Middle East Journal of Applied Sciences
ISSN 2077-4613
Volume : 08 | Issue :03 |July-Sept.| 2018
Pages: 971-982

Utilization of Microwave for enhancement of content and quality of rosemary and fennel volatile oils

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Received: 15 July 2018 / Accepted: 05 Sept. 2018 / Publication date: 15 Sept. 2018

ABSTRACT

An efficient microwave irradiation pretreatment (MIP) was performed to maintain high content and high quality volatile oils of rosemary leaves and fennel fruits. Different times and levels of MIP were employed; the findings indicated that the oil yield increased as MIP power increased. Where, the highest yield of rosemary and fennel volatile oil was obtained at 180 and 90 watts for 1 and 5 minutes, respectively, with increment rates 14.91 and 17.14 %, respectively. In general, our data showed that although there are common increasing trend of yield of rosemary and fennel volatile oils with increasing microwave irradiation power and time, the relationship was not linear. The volatile oils were fractionated and identified by using GC/MS technique. The main constituents of rosemary volatile oil extracted by hydrodistillation only were 1,8 cineol, α-pinene and camphor, however, MIP increased the amounts of α-pinene, β-pinene, camphor and α-terpineol. Estragole was the dominant compound in fennel oil extracted by hydrodistillation only, followed by d-limonene and fenchone, however, MIP at 90 watts for 5 min. led to increase camphene, sabinene and β-pinene. Furthermore, no obvious differences were noticed in an antioxidant capacity (IC50 in µg/ml) of oils obtained with hydrodistillation only and the ones treated with MIP method ($p \leq 0.05$) with the superiority to rosemary volatile oil, followed by BHT (as a synthetic antioxidant agent), meanwhile, fennel volatile oil recorded the lowest radical scavenging activity. Thus, MIP could be used as a good method for increasing the aromatic oil yield with maintaining the original quality of aromatic plants.

Keywords: Microwave, irradiation, pretreatment, volatile oils, rosemary, fennel, antioxidant, chemical constituents

Introduction

Volatile oils are known as the etheric oils (volatile and volatile oils) produced by the plant and could be obtained from the roots, stems, leaves, fruits, and/or flowers of plants (Kusuma and Mahfud, 2017). It is noteworthy that aromatic plants contain mixture of components which have health benefits to human. Nowadays, a great interest in evaluation the bioactive potency of ethereal oil extracted from aromatic herbs for their therapeutic properties and benefits as natural preservatives (Djenane *et al.,* 2012 and Costa *et al.,* 2015). Volatile oils have been applied in several industries such as cosmetics, perfumes, antiseptics, medicines, flavoring agents in food or beverages and cigarettes mixing as aromatherapy (Kusuma and Mahfud, 2017).

Fennel, (*Foeniculum vulgare*) belongs to family *Apiaceae*. It is a little group of annual, biennial, or perennial herb (Sharma, 2003). It is widely grown in the subtropical and temperate regions of the world. Germany, France, Russia, India, Italy, and the US are the major fennel producing countries (Azeez, 2008). The fruits of Fennel and its volatile oil are used as a spice for adding flavors to meats, breads, biscuits, candies, and liquors or in the manufacturing of perfumes, soaps, dentifrices, and phytotherapies (Maranca, 1985). Fennel is also used in culinary preparations, confectionery applications, food, beverage and cosmetic industries (Salama *et al.,* 2015). Anethole, estragol, fenchone, d-limonene and α-pinene are the main compounds reported to be present in fennel volatile oil (Senatore *et al.,* 2013). It is recorded that the percentage of these components found in the fennel volatile oil may differ considerably according to the geographical origin and phenological state of the fennel, harvesting season, methods of separation, techniques employed, environmental, genetic and agricultural practices (Marotti *et al.,* 1994, Fuente *et al.,* 2003, Díaz-Maroto *et al.,* 2006, Kandil *et*

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al., 2008 and Telci *et al.,* 2009). Aromatic oil of fennel fruits was found to have different medicinal and antioxidant properties against some diseases (Mirabolghasemi and Alizadeh, 2014 and Kooti *et al.,* 2015).

