2019

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

Dina M. El-Kersh  
*The British University in Egypt, dina.elkersh@bue.edu.eg*

Dalia Rasheed  
*Faculty of Pharmacy 6th of October University*

Manar Eissa  
*International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, Malaysia*

Follow this and additional works at: [https://buescholar.bue.edu.eg/pharmacy](https://buescholar.bue.edu.eg/pharmacy)

Part of the [Natural Products Chemistry and Pharmacognosy Commons](https://buescholar.bue.edu.eg/pharmacy)

**Recommended Citation**

El-Kersh, Dina M.; Rasheed, Dalia; and Eissa, Manar, "Impact of Extraction Technique on the Volatile Oil Contents and Composition of four Ocimum Species; Microwave Assisted Extraction versus Distillation Study" (2019). *Pharmacy*. 495.  
[https://buescholar.bue.edu.eg/pharmacy/495](https://buescholar.bue.edu.eg/pharmacy/495)

This Article is brought to you for free and open access by the Health Sciences at BUE Scholar. It has been accepted for inclusion in Pharmacy by an authorized administrator of BUE Scholar. For more information, please contact bue.scholar@gmail.com.
Impact of Extraction Technique on the Volatile Oil Contents and Composition of four Ocimum Species; Microwave Assisted Extraction versus Distillation Study

Dina M. El-Kersh1, Manar Eissa2, Dalia M. Rasheed3*

1Pharmacognosy Department, Faculty of Pharmacy, The British University in Egypt (BUE), 11837, Egypt
2International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, Malaysia
3Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Central Axis, Part 1/1, 6th of October, Egypt

*Corresponding author: Dalia M. Rasheed, Pharmacognosy Department, Faculty of Pharmacy, October 6 University, Central Axis, Part 1/1, 6th of October, Egypt. Tel.: (+2)37866067 E-mail address Daliarasheed@o6u.edu.eg

Submitted on: 30-06-2019; Revised on: 10-07-2019; Accepted on: 13-07-2019

To cite this article: El-Kersh, D. M.; Eissa, M.; Rasheed, D. M. Impact of Extraction Technique on the Volatile Oil Contents and Composition of four Ocimum Species; Microwave Assisted Extraction versus Distillation Study. J. Adv. Pharm. Res. 2019, 3 (3), 134-142. DOI: 10.21608/aprh.2019.40279

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, Lavender, Ocimum, Mentha, Rosemary, Salvia and Origanum . 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. 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. For these economic and medicinal attributes, numerous Ocimum cultivars, primarily Ocimum basilicum (sweet basil), are currently cultivated worldwide. Previous studies indicated that Ocimum oils are generally enriched in phenylpropanoids and oxygenated monoterpenes viz. β-linalool and caryophyllene when prepared by hydrodistillation.

The composition of volatile oils is generally influenced by ontogenetic, seasonal and environmental variables. Nevertheless, extraction of the volatile oils from their natural sources is a crucial step defining the end product qualitatively and quantitatively. 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 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. Unlike conductive heating methods, microwaves with their electro-magnetic power, allow for heating the whole extracted sample in a uniform and rapid manner. Other benefits of (MAE) include reducing the extraction time from hours to minutes, higher yields as well as energy and plant material saving.

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.

![Figure 1. Microwave assisted extraction kit.](http://aprh.journals.ekb.eg/)

### Hydodistillation (HD)

In a Clevenger apparatus, place (500 g) of each Ocimum sample submerged in distilled water having no xylene. Hydrodistillation was carried out for 4 hours using ca. 6 L of water. (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.

### 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:

\[
\text{Relative Percentage of Volatile Component} = \frac{\text{Component area}}{\text{Total area}} \times 100
\]

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)* |
|----------------------|-------------------------------------|
| O. basilicum         | 0.42 HD 0.44 SD 0.25 MAE            |
| O. africanum         | 0.4 HD 0.4 SD 0.1 MAE               |
| O. americanum        | 0.18 HD 0.2 SD - MAE                |
| O. minimum           | 0.16 HD 0.16 SD - MAE               |

*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, respectively). 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 occurrence 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.

