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Commercial potentialities of essential oil of *Ocimum* members growing in North East India

Janmoni Kalita* and Mohamed Latif Khan
Department of Forestry, North Eastern Regional Institute of Science and Technology (Deemed University), Nirjuli, (Arunachal Pradesh) - India

Abstract

Ocimum L. is a versatile genus and is distributed in tropical and subtropical regions of the world. About 160 species of the genus have been reported in the world where India accounted with nine species. In North East India four species are cultivated. The study on the chemical constitution of essential oil obtained from various species have shown that there exists a number of types available which can be interesting to perfumers and can find utilization in industry. Therefore, more emphasis is given on breeding and to develop synthesizing new types or strain of genus. The present review focuses on taxonomic distribution, essential oil composition, traditional uses, pharmacological activities and agrotechnology of available species of the genus from published literatures which may be helpful in pharmaceutical industry and formulation of drug principles.

Key-Words: *Ocimum,* essential oil, chemical composition, North East India

Introduction

Ocimum L., is a versatile aromatic genus (family-Lamiaceae) well known for medicinal properties and also for economically important essential oils. The genus is very variable and possesses wide range of intra- and inter-specific genetic diversity. The nomenclature of *Ocimum* species and varieties is complicated and confusing and in several instances, the oils extracted from morphologically identical plants (one phenotype) show different physico-chemical properties[1]. Species like *O. sanctum* L. (tulsi), *O. gratissimum* (Ram Tulsi), *O. canum* (Dulal Tulsi), *O. basilicum* (Ban Tulsi), *O. kilimandscharicum* Guerke (Kapoor Tulsi), *O. camphora* and *O. micranthum* are examples of known important species of the genus which grow in different parts of the world and are known to have medicinal properties[2]. The plants are predominantly herbs, shrubs or under importance shrubs, annuals or perennials in habit. They possess glandular hairs or sessile glands secreting strongly scented volatile oils. Flowers appear to be uniform in the appearance throughout the group but they are of great taxonomic importance for the demarcation of species. The seeds contain edible oils and a drying oil similar to linseed oil[3].

*** Corresponding Author**
E.mail: janmoni.kalita@gmail.com
Mob.: + 91-9863919291

From industrial point of view, oil of *Ocimum* species are rich in camphor, citral, geraniol, linalool, linalyl acetate, methyl chavicol, eugeol, thymol, etc. and can be harnessed for successful utilization in industry. In India, the requirements of most of these are met by imports and the demand is on increase[4]. *O. basilicum* and its varieties are the most worked out group so far and its various aspects have been dealt with in detail. Earlier screening and evaluation of basil oil led to the evolution of useful strains which have worldwide acceptability and utilization. Experiments on the essential oils of various *Ocimum* species have indicated that the oil possess biological activities. Of these, antimicrobial, antibacterial, antifungal properties are very important. Efforts are being made to utilize these essential oils on commercial basis. The essential oil constituents responsible for these activities have been chemically defined.

In view of extensive importance of the genus *Ocimum* in North East India, this review is an effort to compile all the information on taxonomic distribution, essential oil composition, traditional uses, pharmacological activities and agro technology of available species of the genus from published literatures which may be helpful in pharmaceutical industry and formulation of drug principles.

North East Indian *Ocimum* – distribution, botany and systematics

About 160 species of *Ocimum* genus are reported to be distributed in tropical and subtropical regions of the world from sea level to 6000 ft.[5]. The maximum numbers of species are from the tropical rain forests of Africa. The geographical distribution of the genus *Ocimum* shows three main centers of biodiversity (a) Tropical and subtropical regions of Africa (b) Tropical Asia and (c) Tropical parts of America (Brazil)[6]. In India nine species have been reported of which three are exotic[1,7,8]. In North East India, species like *O. canum, O. basilicum, O. basilicum* L. var. *purpurascens, O. gratissimum* and *O. sanctum* are cultivated. *O. sanctum* which occupies a wide range of habitats, varying from sea level to 1800 m has been reported to occur throughout North Eastern India. *O. canum* reported to be distributed in Shillong area of Meghalaya (1200-1500 m) and Jorhat area (90 m) of Assam[9]. *O. gratissimum* L., an eugenol containing species was found to occur in suburb areas of Kamrup, Nowgong and of Jorhat districts in Assam (80-100 m)[9]. *O. basilicum* L. var. *purpurascens* (Ram tulsi/Assamese) is reported from Dhemaji district of Assam, the foot hills of Arunachal Pradesh and foot hills of Meghalaya[10, 11]. The species flowers between August to February.

