Chemical Constituents and Antioxidant Capacity of *Ocimum basilicum* and *Ocimum sanctum*

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ABSTRACT: *The chemical constituents of leaves, inflorescence, and flowers from Ocimum basilicum (Thai basil) and Ocimum sanctum (Holy basil) were analysed by gas chromatography-mass spectrometry. The chemical compounds were extracted by hydrodistillation, headspace-solid phase microextraction, and solvent extraction. The main constituents of Ocimum basilicum were identified to consist of estragole (> 35.71%), (E)-β-ocimene (> 1.47%), trans-α-bergamotene (> 0.83%), τ-cadinol (> 0.41%) eucalyptol (> 0.25%) and α-caryophyllene (> 0.07%) while Ocimum sanctum consists mainly of eugenol methyl ether (> 34.34%), (E)-caryophyllene (> 7.91%), germacrene D (> 5.58%), β-elemene (> 4.22%) and copaene (> 1.49%). Ocimum basilicum and Ocimum sanctum leaves contain more chemical constituents followed by inflorescence and flowers. The genetic distance between the two species was calculated to investigate the interspecies relationship and it is 2.86. The calculated genetic distance between the two species showed that Ocimum basilicum and Ocimum sanctum are closely related species and share some of the same traits. The methanol and dichloromethane extracts of Ocimum basilicum leaves showed an IC_{50} value of 88 μg/mL and 1178 μg/mL, respectively, while the methanol and dichloromethane extract of Ocimum sanctum showed a higher 2, 2-diphenyl-1-picrylhydrazil free radicals scavenging activities with an IC_{50} value of 11 μg/mL and 369 μg/mL, respectively. The natural antioxidant level Ocimum sanctum and Ocimum basilicum indicated that they can be used effectively in food preservation.*

KEYWORDS: *Ocimum basilicum; Ocimum sanctum; Free radical scavenging activity; Chemical constituents; Genetic distance.*

INTRODUCTION

The genus *Ocimum* from the Lamiaceae family are annual or perennial aromatic herbs that are native to the tropical and subtropical regions of Asia, Africa and Central South America [1] and they composed of over 65 species [2]. *Ocimum basilicum*, *Ocimum americanum*, *Ocimum gratissimum*, *Ocimum sanctum*, and *Ocimum tenuiflorum* are important species of the genus *Ocimum* which are investigated for their beneficial traits.

Ocimum basilicum L. (*O. basilicum*) or commonly known as Thai basil and *Ocimum sanctum* L. (*O. sanctum*) or Holy basil, are the most widely cultivated *Ocimum* species in Malaysia. *O. basilicum* has an intense aroma that resembles cloves [3] and often being used in confectionery, baked goods, seasonings, spiced meats and sausages, oral care products and fragrance [4]. In Asian cuisines, the leaves are used in flavouring curries,

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noodles, salads, and chickens. *O. basilicum* provides benefits to health as a study had shown that it has the ability to protect the myocardium against isoproterenol-induced infarction in rats [5] and it was reported that *O. basilicum* had reduced systolic and diastolic blood pressure and cardiac hypertrophy in renovascular hypertensive rats [6]. *O. sanctum* is being cultivated in the garden of Hindu homes and temples as a sacred plant and known as "The Incomparable One", "Mother Medicine of Nature" and "The Queen of Herbs" in Ayurveda [7]. *O. sanctum* provides a vast array of health benefits such as prevention of mental stress, anti-malarial against *Plasmodium vivax* and *Plasmodium falciparum* and antibacterial against *E. coli*, *B. anthracis* and *P. aeruginosa in-vitro* [8]. In addition, *O. sanctum* showed significant anti-inflammatory activity against carrageenan- and different another mediator-induced paw edema in rats [9].

Oxidation reaction in food resulted in an unpalatable taste and products that may be harmful for human consumption [10]. In order to decelerate oxidation reaction in food, synthetic antioxidants namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used in the food industries [11]. Despite their effectiveness in delaying the oxidation process, their usage might be a contributing factor to cancer [12]. Due to this, antioxidants from natural resources are widely sought after to replace synthetic antioxidants. Furthermore, the increase in consumption of antioxidant-rich foods was found to correlate with prevention of oxidative stress diseases [13, 14].

Different parts of the plant serve different functions, thus contain different chemical composition and different percentages of constituents. The flower is the plant organs of sexual reproduction and plays an important role in pollination [15]. Leaves function as photosynthetic organs of a plant and have the ability to control water potential in order to allow carbon dioxide intake by opening the stomata [16]. Inflorescence helps in pollen transferring, provide maximum success in reproduction, and provide nutrients in fruits and flower development, supporting the fruits before the dispersal when the fruits are matured and allowing successful fruit and seed dispersal [17].

We report the identification of the chemical constituents from various parts of *O. basilicum* and *O. sanctum* has grown in Malaysia by using different extraction techniques and their scavenging strength against free radicals. We also calculated the genetic distance between the two species to depict their interspecies relationship.

EXPERIMENTAL SECTION

Plant Materials

O. basilicum and *O. sanctum* approximately four months of age were purchased from a local nursery in Sungai Buloh, Selangor, Malaysia. The leaves, flowers, and inflorescence were detached from the stem, cleaned and were dried at room temperature.

Chemicals and materials

Estragole (methyl chavicol) 98%, 2, 2-diphenyl-1-picrylhydrazil (DPPH) and European Pharmacopeia (EP) grade butylated hydroxytoluene (BHT) were purchased from Aldrich, (Steinheim, Germany). C_7-C_{30} saturated alkane analytical standard was purchased from Supelco (Pennsylvania, USA). Methanol, hexane and anhydrous sodium sulfate were purchased from Systerm (Selangor, Malaysia). Analytical grade dichloromethane was purchased from Merck (Darmstadt, Germany). A SPME fibre holder with a 1 cm fibre assembly coated with a 100 µm polydimethylsiloxane (PDMS) was purchased from Supelco (Bellefonte, PA, USA).

