The Pharma Innovation Journal 2017; 6(4): 181-186

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(4): 181-186

www.thepharmajournal.com
Received: 19-02-2017
Accepted: 20-03-2017

Sheelu Monga
Department of Bio & Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

Pradeep Dhanwal
Department of Bio & Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

Ravinder Kumar
Department of Bio & Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

Anil Kumar
Department of Bio & Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

Vinod Chhokar
Department of Bio & Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

Correspondence
Vinod Chhokar
Department of Bio & Nano Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India

Pharmacological and physico-chemical properties of Tulsi (*Ocimum gratissimum* L.): An updated review

Sheelu Monga, Pradeep Dhanwal, Ravinder Kumar, Anil Kumar and Vinod Chhokar

Abstract
Ocimum gratissimum, common name tulsi, is considered as a sacred plant and worshiped in India. It belongs to family Lamiaceae. It is a valuable medicinal plant which has numbers of pharmacological properties. Antitumor and anti-cancer effects have been reported in *in vitro* experiments. It is also recommended for treatment of diseases like bronchitis, bronchial asthma, diarrhea, dysentery chronic fever etc. Ocimum contains eugenol (1-hydroxy-2-methoxy-4-allylbenzene) which can be used for therapeutic purposes. Preclinical studies has revealed that certain compounds present in *Ocimum* like rosmarinic acid, apigenin, myretenal, luteolin, ß-sitosterol and carnosic acid, have antioxidant properties. Contents of secondary metabolites can be increased with the help of different tissue culture techniques. Availability of genome and transcriptome sequences can reveal the gene site of important secondary metabolite pathways.

Keywords: *Ocimum gratissimum,* medicinal plant, basil, essential oils, metabolites

1. Introduction
Ocimum gratissimum also called African basil, holy basil, clove basil or wild basil, is a valuable medicinal plant used since ancient times. It belongs to the family *lamiaceae,* has been mentioned in *Charaka Samhita* (NIIR Board, 2004) [27], an ancient Ayurveda text and marked by its strong aroma and astringent taste It is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity. It is an elixir for cough; the leaves when chewed after meals acts as a digestive agent. *Ocimum* leaves prevent bacterial growth and used as a preservative. The leaves and flower of *Ocimum gratissimum* are traditionally used as digestive, carminative, aromatic, galactogongue, stomachic and tonic agents. *Ocimum gratissimum* have been recommended for the treatment of diarrhea, fever, ophthalmic skin diseases and upper respiratory tract infections and for insect bite. It is also suggested as antimicrobial, antifungal, antibacterial, antimalarial, antiviral, anesthetic, antiprotozoal and anthelmintic agents. It has antidiabetic, antifertility, anti-inflammatory and anti-stress activity (Cohen 2014) [4]. Extracts of its essential oil also have insecticidal and nematicidal properties. The leaves of *O. gratissimum* are nerve tonic and help in sharpen memory. Mucilage extracted from this plant is a good pharmaceutical adjuvant, specifically a disintegrating agent (Ravikumar *et al.,* 2007) [39]. It is also widely used for making indigenous medicinal preparations (Paton *et al.*, 1996) [33].

2. History
Basil belongs to genus *Ocimum*, derived from the Greek word *ozo* which means to smell, in reference to the strong odor of the species within the genus (Mcintosh *et al.,* 1853) [23]. In French, it is frequently given the name “Herbe Royale”, revealing the positive light (Meunscher *et al.,* 1978) [26]. It is sometimes refer as king of herbs. *Ocimum* was discovered by Linnaeus in 1753 in Africa. He listed five species at that time. Paton revised the African species of *Ocimum* and he recognized around 30 species in 1992. There is also another species of *Ocimum* i.e. *Ocimum basilicum* and *basilicum* comes from the Greek word 'Basilicos' meaning 'king' or 'royal’. Its properties were mentioned in the *Charaka Samhita*, an ancient Ayurvedic text. The British used tulsi as a substitute of Bible upon which the Indians would take an oath in a court of law (Stobart *et al.,* 1985) [45].
There are so many names of *Ocimum gratissimum.* It is pronounced differently in different languages. Normally it is called “Tulsi” in most of the states of India. But in Kannada, it is called Vishnu-tulsi; Trittaru in Malyalam; Manjiri in Sanskrit and Thulsai in Tamil etc.

In the other countries, it is called Furanjmishk in Arabic; Ding xiang luo le in Chinese; Basilic africain in French; Baum-Basilikum in German and Indo mebouki in Japanese (Triveni *et al.,* 2013) [47].

