

Progress in Essential Oils

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Sacred Basil, or Tulsi Oil

Sacred basil, holy basil or tulsi (*Ocimum tenuifolium* L. [syn. *O. sanctum* L.]) is a perennial member of the Lamiaceae (Labiatae) family (Paton et al. (1999). It is an aromatic, erect, much-branched subshrub that can grow to a height of 30–60 cm. Like all Lamiaceae, *O. tenuifolium* possesses quadrangular (square) stems and opposite ovate to ellipticaloblong green or purplish leaves with serrated margins borne on relatively long petioles. It possesses white flowers which arise in whorls (arranged in circles) on the terminal apexes of the stems.

Ocimum tenuiflorum is native to the tropical regions of India and possibly Burma and Malaysia; however, it is widely cultivated. In India it has been known since the Vedic period (1,500-600 BC) and is held sacred (hence its name) by Hindus. It can be commonly found planted around Hindu temples and is used in religious rosaries (Das and Vasudevan, 2006). It is also used as a traditional medicinal plant by several ancient systems such as Ayurveda, Siddha, Unani and even those of Greek and Roman medicine in which it is reported to have a wide range of therapeutic uses (Mondal et al., 2009). Ocimum tenuifolium oil is readily available in India, although only in levels of 1–2 metric tonnes annually.

Dutt (1939) reported that the major components of an oil of *O. tenuifolium* of Indian origin were:

 $\begin{array}{l} \beta \text{-caryophyllene (1.7\%)}\\ \text{carvacrol (3.2\%)}\\ \text{methyl eugenol (20.4\%)}\\ \text{eugenol (71.3\%)} \end{array}$

A sample of oil produced from *O*. *tenuiflorum* that was grown by the Applied Scientific Research Corporation of Thailand (Bangkok) was analyzed by Lawrence et al. (1972) using a combination of column and gas chromatographic techniques. All oil components were characterized using infrared spectroscopy. They were as follows:

 α -pinene (0.7%) camphene (0.7%) β -pinene (0.5%) sabinene (0.1%) limonene (0.2%) 1,8-cineole (0.1%) γ -terpinene (0.1%) α -cubebene (0.2%) α -copaene (2.0%) linalool (1.5%) isocaryophyllene (0.7%) β -elemene (5.1%) β -caryophyllene (27.4%) α -amorphene (0.3%) methyl chavicol (9.9%) borneol + α -selinenea (0.3%) germacrene $D^a + \gamma$ -humulene (9.9%) δ -cadinene + γ -cadinene (0.8%) caryophyllene oxide (0.8%)methyl eugenol (37.7%)

^amajor component

Trace amounts (<0.05%) of p-cymene, terpinolene and *cis*-calamenene were also characterized in this oil.

Lal et al. (1978) also analyzed a tulsi oil of Indian origin, which they determined contained the same major components:

 $\begin{array}{l} \alpha \text{-pinene (3.5\%)} \\ \text{camphene (2.0\%)} \\ \beta \text{-pinene (0.4\%)} \\ \text{decanal (0.2\%)} \\ \text{terpinen-4-ol (0.4\%)} \\ \beta \text{-caryophyllene (7.5\%)} \\ \text{carvacrol (3.5\%)} \\ \text{methyl eugenol (4.8\%)} \\ \text{nerol (6.4\%)} \\ \text{eugenol (70.5\%)} \end{array}$

Sobti et al. (1979) reported that the eugenol content of *O. tenuiflorum* oil

produced from Indian selections ranged from 48.9–57.0%.

A clone of *O. tenuiflorum* grown as a Kharif (short duration) crop in the vicinity of Delhi (India) by Pareek et al. (1980) was found to possess an oil content from the whole mature plant of 1.4%, which contained eugenol (52.2%) as the major constituent.

Lawrence et al. (1980) subjected the flowering tops of five taxa of *O. tenuiflorum* (at that time known as *O. sanctum*) that were grown in eastern North Carolina. Analysis of these oils using a combination of analytical techniques revealed that the oils, produced by steam distillation, differed in composition, as can be seen in **T-1**. This study revealed eugenol-, methyl-eugenol- and sesquiterpene-hydrocarbon-rich-forms.

Brophy and Jogia (1984) examined the composition of oils produced from seven cultivars of *O. tenuiflorum* that were grown in various locations in Fiji. The authors found that the oils could be grouped according to their oil compositions irrespective of the oil yield or leaf color (ranges of green and purple were found). Five of the cultivars possessed oil contents of 0.4–0.9% and were found to be rich in methyl eugenol. The other four cultivars that were rich in eugenol possessed oil contents of 0.4–0.6%. The composition of the methyl eugenol-rich oils was determined to be as follows:

 $\begin{array}{l} \alpha \text{-pinene } (0-t) \\ \text{camphene } (0-t) \\ \beta \text{-pinene } (0-t) \\ \text{sabinene } (0-t) \\ \text{myrcene } (0-t) \\ \text{limonene } (0-t) \\ \alpha \text{-copaene } (0-t) \\ \alpha \text{-cubebene } (0-t) \\ \beta \text{-bourbonene } (0-t) \\ \beta \text{-bourbonene } (0-t) \\ \beta \text{-cubebene } (0-t) \\ \beta \text{-caryophyllene } (3.1-10.0\%) \\ \alpha \text{-humulene } (0-0.6\%) \end{array}$

T-1. Comparative percentage composition of the oils of a number of taxa of *Ocimum tenuiflorum*