Extraction Methods

Hydrodistillation

Fresh leaves (100.00 g) and distilled water (500 mL) were placed in a Clevenger type apparatus. The essential oil was isolated by hydrodistillation for 2 h. The essential oil obtained was separated and dried over anhydrous sodium sulfate. The aliquot was collected in triplicates and analysed using Gas Chromatography-Mass Spectrometry (GC-MS).

Solvent Extraction

Fresh leaves (18.00 g), flowers (0.20 g) and inflorescence (5.20 g) were successively extracted with dichloromethane and methanol separately at a ratio (1 g plant materials: 3 mL of solvent) and supernatants were collected after 24 h. The extract was passed through a column of anhydrous sodium sulfate to eliminate humidity. The solvent was removed using a rotatory evaporator. Each extract was analysed in triplicate by GC-MS.

Headspace Solid Phase Microextraction (HS-SPME)

The fibre was exposed to the headspace of a 20 mL capped vial, which contained separately of leaves (1.00 g), flowers (0.02 g) and inflorescence (0.02 g). The SPME system was left for 2 min at 80°C to allow equilibration. Volatile compounds were extracted from the headspace volume of the samples with 15 min extraction time and the extraction temperature was set at 50°C. The trapped volatile compounds were desorbed at 250°C in the GC injection port for 7 min and flushed into the GC column. The contents were analysed in triplicates by GC-MS.

GC-MS Analysis

The extracts were analysed by a Hewlett Packard HP 6890 series mass selective detector linked to a GCMS-QP 2010 Plus Shimadzu gas chromatograph that operates in a splitless injection mode and was fitted with a DB-5 ms column coated with 5% phenyl 95% dimethyl arylene siloxane with a column length of 30.0 m, a diameter of 0.25 mm and film thickness of 0.25 µm. The oven temperature was set at 50°C and the injection temperature was set at 250°C. The flow control mode was at linear velocity and the helium pressure was set at 68.1 kPa. The helium total flow was 58.2 mL/min and the column flow was 1.2 mL/min. The linear velocity was 39.7 cm/s and the purge flow was 3 mL/min with the split ratio of 45. The initial temperature was set at 50 °C and the hold time at 2 min, the temperature was programmed to 180°C at a rate of 3°C/min and held for 3 min, and then programmed at a rate of 8°C/min to a final temperature of 280°C and held for 10 min. The ionization of the sample components was performed in the EI mode at 70 eV. The ion source temperature and interface temperature were kept at 200°C and 300°C, respectively. Mass spectra were scanned from *m/z* 50 to 600 with a scan speed of 1250. The injected volume was 2 µL and the samples were analysed in triplicates.

Kovats Indices

The chemical constituents extracted from the *O. basilicum* and *O. sanctum* were identified by comparing its mass spectra with National Institute of Standards and Technology (NIST) library and further confirmed with Kovats retention indices. The retention indices of the compounds were determined by co-injection of the samples with a solution containing the homologous series of C_7-C_{30} *n*-alkanes [18]. Qualitative analysis was based on comparison of retention times and mass spectra with corresponding data in the literature [19]. Kovats index of the individual volatile compound was calculated, relative to the series of homologous hydrocarbons by using the following equation:

$$I = \left[100\left(t - t_n\right) + 100\left(n\right)\right] / \left[\left(t_{n+1} - t_n\right)\right] \quad (1)$$

Where

I = calculated retention index of the analyte

t = retention time of analyte

t_n = retention time of alkane before analyte

t_{n+1} = retention time of alkane after analyte

n = number of carbon atoms of analyte

Genetic Distance

The genetic distance can be defined as a measure of the evolutionary divergence between copies of homologous genes that share a common ancestor [20]. A statistical method developed by *Nei* [21] has enabled genetic distance (*D*) to be measured based on the identity of genes between populations. Nei had defined the normalized identity of genes between populations and related it to the accumulated number of gene differences per locus. Genetic distance between two populations can be measured by using equation as follows:

$$D = -\log J_{XY} / \sqrt{J_X J_Y} \quad (2)$$

Where

D = genetic distance between X and Y population

$J_{XY} / \sqrt{J_X J_Y}$ = normalized identity of genes between X and Y population

By assuming that X and Y are two different populations, let x_i and y_i be the frequencies of the $_i$th alleles in X and Y, respectively. The probability of the identity of two genes that have been chosen randomly in population X is $jx = \sum x_i^2$, while for population Y it is $jy = \sum y_i^2$. The probability of identity of a gene from X and a gene from Y is $jxy = \sum x_i y_i$. The normalized identity of genes between X and Y with respect to loci is $I_j = jxy / \sqrt{jxjy}$. The normalized identity of genes between X and Y with respect to all loci is $I = J_{XY} / \sqrt{J_X J_Y}$, where J_X, J_Y, and J_{XY} are the arithmetic means of *jx*, *jy* and *jxy*, respectively, over all loci.

Antioxidant Assay on Leaves Extracts

The free radical scavenging capacity of the leaf extracts was determined by using DPPH assay as described by *Brand-Williams et al.* [10]. A series of concentration (6.75 µg/mL to 5000.00 µg/mL) of leaf extracts were prepared respectively with methanol as solvent. The prepared samples were then mixed with 90 mM DPPH in methanol. A blank solution was prepared by using 90 mM DPPH in methanol. The absorbance of mixture at 517 nm was recorded after an incubation period of 30 min by using a microplate reader (TECAN infinite M200 PRO). The condition for the microplate reader was agitated for 5 s in orbital mode with 3 mm amplitude at the speed of 44.3 rpm and a measurement bandwidth of 9 nm. A 96-well flat bottom, polypropylene plate was used. Different concentrations of BHT (6.75 µg/mL to 500.00 µg/mL) were used as positive control. The samples were analysed in triplicates. The free radical scavenging of DPPH percentage was calculated by using the following equation:

$$\text{Free radical scavenging of DPPH}[\%] = \left[\left(A_{\text{blank}} - A_{\text{sample}}\right)/\left(A_{\text{blank}}\right)\right]\times 100 \quad (3)$$

Where

A_{blank} = Absorbance of blank

A_{sample} = Absorbance of sample

After free radical scavenging capacity of DPPH percentage had been calculated, a graph was plotted to determine the IC_{50} value of the extract. The IC_{50} value represents the concentration of the extract that caused 50% inhibition on DPPH assay. Non-linear regression could also estimate IC_{50} value. AntiRadical Power (ARP) can be recalculated to the reciprocal of IC_{50} values (1/ IC_{50}). The higher the antioxidant activity, the higher the antiradical power value is.

