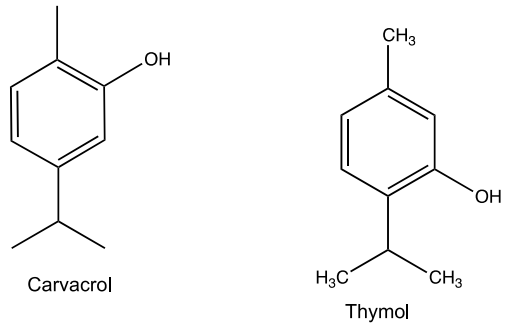

Figure 4. Structures of Carvacrol and Thymol (Carvalho et al., 2003).

used thyme that was purchased from a local market in Davis, California and identified 43 monoterpenes, 16 sesquiterpenes, 14 aromatic compounds, 7 alcohols, 3 aldehydes, 4 ketones and esters, and 3 acids (Lee, et al., 2005) as the aroma compounds. Within the entire list of volatile oils there are six compounds with the highest concentration: thymol (72%), carvacrol (5.7%), linalool (4.0%), α-terpineol (2.4%), 1,8-cineole (2.1%), and borneol (2.0%) (Lee, et al., 2005). Thymol and carvacrol are the most important compounds and collectively they comprise roughly three-quarters of measured volatile oils (**Figure 4**) (Lee, et al., 2005). Many studies have demonstrated that thymol and carvacrol are responsible for much of the medicinal activity of *T. vulgaris*.

Biological Activity

In vitro

In one study, various odiferous angiospermic essential oils were tested for antifungal activity against a toxic strain of *Aspergillus flavus*. *A. flavus* is a type of fungi or mold commonly found in food. The presence of said mold is particularly

troublesome when it in occurs in the post harvest stage of food commodities (Kumar, et al., 2008). In developing countries, spoiled food is not only a loss of profit but also a loss of sustenance. This type of mold produces a particular class of mycotoxins (any toxic substance produced by a fungus) called aflotoxins, which are extremely poisonous and can cause liver damage and cancer. Nearly 4.5 billion people in underdeveloped countries are exposed to these toxins as a result of post harvest fungal infections of their cereals and pulses (Kumar, et al., 2008). This study's aim was to investigate possible compounds in various essential oils that could act as natural preservatives, since many industrial preservatives have their own slew of toxicities. The thyme used was collected from Banaras Hindu University, and the essential oil was collected using Clevenger's hydrodistillation apparatus. The culture was performed on potato dextrose agar (PDA). Dissolved oil was added aseptically to different pre-sterilized Petri dishes that had 9.5 ml of the PDA in order to obtain a concentration of 1.0 and 1.5µl ml^{-1}. A fungal disk of the strain of *A. flavus* was inoculated aseptically to the center of the poured Petri plates. The plates were then incubated at 27±2 °C for seven days, after which the diameter of fungal colonies was measured and the percentage of mycelial inhibition was calculated based on these diameters (Kumar, et al., 2008). *T. vulgaris* essential oil showed strong fungitoxicity and was further analyzed for its minimum inhibitory concentration (MIC) at which it showed absolute fungitoxicity. They performed this analysis by placing varying concentrations of the thyme oil on the PDA plates and incubating them with the fungal strain for another seven days at 27±2 °C. They then investigated thyme oil's ability to arrest aflatoxin elaboration using the same conditions as above except they used SMKY medium (Sucrose, 200 g; $MgSO_4$ $7H_2O$, 0.5 g; KNO_3, 0.3 g; Yeast extract, 7.0g; Distilled water, 1000 ml; pH, 5.6 ± 0.2) in Erlenmeyer flasks that were inoculated with 1 ml spore suspensions. These flasks were incubated for 10 days. After a variety of other steps, the researchers then evaluated the aflatoxin content.

The results indicated that the essential oil of *T. vulgaris* showed complete inhibition of inoculums of the toxigenic strain of the test fungus at 1.0µl ml^{-1} and an MIC of 0.7µl ml^{-1} (Kumar, et al., 2008). *T. vulgaris* showed better antifungicidal activity than most synthetic derivatives, mainly because of its MIC and its broad-spectrum fungitoxicant activity (which was also elucidated in this trial). The authors indicated that thyme would be an exceptional replacement for industrial preservatives mainly because it is a natural compound and it is quite stronger.

In vivo

In an *in vivo* study, scientists investigated the antispasmodic effect of *T. vulgaris* on guinea-pig ileum. This study aimed to evaluate the effectiveness of *T. vulgaris* and its hydroalcoholic extract on the motility properties of the ileum and to determine the mechanisms by which these motility effects are elicited (Babaei et al., 2008). After being excised and prepped, the ileum was placed in a Tyrode solution, which is an isotonic solution that contains: (mM) NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, and Glucose 5.0. Glucose acts as the energy source and bicarbonate acts as the buffer. The tissue bath was maintained at 37°C and whole segments of the tissue (roughly 3 cm long) were set up to record isometric contractions while in 50 mL of the Tyrode solution, which was gassed with 5% CO_2 and 95% O_2. Each strip of tissue was bound on one end to a glass holder and on the other connected by a silk thread to a strain-gauge transducer that allowed the scientists to monitor the

mechanical activity of the tissue. The tissue strip was loaded with a resting tension of 0.5g and after equilibration was ready for field stimulation with rectangular 1 ms pulses of supramaximal voltage delivered at 0.1 Hz through platinum electrode 10mm in length and 10mm apart. This electrical field stimulation of the ilea induced twitch contractions. After a control period, thyme extract was added to the bath in a volume of 0.5 mL at increasing concentrations. The responses of the tissue as a result of the addition of the varying concentrations of the extract were recorded for a five-minute period. Another experiment was performed that looked at the contractions of the ileum in the presence of Acetylcholine, Atropine, Granisetron, Morphine, and Naloxone. The leaves of *T. vulgaris* plant used in this experiment were collected from Iran and the extract was collected via a methanol extraction (Babaei, et al., 2008).

The effect of the extract and of the controls was determined by comparing the frequency and amplitude of the test and control materials. There were six different concentrations of the extract in which the tissue was tested (0.02mg to 6 mg mL^{-1}), which induced concentration-dependent depression of contraction that was evoked by the field stimulation. In comparison to the control substance, there was a significant, dose-dependent difference in the action of *T. vulgaris* on peristalsis. *T. vulgaris extract* displayed a 50% maximum effective concentration value of 1.7 mg mL^{-1} as well as a maximum inhibition of 60.1±2.9% This smooth muscle relaxant effect was determined while in the presence of Acetylcholine, which induces contractions of the isolated tissue. Acetylcholine can be blocked by Atropine and the extract can consequentially inhibit these types of contractions as well (Babaei, et al., 2008). These results and the successful dose-dependent decrease in contractions suggest that thyme essential oil is a potent inhibitor of the contractions in the guinea-pig ileum. Therefore, these results also imply that *T. vulgaris* extract could be useful in the treatment of diarrhea predominant irritable bowel syndrome (Babaei, et al., 2008).

Mechanism of action

The constituents of thyme that confer biological activity and thereby usefulness in medicinal applications and everyday employment include its essential oils; specifically, the phenols of thymol and carvacrol, glycosides, flavonoids, *p*-cymene, borneol, linalool, alcohols, rosmarinic acid, saponins, tannins, and terpenoids (Basch, et al., 2004). Thyme's antimicrobial effects can be attributed to thymol, which expresses activity against strains of fungi and yeast including *Aspergiullus parasiticus, A. flavus and Candida albicans* via suppression of fungal growth and aflatoxin synthesis. Thyme's spasmolytic and antitussive activity is accredited to thymol and carvacrol, while flavonoids relax tracheal and ileal smooth muscles by inhibiting acetylcholine and histamine receptors and possibly through calcium channel antagonism. It has been shown *in vitro* that the extract of thyme and its volatile oil have relaxing effects by inhibiting phasic contractions. Thyme's antioxidant effects are due to a biphenyl compound and flavonoids, which inhibit superoxide anion production and protect red blood cells against oxidative damage. Finally, thyme's anti-inflammatory effects are due to thymol and carvacrol's inhibition of prostaglandin synthesis (Basch, et al., 2004).

Clinical Studies

Many studies have supported the efficacy of Listerine and one of its active components, thymol, in decreasing plaque formation and gingivitis. However, no evidence has been shown to implicate single therapies of thymol to be effective at

Figure 5. Bronchipret, a syrup comprised of *T. vulgaris* and Primula that treats acute bronchitis (Vitamin, 2012).

this same type of inhibition. A study was performed on a group of 110 school children in which they each received a combination product of 1% chlorohexidine and 1% thymol varnish. They were to receive this preparation for two years and the results would be compared to a control group. Analysis revealed that there were significant reductions in bacterial colonization levels and interdental plaque in the treated children (Basch, et al., 2004).

Another clinical trial was performed that compared a drug called Bronchipret to other pharmaceutical drugs that aim to treat acute bronchitis (**Figure 5**) (Ernst, et al., 1997). The use of antibiotics for acute bronchitis accounts for nearly 30% of all antibiotic prescriptions. This leads to the incurrence of large costs, which can be exacerbated when an antibiotic is pronounced ineffective or with absenteeism. Additionally, many traditional antibiotics have adverse side effects. One clinical trial used Bronchipret to investigate whether herbal expectorant remedies were a viable replacement for the traditional antibiotics. Bronchipret contains 60mg of dried Primula extract and 160mg if dried *T.* vulgaris extract. The clinical study involved 771 German physicians who were asked to place their groups of patients into "lots" of five. They were then asked to prescribe one group the Bronchipret tablets and to prescribe the other group any other secretolytic drug that qualified for their condition. The groups were matched according to age, gender, and severity of disease. The researchers investigated two distinct age groups; those under the age of twelve and patients twelve years or older. Patients were seen initially by a physician and thoroughly examined; after which, they were either prescribed Bronchipret or another secretolytic drug. The patients returned ten days later and were examined in the same manner. Each patient was examined for a list of pre-determined criteria prior to taking the drug and after ten days of treatment. Odds ratios were calculated that related to each of these variables for the children and adult groups. The odd ratios depict the likelihood of therapeutic success such that if the odds ratio is low then the probability of a patient profiting from a control medication compared to Bronchipret is low. For example, in the adult group treated with a medication called Ambroxol, the odds ratio for the criteria coughing during the day was 0.51, which indicates a 49% higher chance for someone on Bronchipret to symptomatically improve compared to Ambroxol. Overall, in the adult and child group, for the three control drugs tested, the odds ratio was continually low, suggesting Bronchipret had an overall more effective therapeutic action in treating acute bronchitis. Additionally, the adverse drug reactions (ADRs) to Bronchipret were generally low, especially compared to the other drugs (Ernst, et al., 1997).

Contraindications

Toxicology

The suggested dose, taken orally, should not exceed 10g of the dried leaf with 0.03% phenol per day. Ensuring not to exceed this dose will most likely prevent toxicity, however, each individual will metabolize and respond to the compounds in thyme differently (see subsection Contraindications). Thyme oil, the essential oil and most active compound in thyme, is highly toxic. Symptoms of toxicity implicating exposure or over-exposure include nausea, tachypnea and hypotension (Basch, et al., 2004). The lethal dose (LD) of the essential oil is roughly 2.84g/kg of body weight (this is measured for rats), and in mice, oral doses of concentrated thyme extract equivalent to 4.3-26 g/kg of thyme decreased locomotor activity and respiratory activity (Basch, et al., 2004).

Typically, if one has a known allergy or sensitivity to members of the Lamiaceae (Mint) family or any aspect of thyme, the plant should be avoided. Many contact allergies have been reported, in cases recorded as long ago as the 1940s. Cases usually detail contact dermatitis, allergic alveolitis, and pruritic contact dermatitis provoked by the thyme plant and thymol. If one is allergic to other plants such as birch pollen, celery, or species in the mint family like oregano there is a chance for cross-reactions (Basch, et al., 2004).

Adverse Effects

Traditional and historical use of the thyme flower and leaves appears to generally be safe, especially when used in culinary pursuits. However, caution is advised when attempting to use the product for its medicinal properties. As mentioned in the toxicology subsection, thyme oil or thymol when concentrated is highly toxic and should be diluted if used for topical

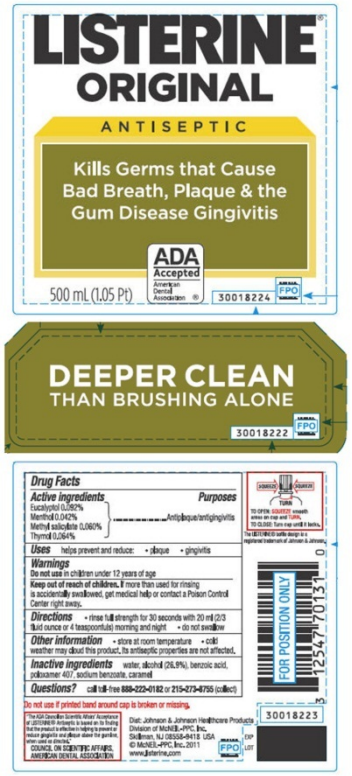

Figure 6. Listerine with thymol (Johnson & Johnson Healthcare Products, 2012).

applications (Basch, et al., 2004). In regards to the neurologic and CNS, one may experience headache or dizziness with the ingestion of thyme, and in some cases the essential oil has caused seizure or coma. There have been reports of conjunctivitis in exposure to thyme dust as well as contact dermatologic reactions. Occupational asthma, as well as allergic alveolitis and rhinitis due to exposure to the dust has also been reported. Most side effects are related to an individual having some sort of established sensitivity to the plant and its constituents, however, a relative of *Thymus*

Figure 7. Typical herbal medicine preparations (Source: (Ltd)).

vulgaris, *Thymus serpyllum* has been shown to have more severe reactions such as those that interrupt TSH and prolactin in rats (Basch, et al., 2004).

Interactions

Some drug interactions include anti-thyrotrophic effects, estradiol and progesterone receptor-binding, and percutaneous absorption of topical thymol. Herbal supplements with estrogen or progesterone may interact with thyme's interaction with these receptors (Basch, et al., 2004).

Current use in Allopathic and CAM Therapies

A common drug or household item is mouthwash. Listerine (**Figure 6**) is the brand name of such oral cleansers, however, many use them without any idea of the true nature of their efficacy. Listerine is an antiseptic and contains a combination of essential oils like eucalyptol, menthol, thymol (found in *T. vulgaris)* and methyl salicylate. Studies have shown Listerine's effectiveness as a broad-spectrum antibiotic against species like *Streptococcus mutans,* herpes simplex virus, and influenza A. It is also effective in the treatment of supragingival plaque and gingivitis (Basch, et al., 2004). Many parents also continually seek after Bronchipret as a natural remedy for their children with bronchitis. A typical CAM remedy for cough, the dried leaves can be used to make a tea; with one teaspoon of dried thyme per cup of water and consumed three times daily. The addition of honey, interestingly enough, increases the effectiveness of the tea (Thymes, 1998). These same proportions (1 teaspoon of dried thyme per cup of boiling water) can be used as a gargle to treat sore throat (Dworkin, 1998). Thyme can also be used to unplug clogged sinuses through steam inhalation. The preparation of such a steam bath includes pouring three cups of boiling water over two tablespoons of crushed thyme leaves in a medium bowl and placing one's face 10-12 inches above the fragrant water with a towel over the head to concentrate the vapors (Shinn, 2011).