Rosemary is an aromatic plant belonging to the *Lamiaceae* family, it has been cultivated since ancient times and recognized as one of the plants with the highest content of antioxidants (Ibaňez *et al.,* 2003). Rosemary (*Rosmarinus officinalis* L.) is a long-lasting evergreen aromatic herb (Bousbia *et al.,* 2009). Before refrigeration was invented, rosemary oil was used for food preservation purposes besides, medical antiseptic, and astringent purposes (Bousbia *et al.,* 2009). Rosemary volatile oil includes phenolic constituents in its composition (Baser and Buchbauer, 2010). Due to this composition, which mainly involves monoterpenes like 1,8-cineole, α-pinene, camphor, camphene, rosemary volatile oil has many therapeutical effects (Katerinopoulos *et al.,* 2005 and Baser and Buchbauer, 2010). Among these effects, the most well-known ones are its antioxidant effects (Estevez *et al.,* 2007 and Valgimigli, 2012).

Steam distillation, hydrodistillation and simultaneous distillation-extraction methods are the most ordinary methods for the isolation of aromatic oils. Volatile oils from aromatic plants can be extracted by using a number of extraction techniques such as conventional methods, which have some disadvantages concerning the quality of the final product. Also, some chemical constituents mainly, volatiles, could be lost, beside, low extraction efficacy, degradation of esters or unsaturated compounds through hydrolytic or thermal effects in addition to the residue of solvent residue in the isolate may be encountered using the previous extraction methods (Ferhat *et al.,* 2007). Moreover, these extraction methods are time-consuming. Therefore, during last decades, an increasing requirement for novel process technologies is needed. Extraction is a new concept to face the challenges of the 21stcentury, to protect both the consumers and environment, and in the meantime enrich competition of industries to be more ecologic, economic, and innovative (Chemat *et al.,* 2012). These abuses of these techniques encourage researchers to energize the optimization, intensification and enhancement of existing and novel "green" extraction techniques. Novel techniques obliged by green extraction concept emerged in lately years for getting natural isolates with a similar or better quality close to that of official methods while reducing operation units, energy consumption, CO_2 emission and harmful solvents in specific cases (Chemat *et al.,* 2012). Novel extraction techniques with the application of supercritical solvents (Meireles, 2003), ultrasound (Lou *et al.,* 2010), microwave treatments (Taghvaei *et al.,* 2014) and alternating electric fields (Zeng *et al.*, 2010).

Novel microwave-assisted extraction (MAE) (Kaufmann and Christen, 2002) methods such as microwave-assisted hydrodistillation (MAHD) (Golmakani and Rezaei, 2008a,b and Wang *et al.,* 2010) and solvent-free microwave extraction (SFME) (Filly *et al.,*2014) have verified to be efficient and fast techniques for extracting aromatic oils from plants.

Among these novel technologies, microwave assisted extraction, which gained many advantages as high efficiency, convenience and low consumed time (Flamini *et al.,* 2007 and Zhai *et al.,* 2009).

Therefore, this investigation has been planned with aim to optimize a green technique as microwave pretreatment for extracting aromatic oils from fennel fruits and rosemary leaves with distinct power degrees of irradiation at different times. In addition to compare the results with those achieved by using a conventional hydrodistillation method with respect to oil yield, chemical constituents besides, antioxidant activity of such volatile oils.

Materials and Methods

Plant Materials

About 20 kg of dried Fennel fruits (*Foeniculum vulgare* Mill. Var. *vulgare*) and 20 kg of dried rosemary leaves (*Rosmarinus officinalis* L.) were obtained from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

Chemicals and reagents

DPPH (2, 2-diphenyl-1-picrylhydrazyl) and BHT (Butylated Hydroxy Toluene) were obtained from Sigma Aldrich, Germany. Methyl alcohol (99%) was obtained from El-Nasr Pharmaceutical Chemical Co., Egypt. Moreover, anhydrous sodium sulphate (99%) was purchased from Elgomhoria

Co., Chemicals trade, Cairo, Egypt.