There are many species of *Ocimum,* which have their different morphological or anatomical characters. They are found in different places and have different living conditions; so that they have different medicinal value. Content of secondary metabolites also differs species to species. Different species of *Ocimum* are *Ocimum americanum*, *Ocimum basilicum*, *Ocimum campechianum*, *Ocimum centraliafricanum*, *Ocimum gratissimum*, *Ocimum kilimandscharicum*, *Ocimum minimum*, *Ocimum viride*, *Ocimum suave*, *Ocimum ovatum*, *Ocimum selloi*, *Ocimum tenuiflorum* and *Ocimum citriodorum* (*O. americanum* × *O. basilicum*) (Joshi *et al.,* 2011) [17]

2.1 Botany of *Ocimum gratissimum* L.

Botanical classification of *Ocimum gratissimum* L.

Kingdom	-	Plantae
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Order	-	Lamiales
Family	-	Lamiaceae
Genus	-	*Ocimum*
Species	-	*O. gratissimum*
Subfamily	-	Nepetoideae
Tribe	-	Ocimeae

Botanical name - *Ocimum gratissimum* L.

3. Morphology and microscopy of *Ocimum gratissimum*

3.1 Leaves, Stomata and Phyllotaxy

It is classified as small herb plant which is branched with small leaves. It grows up to a height of 1-3 m. Leaves have opposite phyllotaxy and petiole is 2-4.5 cm long, slender and pubescent. *O. gratissimum* grows with opposite, light green; silky leaves 3-4 centimeters long and 1–2 centimeters broad as shown in Figure 1. Both the fresh and dried leaves of *Ocimum* have medicinal value. It is little acerbic astringent in taste. Leaves are acute, oblong in shape with entire or serrate margin. They are also pubescent on both sides with minute glands. Stomata are rarely present on the upper surface of the leaf but they are present on the lower surface.

3.2 Flowers, Inflorescence and Floral structure

This plant consists of verticillaster inflorescence consisting of purple to pink colored flowers. Flowers are arranged in a terminal, simple or branched raceme 5-30 cm long, bracts sessile, ovate, 3-12 mm x 1-7 mm, acuminate, caducous; pedicel 1-4 mm long, spreading or ascending, slightly curved; flowers in 6-10-flowered verticillasters, small, hermaphrodite; calyx 2-lipped, 2-3 mm long, pubescent, upper lip rounded and re-curved, reflexed in fruit, lower lip with 4, narrow, pointed teeth, central pair of teeth minute and much shorter than the upper lip; corolla campanulate, 3.5-5 mm long, 2-lipped, greenish-white, pubescent outside, upper lip truncate, 4-fid, lower lip longer, delineate, flat, entire; stamens 4, delineate, in 2 pairs, inserted on the corolla tube, filaments distinctly exerted, upper pair with a bearded tooth at the base; ovary superior, consisting of 2 carpels, each 2-celled, style 2-fid. Flowering of basil started after 136 days; continued until 195 days and seed matured after 259 days.

3.3 Fruit, Stem and Roots

Fruit consisting of 4, dry, 1-seeded nutlets enclosed in the persistent calyx; nutletsubglobose, 1.5 mm long, rugose, brown; outer pericarp not becomes mucilaginous in water. Basil produces small seeds which are reddish black in color. Stem becomes woody in older plants while it is green in newly born plant. Stem becomes woody in winters also. Woody stem is shown in Figure 2. Root of *Ocimum gratissimum* also contains essential oils like eugenol. This content can be increases by *in vitro* culturing of the plant treating with *Agrobacterium rhizogenes*. It can be increased with the help of elicitors also (Sembulingam *et al.,* 1997) [41].

3.4 Microscopy

Two of the leaf epidermal cells are typical of irregular contours, and diacytic stomata, secretory glands most abundant in the leaf and also present in the simple pluricellular hairs on the leaf veins. Its cross section shows the epidermis monoestratificada, a layer of parenchyma fenced in sub-epidermal position, followed by parenchymal pond, and finally the epidermis monoestratificada (Garcia *et al.,* 1998) [10].

4. Medicinal importance in Ayurveda

Basil, or holy basil, is an integral ingredient in many Ayurvedic preparations. Some ayurvedic preparations such as Ayurpanas Dakamuladya Ghrita, Cwasahara and Jwarakunjana - parindra Rasa promotes the health of the respiratory system. Another preparation called Surasa. Mahajwarankuca Rasa is used to maintain normal body temperature. Bhallataka Lauha is used to fight loss of appetite, to improve the stamina and to support the digestive system. To promote the elimination system of the body, there is a preparation called Bhaktavipaka Bati. To maintain the blood pressure and blood sugar levels, that are already within the normal range, Lauha Parppati is used. Vrihat Yogaraja Guggulu is employed to maintain the skeletal and joint system in our body. Rasacekhara Cwitrapanchanana and Durlabha Rasa are used to maintain healthy skin and Mahanila oil to promote healthy hair. For dental hygiene and healthy vision, Vakuladya oil and Maktadi Mahanjana are used respectively. Kumara Kalyana Ghrita is particularly used for dental hygiene promotion in children. (Govindarajan *et al.,* 2005) [13]