In the herbal supplement market, products based on thyme and its extracts are typically those containing the essential oil, thymol. Most products display that the constituents come from Thyme and usually tote the fact that they contain the pure extract. (**Figure 7**) However, as mentioned, earlier, the use of the concentrated extract is quite dangerous and if it is to be used for topical application, it is highly recommended that the oil be diluted first.

Discussion

Upon first inspection, Thyme is a very mundane and common plant. However, upon further investigation one discovers the multifaceted and immense amount of applications that the plant can boast of. Centuries ago, the use of thyme developed a

homology between cultures, which lead to its popularity as a medicinal agent and has maintained its use in traditional medicine, allopathic and homeopathic medicine today. Thyme was commonly prescribed for respiratory ailments like bronchitis and whooping cough or for skin infections and inflammations. The continued use of thyme throughout history for these ailments and many others implicates a level of chemical activity that is biologically important. This turns out to be the case because thyme's chemical composition confirms its antimicrobial and antibiotic properties (as well as others). *T. vulgaris* undoubtedly offers new avenues of drug discovery, especially in this age of over use of antibiotics; exploring its inherent compounds (like thymol and carvacrol) as well as possible synthetic derivatives may allow for the expansion of the applications for which it is currently used. However, any uses that are investigated and potentially proposed for thyme and its derivatives should be carefully studied so as to ensure that its biological activity and application is taken advantage of in a way to prevent its decline into the same category of over-use as many antibiotics have today. It would be a shame to waste such a magnificent plant.

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Uncaria tomentosa (Willd. ex Roem. & Schult.) DC., Rubiaceae

Taryn DeGrazia

Introduction

The plant *Uncaria tomentosa* is part of the Rubiaceae family and the Cinchonoideae subfamily. It is commonly known as Cat's Claw or Una de Gato because of the penduncles (curved thorns) that sprout from the side shoots and resemble the nails of a cat (**Figure 1**). There are currently 34 species in the *Uncaria* genus, only two of which grow in tropical America, the other being Uncaria guinensis (Heitzman et al. 2004; Keplinger et al. 1998). *U. tomentosa* has been used for over 2,000 years by Peruvian tribes for medicinal and religious purposes but has recently grown in popularity in Europe and the Americas (Pilarski et al. 2006). Throughout the past 20 years, *U. tomentosa* has become a plant of interest because it is rich in secondary plant metabolites such as alkaloids, terpenes, quinovic acid glycosides, flavonoids and courmarins. The compounds are commonly used as treatments for wounds, ulcers, fevers, headaches, gastrointestinal illnesses, and bacterial/fungal infections. Typically *U. tomentosa* is applied topically or ingested as a decoction or tea (Heitzman et al. 2004).

Figure 1. A photograph of *Uncaria tomentosa* highlighting the claw-like thorns (Image source: Taylor 2000).

Botanical Description

Uncaria tomentosa, a "woody climber," is a vine indigenous to Central and South American rainforests that can grow up to 30 m tall (Heitzman et al. 2004; Taylor 2004). *U. tomentosa* may be confused with *Uncaria guinensis*, another member of the genus *Uncaria* that grows in the tropical Americas. The two can be distinguished by their flowers*: U. tomentosa* has small, yellow-white flowers while *U. guinensis'* flowers are red-orange (**Figure 2**). Furthermore, the penduncles on *U. guinensis* are more curved than the penduncles of *U. tomentosa* which tend to be straight or mildly sickle-shaped (Taylor 2004; Keplinger et al. 1998). Thorns on *U. tomentosa* are painfully sharp and the leaves have no covering but rather display a waxy appearance (**Figure 3**). Reaching a diameter of over 20 cm, the main vines dangle from the rainforest canopy and can form impenetrable thickets when growing in a secondary forest or on the edges of the rainforest (Keplinger et al. 1998; **Figure 4**).

Traditional Uses

Uncaria tomentosa has traditionally been used in Peru and is still currently used by the 60,000 Ashaninka Indians who live

Figure 2. The *Uncaria tomentosa* flower. (Image source: http://thehealthyhavenblog.com/2011/06/08/cats-claw-for-osteoarthritis-and-rheumatoid-arthritis-support/)

Figure 3. *Uncaria tomentosa* leaves. (Image source: http://www.macapunch.com/ingles/catsclaw.html)

between the Pichis-Palcazu, Ucayali and Perene-Tambo rivers (Keplinger et al. 1998). The Aguaruna, Cashibo, Conibo, and Shipibo tribes in Peru have historically used Cat's Claw as well, however the Ashaninka have reportedly been using it the longest (Taylor 2004). Humans in Ashininka culture are believed to be composed of three elements: ivátsa, a physical being, isancáne, a spiritual being and ineatátsiri, a being who mediates between the physical and spiritual. When all three beings are in harmony, a person is considered to be healthy, however when one element becomes unbalanced, illness arises. The Ashaninka priests use *U. tomentosa* to facilitate the communication between the physical and spiritual worlds, restoring balance and health. It is believed the plant contains magical healing powers, therefore Cat's Claw is used exclusively by priests, highlighting its significance in Ashaninka culture (Heitzman et al. 2004; Keplinger et al. 1998).

The bark and roots of the vine are typically the parts of the plant used to make Cat's Claw decoctions and teas because they contain the oxindole alkaloids which are essential for healing. When a Peruvian man was observed in his traditional preparation of *U. tomentosa*, the process involved boiling approximately 20 g of sliced root bark in a liter of water (**Figure 5**). After forty five minutes of boiling, the remaining liquid was poured off and more water was added to the liquid

Figure 4. Central Vine. (Image source: http://www.lianaecologyproject.com/photos)

Figure 5. Sliced root bark that can be used to make teas and decoctions. (Image source: http://www.sanat.tv/en/plants/uncaria-tomentosa.html)

in order to replace the water losses due to evaporation. The decoction was then ready to be used as a medicinal treatment. *U. tomentosa* can also be applied topically if an external illness is being treated (Keplinger at al.1998).

Cat's Claw has traditionally been used throughout the Americas to treat asthma, cancer, cirrhosis, fever, gastritis, diabetes, rheumatism, dysentery, inflammation of the urinary tract and many other illnesses (Keplinger et al 1998**; Table 1**). Women commonly use *U. tomentosa* after child birth, to correct menstrual irregularity or as a birth control measure. For birth control use, a decoction is made and one cup is consumed daily for three consecutive months. This method is believed to promote sterility for three to four years. In

WORLDWIDE ETHNOMEDICAL USES	
Colombia	for dysentery, gonorrhea
French Guiana	for dysentery
Peru	for abscesses, AIDS, arthritis, asthma, blood cleansing, bone pains, cancer, cirrhosis, diabetes, diarrhea, disease prevention, dysentery, fevers, gastric ulcers, gastritis, gonorrhea, hemorrhages, herpes, immune disorders, inflammations, intestinal affections, menstrual irregularity, kidney cleansing, prostatitis, rheumatism, shingles, skin disorders, stomach disorders, ulcers problems, urinary tract disorders, tumors, wounds
Suriname	for dysentery, intestinal disorders, wounds

Table 1. The most common uses of *Uncaria tomentosa* in Colombia, French Guiana, Peru and Suriname (modeled after Taylor, 2004).

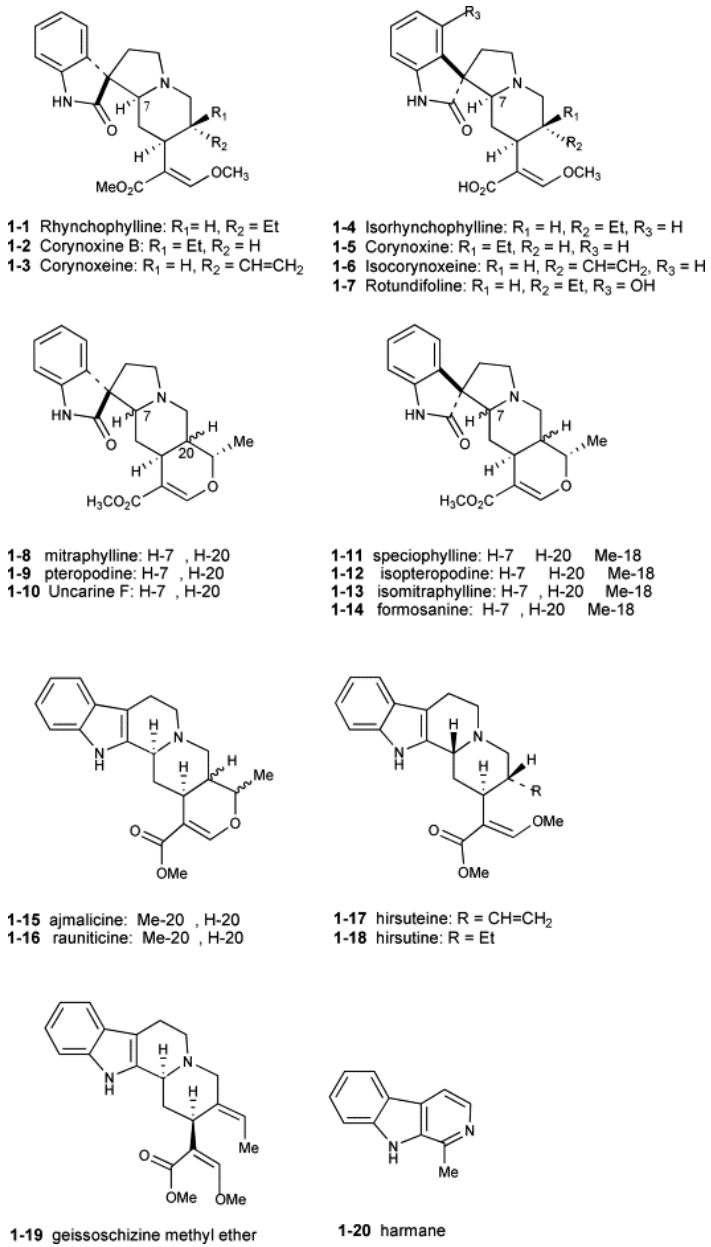

Figure 6. Structures of *Uncaria tomentosa* constituents
(Image source: Heitzman et al. 2004).

general, *U. tomentosa* is regarded as a means of "normalizing the body" (Taylor 2004).

Chemistry and Pharmacology

Uncaria tomentosa is particularly sought after because of the alkaloids and triterpenes found within the plant. An initial report analyzing the leaves and stems identified rhynchophylline and isorhynchophylline as the major alkaloids in addition to the minor alkaloids, mitraphylline, isomitraphylline, dihydrocorynantheine, hirsutine and hirsuteine (**Figure 6**). Two other minor alkaloids, rotundifoline and isorotundifoline, were later identified in the leaves and stem as well. In the bark, the stereoisomeric alkaloids pteropodine, isopteropodine, speciophylline, uncarine F and isomitraphylline were found. The roots revealed six pentacyclic oxindole alkaloids and, in a few cases, two tetracyclic oxindole alkaloids (**Table 2**). The oxindole alkaloids from *Uncaria tomentosa's* roots reportedly have immune-stimulant and antileukemic properties and are widely sought after when preparing remedies. The root's bark has been shown to contain eight types of quinovic acid glycosides, four types of polyhydroxylated triterpenes, the precursor alkaloid 5α-carboxystrictosidine, oleanolic acid and ursolic acid. The quinovic acid is thought to be responsible for the anti-inflammatory and antiviral effects of the plant. (Keplinger et al. 1998; Taylor 2004). Compounds found elsewhere in the plant include flavonoids, glucosinolates, sterols, polyunsaturated fatty acids and carbolines. The anti-inflammatory response to *U. tomentosa* is also thought to be due to the antioxidant chemicals and plant sterols that it contains (Rinner et al. 2009; Taylor 2004). A more complete list of *U. tomentosa* constituents can found in **Table 3**.

Oxindole Alkaloids	Pentacyclic Alkaloids	Tetracyclic Alkaloids
	Pteropodine	Rhynchophylline
	Isopteropodine	Isorhynchophylline
	Speciophylline	Corynoxeine
	Uncarine F	Isocorynoxeine
	Mitraphylline	
Indole Alkaloids	Isomitraphylline Akuammigine	Hirsutine
	Tetrahydroalstonine	Dihydrocorynantheine
	Isoajmalicine	Hirsuteine
		Corynantheine

Table 2. Chart showing the alkaloids present in *Uncaria tomentosa* and organized by whether the compound is a pentacyclic alkaloid or a tetracyclic alkaloid and an oxindole alkaloid or an indole alkaloid (Modeled after Keplinger et al., 1998).

A revolutionary discovery made regarding *Uncaria tomentosa* was that the plant has two different chemotypes: One chemotype containing pentacyclic indole and oxindole alkaloids and the other chemotype containing tetracyclic indole and oxindole alkaloids. While studies have found *U. tomentosa* to possess many health benefits, some antagonistic health effects have been associated with the chemotype containing the tetracyclic alkaloids. The difference is thought to lie in the different types of alkaloids with pentacyclic alkaloids being the "good" alkaloids and tetracyclic alkaloids being the "bad" alkaloids (Keplinger et al. 1998). It is widely

Other identified *Uncaria tomentosa* Constituents	
ajmalicine	mitraphylline
akuammigine	oleanolic acid
campesterol	palmitoleic acid
catechin	procyanidins
carboxyl alkyl esters	pteropodine
chlorogenic acid	quinovic acid glycosides
cinchonain	rhynchophylline
corynantheine	rutin
corynoxeine	sitosterols
daucosterol	speciophylline
epicatechin	stigmasterol
harman	strictosidines
hirsuteine	uncarine A thru F
hirsutine	vaccenic acid
iso-pteropodine	
loganic acid	
lyaloside	

Table 3. Additional compounds found in *Uncaria tomentosa* (Taylor, 2004).

accepted that the oxindole alkaloids from *U. tomentosa* are mainly responsible for the desired medicinal effects associated with the plant, therefore it is important the proper alkaloids are being used when preparing remedies.

Biological Activity

Anticancer

One study involved incubating leukemic cell lines HL60 and U-937 with different concentrations of alkaloids extracted from *U. tomentosa* for 7 days. To measure the antiproliferative effect of the extracts, colorimetric and clonogenic assays were used. Results showed growth of HL60 and U-937 leukemic cells to be inhibited by the pentacyclic oxindole alkaloids (IC_{50} values were in the range 10^{-5} to 10^{-4} mol/l). Uncarine F demonstrated the most significant effect on inhibiting cell growth. Furthermore, uncarine F appeared to selectivity inhibit cell growth of cancerous cells and normal cells (Keplinger et al. 1998).

