

Tinospora cordifolia: the antimicrobial property of the leaves of amruthaballi

Abstract

Medicinal plants are known for their bioactive compound which is responsible for various beneficial characters, in this case, anti-microbial character. In this paper, the anti-microbial character of shade dried and powdered *T. cordifolia* leaves were tested and observed against a test microorganism *Escherichia coli*. Three different solvent extracts, i.e. ethanolic, methanolic and aqueous extracts of the leaf were taken and then tested for their degree of anti-microbial nature against *E. coli* using slip disc method. The degree of the character of the plant extract was inferred by the comparative studies of the zone of inhibition (in mm diameter). The results observed from the experiment prove to us that the natural medicinal character of the climber is an economical alternative form of medicine compared to the currently used ones, with fewer side effects to the consumers and easy availability.

Keywords: *tinospora cordifolia*, amruthaballi, *escherichia coli*; anti-microbial activity, leaf extract, disc diffusion method

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Introduction

From time immemorial, the mankind has depended on the nature and her products for the sustenance and the well-being and the pages of the evolution in the lifestyle of mankind has been decorated with the dominant presence of medicinal plants. Of all the products that the nature has to provide us, the plants of nutritive and therapeutic properties are her jewels and obvious reason, these medicinal plants have been the most exploited and depended sources for man. Medicinal plants are those plants which exhibit medicinal and therapeutic properties in the form of biologically active compounds in the form of secondary metabolites and these metabolites are found either incorporated in the plant parts like leaves or flowers, seed or bark or sometimes found in the form of exopolysaccharides, resins and gums. The recent discovery has pointed that the rhizosphere surrounding the plant roots have therapeutic properties that can be used and exploited by the mankind for his betterment. The medicinal plants have always been the hub and center for cultural and medicinal prosperity in various cultures and of all the cultures, the central and south Asian cultures and spirituality has been deeply rooted and supported by medicinal plants and their products. Various sages and ancient physicians like Susruta, who wrote the "Susruta Samhita" which praises the various medicinal and therapeutic properties of many plants. The ayurvedic scriptures depicts the transfer of the medicinal knowledge from godly sources to sages and thereby passed to human society via physicians in written form and orally transmitted knowledge from gurus (teachers) to their pupils. The practices usually uses well designed concentrations of substances like minerals, metals along with the herbal compounds in set manner for protective and curative effects.¹

The scientific bodies have always been inquisitive and amazed by the medicinal plants for their beyond scientific explanation behavior and have always tried to replicate their effects in the laboratory conditions. Like seen, they have not been able to infuse and bind the ocean similar effects of the medicinal plants in the synthetic and

semi-synthetic form. So the other concept of clubbing this age old medicinal form in the advanced technology methods is now widely followed. This method requires the well informed knowledge of the nature and working of the medicinal plants in their native and in combinational form. The know-hows and the in-depth working principles are important to justify the substantial medicinal and therapeutic value that has been tagged to the plants.²⁻⁴

Out of the many medicinal plants that have been seen throughout the globe, they have geographically found in strong numbers and multiple varieties in the tropical and sub-tropical regions of the Earth. Out of these regions, the Asian and African continents have the most quantity of medicinal plants and the extent of the medicinal properties that these medicinal plants bear are of very high extent. This is evidently seen in the dominant presence of medicinal plants and their compounds in the daily life and cultural practices of the respective places. India has always been known for her rich cultural and spiritual practices and the extent of the effect that the medicinal plants have on the culture, history and in the lives of the people dwelling in India is clearly visible. So the well-known and in-depth knowledge of both the medicinal plants and their interactions with the advanced technology and how these in native and in combined form react with the biological system and bring about the desired medicinal and therapeutic properties.

Tinospora cordifolia is a medicinal plant whose status in the field of natural medicine and Ayurveda is of the highest order. Vernacularly speaking, *Tinospora cordifolia* is known "Guduchi" whose origin is rooted to Sanskrit; and is known as "Amruthaballi" in Kannada and is an important drug of the Indian System of Medicine (ISM). *T. cordifolia* is an esteemed medicinal plant whose uses and application with reference to human benefits have been praised to indescribable heights in various ayurvedic and Vedic scriptures and the practices. The medicinal plant of interest in this paper, *Tinospora cordifolia*, a climber plant of great medicinal property which is widely and popularly used in the ayurvedic and local forms of medicine is

studied in the phytochemical and different components that exhibit the properties that have been celebrated and upheld in the age old traditions and medicinal practices.

Botanical classification

The plant is popularly known as Guduchi, is an herbaceous vine belonging to the Menispermaceae and is found normally deciduous and dry forests. The botanical classification of this medicinal herb is given below;

Kingdom: *Plantae*.

Division: Magnoliophyta.

Class: Magnoliopsida.

Order: Ranunculales.

Family: Menispermaceae.

Genus: *Tinospora*

Species: *T. cordifolia*.

