

## Comparative Study of Volatile Compounds from Genus *Ocimum*

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**Abstract:** There are distinct varieties of basil types in the genus *Ocimum* which makes them very special. Genus *Ocimum* is widespread over Asia, Africa and Central & Southern America. All basil types are members of the *Lamiaceae* family. The colors of the leaves vary from bright green to purple-green and sometimes almost black. Fresh basil leaves have a strong and characteristic aroma, not comparable to any other spice, although there is a hint of clove traceable. *Ocimum Sanctum*, also addressed as *Ocimum Tenuiflorum* is a sacred plant in the Hindu culture and known as Tulasi in Tamil or Holy Basil in English. Meanwhile *Ocimum Basilicum*, known as Common or Sweet Basil has very dark green leaves. The genus *Ocimum* is cultivated for its remarkable essential oil which exhibits many usages such as in medicinal application, herbs, culinary, perfume for herbal toiletries, aromatherapy treatment and as flavoring agent. Due to varying essential oil profiles even within the same species, plants may often be classified as a different species as a result of different scents. In the present study, volatile constituents of *Ocimum Sanctum* and *Ocimum Basilicum* were extracted using various solvents and their chemical constituents were identified and quantified by using GC-MS in optimized conditions. The profiles of extract from both species were compared in an effort to investigate effects of seasonal variation on their chemical compositions. The predominant species in *Ocimum Sanctum* and *Ocimum Basilicum* was found to be methyl eugenol and methyl chavicol, respectively, during different months of analysis.

**Key words:** Holy basil, sweet basil, essential oils, methyl eugenol, methyl chavicol, *Ocimum sanctum*, *Ocimum basilicum*

### INTRODUCTION

The genus *Ocimum* is ranked high among some of the astonishing herbs for having enormous medicinal potentialities. Previous studies show that there are large numbers of species and varieties falls in this genus<sup>[1,3]</sup>. Several authors recognize more than 60-150 species in the genus. Characterizations of each species in this genus (family *Lamiaceae*) are based on the leaves and habitat<sup>[1]</sup>. The shape of the leaves in *Ocimum Sanctum* and its close relatives varies in size of leaves, vein and petioles. The colours of the leaves vary from bright green to dark green and sometime almost black. Though the colours in the plants vary, but the reason behind it, especially in basil, are not being studied yet. Regular occurrence of the interspecific hybridization within the genus, have created taxonomic challenges, leaving very little publications on basil taxonomy which follows the International Code of Botanical nomenclature<sup>[1,2]</sup>. Due to the difficulties in identifying

the species, Massimo *et al.* (2004) has concluded that identification can be optimized by combined analysis of morphological traits, essential oil composition and molecular markers<sup>[2]</sup>.

Essential oils are fragrant, highly concentrated essences of plants which are considered to exemplify the soul or life-force of the plant. Essential oils are approximately 75-100 times more concentrated than dried herbs<sup>[6]</sup>. Essential oils are generally extracted by distillation, expression, solvent extraction, cold pressing, maceration or supercritical carbon dioxide extraction<sup>[7-9]</sup>. It has been reported that the quality and quantity of essential oil produced by plants depends on various factors such as seasonal variation<sup>[10]</sup>, method of harvest<sup>[11]</sup>, leaf development stages<sup>[12]</sup>, climate<sup>[13]</sup> and soil type<sup>[6,13]</sup>. Essential oil's combined effect, which depends on the desired application, is more powerful than that of individual oil. This is commonly known as a synergistic blend. Essential oil exhibits many usages such as in medicinal application<sup>[8,14]</sup>, herbs, culinary

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and are used as perfume for herbal toiletries, aromatherapy treatment<sup>[14]</sup> and also in perfume industry<sup>[8,10]</sup>. *Ocimum Sanctum* has been extensively used in Ayurvedic system of medicine for various ailments including capability lowering plasma glucose<sup>[15]</sup>. Essential oils and herbal extracts have attracted a great deal of scientific research interest due to their potential as natural flavours. A scientifically based guide has been developed to assess the safety of naturally occurring mixtures based on chemical composition, particularly essential oils, for their intended use as flavor ingredients<sup>[16]</sup>. This research reports the seasonal variation of essential oil composition of *Ocimum Basilicum* and *Ocimum Sanctum*.

