



Effects of ultrasonic and microwave pretreatments on the extraction yield, chemical composition and antioxidant activity of hydrodistilled essential oil from anise (*Pimpinella anisum L*)

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Abstract

Aromatic plants are rich in biologically active compounds that act as antioxidant, antiviral and antitumor agents. There were different methods for the extraction of these bioactive compounds. Hydrodistillation (HD) is the most common method to get the essential oils. Ultrasonic and microwave pretreatments improve yields and quality of the bioactive compounds. The aim of the current study was to evaluate the effect of ultrasonic and microwave pretreatments followed by hydrodistillation (US-HD and MW-HD, respectively) on the quality of anise essential oil (EO) compared to conventional hydrodistillation (HD). Power and time of US-HD and MW-HD were optimized. Results revealed that MW-HD and US-HD, at optimum conditions, enhanced the extracted yield of EO compared with HD method, however the highest yield (3.0% w/w) was obtained at 60% of the maximum ultrasonic power for 30 min. The gas chromatography-mass spectrometry (GC-MS) analysis showed that at optimum conditions both extraction techniques improved the content of the phenylpropanoids compounds. There was direct correlation between the free radical scavenging activity of each extracted EO and its content of *trans*-anethole (the principle compound of anise EO). The principal component analysis (PCA) showed significant variations between the EO extracted by the different methods (MW-HD and US-HD) compare to HD. Finally, this study revealed that the best conditions to obtain better chemical composition and antioxidant activity of Egyptian anise EO was applying US-HD extraction at 60% of the maximum ultrasonic power for 30 min.

Keywords: *Pimpinella anisum L*, essential oil, antioxidant activity, Principal component analysis, GC-MS analysis

I. Introduction

Aromatic plants were recommended as natural sources of biologically active compounds that having antioxidant, antiviral and antitumor properties. Anise was known as annual herb belonged to the Apiaceae family, botanically named *Pimpinella anisum*. It was one of the oldest cultivated spices. Since 1500BC, ancient Egyptian had used anise for its pharmaceutical properties such as diuretic, digestive and analgesic against toothache.

The essential oil (EO) of anise seeds was used in food flavouring, perfumes, toothpaste as well as for the symptomatic of gastrointestinal disturbances and catarrh of upper respiratory tract [1]. It was approved

as a traditional herbal medicinal product with therapeutic indications [2].

Recently, there were increasing interest in plant derived antioxidant compounds that were considered as natural and safe [3]. Several herbs and spices that were used as flavouring agents in food products were considered as excellent source of antioxidant compounds [4, 5]. The antioxidant activity of anise EO has been established [6]. The authors suggested that anise EO can be considered as promising alternative to synthetic antioxidant that linked to carcinogenesis and hepatic damage [7].

trans-Anethole was the major active principal compound in the anise EO [8], it might be responsible for its antioxidant activity [9]. *trans*-

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Anethole can mask unpleasant odour. It was used as commodities such as toilet soap and toothpaste [10].

Hydrodistillation (HD), steam and organic solvent distillation are the commonly extraction techniques used for extraction of EOs [11]. Among them hydro-distillation is the more common.

Earlier studies showed that extraction of bioactive compounds by different techniques, greatly affected on their yields and quality [12, 13]. The challenge for extraction of EOs from aromatic plants was to use methodology to increase the total yield of the EOs and keep their functional properties.

Recently, traditional extraction methods of bioactive compounds from aromatic plants were replaced by novel ones such as ultrasound and microwave assisted extraction [14, 15]

Microwave (MW) generates a non ionizing radiation that can penetrate into materials without changing the chemical structure of the target compounds [16]. Several studies proved the benefit of microwave irradiation in extracting the essential oils from aromatic plants [14]. The general conclusion of these studies was that microwave assisted extraction was preferred, offering better recoveries in shorten time compared to traditional extraction method.

The mechanical effect of ultrasound (US) was proposed to increase the penetration of the solvent into the extracted material and enhance the mass transfer process to and from inters face [17] and consequently increased the rate and yield of the extracted compounds [18].

The advantages of ultrasonic pretreatment on hydrodistilled EOs are to increase their yield, improve the quality and the antioxidant activity that making them very favorable to be used in cosmetics, medicines and food as natural preservatives [19]. Combined methodology of ultrasound pretreatment and hydrodistillation (US-HD) for extraction of *cinnamomum cassia* bark oil was evaluated [20]. The results revealed the efficiency of US-HD extraction technique without causing any changes in the EO composition. The same finding was reported by Morsy [21] who proved the advantage of ultrasound as pretreatment on improving the quality of the hydrodistilled essential oil.