Methods

Separation of volatile oils

For comparison hydrodistillation method, as a conventional method, was performed for both investigated plants as described in the European Pharmacopeia (1997). Fifty grams of fennel fruits and 250 ml of distilled water were poured into 500 ml boiling flask. Regarding rosemary leaves, 30 grams of leaves and 300 ml of distilled water were poured into 500 ml boiling flask. The extraction time was 6 h and 3 h for fennel and rosemary, respectively. The extracted volatile oils were collected and desiccate over anhydrous sodium sulphate. The extracted oil was stored in opaque glass bottles, in a refrigerator at 4±1ºC, until used and analysis. Hydrodistillation were carried out in triplicates and the mean values of the obtained oil was recorded.

Oil yield was calculated as follows:-

$$\text{Oil yield}(\%,\ v/w)=\frac{\text{volume of extracted oil (ml)}}{\text{weight of plant material (gm)}}\times 100$$

Microwave pretreatment:

Plant materials (50 g of fennel fruits or 30 g of rosemary leaves) were put inside microwave oven (DAEWOO (KOR-9G2B), DAEWOO Electronics, Korea. Frequency 2.45 M Hz, maximum output power 900W, 10 power levels, and cavity dimensions was 465 x 280 x 382 mm). The samples were exposed to different microwave's power levels, 90, 180, 270 and 360 watts for 1, 3, and 5 min, then submitted to hydrodistillation as previously mentioned. Each experiment was carried out in triplicate for each plant.

Fractionation and identification of individual components of the aromatic oils using the GC/MS technique:

The volatile oils (fennel fruits and rosemary leaves) were analyzed using the GC/MS (Gas Chromatography/Mass Spectrometry) technique. The GC/MS analysis was applied using the GC/MS system (Agilent Technologies), which was equipped with Gas Chromatograph (7890B) and Mass Spectrometer detector (5977A) at Central Laboratories Network, National Research Centre (NRC), Cairo, Egypt. Samples were diluted with hexane (1:19, v/v).The GC was provided with HP-5MS column (30 m x 0.25 mm internal diameter and 0.25 µm film thickness). Helium was the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:30, injection volume of 1 µl and the following temperature program: 40 °C for 1 min, rising at 4 °C /min to 150 °C and kept for 6 min, rising at 4 °C/min to 210 °C and kept for 1 min. The injector and detector were controlled at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV and using a spectral range of m/z 50-550. Identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST (National Institute of Standards and Technology) Mass Spectral Library data.

Antioxidant activity of volatile oils

The antioxidant potential of fennel and rosemary volatile oils were determined by using DPPH method to assess the effect of pretreatment with different microwave power levels for different times on the potency of such oils using the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color, as described by Choi (2010). The DPPH analysis was measured as mentioned by Brand-Williams, *et al.* (1995). Each sample was prepared by adding 25, 50, 100, 150 µL of volatile oil in a test tube followed by addition 2.8 ml of 0.4 g /L DPPH solution (0.020 ± 0.0001 g DPPH in 50 mL methanol as a solvent). The solution was vigorously shaked for 10 s and subsequently preserved in darkness for 15 min at room temperature. Afterword the absorbance was recorded at 517 nm against methanol in a UV–vis Spectrophotometer UNICO model (UV 2000, U.S.A).

The initial absorbance of the DPPH solution was 0.966. BHT solution was considered the reference in concentrations of 25, 50, 100, 150, and 200 ppm. The radical-scavenging activity was expressed as the percentage quenching of the DPPH radical, calculated as follows: -

$$\text{Inhibition of DPPH}(\%) = \frac{A_{blank}\text{-}A_{sample}}{A_{blank}} \times 100$$

Where 'A' is absorbance

The IC_{50} (50% of inhibition) was calculated from a graph plotting percentage inhibition against each volatile oil concentration.
Butylated hydroxy toluene (BHT) was used as a positive control

Statistical analysis

Data are presented as means and standard deviations (SD) where appropriate. They were compared by one way analysis of variance (ANOVA). Where significant differences were observed at 5 %. Statistical software (Assistat Version 7.7, Brazil) was used for all statistical analyses according to Silva and Azevedo (2009).