Habitat and description

As described in the classification, *T. cordifolia* belongs to the *Menispermaceae* family and the shrubaceous deciduous plant that grows to about 3–4 feet in height and is about 1 feet in width. The climbing plant is seen to bear lots of spreading slender branches which grab on to the nearby objects for support. The leaves that are seen are simple, alternate, and exstipulate, with petioles up to 15cm in length, bearing roundish and pulvinate leaves at apex and basal region; the basal region being much longer and partially twisted half way around. The flowers that are seen are observed are small and unisexual; female and male flowers are seen in different plants. On the flowering season, the plants bear no leaves and the flowers bear yellowing green color and the flowers are positioned at the apex and terminal racemes. The differentiations in the sexes are seen in the form that the male flowers are usually clustered and the female flowers are solitary in positioning. The sepals and petals are 6 in number and are usually free or grouped in 2 or 3 numbers. The fruits are found in an aggregate of 1–3 drupes with scarlet or orangish coloring. The seeds are curved and pea sized and are transverse dehiscent in nature. The roots which are present in this plant are seen in both underground and aerial form (Figures 1–3).



Figure 1 The heart shaped leaves of *Tinospora cordifolia*.



Figure 2 The seeds and flowers of *Tinospora cordifolia* plant.



Figure 3 A botanical diagram describing the leaves, flower, seed and various other arrangements of the *Tinospora cordifolia* plant.

History and vedic references

The use of this medicinal plant has been described in detailed manner in Vedic and ayurvedic scriptures. The plant is known as Guduchi or Amrita in Sanskrit which points to the nature of this plant in the rejuvenating and the retainment of youth and life span of the consumer. In other words, the fountain of life force is an apt title for this medicinal plant. The Caraka Samhita, Sushruta Samhita, Bhela Samitha, Kashyapa Samhita and Ashatanghrdayam are few of the noted works that have detailed description of the medicinal plants in the field of spiritual and health field of the biological system that they are introduced to. The influence of Persian, Arabic, folk medicines in the life style of man along with the Vedic and Ayurvedic practices has heavily influenced the normal household life along with the scientific know-hows in the molecular level with the biochemical and phytochemical composition of the plant and the plant compounds has done a great deal in the understanding of this miraculous plant. The traditional and folk medicine with no scientific basis has been strongly advocating the regular use of the medicinal plants in the dietary form or in supplementary form; this is credited to the observational knowledge and the information which is passed from godly beings to the sages and to general bodies through Gurus (teachers).

The abundant medicinal plants and the Vedic scriptures that point to the correct usage of these plant for the optimum beneficial effect has spiked the interest of the science bodies and the further research on these plants on the scientific platform has invidently pointed to the same results that have been preached from time immemorial by the traditional forms of medicine.

Phytochemical composition

The detailed scientific studies have yielded the discovery of

various compounds in the plant extract of various solvent natures that are either directly or indirectly responsible for the expression of biological characters in the plant or host system. The phytochemical composition of *Tinospora cordifolia* has been discussed below.

The different classes of compounds which are found in this plant are classed in groups like alkaloids, steroids, terpenoids, polysaccharides, glycosides and different aromatic and aliphatic compounds that are present in their phytoactive form that are responsible for the wide range of medicinal and therapeutic properties. The presence of these compounds is found in various plant parts but highly concentrated in the stem, leaves and root part of the plant.⁵ The main compound of this plant is berberine and furanolactone and furthermore compounds like tinosporone, tinosporic acid, cordifolisides A to E, giloin,

gilenin, crude giloininand, arabinogalactan polysaccharide, picrotene, bergenin, gilosterol, tinosporol, tinosporidine, sitosterol, cordifol, heptacosanol, octacosonal, tinosporide, columbin, chasmanthin, palmarin, palmatosides C and F, amritosides, cordioside, tinosponone, ecdysterone, makisterone A, hydroxyecdysone, magnoflorine, tembetarine, syringine, glucan polysaccharide, syringine apiosylglycoside, isocolumbin, palmatine, tetrahydropalmatine, jatrorrhizine are few of the compounds that have been isolated from the plant. The presence of three compounds like cycloephordenol, Cyclohexyl-11-heneicosanone and 2-Hydroxy-4-methoxy-benzaldehyde has been isolated from the plant and has been seen to be present in various other plants. The presence of proteins and miscellaneous compounds has been attributed to the medicinal properties of the plant (Table 1) (Figure 4).^{6,7}

Table 1 The table describes the various secondary metabolites present in the plant with the source of location and the biological function they contribute to.¹⁰

