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Impact of Extraction Technique on the Volatile Oil Contents and Composition of four *Ocimum* Species; Microwave Assisted Extraction *versus* Distillation Study

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ABSTRACT

Objectives: The aim of this study is to unravel the variabilities posed by alteration of the extraction technique employed on the contents and composition of essential oils derived from the same plant species **Methods:** Volatile oils of four different *Ocimum* species (*Ocimum basilicum* L., *O. africanum* Lour., *O. americanum* L. and *O. minimum* L. family Lamiaceae) were individually extracted from their fresh aerial parts using green microwave assisted extraction (MAE) method and conventional hydrodistillation (HD) and steam distillation (SD) methods. Extracted volatile oil samples were further analysed by GC-MS. **Results:** Qualitatively, distillation of the *Ocimum* samples resulted in higher yields of volatile oil than MAE (0.16-0.42%, 0.16-0.44% and 0.1-0.25% ml/g fresh weight for HD, SD and MAE, respectively). However, MAE technique was accomplished in a fraction of time (8 minutes) compared to distillation procedures (2 - 4 hours). GC-MS analysis of the *Ocimum* oils extracted using MAE method revealed higher enrichment of marker ingredients, *viz. β*-linalool and eucalyptol, over the distillation methods. Relative percentage of *β*-linalool in oil of *O. basilicum* and *O. africanum* was 76.9 & 72.2% versus 31.2 & 42.9% and 24.7 & 57.2%, whereas that of eucalyptol was 11.1 & 9.4% versus 6.2 & 4.5% and 4.8 & 4.2%, by MAE, SD and HD, respectively. Estragole, a natural volatile having safety concerns, was detected with appreciable amounts in the oil samples obtained by distillation. MAE extraction resulted in less than third the estragole content in oil of *O.basilicum* when compared to (HD) and (SD) methods (10.2%, 36.7% and 33.2%, respectively). **Conclusions:** MAE provides a rapid, power saving and green technique for extraction and preserving the valuable constituents of *Ocimum* essential oils. (MAE) produced an exceptionally *β*-linalool and eucalyptol enriched oil of sweet basil, much suitable for commercial and medicinal uses. Estragole contents were much reduced in (MAE) prepared oil samples comparable to distillation methods, a fact that prioritize selecting this technique for preparing *Ocimum* oils intended for systemic and/or pediatric applications.

Keywords: *Estragole; GC-MS; Microwave assisted extraction; Ocimum, Volatile oil*

INTRODUCTION

Family Lamiaceae (formerly Labiatae) is one of the main plant families which comprises a wide range of genera highly enriched in volatile oils *viz. Thyme, Lavander, Ocimum, Mentha, Rosemary, Salvia* and *Origanum* [1]. The genus *Ocimum* affords various species used for culinary and condiment purposes, and

their essential oils are extensively employed commercially as ingredients in foods, insect repellents, perfumes and cosmetic industries [2]. Medicinally, *Ocimum* herbs and oils are also consumed in folk medicine and aromatherapy for their marked anti-spasmodic, anti-inflammatory, expectorant, sedative and anxiolytic effects [3, 4]. For these economic and medicinal attributes, numerous *Ocimum* cultivars, primarily *Ocimum basilicum* (sweet basil), are currently cultivated worldwide [5]. Previous studies indicated that *Ocimum* oils are generally enriched in phenylpropanoids and oxygenated monoterpenes *viz. β*-linalool and caryophyllene when prepared by hydro-distillation [6].

The composition of volatile oils is generally influenced by ontogenetic, seasonal and environmental variables [7]. Nevertheless, extraction of the volatile oils from their natural sources is a crucial step defining the end product qualitatively and quantitatively [8]. Conventional distillation methods involving prolonged exposure to heat and water as a liquid or vapor phase could be destructive for many of the volatile oil constituents [9] which affects the end product significantly. Microwave assisted extraction (MAE) is a green, solvent free extraction procedure that is considered to be a modified dry distillation technique [10, 11]. Unlike conductive heating methods, microwaves with their electro-magnetic power, allow for heating the whole extracted sample in a uniform and rapid manner [10]. Other benefits of (MAE) include reducing the extraction time from hours to minutes, higher yields as well as energy and plant material saving [12].

The present study is an attempt to compare the oil composition and abundance of bioactive ingredients after different extraction techniques *viz.* distillaion (steam and hydro-distillation; SD and HD, respectively) and (MAE). Four *Ocimum* oils (*Ocimum basilicum* L., *O. africanum* Lour., *O. americanum* L. and *O. minimum* L.) were separately prepared using the three aforementioned techniques and further analysed by gas chromatography coupled to a mass detector (GC-MS).