Compound	1	2	3	4
α -pinene	t	0.2-0.3	0.2	0.1
camphene	_	0t	t	0.1
β-pinene	t	0.8–1.0	1.2	0.1
sabinene	_	0.1-0.2	0.9	t
myrcene	t	0.2-0.3	0.1	t
α -terpinene	_	t	-	t
limonene	t	0.1–0.3	0.1	t
1,8-cineoleª + (Ζ)-β-ocimene	t	4.4-12.1	19.3	0.2
(E)-β-ocimene	-	0.1–0.3	0.2	t
γ-terpinene	t	1.3–5.3	3.2	0.6
p-cymene	t	t	0.1	t
terpinolene	-	t	t	t
1-octen-3-ol	t	t	0.1	-
lpha-cubebene	0.1	t–0.1	t	-
lpha-copaene	2.3	0.2-0.9	0.1	-
camphor	1.1	t–0.8	0.1	t
linalool	2.5	0.4–0.7	0.4	0.7
isocaryophyllene	t	-	0.1	-
bornyl acetate	0.2	0–2.7	1.2	t
β -elemene ^a + <i>trans</i> - α -bergamotene	5.8	0.3–3.7	0.3	6.5
terpinen-4-ol + β -caryophyllene ^a	6.6	2.0–2.8	1.6	33.3
lpha-amorphene	t	0t	0.1	t
methyl chavicol	0.6	1.8–12.2	15.1	2.0
borneol + α -selinene ^a	0.1	t	t	t
α -terpineol + α -humulene ^a	0.1	0.2–1.7	0.9	t
germacrene D ^a + γ -humulene	t	2.0–2.4	0.9	0.1
δ -cadinene ^a + γ -cadinene	t	-	-	0.4
β-bisabolene	-	30.0–33.4	11.0	-
(Z)-α-bisabolene	-	16.3–19.5	7.2	-
<i>cis</i> -calamenene	_	0t	_	_
caryophyllene oxide	10.3	1.0–1.1	0.4	1.4
methyl eugenol	45.4	0-0.5	0.2	t
methyl (E)-cinnamate	1.4	0-0.2	0.1	t
humulene epoxide II	t	0-0.4	-	-
eugenol	0.4	0-8.3	27.4	50.4
lpha-bisabolol	-	0–0.3	-	-
t = trace (<0.05%); ^a major component	t			

$$\begin{split} & \text{isoborneol} + \text{unknown} \ (0\text{--}1.7\%) \\ & \beta\text{-cubebene}^a \ (t\text{--}4.8\%) \\ & \delta\text{-cadinene} \ (0\text{--}2.5\%) \\ & \text{methyl eugenol} \ (77.5\text{--}86.0\%) \end{split}$$

t = trace (<0.05%) ^ashould be germacrene D

The constituents characterized in the two eugenol-rich type were as follows:

 $\begin{array}{l} \beta\mbox{-caryophyllene} (20.7\mbox{-}42.0\%) \\ \alpha\mbox{-humulene} (1.8\mbox{-}3.0\%) \\ \mbox{caryophyllene} \mbox{oxide} (0\mbox{-}4.5\%) \\ \mbox{methyl} \mbox{eugenol} (13.4\mbox{-}20.0\%) \\ \mbox{eugenol} (22.6\mbox{-}62.2\%) \end{array}$

In addition, trace amounts (<0.05%) of a-cubebene and β -bourbonene were characterized in the oil. Tentative identifications of β -copaene (1.5%) and δ -cadinene (<0.05%) were also reported in this oil.

Philip and Damodaran, (1985 and Vimalan et al. 1989), examined oils from four different selections of *O. tenuiflorum* grown at the International Institute of Ayuveda (Coimbatore, India). They found that the oils were either rich in eugenol or methyl eugenol. The two eugenol-rich oils contained: $\begin{array}{l} \beta \text{-caryophyllene} \ (6.7{-}24.8\%) \\ methyl \ eugenol \ (t{-}2.5\%) \\ eugenol \ (71.5{-}82.8\%) \end{array}$

t = trace (<0.1%)

In contrast, the methyl eugenol-rich oils contained:

 $\begin{array}{l} \beta \text{-caryophyllene} \ (17.3\text{--}20.4\%) \\ \text{methyl eugenol} \ (70.9\text{--}72.7\%) \end{array}$

Only a trace (<0.1%) of eugenol was found in either oil. Also, no carvacrol was characterized in any of the oils.

Shafiq Malik et al. (1986) produced an oil in 0.85% yield by steam distillation from the non-flowering plants of *O. tenuiflorum* collected from local nurseries in Lahore (Pakistan). With gas chromatographic separation of the oil conducted on a polar-packed column and retention times as their method of identification, the authors reported that the main components of the oil were:

 $\begin{array}{l} \beta \text{-caryophyllene (1.6\%)} \\ \text{carvacrol (30.4\%)} \\ \text{methyl eugenol (1.8\%)} \\ \text{eugenol (61.2\%)} \end{array}$

This reviewer believes that carvacrol was a misidentification.

Maheshwari et al. (1987) used a combination of column chromatography, thin-layer chromatography, gas chromatography as the separation techniques, and infra-red, ¹H-NMR and MS spectroscopic techniques for characterization to analyze an oil of *O. tenuiflorum* produced from plants grown in India. After separation of eugenol (54%) from the oil, analysis of the non-eugenolic portion of the oil revealed that it contained the following constituents:

nonane^a (0.1%) decane^a (0.1%) α-pinene (1.6%) camphene (0.2%) β-pinene (0.2%) ethylbenzene^a (0.2%) limonene (0.1%) 1,8-cineole (0.4%) (Z)-β-ocimene (0.1%) (E)-β-ocimene (1.1%) p-cymene (0.1%) 3-hexenol^b (0.1%) heptanol + α-cubebene^c (0.1%) linalool oxide^{*f} (0.1%) α-copaene (1.0%) β -bourbonene (0.3%) β -cubebene (0.5%) linalool (0.5%) octanol (0.7%) β-elemene (44.2%) β -caryophyllene (31.3%) β -farnesene° (0.2%) α -humulene (1.9%) selina-4,11-diene (0.6%) borneol^c + α -campholenal (2.0%) germacrene D (1.0%) α -selinene (2.5%) β -selinene (2.3%) $\delta\text{-cadinene}\;(0.6\%)$ myrtenyl formate^{\dagger} (0.1%) cuparene (0.1%)calamenene° (0.1%) geraniol (0.2%) isocaryophyllene oxide (0.1%)caryophyllene oxide (2.5%) methyl eugenol (0.4%) humulene epoxide $^{\circ}(0.1\%)$ eugenol (0.8%) ^aartefact

^b(Z)-isomer ^cmajor component ^{*}correct isomer not identified [†]incorrect identification ^ffuranoid form

In addition, trace amounts (<0.05%) of octane, benzene, toluene, dimethylbenzene isomers, myrcene, an allo-ocimene isomer, octanal, butylbenzene, hexanol, 3-octanol, 1-octen-3-ol, an ocimene epoxide isomer and α -terpineol were found in this same oil; however, it is the belief of this reviewer that the non-terpenoid hydrocarbons were contaminants of the oil.