Results and Discussion

Preliminary study

Various microwave irradiation powers, 90, 180, 270, 360, 450, 540 and 630 watts which comprise 10, 20, 30, 40, 50, 60 and 70% from maximum output power were examined for different times (1, 3 and 5 minutes) as a pretreatment Results indicated that the chosen microwave irradiation power levels were 90, 180and 270 for rosemary leaves for 1, 3 and 5 min., while 360 watt was applied only for 1and 3 min. Meanwhile, power levels applied for fennel fruits were 90 and 180 watt for 1, 3 and 5 min., while 270watt was applied only for 1 and 3min. However, the power level 270 and 360 watt for 5 min. led to burn fennel fruits and rosemary leaves, respectively.

Volatile oil yield

The aromatic oil yield was calculated as (ml/100gm) and the data are given in Table (1) which reflects the effect of microwave power on volatile oil content. It could be noticed that pretreatment with microwave irradiation favorably affected the yield of aromatic oils for both rosemary leaves and fennel fruits. Regarding rosemary fruits, our findings indicated that oil yield increased as a microwave power increased until 180 watts(20% power) for 1 min. where it recorded (1.31%), with 14.91% significant increase compared with 1.14% volatile oil content for control one (Hydrodistillation only), however, no significant differences ($p \leq 0.05$) were observed at other microwave irradiation power used compared to control sample.

Concerning fennel fruits volatile oil, results indicated that the highest yield content was obtained by applying 90 watts (10% power) microwave power for 5 min. with significant difference ($p \leq 0.05$), with an increment percent of 17.14%. The data also ascertained that significant increments in oil yield were observed with the increment in microwave irradiation power and time. As a general, the contents of fennel fruits volatile oil are higher in comparison with the yields obtained by Benmoussa, *et al.* (2016) who found that it constitutes 0.5%. while Miraldi (1999)found that the fennel fruits contained 1to 4% volatile oil, These variations could be due to the phonological state, geographical origin and harvest season as mentioned by Marotti, (1994) and Telci, *et al.* (2009). Concerning the oil yield of rosemary leaves, our findings are higher than those found by Hussain, *et al.* (2010), who purported that it constituted 0.93% (w/w). The difference could be due to the variation in both origin and ecological conditions as stated by Hussain, *et al.* (2010).

Sui, *et al.* (2012) interpreted the action of microwave irradiation in improve content and quality of rosemary leaves volatile oil. They ascertained that the more efficient heating by microwave irradiation the more volatile oil% which can disturb hydrogen bonds, because microwave induced dipole rotation of molecules and migration of dissolved ions. Besides, the ability of microwave irradiation energy to enhance movement of organic solvent in volatile oil and subsequently deliver them to other materials through molecular interaction with electromagnetic field including introduce a rapid transfer of energy to the volatile compound that allow volatilization of components to be extracted.

Table 1: Effect of pretreatment with microwave irradiation on the essential oil yield of rosemary leaves and fennel fruits

Treatment	Time (min.)	Fennel fruits			Rosemary leaves		
		Oil yield % (v/w)	SD	% of I or D	Oil yield % (v/w)	SD	% of I or D
Control		1.40 [d]	±0.01	0.00	1.14[bc]	±0.02	0.00
Microwave Power (90 W)	1	1.43 [d]	±0.02	2.14	1.16[bc]	±0.02	1.75
	3	1.49 [c]	±0.02	6.43	1.14[bc]	±0.02	0.00
	5	1.64 [a]	±0.04	17.14	1.14[bc]	±0.02	0.00
Microwave Power (180 W)	1	1.53 [bc]	±0.02	9.29	1.31[a]	±0.04	14.91
	3	1.53 [bc]	±0.03	9.29	1.21[b]	±0.05	6.14
	5	1.53 [bc]	±0.03	9.29	1.16[bc]	±0.02	1.75
Microwave Power (270 W)	1	1.53 [bc]	±0.04	9.29	1.20[bc]	±0.7	5.26
	3	1.58 [b]	±0.03	12.86	1.14[bc]	±0.02	0.00
	5	--	--	--	1.12[cd]	±0.05	-1.75
Microwave Power (360 W)	1	--	--	--	1.11[d]	±0.04	-2.63
	3	--	--	--	1.02[e]	±0.08	-10.53
	5	--	--	--	--	--	--