## MATERIALS AND METHODS

**Climatic Conditions:** Samples were collected 60.8 m above M.S.L., 3° 06'N and 101° 39'N (station: Petaling Jaya). The average temperature during the collection of *Ocimum Basilicum* is 27.3°C and varies from 26.4-29.1°C, with the highest (29.1°C) in July 2003. Meanwhile the average temperature is 28.2°C when collecting *Ocimum Sanctum* samples.

**Materials:** Samples of *Ocimum Basilicum* were obtained from housing area at Section 17 and SS3, Petaling Jaya. *Ocimum Sanctum* samples were collected from an orchard along Jalan Gasing, Petaling Jaya, Malaysia.

**Isolation of essential oil:** Oils were isolated from fresh leaves. The leaves were cut into small pieces and soaked in n-hexane for an hour and subsequently, filtered by gravity. The extracts were then subjected to further filtration by using a short column silica gel to remove impurities. The volatile constituents extracted are quantified using Gas Chromatograph equipped with Mass Spectrometry (GC-MS).

**GC-MS Analysis:** GC-MS analysis of the samples were carried out using Hewlett Packard HP 6890 Series Mass Selective Detector, equipped with a cross linked methyl silicone gum phase capillary column (25m x 0.32mm). Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 40°C and held for 3 min and the final temperature of the oven was 280°C with rate at 10°C min<sup>-1</sup>. A 2 µL sample was injected with splitless mode. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 eV. The total running time for a sample is 45 min.

**Identification of essential oil constituents:** The chemical components from the essential oil were identified by comparing the retention times of chromatographic peaks with those of authentic compounds run under identical conditions, by comparison to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS. Qualitative and quantitative analysis were carried out to determine the concentration of the essential oils.

## RESULTS AND DISCUSSION

**GC-MS Analysis:** The essential oils of *Ocimum Sanctum* and *Ocimum Basilicum* were subjected to detailed GC-MS analysis in order to determine the impact of the locality and seasonal variations on their volatile constituents. The yield of oils ranged from 0.4-1.7%. Exactly 15 compounds were identified in *Ocimum Basilicum* (Table 1) meanwhile 16 compounds were identified in *Ocimum Sanctum* leaves (Table 2).

Essential oils found in *Ocimum Basilicum* belong to a variety of groups including monoterpene hydrocarbon, oxygenated monoterpenes (e.g., 1,8-cineole, L-camphor), sesquiterpene hydrocarbons (e.g.,  $\beta$ -cubebene,  $\gamma$ -cadinene) and aromatic compounds (e.g., methyl chavicol). From Table 1, the constituents from *Ocimum Basilicum* yielded methyl chavicol in abundance, supports previous findings<sup>[22,24,25]</sup>, proving that this plant belongs to the phenolic chemotype. The amount of methyl chavicol, being high on April 2003, significantly decreased on June 2003, then increases gradually, until November 2003. Meanwhile, linalool was only present (April 2003) when methyl chavicol was relatively high.

The constituents obtained from *Ocimum Sanctum* (Table 2) were characterized by a high content of aromatic compounds, with methyl eugenol as the main constituent. As complementing the results with *Ocimum Basilicum*, the constituents found in *Ocimum Sanctum* belong to a significant group such as monoterpene hydrocarbon (limonene), oxygenated monoterpene (borneol), sesquiterpene hydrocarbons (e.g.  $\alpha$ -copaene,  $\beta$ -caryophyllene), oxygenated sesquiterpenes (elemol) and aromatic compounds (e.g. methyl chavicol, methyl eugenol). The composition of *Ocimum Sanctum* appears to be different from *Ocimum Basilicum*. Methyl chavicol is also present, but at very low percentage in all the months being investigated. The methyl eugenol content is high in September and October 2007 and then dropped extremely low on April and May 2008, then increases in June 2008. It is noticed that the amount of methyl eugenol and  $\beta$ -caryophyllene are consistently vice-versa.