Extensive field survey was made and samples were collected. Identification was done by consulting floras[11,12] and Botanical Survey of India, Itanagar for authentication. Leaf samples were collected and preserved in CTAB buffer solution for DNA extraction. Herbaria of collected specimen were deposited in Department of Forestry, NERIST, Arunachal Pradesh. The taxonomic enumerations of available species are given below.

***Ocimum sanctum* L. (syn. *O. tenuiflorum* L.)**

A perennial with woody root-stock; branchlets purplish. *Leaves* 1-2 by 0.5-1 in., ovate or ovate-oblong, elliptic-oblong, distantly serrate, crenate or entire, acute, pubescent on both sides; minutely gland dotted; petiole 0.1-0.3 in. Flowers 1.8-2 in. long, purplish or crimson in racemed. Often panicled whorl upto 8 in., long; pedicels usually longer than the calyx; bracts reflexed. *Calyx* glabrous within, glandular and pubescent without, enlarged in fruit, upper lip obovate, acute at the base in fruit. *Corolla* 0.15 in. long, upper lip hairy on the back; filaments of the upper stamens bearded at the base. *Nutlets* reddish with black markings[11] (**Fig. 1a).**

***Ocimum canum* Sims. (syn. *O. americanum* L.)**

An erect very pubescent undeshrub. *Leaves* 0.5-1.5 in., elliptic or elliptic-lanceolate, glandular, glabrate on maturity. *Flowers* white or pale purple. *Calyx* densely villous inside, upper lip in fruit sub-orbicular, shorter than the teeth of the lower lip. *Nutlet*s 0.05 in. long black when ripe[11] (**Fig. 1b).**

***Ocimum gratissimum* L.**

A shrub, 4-8 ft., much branched, woody below. *Leaves* 2-4 in., petiole 1-2 in. *Racemes* strict, slender; whorls rather close-set; pedicels shorter and bracts longer than the calyx. *Calyx* pubescent, fruiting ¼ in. long, recurved. *Corolla* hardly exceeding the calyx, pale yellow. Filaments exerted, knee bearded. *Nutlets* subglobose, rugose with glandular depressions[12] **(Fig. 1c).**

***Ocimum basilicum* L.**

The plant is erect, herbaceous, glabrous, pubescent. *Leaves* ovate toothed or entire. *Bracts* petiolate. *Flowers* are white pinkish or purplish. Fruiting *calyx* very shortly pedicelled, two lower teeth ovate-lanceolate awned longer than the rounded upper, lateral smaller than the lower. *Corolla* 1/3-1/2 in. long. *Seeds* are dark brown to black, ellipsoid and mucilaginous. [12,13] (**Fig. 1d).**

O. basilicum* L. var. *purpurascens

A slender much scented and much branched herb, generally purple coloured. *Leaves* 0.5-1.5 by 0.2-0.5 in. ovate, acute, entire, glandular. Flowers pale purple in nearly single racemes; pedicels shorter than the calyx, much deflexed in fruit, upper lip of calyx rounded, shorter than the teeth of the lower lip. *Nutlets* 0.07 in. across, ellipsoid, black, slightly pitted and become very gelatinous in water[11].

Traditional uses

Ocimum species have many uses, but the most common is for culinary purposes. As a fresh herb, they are used to flavor foods such as vegetables, poultry and fish, jelly, honey, tea, and liquor. The flowers are edible and can make an attractive addition to salads and other dishes [14]. The cosmetic industry uses oil in lotions, shampoos, perfumes, and soaps. Extracts of the plants are used in traditional medicines and have been shown different pharmacological activities[15,16,17]. The genus *Ocimum* is widely used in various ailments in North East India is given in **table 1.**

Reported pharmacological activities

O. basilicum

The essential oil showed antibacterial[18], antifungal[19], insecticidal and larvicidal activity[20]. Methyl chavicol and methyl cinnamate obtained from the essential oil of *O. basilicum* were found to be mainly responsible for the insecticidal activity of the oil against *Tribolium castaneum, Sitophilus oryzae, stagobium paniceum* and *Bruchus chinensis*[21].

O. canum

The essential oil from leaves showed antibacterial activity. Antitubercular activity against *Mycobacterium* strain was also reported[22, 23]. The oil showed wide range of antifungal activity[24].

