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

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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, β-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 astrangent 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, galactogogue, stomachic and tonic agents. Ocimum gratissimum have been recommended for the treatment of diarrhea, fever, ophthalmic skin diseases and upper respiratory track infections and for insect bite. It is also suggested as antimicrobial, antifungal, antibacterial, antimalarial, antiviral, anesthetic, antiprotozoal and anthelmintic agents. It has anti-diabetic, anti-fertility, 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) [20]. 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 Furanjimshk 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 centralafricanum, 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.

2. 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 increased by in vitro culturing of the plant 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. Mahayarnuka 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 Parrpatti 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 tulsia are eugenol, rosmnerinic 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) [10]. It has been observed that proportion of Eugenol (Adams et al., 1995) [3] 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-Guaiene (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 carophyllene 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-Terpipene, p-Cymene, Terpin-4-ol, Carvacrol and α-Humulen 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.
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) [45, 25], leaf explants (Phippen et al., 2000) [55], 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 (BA), 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 young inflorescence explants were established on MS medium, supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) or thidiazuron (TDZ) resulting in only non-morphogenetic callus. MS + BAP (1.0 mg/l) produced the maximum number of shoots. Addition of indole-3-acetic acid (IAA) (0.05 mg/l) along with BAP (1.0 mg/l) 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 1.0 mg/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 1.5 mg/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. Upadhyan 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, Botryosphaeria rhodina, Rhizoctonia sp. and two strains of Alternaria sp. (Prabuseenivasan et al., 2006). Ethanol, hot water and cold water extract of O. gratissimum was tested against Colletotrichum species isolated from spool tomatoes. Maximum zone of inhibition was measured in case of hot water extract and then in ethanol 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) \[41\].

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) \[5\]. 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) \[33\]. 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) \[3\]. 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 lipoxigense 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. (Laize 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 feces in the extract treated rats. The extract also inhibits the propulsive movement of the intestinal contents. (Das et al., 2003) \[3\]. 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 spasmyloitic activities (Aziba et al., 1999) \[5\]. 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) \[42\].
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 antiadiabetic, 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

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