Data are means ±SD (n=3)
Oil yield % with different superscript letters in the same column is significantly different at 5% level
(--) not determined
% of I or D: percentage of increase or decrease

Otherwise, Bousbia *et al.* (2009) purported that the different extraction methods (microwave hydrodiffusion and gravity, MHG and hydrodistillation, HD) produce distinguishable physical changes in plant material. The untreated rosemary leaf shows glandular trichomes which have volatile oil even after extraction time. However, after utilizing MHG, for 15 min, cells and cell walls have been affected and it can be observed damages of the oil-containing glands. Glandular trichomes seemed to be destroyed. In contrast, plant material subjected to hydrodistillation (for 3 h) appeared almost the same as untreated material. The changes observed for MHG extraction were appreciably different from those observed by HD, showing clearly that the cells are damaged and broken during microwave treatment.

Regarding the action of microwave irradiation on fennel fruits volatile oil, Benmoussa, *et al.* (2016) investigated the act of microwave irradiation technique on microstructural changes on fennel fruits by using electron microscope, compared with hydrodistillation method. Results ascertained that after hydrodistillation extraction (for 3 h), the fruits became atrophic and appeared wrinkled with only a few ruptures, whereas, after extraction by using solvent-free microwave (for 37 min) and enhanced solvent-free microwave isolation (30 min only), enormous perforation on the fruit outer surface was clearly detected, which is totally disrupted and empty from volatile oil.

The heat transfer in the HD method, was executed by both conduction and convection only, in contrast, in solvent-free microwave isolation techniques, a rapid decompression of the *in situ* water inside the glands could have surpassed their capacity for expansion and caused the damage of cell wall more rapidly compared with HD method.

Our obtained results showed that although there are common increasing trend of yield of rosemary and fennel volatile oils with increasing microwave irradiation power and time, the relationship was not linear.

To complete our investigation, the microwave irradiation power of 180 W for 1 min. for 30 gm of rosemary leaves and 90W for 5 min. for 50gm of fennel fruits were chosen as optimum powers for later experiments, because these microwave irradiation powers gave the highest volatile oil yield, for each plant material.

Chemical constituents of volatile oils

Chemical components of both tested volatile oils were fractionated and identified by GC/MS, and the obtained results are presented in Tables (2 and 3). Twenty-four components were identified for rosemary leaves aromatic oil extracted by HD technique representing 100% of oil components. Oxygenated and hydrocarbon compounds representing 69.95% and 30.05%, respectively. The main compound was 1,8 cineol with 35.44%, followed by α-pinene (12.56%), then camphor (12.52%), borneol (8.02%), camphene (7.42%) and α-terpineol (7.08%). These findings are compatible with those reported by Sienkiewicz *et al.*, (2013) who found that the main constituent in rosemary volatile oil was 1,8 cineol (46.4%), followed by camphor (11.4%), α-pinene (11.0%), β-pinene (9.2%) and camphene (5.2%). However, Bajalan, *et al.* (2017) found that the chief compounds of rosemary aromatic oil were α-pinene (14.69-20.81%), camphene (3.63-6.67%), 1,8-cineol (5.63-26.89%), camphor (1.66-24.82%). These differences could be due to the variation in origin and ecological conditions. In addition, our findings are also compatible with those found by Hussain, *et al.* (2010), who also ascertained that 1,8-cineol is a major component in rosemary foliage volatile oil (38.5%), followed by camphor (17.1%) and α-pinene (12.3%). Similar results were found with Miladi, *et al.* (2013).