The main objective of the present study was to evaluate the impact of microwave and ultrasonication pretreatments on the yield, chemical composition and antioxidant activity of hydrodistilled anise essential oil, in comparison to the conventional hydrodistillation extraction method.

2. Experimental

Plant material and chemicals

Seeds of anise (*Pinmpinella anisum*) were purcha-

-sed from local market in Egypt. 1,1-diphenyl-2-picrylhydrazyl (DPPH), the authentic volatile compounds and standard n-paraffin (C₈-C₂₂) were purchased from Sigma Aldrich chemical Co. (St. Louis, Mo, USA) and Merck (Darmstadt, Germany). All other chemicals of analytical grade and the solvent were purified and distilled before use.

Extraction and gas chromatography-mass spectrometry (GC-MS) analysis of anise seeds essential oil

Ultrasonic pretreatment

Anise seeds (50 g) were placed in a 1 L beaker. The ultrasound pretreatment was carried out using solid to deionized water ratio 1:12 (w/v). The mixture was subjected to ultrasonic radiation using ultrasound generator (Daigger, Model GEX 750, 20 HZ, USA). The ultrasound pretreatment was carried out at 40% (US1) and 60% (US2) of the maximum output ultrasonic power for 20, 40 and 60 min (US1) and 20, 25 and 30 min (US2) at the room temperature.

Microwave pretreatment

Anise seeds (50 g) were taken in a conical flask (500 mL) and deionized water was added in the ratio 1:3 (w/v). The anise seeds was treated with microwave (DAEWO, Mod: KOG-181G, Made in Korea) energy at different microwave irradiation power 200, 300 and 400 W (MW1, MW2 and MW3, respectively) for different time intervals 6, 8, 10, 12, 14, 16 and 18 min (MW1) ; 4,6,8 and 10 min (MW2) and 4,6,8 and 10 min (MW3).

Hydrodistillation

Fifty gram of untreated anise seeds and anise seeds pretreated as mentioned above were separately taken in a round bottom flask (2L), deionized water was added to a total volume 1000 mL and subjected to hydrodistillation for 2 h. The hydrodistilled EO obtained from anise seeds pretreatment by microwave (MW-HD), ultrasonication (US-HD) and that obtained by conventional hydrodistillation (untreated) were subjected to gas chromatography - Mass spectrometry (GC-MS) analysis according to Fadel et al. [22] by using gas chromatography (Hewlett-Packard model 5890) coupled to a mass spectrometer (Hewlett-Packard-MS 5970).

DPPH free radical-scavenging activity

The antioxidant activity of the EOs extracted by MW-HD, US-HD and HD was evaluated by using DPPH assay. DPPH radical-scavenging activity was determined according to Brand-Williams et al. [23], with a slight modification. Briefly, 100 µL of anise seeds EO, dissolved in 1 mL ethanol, were mixed with 2 mL of DPPH solution (0.1 mmol/L). The

mixture was kept in the dark at room temperature for 30 min and then the absorbance at 517 nm was measured using a spectrophotometer (Model UV-1601, SHIMADZU, Kyoto, Japan). The control sample was conducted in the same manner, but the EO was replaced by ethanol (95%). The DPPH free radical scavenging activity of each sample was calculated as follows:

$$\text{Radical scavenging activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A_{control} is the absorbance at 517 nm of DPPH without sample and A_{sample} is the absorbance at 517 nm of the reaction mixture containing DPPH and anise seeds EO.

Statistical analysis

All experiments were conducted in triplicate and expressed as mean \pm standard deviation (SD). Statistical difference within samples was compared using one-way analysis of variance (ANOVA). Principal component analysis (PCA) was performed to interpret data structure. PCA is used as a tool for screening, extracting and compressing data. PCA employs a mathematical procedure response variable into a new set of non correlated variable called principal components [24].