RESULTS AND DISCUSSION

Chemical Composition of O. basilicum

In the present study, the chemical constituents of *O. basilicum* and *O. sanctum* has grown in Malaysia were investigated to determine their chemical composition. The chemical constituents of both species were analysed by GC-MS and were further confirmed with Kovats indices [19]. Kovats index is a system of retention indices which the data is accepted for identification of chemical compounds by gas chromatography [22]. The extraction techniques that were applied in extracting the chemical constituents are solvent extraction using methanol and dichloromethane, hydrodistillation and HS-SPME. In addition, the chemical constituents of different parts of the plant, namely, leaves, flowers, and inflorescence were investigated to determine the major compounds of each part. The chemical constituents identified from Malaysian grown *O. basilicum* are listed in Table 1.

A total of 47 chemical compounds was identified in *O. basilicum*. The percentage yields of leaves extract obtained by hydrodistillation, dichloromethane extraction and methanol extraction were 0.10% (v/w), 0.13% (w/w) and 0.83% (w/w), respectively.

HS-SPME analysis of the volatiles from *O. basilicum* leaves showed estragole (59.67%), eucalyptol (9.02%), *trans*-*α*-bergamotene (8.60%), *α*-caryophyllene (2.65%) and camphor (1.62%) as the major compounds. Hydrodistillation of *O. basilicum* leaves yielded estragole (35.71%), eucalyptol (13.26%), *(E)*-*β*-ocimene (7.99%), *trans*-*α*-bergamotene (5.82%), and *τ*-cadinol (5.71%) as the main components. The leaves methanol extract of *O. basilicum* yielded estragole (82.69%), *(Z)*-*β*-ocimene (1.26%), *trans*-*α*-bergamotene (0.83%) and eugenol methyl ether (0.40%) as the major compounds. The leaves dichloromethane extract of *O. basilicum* yielded estragole (73.16%), eucalyptol (6.17%), *trans*-*α*-bergamotene (5.26%), *(E)*-*β*-ocimene (4.52%) and *τ*-cadinol (2.56%) as main components. Hydrodistillation was conducted only on leaves due to insufficient material of flowers and inflorescence as only one plant was used throughout the entire research in order to avoid inconsistent parameters that might affect the chemical composition of the plant such as a difference in geographical origin or nutrient availability [23, 24].

Extractions of *O. basilicum* flowers were conducted using dichloromethane extraction, methanol extraction and HS-SPME. The percentage yield of flower extracts obtained by dichloromethane extraction and methanol extraction was 0.05% (w/w) and 0.34% (w/w), respectively. Estragole (98.88%) and *(Z)*-*β*-farnesene (1.11%) were the two main compounds identified from dichloromethane extraction while estragole (99.22%) and *(Z)*-*β*-farnesene (0.77%) were identified from methanol extract. The major compounds that were identified in HS-SPME analysis were estragole (88.18%), *trans*-*α*-bergamotene (2.82%), *(E)*-*β*-ocimene (1.47%), eugenol methyl ether (0.72%) and *α*-bulnesene (0.51%).

Table 1: Chemical constituents of leaves (L), inflorescence (I) and flowers (F) from O. basilicum.