O. gratissimum

The essential oil showed antibacterial, antifungal, hypoglycaemic, antipyretic, anti-nociceptive, antioxidant, anti-inflammatory, anthelmintic, anti-carcinogenic, free radical scavenging, radio protective, antidermatophytic activities and numerous others pharmacological use [25-37]. Earlier reports had also shown the smooth muscle contracting and antimutagenic activity[37] as well as its anti-diarrhoeal effects in experimental animals[38], high antiviral indices against HIV-1 and HIV-2[39].

O. sanctum

The combination of essential oils of *O. sanctum* (0.01%), *O. basilicum* (0.02%) and *Eucalyptus globules* (0.02%) has been reported to show 100% mosquito larvicidal activity, while the individual oil showed only 16, 8 and 16 % activity respectively. The oil from the leaves showed antifungal activity[27]. The essential oil from leaves also showed antimicrobial and antimycotic effects *in vivo*[27].

Essential oil of North East Indian *Ocimum*

It has been reported that the wild growing *O. canum* yielded average oil (0.67%) and substantial percentage of methyl chavicol (77%) and linalool (18%) in North East India[9]. The oil resembled to that of European type of sweet basil oil in having methyl chavicol and linalool as major constituents[9]. This type of oil was considered as superior in quality. *O. gratissimum* yielded 0.54% oil where major component reported to be eugenol (74.4%) in the oil. This eugenol type of *O. gratissimum* differs from the other two varieties of the species reported as thymol and citral type[9]. *O. sanctum* yielded about 0.7 percent volatile oil which contains around 71% of eugenol[9] (**Table 2**).

O. basilicum is highly polymorphic. The species characterized by great variability in chemical compositions. In North East India, three chemotypes characterized by high content of Camphor, Methyl Chavicol and Linalool, Methyl cinnamate and Linalool respectively have been reported. These chemotypes were distinguishable one from another, based on characterized of epidermal cells and stomata[40].

In camphor type, forty-four components, representing about 92% of the total oil, were identified of which Camphor (42.1%), followed by Limonene (7.6%) and β-selinene (5.6%), were the major component of the oil[41]. In methyl chavicol type, 34 components representing 99.5% of the total oil obtained from aerial parts of the plant at flowering stage were identified. The content of methyl chavicol was 74.7% followed by linalool 14.3% were detected as major compounds[42]. In terms of the individual plant parts, total percentage occurrence of these two compounds together was found maximum in the oil of leaves (96.52%), while minimum in the inflorescence (91.50%). However, as regards to the growth stages, percentage occurrence of these two components together in the oil obtained from aerial parts of the plant was found maximum at its full-blossoming stage (95.16%) followed by vegetative (94.97%), flower abscission (94.16%) and flower initiation (93.01%) stages respectively[42] (**Table 3**). In methyl cinnamate and linalool type, oil obtained from *Ocimum basilicum* var. *purpurascens* yielded 0.93% of which methyl cinnamate content is reported 60.2% and linalool 18.5%. The highest oil content was recorded when the inflorescence was in full blooming stage. Essential oil recovered from flower parts had higher percentage of main components viz. methyl cinnamate (60.2%) and linalool (18.54%) as compared to leaf (53.8 and 13.4%), which in turn was markedly superior to stem (44.3 and 9.9%), respectively[43] (**Table 3, 4**).

Commercial prospects of genus *Ocimum* in North East India

Basil is the source of natural products. The essential oil of basil is complex and has variable composition. Within the species, several different chemical groups exist, and furthermore soil and time of harvest influence the quantity and the composition of the essential oil. The most important aroma components are 1, 8 cineole, linalool, methyl chavicol and eugenol[41-44,45]. Monoterpenes (ocimene, geraniol, camphor), sesquiterpenes (bisabolene, caryophyllene), phenylpropanoids (methyl cinnamate, methyl eugenol), alpha-pinene, beta-pinene, limonene are present in varying amounts and strongly influence the flavor[46-48] (**Fig. 2**). *O. basilicum* contains primarily phenol derivatives, such as eugenol, methyl eugenol, chavicol, estragole, and methyl cinnamate often combined with various amounts of linalool[49-50].