5. Chemical composition of *Ocimum gratissimum*

Ocimum gratissimum have great medicinal properties. Medicinal properties of this plant is all because of the secondary metabolite and essential oil present in the leaves, stem and roots. Major metabolites in tulsi are eugenol, rosmerinic acid, apigenin and carnosic acid etc. Thymol and flavonoids in the form of orintin and vicenin are also present in great amount. It also contains terpenes, lactone and xanthenes (Ijaduola *et al.,* 1980) [16]. It has been observed that proportion of Eugenol (Adams *et al.,* 1995) [1] is maximum (57.82%) amongst all the constituents present in basil, followed by (Z)-α-Bisabolene (17.19%) and Thymol (9.80%). γ-Terpinene (3.06%), β-Caryophyllene (3.03%), *p*-Cymene (2.11%) and *cis*-β-Guaieno (1.06%) are the other main constituents of basil (Van *et al*. 1963) [49]. However, a number of constituents which comprises in very low percentage in basil are caryophyllene oxide (0.82%), germacrene D (0.79%), (E)-β-Ocimene (0.49%), α-Selinena (0.45%), (E)-β-Farnesene (0.39%) and myrcene (0.34%). Other constituents like α-Terpinene, *p*-Cymenene, Terpin-4-ol, Carvacrol and α-Humulene are also present in very minute amount. Nutritional composition of *Ocimum gratissimum* has also been investigated by Vieira *et al* (2001) [51] and presented in Table 1

Table 1: Nutritional value of *Ocimum gratissimum*

Energy	94 Kj	Folate	68 µg
Carbohydrates	2.65 g	Choline	11.4 mg
Dietary fiber	1.6 g	Vitamin C	18 mg
Fat	0.64 g	Vitamin E	0.8 mg
Protein	3.15 g	Vitamin K	414.8 µg
Water	92.06 g	Calcium	177 mg
Vitamin A equivalent	264 µg	Iron	3.17 mg
β-carotene	3142 µg	Magnesium	64 mg
Thiamine	0.034 mg	Manganese	1.148 mg
Riboflavin	0.076 mg	Phosphorus	56 mg
Niacin	0.902 mg	Potassium	295 mg
Pantothenic acid	0.209 mg	Sodium	4 mg
Vitamin B_6	0.155 mg	Zinc	0.81 mg

6. Tissue culture of *Ocimum gratissimum*

Medicinal and aromatic plants are an important source of medicines and play a significant role in world health care system. Today medicinal plants are important to the global economy, as well as source of income for rural people in developing countries. About 70% - 80% of the people worldwide rely on herbal medicines derived from plants for their primary healthcare needs. This awakening has led to a sudden rise in demand for herbal medicines. Generally, herbal preparations are produced from field-grown plants and are susceptible to infestation by bacteria, fungi, and insects that can alter the medicinal content and properties of the preparations. There is significant evidence to show that the supply of plants for traditional medicines is failing to satisfy the demand. To meet the demand of traditional medicines, plant tissue culture technique can be used to grow the plants at large extent in a limited time.

Modern techniques of plant tissue culture provide new area for enhancing the production of pharmaceuticals, nutraceuticals, and other important secondary metabolites (Hansen and Wright, 1999) [14]. Currently plant tissue culture technology use transcription factors as a new molecular tool in the field of plant metabolic engineering to enhance secondary metabolites production (Gantet and Memelink, 2002) [9]. These days, researchers scale up the production of compounds which are beneficial in antitumor, antiviral, hypoglycemic, anti-inflammatory, anti-parasitic, antimicrobial, tranquilizer and immunomodulation activities (Vanisree *et al.,* 2004) [50]. Because of the medicinal properties of *Ocimum* genus, many *in vitro* studies have been conducted by using different explants like nodal segments (Shahzad *et al.,* 2000; Monga *et al*. 2014) [42, 25], leaf explants (Phippen *et al.,* 2000) [35], young inflorescence (Singh and Sehgal, 1999) [44] and axillary buds (Egum *et al.,* 2002) [6]. Single node explants were inoculated on basal MS medium which includes 3 % sucrose, supplemented with different concentrations and combinations of 6-benzylaminopurine (BAP), kinetin (KN), indole-3-acetic acid (IAA) for direct plant regeneration. Maximum numbers of shoot (14.3±1.5) were observed on medium having 0.5 mg/l of BAP after 4 weeks of culturing (Gopi *et al.,* 2006) [12].