Table 1: Chemical constituents of the essential oils of *Ocimum Basilicum* species<sup>[32]</sup>

No	Compound	Molar Mass (g mol <sup>-1</sup> )	Area (%)				
			April 2003	June 2003	July 2003	August 2003	November 2003
1	1,8-cineole	154	0.96	0.7	*	0.49	0.83
2	$\beta$ -ocimene	136	2.7	2.12	0.97	0.88	1.18
3	Linalool	154	1.02	*	*	*	*
4	L-camphor	152	1.09	1.1	0.39	0.3	0.54
5	Methyl chavicol (estragole)	148	80.95	30.87	58.1	51.05	67.06
6	Eugenol	164	*	*	*	*	12.82
7	$\beta$ -elemene	204	*	0.1	*	*	3.09
8	Methyl eugenol	178	*	*	0.71	*	*
9	$\beta$ -caryophyllene	204	1.88	*	*	*	1.48
10	$\alpha$ -humulene	204	1.76	2.24	0.56	*	0.85
11	Germacrene-D	204	0.73	0.7	0.31	*	0.42
12	Bicyclogermacrene	204	2.19	1.85	0.81	*	0.72
13	$\gamma$ -cadinene	204	*	*	0.69	*	0.61
14	$\alpha$ -amorphene	204	1.05	3.49	*	*	*
15	$\beta$ -cubebene	204	*	*	2.44	1.42	*

\*not detected

Table 2: Chemical constituents of the essential oils of *Ocimum Sanctum* species

No	Compound	Molar mass (g mol <sup>-1</sup> )	Area (%)				
			Sept 2007	October 2007	April 2008	May 2008	June 2008
1	$\alpha$ -humulene	204	0.71	*	*	*	*
2	Germacrene D	204	2.78	2.11	*	4.81	4.91
3	Indole	117	1.64	*	*	*	*
4	$\beta$ -elemene	204	4.44	4.96	5.38	6.35	1.59
5	$\alpha$ -copaene	204	0.88	*	*	0.56	*
6	$\beta$ -caryophyllene	204	8.52	10.62	53.63	46.22	8.71
7	Methyl chavicol	148	4.67	2.52	*	4.4	*
8	Methyl eugenol	178	74.27	76.27	37.95	36.47	62.29
9	Borneol	154	1.44	*	*	*	2.74
10	Limonene	136	*	*	*	1.19	*
11	Elemol	222	0.67	*	*	*	*
12	Eugenol	164	*	*	*	*	2.79
13	Farnesol	222	*	3.52	*	*	4.19
14	$\alpha$ -farnesene	204	*	*	*	*	11.27
15	Hedycaryol	222	*	*	*	*	0.72
16	Camphene	136	*	*	*	*	0.79

\*not detected

As indicated above, essential oils obtained from *Ocimum Basilicum* and *Ocimum Sanctum* species showed significant variability in their chemical composition. However, literature review showed variation between chemical compositions, depending of location, seasonal variation and stages of development. *Ocimum Sanctum* grown in India, in the field, under natural conditions<sup>[12]</sup> gave highest percentage of methyl eugenol in young leaves (5-10 days old). Previous publication suggests that essential oil may be isolated from *Ocimum Sanctum*<sup>[10]</sup> but with absolute seasonal variation. Eugenol is the main component of *Ocimum Sanctum* grown in Bangladesh<sup>[3]</sup>, Germany<sup>[17]</sup>, Cuba<sup>[18]</sup>, Northeastern Brazil<sup>[19]</sup>, methyl eugenol from India<sup>[11]</sup>. Detailed morphological characters developed<sup>[20]</sup> can be used as reference to classify various types of *Ocimum Sanctum*. Meanwhile the essential oil of *Ocimum*