3. Results and discussions

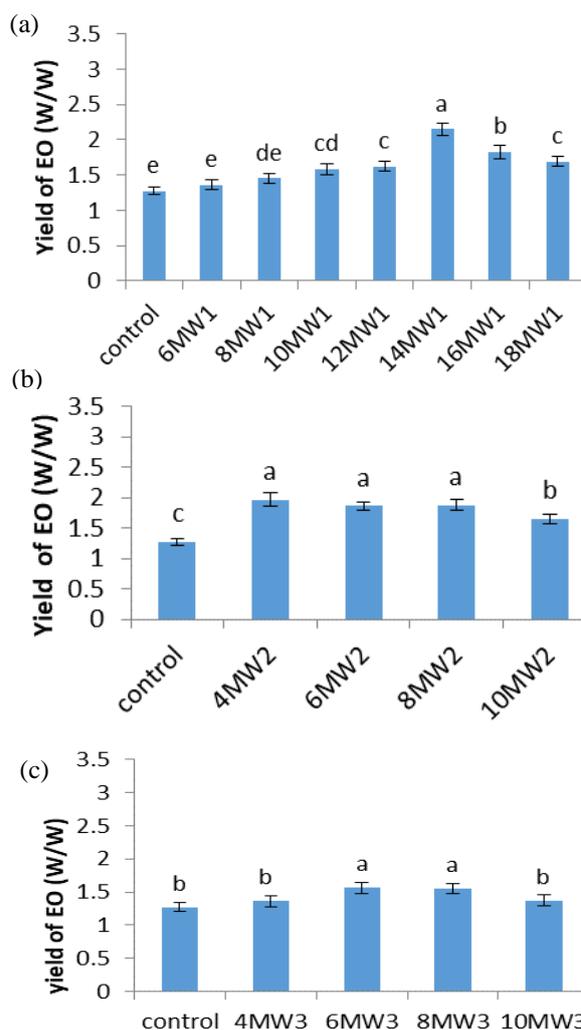
Several studies confirmed the ultrasonication and microwave irradiation pretreatments as promising techniques for extraction of high yield and quality hydrodistilled essential oils [14]. The main parameters affect on the efficiency of the microwave or ultrasonic pretreatments were power and time.

In present study the impact of each of these parameters on the yield and composition of the EO was evaluated.

Effect of time and power of microwave pretreatment on yield of essential oil

Figure 1 a, b, c showed the effect of varying the power and time of microwave pretreatment on the total yield of hydrodistilled EO. In general, it was obvious that the yield of the EO extracted from the treated samples showed higher values than the hydrodistilled (HD) oil (control). The direct effect of microwave energy on plant material was to lose the matrix of the cell walls, which leads to increased release of the components to the solvent medium and thus enhances the extraction yield [25]. As shown in Figure 1 a, the yield of the EO extracted by microwave treatment, at 200 W (W1), combined with hydrosdistillation (MW-HD) showed a gradual increase by increasing pretreatment time up to 14 min followed by a significant decrease at prolonged time. At higher microwave power (300 and 400 W, W2 and W3, respectively), the highest yield of EO was

obtained at shorter time (4 and 8 min) compared with that at low microwave power (W1). However, as shown in Figure 1 a, b, c, there was a negative relation between increasing the microwave power and the yield of EO. The too high microwave power and prolonged exposure time resulted in high intensity of heating to the medium and causing degradation and loss of volatile compounds [14, 26, 27 and 28]. Figure 1 a, b, c demonstrated that the prolonged pretreatment time resulted in a significant decrease in the yield of the extracted essential oil. The above results revealed that the optimum yield of anise EO was obtained by microwave pretreatment at 200 W for 14 min.

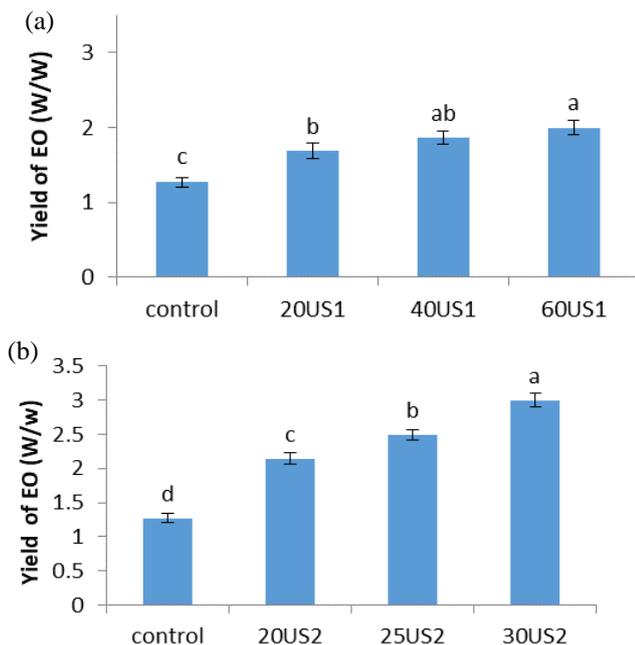


Values followed by different superscript letters are significantly different ($p < 0.05$).