MATERIAL AND METHODS

Plant material

Fresh aerial parts (leaves and stems) of *Ocimum basilicum* L., *O. africanum* Lour., *O. americanum* L. and *O. minimum* L. were collected during early Spring from the Experimental Station of Medicinal and Aromatic Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza, Egypt. The plants were identified by Dr. Gemma L. C. Bramley, Royal Botanic Gardens, Surrey, UK. Voucher specimens of the examined plants (number OB-201323) were deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University. The four *Ocimum* samples were cut to pieces manually, weighed and individually extracted by hydrodistillation (HD), steam distillation (SD) and microwave assisted extraction (MAE) techniques. *Ocimum* volatile oils were isolated separately in well closed vials containing anhydrous sodium sulphate to remove any traces of water to protect the oil from hydrolysis, and vials were stored in the refrigerator at 4°C till further GC-MS analysis.

Microwave assisted extraction (MAE)

A microwave essential oil distiller (OilexTech®, USA) with a specific extraction kit **(Figure 1)** was used for preparing the volatile oils. *Ca.* (100 g) of each *Ocimum* sample was placed in the distillation kit container, where a cone of ice fixed in the cover of the container, was placed inside the kit acting as a condenser. Microwave assisted extraction was carried out for 8 minutes with only 80% of microwave radiation power [13].

Figure 1. Microwave assisted extraction kit.

Hydrodistillation (HD)

In a Clevenger apparatus, place (500 g) of each *Ocimum* sample submerged in distilled water having no xylene. Hydrodistilltaion was carried out for 4 hours using *ca.* 6 L of water [14]. (HD) technique was applied for preparation of volatile oils of the four species of *Ocimum* used in this study.

Steam distillation (SD)

Steam distillation was held in a similar way to hydrodistillation. The only difference was that the plant sample (250 g) was held in a separate rounded flask above the flask containing boiling water, where the steam was forced to move through the plant sample. The procedure was carried out for 2 hours for each *Ocimum* sample, where there was no direct contact between the plant sample and the boiling water [15].

GC-MS volatile oil analysis

The mass spectra were recorded using Shimadzu GCMS-QP2010 (Tokyo, Japan) equipped with Rtx-5MS fused bonded column 30 meters long (0.25 mm i.d. x 0.25 µm film thickness, Restek, USA)

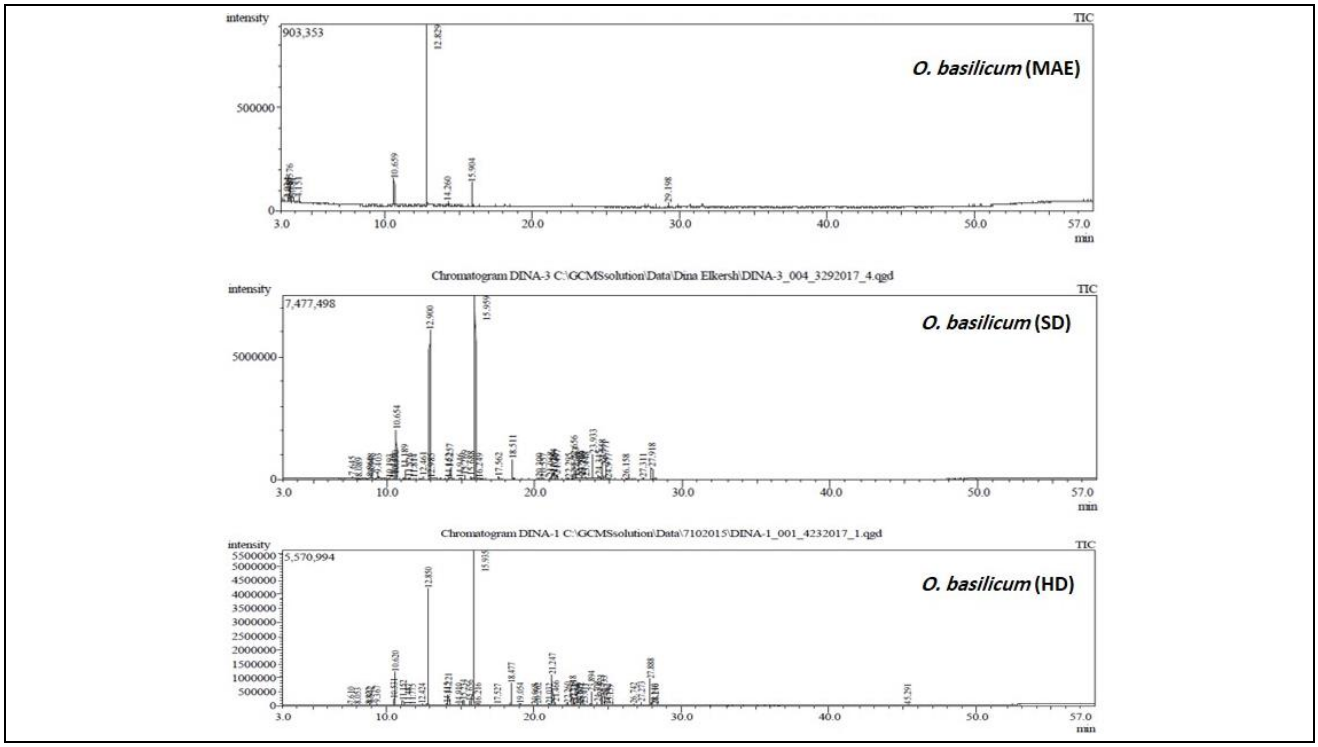

Figure 2. GC-MS chromatograms of *Ocimum basilicum* oils extracted by microwave assisted extraction (MAE), steam distillation (SD) and hydrodistillation (HD).