active component types	Compounds	Source	Reported biological effects in animals	In humans, cell lines	References
Glycosides	Berberine Choline Palmatine Tembetarine Magnoflorine Tetrahydropalmatine Tinosporin Isocolumbin Tetrahydropalmatine Jatrorrhizine Aporphine alkaloids, N-formylasimilobine 2-0-p-D-glucopyranosyl-(1 →2)-j3-D-glucopyranoside (tinoscorside A, 1) Aporphine alkaloids, N-acetylasimilobine 2-0-p-D-glucopyranosyl-(1 →2)-j3-D-glucopyranoside (tinoscorside B, 2)	Stem root	Isoquinoline alkaloids have anti-cataract potential in rats. Anti-oxidant activity in mice, anti-cancer in (EAC) mice, hypoglycemic activity in Rlhmsf rat insulinoma cell line	Anti-cancer, anti viral infections, inflammation And immuno-modulatory roles. Neurological, psychiatric conditions, ehriich ascites carcinoma anti-diabetes	4,5,7-10
	I S-norclerodans glucoside Furanoid diterpene glucoside Tinocordiside Tinoordifolioside Cordioside Palmatosides	Stem	Cytotoxic action, protection against iron-mediated lipid peroxidation of rat brain homogenate, anti-oxidant and hydroxyl radical scavenging activities in Swiss albino mice	Treats neurological disorders like ALS, parkinsons, dementia, motor and cognitive deficits, and neuron loss in spine and hypothalamus. Immunomodulation: IgG increase ¹¹⁻²⁰ and macrophage activation. Inhibits NF-kB and act as nitric oxide scavengers to show anti-cancer activities	
Diterpenoid lactones	Furanolactone Clerodane derivatives E(5R,10R> 4R SR dihydroxy-2S-3R: 15,16-diepoxy-cleroda-13 [15), 14-dieno-17,12S: 18,1 S-dilactonej Tinosporides	Whole plant	Chemopreventive potential diethylnitrosamine (DEN) induced hepatocellular carcinoma (HCC) in rat.	Vasorelaxant: relaxes Norepinephrine induced contractions. Inhibits Ca ⁺ influx. Anti-inflammatory, anti-viral. Induce apoptosis in leukemia by activating caspase-3 and bax, inhibits bcl-	21-26
Steroids	hydroxy ecdysone Ecdysterone Giloinsterol	Stems aerial parts	Beta-Ecdysone shows anabolic and anti-osteoporotic effects in mammals	IgA neuropathy, glucocorticoid induced osteoporosis in early inflammatory arthritis, induce cell cycle arrest in G2/M phase and apoptosis through c-Myc suppression. Inhibits TNF-α, IL-1β, IL-6 and Cox-2. Activate NF-kB	27-30

Table Continued...

active component types	Compounds	Source	Reported biological effects in animals	In humans, cell lines	References
Aliphatic compounds	Ootaoosanol Heptacosanol Nonacosan-15-one dichlromethane	Whole plant	Radiosensitizing activity in ehrlich ascites carcinoma mice. Modulating the pro-inflammatory cytokines. Inhibits proliferation of endothelial cells and Ehrlich ascites tumor cells	Anti-nociceptive and anti-inflammatory. Protection against 6-hydroxydopamine induced parkinsonism in rats. Down-regulate VEGF and inhibits TNF- α from binding to the DNA	8,31-34
Other	3, (a,4-di hydroxyl -3-methyl-benzyl)-4-(4-hydroxy-3-methoxy-benzyl)-tetrahydrofuran Jatrorrhizene N-trans-feruloyl tyramine Giloin Tinosporic acid	Root whole plant	Insulin mimicking and insulin releasing effect. Enhanced phagocytic activity of milk polymorphonuclear cells in bovine subclinical mastitic	Protease inhibitors for HIV and drug resistant HIV. Tyramine is a neuro-modulator. Used to treat anxiety and depression by inactivating neurotransmitters	10,35-39

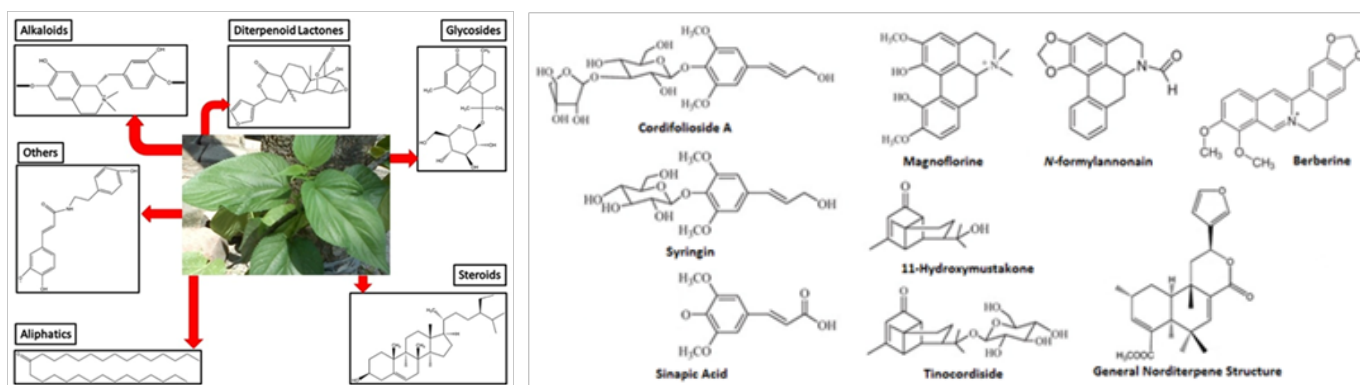


Figure 4 The above are few of the many biologically active compounds present in the plant that is responsible for the various medicinal and therapeutic properties.

The above mentioned compounds are few of the many compounds present in the plant that contribute to the medicinal and therapeutic properties of *T. cordifolia* plant. The plant contains a high amount of fiber totaling to an total estimate of 15.9% and the protein content to about 4.5–11.2%, the total carbohydrate estimate to about 61.7 & and a low fat amount estimating to about 3.2% and the mineral content totaling to about 0.845% of potassium, 0.006% chromium, 0.28% of Iron and 0.131% of calcium. All these compounds are involved directly or indirectly in the pathways or regulatory, metabolic and cellular nature.