Peak #	*Compounds	Retention Index (RI)	Relative Area (%)									
			Hydrodistillation	Solvent Extraction (Dichloromethane)			Solvent Extraction (Methanol)			HS-SPME		
			L	L	F	I	L	F	I	L	F	I
1	α-thujene	932	n.d	n.d	n.d	0.05 ± 0.04	n.d	n.d	n.d	n.d	n.d	n.d
2	α-pinene	936	0.94 ± 0.03	n.d	n.d	n.d	n.d	n.d	n.d	0.32 ± 0.07	n.d	n.d
3	α-fenchene	944	0.01 ± 0.00	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
4	camphene	947	0.23 + 0.40	n.d	n.d	n.d	n.d	n.d	n.d	0.09 ± 0.01	n.d	n.d
5	sabinene	972	0.30 ± 0.40	n.d	n.d	0.03 ± 0.01	n.d	n.d	n.d	0.35 ± 0.08	n.d	n.d
6	β-pinene	976	1.26 ± 0.42	0.80 ± 0.19	n.d	0.12 ± 0.03	n.d	n.d	n.d	0.66 ± 0.20	n.d	n.d
7	β-myrcene	988	1.59 ± 0.01	0.57± 0.14	n.d	0.14 ± 0.40	n.d	n.d	n.d	0.55 ± 0.09	n.d	0.02 ± 0.01
8	(Z)-3-Hexen-1-ol acetate	1004	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.03 ± 0.01	n.d
9	Limonene	1026	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.04 ± 0.02	0.06 ± 0.01
10	eucalyptol	1031	13.26 ± 0.84	6.17 ± 0.81	n.d	1.71 ± 0.38	n.d	n.d	0.42 ± 0.01	9.02 ± 1.38	0.25 ± 0.12	0.44 ± 0.13
11	(Z)-β-ocimene	1034	n.d	n.d	n.d	n.d	1.26 ± 0.08	n.d	3.89 ± 0.06	0.27 ± 0.06	0.03 ± 0.01	0.03 ± 0.01
12	(E)-β-ocimene	1046	7.99 ± 0.43	4.52 ± 0.94	n.d	6.59 ± 0.60	n.d	n.d	n.d	2.44 ± 0.62	1.47 ± 0.47	1.70 ± 0.45
14	γ-terpinene	1056	0.13 ± 0.00	n.d	n.d	n.d	n.d	n.d	n.d	0.05 ± 0.01	n.d	n.d
15	*trans*-β-terpineol	1069	0.20 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	0.11 ± 0.01	n.d	n.d
16	terpinolene	1082	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.16 ± 0.03	0.06 ± 0.02	0.08 ± 0.02
17	2-carene	1083	0.50 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	0.03 ± 0.01	n.d	n.d
18	linalool	1100	1.06 ± 0.01	0.44 ± 0.12	n.d	1.84 ± 0.23	n.d	n.d	n.d	0.41 ± 0.18	0.50 ± 0.21	0.81 ± 0.16
19	camphor	1145	3.15 ± 0.06	1.24 ± 0.25	n.d	1.13 ± 0.14	n.d	n.d	0.30 ± 0.01	1.62 ± 0.35	0.43 ± 0.25	0.55 ± 0.27
20	borneol	1171	n.d	n.d	n.d	0.43 ± 0.08	n.d	n.d	n.d	n.d	0.1 ± 0.07	0.37 ± 0.15
21	estragole	1205	35.71 ± 1.81	73.16 ± 2.24	98.88 ± 0.13	57.85 ± 3.53	82.69 ± 1.14	99.22 ± 0.12	77.65 ± 0.17	59.67 ± 0.31	88.18 ± 4.51	86.39 ± 2.38
22	chavicol	1251	n.d	n.d	n.d	0.50 ± 0.08	n.d	n.d	n.d	n.d	n.d	n.d
23	bornyl acetate	1282	0.27 ± 0.00	n.d	n.d	0.53 ± 0.05	n.d	n.d	n.d	0.03 ± 0.01	0.16 ± 0.14	0.05 ± 0.03
24	α-terpineol acetate	1340	0.36 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	0.23 ± 0.12	n.d	n.d
26	copaene	1369	0.14 ± 0.00	n.d	n.d	n.d	n.d	n.d	n.d	0.14 ± 0.06	n.d	n.d
27	β-cubebene	1390	0.28 ± 0.01	0.73 ± 0.47	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
31	eugenol methyl ether	1398	2.85 ± 0.04	0.76 ± 0.14	n.d	0.34 ± 0.06	0.40 ± 0.07	n.d	n.d	0.77 ± 0.10	0.72 ± 0.79	0.53 ± 0.44
32	*cis*-α-bergamotene	1409	0.12 ± 0.00	n.d	n.d	7.24 ± 0.12	n.d	n.d	n.d	0.15 ± 0.06	n.d	0.03 ± 0.01

Table 1: (Continue)

Peak #	*Compounds	Retention Index (RI)	Relative Area (%)									
			Hydrodistillation	Solvent Extraction (Dichloromethane)			Solvent Extraction (Methanol)			HS-SPME		
			L	L	F	I	L	F	I	L	F	I
33	(*E*)-caryophyllene	1414	1.37 ± 0.03	0.75 ± 0.15	n.d	0.65 ± 0.05	n.d	n.d	n.d	0.86 ± 0.40	0.19 ± 0.13	n.d
34	*β*-cedrene	1418	0.10 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	0.10 ± 0.04	n.d	n.d
35	*trans*-*α*-bergamotene	1431	5.82 ± 0.06	5.26 ± 0.40	n.d	7.24 ± 0.12	0.83 ± 0.03	n.d	2.35 ± 0.03	8.60 ± 2.99	2.82 ± 2.12	1.44 ± 2.22
36	(*Z*)-β-farnesene	1437	0.16 ± 0.00	0.39 ± 0.08	1.11 ± 0.13	n.d	n.d	0.77 ± 0.12	n.d	0.17 ± 0.06	0.18 ± 0.13	0.22 ± 0.04
37	*α*-cubebene	1441	0.53 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	1.86 ± 1.57
38	*ε*-muurolene	1446	0.16 ± 0.00	1.98 ± 0.36	n.d	0.78 ± 0.04	n.d	n.d	n.d	n.d	0.30 ± 0.18	n.d
39	*α*-caryophyllene	1451	n.d	n.d	n.d	0.27 ± 0.02	n.d	n.d	n.d	2.65 ± 1.50	0.09 ± 0.06	0.07 ± 0.05
40	*β*-farnesene	1462	n.d	n.d	n.d	0.52 ± 0.02	n.d	n.d	n.d	0.65 ± 0.22	n.d	n.d
43	*α*-amorphene	1483	0.11 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d
47	*α*-bulnesene	1496	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.48 ± 0.15	0.51 ± 0.47	0.59 ± 0.13
44	geranyl propionate	1500	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.24 ± 0.08	0.33 ± 0.14
45	*β*-bisabolene	1502	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.18 ± 0.06	n.d	n.d
46	linalyl isovalerate	1504	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.43 ± 0.14	0.40 ± 0.10
48	*δ*-cadinene	1512	0.38 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.09 ± 0.06
49	(-)-calamenene	1515	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.29 ± 0.11	n.d	n.d
50	*β*-sesquiphellandrene	1519	0.45 ± 0.01	n.d	n.d	0.40 ± 0.03	n.d	n.d	n.d	0.43 ± 0.15	0.15 ± 0.10	0.19 ± 0.04
52	*α*-cadinene	1535	n.d	n.d	n.d	n.d	n.d	n.d	n.d	0.08 ± 0.02	n.d	n.d
53	*τ*-cadinol	1637	5.71 ± 0.05	2.56 ± 0.50	n.d	1.06 ± 0.05	n.d	n.d	n.d	1.75 ± 0.34	0.41 ± 0.21	0.65 ± 0.26
54	*α*-cadinol	1648	0.82 ± 0.02	n.d	n.d	n.d	n.d	n.d	n.d	0.06 ± 0.03	n.d	n.d
57	*α*-bisabolol	1682	0.29 ± 0.01	n.d	n.d	n.d	n.d	n.d	n.d	0.03 ± 0.01	n.d	0.02 ± 0.00
	Total		86.25	99.33	99.99	89.42	85.18	99.99	84.61	93.40	97.29	96.92