Figure 1. Effect of time and power of microwave pretreatment on the yield of the hydrodistilled anise EO. (a): microwave power 200W (W1) at 6, 8, 10, 12, 14, 16 and 18 min. (b) : microwave power 300W (W2) at 4, 6, 8 and 10 min. (c) microwave power 400W (W3) at 4, 6, 8 and 10 min. control (HD): conventional hydrodistillation.

Effect of time and power of ultrasound pretreatment on yield of essential oil

The power and time of ultrasound pretreatment before hydrosistillation affect oil extraction [20, 29]. The current study demonstrated that the extracted oil from anise seeds by ultrasound pretreatment followed by hydrodistillation (US-HD) showed higher yield than that obtained by hydrodistillation HD only (control) at each power 40 and 60% of the maximum output ultrasonic power, (US1 and US2, respectively) at different pretreatment times (Figure 2 a, b). The result revealed that increasing pretreatment time enhanced the yield of the extracted essential oil and increasing the power from US1 to US2 significantly improved the yield of the extracted oil (Figure 2 a, b). These results were in agreement with Gholivand et al. [30] who reported that increasing the ultrasound power generated bubbles that lead to collapsed and disintegrated cell wall and improved the extraction efficiency. The effect of ultrasound pretreatment on essential oil yield at different ultrasound power was investigated [20] and the results showed that increasing the ultrasound power from 300 - 600 W significantly increased the EO yield. Other study enhanced the yield of cardamon EO using US-HD technique compared to conventional hydrodistillation



Values followed by different superscript letters are significantly different ($p < 0.05$).

Figure 2. Effect of time and power of ultrasonic pretreatment on the yield of the hydrodistilled anise EO. (a): ultrasonic power 40% of the maximum power (US1) at 20, 40 and 60 min. (b) : ultrasonic power 60% of the maximum power (US2) at 20, 25 and 30 min. control (HD): conventional hydrodistillation.

(HD) [21]. The current study showed in Fig 2 a, b that, the optimum yield (3.0%) of essential oil was obtained after ultrasound pretreatment for 30 min at 60 % of the maximal output of ultrasound power (30US2-HD), it showed 135 % more oil yield compared with the yield of the untreated sample (HD).

Effect of microwave and ultrasound pretreatment on the quality of hydrodistilled anise seed oil

The quality of the EO is mainly correlated to its chemical composition [11], and therefore the technique used to extract the EO should not adversely affect its chemical composition.

GC-MS analysis revealed the identification of 6 volatile compounds in the anise EO extracted by MW-HD, US-HD and HD, namely; methylchavicol (1), anisaldehyde (2), *trans*-anethole (3), γ -himachalene (4), β -bisabolene (5) and *trans*-pseudoisoeugenol-2-methylbutyrate (6) (Tables 1 and 2). These compounds were represented in all investigated samples but there were significant variations among them. The identified compounds are belonged to two chemical classes; phenylpropanoids, including compounds 1, 2, 3 and 6 and sesquiterpene hydrocarbons; compounds 4 and 5. Phenylpropanoids considered as the phytochemical markers for the genus *pimpinella* [31]. Whereas, sesquiterpenes have less contribution to the quality of anise EO [20]. *trans*-Anethole (3) was the major compound in all investigated samples followed by *trans*-pseudoisoeugenol-2-methylbutyrate (6) (Tables 1 and 2). These compounds were previously reported as principal compounds in anise EO [8, 22, 32 and 33].