Using a combination of GC/MS and ¹H-NMR spectroscopy as their method of analysis and compound characterization, Sukari and Takahashi (1988) reported that an oil of *O. tenuiflorum* produced from plants grown in Malaysia contained the following major components:

 $\begin{array}{l} \beta \text{-caryophyllene (3.0\%)} \\ methyl eugenol (86.0\%) \\ eugenol (0.5\%) \end{array}$

Gulati and Sinha (1989) reported that an Indian oil of *O. tenuiflorum* contained the following components:

1,8-cineole (0.1%)linalool (0.2%)camphor (0.3%)citronellal (0.1%)isoborneol (0.5%)borneol (0.3%) α -terpineol (0.2%)citronellol (0.1%) geraniol (0.5%)citral^{*} (0.2%)thymol (0.1%)eugenol (54.0%)methyl eugenol (18.0%) β -caryophyllene (15.0%) α -humulene (0.8%)methyl isoeugenol^{*} (0.8%)eugenyl acetate (3.0%) β -elemene (0.3%) γ -cadinene (1.0%)farnesol^{*} (0.1%)

° correct isomer not identified

Trace amounts (<0.05%) of α -pinene, camphene, β -pinene, terpinolene, bornyl acetate, an isomer of isoeugenol and γ -elemene were also reported to be characterized in this same oil.

Laakso et al. (1990) used a combination of GC/MS and GC-FTIR to analyze an oil of *O. tenuiflorum* that was produced from plants grown in an experimental garden at the University of Erlangen (Germany). The authors compared the compositions of oils produced by water distillation and steam distillation. The constituents found in both oils ranged as follows:

α-pinene (0.1%) β-pinene (0.3-0.4%) sabinene (0.1%) myrcene (0.1-0.2%) limonene (0.2%)1,8-cineole (5.6-11.0%) (Z)-β-ocimene (0.1-0.2%) (E)-β-ocimene (4.0-4.7%) trans-sabinene hydrate (0.2%) trans- α -bergamotene (0.9–1.9%) β -caryophyllene (1.4–2.5%) (E)-β-farnesene (0.3-0.6%) α-humulene (2.0-3.5%) methyl eugenol (11.6-14.4%) α -terpineol (0.3–0.5%) germacrene D (2.4-4.5%) β -bisabolene (7.6–15.4%) α-bisabolene[°] (9.4–19.6%) eugenol (24.2–38.2%) α -bisabolol (0.4–0.5%)

° correct isomer not identified

An oil of *O. tenuiflorum* was the subject of analysis by Skaltsa-Diamantidis et al. (1990). The constituents characterized in this oil were as follows:

 $\begin{array}{l} myrcene \; (0.1\%) \\ octanal \; (0.1\%) \\ linalool \; (0.1\%) \\ octanol \; (0.1\%) \\ \beta\mbox{-caryophyllene } (33.7\%) \end{array}$

 $\begin{array}{l} \alpha \text{-humulene (2.5\%)} \\ \beta \text{-farnesene}^* (0.1\%) \\ \beta \text{-selinene (1.0\%)} \\ \beta \text{-elemene (12.2\%)} \\ allo-aromadendrene (0.3\%) \\ caryophyllene oxide (13.5\%) \\ eugenol (23.9\%) \\ T\text{-muurolol (0.3\%)} \\ \hline \end{array}$

° correct isomer not identified

Trace amounts (<0.05%) of α -pinene, camphene, β -pinene, limonene, p-cymene, 3-hexenol isomer, 3-octanol, menthone, decanal, isomenthone, camphor, theaspirane I, benzaldehyde, theaspirane II, γ -elemene and menthol were also reported as constituents of this same oil.

A germplasm collection of genotypes of *O. tenuiflorum* in India was determined by Gupta (1992) to range in oil and eugenol content by 0.1–0.4% and 30–45%, respectively.

Ocimum tenuiflorum leaves that were collected in northern Queensland (Australia) were steam-distilled with cohobation to yield an oil of 4.1% based on the dry weight of leaves. Analysis of this oil by Brophy et al. (1993) revealed that it possessed the following composition:

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\begin{array}{l} \alpha \text{-pinene} \ (0.1\%) \\ \text{camphene} \ (0.2\%) \\ \text{limonene} \ (0.4\%) \\ \text{terpinolene} \ (0.1\%) \\ \text{camphor} \ (4.4\%) \\ \text{linalool} \ (0.1\%) \\ \beta \text{-caryophyllene} \ (5.3\%) \\ \text{methyl chavicol} \ (87.7\%) \\ \alpha \text{-humulene} \ (0.9\%) \\ \text{borneol} \ (0.6\%) \\ \text{caryophyllene} \ oxide \ (0.2\%) \\ \text{methyl eugenol} \ (0.1\%) \end{array}
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Trace amounts (<0.05%) of myrcene and (E)-anethole were also found as components of this same oil.

Pino et al. (1998) used GC-FID and GC/MS to analyze an oil of *O. tenuiflo-rum* that was produced in 1.6% yield by water distillation from the aerial parts of plants grown in an experimental garden near Havana (Cuba). The composition of this oil was determined to be as follows:

myrcene (0.1%) p-cymene (0.3%) 1,8-cineole (0.1%) limonene (0.2%) (E)- β -ocimene (0.1%) γ -terpinene (0.1%) linalool (0.2%) borneol (0.3%) menthol (0.2%)terpinen-4-ol (0.1%) thymol (2.4%) eugenol (34.3%) β -elemene (18.0%) β -caryophyllene (23.1%) γ -elemene (0.1%) α -humulene (2.0%) α -amorphene (0.1%) germacrene D (0.1%) (Z)- α -bisabolene (2.2%) β -bisabolene (1.1%) cis-calamenene (0.1%) δ -cadinene (0.3%) γ -cadinene (0.1%) elemol(0.9%)caryophyllene oxide (3.8%) cubenol (0.1%) T-cadinol (0.2%) β -eudesmol (0.1%)

Trace amounts (<0.1%) of α -thujene, α -pinene, camphene, sabinene, β -pinene, p-cymenene, *cis*-linalool oxide (furanoid form), isomenthone, α -terpineol, α -muurolene, (E)-nerolidol and caryophylla-3,8(13)-dien-5 β -ol were also characterized in this oil.