NF-kB, nuclear factor kappa-B; VEGF, vascular endothelial cell growth factor; TNF, tumor necrosis factor; IL, interleukin; COX, cytochrome oxidase; ALS, amyotrophic lateral sclerosis; IgG, immunoglobulin G; IGA, immunoglobulin A

The paper has discussed of the many biochemical compounds that have been found in the medicinal plant of interest and the root cause of these compounds can be found to be traced to the photosynthesis which are the unique property of the green plants by which they synthesize their energy from the inorganic minerals and water from the soil in the presence of sunlight. Though at the beginning the relation between the biochemical compounds that is responsible for the medicinal properties and the process of photosynthesis cannot be clearly stated; the close observation of the biochemical nature of the phytochemicals can be linked to intermediate, by products and the end products of the photosynthesis and biochemical cycles that the plant deploy to sustain themselves.⁸

Medicinal and therapeutic properties

The plant has been titled to many properties that have been used from time immemorial and few of them include curative properties against Jaundice, fever, gout, urinary and upper respiratory infections and preventive measures against skin infections, chronic diarrhoea, bleeding piles, dysentery, itching and erysipelas. The plant is known for its potent aphrodisiac nature and its rejuvenating nature.⁹ The plant extract influences the secretion of bile liquids and is known to enrich the blood constituents. The effect that the plant extract has in both adult and children systems is similar and this is a good factor in the administration of the plant drug irrelevant to the host age which is not the case in synthetic drugs which base the age of the consumer to be a major factor in drug administration.^{8,10,11} The plants show anti-diabetic properties due to the presence of tannins, alkaloids, flavonoids, glycosides, saponins and steroids. The plant has seen to have effect on both the sexual arousal and the sexual performance of the biological systems and these drugs have stimulatory effect on the copulatory behavior and thereby entitled to aphrodisiac activity. The most admirable character of the plant extract is the effect it bears in the cognitive field; its ability to affect the learning and memory cortex of the human intelligence is an admirable trait and this trait has attracted the interest of various scientific and industrial bodies. The plant also bears anti-oxidant, anti-inflammatory, anti-tuberculosis; wound healing, immunomodulatory and immunoprotective, hepatoprotective, anti-osteoporotic, anti-cancer, anti-tumor, anti-malaria, cardio protective nature and many more properties that make

them a topic of great importance and interest.^{5-7,12-19} Their effect on the fat and lipid metabolism and the rate of blood fat deposition is observed to be a direct or indirect reason for the expression of many activities mentioned above. Many of these properties have been studied in detailed manner in animal models like rats and mice for the in-depth knowledge of the manner and pathway that is opted to achieve the biological process.

This experiment concentrates on the anti-bacterial property of the leaves of *Tinospora cordifolia* against a human clinical pathogen namely *Escherichia coli*.²⁹ There has been an alarming rate in number of the microorganisms that have become increasing potent and resistant towards the synthetic and semi-synthetic drugs that the man has become used to. This is mainly due to the indiscriminating and rather repeated usage of the drugs and antibiotics for treating the infections and discomforts that can rather be left on its natural course for cure. This repeated use has made the microorganisms to employ ways and mechanisms to escape the effect of the drug at cellular and metabolic levels and thereby leading to the emergence of multiple drug resistant strains of the pathogens which at natural course of existence are rather harmless or weak pathogens.¹⁹ So the reversion in the application of medicinal products to counter the effect of the pathogens is gaining the interest and attention of scientific and industrial bodies. It is presence of biologically active phytochemicals like berberine, an alkaloid compound and the bitter compounds like tinosporin, tinosporic acid, tinosporal, a complex mixture of fatty acids and essential oils that are responsible for the expression of the antibacterial or the general antimicrobial activity of the plant extract.^{8,10,15,18,20,21}

The expression of biological processes mentioned above have endured that the medicinal status of this plant is of high orders and the socio-ethnic status is also no less than that of the medicinal status. The dietary and nutritional values along with the medicinal and therapeutic values of the plant are added bonus to the immense amount of the physiological and pharmacological activities of this plant. The properties attributed to the plant by the ayurvedic and traditional scriptures are evidence to the therapeutic properties that have been celebrated by the folk medicine and god-like status given to the plant and thereby attracting the interest of industrial and pharmacological companies.

Mode of action of medicinal plants

Human have always relied on nature and her remedies to relieve himself of bodily and mental discomforts. The primary source of all natural drugs is traced to the medicinal plants and few microorganisms. From time immemorial, various traditional and folk forms of medicine have appreciated the medicinal plants for their miraculous therapeutic properties that have attracted the interest of the modern medicine and industries.²² Upon extensive studies on the medicinal plants, it has been observed that the presence of low molecular weight compounds of wide structural diversity found in almost all medicinal plants are responsible for the expression of medicinal properties. These compounds are termed as secondary metabolites and these compounds are very important for the survival and protection of the plant. The compounds are secreted in small amount compared to other compounds which are involved in the metabolic and physiological pathways; but their involvement in the protection aspect is of at most importance and these compounds are involved in many pathways like cellular signaling pathways, either directly or indirectly involved in processes like pollination, repelling insects or harmful organisms.²³