Axillary shoot bud proliferation was initiated from nodal explants cultured on MS medium supplemented with various concentrations of N6- benzyladenine (BA) (0.5 - 3.0 mg/l), Kinetin (KN) (0.5 - 3.0 mg/l) and 2-isoPentenyladenine (2-iP) (0.5 - 3.0 mg/l). Maximum numbers of shoots (5.17 ± 0.04) were observed on medium containing 1.0 mg/l BA. After hardening of the plants, genetic fidelity was assessed by the use of RAPD markers and found that no genetic alteration in the micropropagated plants (Saha *et al.,* 2016) [40]. *In vitro* micropropagation of young inflorescence explants were established on MS medium, supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) or thidiazuron (TDZ) resulted in only non-morphogenetic callus. MS + BAP (1.0 mgl−1) produced the maximum number of shoots. Addition of indole-3-acetic acid (IAA) (0.05 mgl−1) along with BAP (1.0 mgl−1) showed a remarkable increase in the number of shoots (Singh and Sehgal, 1999) [44]. The leaf explants cultured on basal medium were supplemented various concentration of cytokinins and auxins. Best response of shoot induction was observed using 1.0mg/l 6-benzylaminopurine (BA) in combination with 0.5 mg/l Indole Acetic Acid (IAA). This medium showed 82% shoot bud proliferation with 23.8±0.23 mean number of shoots and the rooting was observed on 1.5mg/l of indole butyric acid (IBA) supplemented medium with survival rate of 90% (Mishra 2015) [24].

7 Cultivation of *Ocimum gratissimum*

O. gratissimum is found throughout the tropical and subtropical regions, both wild and cultivated. Most culinary and ornamental basils are of species *Ocimum*, but other species are also grown (Matias *et al*., 2010) [22]. This herb is harvested at full bloom for extraction of essential oils from the flowering tops. Basil is very sensitive against cold, with best growth measure in hot and dry conditions. It is best grown on drained soil (Lerner *et al*. 1996) [18], which is slightly acidic with pH ranging from 5.5-6.5. The minimum temperature in which it can be grown properly is 17 °C and the maximum temperature is 39.2 °C. It requires relative humidity of 94%. In northern Europe, Canada, northern states of U.S., and south island of New Zealand, the climate is very cold, therefore *Ocimum gratissimum* is grown in a green house, and then it is planted out in late spring or early summer. *Ocimum gratissimum* is grown commercially by home gardeners and by gourmet cooks. Once a stem produces flowers, foliage production stops on that stem and becomes woody. The production of essential oil declines. To prevent this, a basil-grower may pinch off any flower stems before they are fully mature. Once the plant is allowed to flower, it may produce seed pods containing small reddish black seeds, which can be saved and planted in upcoming years. Use of raised-beds with plastic row covers is preferred to avoid weeds. These practices can improve soil drainage, conserve water, reduce the need for weed control and keep soil from splashing into leaves (Loughrin *et al*., 2001) [19].

8. Genome and transcriptome sequencing of Tulsi (*Ocimum Tenuiflorum*)

Genome and transcriptome sequencing of important medicinal plants is a good approach for gene discovery and biochemical pathway discovery of medicinally important secondary metabolites (Gongora-castillo *et al.,* 2012) [11]. Upadhyay *et al.,* (2015) [48] conducted whole transcriptome sequencing of *Ocimum Tenuiflorum* using the Illumina Hiseq 1000 platform, resulting in an assembled genome of 374 Mb, with genome coverage of 61 % (612 Mb estimated genome size). In the initial genome draft of *O. tenuiflorum* genome, 36768 putative gene models were identified. 16384 gene models were observed by the process of refined gene prediction, which have expression evidence. A total of 19384 gene models have been identified by without any RNA or protein evidence. The repeat content of the genome was identified as, 78224 repeat regions, with a GC content of 36.1 %, adding to 160889218

bp (160 Mb), which constituted 42.9 % of assembled genome which is 374806882 bp (374 Mb) long. Upadhyay *et al* (2015) [48] also compared transcriptomes of two subtypes, Krishna and Rama Tulsi, from leaf samples. Large numbers of genes were identified, which involved in the production of secondary metabolites of pharmaceutical concern such as apigenin, luteolin, rosmarinic acid pathway, eugenol, and ursolic acid.