Fresh leaves of *O. tenuiflorum* that were harvested from cultivated plants grown in the New Delhi (India) area were subjected to hydrodistillation to yield an oil of 0.3%. Analysis of this oil by Raju et al. (1999) using GC-FID and GC/MS revealed that it possessed the following composition:

 α -pinene (0.2%) camphene (0.1%) β -pinene (0.1%) α -phellandrene (0.1%) 1,8-cineole (0.7%) borneol (0.3%)eugenol (53.4%) α -elemene[†] (0.3%) β -elemene (6.2%) β -caryophyllene (31.7%) α -humulene (1.7%) germacrene D (0.1%)valencene (0.1%) bicyclogermacrene (1.0%) δ -cadinene (0.2%) elemol (1.0%) caryophyllene oxide (1.5%)

[†]incorrect identification based on GC elution order

In addition, trace amounts (<0.1%) of sabinene, γ -terpinene, linalool, γ -gurjunene, epi-globulol, ledol and a humulene epoxide isomer were also characterized in this oil.

Lacerda Machado et al. (1999) compared the compositions of oils produced separately from the leaves and inflorescences of *O. tenuiflorum* grown in Fortaleza (Brazil). Steam distillation of the leaves and inflorescences resulted in oil yields of 2.5% and 0.3%, respectively, while microwave distillation resulted in oil yields of 1.1% and 0.3%, respectively. The main components of the leaf oil were determined by GC/MS only to be as follows:

 $\begin{array}{l} & \text{borneol} \; (0.4\%) \\ & \text{eugenol} \; (79.0\%) \\ & \beta \text{-elemene} \; (5.0\%) \\ & \beta \text{-caryophyllene} \; (9.8\%) \\ & \alpha \text{-humulene} \; (0.7\%) \\ & \text{germacrene A} \; (4.7\%) \end{array}$

In contrast, the composition of the inflorescence oil was as follows:

 $\begin{array}{l} (E)\text{-}\beta\text{-}ocimene~(0.3\%)\\ eugenol~(17.6\%) \end{array}$

β-elemene (5.8%) isocaryophyllene (0.9%) β-caryophyllene (40.7%) α-humulene (2.9%) β-selinene (1.3%) germacrene A (4.4%) globulol (0.6%) caryophyllene oxide (18.5%) selin-ll-en-4α-ol (0.5%)

Bradu et al. (2000) reported that oils of *O. sanctum* (syn. *O. tenuiflorum*) produced from plants grown in Kerala possessed 0.5–1.0% with eugenol (35– 40%) being the major constituent.

An oil produced from *O. tenuiflorum* cultivated in the experimental garden of the university of Benin (Lomé, Togo) was analyzed using GC/MS only by Chaumont et al. (2001). The components characterized in this oil were as follows:

 $\begin{array}{l} \alpha \text{-pinene} \ (0.5\%) \\ \text{sabinene} \ (0.6\%) \\ \beta \text{-pinene} \ (0.9\%) \\ \text{myrcene} \ (0.6\%) \\ (E) \text{-}\beta \text{-crimene} \ (3.2\%) \\ 1,8 \text{-crineole} \ (6.2\%) \\ \text{terpinolene} \ (1.0\%) \\ \text{methyl chavicol} \ (7.4\%) \\ \text{methyl eugenol} \ (74.5\%) \\ \alpha \text{-farmesene}^* \ (4.1\%) \\ \text{germacrene D} \ (0.6\%) \\ \alpha \text{-amorphene}^\dagger \ (0.6\%) \end{array}$

 $^{\dagger}\text{incorrect}$ identification based on GC elution order $^{\circ}\text{correct}$ isomer not identified

Both the green and purple leafed forms of *O. tenuiflorum* that were grown in Chittagong (Bangladesh) were hydrodistilled separately to produce oils 0.26% (green) and 0.52% (purple). Analysis of these oils by GC-FID and GC/MS by Mondello et al. (2002) revealed that they had qualitatively similar-compositions as shown in **T-2**.

Jirovetz et al. (2003) produced an oil from the fresh leaves of *O. tenuiflorum* of Kerala (India) origin using hydrodistillation. For some strange reason the oils were only analyzed as headspace volatiles using solid-phase microextration (SPME). The SPME needle, which was coated with 2 cm (50/30 μ m) divinylbenzene/carboxen/polydimethyl methylsiloxane, was introduced via a septum into the headspace above the oil at room temperature for 30 minutes (an equilibrated headspace). Thermal desorption followed by GC-FID and GC/ MS analysis revealed that the headspace

T-2. Comparative percentage composition of oils of the green and purple forms of *Ocimum tenuiflorum* of Bangladesh origin

Compound	Green form oil	Purple form oil
α -pinene	0.1	0.1
camphene	t	0.1
sabinene	0.1	_
β-pinene	0.7	0.1
myrcene	0.1	t
octanal	0.1	0.2
p-cymene	0.3	t
limonene	3.8	0.6
1,8-cineole	0.1	0.1
(E)-β-ocimene	0.1	0.3
γ-terpinene	0.2	t
octanol	0.3	0.2
linalool	0.1	0.1
borneol	0.2	0.4
geraniol	0.1	-
eugenol	41.7	77.5
β-elemene	0.2	0.1
isocaryophyllene	5.3	2.1
methyl eugenol	t	0.1
β-caryophyllene	24.4	10.7
α -santalene	0.1	0.1
α -humulene	1.4	0.6
<i>cis</i> -muurola-4(14),5-diene	0.1	t
β-selinene	2.0	0.9
α -selinene	2.4	1.2
germacrene A	2.9	0.6
7-epi-α-selinene	0.1	t
δ-cadinene	0.3	0.1
<i>cis</i> -sesquisabinene hydrate	0.2	-
elemol	0.1	0.1
caryophyllene oxide	5.1	1.1
humulene epoxide II	0.1	0.1
tetradecanal	0.3	-
T-cadinol	0.2	-
selin-II-en-4a-ol	0.7	0.4
epi-a-bisabolol	-	0.6
14-hydroxy- α -humulene *	0.3	0.1
*tentative identification t = trace (<0.05%)		