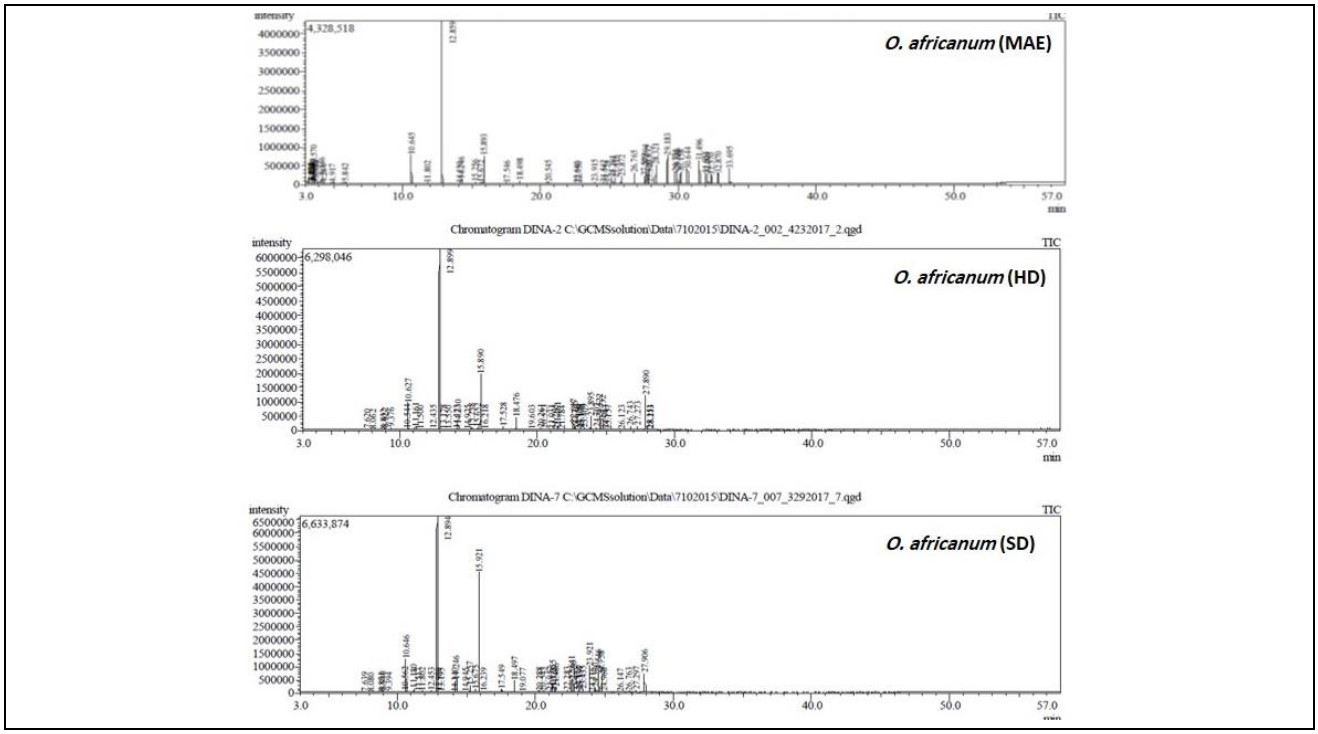

Figure 3. GC-MS chromatograms of *Ocimum africanum* oils extracted by microwave assisted extraction (MAE), steam distillation (SD) and hydrodistillation (HD).