Another attempt of genome sequencing of *Ocimum tenuiflorum* was made by Rastogi *et al*., (2015). In this study, nuclear and chloroplast genomes were sequenced combining the sequence data from 4 libraries and three NGS platforms. The saturated draft assembly of the genome was about 386 Mb, beside the plastid genome of 142,245 bp, the smallest in Lamiaceae family. Phylogenetic analysis for chloroplast proteome found the nearest neighbor is *Salvia miltiorrhiza.* From the analysis of the assembly, 53,480 protein coding genes were identified. Two libraries of Illumina HiSeq2000, one library of 454 GS FLX and one mate-pair library of SOLiD 5500XL were constructed. The assembled de novo genome of Holy basil, in this study, represents the smallest nuclear genome in the Lamiaceae family and smallest chloroplast genome in the order Lamiales. The genome of *O. sanctum* seems to be compact with repeat sequences are relatively less. The information of genome sequence will also speed up the identification of genes involved in important secondary metabolite synthesis.

9. Pharmacological studies

9.1 Antifungal activity

An antifungal activity is found in the essential oil that can be obtained by steam-distillation (1.1% w/v) of the aerial parts of *O. gratissimum.* The results showed that the essential oil inhibit the growth of all fungi tested, including the phytopathogens, *Botryospaeria rhodina, Rhizoctonia* sp. and two strains of *Alternaria* sp. (Prabuseenivasan *et al.,* 2006). Ethanolic, hot water and cold water extract of *O. gratissimum* was tested against *Colletotrichum* species isolated from spoilt tomatoes. Maximum zone of inhibition was measured in case of hot water extract and then in ethanolic extract and least in cold water extract (Orji *et al.,* 2015) [31]. Antifungal activities against, *Microsporum canis, M. gypseum, Trichophyton rubrum and T. mentagrophytes. Trichophyton rubrum,* the most common dermatophytes in Brazil was carried out and found that hexane extract of *O. gratissimum* and eugenol is very effective against the dermatophyte (Silva *et al.,* 2010) [43].

9.2 Antibacterial activity

Different extracts from the leaves of *Ocimum gratissimum,* show antibacterial activity when tested against *Staphylococcus aureus, Salmonella typhi* and *Salmonella typhimurium,* pathogenic bacteria which causes diarrhea. Extract included cold water extract, hot water extract and steam distillation extract. Only steam distillation extract has inhibitory effects on the selected bacteria and the minimum inhibitory conc. ranged from 0.1% for *S. aureus* to 0.01% for *E. coli* and *S. typhimurium,* and 0.001% for *S. typhi* (Adebolu *et al*., 2005) [2]. *Ocimum gratissimum*, ethanolic extract was tested for anti-microbial activity against *Actinobacillus actinomycetemcomitans* in human dental plaque and compared with 0.2% chlorhexidine as the positive control and dimethyl sulfoxide (DMSO) as the negative control. Maximum antimicrobial potential was at 0.6% concentration level (Eswar *et al*., 2016) [7]. Antimicrobial activity was carried out against *Aggregatibacter actinomycetemcomitans, Prevotella intermedia*, and *Porphyromonas gingivalis* and found that 0.5 and 1.0 % extract showed maximum zone of inhibition. Doxycycline was taken as positive control and DMSO as negative control (Mallikarjun *et al*., 2016) [21].

9.3 Ovicidal activity

The main component of ovicidal activity present in the essential oil of *Ocimum gratissimum* is eugenol. It was evaluated against *Haemonchus contortus,* a gastrointestinal parasite of small ruminants. The essential oil and eugenol showed maximum inhibition at 0.5% conc. These results suggest a possible utilization of essential oil of *O. gratissimum* as an aid to control gastrointestinal helmintosis of small ruminants (Pessoa *et al.,* 2002) [34].

9.4 Larvicidal, pupicidal and adulticidal potential

larvicidal, pupicidal and adulticidal activities of acetone, hexane and chloroform extracts of *Ocimum gratissimum* investigated against filariasis mosquito vector *Culex quinquefasciatus.* Results suggested that *O. gratissimum* chloroform extract is a best controlling agent for *Cx. Quinquefasciatus* among all the extracts (Pratheeba *et al.,* 2015) [37]. Pupicidal and larvicidal mortality was recorded in the same extract exposure at 24 hrs is of 2.6916 mg/ml and 2.8916 mg/ml respectively.

9.6. Wound Healing activity

Wound healing effects of *Ocimum gratissimum* were investigated using incisional wound model in rats and found that *O. gratissimum* have wound healing potential (Eyo *et al.,* 2014) [8]. The ability to increase the vascular permeability of *O. gratissimum* may be one of the factors that contribute to its wound healing property (Orafidiya *et al.,* 2005) [30].