Also, the same Table (2) displayed that the pretreatment with microwave at 180 watt for one min. affected the chemical components of rosemary volatile oil, where 23 compounds were isolated and identified representing 100%of oil components. Oxygenated and hydrocarbon compounds representing 68.46% and 31.54%, respectively.

Table 2: Effect of microwave irradiation pretreatment on chemical constituents of rosemary essential oil

Peak no.	Compounds	RT	RRT	Area %	
				Control	M.P.
1	Tricyclene	7.60	0.66	0.36	0.39
2	α-Pinene	8.01	0.70	12.56	12.78
3	Camphene	8.47	0.74	7.42	7.52
4	β-Pinene	9.40	0.82	1.53	1.62
5	1-Octen-3-ol	9.71	0.85	0.33	-
6	β-Myrcene	10.00	0.87	1.32	1.50
7	Limonene	10.40	0.91	0.25	-
8	α-Terpinolene	10.85	0.94	0.69	0.81
9	*p*-Cymene	11.20	0.97	5.20	5.71
10	1,8 Cineol	11.49	1.00*	35.44	33.87
11	Isopinocarveol	15.24	1.33	0.36	0.34
12	Camphor	15.42	1.34	12.52	11.14
13	trans-Pinocamphone	15.97	1.39	0.50	0.47
14	Pinocarvone	16.05	1.40	0.74	0.67
15	Borneol	16.20	1.41	8.02	7.64
16	Terpinen-4-ol	16.60	1.44	2.15	1.96
17	α-Terpineol	17.12	1.49	7.08	6.47
18	Estragole	17.36	1.51	-	2.58
19	Verbenone	17.74	1.54	0.75	0.57
20	Bornyl acetate	20.36	1.77	0.46	0.50
21	Methyleugenol	24.43	2.13	0.33	0.37
22	Caryophyllene	24.68	2.15	0.72	1.21
23	Caryophyllene oxide	29.73	2.59	0.29	0.39
24	Epicubenol	30.87	2.69	0.36	0.54
25	Cadinol	31.87	2.77	0.62	0.95
	Total identified (%)			100.00	100.00
	Total non-oxygenated compounds (%)			30.05	31.54
	Total oxygenated compounds (%)			69.95	68.46

RT: Retention time (min.)
RRT*: relative retention time: The retention times of 1,8 cineol(11.49min.) was taken as standard retention time equal one.
Control: sample extracted by hydrodistillation only without microwave pretreatment
MP: sample treated with 180 watts Microwave irradiation pretreatment for 1 min.

The microwave primary treatments of rosemary foliage increased amounts of some hydrocarbons such as tricyclene, α-pinene, camphene, β-pinene, β-myrcene, β-terpinolene, p-cymene, caryophyllene, and other oxygenated compounds like bornyl acetate, methyl eugenol, caryophyllene oxide, epicubenol and cadinol and decreased amounts of some oxygenated compounds like eucalyptol

(1,8 cineol), isopinocarveol, camphor, trans-pinocamphone, pinocarvone, endo-borneol, terpinen-4-ol, α-terpineol and verbenone in contrast with those obtained by HD without microwave pretreatment (control sample).

Regarding the main component (1,8 cineol), microwave irradiation led to a decrement from (35.44%) to (33.87) by 4.43%, also camphor decreased by 11.02%, while α-pinene increased by 1.72%, compared with control sample. In general, the results indicated that microwave irradiation of rosemary oil led to disappear of1-Octen-3-ol and limonene, simultaneously estragole, which was not present in control oil, appeared in microwave treated oil.