**Compounds identified by using Mass Spectra (MS) data and confirmed by Kovats Index; n.d = not detected*
HS-SPME = Headspace-Solid Microextraction; L = Leaves; F = Flowers; I = Inflorescence

Extraction of *O. basilicum* inflorescence was conducted using HS-SPME, dichloromethane and methanol extraction. The percentage yields of inflorescence extract obtained by dichloromethane extraction and methanol extraction were 0.45% (w/w) and 2.75% (w/w), respectively. The major compounds that were identified in the dichloromethane extract of *O. basilicum* inflorescence were estragole (57.85%),

trans-*α*-bergamotene (7.24%), *(E)*-*β*-ocimine (6.59%), linalool (1.84%) and eucalyptol (1.71%). Five compounds that were identified from the methanol extract of *O. basilicum* inflorescence were estragole (77.65%), *(Z)*-*β*-ocimene (3.89%), *trans*-*α*-bergamotene (2.35%), eucalyptol (0.42%) and camphor (0.30%) while estragole (86.39%), *α*-cubebene (1.86%) and *(E)*-*β*-ocimene (1.70%), *trans*-*α*-bergamotene (1.44%) and linalool (0.81%) were the main components identified in HS-SPME analysis.

Chemical Composition of O. sanctum

A total of 34 chemical compounds was identified in *O. sanctum.* The percentage yield of *O. sanctum* leaves extracts obtained by hydrodistillation, dichloromethane extraction, and methanol extraction were 0.20% (v/w), 0.21 % (w/w) and 0.36 (w/w), respectively. The chemical constituents identified from Malaysian grown *O. sanctum* is listed in Table 2.

HS-SPME analysis of the volatiles from *O. sanctum* leaves showed eugenol methyl ether (34.34%), caryophyllene (22.15%), germacrene D (11.54%), *β*-elemene (9.16%) and copaene (4.62%) as the major compounds. With a slight difference in percentage, the profile of hydrodistillation of *O. sanctum* leaves are characterized by the presence of eugenol methyl ether (39.90%), caryophyllene (27.51%), germacrene D (9.62%), *β*-elemene (4.59%) and copaene (4.22%) as the major compounds. The methanolic extract of *O. sanctum* leaves yielded eugenol methyl ether (57.46%), caryophyllene (18.02%), germacrene D (5.58%), β-elemene (5.26%) and copaene (1.80%) while the dichloromethane extract on leaves of *O. sanctum* yielded eugenol methyl ether (50.12%), caryophyllene (29.95%), germacrene D (6.58%), *γ*-muurolene (5.18%) and copaene (3.51%) as the main constituents.

The percentage yield of *O. sanctum* flower extract obtained from dichloromethane extraction and methanol extraction was 4.28 % (w/w) and 9.04 % (w/w) respectively. The main constituent of *O. sanctum* flowers from methanol extract were eugenol methyl ether (74.51%), caryophyllene (13.76%), germacrene D (9.34%), and copaene (2.39%) while eugenol methyl ether (62.94%), caryophyllene (13.35%), germacrene D (8.29%) and *β*-elemene (4.22%) were the main constituents in *O. sanctum* flowers dichloromethane extract. HS-SPME analysis of *O. sanctum* flowers yielded eugenol methyl ether (62.44%), germacrene D (12.73%), caryophyllene (11.09%), *β*-elemene (6.61%) and copaene (1.49%) as the main components.

The percentage yields of inflorescence extract obtained by dichloromethane extraction and methanol extraction were 11.76% (w/w) and 2.23 % (w/w) respectively. The methanolic extract of *O. sanctum* inflorescence yielded eugenol methyl ether (59.84%), germacrene D (11.01%), caryophyllene (9.77%), *β*-elemene (5.28%) and *β*-cubebene (3.67%) as the main components while the dichloromethane extract of *O. sanctum* inflorescence yielded eugenol methyl ether (51.68%), caryophyllene (16.58%), germacrene D (13.71%), *β*-elemene (9.60%) and copaene (5.08%) as the main components. HS-SPME analysis of *O. sanctum* inflorescence yielded eugenol methyl ether (63.96%), germacrene D (13.71%), caryophyllene (7.91%), *β*-elemene (7.70%), and copaene (2.35%) as major compounds.

O. basilicum and *O. sanctum* were found rich in phenylpropene and terpenes. Estragole is the main constituent in *O. basilicum* while eugenol methyl ether is the main constituent in *O. sanctum. O. basilicum* leaves contain the most chemical constituents, followed by inflorescence and flowers with estragole as the main constituent in all three parts of the plant. A similar trend was observed in *O. sanctum* as the leaves contain more chemical constituents compared to inflorescence and flowers with eugenol methyl ether as the main constituent in all three parts of the plant.

Based on the present study, more identified chemical constituents were extracted by using hydrodistillation and HS-SPME. This finding can be explained by taking into account the solubility of the chemical constituents in different extraction procedures. The solubility of the chemical constituents in different extraction procedures is affected by the polarity of the solvent used [25]. Dichloromethane and methanol used in solvent extraction and water used in hydrodistillation are polar solvents while PDMS used in HS-SPME is non-polar fibre. The least polar solvents are frequently considered to be suitable for the extraction of lipophilic phenols unless very high pressure is applied [26], while polar solvents are commonly suitable for the extraction of polyphenols [27]. The chemical compounds in methanol and

Table 2: Chemical constituents of leaves (L), inflorescences (I) and flowers (F) from O. sanctum.