9.7 Anti-Inflammatory activity

The study reported the inhibitory effect produced by chemical constituents of essential oils of *Ocimum gratissimum* used in traditional medicine as anti-inflammatory and analgesic drugs, *in vitro*, on soybean lipoxygenase L-1 and cyclooxygenase function of prostaglandin H synthase, the two enzymes, which are involved in the production of mediators of inflammation. (Tanko *et al.,* 2008) [46]

9.8 Miscellaneous activities

Hydro-alcoholic extract of basil indicated good leishmanicidal activity against *Leishmania amazonensis* compared to that of *Trypanosoma cruzi*. (Luize *et al.,* 2005) [20]. The liquid extract of the leaves of *Ocimum gratissimum* show antidiarrheal effects. The extract inhibited castor oil induced diarrhea in rats judged by decrease in the number of wet feaces in the extract treated rats. The extract also inhibits the propulsive movement of the intestinal contents. (Das *et al.,* 2003) [5]. Essential oils of *O. gratissimum* have relaxant action due to direct effect on the smooth muscle of ileum rather than an indirect action on neurotransmitter release (Nwinyi *et al.,*, 2009) [28]. The result of analgesic activity of *Ocimum gratissimum* showed that the extract produced a prolongation of reaction time of 85% over 20 min observation times with no over signs of toxicity. The results revealed the analgesic and spasmolytic activities (Aziba *et al.,* 1999) [3]. The hyposensitive effect that seems related to an active vascular relaxation was induced by intravenous treatment of conscious deoxycorticosterone acetate salt hypersensitive rats with the essential oils of *O. gratissimum* (Patil *et al.,* 2010) [32]

Fig 1: leaves of *Ocimum gratissimum*

Fig 2: *Ocimum gratissimum* with woody stem.

11. Conclusion

Ocimum has been used from many decades in Ayurveda because of its pharmacological importance. Basil, or Holy basil, is an integral ingredient in many Ayurveda preparations. It is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity. It is an elixir for cough; the leaves when chewed after meals acts as a digestive agent. *O. gratissimum* have lots of pharmacological properties i.e. antimicrobial, antifungal, antibacterial, antimalarial, antiviral, anesthetic, antiprotozoal, and anthelmintic agents. It also has antidiabetic, antifertility, anti-inflammatory and antistress. It can also be used to treat breast cancer very effectively. *Ocimum gratissimum* have been recommended for the treatment of diarrhea, fever, ophthalmic skin diseases and upper respiratory tract infections and for insect bite. Genome sequencing of *Ocimum* disclose the strong medicinal properties of the plant. The availability of the genome sequence will help to study the functional gene site of the important metabolic pathways.

12. Reference

1. Adams RP. Identification of Essential oil Component by Chromatography/mass Spectroscopy. Edn 4, Allured Publishing Co., Carol Steam, Ilinois, 1995.
2. Adebolu TT, Oladimeji SA. Antimicrobial activity of leaf extracts of *Ocimum gratissimum* on selected diarrhoea causing bacteria in southwestern Nigeria. African Journal. of Biotechnology 2005; 4(7):682-684.
3. Aziba PI, Bass D, Elegbe Y. Pharmacological investigation of *Ocimum gratissimum* in rodents. Phytotherapy Research 1999; 13:427-9.