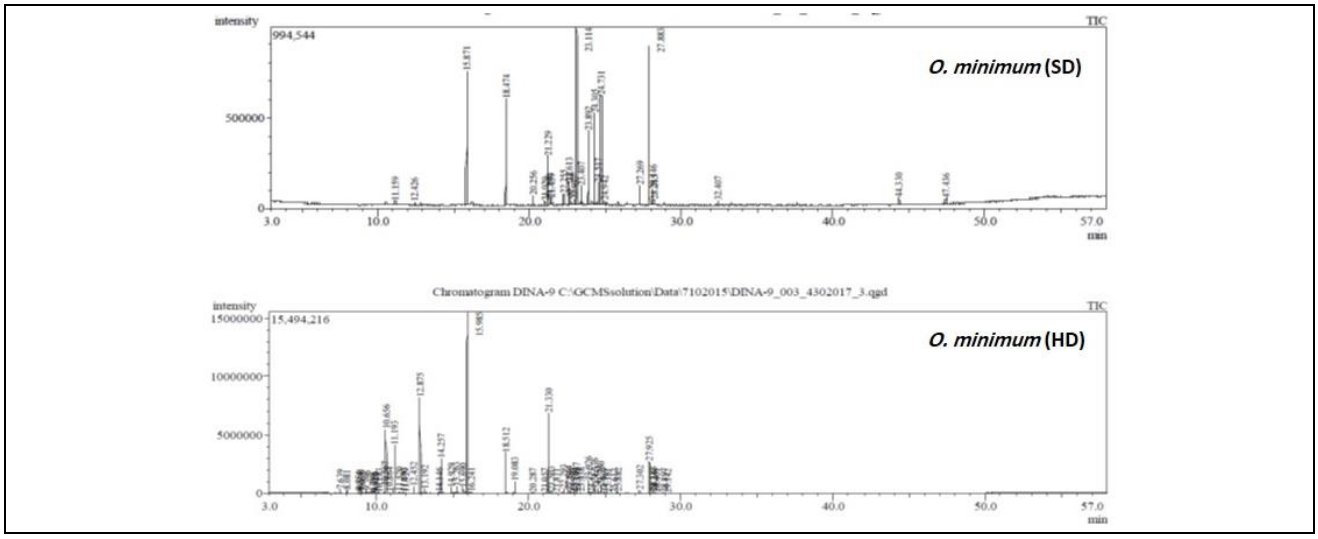

Figure 4. GC-MS chromatograms of *Ocimum minimum* oils extracted by steam distillation (SD) and hydrodistillation (HD).

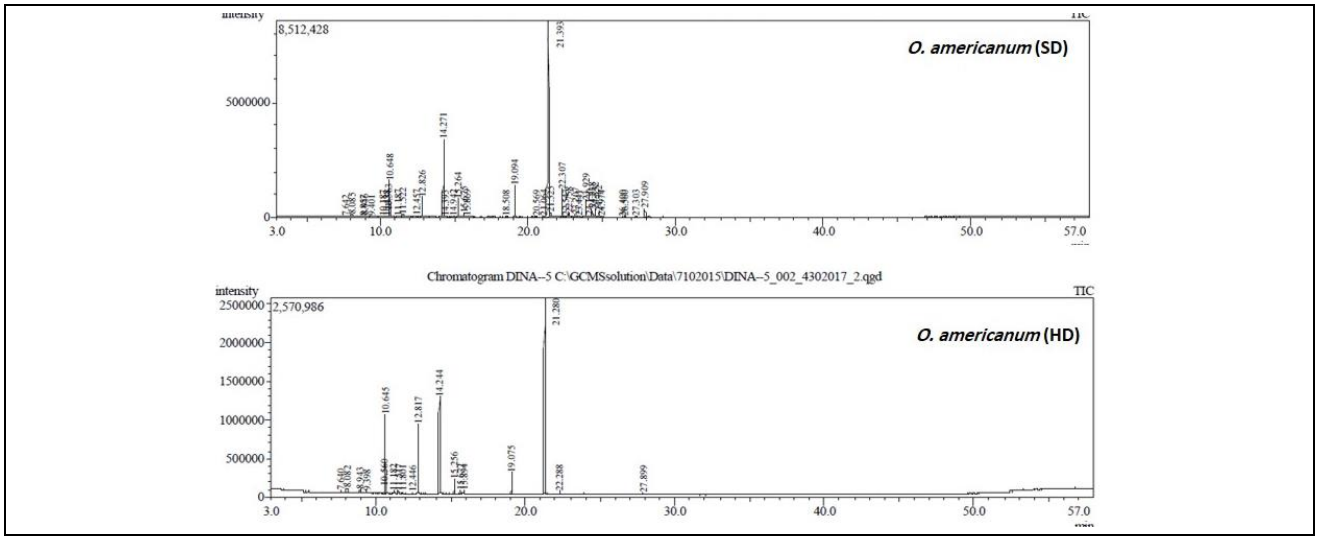

Figure 5. GC-MS chromatograms of *Ocimum americanum* oils extracted by steam distillation (SD) and hydrodistillation (HD).

with a split injector (split ratio 15:1). The capillary column was directly coupled to a quadrupole mass spectrometer (SSQ 7000; Thermo-Finnigan, Bremen, Germany). The program conditions were as follows: The initial column oven temperature was kept at 45°C for 2 min (isothermal) then raised to 300°C at a rate of 5°C/min. The column oven temperature was kept constant at 300°C for 5 min whereas the injector temperature was 250 °C. Helium carrier gas flow rate was 1.41 ml/min. All the mass spectra were recorded applying the following condition: filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200°C. Injection volume was 0.5 µl (10 % v/v of volatile oil dilution by *n*-hexane). Volatile components were identified by their linear retention indices relative to a homologous *n*-alkanes series (C_8–C_{20}), and by comparing the components fragmentation pattern with those of NIST database (National Institute of Standards and Technology, WILEY library database) and with the data of previous

literatures. Quantification of each volatile component was carried out by relative area method using the equation:

Relative Percentage of Volatile Component = Component area/Total area X100

RESULTS AND DISCUSSION

The present study reveals the differences in volatile oil composition arising from altering the extraction procedure for the same specimen in four *Ocimum* species. Extraction of volatile oils using (MAE) technique was only successful with both *O. basilicum* and *O. africanum*., while the other species, *O. minimum* and *O. americanum*, failed to produce enough volatile oil for GC-MS analysis under same experimental conditions. Percentages of the volatile oils yielded the examined *ocimum.* samples are presented in **Table 1**.

Table 1. Percentage of volatile oil yields extracted from fresh aerial parts of four *Ocimum* species by hydodistillation (HD), steam distillation (SD) and microwave assisted extraction (MAE)

Ocimum Species	% yield of oil (ml/g fresh weight)*		
	HD	SD	MAE
O.basilicum	0.42	0.44	0.25
O. africanum	0.4	0.4	0.1
O. americanum	0.18	0.2	-
O. minimum	0.16	0.16	-

**Sample weight used for extraction of volatile oils using HD, SD and MAE was 500, 250 and 100 g, respectively for each species.*

Qualitatively, distillation of the *Ocimum* samples resulted in higher yields of volatile oil than MAE (0.16-0.42%, 0.16-0.44% and 0.1-0.25% ml/g fresh weight for HD, SD and MAE, respectivelydf). Nevertheless, it should be noted that (MAE) technique was successful in producing oils from *Ocimum basilicum* and *O. africanum* when applied for 8 minutes only relative to 2 or 4 hours required for distillation procedures.

GC-MS analysis of volatile oils isolated using different extraction methods HD, SD and MAE from the aerial parts of the four species of *Ocimum viz. O. basilicum, O. africanum, O. americanum* and *O. minimum* are presented in **Table 2**. Representative GC-MS chromatograms of *Ocimum* volatile oils analysed after different extraction techniques are presented in **(Figures 2-5)**. A total of 58 volatiles were identified from all the samples collectively, with 41-45 ingredients appearing in oils of *O. basilicum, O. africanum* and *O. minimum*, and only 35 compound in *O. americanum.* Structures of the major identified volatile constituents of the analysed oils with relevant discussion throughout the manuscript are illustrated in (**Figure 6**). Bioactive and marker components in the volatile oils of the same species varied significantly according to the extraction technique employed, being more enriched, less abundant or even absent in one of the procedures. Generally, the number of volatiles detected in the oils extracted using (MAE) method were less than distillation derived volatiles, where only 16 ingredient were identified collectively. This alteration in the volatile blend observed in the same species will definitely have an impact on the organoleptic, chemical and biological properties of the produced oils .

O. basilicum, the most commonly consumed basil species, revealed only 4 volatiles in the (MAE) oil sample comparable to 39 and 38 volatiles by (SD) and (HD) techniques, respectively (**Figure 2)**. *β*-Linalool was markedly distinguished in *O. basilicum* oil when extracted using (MAE) technique relative to distilled oil samples (relative percentage =76.9%, 31.2% and 24.7% by MAE, SD and HD, respectively). *β*-Linalool is a chief volatile compound marker to basil and lavender oils, with characteristic antibacterial, antioxidant, cytotoxic and anticonvulsant activities [16-18]. Both *O.basilicum* and *O. africanum* oils obtained by (MAE) were distinctly enriched in *β*-linalool content (76.9% and 72.2%, respectively) compared to distillation methods (**Figure 3)**, which favors (MAE) technique for production of natural linalool from these essential oils.

1,8-Cineole (eucalyptol), was also detected at higher contents in the oils of *O. basilicum, O. africanum* extracted by MAE relative to (SD) and (HD) extracted oils (relative percentage =11.1 & 9.4% versus 6.2 & 4.5% and 4.8 & 4.2%, by MAE, SD and HD, respectively). Eucalyptol possess strong evident anti-inflammatory properties, notably for pancreatitis [19]. On the contrary, lower percentage of estragol were detected in the oils of *O. basilicum* and *O. africanum* extracted by MAE (*ca.* 10% for both oils) *versus* distillation methods (33.1% &19.6 and 36.7% & 10% for SD and HD, respectively).