T-3. Comparative percentage composition of oils produced from three developmental stages of *Ocimum tenuiflorum*

Compound	Pre-flowering stage oil	Flowering stage oil	Fruiting stage oil
ethyl 2-methylbutyrate	t	0t	0.4
ethyl isovalerate	0.3	0.2-4.6	2.2
α-pinene	0.6	0.5–1.4	0.7
camphene	0.2	0t	0.1
sabinene	0.4	0.5–1.0	0.3
β-pinene	1.1	1.8–2.9	1.3
myrcene	0.8	0.8–0.9	0.5

T-3. Comparative percentage composition of oils produced from three developmental stages of *Ocimum tenuiflorum* (Cont.)

Compound	Pre-flowering stage oil	Flowering stage oil	Fruiting stage oil
α -terpinene	t	0.2–0.3	0.2
p-cymene	0.1	t-0.2	0.3
limonene	0.3	0.3–0.4	0.2
1,8-cineole	8.9	32.2–33	15.3
(E)-β-ocimene	1.7	1.4–1.7	1.7
γ-terpinene	0.1	0.2	0.1
<i>trans</i> -sabinene hydrate	0.3	0.6–1.3	0.3
<i>cis</i> -linalool oxide ^f	0.1	0-t	t
<i>trans</i> -linalool oxide ^f	0.1	0.3–0.4	0.1
terpinolene	0.1	0-t	0.2
linalool	19.2	0.6	6.3
camphor	0.1	0.1	0.1
δ-terpineol	0.2	0.1–0.2	0.4
borneol	2.4	0.2–0.4	1.0
terpinen-4-ol	0.2	0.4	0.3
α-terpineol	1.7	5.1–7.0	3.9
methyl chavicol	1.9	10.2–12.5	5.2
myrtenol	t	t	t
α -fenchyl acetate	0.1	t	0.1
isobornyl formate	0.1	t	0.1
chavicol	0.9	0.2–1.0	0.5
bornyl acetate	0.4	0-t	0.6
carvacrol	t	_	t
eugenol	8.9	4.1-4.6	7.1
α-cubebene	0.1	t–0.1	t
β-damascenone*	0.1	0.1	0.1
α-copaene	0.3	0.2	0.3
β-bourbonene	0.1	t	0.2
β-elemene	1.2	0.3	0.9
<i>cis</i> -α-bergamotene	0.2	0.2	0.2
β-caryophyllene	0.9	1.2-1.4	1.4
<i>trans</i> - α -bergamotene	1.6	2.3-3.0	2.0
(Z)-β-farnesene	1.1	t	0.9
α-humulene	0.7	0.6	0.9
ar-curcumene	0.3	t-0.4	t
γ-muurolene	1.4	0.5–0.9	1.4
(E)-β-farnesene	0.1	t	0.4
bicyclogermacrene	0.1	t	0.1
α -selinene	0.3	t	0.1
β-bisabolene	15.4	13.0–14.4	20.4
β -sesquiphellandrene	0.2	0.1	0.2
(E)- $lpha$ -bisabolene	4.9	4.1–4.3	6.9
(E)-nerolidol	0.2	0—t	t
spathulenol	1.3	0.1–1.1	0.4
caryophyllene oxide	1.0	0.1–0.7	1.1
salvial-4(14)-en-l-one	0.2	0.1	0.2
humulene epoxide II	1.1	0.3–0.6	1.1
1,10-di-epi-cubenol	0.8	t0.2	0.5
T-cadinol	3.6	0.2–0.3	1.1
lpha-bisabolol	1.4	0-0.4	1.0
t=trace (<0.05%) *correct isomer not identified			

*correct isomer not identified ffuranoid form

volatiles of this oil were found to be as follows:

l-hexen-3-ol (0.1%) hexanal (0.1%) (E)-2-hexenal (0.1%) (Z)-3-hexenol (0.5%)(E)-2-hexenol (0.1%) hexanol (0.1%)l-octen-3-ol (0.2%) 3-octanol (0.1%) camphene (0.1%)limonene (0.1%)fenchone (0.2%) linalool (0.5%)2-phenethyl alcohol (0.9%) nonanol (0.1%)borneol (0.3%) α -terpineol (0.1%) methyl chavicol (0.2%) geraniol (0.1%) myrtenol (0.1%) nonanoic acid (0.2%)thymol (0.4%) carvaerol (0.1%) eugenol (1.7%) α -copaene (0.2%) methyl eugenol (56.2%) $\beta\text{-bourbonene}\;(0.1\%)$ β -cubebene (0.1%) β -elemene (1.7%) α -gurjunene (0.3%) β -caryophyllene (16.6%) β -gurjunene (0.2%) α -bergamotene° (0.2%) aromadendrene (1.0%)amorphene[°] (0.1%) (E)- β -farmesene (0.1%) α -humulene (0.7%) allo-aromadendrene (0.1%) α -guaiene (0.2%) γ -muurolene (0.1%) germacrene D (5.1%) β -selinene (0.3%) ledene (1.9%) α -selinene (0.1%) (E,E)- α -farmesene (0.1%) $\alpha\text{-cadinene}\;(0.2\%)$ γ -cadinene (0.7%) α -bisabolene° (0.1%) δ -cadinene (0.2%) germacrene B (0.4%) nerolidol° (0.1%) globulol (1.0%) spathulenol (0.6%) caryophyllene oxide (1.1%)

°correct isomer not identified

Trace amounts (0.05%) of 2-hexanol, (E,E)-2,4-hexadienal, (Z)-3-hexenyl acetate, nonanal, sabinene, β -pinene, p-cymene, β -phellandrene, (Z)- β ocimene, γ -terpinene, 1,8-cineole, *cis*-linalool oxide (furanoid form), α -fenchol, camphor, *trans*-verbenol, *cis*-verbenol, terpinen-4-ol, myrtenol, nerol, α -cubebene and bicyclogermacrene were also characterized in the headspace of *O. tenuiflorum*.