In another study, *Uncaria tomentosa* was observed to have a positive effect on myeloid progenitor cells (cells that can develop into other types of blood cells) when tested *in vivo* using mice. The mice were given 5 and 15 mg of *U. tomentosa* orally for four days along with Filgrastim. One of the worst side effects of chemotherapy is the loss of healthy white blood cells, therefore this study is significant because it implies that *U. tomentosa* may have the potential to be used alongside chemotherapy treatments as a means of enhancing the proliferation of healthy cells (Farias et al. 2011).

Yet another study was conducted supporting the use of *U. tomentosa* as a complementary treatment to chemotherapy. Four types of extracts where made by dissolving Cat's Claw in various solvents, then cell growth was observed in the presence of each extract. The constituents reduced adverse effects of chemotherapy by producing reactive oxygen species, releasing cytochrome C and activating caspases in human leukemia cells. However, the finding of most significance to this study was the induction of apoptosis in HL60 cells, making *U. tomentosa* a key ingredient to consider when developing chemopreventative medicines (Cheng et al. 2007). In addition, the impact of aqueous *Uncaria tomentosa* extracts on the growth activity of human leukemia cell lines K562 and HL60 and a human EBV-transformed B lymphoma cell line was analyzed. The *U. tomentosa* extracts were found to significantly inhibit growth of HL60 cells, however growth of K562 cells was only moderately inhibited. The study found that the *U. tomentosa* extracts induced apoptosis, the mechanism of action by which the plant is believed to be able to treat cancer (Heitzman et al. 2004). Finally, a study performed on human medullary thyroid carcinoma cells exhibited a significant pro-apoptotic effect as well when treated with the alkoloids isopterpodine and pteropodine (Rinner et al. 2009).

Anti-inflammatory

A recent experiment on rats deduced that quinovic acid-3-β-O-(β-d-quinovopyranosyl)-(27→1)-β-d-glucopyranosyl ester decreased the inflammatory response by 33% at a dosage of 20 mg/kg p.o (Keplinger et al. 1998). However, no individual compound can be accredited for the anti-inflammatory activity. There appears to be a synergistic effect due to the combination of different compounds found in Uncaria tomentosa. When mitraphylline was isolated from U. tomentosa bark and given orally to mice, it displayed activity against cytokines, which are responsible for the inflammation process, indicating mitraphylline is one potential anti-

inflammatory compound. Overall, the study concluded that the compound mitraphylline did cause a decrease in inflammation (Rojas-Duran et al. 2012).

Antiviral

When applied to HeLa cells, the six quinovic acid glycosides extracted from *Uncaria tomentosa* bark showed reduced activity of the rhinovirus type 1B infection (Heitzman et al. 2004). The glycosides also showed antiviral activity against two types of RNA viral infections (Keplinger et al. 1998). These studies provide strong support for the use of U. tomentosa against viral infections.

Immunostimulant

Uncaria tomentosa has shown high amounts of immunostimulant activity during *in vitro* and *in vivo* studies of phagocytosis. For example, in the late 1900s research was published claiming that *U. tomentosa* increased immune function by 50% (Taylor 2004). The phagocytosis of human granulocytes and macrophages may increase due to the pentacyclic oxindole alkaloids found in *U. tomentosa*. A granulocyte-smear test, chemoluminescence model and a carbon-clearance test were performed with U. tomentosa extracts and pure alkaloids isolated from the plant. Pteropodine, isomitraphylline and isorhynchophylline enhanced phagocytosis but the isopteropodine was found to have the strongest effect on immune stimulation. Mitraphylline and rhynchophylline did not produce any stimulating effects on the immune system. A catechin, or antioxidant, had to be added to the inactive alkaloids in order to see any effect in the carbon-clearance test. These constituents may also inhibit the proliferation of myeloid cell lines in addition to stimulating the immune system (Heitzman et al. 2004; Keplinger et al. 1998).

The effects of aqueous extracts on the immune system were also studied in vivo by conducting a mouse feeding study. It was determined that immune cells such as B, T and NK cells, granulocytes and memory lymphocytes increased in number. This effect extended the cell lifetime but did not seem to increase the rate of cell growth. The antioxidants in U. tomentosa, such as the proanthocyanidins and cinchonains, have demonstrated protection against oxidative stress and NF-jB activation which may counteract apoptosis and increase DNA repair (Heitzman et el. 2004). In another in vivo study on mice, an aqueous-ethanol extract of *U. tomentosa* was found to reduce glycemic levels and prevent the progression of immune-mediated diabetes. The compounds extracted from the Cat's Claw appeared to interfere with distinct pathways that fuel diabetes (Dominguez et al. 2011).

Antimutagenic

In vitro studies on *S. typhimurium* using the Salmonella microsome test (Ames test) led to the conclusion that *Uncaria tomentosa* extracts and fractions are effective in protecting against photomutagenesis (Heitzman et al. 2004). U. tomentosa does not appear to cause mutations but, based on the findings, can in fact protect against mutagenic agents.

Contraception

Testing done on 29 rats with experimental endometriosis demonstrated the contraceptive effect of Uncaria tomentosa. The test group of rats received 32 mg/ml of *U. tomentosa* extract daily for fourteen days and then the ovaries were removed and analyzed. By analyzing the cells it was

determined that the extracts displayed a significant contraceptive effect (Nogueira et al. 2011). Further studies performed on rats treated with U. tomentosa showed a decrease in uterine cell growth. These findings supported the use of *U. tomentosa* as an alternative treatment for endometriosis (Nogueira et al. 2011).

Rejuvenation

Quinic acid, one of *Uncaria tomentosa's* active compounds, could have a rejuvenating effect on neurons and lead to future Alzheimer's treatments. When tested on *Caenorhabditis elegans* under deleterious conditions (heat stress and oxidative stress) the experiment yielded promising results; quinic acid enhanced DNA repair and was able to generate protective effects in the neurons (Zhang et al. 2012).

Clinical Studies

Because of its anti-inflammatory properties, *Uncaria tomentosa* is believed to be a good remedy for rheumatoid arthritis. In a clinical trial, the occurrence of painful joints was reduced by 53.2% in the group treated with U. tomentosa. This was compared to the control group which did not receive the botanical treatment and had a 24.1% reduction in painful joints (Heitzman et al. 2004). *U. tomentosa* could prove to be a viable source of pain relief for those suffering of rheumatoid arthritis. However, these controlled studies were small and the results are inconclusive due to an insufficient amount of clinical data (Rinner et al. 2009).

Another clinical trial examined the effects of *U. tomentosa* treatments in conjunction with chemotherapy which was being administered to breast cancer patients. Randomized patients with Invasive Ductal Carcinoma-Stage II who were receiving the FAC (Fluorouracil, Doxorubicin, Cyclophosphamide) treatment were administered U. tormentosa along with their regular dosage. Results showed that *U. tormentosa* reduced neutropenia, or low white blood cell count, and restored cellular DNA damage caused by the chemotherapy. Thus, it was concluded that *U. tomentosa* was successful in reducing the adverse effects of chemotherapy and was an effective adjuvant breast cancer treatment (Santos Araújo Mdo et al. 2012).

One study compared two 35 year old men, one who was a habitual smoker and the other who had never smoked. A decoction was made of Uncaria tomentosa in accordance with traditional practices and both participants consumed the decoction for 15 consecutive days. Results found that mutagenicity of the smoker's urine significantly decreased. This demonstrates the anitmutagenic properties of Cat's Claw and the benefits that can be attained by consuming it (Rizzi 1993).

Contraindications

Toxicity of *Uncaria tomentosa* was tested by examining the effects of an aqueous extract on Chinese hamster cells. The Neutral red assay, total protein content, and tetrazolium assay supported the claim that the herb is not toxic and was indeed found to be non-toxic in vitro at the concentrations tested. In another study of cytotoxicity, uncarines C, D and E were specifically isolated from the inner bark. After being assayed against SK- MEL, KB, BT-549, SK-OV-3 and VERO cell lines, it was determined that uncarine D was the most cytotoxic. IC_{50} ranged from 30 to 40 lg/ml in all cell lines (Heitzman et al. 2004).

Acute toxicity was measured in a study with mice where lethargy and piloerection ("goose bumps") were observed not long after the administration of high doses of Uncaria tomentosa. Other symptoms included hemorrhaging of the stomach and intestines, pallor of the liver and spleen and death. The mice that survived, however, completed their recovery within five days of being exposed to the *U. tomentosa* extract. In another study where 1000 mg/kg of aqueous extract was administered daily to mice for 28 days, minor but statistically significant results were collected. The mice showed an increase in the percentage of lymphocytes expressed and a decrease in the percentage of neutrophil granulocytes. In both male and female mice an increase in the relative weight of the kidneys occured, however there were no deaths during the experiment (Keplinger et al. 1998).

It is suspected that *U. tomentosa* may potentially interact with protease inhibitors or non-nucleoside reverse transcriptase inhibitors present in other drugs. If taking Cat's Claw simultaneously with other treatments, users should be aware of the likelihood this reaction may occur. The possibility that *U. tomentosa* could induce pharmacokinetic interactions with other drugs first came to light during *in vitro* study reports (Muller et al. 2011). *U. tomentosa* may however protect against the gastrointestinal damage that accompanies NSAIDs (Taylor 2004).

Most manufacturers of *U. tomentosa* are unaware of the two types of Uncaria plants that come from Peru and have never bothered to consult a Peruvian authority. Thus, companies are unknowingly packaging supplements that contain the wrong type of Uncaria. This adulteration could pose negative health effects instead of the desired positive ones. One study analyzed approximately fifty Cat's Claw products sold in the United States, Peru and Central America and found varying amounts of pentacyclic and tetracyclic alkaloids in the products, indicating the presence of both species. In one such product, up to 80% of the total alkaloids were found to be tetracyclic alkaloids, the "bad" alkaloids (Keplinger et al. 1998).

Thus far, scientific findings have been not been extensive enough to conclusively determine whether or not *U. tomentosa* can be used as an effective treatment for any health condition. Because *U. tomentosa* has been traditionally used a method of birth control, women who are pregnant or trying to become pregnant should not take *U. tomentosa* supplements. Also, due to findings that some plant properties may enhance the immune system, it is still unknown what effect *U. tomentosa* may have on people with conditions affecting the immune system such as HIV/AIDS. Slight toxicity was observed in rats, however this was only at the highest concentrations of *U. tomentosa* (Heitzman et al. 2004).

Current Uses in Allpathic and CAM Therapies

Commercial interest was taken in *Uncaria tomentosa* at least a quarter of a century ago when rumors of its miraculous healing powers began circulating. Klaus Keplinger was the first to truly investigate its medicinal potential and his research eventually led to the production of *U. tomentosa* in Germany, Austria and the United States (Taylor 2004). Since the production of Cat's Claw, the commercial use of U. tomentosa has taken off and there are over 50 dietary supplement manufacturers in the United States who produce products containing the plant or its extracts (Keplinger et al. 1998). Capsule and tablets containing *U. tomentosa* extracts can be found in most health food stores and are typically used as a general tonic, for inflammation reduction, or as a treatment of bowel problems (**Figure 7**). For those suffering

Figure 7. An example of how *Uncaria tomentosa* is marketed (Image source: iherb.com).

from the side effects of chemotherapy, Cat's Claw has been a popular herbal supplement due to promising but inconclusive studies (Taylor 2004). As with any natural supplement, the best source is always the unmanufactured herb itself.

Discussion

Uncaria tomentosa has traditionally been a way for Peruvian tribal priests, such as the Ashaninka priests, to facilitate the relationship between the spititual and physical worlds and restore health. *U. tomentosa* may be a viable treatment for osteoarthritis and rheumatoid arthritis, though it has only been tested in small-scale human trials. Cat's Claw also has the potential to stimulate the immune system based on laboratory evidence. Unfortunately, these results have not been translated to humans and the results are inconclusive. Clinical trials on *U. tomentosa* as a chemotherapy supplement appear promising and commercially available Cat's Claw extracts are popularly used as adjunct cancer treatments even though no concrete conclusions have been formed. Alas, further studies on larger groups of patients are required to validate these claims as well. Minor studies have also been conducted to evalute *U. tomentosa's* effects on inflammation, mutagenicity, contraception and viral infections, highlighting the plant's broad spectrum of applications.

Cat's Claw is an herbal remedy that has grown in popularity among the public and the scientific world, however, much more research must be conducted on the plant for it to be regarded as a viable treatment for any illnesses. As of yet, the abilities of Uncaria tomentosa have barely been uncovered and the room for growth is endless. Promising areas for future research include Alzheimers and diabetes, two fields with very few, if any, available treatments. Natural remedies, such as U. tomentosa, tend to be overlooked but their chemistry is unlike anything that can be generated in the lab. It is evident from the culmination of many findings that the Ashaninkas belief Cat's Claw is a powerful plant with many medical applications is actually rooted in the plant's phytochemistry.

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Urtica dioica L., Urticaceae

Sarah Henkel

Introduction

Urtica dioica L., of the Urticaceae family, is commonly known as a stinging nettle (Lewis and Elvin-Lewis, 2003). Other common names include burn weed and burn nettle (Olsen, 1921). This plant is used as a treatment in a wide variety of ailments, both in traditional and modern medicine, such as bladder and kidney disorders, diabetes, various allergies, anemia, internal bleeding, osteoarthritis, and musculoskeletal aches. In some places, the plant is even eaten as food. Recent medical research has narrowed the use of the plant, making it more medicinally effective. The basic components of *U. dioica* are vitamin C, carotene, polysaccharides, beta-sitosterol, quercetin, rutin, and kaempferol (Rodriguez-Fragoso et al., 2008).

Description

Urtica dioica plants grow in a wide variety of areas and are spread throughout Europe, Asia, Denmark, America, and North Africa, in areas where moist soil is available. This green, leafy plant grows to be in between three and seven feet tall, with leaves reaching between one and six inches in length. These leaves are arranged on opposite sides of the wiry stalk and continue up the whole length of the plant.

Although the plant bears green leaves and numerous brown or green flowers, its extensive root system and rhizomes are a bright yellow color. The leaves and stems have visible hairs, which contain the chemicals responsible for the plant's characteristic sting. These hairs provide the plant protection and can grow to be more than one meter long. They are also capable of branching. The stings of this plant cause multiple, painful blisters. The stinging nettle is often found in areas of dense growth and is capable of cohabitating with other plants, due to its strong root system (Olsen, 1921). The plants are pollinated by a variety of different insects. In **Figure 1**, the green flowers and leaves of the plant can be seen**. Figure 2** shows the stinging hairs on the plant, while **Figure 3** shows the results of a sting on human skin.