These findings are in the same line with those reported by Sui, *et al.* (2012) who found that the amounts of limonene and 1,8 cineol decreased and the amount of myrcene increased in rosemary volatile oil obtained from microwave pretreated leaves compared with that gained by hydrodistillation. Also, the results of Bousbia, *et al.* (2009), who found that the amounts of camphene, p-cymene, caryophellene oxide, bornyl acetate and methyl eugenol increased in rosemary volatile oil attained by MHG technique compared with hydrodistillation technique only. It is noteworthy that the different in amounts of some components between conventional hydrodistillation and pretreatment with microwave irradiation may be referred to three reasons, short extraction time of microwave pretreatment. Where, some components were not completely collected, Microwave radiation can cause a rather high temperature in the plant body for very short periods of time, resulting in decomposition of some compounds, hydrodistillation extraction may cause impairment of some components due to thermal and hydrolytic reactions (i.e., hydrolysis, trans-esterification or oxidation).
Regarding the chemical components of fennel volatile oil, Table (3) showed that 17 compounds were isolated and identified for fennel volatile oil distilled by water distillation technique representing 100%. Oxygenated and hydrocarbon compounds representing 80.23% and 19.77% of oil components, respectively. The main compound was estragole, which known as methyl chavicol, (71.75%), followed by d-limonene (14.17%) then fenchone (6.23%). These findings are in the same line with those reported by Miraldi (1999),who studied the composition of 10 oil samples of *Foeniculum vulgare* Miller fruits of varied origin, and found that the chief component was estragole ranged between 1.4-93.9% according to cultivar then limonene (trace-22.4%) and fenchone (0.7-10.4%).
Also, Shahat, *et al.,* (2011) showed the same range concerning chemical components of fennel fruits volatile oils var. *vulgare*. They indicated that this variety is completely different than other varieties like *dulce* and *azoricum* which exhibited great variations in the chemical composition.

Also, the same Table (3) indicated that the pretreatment with microwave at 90 watt for 5 min. affected the chemical components of fennel volatile oil, where 16 components were identified representing 100% of whole oil components. Oxygenated and hydrocarbon compounds representing 81.01%and 18.99%, respectively. Where, α-terpineol was disappeared as a result of microwave irradiation pretreatment.

The microwave primary treatments of fennel fruits increased amounts of camphene, sabinene, β-pinene, p-cymene, fenchone, borneol and estragole and decreased amounts of α-pinene, β-myrcene, α-phellandrene, limonene, eucalyptol, cis-ocimene, γ-trpinene, camphor, terpinene-4-ol and α-terpineol compared with those gained by water distillation without microwave pretreatment. These results are in accordance with those reported by Benmoussa *et al.* (2016), who found that the amount of α-pinene, eucalyptol and camphor decreased in fennel volatile oil as a result of solvent free microwave separation technique and enhanced solvent free microwave separation technique than those obtained by HD technique. They also concluded that using microwave irradiation produced the better volatile oil, concentrated in oxygenated monoterpenes from aromatic plant compared to using the water distillation method only.

Table 3: Effect of microwave irradiation pretreatment on chemical constituents of fennel essential oil

Peak no.	Compounds	RT	RRT	Area %	
				Control	M.P.
1	α-Pinene	7.98	0.45	2.21	2.07
2	Camphene	8.45	0.48	0.08	0.09
3	Sabinene	9.32	0.53	0.57	0.58
4	β-Pinene	9.39	0.53	0.16	0.18
5	β-Myrcene	9.98	0.57	0.54	0.54
6	α-Phellandrene	10.39	0.59	0.27	0.26
7	β-Cymene	11.11	0.63	0.49	0.54
8	D-Limonene	11.27	0.64	14.17	13.54
9	Eucalyptol	11.34	0.64	1.39	1.21
10	cis-Ocimene	11.65	0.66	0.72	0.65
11	γ-Terpinene	12.34	0.70	0.56	0.55
12	Fenchone	13.37	0.76	6.23	6.51
13	Camphor	15.37	0.87	0.50	0.36
14	Borneol	16.18	0.92	0.11	0.16
15	Terpinen-4-ol	16.62	0.94	0.13	0.12
16	α-Terpineol	17.14	0.97	0.12	-
17	Estragole	17.60	1.00*	71.75	72.65
	Total identified (%)			100	100
	Total non-oxygenated compounds (%)			19.77	18.99
	Total oxygenated compounds (%)			80.23	81.01

RT: Retention time (min.)
RRT: relative retention time. The retention time of Estragole (17.60 min.) was taken as standard retention time equal one.
Control: sample extracted by hydrodistillation only without microwave pretreatment
MP: sample treated with 90 watts Microwave irradiation pretreatment for 5 min.