The essential oils of basil have antioxidant compounds. Leea *et al*., (2005) reported eugenol, thymol, carvacrol and 4-allylphenol had stronger antioxidant activities than other volatile components. It has been experimentally established that eugenol, which forms a predominant constituents of the genetically upgraded F1 hybrid of *O. gratissimum* termed as "Clocimum-2", gets progressively converted into lignin when the hybrid plant becomes over mature and attains the fruiting stage or even beyond this stage. A stage comes when the eugenol content gets more or less totally converted into lignin and lignified cells get

increasingly pronounced with the increased deposition of lignin. Eugenol and lignin contents were inversely proportional to each other. The hybrid variety 'Clocimum-2' rich in eugenol, not only forms a dependable alternative source of clove oil but also an excellent starting material for the isolation of eugenol, isoeugenol and synthesis of vanillin. Most of the *Ocimum* species, both the sanctum and basilicum group provide a source of useful compounds of perfumery cum flavoring as well as pharmaceutical value. The pleasant odour and high volatility of aroma chemicals like camphor cineole, citronellol, geraneol, linalool, methyl chavicol etc. are utilized in consumer industries like the manufacture of perfumers, and cosmetic as well as in medicine and food adjuncts (**Table 5**).

Camphor has got diverse industrial applications like manufacture of celluloides and other nitro celluloid compounds. In medicine, camphor has anti-pyritic, carminative, and counter-irritant properties besides its use in the preparations of ammoniated camphor, camphor water, camphor liniment, camphorated tinctures and in various dental and oral preparations.

Apart from being carminative, antiseptic, anti-putrescent and host of other medicinal properties, a number of its attractive attributes such as pleasant odour or an appetizing flavor had traditionally been used in perfumes, food adjuncts etc. Application of perfumes is not only limited to products like cosmetics, soaps, detergents and agarbattis but with every passing day the list of consumer products wherein perfumes are used, is fast-mounting up. Involvement of breeding strategies to effect a genetic response for higher yield potential and in consequence genetically upgrade an *Ocimum* species had been the prime contributing factor for improvement studies.

Agrotechnology

1. **Climate and soil requirement**- *Ocimum* species can thrive on well-drained soil. Generally tropical and subtropical climate is suitable for the cultivation.

2. **Seed propagation**- Since *Ocimum* species is generally highly cross pollinated, a certain amount of heterozygosity is essential for vigour and yield attributes. These characters are mostly controlled by polygenes whose effect is considered additive.

3. **Nursery raising** - Seed sowing is carried out in well prepared seed beds in the third week of February, extended to middle of March, depending on the season. A spray of 2% urea solution on nursery beds two weeks before transplanting provides vigour to nursery growth.

4. **Transplanting-** Transplantation is done in March end or first week of April. A spacing of 45-60 cm is found suitable for most of the *Ocimum* species.

5. **Manuring-** Application of 60 kg N/ha with 40 kg P_2O_5 and 40 kg K20 /ha uniformly applied split-wise throughout the growth period of the crop.

6. **Irrigation-** Basil is not tolerant to water stress. A regular and even supply of moisture via trickle or overhead irrigation is necessary. If trickle irrigation is employed, care must be taken during harvest so no damage is done to the irrigation line[51-52].

7. **Insects and diseases-** Several insects and diseases may infest basil, but no pesticides are currently available for use on basil. While basil is susceptible to at least 30 viruses, including alfalfa mosaic alfamo virus, cucumber leaf spot carmovirus, and tobacco mild green mosaic dianthovirus, the plant is resistant to more than 40 viruses[51-52.]

6**. Harvesting-** Harvesting is done at the flowering stage or initiation of flowering.

Discussion

The study on the chemical constitution of essential oil obtained from various species have shown that there exists a number of types available which can be interesting to perfumers and can find utilization in industry. Therefore, more emphasis is given on breeding and to develop synthesizing new types or strain of genus. Clocimum is such new hybrid strain of *Ocimum gratissimum* which is a source of clove type oil, rich in eugenol. Improved strain of clocimum had 70-75 % eugenol compared to 45-60 % in the original races. *Ocimum* is highly cross pollinated. Therefore, a certain amount of heterozygosity is essential for vigour, high oil yield and high eugenol content. These characters appear to be controlled by polygenes whose effect is additive. Therefore a programme of exploration, introduction, and improvement of *Ocimum* species for their better utilization in industry is essential. It has been noted that under present day stress any naturally sourced aroma chemical would have added importance if it exhibited pharmaceutical and antimicrobial activity. The summery of all the above observations, focuses the exploration of more and more species of genus *Ocimum* whether indigenous or toxic and organized cultivation and commercial utilization.