Figure 1. The leaves, flowers, and stem of *Urtica dioica*. (Source:http:// www.fitoterapia.net)

Figure 2. The stinging hairs on the nettle stalk. (Source: http://www.acupuncturebrooklyn.com/altern ative-health/nettles)

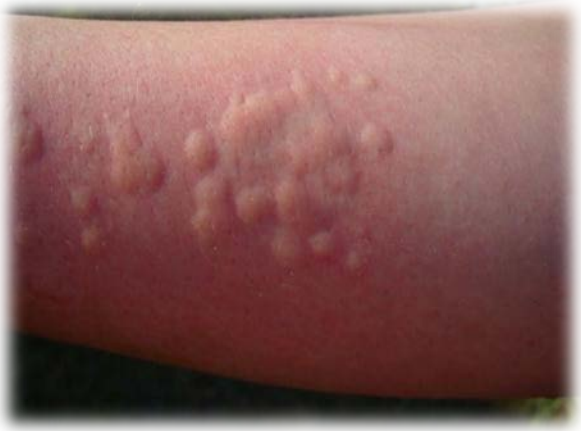

Figure 3. A rash resulting from contact with stinging nettles' hairs. (Source: http://creationwiki.org/File: Nettle_sting.jpg)

Traditional Uses

Medical Uses

Traditional medical uses of the stinging nettle are thought to have originated in Europe, especially in Germany. *Urtica dioica* is one of the plants mentioned in the 10th century book "Nine Herbs Charm." Many forms of traditional medicine used *U. dioica* in a variety of ways to treat GI ailments, kidney disorders, musculo- skeletal aches, osteoarthritis, and rheumatoid arthritis. However, recent studies have provided scientific evidence to support the use of stinging nettle in only a few of these ailments which will be discussed later in this paper (Rodriguez-Fragoso et al., 2008).

The use of *U. dioica* has been recorded in Egypt over 2,000 years ago for the treatment of arthritis and lumbago pains. It was also one component used in a mixture of Passover herbs. The plant can still be found there today (Kavalali, 2003).

U. dioica was also used by the ancient Greeks and Romans back in BCE times. Theophrastus, a student of Aristotle, describes the medical warming uses of the stinging nettle plant in his book *De historia et causis plantarum*. Pliny the Elder also recorded the medicinal uses of the stinging nettle in his *The Natural History of Pliny*. According to him, the plant should be eaten in order to prevent disease during the winter (Kavalali, 2003).

Food Uses

Records show that many cultures utilizing *U. dioica* for medical purposes also utilized the plant as a source of food, including the Native Americans. The leaves of the plant were first boiled in water to remove the stinging chemicals and then eaten as cooked greens. In some cultures, the water was saved and used for medical purposes, while in others it was

simply discarded (Rodriguez-Fragoso et al., 2008). Nettles have also been used to make nettle soup, nettle tea, and nettle ale (Kavalali, 2003).

Chemistry & Pharmacology

There are approximately fifty active chemical compounds in the *Urtica dioica* plant including sterines, coumarins, simple phenols, triterpenic acids, lignans, hydroxyl fatty acids, and ceramides (Kavalali, 2003). However, most of the medicinal uses of *U. dioica* come from chemicals located in the stinging hairs of the plant. Here, there is thought to be three significant chemicals responsible for the plant's smooth muscle stimulation: acetylcholine, histamine, and 5-hydroxytryptamine. The presence of 5-hydroxytryptamine was just recently accepted and is based on the result of six properties: the constriction of blood vessels in a rabbit's ear, the lowering of a rabbit's blood pressure, complete solubility in ether, simulation of a guinea-pig's and rabbit's small intestine and instability in the boiling of an alkaline solution (Collier and Chesher, 1956). The chemical structures of these three smooth muscle stimulators can be seen in **Figure 4**.

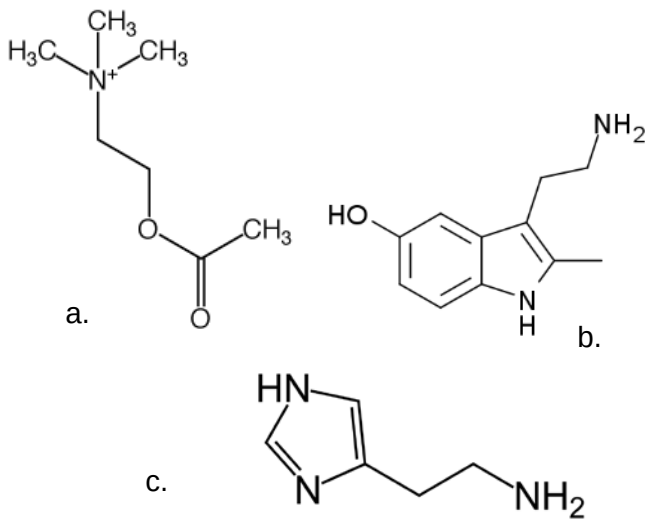

Figure 4. The chemical structures of three compounds present in the stinging nettle's hairs. (a) Acetylcholine, (b) 5-hydroxytryptamine, (c) Histamine.

Biological Activity

Urtica dioica has many different biological activities. In an experiment by Gulcin *et al* (2003), scientists used water extract of nettle (WEN) to test the nettle's antioxidant, antimicrobial, and antiulcer biological activity.

Gulcin *et al* (2004) first tested the plant's antioxidant activity. The reason scientists are so interested in its antioxidant activity is because many common antioxidants have recently been shown to cause liver damage and cancer in some lab animals. Researchers are looking for more effective and natural antioxidants. In this experiment, *U. dioica's* antioxidant activity was measured by reducing power, superoxide anion scavenging activity, and metal chelating activity. These measurements showed that the higher the concentration of WEN, the higher its antioxidant properties. Also, the power of the stinging nettle's antioxidants was shown to be higher than that of the common ALPHA tocopherol. No *in vivo* experiments were done in this study.

In order to test the antimicrobial activity of *U. dioica*, agar with WEN was infected by ten different microbial agents: *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Citrobacter koseri*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Micrococcus luteus*, *Staphylococcus epidermidis*, and *Candida albicans*. Then results were recorded by measuring zones of growth inhibition on the

agar plate. Each microbial agent was inhibited at least slightly, with the most significant inhibition being with *Staphylococcus epidermis* and *Miccrococcus luteus*. No *in vivo* experiments were done in this study.

Forty albino male rats were used for an *in vivo* study of the antiulcer activities of *Urtica dioica* in this study also. Using the ethanol-induced ulcer model, researchers injected the rats with 10 mg/kg WEN and then thirty minutes later with ethanol. One hour after the injection of ethanol the rats were killed and their stomachs examined. The larger amounts of WEN the rats, received, the fewer ulcers they had.

Clinical Studies

Numerous clinical studies on *Urtica dioica* have been conducted in the past and are still being conducted today. In this monograph, a focus is placed on three different conditions for which *U. dioica* has been tested as a treatment. First, *U. dioica* has been proven to treat lower urinary tract infections in several clinical trials (Lopatkin 2007, Lopatkin 2006, Safarinejad 2005). Sometimes *U. dioica* was used in combination with other chemicals or drugs, but in each of these three studies the administered drugs proved effective. Another ailment for which *U. dioica* has been used to treat is that of benign prostatic hyperplasia or benign prostatic syndrome. In several clinical trials, *U. dioica* has been proven to relieve irritative symptoms (Popa 2005, Schneider 2004). Other clinical trials studied the effect of *U. dioica* on gingivitis, but the plant did not offer any effective treatment (Van der Weijden, 1998).

Contraindications

So far, no negative interactions with other drugs have been recorded. However, it is recommended that pregnant women and breast-feeding women do not use any form of *Urtica dioica*. The plant and its drug derivatives are known to cause gastrointestinal issues, skin irritation, and sometimes diarrhea (Anderson, 2003). Also, one molecular contraindication is that the extract of the plant's leaf can reduce the amount of TNF-alpha and IL-1 beta in humans (Oberteis, 1996).

Current Use in Allopathic & CAM Therapies

Urtica dioica can be found in several drug therapies today. One drug marketed containing stinging nettle is known as Bazoton, which is taken in 300 milligram capsules, twice daily, for up to sixth months. One CAM therapy used today, for the treatment of allergic rhinitis, calls for the patient to take 600 milligrams of freeze dried nettles as soon as symptoms appear. Many nettle supplements exist, in which patients can buy a bottle of nettle capsules over the counter in pharmacies and online ("Nettle," 2010).

Discussion

The *Urtica dioica* plant has been utilized for both medical and food purposes throughout history. Today, modern science has found many of the key elements in the stinging nettle that make it useful as a medicine. For thousands of years, humans have utilized this plant and as science continues to narrow down the useful chemicals and possible dangers of the plant, its popularity as a drug is likely to grow.

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Vaccinium macrocarpon Aiton, Ericaceae

Sarah McMullin

Introduction

Vaccinium macrocarpon Aiton, or American Cranberry, is a member of the Ericaceae family. Sometimes the plant is placed in a genus its own, called, Oxycoceus (TWC staff, 2007). It is one of only three species of fruit native to North America, the other two being the blueberry and the bilberry, which are part of the same genus, *Vaccinium* (Lynch, 2004).). It grew wild until 1816 when it was cultivated with difficulty by Henry Hall (History, 2011). Now US farmers grow over 40,000 acres of cranberries every year, primarily in Wisconsin, Massachusetts, and New Jersey (Wisconsin produces over 50% of the national total) (Keough, 2011). Cranberries are usually commercially harvested by flooding the bog where they grow, beating the berries off the bushes and then collecting the berries that float on top of the water (CCCGA, 2011) (See **Figure 1**). In 2011 growers in the United States are projected to produce 7.5 million barrels of cranberries, and they received on average $46.50 a barrel for their harvested cranberries in 2010 (Keough, 2011).

Cranberries were used as a food source and as part of traditional medicine by Native Americans before Europeans arrived in North America. It is even thought that cranberry sauce sweetened with maple sugar was part of the first Thanksgiving feast between the Native Americans and the Pilgrims. Cranberries continue to be very important in food, especially around the holiday season. They are seen on shelves in virtually all grocery stores near Thanksgiving and in the freezer aisle and as juice, all year-round. Cranberry

Figure 1. Workers harvesting cranberries from bog. (Photo courtesy of Wisconsin Farm Bureau Federation. < http://www.history.org/foundation/journal/holiday06/cran.cfm>)

production in the United States is projected to be up 10% from 2010 production this year (Keough, 2011).

While *V. macrocarpon* juice has been used under anecdotal evidence since before colonization as a prophylactic prevention for urinary tract infections, recently clinical trials have been adding hard scientific evidence its efficacy for this use (The Natural Standard Research Collaboration, 2011). The scientific community's interest in *V. macrocarpon* has even extended beyond this common treatment to explore other possible medicinal properties it might have, including possible compounds that treat heart disease and cancer.

Figure 2. *Vaccinium macrocarpon* sketch. (Source: Britton, N.L., and A. Brown. http://plants.usda.gov/java/profile?symbol=VAMA&photoID=oxma6_001_avd.tif)

Botanical Description

Cranberry plants are low growing perennial evergreen shrubs, less than 12 inches (30cm) high, that grow in moist bog-like acidic soil (pH 4.0 to 6.1) in partial shade. They are native to cool temperate boreal forests in the Northeastern United States and Southeastern Canada (TWC Staff, 2007). They are close relatives of the blueberry plant, which is also a member

Figure 3. *V. macrocarpon* in bloom. (Photo courtesy of Tom Harville. http://www.ncwildflower.org/index.php/plants/details/Vaccinium-macrocarpon/)

of the *Vaccinium* genus (**Figure 2**). The plants spread by rhizomes (TWC Staff, 2007), horizontal stems that grow new stems up to the surface from nodes. This allows the plants to spread very quickly but can make them difficult to transplant (Beaulieu, 2011). The leaves are very small, ¼ inch to ½ inch (0.64cm to 1.28cm) long, leathery and oval shaped. They are shed in late summer every two years. The flowers have four petals and are white to pink in color. They hang inverted and flower for two to four weeks in late June to early July (Reiger, 1990). As the flower ages its petals curve back (**Figure 3**). Early settlers called the berries "craneberries", because the early flowers' (before they expanded) shape resembled a crane. The flower discourages self-pollination because the five

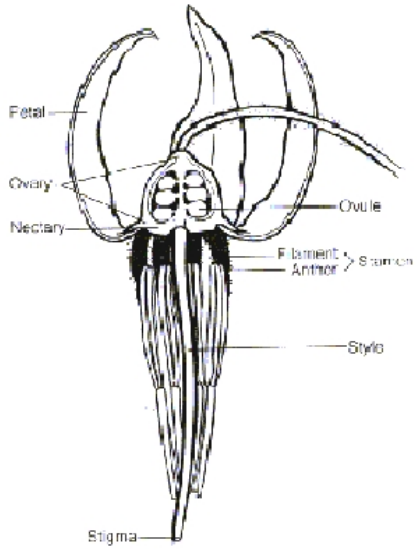

Figure 4. Reproductive parts of *V. macrocarpon* flower. (Photo courtesy of K.S. Delaplane & D.F. Mayer http://ag.udel.edu/enwc/faculty/dmcaron/Pollination/cranberry.html)

to eight stamens are exposed first, releasing their pollen before the single stigma is exposed. The pollen is heavy and not easily transported by wind, so insects, especially bees, are very important for pollination (Delaplane, 2011) (**Figure 4**). The flower takes on more of a pink color if it is not pollinated. The flowers produce some nectar, which is what attracts pollinators. The fruit is a small "false" berry (epigynous), which starts out green then changes to white and then bright to dark red as it matures. The berry matures in 60-120 days after fertilization, and is very tart to taste (Rieger, 1990) (**Figure 5**).

Traditional uses

Native Americans and Canadians called cranberries sassamanash and used them most often to treat bladder and

Figure 5. Ripe *V. macrocarpon* on bush. (Source: <http://www.justbio.ca/products/vacciniummacrocarpon>)

kidney problems (Block, 2011). They were also used for stomach problems, wounds, blood poisoning and appetite loss. Cranberries were used as a poultice to spread on the skin for wound healing and treating inflammation, and mixed with cornmeal to cure blood poisoning. An infusion of branches was made to treat pleurisy, chest pain caused by inflammation in the lining of the lungs and chest (Speck, 1917) (Dugdale, 2011). An infusion of the fruit could be made to treat nausea. (Smith, 1932). A decoction of the fruit could also be made to treat diarrhea (Lantis, 1959).

V. macrocarpon is high in vitamin C and therefore was very valuable to early explorers in preventing scurvy, a debilitating and often deadly disease caused by lack of vitamin C in the diet. The fruit could be sun or fire dried and kept over winter as a food source which was easily portable and could be taken on hunting expeditions (Waugh, 1916). Cranberries have also been used to treat type 2 diabetes (NIH, 2011), and have also

been used for infections, especially urinary tract infections, for hundreds of years, especially before the discovery of antibiotics (Lynch, 2004).

Native Americans made a sauce with the berries that was sweetened with maple syrup, and it is thought that this was likely part of the first Thanksgiving celebration. The berries were eaten either raw or cooked (Densmore, 1928), and were also used to make a special very high-energy food called pemmican which consists of dried meat or fish and berries pounded into a pulp, shaped into a cake and dried. It could then easily be stored and transported. It was taken along on canoe trips and became very important to the Métis people (Native people who intermarried with early French settlers in Canada). They often sold it as food to European traders along the French Fur Trade Route. (Block, 2011)

Traditional non-medical uses for cranberries include use of the juice for dying fabrics, such as blankets and rugs (Caruso, 2000), and the making of jewelry from firm ripe berries strung together (Leighton, 1985).