No matter what the build-up of the host, the plant or the structural diversity of the secondary metabolite, the general mode of working

is that these secondary metabolites target the cell surface receptors that include ion channels, enzyme linked receptors, neuroreceptors, ion pumps or receptors linked to cytoskeleton structures. This process is rather explained in detailed manner using the selective process of evolutionary molecular modeling and how these structures evolve along the timeline of evolution and yet they maintain their therapeutic properties. Upon closer analysis of the working of these compounds, it has seen that the optimum effect of these compounds is due to the synergic effect of the multiple biologically active phytochemicals and their interactions that are yet to correctly known and understood. The mediation of the biological activities can be attributed to the modifications that occur at the protein and DNA bases and these modifications are due to reactive group and side chains that the secondary metabolites contain. These groups and side chains that are responsible for such modifications include aldehydes, SH-groups, epoxides, double bonds with enon configuration, triple bonds and these side chains and biologically active groups either form covalent bonds with the amino acids residues of the protein or the DNA bases.²² These bonds bring about conformational changes and thereby bringing about the biological process under physiological conditions. The modification at transcriptional and translational level is also observed to be the reason for the expression of the biological activities. The alterations at the genes involved in the transcription and translation process and the modification of the transcription factors are also seen to be involved in bringing about the desired change either by the up-regulation or down-regulation of the gene that undergo the modification. Upon the advent of the softwares and sequencing techniques using bioinformatics, it is evident that these secondary metabolites affect multiple proteins and genes mediating single or multiple processes. This is in favor to the complex process of epigenetics that is observed in human system and higher vertebrates.²⁴

These secondary metabolites have an affinity to the cellular membranes that maintain the cellular concentration, osmotic pressure and the influx-out flux of the cellular contents and alter their structural and functional being and thereby bringing about cell death or disability. The activity at the ion channel and pump systems is altered and thereby disturbing or altering the ionic and osmotic gradient in the cellular environment. Certain classes of secondary metabolites alter the polymerization and depolymerization of the compounds of the plasma membrane, the synthesis and activation of collagen and collagen related enzyme and thereby altering the cellular density and elasticity. Certain secondary metabolites get involved in the metabolic pathways as a substituent compound to primary metabolites like acetic acid, fumeric acid, malic acid and furthermore.^{22,25}

Certain secondary metabolites of the class ergot alkaloids which are of microbial origin affect and modulate the activity of noradrenaline, serotonin and dopamine receptors and influence the agonistic and antagonistic behavior of the cell which is either way responsible for the antimicrobial nature and process like muscle contraction and lactation are also mediated by this class of secondary metabolites.²⁶ These compounds have the ability to interfere with the telomere and telomerase activity which plays an effective role in the control of cancer. The control of cellular proliferation and block in the cell division by blocking important enzymes and transcription factors that are involved in the process are few of the pathways and factors that are targeted by the secondary metabolites in controlling and eliminating the cancer.²³

Thought the rough outline of the various pathways and mechanisms that the secondary metabolite employs to bring about the necessary biological modifications and relief measures have been

studied, the entire process of how these compounds bring about the wide array of characteristics is still not correctly brought to light and further research are solely conducted in finding new characteristics or behaviors of these medicinal plants and further knowledge on how these compounds work in a biological system and in what manner these compounds interact with other compounds in synergic manner to bring about the therapeutic behavior.^{22,24}

ILL-effects of the plant product

No proper clinical and research evidence has been observed or reported in reference to the adverse effects that the plant exerts. The animal trials on the toxicology aspect of the plant extract are still in the infancy stages and the information is weakly credited to. It is seen that in healthy mice and rabbit models, a high oral dose is required to show adverse effect; doses exceeding 1000mg/kg of the whole plant extract in case of rats and 1.6g/kg of stem extract in case of rabbit showed adverse effects. At extreme cases, the animal models that were subjected to the plant extract showed fatal symptoms.^{5,13,26}

It is also seen that the usage of this plant extract in case of lactating mothers and patients suffering from autoimmune disease is not advised and thereby caution is advised in the usage of the plant and the plant products are to be used on the terms of the physician's prescription.¹⁴

Diseases affecting *Tinospora cordifolia*

The *Tinospora cordifolia* plant had been appreciated to high levels for the medicinal, therapeutic, curative, healing and relieving nature. Though the plant has a strong defense against microorganisms, pests and insects, the plant in itself is not invincible and is bound to be affected by various diseases and one of the diseases that prominently affect the plant have been mentioned below. The new flat stem disease that infects the branches and the infected plant bears small leaves and it was observed that the same nodal region bears multiple new leaves ranging to about 20 to 30 in number and the older leaves are fewer in number and compared to normal plant the internodal region is shorter in the winter and the causal organism of this disease is phytoplasma. The differences in the infected and normal plant is not just restricted to the physical appearance but the irregular arrangement the phloem and xylem systems are visibly observed and the sieve components and the vascular bundle bear altering arrangements to the normal cells²⁷ (Figure 5).



Figure 5 The above figure shows the differences between normal and infected plants at the external appearance and at the cellular orientation wise. Box (A) depicts appearance of healthy plant; box (B) and (C) depicts the appearance of an infected plant. Box (D) depicts the vascular bundle arrangement in healthy plant and box (E) depicts the xylem and phloem arrangement in an infected plant and the circled area indicates the presence of phytoplasma. The cells were stained using Diene's staining method.⁹

Materials and methods

Materials

The *Tinospora cordifolia* leaves were collected from Dixit clinical from Vijaynagar in Mysore, Karnataka. The leaves of healthy nature were selected and used for the experimental purpose.

Methodology

Plant extract: The leaves of the climber that were selected was washed thoroughly and wiped carefully using cotton cloth without damaging the leaf. The wiped leaves were then shade dried for complete drying and upon drying the leaves were finely powdered using mortar.