Estragole (methyl chavicol, *p*-allylanisole) is an anethole isomer that has been listed as "genotoxic carcinogens" [20]. Although estragole has natural occurence in *Ocimum* species and other members of family Lamiaceae [21] but it is obvious that prolonged extraction periods and application of heat can result in elevating its content [22]. *The committee on herbal medicinal products* (HMPs) also has released a public statement about the potential genotoxic carcinogenicity of estragole and they recommended restricted consumption for children and nursing women [23].

Table 2. Relative percentages of volatile components analysed using GC-MS in the oils of aerial parts of *O. basilicum*, *O. africanum*, *O. americanum* and *O. minimum* extracted by steam distillation, microwave assisted extraction and hydro-distillation (SD, MAE and HD, respectively)

Peak	Rt. (min)	RI[a]	RI[b]	Name	*O. basilicum*			*O. minimum*		*O. africanum*			*O. americanum*	
					SD	MAE	HD	SD	HD	SD	MAE	HD	SD	HD
1	7.65	924	936	*α*-Pinene	0.33	-	0.18	-	0.29	0.1	-	0.15	0.07	0.32
2	8.09	940	943	Camphene	0.17	-	0.13	-	0.27	0.05	-	0.07	0.28	0.74
3	8.85	969	983	*α*-Thujene	0.22	-	0.12	-	0.15	0.1	-	0.12	0.07	-
4	8.95	971	983	*β*-pinene	0.64	-	0.32	-	0.39	0.25	-	0.32	0.19	0.55
5	9.07	977	977	Sabinene	-	-	-	-	0.13	-	-	-	-	-
6	9.4	988	991	*β*-Myrcene	0.6	-	0.33	-	0.22	0.17	-	0.34	0.08	0.22
7	9.92	1007	1003	Cis-3-Hexenyl acetate	-	-	-	-	0.06	-	-	-	-	-
8	9.98	1009	1011	3-Carene	-	-	-	-	0.12	-	-	-	-	-
9	10.43	1023	1029	*p*-Cymene	0.09	-	-	-	-	-	-	-	0.06	-
10	10.57	1027	1031	D-Limonene	0.53	-	0.97	-	0.76	0.32	-	0.26	0.79	1.29
11	**10.65**	**1029**	**1034**	**1,8 Cineole (Eucalyptol)**	**6.18**	**11.12**	**4.82**	**-**	**6.44**	**4.52**	**9.4**	**4.2**	**4.45**	**13.71**
12	11.19	1047	1037	*β*-Ocimene	1.17	-	0.67	0.2	4.82	0.58	-	0.47	0.13	0.41
13	11.53	1057	1062	*γ*-Terpinene	0.17	-	0.11	-	0.16	0.15	-	0.06	0.32	0.38
14	11.81	1067	1068	(z)-Sabinene-hydrate	0.61	-	0.13	-	-	0.19	0.32	-	-	0.31
15	12.46	1087	1084	*α*-Terpinolene	0.36	-	0.2	0.27	0.75	0.23	-	0.18	0.27	0.21
16	**12.9**	**1101**	**1098**	***β*-Linalool**	**31.25**	**76.93**	**24.75**	**-**	**14**	**42.98**	**72.2**	**57.27**	**2.49**	**12.63**
17	12.99	1105	1104	Nonanal	0.09	-	-	-	-	0.14	-	-	-	-
18	13.19	1112	1109	1-Octenyl acetate	-	-	-	-	0.33	0.07	-	0.12	-	-
19	14.11	1142	1144	*α*-campholenal	0.13	-	0.31	-	0.07	0.09	0.12	0.19	-	-
20	14.26	1145	1143	Camphor	1.5	1.77	1.76	-	3.99	1.88	1.58	0.97	11.83	18.28
21	14.4	1151	1156	Isoborneol	-	-	-	-	-	-	-	-	0.08	-
22	14.95	1168	1166	Phellandrene-8-*α*-ol	0.16	-	0.25	-	0.66	-	-	-	0.15	-
23	15.23	1178	1177	Terpinen-4-ol	-	-	0.84	-	0.69	1.06	0.74	0.42	2.43	2.8
24	15.69	1191	1190	*α*-Terpineol	0.47	-	0.68	-	0.7	0.58	0.51	0.57	0.51	0.53
25	**15.96**	**1200**	**1195**	**Estragole**	**33.16**	**10.18**	**36.7**	**11.73**	**33.58**	**19.6**	**10.3**	**10.06**	**0.13**	**0.57**
26	16.25	1210	1211	3-Octyl acetate	0.12	-	0.16	-	-	0.13	-	0.19	-	-
27	17.56	1256	1249	Linalyl acetate	0.25	-	0.16	-	-	0.51	0.32	0.54	-	-
28	18.51	1289	1283	Bornyl acetate	2.74	-	3.44	**9.44**	**4.52**	1.76	1.33	2.15	-	-
29	19.05	1309	1301	(z) Methyl cinnamate	0.86	-	5.86	-	-	1.5	-	-	0.12	-
30	19.6	1329	1324	Limonene aldehyde	-	-	-	-	-	-	-	0.11	-	-
31	20.07	1345	1337	exo-2-hydroxycineole acetate	-	-	0.1	-	-	-	-	-	-	-
32	20.26	1351	1354	*α*-Cubebene	0.23	-	0.28	0.87	0.2	0.21	-	0.32	-	-
33	20.56	1362	1356	Eugenol	0.17	-	-	-	-	0.22	0.7	0.23	0.11	-
34	21.07	1378	1375	*α*-copaene	0.67	-	0.8	2.12	0.03	0.34	-	0.18	0.18	-
35	**21.23**	**1385**	**1379**	**(*E*)-Methyl cinnamate**	**-**	**-**	**-**	**4.63**	**14.96**	**-**	**-**	**-**	**61.22**	**46.16**
36	21.34	1389	1382	*β*-bourbonene	0.16	-	-	0.47	-	0.21	-	0.11	-	-
37	21.46	1393	1392	*β*-cubebene	-	-	-	0.39	0.33	1.12	-	0.75	0.89	-
38	21.5	1394	1398	*β*-elemene	0.99	-	0.83	-	-	-	-	-	-	-
39	21.8	1406	1397	Methyl eugenole	-	-	-	-	0.05	-	-	0.08	-	-