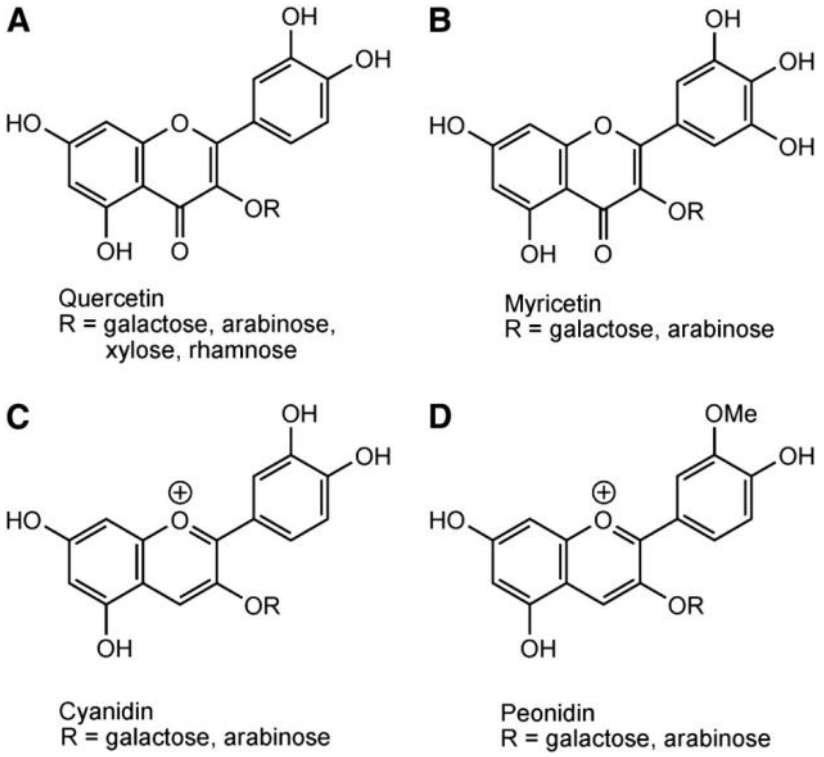

Figure 6. Compounds found in cranberry. (Courtesy of Catherine C. Netto. <http://jn.nutrition.org/content/137/1/186S/F1.expansion.html>)

Chemistry and Pharmacology

The major active constituents of *Vaccinium macrocarpon* are members of the flavonoid group of compounds. Flavonoids are water-soluble polyphenols made by plants. They consist of two benzene rings connected by another three-carbon ring that often contains oxygen (**Figure 6**). These compounds are responsible for plant color (Top Cultures, 2011). *Vaccinium macrocarpon* has one of the highest phenolic contents in fruit. These include: phenolic acids, anthocyanins, flavonols, and flavan-3-ols, procyanidins and proanthocyanidins (Vvedenskaya, 2003). The most prevalent flavonol is quercetin. Most flavonoids, except flavanols (catechins and protoanthocyanidins), are usually bound to a sugar, making a flavonoid glycoside (Higdon, 2005). The bioavailability of these compounds is strongly dependent on the structure of the bound sugar. These flavonols are strong free radical neutralizers. Free radicals are very dangerous and can kill cells, and damage DNA and proteins, which can cause cancer (University of Maryland, 2011). The stability of these flavonols caused by their aromaticity allows them to absorb the reactive extra electron into their rings, thereby stopping its ability to react with parts of the cell.

Biological Activity

Flavonoids are the main active chemical constituents of *Vaccinium macrocarpon*. They have been shown to have many positive effects against disease. Studies have shown that flavonoids are able to induce apoptosis, programmed cell death, in tumor cells. MacLean et al. (2011) showed that whole cranberry extract induced apoptosis in human prostate adenocarcinoma through caspase-8 activation. They found that it was the proanocyanidin and flavonol-enriched fractions of the extract that increased caspase-8 and 9 activities, suggesting that it is these compounds that are involved in apoptosis activation. Caspase-8 cleaves the Bid protein, which causes release of cytocrome-c from the mitochondria. (MacLean et. al., 2011) Cytocrome C initiates a cascade of events that destroy the cell from the inside out and package it into vesicles for disposal by macrophages (apoptosis). This is a very important mechanism in which cancer cells often have a defect. Normally if a cell senses a defect it will intrinsically initiate this apoptosis pathway. Cancer cells often over express BCL-2, which has been suggested to block cytochrome-c release (Yang, 1997). Kuida et al. also showed that mice lacking caspase 9 die early because of lack of apoptosis. (Kuida, 1998) Ferguson et al. showed that a fraction taken from cranberry presscake, the wet hulls left after juice is squeezed out, containing flavonoids was active against breast and prostate cancer. This fraction slowed proliferation and induced cell-cycle arrest and apoptosis in mouse tumors (Ferguson, 2004).

Another amazing property cranberries possess was shown in a 2011 study by Dohadwala et al. Extra-strength cranberry juice, provided by Ocean Spray, was given to patients with coronary artery disease, and was shown to provide an acute decrease in carotid femoral pulse wave velocity, which is directly related to arterial stiffness (Dohadwala et. al., 2011), and is associated with increased risk for cardiovascular events including heart attack. The mechanism that caused the decreased stiffness is still unknown, but future studies hope to discover this information.

A huge problem associated with bacterial infection is the colonization of many bacteria into biofilms. *Pseudomonas aeruginosa* is a type of bacteria that normally lives in soil, water or plants and causes many opportunist infections in humans with compromised immune systems (Todar, 2011). It uses flagellum-mediated swimming to move towards surfaces to attach itself and to swarm with other bacteria. They also use quorum sensing to move together as a group. O'May and Tukenjkji showed that proanthocyanidins from *V. macrocarpon* inhibit this swarming ability. This means that the bacteria were unable to form a colony on a surface. Cranberry extracts as low as 20 micrograms per milliliter almost completely blocked swarming. However, there was no effect on the growth rate of bacteria. These particular bacteria produce a biosurfactant, similar to a soap, to reduce surface tension so the bacteria can move across a liquid's surface. When surfactant, rhamnolipid, was added after cranberry treatment the bacteria regained some of its swarming ability. This suggests that the *V. marcrocarpon* proanthocyanidins somehow interferes with the biosurfactant production. It is also possible that a compound in cranberries interferes with bacteria quorum sensing ability (O'May, 2011).

In 1991 Ofek et. al. presented their findings in the *New England Journal of Medicine* in which they stated that cranberry, and other members of the *Vaccinium* genus, blocked the colonizing ability of *E. coli* bacteria in the urinary tract. They showed that an isolate from cranberry juice inhibited adhesion of bacteria to the mucosal cells of mouse bladders, which prevented urinary tract infection. The isolate seemed to act on the adhesins on the pili which extend off the

surface of *Escherichia coli* cells (Ofek, 1991). Twenty years later Gupta et al. showed that it was the proanthocyanidin component of *V. macrocarpon* that produces this adhesion inhibition. An isolation that contained 10-50 micrograms per milliliter of proanthocyanidin inhibited 70% of adhesion in both sensitive and multidrug resistant E. coli. It is theorized that this inhibition is associated with the A-type linkages of proanthocyanidin, but the specific mechanism in not certain (Gupta, 2011).

Clinical Studies

Many clinical trials have been undertaken to examine whether *Vaccinium macrocarpon* can reduce the number of recurrent urinary tract infections in patients, and most have proved inconclusive. One study looked at women over the age of 45 who suffered from recurrent urinary tract infections. The most common treatment for people with recurrent UTIs is low-dose, long-term, antibiotic prophylaxis. Many clinical trials have shown this to be very effective, but there are significant side effects including fungal infections and a high risk for the development of antibiotic resistance. The study by McMurdo et al. compared the use of this prophylactic antibiotic treatment to daily treatment with 500mg of cranberry extract. The median time for UTI recurrence in the antibiotic group was 91 days, compared to 84.5 days in the cranberry group. The difference was not statistically significant and the women taking cranberry extract had fewer and less severe side effects and did not develop antibiotic resistant bacteria. It was concluded that women should talk to their doctors about possibly taking *Vaccinium macrocarpon* instead of antibiotics to prevent infection, because it is natural, much cheaper, has fewer side effects and works almost as well (McMurdo et. al., 2008).

Another earlier clinical trial by Kontiokari et al. also showed the effectiveness of cranberry juice in preventing recurrent UTIs. It compared women who drank 50mL of cranberry-lingdonberry concentrate daily for six months to those who received no intervention. The recurrent rate in the cranberry group was 16% compared to 36% in the control group. This is statistically significant and shows that drinking cranberry juice has some effect on the likelihood of a urinary tract infection returning. (Kontiokari et. al., 2001)

Another study using female children was also promising. Children who had suffered more than one urinary tract infection in the previous year were given either daily cranberry juice or one of two controls (*Lactobacillus* or no treatment). Only 18.5% of the children in the cranberry group suffered an infection during the trial, compared to 42.3% and 48.1% in the controls. There were no negative reactions except for a few children who did not like the taste of cranberry juice. (Ferrara et. al., 2009)

Despite these studies showing promising results, a more recent study of 319 women between 18 and 40 years of age by Barbosa-Cesnik et al. was less positive. The participants in this study drank either 8oz of a low calorie 27% cranberry juice cocktail, or an 8oz of a placebo twice a day for six months. The placebo group had a recurrence rate of 16% while the cranberry group had a recurrence rate of 19%, indicating that cranberry juice gave no protection against recurrent UTIs (Barbosa-Cesnik et. al., 2011). It is possible however, that the low concentration (27%) of cranberry juice in the cocktail did not contain enough of the active *V. macrocarpon* flavonoids to obtain good results. Most studies showing positive results used extra-strength cranberry juice, which has a higher percentage of active compounds, or cranberry extract pills, which are equivalent to several glasses of juice.

Contraindications

Vaccinium macrocarpon is generally regarded as safe and no significant drug interactions have been reported, but there is a lack of solid long-term safety information (Lynch, 2004). There is a possibility that cranberry may increase the amount of time Warfarin, a drug prescribed to slow blood clotting, remains in the body. This could lead to bruising or bleeding (NIH, 2011). Some very small studies have shown the possibility that cranberry juice can increase oxalate levels in the urine, which is the main cause of kidney stones. Large doses of cranberry can also lead to diarrhea (Burak, 2010). Diabetics should also be careful when they consume large quantities because its high sugar content could result in high blood sugar.

Current Use in Allopathic and CAM Therapies

Many cranberry supplements can be purchased in pharmacies. Most are in pill form and are advertised for improved urinary tract health and antioxidant benefits (Enzymatic Therapy, 2011). Urinary tract infections account for approximately 8 million outpatient and emergency room visits per year, and combined direct and indirect cost associated with UTIs in America is $1.6 billion (Bowen, 2007). The recommended dosage of unsweetened cranberry juice for UTI prevention is 8oz, three times per day, or one 300-400mg tablet of concentrated cranberry extract (Lynch, 2004). One 64oz bottle of Ocean Spray Pure Cranberry Juice Costs approximately $6 (Ocean Spray, 2011), and a thirty day supply of extract tablets costs between $10-$15 (Lynch, 2004). A 60 day supply of the most common antibiotic prescribed for prophylaxis, Trimethoprim (Regional Drug and Therapeutics Center, 2004), costs approximately $33 at healthwarehouse.com (Health Warehouse, 2011); this is slightly more than cranberry extract, but more importantly it puts patients at risk for many side effects, including antibiotic resistance. The National Institute of Health rates cranberry juice and supplements as "possibly effective" for preventing urinary tract infections, and it is often recommended first by physicians for prevention of, or to complement antibiotic treatment of, UTIs (NIH, 2011). The American Institute for Cancer Research also suggests eating cranberries to lower the risk of certain cancers, and there have been many news stories over the past few years promoting this (American Institute for Cancer Research, 2011). Since cranberry juice and cranberry extract pills do not require a prescription, it can be assumed that many people may take cranberry juice as a preventative measure for cancer and UTIs without consulting a doctor.

Discussion

Vaccinium macrocarpon has played an important role for centuries in food and complementary and alternative medicine, among Native Americans and then European settlers. It had been used under circumstantial evidence for treatment of urinary tract infections for years, especially before Alexander Flemming discovered antibiotics in 1928 (Bellis, 2011). While antibiotics are affective against the bacteria causing UTI's, the infection often recurs, which is where *V. macrocarpon* becomes important yet again. Studies have shown *V. macrocarpon* to be effective at significantly reducing the recurrence rate of these infections, while producing virtually no side effects. These promising studies have encouraged researchers to explore cranberries for many other disease fighting properties in the past few years, including the treatment and prevention of cancer and heart disease. Many papers have also been published this year exploring more unorthodox possible uses for this remarkable

berry such as, antifungal, wound healing, osteoclast inhibition in people with periodontitis, and blood pressure reduction properties. A few researchers are also looking deeper into the plant to learn more about its genetic makeup (Google Scholar, 2011).

It is exciting to imagine what new treatments might emerge in the next few years thanks to *V. macrocarpon.* Perhaps it will be the answer to some of the chronic diseases that seem to be only on the rise. Hopefully the positive results of clinical trials seen in this plant will encourage the study of more plants with traditional medicinal value.

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Vaccinium myrtillus L., Ericaceae

Tiffany Baerwaldt

Introduction

Vaccinium myrtillus L., commonly known as bilberry, is a member of the Ericaceae family. It is also known as whortleberry, European blueberry, huckleberry, black whortles, trackleberry, and bleaberry. The name Bilberry is derived from the Danish "bollebar," meaning dark berry (Grieve, 1959). It belongs to a large genus (*Vaccinium*) of plants that also contains blueberry (*Vaccinium corymbosum*) and cranberry (*Vaccinium macrocarpon*). Bilberry is sometimes referred to as "blueberry" because both share many of the same characteristics and features, but the true blueberry is native to the United States (Benzie and Wachtel-Galor, 2011).