Microwave pretreatment

The effect of microwave pretreatment (MW-HD), at different times and powers, on the composition of anise EO compared with hydrodistilled EO (HD) was studied. As shown in Table 1, the total content of the volatile compounds isolated by MW-HD extraction at 200 W was increased by increasing the pretreatment time up to 16 min. This increase is mainly attributed to the increase in the amount of the phenylpropanoids which showed more than 30% increase, compared with the hydrodistilled EO. Moradi et al. [27] reported that oxygenated compounds can be used as measurements of essential oil quality. Results of the present study revealed that *trans*-Anethole, the most abundant compound in anise EO [20, 22], was increased from 6.87×10^6 HD to 9.25×10^6 in the 16 MW1 oil sample. Earlier studies showed that microwave assisted hydrodistillation of coriander increased the content of carvon from 45 (by

Table 1. Chemical composition of anise seeds oil extracted by MW-HD and HD extraction

No.	KI ^a	Volatile compounds	Control (HD)	Peak area ^b x 10 ⁶														
				MW1-HD						MW2-HD				MW3-HD				
				6MW1	8MW1	10MW1	12MW1	14MW1	16MW1	18MW1	4MW2	6MW2	8MW2	10MW2	4MW3	6MW3	8MW3	10MW3
1	1204	Methylchavicol	0.05 ^a	0.05 ^a	0.09 ^b	0.07 ^b	0.06 ^{ab}	0.06 ^{ab}	0.07 ^b	0.01 ^c	0.05 ^a	0.06 ^{ab}	0.07 ^b	0.06 ^{ab}	0.07 ^b	0.07 ^b	0.06 ^{ab}	0.04 ^a
2	1260	Anisaldehyde	0.01 ^a	0.01 ^a	0.02 ^a	0.01 ^a	0.01 ^a	0.01 ^a	0.02 ^a	---	0.01 ^a	0.02 ^a	0.01 ^a	0.01 ^a	0.02 ^a	0.02 ^a	0.02 ^a	0.01 ^a
3	1300	<i>trans</i> -Anethole	6.87 ^a	5.01 ^b	6.46 ^a	8.78 ^c	8.68 ^c	8.42 ^c	9.25 ^{cd}	2.6 ^e	6.82 ^a	9.22 ^c	9.70 ^c	8.39 ^c	9.35 ^{cd}	10.07 ^d	7.78 ^{ac}	6.32 ^a
4	1494	γ -Himachalene	0.09 ^a	0.08 ^a	0.14 ^b	0.10 ^a	0.09 ^a	0.12 ^b	0.13 ^b	0.03 ^c	0.10 ^a	0.14 ^b	0.13 ^b	0.11 ^{ab}	0.14 ^b	0.15 ^b	0.12 ^b	0.10 ^a
5	1506	β -Bisabolene	0.02 ^a	0.01 ^b	0.02 ^a	0.02 ^a	0.01 ^b	0.03 ^{ac}	0.03 ^{ac}	---	0.02 ^a	0.02 ^a	0.03 ^c	0.02 ^a	0.03 ^c	0.02 ^a	0.02 ^a	0.02 ^a
6	1844	<i>trans</i> -Pseudoisoeugenol-2-methylbutyrate	0.26 ^a	0.17 ^b	0.15 ^b	0.20 ^{bc}	0.23 ^{ac}	0.27 ^a	0.28 ^a	0.07 ^d	0.24 ^{ac}	0.34 ^e	0.34 ^e	0.32 ^e	0.34 ^e	0.35 ^e	0.25 ^a	0.25 ^a
Total			7.30 ^a	5.33 ^b	6.88 ^a	9.18 ^c	9.08 ^c	8.91 ^{cd}	9.78 ^e	2.71 ^f	7.24 ^a	9.80 ^e	10.28 ^{eg}	8.91 ^{cd}	9.95 ^e	10.68 ^g	8.25 ^d	6.74 ^a
Phenylpropanoids			7.19 ^a	5.24 ^b	6.72 ^a	9.06 ^c	8.98 ^c	8.76 ^{cd}	9.62 ^e	2.68 ^f	7.12 ^a	9.64 ^e	10.12 ^{eg}	8.78 ^{cd}	9.78 ^e	10.51 ^g	8.11 ^d	6.62 ^a
Sesquiterpene hydrocarbons			0.11 ^a	0.09 ^b	0.16 ^c	0.12 ^{ad}	0.10 ^{ab}	0.15 ^{ce}	0.16 ^c	0.03 ^f	0.12 ^a	0.16 ^c	0.16 ^c	0.13 ^d	0.17 ^c	0.17 ^c	0.14 ^{cd}	0.12 ^{ad}

^aRetention indices of volatile compounds on DB-5 columns.