Peak #	*Compounds	Retention Indices (RI)	Relative Area (%)									
			Hydrodi stillation	Solvent Extraction (Dichloromethane)			Solvent Extraction (Methanol)			HS-SPME		
			L	L	F	I	L	F	I	L	F	I
1	*α*-thujene	931	0.26 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	camphene	948	0.24 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	*β*-pinene	976	0.65 ± 0.86	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8	(*Z*)-3-Hexen-1-ol acetate	1005	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.26 ± 0.07	n.d.	n.d.
9	limonene	1036	0.08 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	0.13 ± 0.01	0.35 ± 0.03	n.d.	n.d.
10	eucalyptol	1040	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.01	n.d.	n.d.
12	(*E*)-*β*-ocimene	1046	n.d.	n.d.	n.d.	0.54 ± 0.02	n.d.	n.d.	n.d.	0.02 ± 0.00	n.d.	n.d.
13	benzeneacetaldehyde	1051	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.22 ± 0.07	0.21 ± 0.13
14	*γ*-terpinene	1062	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03 ± 0.00	n.d.	n.d.
18	linalool	1099	0.09 ± 0.02	n.d.	n.d.	0.88 ± 0.17	n.d.	n.d.	2.19 ± 0.60	0.51 ± 0.08	1.14 ± 0.14	0.75 ± 0.18
19	camphor	1154	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.03 ± 0.01	n.d.	n.d.
20	borneol	1179	0.31 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	1.73 ± 0.02	0.57 ± 0.23	0.87 ± 0.08	0.86 ± 0.60
21	estragole	1203	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.04 ± 0.01	n.d.	n.d.
25	*α*-cubebene	1349	0.19 ± 0.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.43 ± 0.03	n.d.	0.13 ± 0.01
26	copaene	1379	4.22 ± 0.09	3.51 ± 0.13	n.d.	5.08 ± 0.07	1.80 ± 0.19	2.39 ± 0.83	3.31 ± 0.14	4.62 ± 0.19	1.49 ± 0.00	2.35 ± 0.27
27	*β*-cubebene	1383	2.13 ± 0.09	n.d.	n.d.	n.d.	1.76 ± 0.47	n.d.	3.67 ± 0.66	n.d.	n.d.	n.d.
28	*β*-elemene	1384	4.59 ± 0.11	n.d.	4.22 ± 0.85	9.60 ± 0.46	5.26 ± 0.76	n.d.	5.28 ± 4.90	9.16 ± 0.45	6.61 ± 0.33	7.70 ± 1.27
29	*β*-bourbonene	1387	2.16 ± 0.09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.28 ± 0.11
30	*γ*-muurolene	1392	n.d.	5.18 ± 0.58	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31	eugenol methy ether	1403	39.90 ± 0.56	50.12 ± 0.80	62.94 ± 4.48	51.68 ± 1.19	57.46 ± 8.69	74.51 ± 2.27	59.84 ± 4.92	34.34 ± 2.60	62.44 ± 0.22	63.96 ± 3.83
33	(*E*)-caryophyllene	1417	27.51 ± 0.74	29.95 ± 0.11	13.35 ± 0.09	16.58 ± 0.17	18.02 ± 3.06	13.76 ± 0.04	9.77 ± 0.88	22.15 ± 1.61	11.09 ± 0.74	7.91 ± 1.43
35	*trans*-α-bergamotene	1429	0.21 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
38	*ε*-muurolene	1446	0.45 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	0.60 ± 0.15	n.d.	n.d.	n.d.
39	*α*-caryophyllene	1459	2.82 ± 0.02	1.43 ± 0.04	n.d.	n.d.	0.93 ± 0.99	n.d.	0.60 ± 0.09	2.66 ± 0.08	0.76 ± 0.01	0.49 ± 0.11
41	*γ*-gurjunene	1479	0.24 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2: (Continue)

Peak #	*Compounds	Retention Indices (RI)	Relative Area (%)									
			Hydrodistillation	Solvent Extraction (Dichloromethane)			Solvent Extraction (Methanol)			HS-SPME		
			L	L	F	I	L	F	I	L	F	I
43	α-amorphene	1480	0.43 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.34 ± 0.04	n.d.	n.d
42	germacrene D	1484	9.62 ± 0.16	6.58 ± 0.18	8.29 ± 0.27	13.71 ± 0.40	5.58 ± 0.69	9.34 ± 1.48	11.01 ± 0.40	11.54 ± 0.58	12.73 ± 0.76	13.71 ± 1.35
47	α-bulnesene	1511	0.09 ± 0.01	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
51	eugenol acetate	1525	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.10 ± 0.14	n.d.	n.d.	n.d.
48	δ-cadinene	1535	1.08 ± 0.03	n.d.	n.d.	0.58 ± 0.03	n.d.	n.d.	0.77 ± 0.16	1.05 ± 0.05	1.32 ± 0.01	1.07 ± 0.08
55	caryophyllene oxide	1574	0.75 ± 0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.18 ± 0.02	n.d.	n.d.
56	isocaryophyllene	1580	n.d.	0.62 ± 0.16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
53	τ-cadinol	1643	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.00 ± 0.07	n.d.
54	α-cadinol	1649	0.12 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Total		98.14	97.39	88.8	98.65	90.81	100.00	100.00	88.39	99.67	99.42

**Compounds identified by using Mass Spectra (MS) data and confirmed by Kovats Index; n.d = not detected*
HS-SPME = Headspace-Solid Microextraction; L = Leaves; F = Flowers; I = Inflorescences

dichloromethane extract might be lost, especially during the solvent removal process, due to high volatile properties and having a small molecular weight [28].

It is worth mentioning that solvent extraction is applicable in small or large batches with an easy and simple method. Hydrodistillation required largest amount of material, followed by solvent extraction and HS-SPME required the least amount of material. Therefore, hydrodistillation is a more suitable method providing abundant access to material plants while HS-SPME is much preferable when the plant material is limited. HS-SPME requires no solvent in extracting thus an advantage as there will be no solvent peak present in the chromatogram [29].