*Basilicum* high during the winter season, giving high percentage of linalool (60.6%)<sup>[21]</sup>, 52.6 and 58.26% estragole, from leaves and flowers, respectively, of *Ocimum Basilicum*<sup>[22]</sup>. Higher solar irradiance level increased the contents of linalool and eugenol, whereas methyl eugenol was increased by lower irradiance in *Ocimum Basilicum*<sup>[23]</sup>. Seven types of chemotypes presented by Isa Telci *et al.* (2006) also included methyl chavicol (68.3%) for *Ocimum Basilicum* varieties grown in Turkey<sup>[24]</sup>. A research using three cultivars of *Ocimum Basilicum* conducted in Southern Italy<sup>[25]</sup> yielded high amount of methyl chavicol in Napoletano a foglia di lattuga type.

**Biological pathway:** Methyl chavicol and methyl eugenol, which are found abundantly in *Ocimum Basilicum* and *Ocimum Sanctum*, respectively, are

volatile essential oil used extensively in various application including perfumes<sup>[8,10]</sup>, food seasoning and flavouring<sup>[6]</sup>, aromatherapy<sup>[14]</sup> and medicinal application<sup>[8,14]</sup>. Methyl chavicol stimulates liver regeneration, shows hypothermic and DNA binding activities. Methyl eugenol also shows DNA binding activities, spasmolytic and gives muscle relaxant effects<sup>[6]</sup>.

Since both methyl chavicol and methyl eugenol have high economic value, researches have tried to understand the biosynthetic pathway that produces this compound in the plant, which however, still remains fully unexplored. *Chavicol O*-methyltransferase (CVOMT) identified in crude protein extracts of sweet basil<sup>[26]</sup>, is responsible for the conversion of chavicol to methyl chavicol. Analysis shows that eugenol is being transformed into methyl eugenol by *eugenol O*-methyltransferase (EOMT). Both CVOMT and EOMT, which are enzymes, use *S*-adenosylmethionine (*SAM*) as the methyl donor<sup>[26-30]</sup>. But the enzyme involved in basil is different from those involved in *Clarkia breweri* plant, which uses (*iso*)*eugenol O*-methyltransferase (IEMT)<sup>[29,30]</sup>.

CVOMT methylates the 4-hydroxyl group of chavicol to produce methyl chavicol. EOMT methylates the 4-hydroxyl group of eugenol to methyl eugenol.

Chavicol was not present at any of the months being identified because it has all been converted to methyl chavicol. Methyl chavicol was found to characterize *Ocimum Basilicum* in abundance except in June 2003. This finding is consistent to previous publication<sup>[22,24,25]</sup>. *Ocimum Basilicum* species, on various chemotypes being studied, which originates from Yemen and Thailand, also contain methyl chavicol as the major compound<sup>[31]</sup>. Meanwhile, eugenol is not detected during several months, except June 2008, due to same prior discussed biosynthesis pathway. At present, the enzymatic activity involved in the biochemical translation is still being studied in detail<sup>[29]</sup>.

## CONCLUSION

The application of GC-MS for the analysis of essential oil allowed the detection of significant differences in the proportions of volatile compounds from *Ocimum Basilicum* and *Ocimum Sanctum* tested in relation to many factors that contribute to the fragrances present. From our study, *Ocimum Basilicum* should be harvested in April for methyl chavicol and *Ocimum Sanctum* in the month of October, for methyl eugenol under the climatic conditions in Malaysia. At the same time, present study indicates that *Ocimum Basilicum* are

rich in methyl chavicol and *Ocimum Sanctum* belongs to methyl eugenol rich type.

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