Bilberry is a perennial shrub whose fruit is purple or bluish black in color and is sweet tasting. It is native to northern Europe but can also be found in the mountainous regions of the northern hemisphere. Bilberry was used traditionally as food and also as medicine for ulcers, diarrhea, dysentery, diabetes, urinary infections, and vision, and its extracts were also used for bilberry's anti-inflammatory properties. Dioscorides highly regarded the bilberry plant and its fruit, and bilberry fruit was also noted in Ayurvedic medicine as having use for oral infections and teeth cleaning. The leaves of the bilberry plant contain many active constituents including tannins, polyphenolics acids, flavanoids, arbutin, and quercetin. Its berries contain anthocyanoside flavanoids, as well as polyphenols and anthocyanins, which contribute to much of its pharmacological activities. Bilberry has antioxidant, vaso-protective, antimicrobial, and lipid-lowering

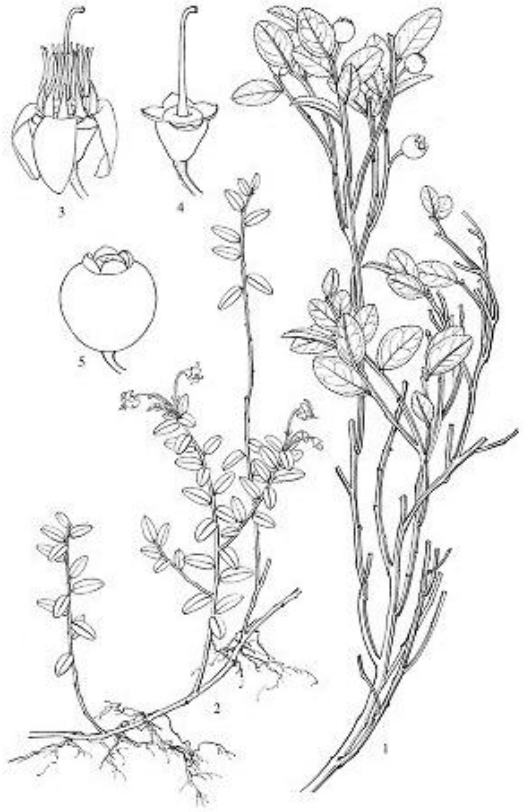

Figure 1. Line drawing of *Vaccinium myrtillus* L. (Source: Flora of China Illustrations vol. 14, fig. 688, 1 http://tropicos.org/Image/73875)

properties, as demonstrated in many recent experiments. Bilberry also has a long history of use as for various eye conditions. It has corneal renewing effects, and is believed to

Figure 2. Photograph of *Vaccinium myrtillus* L. (Source: http://www.zufglobus.com/xzcv/Page/index/135).

improve human night vision and has promise for the treatment of cataracts and prevention of glaucoma. Bilberry is most commonly used for improvement of night vision and general eye health. Bilberry is generally considered as safe when used properly, and is currently used as an antioxidant in the form of an extract, as a vitamin supplement for vision improvement, vascular problems, diabetes, and as a tea for vision health as well as antioxidant support for the whole body.

Botanical Description

Vaccinium myrtillus is a colony-forming, perennial, rhizomatous shrub ranging from 5-120 cm high. Its twigs and leaves are sharp-edged and green. It has alternating leaves that are broadly elliptical and approximately 19-27 by 7-11 mm in size. The leaves lack in rigor and firmness and are glandular beneath (**Figure 1**). The calyx is green and has a smooth surface without hairs or projections. The corolla, or part of the flower that consists of the petals, is thin, globose, and pink, cream, or greenish white in color (Vander Kloet and Dickinson, 1999, 242). The fruit of the bilberry plant are berries that are purple black or bluish black in color, and 7-9 mm in diameter. The berries grow in small clusters (**Figure 2**). The meat of the fruit is purple, unlike the American blueberry, which is usually cream or white (Thorne Research Inc, 2001, 500). The bilberry seedling takes approximately 6-10 weeks to develop its first leaves and shoot, and at 3 months the juvenile plant begins to show the features indicative of the adult plant (Ritchie, 1956, 297) (**Figure 3**).

V. myrtillus is native to northern Europe and is also abundant in the mountains and forests of the northern hemisphere, including northern and central Asia and in northern North America (Ritchie, 1956). Bilberry has been shown to have its highest vegetative and reproductive performance in woodlands, but it may also occur in mountainous heath soils, upland heaths, boggy communities, degraded meadows, open coniferous forests, pine forests, parklands, disturbed or open birchwoods, hummocky seepage slopes, podsols and

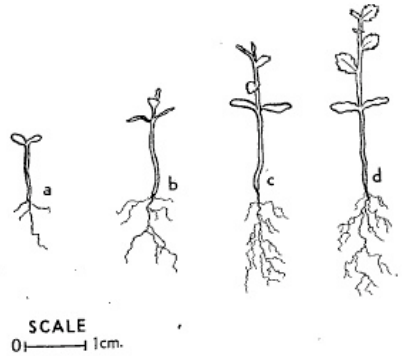

Fig. 3. Diagrams of seedlings to illustrate the early stages of development; (*a*) at 2 weeks, (*b*) at 7 weeks, (*c*) at 11 weeks and (*d*) at 16 weeks.

Figure 3. Diagrams of bilberry seedlings to illustrate the early stages of development. (Source: Ritchie, 1956)

moraines. Bilberry can flourish at altitudes from sea level to 2600 meters above sea level. The abundance of the bilberry plant as well as its size, shape, and color is determined by soil conditions such as the drainage factor and amount of organic matter in the soil. Climate conditions such as the presence of strong winds may also impact the morphological features of bilberry. One study determined that the tallest bilberry plants with the greatest density are usually found in mountain habitats, while populations in lowland forests are generally lower in height and grow in more erratic patches (Rolson et al. 2011, 238). However, results from previous studies demonstrated that the height of bilberry decreases with altitude, which could be the result of other environmental factors influencing the morphological features of the plant (Woodward, 1986).

Bilberry also interacts with various biotic factors that enhance its distribution and pollination. According to Ritchie (1956, 294), "When other more palatable plants are unavailable, sheep will graze bilberry, and areas in Derbyshire have been examined where severe cutting back of the young green shoots was evident. Observation suggests that this has the effect of stimulating further growth and branching producing a densely busy habit." It was also established that bilberry is pollinated by insects, birds, and through self-pollination by gravity.

Traditional Uses

Due to its rich, sweet and enjoyable flavor, as well as its nutritive value, bilberry has been used as food and as medicine for centuries. In addition to its use in tarts, pies, and preserves, bilberry was traditionally used in juices, jams, and wine as a colorant, and was also used to dye wool due to the presence of tannins in the fruit. In addition, according to "A Modern Herbal" written by Grieve in 1959, bilberry was used widely in the ancient world, and was highly regarded by Dioscorides. It was also noted that a decoction of the leaves or bark of the root was used as a local application to ulcers, and could also be applied to ulceration of the mouth and throat. The fruit's astringent properties were used for diarrhea and dysentery in the form of syrup (Grieve, 1959). The fruit was also used for scurvy, tuberculosis, and urinary complaints (Grieve, 1959), and a tea made from the leaves was used as remedy for the treatment of diabetes (Cignarella et al. 1996, 311-312). Bilberry fruit was also used traditionally in Europe for discharges, as a lactation inhibitor (Grieve, 1959), in anti-inflammatory mixtures, and is commonly used as medicine for improving vision (Song et al. 2010, 520). Traditional herbal preparations are still used to enhance circulation and improve eye conditions, and dried berries are sold for the traditional treatment of mild diarrhea and inflammation of the mouth and throat (Grieve, 1959).

Bilberry fruit was also included in Ayurvedic medical practices. Deformities of the oral cavity, plaques, and infections were managed in ancient India, and it was noted that bilberry fruit and hawthorn berry stabilize collagen, strengthening the gum tissue (Singh and Purohit, 2011, 66).

Chemistry and Pharmacology

Bilberry leaves contain several active constituents including tannins, polyphenolic acids, flavanoids, arbutin, cathechins, iridoids, and quercetin (Rolson et al. 2010, 238). In addition, bilberry berries contain anthocyanoside flavonoids, or anthocyanins, vitamins, sugars, and pectins (Thorne Research Inc, 2001, 500). Polyphenols and anthocyanins are the main components in bilberry, and many of the pharmacological activities of bilberry can be attributed to anthocyanins (Song et al. 2010, 520). Bilberry is one of the richest natural sources of anthocyanins (Benzie and Wachtel-Galor, 2011, 55). Anthocyanins are water soluble, glycosylated, nonacetylated polyphenolic compounds responsible for the red, blue, and purple pigments of fruits. These pigments are absorbed by the gastrointestinal tract after consumption, and have been shown to have tumor suppressive, anti-inflammatory, anti-viral, and anti-diabetic properties. Anthocyanins are also potent antioxidant and reactive oxygen species scavengers, and the anthocyanin concentration of fruit is highly correlated with the oxygen radical absorbance capacity (Bell and Gochenaur, 2006, 1164).

In a study conducted to evaluate the morphological and chemical variability of wild populations of bilberry in Poland, the content of phenols in bilberry leaves and fruits were determined. It was found that the content of tannins in bilberry leaves can reach 7.5-12%, and the content of anthocyanins in bilberry fruit can amount from 0.5 to 0.399%

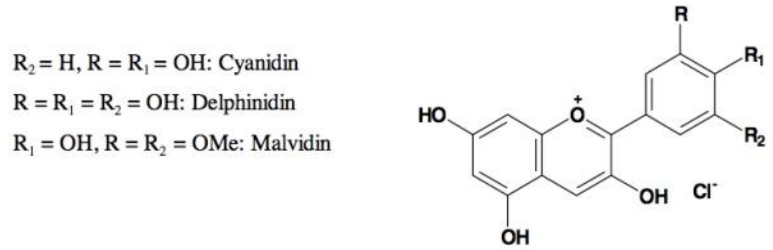

Figure 4. Chemical composition of bilberry fruit extract: Anthocyanins. (Source: National Toxicology Program, 1999. Bilberry Fruit Extract 84082-34-8 http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Bilberry.pdf)

(Rolson et al. 2010, 241). Polyphenols account for approximately 50-80% of the total polyphenol content in bilberries (Karlsen et al. 2009, 346). A concentration of 30mg/g of quercetin in bilberries has been reported (Erlund et al. 2003, 37). Another study determined and summarized the content of the major polyphenol classes found in bilberry, which include polyphenolic acids, tannins, arbutin, flavanoids, and anthocyanins (Karlsen et al. 2009, 352) The main anthocyanins in bilberry and their relative concentrations include delphinidins, cyanidins, petunidins, malvidins, and peonidins (Benzie and Wachtel-Galor, 2011, 57). The chemical composition of 25% bilberry extract, which is considered the standardized amount of anthocyanins in bilberry extract, contains a natural mixture of glycosides of aglycone anthocyanins, cyanidin, delphindin, and malydin (National Toxicology Program, 1999) (**Figure 4**).

Biological Activity

The anthocyanins present in bilberry are safe and proven antioxidants and chemoprotective agents. A variety of *in vitro*,

in vivo, and human studies have demonstrated that anthocyanins have antioxidant effects, promote healthy vision, urinary tract health, and skin health. Anthocyanins also exhibit cardiovascular, neuroprotective, anticarcinogenic potential, and antidiabetic properties and health benefits (Zafra-Stone et al. 2007).

A study that aimed at examining whether bilberry extract alleviates pruritus, or the sensation that causes the reflex to scratch, in mice with chronic allergic contact dermatitis concluded that anthocyanins from bilberry extract may be beneficial in the treatment of chronic pruritus. Chronic pruritus occurs in patients with anti-inflammatory skin diseases such as atopic dermatitis, which is the most common chronic inflammatory skin disorder and always induces pruritus. The daily intake of bilberry may provide a useful treatment and source of relief for pruritus in these patients (Yamaura et al. 2011).

In an *in vivo* study using type 2 diabetic mice, the effect of dietary bilberry extract on hyperglycemia and insulin sensitivity was examined. It was found that dietary bilberry extract significantly reduced the blood glucose concentration and enhanced insulin sensitivity. The upregulation of glucose transporter in white adipose tissue, skeletal muscle, and the liver of diabetic mice fed with bilberry extract was also shown. Acetyl-CoA carboxylase was also inactivated and PPARα, acyl-CoA oxidase, and carnitine palmitoyltransferase-1A were upregulated in the liver. The result of these changes was improvement in hyperglycemia and insulin sensitivity in type 2 diabetes, which provides a biochemical basis for the use of bilberry fruits and implications for the prevention and treatment of type 2 diabetes (Takikawa et al. 2010).

Bell and Gochenaur (2006) recognized that plant pigment anthocyanins are especially potent oxygen radical scavengers, and conducted an *in vitro* analysis of the direct vasoactive or vasoprotective properties of berry pigment extracts using coronary artery rings from mature female pigs. Their results suggest that such extracts could have significant beneficial effects in the treatment of vascular disease.

Huttunen et al. (2011) found that the antimicrobial activity of juice fractions including bilberries was notable against *Streptococcus pneumonia* binding to human bronchial cells, and that pneumococcal growth was completely inhibited at a concentration of ~86 mg/g. In summary, the results indicate that bilberry is a novel source for both antiadhesive and antimicrobial agents against pneumococci.

An *in vitro* study on the protective effect of bilberry extracts on cultured human limbal epithelial cells found that bilberry extract may be beneficial for the physiological renewal and homeostasis of corneal epithelial cells (Song et al. 2010, 520). Another study was conducted that generated a mouse model of endotoxin-induced uvetis that showed retinal inflammation and swelling of the middle layer of the eye. Mice that were pretreated with an anthocyanin-rich bilberry extract were analyzed, and the effect on the retina was documented. The anthocyanin-rich bilberry extract prevented the impairment of photoreceptor cell function, endotoxin-induced uvetis decreased, and the shortening of outer segments in photoreceptor cells were suppressed at the cellular level. The extract induced inflammation-related rhodopsin decrease, and the extract ameliorated the intracellular elevation of reactive oxygen in the inflamed retina. It was determined that anthocyanin-rich bilberry extract has a protective effect on visual function when the retina is inflamed (Miyake et al. 2011).

Cignarella et al. (1996) discovered that bilberry leaves have lipid-lowering properties by administering bilberry leaf

extract to streptozotocin-diabetic rats. Plasma glucose levels consistently dropped about 26% at two different stages of diabetes. The findings indicated that the active constituents of bilberry leaves, which include anthocyanins, might prove useful for the treatment of dyslipidaemia, or an abnormal amount of lipids in the blood.

Clinical Studies

Human data with respect to anthocyanins from bilberry are lacking, however dietary polyphenols present in bilberry fruit have been associated with the prevention and treatment of chronic inflammatory diseases, including cardiovascular disease (CVD). CVD has many risk factors including obesity, diabetes, hypertension, elevated lipid levels, and inflammation, and a novel treatment is therefore necessary. A clinical study on the effect of polyphenols in bilberry juice determined that a supplementation with bilberry juice resulted in significant decreases in plasma concentrations of C-reactive protein, interleukin, and monokine. Increases in plasma quercetin and *p*-coumaric acid were also observed in the bilberry group. These findings suggest that the supplementation with bilberry polyphenols may modulate the inflammation process (Karlsen et al. 2010, 345).

Quercetin, a polyphenolic flavanoid found in bilberry, exhibits antioxidative, anticarcinogenic, and enzyme-inhibiting activities. A study by Erlund et al. (2003) aimed to determine the impact of daily consumption of 100g of berries (black currants, lingonberries, and bilberries) on serum quercetin concentrations in healthy, middle-aged men, and found that in the berry group, the mean calculated intake of quercetin was significantly higher at the end of the 8 week study compared to the baseline group. Concentrations of quercetin were 32-51% higher in the berry group during the berry consumption period. The results of this study recommend the increased intake of berries due to their bioavailable quercetin content in addition to the presence of other beneficial compounds.