Antioxidant activity

The antioxidant capacity of rosemary and fennel volatile oils were assessed by DPPH *in vitro* assay, and the values for 50% scavenging (IC_{50}) were calculated and the data are presented in Table (4). It could be noticed that rosemary leaves volatile oils significantly showed the strongest antioxidant activity with IC_{50}= 59.83 µg/ml, significantly followed by the synthetic antioxidant agent (BHT) which recorded (129.45 µg/ml), meanwhile, the fennel fruits volatile oil significantly ($p \leq 0.05$) had the lowest activity with IC_{50}= 235.64 µg/ml. Our findings are higher than those found by Miladi, *et al.* (2013), who purported that IC_{50} for rosemary oil was 189 µg/ml, while, Hussain, *et al.* (2010) showed that it exhibited 20.9 µg/ml, while, Moghadam, (2015) reported that it was 13 µg/ml, these variations could be referred to variations in cultivar, environmental conditions and techniques.

Table 4: Effect of microwave irradiation pretreatment on antioxidant capacity IC_{50} of rosemary and fennel essential oils

Essential oil samples	IC_{50} (µg/ml)	SD
Rosemary leaves	59.83^{c}	± 1.21
Microwave pre-treated rosemary leaves*	58.60^{c}	± 1.54
Fennel fruits	235.64^{a}	± 0.25
Microwave pre-treated fennel fruits**	233.43^{a}	± 1.46
BHT	129.45^{b}	± 1.97

SD: standard deviation
Data are means ±SD (n=3)
Different superscript letters are significantly different at 5% level
* Sample was treated with 180 watts of microwave irradiation for 1 min.
** Sample was treated with 90 watts of microwave irradiation for 5 min.

Otherwise, our results showed higher antiradical scavenging activity for fennel volatile oil, in contrast with those found by El-Ouariachi, *et al.* (2014), who stated that fennel volatile oil exhibited lower antioxidant capacity (IC_{50}= 900 µg/ml), than ascorbic acid (0.97 µg/ml). However, it is lower than the findings of Viuda-Martos, *et al.* (2011),who reported that it recorded 179.56 µg/ml, also, the findings of Shahat, *et al.*(2011) was higher than the previous results (IC_{50}= 15.33 µg/ml).

The antioxidant capacity of rosemary volatile oil might be ascribed to the synergistic effect of some inferior components present in the rosemary volatile oil, where Hussain, *et al.* (2010) proved practically that 1,8 cineol (the main component in such oil) had poor antioxidant activity compared with rosemary volatile oil and BHT. Meanwhile, Ruberto and Barratta (2000) pointed out that the phenolic compounds of monoterpenes and / or sesquiterpenes had high antioxidant activity, so, this can explain the lower antioxidant capacity of fennel volatile oil compared with rosemary volatile oil (the contents of camphor were 0.5 vs. 12.52%, respectively.) Also, Viuda-Martos, *et al.* (2011) showed that these compounds prevent lipid peroxidation by acting as chain-breaking peroxyl-radical scavengers, beside, phenols directly scavenge reactive oxygen species (hydroxyl radical, peroxy nitrite and hypochlorous acid).

Regarding the action of microwave irradiation pretreatment on the antioxidant potency of such volatile oils, statistical analysis showed no significant differences ($p \leq 0.05$) between each aromatic oil isolated by HD only and that exposed to microwave irradiation pretreatment as indicated in table (4).

Thus, it could be concluded that utilizing microwave irradiation pretreatment did not harmful the powerful of such volatile oils. These results are in accordance with those reported by Farag and El-Khawas (1998) who reported that microwave treatments did not affect the antioxidant activity of essential oils.

Conclusion

Our study purported out that microwave irradiation is considered a good pretreatment method that can maintain high amount of rosemary and fennel volatile oils at mild powers for each. Simultaneously, increasing some important constituents and keeping the antioxidant potency of volatile oils.

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