Kicel et al. (2005) examined the oils produced from *O. tenuiflorum* plants grown in the Botanical Garden in Wroclaw (Poland) that were harvested at four different development stages (preflowering, flowering, end of flowering and fruit formation the oil yields for the four stages [as above]) were 0.68%, 0.59%, 0.83% and 0.69%, respectively. Analysis of these oils using fractional distillation, flash chromatography GC-FID, GC/MS and ¹H-NMR resulted in the characterization of 57 constituents. A summary of the findings can be seen in **T-3**.

Kothari et al. (2005) produced oils from the whole plant as well as plant parts such as leaves, stems and inflorescences of *O. tenuiflorum* grown at CIMAP (Hyderabad, India) using hydrodistillation. The oil yields from the whole plant, leaves, stems and inflorescences were 0.56%, 0.65%, 0.05% and 0.73%, respectively. The results of the GC-FID and GC/MS analyses of these oils can be found in **T-4**.

An oil produced in 2.1% yield from *O. tenuiflorum* plants grown in the medicinal plant garden of the University Federal do Ceara (Fortaleza, Brazil) by Salles Trevisan et al. (2006) was determined by GC/MS only to contain the following main components:

 $borneol (1.0\%) \\ eugenol (59.4\%) \\ \beta-caryophyllene (29.4\%) \\ \alpha-humulene (1.5\%) \\ \alpha-selinene (0.7\%) \\ germacrene A (8.1\%)$

An oil from the fresh leaves of *O*. *tenuiflorum* grown in the Delhi (India) area was screened for its antimicrobial properties by Mondal et al. (2007). The main components of this oil were determined to be:

 $\begin{array}{l} \mbox{eugenol} (57.9\%) \\ \beta\mbox{-caryophyllene} (15.3\%) \\ \beta\mbox{-elemene} (7.6\%) \\ \mbox{germacrene} D (9.1\%) \\ \mbox{caryophyllene} oxide (3.2\%) \end{array}$

Bunrathep et al. (2007) examined the composition of (0.06% yield) two oils produced in the laboratory from

T-4. Comparative percentage composition of oils produced from the whole plant, leaves, stems and inflorescences of *Ocimum tenuiflorum*

Compound	Whole plant oil	Leaf oil	Stem oil	Inflorescence oil
(Z)-3-hexenol	0.2	0.1	0.7	0.4
ethyl 2-methylbutyrate	t	t	t	_
α -pinene	0.1	0.1	0.1	0.1
β-pinene	0.1	0.1	0.1	0.1
myrcene	0.1	0.1	0.1	0.1
limonene	0.1	t	t	0.1
(E)-β-ocimene	0.1	t	t	0.1
γ-terpinene	0.1	0.1	t	0.1
<i>trans</i> -linalool oxide ^f	0.1	0.1	t	0.1
linalool	t	0.1	0.1	t
eugenol	0.9	0.8	0.7	0.2
methyl eugenol	72.5	75.3	83.7	65.2
β-elemene	0.5	2.8	t	0.3
(E)-cinnamyl acetate	3.4	3.4	0.9	7.7
β-caryophyllene	5.5	6.4	2.7	12.0
α -guaiene	t	t	t	0.1
α -humulene	0.4	0.6	0.2	0.8
β-selinene	0.1	0.1	t	0.1
α-muurolene	0.2	0.4	0.1	0.2
δ-cadinene	0.1	0.2	0.1	0.1
nerolidol [*]	0.1	0.1	t	0.1
caryophyllene oxide	1.5	0.8	1.7	3.0
guaiol	0.1	0.2	0.1	0.1
T-cadinol	0.3	0.3	0.4	0.2
β-eudesmol	0.1	0.2	0.4	0.1
α-bisabolol	t	0.1	t	0.1
(E,Z)-farnesol	0.1	0.5	0.9	0.7
t=trace (<0.05%) ^f furanoid form				

*correct isomer not identified

endemic plants of white and red kaprao or *O. tenuiflorum* growing in the experimental garden of Rangsit University (Pathumthani, Thailand), using GC/MS as the only analytical method the oil compositions were found to range as follows:

α-thujene (0.5-0.7%) camphene (0.4-0.7%) sabinene (0.1–0.2%) β-pinene (0.3–0.5%) limonene (0.2-0.4%) linalool (0-0.3%) borneol (0.4%) eugenol (1.7–2.0%) α -bulnesene[†] (1.6–3.4%) $\alpha\text{-copaene}\;(0.4\text{--}0.6\%)$ β -elemene (0.8–1.2%) methyl eugenol (47.2-53.7%) β-caryophyllene (35.2-37.8%) α-humulene (1.8–1.9%) γ -muurolene (2.1–3.5%) [†]incorrect identification

Oils produced from the 'Shyama' and 'Rama' cultivars of *O. tenuiflorum* were analyzed by Awasthi and

Dixit (2007) using GC-FID and GC/ MS. The oil from the 'Shyama' cultivar was found to contain the following constituents:

heptane (0.1%)octane (0.1%)(Z)-3-hexenol (0.1%)nonane (0.1%)camphene (0.3%) β -pinene (0.2%)sabinene (0.1%)(Z)- β -ocimene (0.1%)trans-linalool oxide^{*} (0.3%)linalool (0.2%)borneol (1.0%) α -terpineol (0.2%)octyl acetate (0.2%)geraniol (0.3%)eugenol (0.6%) β -elemene (4.3%) isocaryophyllene (0.5%) methyl eugenol (67.8%) β -caryophyllene (17.1%) α -humulene (1.0%) germacrene D (1.4%) β -selinene (0.2%) elemol (0.5%)(E)-nerolidol (0.1%) caryophyllene oxide (0.6%) humulene epoxide° (0.1%)T-cadinol (0.1%) α -eudesmol (0.2%)

° correct isomer not identified

Trace amounts (<0.04%) of terpinen-4-ol, p-cymen-8-ol, geranyl formate, α-santalene, germacrene A, cis-sesquisabinene hydrate, tetradecanol, epi-α-bisabolol and 14-hydroxy-αhumulene were also characterized in this oil.