Test microorganism: Human pathogenic bacteria, *Escherichia coli*, a Gram negative rod aerophilic bacterium was collected from JSS Medical College, Mysore, India (Anil Kumar Sir). The plates obtained were cultured on Nutrient Agar Medium. This parent culture was sub cultured in NA medium for 48 hours to obtain stable strains. This test microorganism was then used on the plant extract to test the degree of antimicrobial character exhibited by the extract.

Preparation of plant extract: About 60g of shade dried powdered *T. cordifolia* leaf was taken and divided into 3 groups of each 20g of leaf powder. Each group was then subjected to polar and aqueous extract. The leaf powder was soaked in 50ml of the respective solvents like ethanol, methanol and aqueous extract.

The soaked plant extracts were then incubated at room temperature for two days. The obtained concentrated yield is of crude nature and this is further used to test the anti-microbial nature of the neem leaf extract against the test microorganisms.

Determination of anti-microbial nature of the plant extracts

The anti-microbial nature of the plant extract was tested by slip disc method. In this method, each Petri plate which was swabbed with the test microorganism was placed with Whatmann no.1 filter paper discs of different concentrations. Now these plates are placed in the incubator for 48hr. The zone of inhibition (in mm diameter) was measured at regular time intervals of 0hr., 12 hr., 24hr. and 48hr. Control was simultaneously taken and the results were tabulated and inferred. The comparative potent of the anti-microbial nature of the different solvent extracts of the leaves were then evaluated by comparing the ZOI exhibited.

Result and discussion

The antibacterial extent of the leaves was seen in the form of zone of inhibition observed in the Petri plates (Table 2).

Table 2 shows the varying zone of inhibitions that the leaf extract showed against the human pathogen *Escherichia coli* in ethanolic, methanolic and aqueous extract. The tabulations were done in four differentiating concentrations namely 0mg/ml, 25mg/ml, 50mg/ml and 100mg/ml of plant extract in respective solvents.

It is seen that the ethanolic and methanolic extracts of the leaf showed good zone of inhibition at 50mg/ml and 75mg/ml in case of ethanolic extract and 25mg/ml and 50mg/ml in case of methanolic extract. In both the solvents even at 0mg/ml zone of inhibition was observed at 12, 24 and 48hours with a marginal difference of 0.1 or 0.2mm. This can be attributed to the inbuilt and natural antimicrobial and microbicidal nature of the ethanol and methanol solvent.²⁸⁻³⁵ Thereby the inbuilt solvent nature further supports the antibacterial

nature of the plant extract. The aqueous extract showed very marginal zone of inhibition that could not be measured in accurate measurement. This can be due to resistance of the microorganism

against the aqueous extract and this can be attributed to the possible error in the handling of the plant extract or the organisms and the possibility of contamination.

Table 2 The antibacterial extent of the leaves was seen in the form of zone of inhibition observed in the Petri plates

Sl. No	Solvent used	Solvent conc	Micro organism	Zone of Inhibition Exhibited			
				T0	T12	T24	T48
1	Ethanol	0mg/ml	<i>E.coli</i>	0 mm	1.1mm	1mm	1mm
		25mg/ml	<i>E.coli</i>	0mm	1.4mm	1.5mm	1.6mm
		50mg/ml	<i>E.coli</i>	0mm	1.7mm	1.6mm	1.7mm
		75mg/ml	<i>E.coli</i>	0mm	1.9mm	2mm	2.1mm
2	Methanol	0mg/ml	<i>E.coli</i>	0mm	0.9mm	0.7mm	0.8mm
		25mg/ml	<i>E.coli</i>	0mm	1.5mm	1.9mm	1.7mm
		50mg/ml	<i>E.coli</i>	0mm	1.2mm	1.5mm	1.8mm
		75mg/ml	<i>E.coli</i>	0mm	1.7mm	1.4mm	1.2mm
3	Distilled water	0mg/ml	<i>E.coli</i>	0mm	*	*	*
		25mg/ml	<i>E.coli</i>	0mm	*	*	*
		50mg/ml	<i>E.coli</i>	0mm	*	*	*
		75mg/ml	<i>E.coli</i>	0mm	*	*	*

The results obtained in the course of the experiment was just a rough estimate in finding the medicinal property in terms of antibacterial and antagonistic activity of the plant and the extent to which these medicinal plants exert their medicinal and therapeutic nature. The observed results can be observed due to the presence of various classes of phytochemical compounds like alkaloids, terpenoids, glycosides, flavonoids, essential oils and many more biochemical substances that affect the host or effect the target microorganism. The phytochemical screening of the extracts of test medicinal plant have shown the presence of phytochemicals like tannins, phylobatannins, alkaloids, phenolic compounds, flavonoids, steroids, sugars, resins, aromatic compounds, minerals and various primary and modified compounds that contribute to the expression of properties like anti-microbial, anti-inflammatory, Anticancerous, protection against heart disease, infections, bodily discomforts and harmful radiations and chemicals and various other properties that have not yet properly understood and discovered.

Conclusion

Man has always relied and depended on nature and her miraculous remedies in the form of medicinal plants to relieve him of the many ailments that his mortal body was and presently is subjected. Of the many flora that inhabit the same planet as we do, only a fraction of them have been titled to have medicinal properties and extensive studies have been conducted in scientific perspective to estimate the degree of medicinal properties. The obtained results have just encouraged the utmost importance of these plants in the social, economic, cultural and health parameters.