40	22.3	1424	1420	(*E*)-Caryophyllene	0.14	-	0.4	1.07	0.52	0.34	-	-	4.06	0.59
41	22.66	1438	1436	*α*-Bergamotene	2.71	-	1.48	2.04	0.33	1.97	0.27	1.19	-	-
42	23.14	1457	1459	*β*-(*E*) Farnesene	0.42	-	0.06	**16.78**	0.69	0.3	-	0.21	-	-
43	23.21	1459	1455	*α*-Humulene	0.43	-	0.31	-	0.14	0.52	-	0.38	0.36	-
44	23.41	1469	1475	epi-Bicyclosesquiphellandrene	-	-	0.21	1.63	0.13	0.45	-	0.29	0.14	-
45	23.45	1470	1462	*β*-Santalene	0.31	-	-	-	-	-	-	-	-	-
46	23.93	1488	1490	Sesquiphellandrene	3.77	-	2.39	7.41	1.48	4.29	0.76	2.5	2.58	-
47	24.12	1497	1497	*α*-Selinene	-	-	-	-	0.08	0.16	-	0.12	0.24	-
48	24.31	1504	1504	*α*-Bisabolene	-	-	0.6	**8.75**	-	-	-	-	1.33	-
49	24.35	1505	1505	*δ*-Guaiene	0.68	-	-	-	1.28	-	-	-	-	-
50	24.52	1512	1512	*ϒ*-Cadinene	3.55	-	2.46	3.32	0.64	5.62	0.27	3.29	1.8	-
51	24.77	1521	1524	*δ*-Cadinene	2.13	-	6.39	26	0.88	2.86	0.45	6.68	0.81	-
52	24.94	1529	1529	*β*-Sesquiphellandrene	-	-	-	0.86	0.34	0.47	0.73	2.83	0.12	-
53	26.16	1576	1576	Spatulenol	0.08	-	-	-	-	0.35	-	0.84	-	-
54	25.9	1565	1566	Nerolidol	-	-	-	-	0.14	-	-	-	-	-
55	26.74	1599	1582	Caryophyllene oxide	-	-	0.35	-	-	-	-	-	0.23	-
56	27.3	1623	1627	Epicubenol	-	-	-	-	0.43	0.44	-	0.85	0.17	-
57	27.92	1650	1648	*τ*-Cadinol	1.76	-	0.29	-	-	3.17	-	-	1.31	0.3
58	28.22	1663	1660	*β*-Guaiene	-	-	0.16	2.02	4.25	-	-	0.39	-	-
Total number of identified volatiles					**39**	**4**	**38**	**19**	**41**	**44**	**16**	**39**	**35**	**18**
Total relative percentage					**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

RI[a]: Retention indeces calculated from retention time in relation to n-alkanes series on 30m DB-5- capillary column.
RI[b]: Linear retention indeces reported from previous literature. RI: Retention index. (-): Absent.
(Rt): Retention time, (SD): Steam distillation. (MAE): Microwave assisted extraction. (HD): Hydrodistillation.
Bold values are the major constituents in the volatile oil.