In contrast, the oil from the 'Rama' cultivar was found to possess the following composition:

myrcene (0.1%)trans-linalool oxide° (0.1%)borneol (0.1%)octyl acetate (0.1%)geraniol (0.1%)eugenol (46.2%) geranyl acetate (0.9%) β -elemene (16.3%) isocaryophyllene (0.4%) caryophyllene (27.6%) α -santalene (0.1%) α -humulene (1.7%) germacrene D (0.1%) β -selinene (0.1%) bicyclogermacrene (0.3%) germacrene A (0.4%) γ -cadinene (0.1%) 7-epi- α -selinene (0.1%) δ -cadinene (0.1%) cis-sesquisabinene hydrate (0.1%)elemol (1.1%) (E)-nerolidol (0.1%) caryophyllene oxide (1.5%) humulene epoxide $^{\circ}(0.1\%)$ tetradecanol (0.2%) T-cadinol (0.2%) epi- α -bisabolol (0.1%)

° correct isomer not identified

Omer et al. (2008) examined the oil composition of a cultivar of O. tenuiflorum (not O. tenuiflorum, as noted by the authors) produced from plants grown at Shalakan (Kalubia Governorate, Egypt) harvested after 90 and 180 days (grown using saline irrigation), respectively. The T-5. Comparative percentage composition of Ocimum tenuifolium oils produced after 90 and 180 days of growth

Compound	90 day oil	180 day oil
α -pinene	0.3	0.5
sabinene	0.4	0.4
myrcene	0.4	0.5
1,8-cineole	8.3	8.3
lpha-terpinene [†]	0.8	1.0
linalool	55.3	39.4
camphor	0.2	0.5
terpinen-4-ol	4.5	5.8
α-terpineol	_	2.9
methyl chavicol	6.7	-
nerol	0.5	8.0
geraniol	0.3	1.8
citral†*	6.8	4.3
neryl acetate	0.3	0.4
γ-elemene	1.1	2.1
β-caryophyllene	1.3	2.1
<i>trans</i> -α-bergamotene	-	4.4
1-epi-bicyclophellandrene	0.3	0.1
muurolene [*]	0.1	3.9
lpha-humulene	0.8	1.5
$lpha$ -cubebene †	-	1.6
germacrene D	1.7	0.4
lpha-guaiene	0.5	0.6
cadinene [*]	1.5	-
(Z)-α-bisabolene	0.9	1.4
spathulenol	0.2	-
isocubenol [‡]	0.3	0.6
T-cadinol	4.9	4.9
oil yield(%)	0.92	1.29
*correct isomer not identified		

[†]incorrect identification based on GC elution order [‡]unknown compound

oils which were analyzed using GC/MS only were found to be rich in linalool as can be seen in **T-5**.

Lal et al. (2008) reported the registration of a new cultivar of O. tenuiflorum which they called 'Angana, 'which was developed from the 'Shyama' cultivar. The main components of oil of this newly developed cultivar were:

 α -pinene (0.1%) camphene (0.1%) sabinene (0.1%) limonene (0.8%) p-cymene (0.1%) 1.8-cineole (2.5%) linalool (1.9%) eugenol (40.4%) β -elemene (14.1%) β -caryophyllene (9.1%) germacrene D (16.7%)

An oil that was produced from O. tenuiflorum by hydrodistillation from plants collected from the vicinity of Ile-Ife (Nigeria) was screened for its potential lavicidal action against the fourth instar Aedes aegypti mosquitos by Gbolade and Lockwood (2008). The oil, which was analyzed by both GC-FID and GC/MS, was found to possess the following components:

 α -pinene (1.9%) camphene (2.0%)myrcene (0.3%) β -pinene (1.2%) p-menthatriene^{\dagger} (0.2%) limonene (1.0%) borneol (4.8%) eugenol (0.4%) germacrene B (0.4%) methyl eugenol (44.7%) T-6. Comparative percentage composition of the volatiles of the extracts of *Ocimum tenuiflorum* leaves harvested at two different times

Compound	September 2007 volatiles	June 2008 volatiles
camphene	_	0.8
borneol	1.4	2.7
methyl chavicol	4.7	_
eugenol	_	2.8
indole [†]	1.6	_
methyl eugenol	74.3	62.3
α-copaene	0.9	_
β-elemene	4.4	1.6
β-caryophyllene	8.5	8.7
α -farnesene*	_	11.3
lpha-humulene	0.7	_
germacrene D	2.8	4.9
elemol	0.7	_
hedycaryol [†]	_	0.7
farnesol [*]	_	4.2
[†] probably misidentification *correct isomer not identified		

 $\begin{array}{l} \alpha \text{-copaene} \ (0.7\%) \\ \beta \text{-elemene} \ (8.8\%) \\ \text{isocaryophyllene}^a \ (16.8\%) \\ \text{germacrene} \ D \ (1.2\%) \\ (E) \text{-}\beta \text{-ocimene}^\dagger \ (1.2\%) \end{array}$

 $\begin{array}{l} \mbox{caryophyllene oxide (2.7\%)} \\ \mbox{α-bisabolol (0.1\%)$} \end{array}$

 $^{\dagger}incorrect$ identification based on GC elution order $^{a}probably$ misidentification of $\beta\text{-caryophyllene}$

Dohi et al. (2009) screened an Indian oil of *O. tenuiflorum* for its acetylcholinesterase activity. The main components of this oil were reported to be as follows:

 $\begin{array}{l} \alpha \text{-pinene} \ (0.1\%) \\ \\ \text{limonene} \ (0.1\%) \\ \\ 1,8\text{-cineole} \ (0.4\%) \\ \\ \\ \text{linalool} \ (0.1\%) \\ \\ \text{eugenol} \ (59.0\%) \\ \\ \beta \text{-caryophyllene} \ (33.0\%) \\ \\ \alpha \text{-humulene} \ (3.0\%) \end{array}$

Vani et al. (2009) collected *O. tenuiflorum* from an orchard in Petaling Jaya (Malaysia) and subjected fresh leaves harvested over a growing season to static hexane extraction for an hour once they were cut up into small pieces. After filtration initially by gravity and finally through a silica gel column to remove the high boiling imparities, the volatiles from each extraction were separately analyzed by GC/MS only. A comparison between the extract volatiles of leaves harvested in September 2007 and June 2008 can be seen summarized in **T-6**.