^bAverage of three triplicates of total ion chromatograms (TIC) of GC-MS. Mean values in the same row of each volatile compound followed by different superscript lower case letters are significantly different at $P < 0.05$.

MW1-HD: microwave pretreatment prior to hydrodistillation at 200 W for different times 6, 8, 10, 12, 14, 16 and 18 min; MW2-HD: microwave pretreatment prior to hydrodistillation at 300 W for 4, 6, 8 and 10 min; MW3-HD: microwave pretreatment prior to hydrodistillation at 400 W for 4, 6, 8 and 10 min; control (HD): conventional hydrodistillation.

Table 2. Chemical composition of anise seeds oil extracted by US-HD and HD extraction

No.	KI ^a	Volatile compounds	Peak area ^b x 10 ⁶						
			Control (HD)	US1-HD			US2-HD		
				20US1	40US1	60US1	20US2	25US2	30US2
1	1204	Methylchavicol	0.05 ^a	0.06 ^{ab}	0.07 ^b	0.06 ^b	0.07 ^b	0.07 ^b	0.06 ^{ab}
2	1260	Anisaldehyde	0.01 ^a	0.02 ^a	0.02 ^a	---	---	---	---
3	1300	<i>trans</i> -Anethole	6.87 ^a	10.70 ^b	10.29 ^{bc}	9.31 ^{cd}	10.66 ^{bd}	10.89 ^b	9.63 ^{bd}
4	1494	γ -Himachalene	0.09 ^a	0.11 ^{ab}	0.09 ^a	0.10 ^a	0.13 ^c	0.12 ^{bc}	0.12 ^{bc}
5	1506	β -Bisabolene	0.02 ^a	0.02 ^a	0.02 ^a	0.02 ^a	0.03 ^a	0.03 ^a	0.03 ^a
6	1844	<i>trans</i> -Pseudoisoeugenol-2-methylbutyrate	0.26 ^a	0.17 ^b	0.22 ^c	0.24 ^{ac}	0.30 ^d	0.26 ^a	0.30 ^d
		Total	7.30 ^a	11.08 ^b	10.71 ^{bc}	9.72 ^d	11.19 ^b	11.37 ^b	10.14 ^{cd}
		Phenylpropanoids	7.19 ^a	10.96 ^b	10.59 ^{bc}	9.61 ^d	11.03 ^b	11.22 ^b	9.99 ^{cd}
		Sesquiterpene hydrocarbons	0.11 ^a	0.13 ^b	0.11 ^a	0.12 ^{ab}	0.11 ^a	0.15 ^c	0.15 ^c

^aRetention indices of volatile compounds on DB-5 columns.

^bAverage of three triplicates of total ion chromatograms (TIC) of GC-MS. Mean values in the same row of each volatile compound followed by different superscript lower case letters are significantly different at $P < 0.05$. US1-HD: ultrasonic pretreatment prior to hydrodistillation at 40% of the maximum ultrasonic power for 20, 40 and 60 min.; US2-HD: ultrasonic pretreatment prior to hydrodistillation at 60% of the maximum ultrasonic power for 20, 25 and 30 min.; control (HD): conventional hydrodistillation.

conventional HD) to 69% of the total identified compounds [34]. The current results showed (Table 1) that the increase in treatment time to 18 min resulted in a significant decrease in the phenylpropanoid content. Previous results confirmed this finding, where prolonged exposure to MW irradiation resulted in degradation in the structure of the extracted compounds [28] and thereby decreased their contents. In the same time Kumar et al. [14] studied the effect of microwave pretreatment of cinnamon bark powder prior to hydrodistillation. They reported that the recovery of cinamaldehyde, the major compound, increased at the first 5 min of treatment and decreased by prolonged the pretreatment time.

The effect of pretreatment time on the composition of EO extracted by MW-HD at higher power, 300 and 400 W, showed similar trend. However, the increase in pretreatment power to 300 and 400 W resulted in a significant increase in the yield of the phenylpropanoids from 7.19×10^6 (HD) to 10.12×10^6 and 10.51×10^6 at 8 and 6 min, respectively and consequently the total content of the volatile compounds showed the same trend. The above results revealed that the optimum conditions for extraction of high quality anise seeds oil are microwave pretreatment at 400 W for 6 min.