The flavanoid anthocyanosides found in bilberry are potent antioxidants and have effects on the eye and vascular tissues. In a study of 50 patients with senile cataracts, a combination of bilberry extract with 25% anthocyanosides and vitamin E was given for four months. Taken twice daily, the extract stopped the progression of cataracts in 96% of the patients treated compared to 76% in the control group (Head, 2001). Consumption of bilberry extract may also have potential for protection against the development of glaucoma, or damage of the optic nerve. Eight patients with glaucoma were given a dose of 200mg *Vaccinium myrtillus* anthocyanosides and demonstrated improvement due to bilberry's collagen-enhancing and antioxidant properties (Thorne Research Inc, 2001).

Contraindications

Bilberry is classified as a Class 1 herb by the American Herbal Products Association, and is therefore considered safe when consumed appropriately. It has no reported mutagenic activity and there are no cited contraindications to its use (Benzie and Wachtel-Galor, 2011, 56). Dosages as high as 400 mg/kg body weight have been administered to rats without toxicity. Long-term oral administration in humans of doses equivalent to 180 mg/kg anthocyanosides per day for six months produced no toxic effects (Thorne Research Inc, 2001).

According to the University of Maryland Medical Center (UMMC), bilberry fruit and extract are generally considered as safe, and have no known side effects. However, as with any substance, bilberry leaf and extract should not be taken in

large excessive quantities for extended periods of time, especially because bilberries contain tannins, which may cause severe weight loss, muscle spasms, and possible death. The UMMC also notes that taking bilberry extract may change the way other remedies, medicines, and vitamins work and operate with one another. Using these products together may cause harmful effects and lead to dangerous consequences. The UMMC also acknowledges that the anticoagulant properties of anthocyanosides in bilberry may increase the risk of bleeding if taken with blood-thinning medication. The UMMC also proclaims that because bilberry may lower blood sugar, it could have synergistic effects with diabetes medication (UMMC, 2011).

Current Use in Allopathic and CAM Therapies

Currently, bilberry is used in products such as OptiBerry, a multiple-berry extract formulated for whole body antioxidant support. Its suggested uses include enhanced antioxidant protection, cardiovascular support, skin support, healthy cellular function, and healthy cognitive function. The recommended dose for the 50 mg capsules is 1-2 capsules per day, taken with a meal (Pure Encapsulations, 2011). OptiBerry has been demonstrated as being safer and more potent compared to the extracts of the individual berries used in the OptiBerry blend. OptiBerry also demonstrated superior bioavailability, antioxidative, antiangiogenic, antibacterial, anti-inflammatory, and antiatherosclerotic properties (Zafra-Stone et al. 2007, 675).

Bilberry leaf and berry is also sold as a liquid extract and as vitamin supplement for its antioxidant and vision-improving effects. Among many other supplement producers, Puritan's Pride sells bilberry as a supplement claiming to contain 1,000 mg bilberry extract. They suggest taking one soft gel once or twice daily, preferably with a meal. Bilberry tea, prepared similarly to most packaged tea bags, is also available and promoted for vision health and support. Skin Actives Scientific, a skin care manufacturer, also markets bilberry for use as a face and body cream. Standardized for anthocyanins, which give this extract its color, the extract is said to have vasoprotective, anti-inflammatory, antioxidant and healing activity.

Discussion

Vaccinium myrtillus L. has been used for centuries in northern Europe as a functional food product as well as in jams, preserves, and baked goods such as pies and tarts. The use of bilberry across the northern hemisphere provides strong evidence for its efficacious medicinal properties. Bilberry is one of the richest natural sources of anthocyanins, which are responsible for bilberry's high antioxidant content, anti-inflammatory, antimicrobial, and lipid-lowering effects, and for the promotion of vision health and improvement. Therefore, bilberry has high potential in the treatment and prevention of conditions associated with diabetes, hyperglycemia, cardiovascular disease, inflammation, cancer, and eye conditions. However, human data with respect to anthocyanins from bilberry are lacking. The antioxidant effects of the anthocyanins present in bilberries give incentive for further clinical studies that aim to explore the additional health benefits from bilberry's antioxidant properties. The vasodilatory and anti-inflammatory effects of bilberry may also provide further therapies for age-related degenerative diseases. Due to its many biological activities, bilberry should be regarded as a functional food whose constituents hold the potential to provide therapies for a number of conditions whose current treatments are limited. More research on

bilberry and its constituents is necessary in order to fully recognize and benefit from the medicinal properties of the bilberry plant.

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Zingiber officinale Roscoe, Zingiberaceae

Sarah Mosby

Introduction

Zingiber officinale Roscoe, of the Zingiberaceae family, has played an integral part in human history, culture, and medicine, and it remains the most widely cultivated spice (Ravindran 2005, 7). The knotted rhizome (*Zingiberis rhizoma*) of the plant is commonly as ginger or ginger "root, though the latter is a misnomer. Ginger has been used in traditional healing remedies believed to affect nearly every human body system, and many of its medicinal uses have been verified as effective by *in vitro*, *in vivo*, and clinical studies. The use of ginger in complementary and alternative medicine (CAM) is growing and today includes use as a carminative, anti-emetic, anti-inflammation agent, and analgesic. It shows promise in biomedicine in treatments of arthritis, nausea and vomiting, and cancer.

Botanical Description

Zingiber officinale belongs to the tropical/sub-tropical family Zingiberaceae and the order Zingiberales. The specific origin of Z. officinale species is unknown, but it is most likely to have origins in tropical or sub-tropical India. Variants of the species are dependent on the environment and *Z. officinale* var. *cholmondeleyi* F. M. Bailey, *Z. officinale* var. *macrorhizonum* Makino, *Z. officinale* var. *rubens* Makino, *Z. officinale* var. *rubrum* Theilade, *and Z. officinale* var. *sichuanense* Z. Y. Zhu et al. (Tropicos 2011).

Z. officinale is a perennial herb, making it useful as an aesthetic plant, and can attain a stem length of 1.5 m. Branching out from the stem are smooth, pale green, lanceolate leaves that can grow to 5-30 cm long, 8-20 cm wide, and up to 2 cm thick. A few shorter stems bear an ovate-oblong spiked flower: a purple anterior petaloid divided into three rounded lobes surrounds a tufted stigma and a fruit capsule with small arillate seeds (**Figure 1**). These branches extend horizontally from the stem of the plant, which projects vertically from the rhizome (WHO 2011).

Figure 1. The oblong flower of *Z. officinale.*
(Source: http://www.types-of-flowers.org/pictures/ginger_flower.jpeg)

Figure 2. The knotted rhizome known as ginger is used in food and medicine around the world. (Source: precision nutrition.com)

The dried rhizome of *Z. officinale* is scientifically referred to as *Zingiberis rhizoma* (**Figure 2**). The subterranean rhizomes are thickly lobed and tuberous with a yellowish-brown to pale buff external surface with few scales. When the skin is peeled, the aromatic rhizomes show fine longitudinal striations in the pale yellow to white-colored flesh (Pakrashi 2003, 16-18).

The optimal conditions for cultivation of ginger include an elevation of 300-900m and at least 1.98 meter of rainfall per year (Pakrashi 2003, 6). Today, ginger is cultivated in Asia, Africa, the Americas and Oceania. Propagated by planting of rootstalk cutting, cultivation of ginger ideally begins in April.

Country	Production (tons)	Country	Production (tons)
India	380100	Mauritius	616
China	331393	Dominican Republic	600
Indonesia	192500	Jamaica	459
Nepal	174268	Guyana	424
Thailand	170125	Kenya	245
Nigeria	152106	Dominica	169
Bangladesh	72608	Trinidad and Tobago	145
Japan	52000	Uganda	144
Philippines	27415	Reunion	107
Cameroon	12000	Puerto Rico	100
Malaysia	11200	Pakistan	94
Sri Lanka	10780	Ghana	81
Cote d'Ivoire	7680	Madagascar	29
Ethiopia	6834	Saint Lucia	14
Bhutan	3766	United Republic of Tanzania	10
Fiji	3041	Africa	179852
Republic of Korea	3000	Americas	3832
Costa Rica	1105	Asia	1429249
USA	816	World	1615974

Table 1. World production of ginger. India remains the earliest and largest producer of ginger (Source: FAO Statistics Division 2011.)

The rhizomes are harvested in stages dependent on their intended use. “Green ginger” is harvested before five months have passes since sowing, and “dry ginger” is harvested between 7-8 months after planting. Green ginger is used more often as a preservative, whereas dry ginger is more common in food preparation; both are commercially distributed (Pakrashi 2003, 8-10).

Though native to Asia, ginger is commercially grown and harvested in 34 countries spanning four continents. The majority of production and world trade still occurs in the Asian countries of India, China, Indonesia, Nepal, and Thailand, but Nigeria and Cameroon maintain significant levels of production as well. **Table 1** shows the most recent statistics of world production. India was the earliest producer of ginger and remains the largest producer today contributing to 30-40% of the world's production. India exports both dried rhizomes and extracted ginger oil, but only 10-15% of ginger produced in India is exported. China is the largest consumer of ginger, and contributes significantly to world ginger production (Ravindran 2005, 9-10). Like other species of the Zingiberaceae family–turmeric and cardamom–, ginger has a long history of consumption as a spice and food additive as well as a medicinal role.

Traditional Uses

Ginger has been used by humans since antiquity, and it remains the world's most widely cultivated spice. In its country of origin, ginger's use is prehistoric. It was renowned as *mahabheshaj*, *mahaoushadi*, meaning "the great cure, the great medicine," and was one of the most essential components of household folk medicines. Ginger's use in China dates back to at least 500 BC, as recorded by Confucius. The trade of ginger began with maritime circulation throughout India, China and Indochina, and by the 8th century AD, ginger was commonly traded throughout the Mediterranean. In second century Rome, ginger was one of the few items on which was imposed an import levy at Alexandria, evidence of its extensive trade (Ravindran 2005, 2-5). It was well known in Europe by the 11th century, where the Greeks and Romans considered it a favorite spice, second to black pepper (Pakrashi 2003, 2).

Ginger is mentioned in the Koran, although not in the Christian Bible, and has long been incorporated into folklore, indicating its historical culinary and medicinal importance. Westerners are most likely familiar with the story of the Gingerbread man, a common child's folktale. Gingerbread became popular in Europe under Henry VIII's recommendation to consume ginger in preventive measures against the plague. In eastern Africa, ginger is associated with auspiciousness and is essential to the Savaras tribe for religious and marital ceremonies (Pakrashi 2003, 2; Ravindran 2005, 4). Another interesting example of ginger in culture is the Chinese Red Egg and Ginger party. It is traditional for parents or grandparents to welcome a newborn into the world with a celebration, and these celebrations often include "announcement packages" of red-dyed hard-boiled eggs and ginger root. This tradition is carried out today often with banquets or, in America, buffet-style lunches, when the baby is around one-hundred days old (Gong 2005, 164).

Culinary

The use of ginger as a cooking spice or condiment dates back 4,400 years to Asia, and it remains an integral part of Asian culinary use today. It has a distinctive, hot, pungent aroma and flavor that is essential to many modern and traditional Asian cuisines such as curry and chutney. The spice and flavor of ginger can be elicited from fresh or dried ginger root; the fleshy rhizome is first washed and peeled, then sliced, grated, or cooked whole. Ginger pickled in sweet vinegar is a common palette-cleanser in Asia, accompanying meals such as sushi, while crystallized or preserved ginger is consumed as a

confection. Ground ginger is a convenient kitchen spice that is available in most commercial grocery markets.

Ginger was one of the first spices of the orient to arrive in the West through trade. In Western food and culture, ginger is well-known as the spice of gingerbread, ginger beer, and ginger ale. The origins of gingerbread may date back to the ancient Greeks, who wrapped ginger in bread and consumed it after meals as a digestive aid (O'Hara 1998, 530). The spice is also incorporated into biscuits, cakes, puddings, soups, and pickles (Ravindran 2005, 12).

Medicinal

Many Zingiberaceous plants play a role in traditional medicine, especially in Asian medical systems such as Ayurvedic and Traditional Chinese Medicine. Across medical systems, ginger has been used medically as a carminative and a stimulant in the digestive tract (Tuntiwachwuttikul 1986, 191). The medicinal use of ginger dates back 2,500 years to ancient Chinese and Indian civilizations for treatments of many ailments including nausea, rheumatism, and headaches.

Ayurveda

In Ayurvedic medicine, *Zingiber officinale* is referred to by many Sanskrit names, most notably by Ardrak. Ardrak relieves the three dosha (energies of health), vatakapha and pitta, and thus can help maintain balance of dosha in multiple systems of the body (Garodia et al. 2007, 2). Uses of Ardrak in Ayurvedic medicine are numerous and include external application as well as ingestion. Externally, ginger can be prepared as a paste with milk or water to reduce infantile cold and swelling. The ginger powder and oil are also used in massage therapy to alleviate chills, cold and stiffness, excessive swelling and hypothermia (Gogte 2000). Ginger's balancing action against "cold" is appropriate for its characteristically "hot" aroma and flavor; and it is advised not to use ginger in the summer and autumn seasons.

Internally, ginger is believed to act upon the nervous system as a nerve-stimulant to improve impulse transmission and relieve pain. Prepared with castor oil, ginger powder is useful against rheumatoid arthritis. The stimulating and anti-inflammatory action extends to the heart and circulatory system, where it also acts as a blood-purifier. Consumption of ginger prepared with honey is an Ayurvedic remedy for asthmatic bronchitis, hiccups, coughs, and respiratory colds. In the reproductive system, ginger acts as an aphrodisiac and sex stimulant. Most importantly, however, are ginger's uses in the digestive system. Ginger improves appetite and digestion and is antiflatulent, antihaemorrhoidal, and antispasmodic. It is also used to treat anorexia, nausea, vomiting, abdominal pain, jaundice, and piles (Gogte 2000).

Traditional Chinese Medicine (TCM)

The use of ginger in TCM is comparable to that of Ayurveda. Ginger has traditionally been thought of as 'hot' and 'spicy,' both of which can treat coldness and weakness by mobilizing the body's reserves of *qi*. The Chinese distinguish between fresh, roasted, and dried ginger, attributing each with different healing properties. Fresh ginger is regarded as "mildly warm", dried ginger "warm," and roasted ginger "hot." All forms of ginger, however, have an "opening up" or "loosening" effect, enhancing the body's ability to integrate external stimuli. This assimilating property pertains to effects of certain foods and relates to ginger's perceived power in the digestive system (Pokert and Ullmann 1988, 218). Ginger is generally included in prescriptions for digestive ailments, appetite disorders, and

queasiness, and is useful in TCM because many of the remedies involve absorption through the GI tract. In addition, Chinese meals are extremely rich and are eaten late in the evening, thus the incorporation of ginger may be important in aiding digestion.