Though the field of experimental biology is rather new to medicinal properties of the plants, the pages of history are no stranger to the miraculous properties of these medicinal plants. The observational biology has played an important role in the ancient and folk medicinal practices. The ancient Egyptians, Chinese, Indian, Greek, Roman and Hebrew have given high status of importance to these medicinal

properties and these can be confirmed by the various scriptures and sacred places which have been etched with stories and writings praising the medicinal plants to heaven heights. Though the practices may not hold any importance to advanced and synthetic medicinal properties, the close observation of such practices show deep knowledge of botanical and medical knowledge. Various botanists and personal of biology like Theophrastus, Mithridates, Dioscorides, Pliny, Galen, Avicenna, Nicholas Culpeper, Samuel Hehnemann, Carolus Linnaceus, Charaka and many more have voiced their acceptance of the medicinal properties of plants and their importance to mankind via their literature works and field work.¹⁰

Of all the advantages that man has obtained from the advancement in technology and industrialization, man has lost one of the most important matters and that is health. The constant pressure and ever imposed stress has man on wheels and the society implications on the outwardly appearance and beauty have made the importance and necessity of inner health and beauty near to nil. The result of this fast life is the declining in the lifespan, life style and quality of life and this is just not in case of adults but the effect of this fast and polluted life is seen in children and young adults as well. The quality of life that our ancestors led are now reduced to evidences on the pages of history.

The decline in the health of both man and nature has drawn the attention of many and efforts have been made to improve and revive the lost wealth of age old practices. The first step in the revival process is the knowledge and wealth of the natural chemists, the medicinal plants. From time immemorial, man has depended upon medicinal plants for relief, protection and as nutrient supplement and many forms of traditional medicines gives such medicinal plants godly status. Inspired by such miraculous medicinal and therapeutic properties, the scientific body set out to test these medicinal properties of the medicinal plants that have been celebrated in the Ayurvedic and folk medicinal scriptures and the result of such scientific experiments did not land far from the theorized results in the scriptures. The scientific explanation served to be supportive evidence to the folk medicine and

thereby further strengthening the viability and effectiveness of the medicinal plants and the practices that revolve around the medicinal plants.²

The above experiment is just a small effort in knowing the medicinal plant of interest and a basic platform for further experiments that can be done to support the medicinal plant. The further scope of this experimentation is the use these medicinal plants in their crude form as well as in forms that are supported by modern medicinal practices and protocols to enhance the already existing medicinal property as way of improvising the available resources in reaching the increasing population. The more the knowledge of these miraculous plants gets discovered, the more remains to be discovered. The basic working of these phytochemicals is the unknown manner at which these compounds interact with each other and with the compounds and cells that are present in the cell. The manner through which these compounds behave with toxic and harm less cells and compounds, the manner by which the differentiation of good and evil is attained by these phytochemicals and their behaviors in a living biological system is unknown; and this is one of the main fundamental reason as to why the synthetic drugs have not been able to match the efficiency and efficacy of the natural drugs and the plants that house such miraculous compounds. Taxonomically speaking, each plant varies in the expression, utilization and the degree of expression of primary and secondary compounds and this makes each plant, their plant extract and the compound themselves very unique and thereby their value in economical, medicinal, ecological and cultural fields irreplaceable and priceless. The synergic action of these compounds has always been interest attracting to plant biochemists and pharmaceutical companies which search for new and more substantial sources of medicine.^{1,3} It is also said that the nature exhibited outwardly is dependent on the genetic makeup of the plant and the knowledge about such genetic makeup helps us understand the plants and their nature better. This understanding at genetic level is better achieved by using biotechnology techniques and genetic techniques like PCR, RFLP, Genome studies and various other scientific techniques.¹⁶ The reversion back to the age old medicines to face and counter act the current and challenging problems is the testimony in the strength and endurance that the medicinal plants possess and hold on to.

The age old knowledge with the touch of modern technologies is theoretically said to the new hope of medicine where the base is founded the phytochemicals from the plants and the alterations to the mode of delivery are formed by the advanced technology. The efforts made in achieved in this field to achieve desirable results are in their infancy stage and further work and efforts are to be put in to achieve the final picture. The understanding and knowledge of medicinal plants and the age old practice is the primary and most important step in achieving the final result. This effort is to bring age old traditions with required changes in accordance to the current situation using technologies.

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Conflict of interest

The author declares no conflict of interest.