An oil of *O. tenuiflorum* that was screened for its antifungal activity by

Amber et al. (2010) was found, by GC/ MS alone, to contain the following components:

 † incorrect identification

Munoz-Acevedo et al. (2011) analyzed an oil produced from the fresh leaves and flowers of *O. tenuiflorum* grown in an experimental garden at CENIVAM agro-industrial complex of the Industrial University of Santander (Bucaramanga, Colombia). Using a combination of GC-FID and GC/MS, the oil composition was determined to be as follows:

```
(E)-3-hexenol<sup>a</sup> (0.6%)
\beta-pinene (1.0%)
myrcene (0.6%)
1,8-cineole (8.8%)
(E)-\beta-ocimene (2.4%)
fenchone (8.8%)
linalool (19.0%)
camphor (1.8\%)
\alpha-terpineol (1.4%)
geraniol (0.9%)
bornyl acetate (0.6%)
eugenol (46.7%)
\beta-elemene (1.0%)
(E)-\beta-farmesene (1.8%)
\alpha-humulene (0.5%)
germacrene D (2.2%)
bicyclogermacrene (1.0\%)
\alpha\text{-bulnesene}\;(0.6\%)
\gamma-cadinene (1.1%)
T-cadinol (2.0%)
```

^aprobably (Z)-3-hexenol

The freshly harvested plants of a purple and green cultivars of *O. tenuifo-lium* grown at the CIMAP experimental farm in Pantnagar (Uttarakhand, India) were water distilled in the laboratory to yield 0.28% and 0.20% oils, respectively. The oils were analyzed using GC-FID and GC/MS by Padalia and Verma (2011), and as there were only quantitative differences in the oils, their compositions are summarized as follows:

 α -thujene (0-t) α-pinene (t-0.2%) camphene (t-0.2%) β -pinene (t=0.1%) 1.8-cineole (t-0.2%) (E)-β-ocimene (0.9-5.8%) linalool (0.2-0.4%) borneol (0.2-0.8%) eugenol (67.4-72.8%) methyl (E)-cinnamate (0.5–0.7%) methyl eugenol (t-0.2%) β -bourbonene (t-0.8%) β-elemene (10.9–11.0%) β-caryophyllene (7.3–8.4%) β-gurjunene (t-0.1%) α-humulene (t-0.5%) (E)- β -farnesene (t-0.6%) germacrene D (2.2-2.4%) β-selinene (0.1–0.2%) α-selinene (0.1–0.3%) bicyclogermacrene (0-0.1%) caryophyllene oxide (0.3%)

In addition, trace amounts of myrcene, α -terpinene, limonene, terpinolene, *cis*-sabinene hydrate, *trans*-p-menth-2-en-l-ol, terpinen-4-ol, α -terpineol, α -copaene, γ -muurolene, cubebol, 10-epi- γ -eudesmol and β -eudesmol was found in one or both of the oils.

The main components of the oils of four accessions of *O. tenuiflorum* that were grown in an experimental garden of the University of Zagreb (Croatia) were determined using GC/MS only and retention indices by Carovic-Stanko et al. (2011). The oils of three of the accessions were found to possess the following major components:

 $\begin{array}{l} 1,8\text{-cineole}\;(21.8-24.9\%)\\ \\ \text{linalool}\;(0-3.7\%)\\ \\ \text{methyl chavicol}\;(14.7-21.6\%)\\ \\ \text{eugenol}\;(4.8-7.6\%)\\ \\ \\ \alpha\text{-bergamotene}^*\;(2.9-4.4\%)\\ \\ \\ \beta\text{-bisabolene}\;(24.6-26.7\%)\\ \\ \\ \text{methyl}\;(E)\text{-cinnamate}\;(0-0.1\%)\\ \\ \\ \text{caryophyllene}\;oxide\;(2.8-3.6\%) \end{array}$

° correct isomer not identified

The major components of the oil of the other accession were as follows:

 $\begin{array}{l} 1,8\text{-cineole}\ (5.1\%)\\ \text{linalool}\ (2.8\%)\\ \text{methyl chavicol}\ (3.4\%)\\ \text{eugenol}\ (2.9\%)\\ \alpha\text{-bergamotene}^*\ (6.6\%)\\ \beta\text{-bisabolene}\ (52.0\%)\\ \text{caryophyllene oxide}\ (4.2\%) \end{array}$

Ocimum tenuiflorum plants were collected from a nursery in Greater Noida (Uttar Pradesh, India) and the fresh leaves were subjected to hydrodistillation. Analysis of the oil by Dohare et al. (2012) using GC-FID and GC/MS revealed that it contained the following components:

```
\alpha-pinene (4.2%)
\alpha-thujene (3.1%)
camphene (1.8\%)
\beta-pinene (4.9%)
myrcene (0.1\%)
limonene (0.4%)
1,8-cineole (0.3%)
cis-α-terpineol<sup>a</sup> (0.8%)
sabinene<sup>\dagger</sup> (0.9%)
borneol (2.0\%)
bornyl acetate (14.5%)
camphor (9.0%)
eugenol (27.4%)
selinene<sup>°</sup> (5.9%)
cubenol (0.1\%)
caryophyllene oxide (0.4%)
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^{\dagger} \rm incorrect identification based on GC elution order ^{\circ} \rm correct isomer not identified
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^{\mathrm{a}}\alpha\text{-}\mathrm{terpineol} does not exist in geometric isomeric forms
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In addition there were a number of misidentified components that will not be included in this review.

An oil of *O. tenuiflorum* that is available commercially was reported (Vossen, 2012) to possess the following constituents:

 α -pinene (0.2%) limonene (0.2%) 1.8-cineole (0.8%) linalool (0.8%) methyl chavicol (0.3%) eugenol (50.0%) α -copaene (0.5%) isocaryophyllene (0.2%) β -caryophyllene (38.1%) α -humulene (2.6%) δ -cadinene (0.2%) elemol (4.1%) γ-eudesmol (0.3%) α -muurolol (0.6%) β -eudesmol (0.2%) α -cadinol (0.5%)

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