Genetic distance

The genetic distance between the species was calculated by using Nei's statistical method to investigate the interspecies relationship between *O. basilicum* and *O. sanctum*. Nei had reported that when the two populations have the same alleles in identical frequencies, the normalized identity of genes between the two populations with respect to the locus is unity. On the contrary, when the two populations have different alleles, it is zero. Based on compounds identified by GC-MS when hydrodistillation was carried out, the genetic distance between *O. basilicum* and *O. sanctum* was calculated [30]. X in the equation represents *O. basilicum*, while Y represents *O. sanctum*. The probability of identity of two genes that have been chosen randomly in *O. basilicum* is jx = 1618.11, while for *O. sanctum* is jy = 2500.06. The probability of identity of a gene from *O. basilicum* and a gene from *O. sanctum* is jxy = 115.06. The normalized identity of genes between *O. basilicum* and *O. sanctum* with respect to all loci is $I = J_{XY}/\sqrt{J_X J_Y} = 0.0572$, where J_X, J_Y, and J_{XY} are the arithmetic means of jx, jy and jxy, respectively, over all loci. Based on the present study, the calculated genetic distance between the two species is 2.86. Therefore, *O. basilicum* and *O. sanctum* is related to each other, shared some similar alleles and these two species have a recent same ancestor.

Antioxidant activities of O. basilicum and O. sanctum leaves

The antioxidant activities of the various extracts of *O. basilicum and O. sanctum* leaves were determined

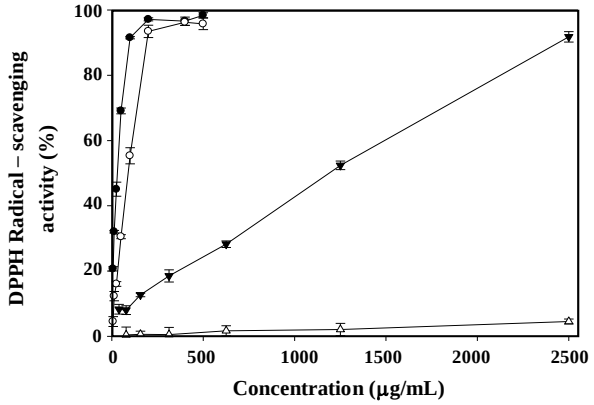

Fig. 1: Antioxidant capacity of O. basilicum leaves of (○) methanol extract, (▼) dichloromethane extract and (Δ) hydrodistillation extract as compared to (●) BHT standard.

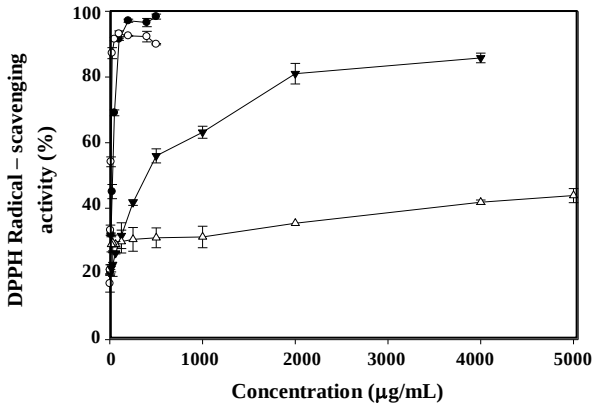

Fig. 2: Antioxidant capacity of O. sanctum leaves of (○) methanol extract, (▼) dichloromethane extract and (Δ) hydrodistillation extract as compared to (●) BHT standard.

DPPH radical scavenging assay. The violet colour of DPPH disappeared and turned to a pale yellow colour when the solution was tested with the methanol and dichloromethane extracts of *O. basilicum* and *O. sanctum* leaves. However, a similar observation was not obtained when the solution was tested with the leaves hydrodistillation extract of the two species as the violet colour of DPPH changed to a lighter shade of violet. There is a significant positive relationship between the concentration of *O. basilicum* and *O. sanctum* leaves extracts with the DPPH radical scavenging activity ($p < 0.05$). *O. basilicum* leaves methanol extract showed a significantly lower IC_{50} value of 88 µg/mL as compared to its dichloromethane extract with a value of 1178 µg/mL. BHT was used as positive control in this investigation showed an IC_{50} value of 29 µg/mL. The hydrodistillation extract could not provide IC_{50} value even though the concentration had been increased to 2500 µg/mL. Fig. 1 shows the graph of the antioxidant activity of *O. basilicum* leaves extracts.

The hydrodistillation extract was expected to have lower radical scavenging activity due to lower concentration of oxygenated compounds and the presence of hydrocarbons [31] as compared to the methanol and dichloromethane extracts of *O. basilicum* leaves. It was reported that compounds with hydroxyl groups sterically hindered by a *t*-butyl group do affect the antioxidant activity [32]. *O. sanctum* leaves methanol extract showed a lower IC_{50} value of 11 µg/mL compared to its dichloromethane extract which was 369 µg/mL while similar to hydrodistillation leaves extract of *O. basilicum*, hydrodistillation extract of *O. sanctum* showed a very weak free radical scavenging activity, even though the concentration had been increased to 5000 µg/mL. Fig. 2 shows the antioxidant activity of *O. sanctum* leaves extracts.

The results showed that *O. sanctum* had a stronger free radical scavenging capacity compared to *O. basilicum*. The major compounds that contributed to the free radical scavenging activities of *O. basilicum* and *O. sanctum* were possibly eugenol methyl ether, estragole, *β*-ocimene and *α*-caryophyllene [33]. Fig. 3 shows the IC_{50} values of BHT, the methanol extract of *O. basilicum* leaves, the methanol extract of *O. sanctum*, the dichloromethane extract of *O. basilicum* leaves and the dichloromethane extract of *O. sanctum*.

The lower IC_{50} value of *O. sanctum* leaves methanol extract compared to BHT showed that the extract has stronger DPPH scavenging activity compared to BHT. The possible reason behind this finding is due to the presence of eugenol methyl ether. Eugenol methyl ether has stronger DPPH scavenging activity compared to BHT and it was reported that the essential oil composition of three *Melaleuca* species with eugenol methyl ether identified as the principal component, showed lower IC_{50} values (37.30 ± 0.90 µg/mL, 37.80 ± 1.60 µg/mL and 39.10 ± 0.30 µg/mL) compared to BHT (41.50 ± 0.50 µg/mL) [34]. Fig. 4 shows the ARP values of BHT, the methanol extract of *O. basilicum* leaves, the methanol extract of *O. sanctum*, the dichloromethane extract of *O. basilicum* leaves and the dichloromethane extract of *O. sanctum*.