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

| Peak | Rt. (min) | RIa | RIb | Name | O. basilicum SD MAE HD | O. minimum SD HD | O. africanaum SD MAE HD | O. americanum 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       | 986 | 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| α-Cymene | 0.09 - - - - - |                   |                        |                      |
| 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 | 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-Terpine | 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 | - - - - - - - |                   |                        |                      |
| 22   | 14.95     | 1168| 1166| Phellandrene-8-α-ol | 0.16 - 0.25 - 0.66 - - - |                   |                        |                      |
| 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-Terpinol | 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 exo-2-hydroxycineole acetate | - - - - - - - |                   |                        |                      |
| 31   | 20.07     | 1345| 1337| - - - - 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-copane (E)-Methyl cinnamate | 0.67 - 0.8 2.12 0.03 0.34 - 0.18 0.18 - |                   |                        |                      |
| 35   | 21.23     | 1385| 1379| - - - - 4.63 14.96 - - - |                   |                        |                      |
| 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 - |                   |                        |                      |

http://aprh.journals.ekb.eg/  139
| No | RIa | RIb | Retention time | (SD) | (MAE) | (HD) | Total number of identified volatiles | Total relative percentage |
|----|-----|-----|----------------|------|-------|------|------------------------------------|---------------------------|
| 40 | 22.3 | 424 | 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 epiphenone | 0.43 | - | 0.31 | - | 0.14 | 0.52 | - | 0.38 | 0.36 | - |
| 44 | 23.41 | 1469 | 1475 | 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 | γ-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

**Total relative percentage**: 100

**RIa**: Retention indexes calculated from retention time in relation to n-alkanes series on 30m DB-5 capillary column.

**RIb**: Linear retention indexes reported from previous literature. RI: Retention index. (Rt): 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](http://aprh.journals.ekb.eg/)
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 (estrageol), 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 generally 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 exceptionally 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 comparable to distillation methods. Therefore (MAE) technique would be generally recommended over both distillation methods for extraction of essential oils of *Ocimum* species.

**Acknowledgement**

The authors would like to thank Salma Ibrahim, Nada Rushdy, Nourhan Mohsen, Menna-Allah Othman, Nourhan Faisal, Amr Abdel Hafez, Mohamed Mazroua, Moataz Khaled and Merna Alexan "Year 5 students, Class 2016-2017, Faculty of Pharmacy, The British University in Egypt (BUE)" for their assistance in the practical work.

**Conflict of Interest**

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

**REFERENCES**

1. Bhargava, V.; Patel, S.; Desai K. Importance of terpenoids and essential oils in chemotaxonomic approach. *Int. j. herb. med.* 2013, *6* (2),14-21.
2. Hiltunen, R.; Holm, Y. *The genus Ocimum (Medicinal and Aromatic Plants-Industrial Profiles)*. Singapur: Harwood Academic Publishers, 2006, 39-57.
3. Bhasin M. *Ocimum-Taxonomy, medicinal potentialities and economic value of essential oil. Journal of Biosphere.* 2012, *1*, 46-50.
4. Tewari, D.; Sah, A.; Pandey, H.; Meena, H.; Meena R, Ramaswamy, R.S.; Reddy, R.C.; Deo, Y.K.; Bandari, S.; Bhadra, D.; Dev P.; Murthy, P.H. A review on phytoconstituents of *Ocimum* (Tulsi). *Int. j. Ayurvedic med.* 2012, *3* (1),1-9.
5. Lachowicz KJ, Jones GP, Briggs DR, Bienvenu FE, Palmer MV, Mishra V, Hunter MM. Characteristics of Plants and Plant Extracts from Five Varieties of Basil (*Ocimum basilicum L.*) Grown in Australia. *J. Agric. Food Chem.* 1997, *45* (7), 2660-2665.
6. Padalia, R.C.; Verma, R.S. Comparative volatile oil composition of four *Ocimum* species from northern India. *Nat. Prod. Res.* 2011, *25* (6), 569-575.
7. Lawrence, B.M. *Essential oil production: A discussion of influencing factors. ACS Publications; 1986.*
8. Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MH, Ghafoor K, Norulaini NA, Omar AK. Techniques for extraction of bioactive compounds from plant materials: A Review. *J. Food Eng.* 2013, *117* (4), 426-436.
9. Kimbaris, A.C.; Siatis, N.G.; Daferera, D.J. Tarantilis, P.A.; Pappas, C.S.; Polissiou, MG.