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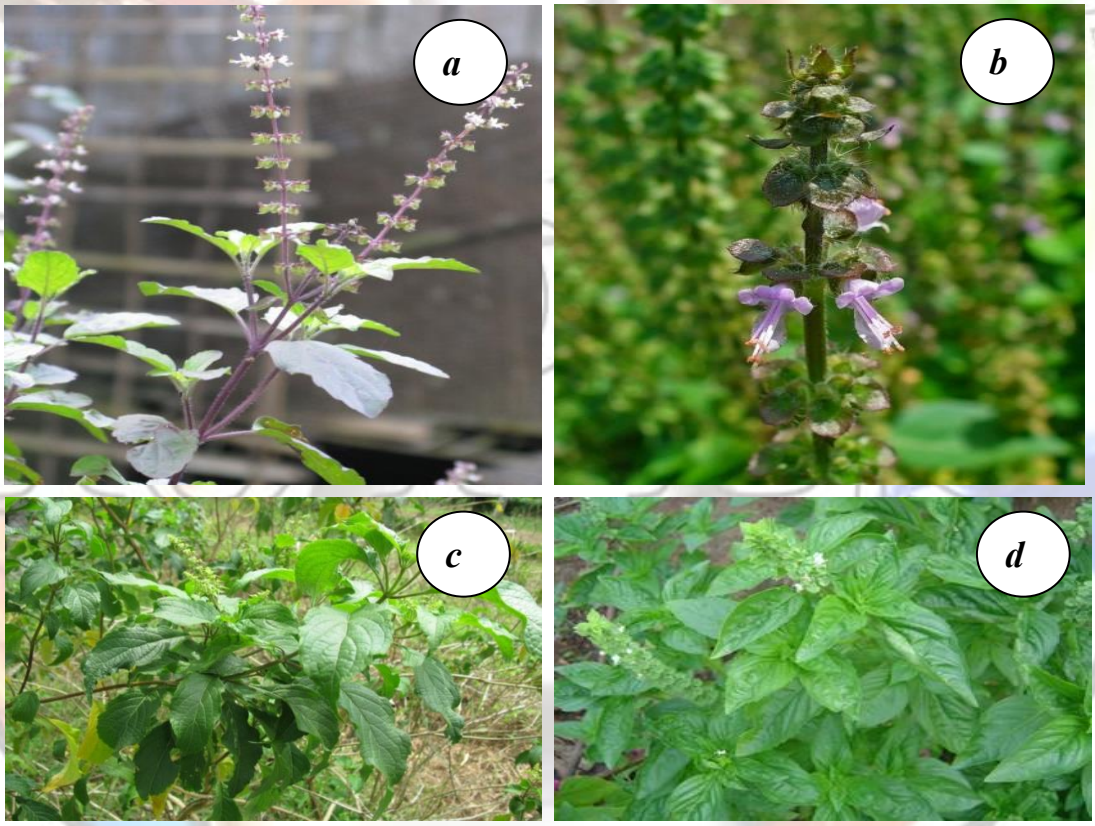
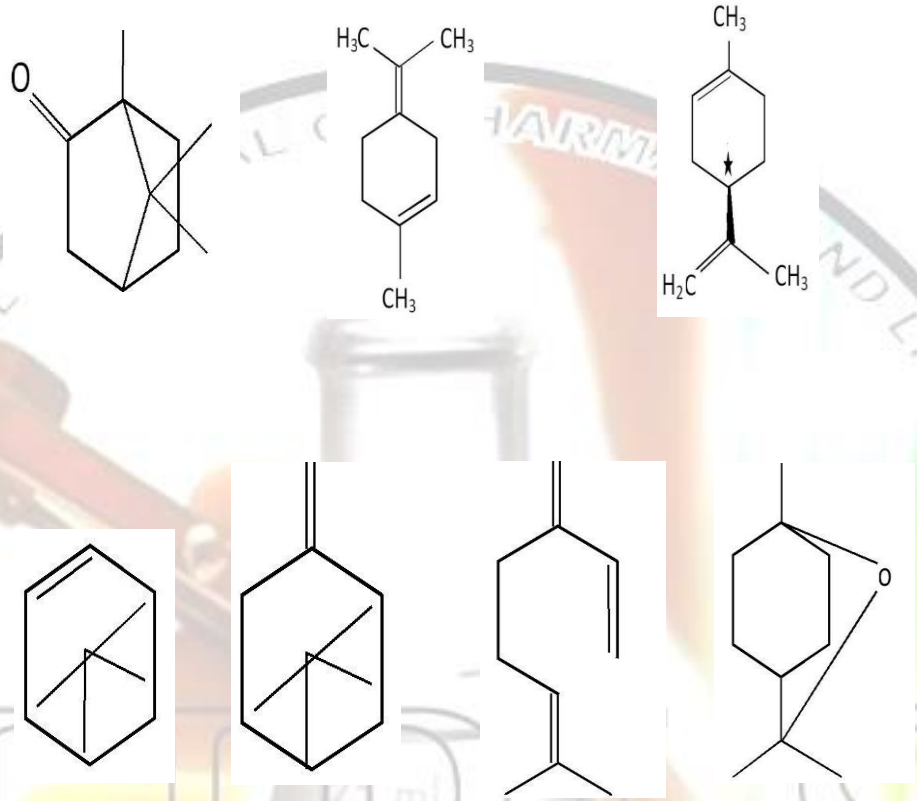