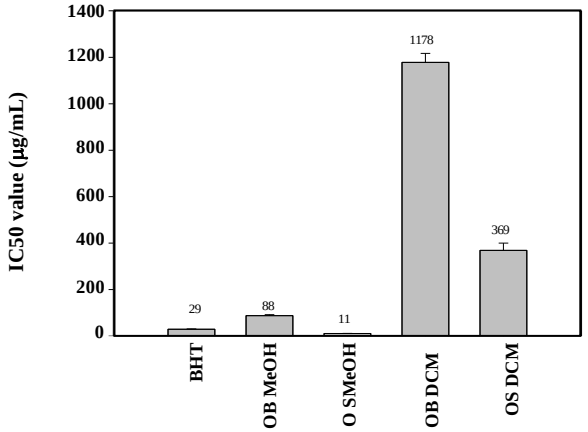

Fig. 3: IC_{50} values of BHT, methanol extract of O. basilicum leaves (OB MeOH), methanol extract of O. sanctum (OS MeOH), dichloromethane extract of O. basilicum leaves (OB DCM) and dichloromethane extract of O. sanctum (OS DCM).

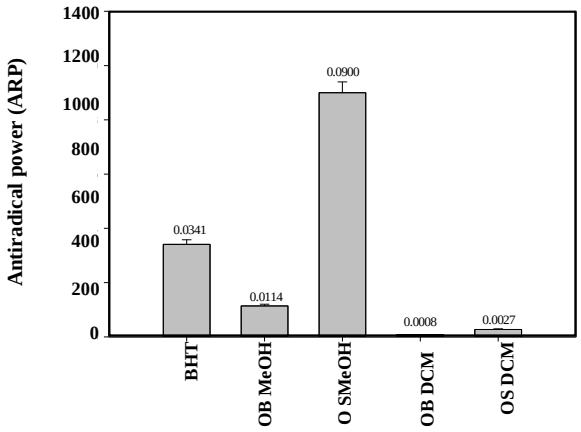

Fig. 4: ARP values of BHT, methanol extract of O. basilicum leaves (OB MeOH), methanol extract of O. sanctum (OS MeOH), dichloromethane extract of O. basilicum leaves (OB DCM) and dichloromethane extract of O. sanctum (OS DCM).

The higher the value of ARP, the higher is the free radical scavenging activity and the lower is the IC_{50} value [35]. The ARP values vary from 0.0008 to 0.0900 with *O. sanctum* methanol leaves extract as the highest and *O. basilicum* leaves dichloromethane as the lowest. Therefore, after considering the IC_{50} and ARP values the overall trend of antioxidant action is reflected as methanol extract of *O. sanctum* leaves< BHT< methanol extract of *O. basilicum* leaves < dichloromethane extract of *O. sanctum* leaves< dichloromethane extract of *O. basilicum* leaves.

Even though synthetic antioxidants are extremely effective as an antioxidant, their adverse effects on health remain the leading concern in their usage. BHA and BHT are competent antioxidants at the lower range of concentrations, nevertheless at high concentrations, they are pro-oxidant [36, 37]. BHT has damaging effects on the liver [38] and enhanced the cell death of lung tumour cells [39]. Besides that, TBHQ was proven to be cytotoxic in human monocytic leukaemia U937 cells [40]. It was also reported that BHT and PG restrain humoral immunity by suppressing regulation of T cells or action of macrophages on B cells [41]. Regulations on the usage level of the synthetic antioxidants are implemented due to the health concern and it varies for different countries. Food and Drug Administration (FDA) and U.S. Department of Agriculture (USDA) allowed maximum usage level of 0.02% and 0.01% of weight fat, respectively, for general use individually of BHT, BHA, TBHQ, and PG. On the other hand, Europe, United Kingdom, Norway, Denmark, Sweden, Switzerland, and Japan have banned the usage of TBHQ as a food additive in their countries [42]. Besides that, antioxidant is one of the dietary factors that are vital in preventing cancer. *O. basilicum* and *O. sanctum* leaves were reported to have reduced the number of azoxymethane induced Aberrant Crypt Foci in Fisher 344 male rats. Therefore, the *O. basilicum* and *O. sanctum* leaves have potential in being chemopreventive agents [43].

CONCLUSIONS

The present study showed that *O. basilicum* has grown in Malaysia is rich in estragole, eucalyptol, *(E)-β*-ocimene, *α-trans*-bergamotene, *α*-caryophyllene, and *τ*-cadinol while *O. sanctum* grown in Malaysia is rich in eugenol methyl ether, caryophyllene, germacrene D, *β*-elemene and copaene. The compounds found in both species are useful in food and flavour industries. HS-SPME and hydrodistillation are able to extract more chemical constituents from these two species as compared to solvent extraction. The chemical constituents extracted depend highly on the extraction methods as the solubility of the chemical constituents in different extraction procedures affected by the polarity of solvent used. Solvent extraction is an easy and simple method. Hydrodistillation is a more suitable method providing abundant access to material plants while HS-SPME is much preferable when the plant material is limited. The leaves of *O. basilicum* and *O. sanctum* was found to consist more chemical constituents compared

to inflorescence and flowers, thus explains the stronger aroma produced by the leaves. Based on the calculated genetic distance between *O. basilicum* and *O. sanctum*, we found that these two species are related to each other and share some of the same alleles with identical frequencies. *O. sanctum* exhibited a higher radical scavenging capacity as compared to *O. basilicum* and BHT. These results indicate that *O. sanctum* can be an effective potential source of natural antioxidants. Therefore, supplementing a balanced diet with *O. sanctum* would have beneficial health effects that can be expected to lower the risk of getting oxidative stress related diseases. Even though *O. basilicum* showed a lower free radical scavenging activity, it still contains a considerable amount of activity. Furthermore, the addition of synthetic antioxidants in food processing has created health concerns. Thus, replacing them with natural oxidation inhibitors or using ingredients that naturally possess antioxidant activity would be preferable.

Acknowledgments

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