*http://aprh.journals.ekb.eg/*

141
Comparison of distillation and ultrasound-assisted extraction methods for the isolation of sensitive aroma compounds from garlic (Allium sativum). Ultrasound Sonochem. 2006, 13 (1), 54-60.

10. Kaufmann, B.; Christen P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. Phytochem Anal. 2002, 13 (2), 105-113.

11. Mandal, V.; Mohan, Y.; Hemalatha, S. Microwave assisted extraction—an innovative and promising extraction tool for medicinal plant research. Pharmacogn Rev. 2007, 1 (1), 7-18.

12. Cardoso-Ugarte GA, Juárez-Becerra GP, SosaMorales ME, López-Malo A. Microwave-assisted extraction of essential oils from herbs. J Microw Power Electromagn Energy. 2013, 47 (1), 63-72.

13. Smith, C.J.; Lebsack, J.; Hackleman, D. Microwave Extraction of Essential Oil from Peppermint-Field Trial. 2012. https://ir.library.oregonstate.edu/concern/default/kd17ct45p

14. Européenne P. Conseil de l’Europe. Maisonneuve SA Editions, Sainte Ruffine. 1996, 45-49.

15. Muráriková A, Ťažký A, Neugebauerová J, Planková A, Jampilek J, Mučaji P, Mikuš P. Characterization of essential oil composition in different basil species and pot cultures by a GC-MS method. Molecules. 2017, 22 (7), 1221.

16. Alviano W, Mendonça-Filho R, Alviano D, Bizzo H, Souto-Padrón T, Rodrigues M, Bolognese AM, Alviano CS, Souza MM. Antimicrobial activity of Croton cajucara Bentham linalool-rich essential oil on artificial biofilms and planktonic microorganisms. Oral Microbio. (8)Immunol. 2005, 20 (2), 101-105.

17. Liu K, Chen Q, Liu Y, Zhou X, Wang X. Isolation and biological activities of decanal, linalool, valencene, and octanal from sweet orange oil. J. Food Sci. 2012, 77 (11), C1156-C1161.

18. Peana AT, D’Aquila PS, Panin F, Serra G, Pippia P, Moretti MDL. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine. 2002, 9 (8), 721-726.

19. Lima PR, de Melo TS, Carvalho KMMB, de Oliveira ÍB, Arruda BR, de Castro Brito GA, Rao VS, Santos FA. 1, 8-cineole (eucalyptol) ameliorates cerulein-induced acute pancreatitis via modulation of cytokines, oxidative stress and NF-kB activity in mice. Life Sci. 2013, 92 (24-26), 1195-1201.

20. Miller J, Miller E. The metabolic activation and nucleic acid adducts of naturally-occurring carcinogens: recent results with ethyl carbamate and the spice flavors safrole and estragole. Br. J. Cancer. 1983, 48 (1), 1.

21. De Vincenzi, M.; Silano, M.; Maialetti, F.; Scaccio, B. Constituents of aromatic plants: II. Estragole. Fitoterapia. 2000, 71 (6), 725-729.

22. Rodríguez-Solana, R.; Salgado, J.M.; Domínguez, J.M.; Cortés-Díéguez, S. Characterization of fennel extracts and quantification of estragole: Optimization and comparison of accelerated solvent extraction and Soxhlet techniques. Ind. Crops. Prod. 2014, 52, 528-536.

23. EMEA E. Committee on herbal medicinal products (HMPC). Guideline on the assessment of clinical safety and efficacy in the preparation of community herbal monographs for well-established and of community herbal monographs/entries to the community list for traditional herbal medicinal products/substances/preparations. 2006.

24. Lawrence, B. Further examination of the variation of Ocimum basilicum L. Developments in Food Science. 1988.