4. Cohen MM. Tulsi - *Ocimum sanctum*: A herb for all reasons. J Ayurveda Integr Med. 2014; 5(4):251-259.
5. Das S, Prakash R, Devaraj SN. Antidiarrhoeal effects of methanolic root extract of *Hemidesmus indicus* (Indian sarsaparilla)--an *in vitro* and *in vivo* study. Indian Journal of Experimental Biology. 2003; 41(4):363-6.
6. Egum F, Amin N, Azad MAK. *In vitro* rapid clonal propagation of *Ocimum basilicum* L. Plant Tissue Culture 2002; 12:27-35.
7. Eswar P, Devaraj CG, Agarwal P. Anti-microbial Activity of Tulsi {*Ocimum Sanctum* (Linn.)} Extract on a periodontal pathogen in human dental plaque: An *In vitro* Study. Journal of Clinical and Diagnostic Research. 2016; 10(3):ZC53-ZC56.
8. Eyo LE, Uzoibiam BO, Ogbanya KC, Nnaji TO. Comparative evaluation of wound healing effects of *Ocimum gratissimum*, *Vernonia amygdaline* and *Zingiber officinalis* extracts on incision wound model in rats. Pharmacology online 2014; 3:44-50.
9. Gantet P, Memelink J. Transcription factors: Tools to engineer the production of pharmacologically active plant metabolites. Trends in Pharmacological Sciences. 2002; 23:563-569.
10. Garcia LD, Sandra PT, Crespo M, Fuentes LL. Pharmacognostic study of *Ocimum gratissimum* L. (Cimarron oregano). Revista Cubana de Plantas Medicinales. 1998; 3:31-36.
11. Gongora-castillo E, Fedewa G, Yeo Y, Chappell J, Dellapenna D, Buell CR. Genomic approaches for interrogating the biochemistry of medicinal plant species. Methods in Enzymology 2012; 517:139-59.
12. Gopi C, Sekhar YN, Ponmurugan P. *In vitro* multiplication of *Ocimum gratissimum* L. through direct regeneration. African Journal of Biotechnology 2006; 5(9):723-726.
13. Govindarajan R, Vijayakumar M, Pushpangadan P. Antioxidant approach to disease management and the role of 'Rasayana' herbs of Ayurveda. Journal of Ethnopharmacology 2005; 99:165-178.
14. Hansen G, Wright MS. Recent advances in transformation of plants. Trends in Plant Science 1999; 4:226-231.
15. Igbinosa EO, Uzunuigbe EO, Igbinosa IH, Odjadjare EE, Igiehon NO, Emuedo OA. *In Vitro* Assessment of antioxidant, phytochemical and nutritional properties of extracts from the leaves of *Ocimum Gratissimum* (Linn). African Journal of Traditional, Complementary and Alternative medicines 2013; 10(5):292-298.
16. Ijaduola G, Anyiwo I, Thomas C. *Ocimum gratissimum* and blood coagulation. Journal of Research in Ethinomedian 1980; 1:19-21.
17. Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K *et al*. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). Journal of Microbiology and Antimicrobials. 2011; 3(1):1-7.
18. Lerner BR, Dana MN. Herb Gardening, Purdue University Cooperative Extension Service Publication HO-28, West Lafayette, IN, 1996.
19. Loughrin JH, Kasperbauer MJ. Light reflect from colored mulches affects aroma and phenol content of sweet basil (*Ocimum basilicum* L.) leaves. Journal of Agricultural and Food Chemistry 2001; 49:1331-1335.
20. Luize PS, Tiuman TS, Morello LG, Maza PK, Ueda-Nakamura T, Filho BPD *et al*. Effects of medicinal plant extracts on growth of *Leishmania* (L.) *amazonensis* and *Trypanosoma cruzi*. Brazilian Journal of Pharmaceutical Sciences. 2005; 41:85-95.
21. Mallikarjun S, Rao A, Rajesh G, Shenoy R, Pai M.