References

- Gurib-Fakim A. Medicinal plants: Tradition of yesterday and drugs of tomorrow. *Mol Aspects Med.* 2006;27(1):1–93.
- Thomas WAR. *Medicines from the Earth.* Maidenhead, United Kingdom, UK: McGraw–Hill Book Co; 1978.
- Eisner T. Prospecting for nature's chemical riches. *Issues Sci Technol.* 1989;6(2):31–34.
- Rios JL, Recio MC, Villar A. Screening methods for natural products with antimicrobial activity. A review of the Literature. *J Ethnopharm.* 1988;23(2–3):127–149.
- Kirti Sinha, Mishra NP, Singh J, et al. Tinospora cordifolia (Guduchi), a reservoir plant for therapeutic applications: A review. *Indian Journal of Traditional Knowledge.* 2004;3(3):257–270.
- Jamal A, Abdul RK, MohammadKA, et al. Phytochemical, antioxidant and antiproliferative studies of some medicinal plants from indian subcontinent. *British Journal of Pharmaceutical Research.* 2016;11(6):1–11.
- Sharma U, Bala M, Kumar N, et al. Immunomodulatory active compounds from Tinospora cordifolia. *J Ethnopharmacol.* 2012;141(3):318–326.
- Reader's Digest. Magic and medicine of plants. *Reader's Digest Assoc.* 1986. p. 42–44.
- Gaur LB, Singh SP, Gaur SC, et al. A Basic Information, Cultivation and Medicinal Use of Tinospora cordifolia. *Pop Kheti.* 2014;2(3):188–192.
- Reader's Digest. Magic and medicine of plants. *Reader's Digest Assoc.* 1986. p. 51–73.
- Shanthi V, Nelson R. Antibacterial activity of T. cordifolia (Willd) Hook. F. Thoms on urinary tract pathogens. *IJCMAS.* 2013;2(6):190–194.
- Wichtl M, Bisset NG. *Herbal drugs and phytopharmaceuticals.* USA: CRC press; 2000. 708 p.
- Kavya B, Kavya N, Ramarao V, et al. Tinospora cordifolia (Willd) Miers: Nutritional, Ethanomedial and therapeutic Utility. *Int J Res Ayurvedic Pharm.* 2015;6(2):195–198.
- Jitendra M, Madan MS, Amla B. Tinospora cordifolia: a multipurpose medicinal plant– A review. *Journal of Medicinal Plants Studies.* 2014;2(2):32–47.
- Saha S, Ghosh S. Tinospora cordifolia: One plant, many roles. *Ancient Science of Life.* 2012;31(4):151–157.
- Bala M, Pratap K, Verma PK, et al. Validation of ethnomedicinal potential of Tinospora cordifolia for anticancer and immunomodulatory activities and quantification of bioactive molecules by HPTLC. *J Ethnopharmacol.* 2015;175(4):131–137.
- Narayanan AS, Raja SS, Ponmurugan K, et al. Antibacterial activity of selected medicinal plants against multiple antibiotic resistant uropathogens: a study from Kolli Hills, Tamil Nadu, India. *Benef Microbes.* 2011;2(3):235–243.
- Veeramuthu D, Savarimuthu I, Kedike B. Antimicrobial activity of Tinospora cordifolia: an ethnomedicinal plant. *Asian Journal of Traditional medicines.* 2012;7(2):59–65.
- Bonvicini F, Mandrone M, Antognoni F, et al. Ethanolic extracts of Tinospora cordifolia and Alstonia scholaris show antimicrobial activity towards clinical isolates of methicillin-resistant and carbapenemase-producing bacteria. *Nat Prod Res.* 2010;28(18):1438–1445.
- Mahesh B, Sathish. Anti-microbial Activity of some important medicinal plants Against Plant and Human pathogens. *World Journal of Agricultural Sciences.* 2008;4:839–843.
- Mahesh B, Satish S. Antimicrobial important medicinal plant against plant and human pathogens. *World journal of Agricultural sciences.* 2008;4:839–843.
- Wink M. Modes of Action of Herbal Medicines and Plant Secondary Metabolites. *Medicines.* 2015;2(3):251–286.

23. Seigler DS. *Plant secondary metabolism*. USA: Kluwer Academic Publishers; 1995.
24. Wink M. *Functions of plant secondary metabolites and their exploitation in biotechnology. Annual plant review*. UK: Wiley-Blackwell; 2010.
25. Wink M. Plant secondary metabolism; Diversity, function and its evolution. *Nat Prod Commun*. 2008;3:1205–1216.
26. Spandana U, Shaik Liakhat A, Nirmala T, et al. A review on *Tinospora cordifolia*. *IJCPRR*. 2013;4(2):61–68.
27. Somasekhara Achar KG, Parashurama TR, Shivanna MB. A New Flat Stem Disease of *Tinospora cordifolia* caused by Phytoplasma. *Sch Acad J Biosci*. 2015;3(11):957–959.
28. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of Medicinal plants and natural products. *Indian Journal of Pharmacology*. 2000;32:81–118.
29. Reddy NM, Rajasekhar Reddy N. *Tinospora cordifolia* chemical constituents and Medicinal properties: A review. *Sch Acad J Pharm*. 2015;4(8):364–369.
30. Nagaprashanthi CH, Rafi Khan P, Gopi Chand K, et al. *In vitro* Antimicrobial Activity of *Tinospora cordifolia* and its phytochemical screening. *International Journal of PharmTech Research*. 2012;4(3):1004–1008.
31. Pandey MM, Rastogi S, Rawat AK. Indian herbal drug for general healthcare: An overview. *Internet J Altern Med*. 2008;6(1).
32. Tyler VE. Phytomedicines: back to the future. *J Nat Prod*. 1999;62(11):1589–1592.
33. Padua de LS, Bunyapraphatsara N, Lemmens RHMJ. *Plant Resources of South-East Asia, No. 12(1). Medicinal and Poisonous Plants 1*. The Netherlands: Backhuys Publishers; 1999.
34. Fabry W, Okemo PO, Ansorg R. Antibacterial Activity of East African Medicinal plants. *J Ethnopharmacol*. 1998;60(1):79–84.
35. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. *J Ethnopharm*. 2005;100(1–2):80–84.