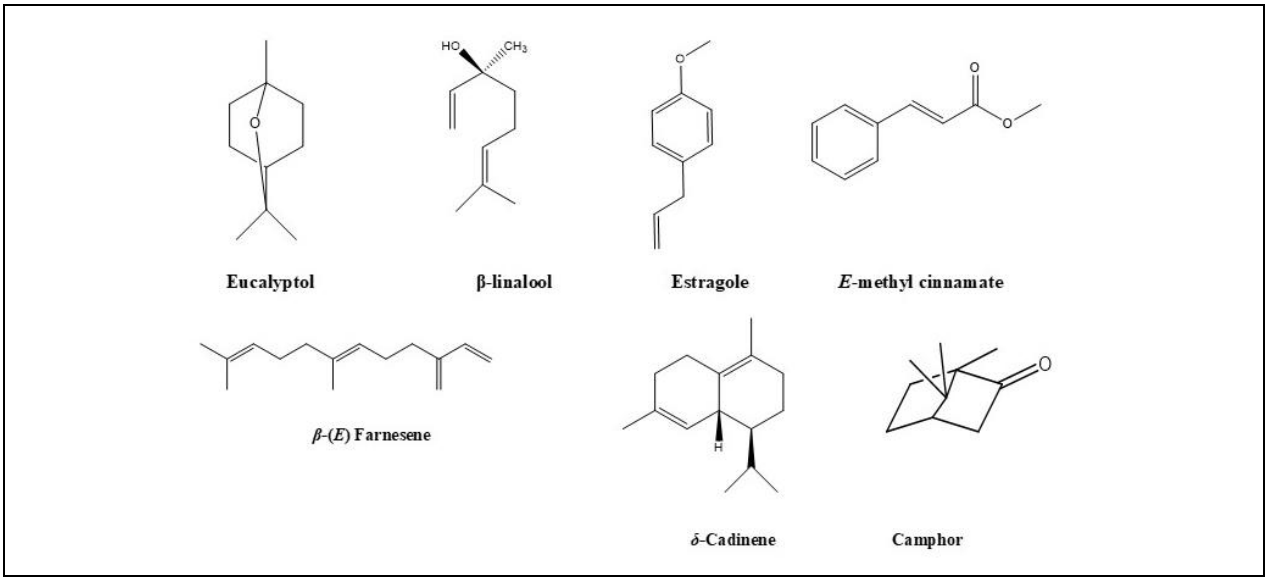

Figure 6. Structures of the main volatiles identified in the essential oils of *Ocimum* species

As for *O. minimum* volatile oil composition, a total of 41 compounds were identified in the sample prepared by HD method *versus* 19 compounds only by (SD) (**Figure 4**). Bornyl acetate and (*E*)-methyl cinnamate attained were major constituents identified in the distilled oils (relative percentage =9.4% & 4.5% *vs.* 4.6% & 14.9% in SD & HD, respectively). Estragole contents were higher in the oil samples extracted by (HD) than (SD) (relative percentage =33.6% *vs.* 11.7%, respectively). Analysis results also revealed some prominent volatiles abundance in either distillation techniques only, *viz* δ-Cadinene, β-(E) farnesene and α-Bisabolene (relative percentage =26%, 16.7% & 8.7%, respectively) in (SD) samples, whereas eucalyptol, β-linalool and camphor were only detectable after (HD) extraction (relative percentage =6.4%, 14% and 3.9%, respectively).

Volatile oil of *O. americanum* exhibited only 35 volatile constituents after (SD) extraction *versus* 18 constituent by (HD) extraction (**Figure 5**). (*E*)-Methyl cinnamate dominated the volatile composition of the oil (relative percentage = 61.2% and 46.2% by SD and HD, respectively), while β-linalool and eucalyptol appeared to be more abundant in (HD) samples (relative percentage = 12.6% & 13.7% compared to 2.5% & 4.5% in SD samples).

Based on the essential oil composition, *Ocimum* species can be categorized into four major chemotypes *viz.* methyl chavicol (estragole), linalool, methyl eugenol or methyl cinnamate enriched oils [24]. (*E*)-Methyl cinnamates was only detected in *O. minimum* and *O. americanum* species (**Figures 4 & 5**), which were not successful for (MAE) procedure, and generallly the distillation procedure seems suitable for its recovery in the volatile oils (relative percentage= 4.6 & 14.9% and 61.2 & 46.2% in *O. minimum* and *O. americanum* by SD and HD, respectively).

CONCLUSION

The present study strongly emphasizes that besides genetic variabilities, the extraction technique employed has a strong impact on the characteristics and the anticipated biological activities of the produced oil. The variation in volatile percentiles among different extraction methods could be attributed primarily to the direct contact with water and prolonged exposure to high temperatures for heating in both (HD) and (SD) methods. The study findings opt (MAE) for extraction of essential oils, whenever applicable, as a rapid, power saving and green technique that preserves the genuine composition of the oils. (MAE) produced an exeptionally β-linalool and eucalyptol enriched oil of sweet basil, much suitable for commercial and medicinal uses. In terms of oil safety and convenience for medicinal and systemic applications, estragole contents were much reduced in (MAE) prepared oil samples comaprable to distillation methods. Therefore (MAE) technique would be generally recommended over both distillation methods for extraction of essential oils of *Ocimum* species.

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Conflict of Interest

The authors declare that they do not have any conflict of interest.

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