Antimicrobial efficacy of Tulsi leaf (*Ocimum sanctum*) extract on periodontal pathogens: An *in vitro* study. Journal of Indian Society of Periodontology. 2016; 20:145-50.
22. Matias EFF, Santos KKA, Costa JGC, Coutinho HDM. Screening for *in vitro* phototoxic activity of methanol extracts of *Croton campestris* A., *Ocimum gratissimum* L. & *Cordia verbenaceae* DC. Indian Journal of Medical Research 2010; 132(5):520-522.
23. McIntosh, Charles, The Book of the Garden, William Blackwood & Sons, Edinburgh & London, 1853, 2.
24. Mishra T. Protocol Establishment for Multiplication and Regeneration of 'Holy Basil' (Ocimum sanctum Linn). An Important Medicinal plant with High Religious Value in India. Journal of Medicinal Plants Studies. 2015; 3(4):16-19.
25. Monga S, Sethi N, Kaura S, Parle M, Lohan S. Effect of 6-benzyl amino purine hormone on the shooting growth of *Ocimum gratissimum* L. International Research Journal of Pharmacy. 2014; 5(2):106-108.
26. Muenscher, Conrad W, Rice MA. Garden Spice and Wild Pot-Herbs. Ithaca, NY, Cornell University Press, 1978.
27. NIIR Board, National Institute of Industrial Research (India). Compendium of Medicinal Plants. National Institute of Industrial Research; 2004, 320.
28. Nwinyi OC, Chinedu NS, Ajani OA, Chinwe I, Ogunniran KO. Antibacterial effects of extracts of *Ocimum gratissimum* and *Piper guineense* on *Escherichia coli* and *Staphylococcus aureus*. African Journal of Food Science 2009; 3(3):077-081.
29. Orafidiya LO, Agbani EO, Adelusola KA. A study on the effect of the essential oil of *Ocimun gratissimum* Linn. on cyclophosphamide induced inhibited hair growth in pulp rats. International Journal of Aromatherapy 2004; 14:119-28.
30. Orafidiya LO, Fakoya FA, Agbani EO, Iwalewa EO. Vascular permeability- increasing effect of the leaf essential oil of *Ocimum gratissimum* Linn as a mechanism for its wound healing property. The African Journal of Traditional, Complementary and Alternative Medicines 2005; 2:253-8.
31. Orji JO, Nwuzo AC, Ejikeugwu PC, Ugbo EN, Moses IB, Nwakaeze EA *et al*. Antifungal activities of *Ocimum gratissimum* and *Gongronema latifolium* leaves on *Colletotrichum* species isolated from spoilt tomatoes. International Journal of Pharmaceutical Science Invention. 2015; 4(5):42-45.
32. Patil NR, Appala RS, Chimkode R, Tripathi R, Tripathi A, Agrawal N. Antihypertensive activity of leaves extracts of *Ocimum gratissimum* Linn. Advances in Pharmacology and Toxicology 2010; 11(3):103-107.
33. Paton A. Basil Taxonomy. Kew Scientist, 1996; 9:7.
34. Pessoa LM, Morais SM, Bevilaqua CML, Luciano JHS Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against Haemonchus contortus. Veterinary Parasitol, 2002; 109:59-63.
35. Phippen WB, Simon JE. Shoot regeneration of young leaf explants from basil (*Ocimum basilicum* L.), *In Vitro*. Cellular Development Biology-Plant, 2000; 36:250-254.
36. Prabuseenivasan S, Jayakumar M, Ignacimuthu, S. *In vitro* antibacterial activity of some plant essential oils. BMC Complementary and Alternative Medicine, 2006; 6(1):1.
37. Pratheeba T, Ragavendran C, Natarajan D. Larvicidal, pupicidal and adulticidal potential of *Ocimum gratissimum* plant leaf extracts against filariasis inducing vector. International Journal of Mosquito Research, 2015; 2(2):01-08.
38. Rastogi S, Kalra A, Gupta V, Khan F, Lal RK, Tripathi AK. Unraveling the genome of Holy basil: an "incomparable elixir of life" of traditional Indian medicine. BMC genomics, 2005; 16(1):1.
39. Ravikumar, Shirwaikar AA, Shirwaikar A, Prabu SL, Mahalaxmi R, Rajendran K. Studies of disintegrant properties of seed mucilage of *Ocimum gratissimum*. Indian Journal of Pharmaceutical Science, 2007; 69:753-758.
40. Saha S, Adhikari S, Dey T, Ghosh P. RAPD and ISSR based evaluation of genetic stability of micropropagated plantlets of *Morus alba* L. variety S-1. Meta Gene. 2016; 7:7-15.
41. Sembulingam K, Sembulingam P, Namasivayam A. Effect of *Ocimum Sanctum* Linn On Noise Induced Changes In Plasma Corticosterone Level. Indian Journal of Physiology and Pharmacology, 1997; 41(2):139-143.
42. Shahzad A, Siddiqui SA. *In vitro* organogenesis in *Ocimum sanctum* L. - A multipurpose herb. Phytomorphology, 2000; 50(1):27-35.
43. Silva LL, Heldwein CG, Reetz LGB, Hörner R, Mallmann CA, Heinzmann BM. Chemical composition, antibacterial activity, in vitro and brine-shrimp toxicity of the essential oil from inflorescences of Ocimum gratissimum L. Braz J Pharmacogn, 2010; 20:700-705.
44. Singh NK, Sehgal CB. Micropropagation of 'Holy Basil' (*Ocimum sanctum* Linn.) from young inflorescences of mature plants. Plant growth regulation, 1999; 29(3):161-166.
45. Stobart AK, Griffith WT, Bukhari IA, Sherwood RP. The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiologia Plantarum, 1985; 63:293-298.
46. Tanko Y, Magaji GM, Yerima M, Magaji RA, Mohammed A. Anti-nociceptive and anti-inflammatory activities of aqueous leaves extract of *Ocimum gratissimum* (Labiate) in rodents. The African Journal of Traditional, Complementary and Alternative Medicines, 2008; 5(2):141-6.
47. Triveni Kumar K, Singh AK, Kumar R, Gupta V, Tripathi K. *Ocimum sanctum* Linn: A Review on Phytopharmacology and Therapeutic Potential of Tulsi. International Journal of Pharmaceutical and Phytopharmacological Research, 2013; 3(2):148-151.
48. Upadhyay AK, Chacko AR, Gandhimathi A, Ghosh P, Harini PK, Joseph AP *et al*. Genome sequencing of herb Tulsi (*Ocimum tenuiflorum*) unravels key genes behind its strong medicinal properties. BMC plant biology. 2015; 15:212.
49. Van DDH, Kratz PDJA. Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography A. 1963; 11:463-471.
50. Vanisree M, Lee CY, Lo SF, Nalawade SM, Lin CY, Tsay HS. Studies on the production of some important secondary metabolites. Botanical Bulletin- Academia Sinica Taipei. 2004; 45(1):1-22.
51. Vieira RF, Grayer RJ, Paton A, Simon JE. Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD markers. Biochemical Systematics and Ecology. 2001; 29:287-304.