Western Medicine

Like ancient Asian peoples, Europeans took notice of ginger's activity on the digestive system (Alcock 2006). The laxative properties are described by the first century physician scholar Dioscorides, writer of *De Materia Medica* (Alcock 2006). Ginger is also described by author Charles Lamb to "make [millet] go down the more glibly" in his 18th-century works (Lamb 1994, 351). Henry VIII recommended the use of ginger in curing or preventing the plague, as by 1416, preserved ginger was a common import from China (Ravindran 2005, 279).

Chemistry and Pharmacology

The wealth and variety of chemicals present in rhizomes of *Zingiber officinale* are responsible for ginger's taste, aroma, and healing properties. Specific constituents of ginger extracts are dependent upon the origin of the variant and the condition of the rhizomes (fresh or dried). According to a phytochemical review of ginger, 63 compounds have been identified in fresh ginger compared to 115 compounds identified in dry ginger, and 45 of these compounds are shared by fresh and dry ginger. The compounds responsible for the pungency of fresh ginger, gingerols, become the dehydrated shogaols responsible for the pungency of dried ginger (Ali et al. 2008, 410).

The most medicinally active chemicals present in ginger rhizomes are components of the volatile oil and non-volatile pungent principles (gingerols and shogaols). Other components include fats, waxes, carbohydrates, vitamins such as Nicacin and Vitamin A, minerals, and zingibain, a proteolytic enzyme (Shukla and Singh 2006, 684; Murray 2004, 134).

Volatile Oil

There are over 50 components of ginger's volatile essential oil, which is present in amounts of 1-3% and is responsible for the aroma of fresh ginger (Dewick 2009, 168). Most of the components are monoterpenoids [β-phellandrene, (+)-camphene, cineole, geraniol, curcumene, citral, terpineol, borneol] and sesquiterpenoids [α-zingiberene (30–70%), β-sesquiphellandrene (15–20%), β-bisabolene (10–15%), (E-E)-α-farnesene, *ar*-curcumene, zingiberol] (Ali et al. 2008, 410).

Pungent Compounds

The non-volatile pungent principles are responsible for the hot taste sensation of ginger. These include the biologically active chemicals gingerols, shogaols, paradols, gingerdiols, and gingerones as well as derivatives.

Gingerols

The pungent taste of fresh ginger rhizomes is mainly due to the presence of homologous, phenolic ketones called gingerols (**Figure 3**). The gingerols are differentiated by the number of carbons in their C-5 side chains (Jolad et al. 2004). The compound [6]-gingerol is the most abundant at portions of 28-34%, followed by [10]-gingerol and [8]-gingerol. Gingerols

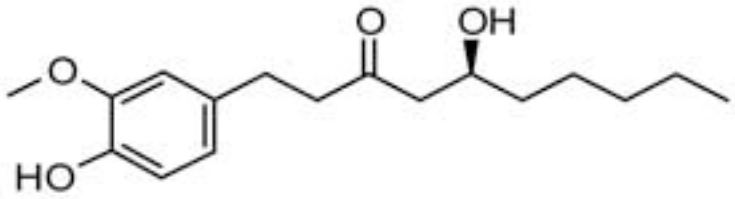

Figure 3. [6]-gingerol is the main pungent principle of fresh ginger. (Source: http://www.dalton.com/images/prod/23513-14-6.jpg)

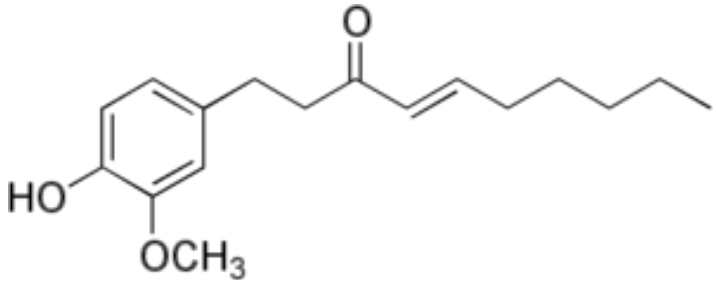

Figure 4. [6]-shogaol is the main pungent principle of dried ginger, it is the product of dehydration of [6]-gingerol. (Source: http://upload.wikimedia.org/wikipedia/commons/thumb/0/05/Shogaol.png/300px-Shogaol.png)

degrade into shogaols or paradols under pH-specific conditions (for shogaols: when pH is between 2.5 and 7.2) upon the addition of heat. Studies on isolations of [6]-gingerol have suggested antipyretic, antioxidant, and anti-inflammatory effects (Young et al. 2005, 207).

Shogaols

Shogaols (**Figure 4**) are the products of dehydration of gingerols and are thus found in the dried rhizomes of Zingiber officinale and in non-fresh ginger powder. These compounds are analogous to gingerols and contribute to the pungency of dried ginger. The structural difference from gingerols is ta double bond between C-4/C-5 and the absence of the hydroxide on the C-5 carbon (Jolad et al. 2004).

Biological Activity

Results of in vitro and in vivo studies on ginger root extract are often contradictory. One possible reason for discrepancies is the variability in chemical composition of the studied ginger, as it is dependent on whether the rhizome is fresh or dried. Besides the differences in composition of the ginger powders and extracts, another source of conflicting results could lie in the doses administered, the treatment duration, and the quality of the ginger products. As a result, standardization of ginger products is essential in researching its medicinal effects (Schwertner et al. 2006).

Anti-inflammatory and anti-pyretic

Anti-inflammatory action has been demonstrated *in vitro* and *in vivo* since the 1980s. Anti-inflammatory and soothing action from gingerols and shogaols may be the result of dual inhibition of both cyclooxygenase and lipooxygenase pathways that are involved in inflammatory processes (Yip and Tam 2008, 137; Jolad et al. 2004, 1937). Specific constituents such as gingerdiones and shogaols have properties mimicking dual-acting non-steroidal anti-inflammatory drugs *in vitro,* and the principle pungent ketones [6]-gingerol, [6]-pearadol, and zerumbone have been shown to have strong anti-inflammatory activity (Shukla and Singh 2007, 687). *In vivo,* anti-inflammatory and analgesic activity of [6]-gingerol was shown by significantly reduced writing of animals (Young 2005, 208, Chrubasik 2005, 691).

Other demonstrations of anti-inflammatory action include ginger's action against cytokines secreted at inflammation sites, ginger's modulation of some pathways activated in chronic inflammation, and ginger extract's suppression of pro-inflammatory cytokines and chemokine expression (Ali et al. 2008, 413-414).

Ginger plant extract has also been found to reduce swelling in a nearly identical fashion to aspirin. In a study of rats, dosages of 50 mg/kg of ginger extract reduced swelling in rats' paws by 22%, while an equal dose of acetylsalicylic acid reduced swelling by 23% (Mascolo et al. 1989). This study also demonstrated that anti-pyretic effects of ginger extract are similar to the effects of equal dosages of acetylsalicylic acid.

Anti-emetic

Both *in vivo* and *in vitro* studies suggest that ginger is an effective treatment for nausea and vomiting. The mechanism of action for the carminative and anti-emetic effects has not been identified. However, it has been suggested that [6]-gingerol may enhance gastrointestinal transport; that other ginger compounds have anti-hydroxytryptamine activity *in vivo*; that galanolactone, a constituent of ginger, is a competitive antagonist at ileal 5-HT_3 receptors; or that ginger affects emesis through the central nervous system (Ernst and Pittler 2000, 368). Current antiemetics act as ilieal 5-HT_3 receptor antagonists. *In vivo*, ginger juice inhibited cisplatin (an anticancer drug) -induced emesis in rats and in dogs (Sharma 1998, 54; Sharma 1997, 95).

Hypoglycaemic

Mascolo et al. found ginger extract to exhibit potential hypoglycaemic activity *in vivo* (Mascolo et al. 1989).

Anti-fungal

Of three plant extracts (ginger, onion, and garlic), an ethanolic extraction of ginger was found to be the most effective in inhibiting fungal growth (Tagoe et al. 2011, 286). The isolated compound gingerenone A exhibited complete inhibition of hyphae invation of *Pyricularia oryzae* and moderate inhibition of *Eimeria tenella* at concentrations of 10 ppm (Endo et al 1989, 798). Ginger oil and oleoresin show moderate to good inhibition of fungal growth in both food poison and inverted petri plate methods (Singh et al 2008, 3301). An antifungal protein was also identified from ginger extract, showing strong antifungal activity similar to the potency of *Asparagus officinalis* seeds (Wang et al. 2005, 103).

Anti-bacterial

Ginger extract has been shown to inhibit both Gram-positive and Gram-negative bacteria growth (Mascolo et al. 1989; Gao et al. 2010, 43). Essential oil and oleoresins of ginger exhibit inhibitory effects towards bacterial growth comparable to streptomycin and chloramphenicol. Oleoresins were shown to be less effective than the essential oil. This activity is most likely due to the phenolic compounds of the oil and oleoresin, and it is likely that the efficacy is synergistic of many of these phenolic constituents of ginger (eugenol, shogaols, zingerone, gingerdiols, gingerols) (Singh et al. 2008, 3300).

In one recent study, freeze-dried ginger was observed to have a significant effect on the relative radiation sensitivity of pork inoculated with *Escherichia coli*, providing supportive evidence of antibacterial properties of ginger (Yun et al. 2011). In a comparison of onion juice and ginger juice, however, ginger juice was found not to be effective against multi-drug resistant bacteria (Adeshina et al. 2011, 293).

Antioxidant

The antioxidant activity of ginger has been demonstrated in many *in vitro* and *in vivo* studies, and this activity may explain the protective actions of ginger against toxicity and radiation (Ali et al. 2008, 416). Bioactive components of ginger extract such as [6]-gingerol, zingerone, and dehydrozingerone have been shown to have potent antioxidant and tyrosinase inhibition activities, and zingerone may act as a scavenger of superoxide anions (Chrubasik 2005, 689; Shukla and Singh 2006, 686).

Cancer preventative effects

Cancer preventative effects of ginger have been found in studies relating to skin, gastrointestinal, colon, and breast cancer. Alcoholic extracts of ginger have been found to be cytotoxic to certain tumor cells in vitro. Both [6]-gingerol and [6]-paradol showed suppressive effects on proliferation of human cancer cells through the induction of apoptosis. Another chemical constituent, zerumbone, inhibits activation of NK-κB-regulated gene expression that is induced by carcinogens. *In vivo*, ginger and specifically [6]-gingerol were shown to suppress promotion of skin carcinogenesis in laboratory mice. Animals treated with ginger extract also showed significantly lower tumor body burdens compared to controls (Shukla and Singh 2006, 686-688). The essential oil of ginger also showed antitumor activity (Chrubasik 2005, 689).

Clinical Studies

Nausea and Vomiting

In a review of the antiemetic effects of ginger, it was reported that 1 g of ginger powder daily alleviated clinical nausea of diverse causes such as pregnancy-induced nausea, drug-induced nausea, and motion sickness (Ernst and Pittler 2000, 368). Ginger has been shown to be more effective in reducing severity of nausea in pregnant women than vitamin B6 or placebo (Ensiyeh 2009; Ozgoli et al. 2009, 245).

Rheumatism

Relief from rheumatism from ginger was shown to be comparable to that from ibuprofen (Haghighi et al. 2005). In osteoarthritis patients, reduction in knee pain on standing was superior for those taking ginger extract than placebo (Altman and Marcussen 2001).

Contraindications

Ginger root extract is generally recognized as safe (GRAS) by the FDA, but pregnant women are discouraged from use due to lack of relevant research. Some adverse effects have been reported by a study of pregnant rats including elevated birth rate of the fetal rat (Ali et al. 2004). However, the overall low toxicity of ginger and its chemical constituents have been confirmed by multiple studies (Weidner et al. 2000, Chang et al. 1995, Ali et al. 2004). Minor adverse effects observed in research include heartburn, mild diarrhea, and IGE-mediated allergy (Ali et al. 2004). Dangerously toxic effects have only been observed in extremely or unnaturally high dosages.

The potential interaction of ginger root extract with warfarin has been studied due to conflicting evidence of blood-thinning properties of gingerols, but no evidence suggests a significant effect of ginger on warfarin. Ginger root extract does have a synergistic interaction similar to aspirin with nifedipine on anti-platelet aggregation (Ali et al. 2008).

Current Use in Allopathic and CAM Therapies

Ginger extracts are commercially available around the world from multiple distributors in the form of capsules. However, the standardization of ginger root extract remains an unsolved problem. In a study of ten ginger dietary supplements, only five manufacturers listed a dosage recommendation. Of the recommendations, the serving size varied widely. Listing of expiration dates, ingredients, or methods of standardizations were not consistently present from all manufacturers. Finally, the composition of the ginger supplements was varied significantly (Schwertner et al. 2006). These results expose a problem characteristic not only to ginger supplements but also to much of the natural product market. The verification of the chemical constituents of ginger extracts is especially important due to the variability of chemical composition based on preparation methods.

Ginger is also used medicinally as a tea, where the fresh or dried rhizome is chopped and steeped in water. Ginger tea and ginger capsules are the most used natural product by pregnant women suffering from nausea, vomiting, and "morning sickness" symptoms (Hollyer 2002). Most women in this study who did not use CAM to alleviate nausea and vomiting reasoned that they wished to know more about the safety of ginger extract and other natural products. The safety of medicinal use of ginger in pregnant women has yet to be intensively studied.

Massage therapists and aromatherapists take advantage of the healing properties of ginger essential oil. Few of the ginger remedies using used in these manners have been clinically evaluated for safety in efficacy, though their uses are extensive. In one study, however, both ginger essential oil and ginger powder were shown to relieve pain when used in massage therapy (Yip and Tam 2008, 137).

Discussion

The use of ginger for multiple millennia serves as support to its efficacy and pertinence in human health. Because of ginger's low cost and low toxicity, there are few, if any, deterrents to pursuing research on the chemical components of ginger and their roles in both biomedicine and CAM. Because ginger is not found growing wild, as long as cultivation promotes biological variation, there is little danger in continuing the large-scale cultivation and harvesting of ginger. The variability of the chemical makeup of ginger so dependent on its preparation is a prime example of a research area for ethnobotanists. Ethnobotanical approaches could potentially expose different methods of preparing and storing the rhizome and their applications to different treatments.

Especially of clinical interest is the role of ginger extract in cancer treatments. Because of the anti-emetic properties, ginger would be useful in relieving nausea induced by chemotherapy. Simultaneously, the antioxidant activity of ginger extract undoubtedly plays a role in reducing the oxidative stress that may accelerate tumor growth. Further research is necessary in this field as some studies have found conflicting evidence into carcinogenic properties of ginger's chemical constituents. Ginger also shows promising results in clinical studies with osteoarthritis, and there is substantial support for ginger's effectiveness as an antiemetic for diverse causes.

Until ginger rhizome extracts are standardized for maximum effectiveness, the biomedical future of ginger extract seems to be more promising than ginger's use as a natural product. For one, the healing properties of gingerols are not found in ginger powder unless it is a fresh ginger preparation, therefore the use of dried ginger or ground ginger in the kitchen is not likely to provide benefits in consumption. As ethnobotany becomes

more closely tied to biomedical and pharmacological research, *Zingiber officinale* will remain a prime investigational target.

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