Fig. 1: *Ocimum* species cultivated in North East India (a) *O. sanctum* (b) *O. canum* (c) *O. gratissimum* (d) *O. basilicum*



Row 1: Camphor **Linalool** **1,8 -Cineole**
Row 2: Alpha- pinene **Beta-pinene** **Myrecene** **Limonene**

Fig. 2: Chemical constituents of some isolated compounds from *Ocimum* species (Dolly *et al*., 2012)

Table 1: *Ocimum* species used in different ailments of North East India

Plant species	Parts Used	Mode of administration	Uses	References
O. basilicum	Shoot	Extract mixed with honey	Fever & cough	[54]
O. canum	Leaf	Extract, decoction or extract with honey	Dysentery, mouth ulcer Fever, cough	[54]
O. gratissimum	Leaf	Crushed	Stopping bleeding of fresh wounds	[54]
O. sanctum	Leaf	Leaf mixed with seed & leaf of *Caesalpinia bonduc* and roots of *Mimusa pudica* is made into paste and given 1 or 2 spoons thrice daily	Indigestion & liver trouble	[55]
	Leaf	Decoction	Cough	[56]
	Leaf	Extract	Cold, lung congestion in children	[54]
	Leaf	Juice mixed with honey	Cough & cold	[57], [58]
	Leaf	Juice mixed with mixture from grinding of fruit of *Musa paradisiaca* and *Garcinia*	Blood dysentery	[59]

		kowa, given at doses of 2 teaspoonful 3 times daily		
	Leaf, inflorescence	Pounded together with common salt	Stomach trouble	[60]

Table 2: Major oil constituents of *Ocimum* species of North East India

Source	Parts used	Average oil percentage (on fresh weight basis)	Percentage of major constituents		
			Methyl chavicol	Linalool	Eugenol
O. canum L.	Leaf	0.67	77.0	18.0	-
O. gratissimum L.	Leaf	0.54	-	-	74.4
O.sanctum	Leaf	0.7	-	-	71.0

Table 3: Oil components of three different chemotypes of *O. basilicum* of North East India

Components	Chemotype I (%)	Chemotype II (%)	Chemotype III (%)
Camphor	42.1	trace	-
Methyl chavicol	-	74.7	-
Methyl cinnamate	-	-	60.21
Linalool	trace	14.3	18.54

Table 4: Major chemical constituents of the essential oil in different parts of *O. basilicum* var. *purpurascens* [3]

Plant parts	Oil (W/W) %	Major components in the Oil (%)	
		Methyl cinnamte	Linalool
Stem	0.06	44.35	9.97
Leaf	0.45	53.85	13.47
Flower	0.79	**60.21**	**18.54**

Table 5: Aroma chemicals present in *Ocimum* species and their industrial importance

Aroma chemicals	Parts used	Major source	Industrial importance	Pharmaceutical properties
Linalool	Whole herb	Ocimum	Perfumery & pharmaceutical value	Antiseptic
Camphor	-do-	-do-	-do-	Respiratory disorders
Borneol	-do-	-do-	-do-	Antiseptic
Nerol	-do-	-do-	-do-	Sedative, antiseptic
Methyl cinnamate	-do-	-do-	Perfumery	-
Pinene	-do-	-do-	-do-	Antiseptic, insecticidal
Eugenol	-do-	-do-	-do-	Antiseptic
Geraniol	-do-	-do-	Perfumery, flavor & pharmaceutical value	Antiseptic
Methyl chavicol	-do-	-do-	-do-	-
Cineol	-do-	-do-	Flavor & pharmaceutical value	Expectorant
Thymol	-do-	-do-	-do-	Antiseptic
Linalyl acetate	-do-	-do-	-do-	Sedative, antifungal
Bornyl acetate	-do-	-do-	-do-	Sedative, antifungal