Ultrasonic pretreatment

The effect of applying US-HD extraction (40% and 60% of the maximum output ultrasonic power) for different pretreatment time, on the essential oil composition was studied. The results revealed a significant increase in total volatile compounds compared with HD extraction (Table 2). This increase is mainly attributed to the increase in the phenylpropanoid content. As shown in Table 2, extending the pretreatment time at the 40% and 60% of the maximum ultrasonic power (US1 and US2, respectively) up to 60 and 30 min (60US1 and 30US2) was accompanied by a noticeable decrease in the total content of the phenylpropanoids. On contrary, samples 60US1 and 30US2 showed 9.0 and 36.0% increases in the total content of sesquiterpene hydrocarbons, compared with the control sample (hydrodistilled sample).

The above results are in agreement with the results reported by Jadhav et al. [20], who evaluated the effect of US-HD extraction on the composition of the essential oil compared with HD extraction. They observed higher yield of the oxygenated compounds in the essential oil extracted by US-HD (85.32%) compared with that in the HD distilled oil (81.67%). The increase in total content of the sesquiterpenes at longer time may correlate to the decomposition of the oxygenated compounds and transformation into sesquiterpene hydrocarbons [20].

Radical scavenging activity

Previous studies proved the antioxidant activity of anise essential oil [4]. It showed higher free radical scavenging ability than the synthetic antioxidant BHA and BHT [6]. *trans*-Anethole, the predominant volatile compound in anise seed oil (Table 1 and 2) had been reported to have free radical scavenging activity [9]. In present study the DPPH radical assay was used to evaluate the effect of the MW-HD, US-HD and HD extraction on the free radical scavenging ability of anise EO. In general, Figures 3 and 4 show that MW-HD and US-HD significantly improved the antioxidant activity of anise EO compared with HD

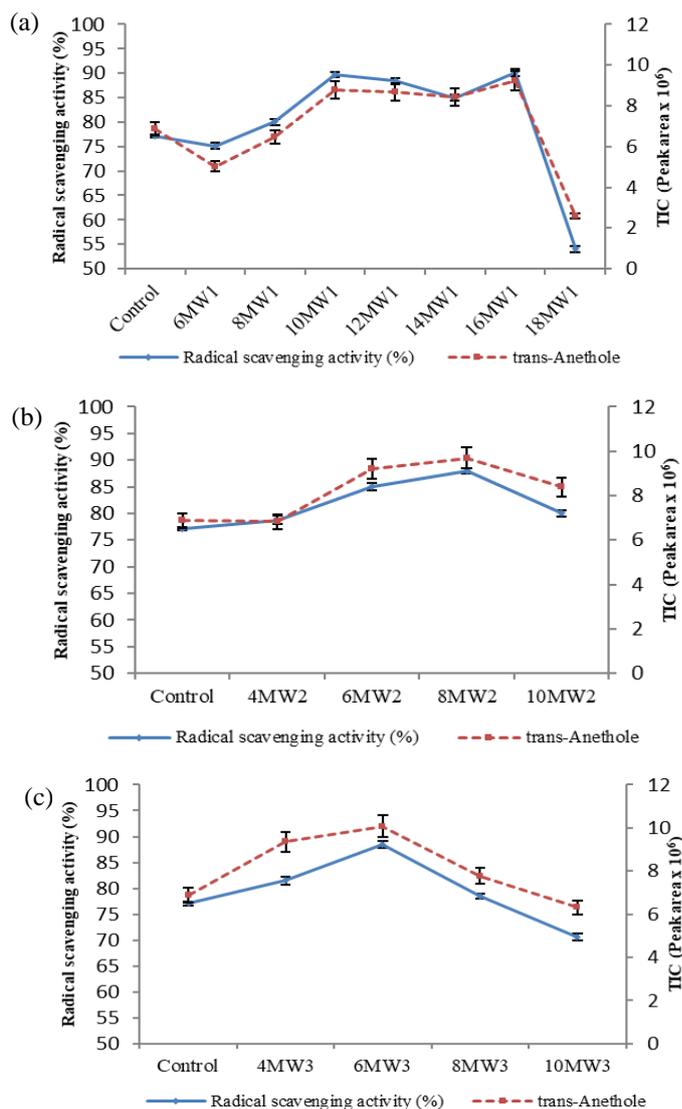


Figure 3. Effect of time and power of microwave pretreatment on the DPPH scavenging activity and *trans*-anethole content of the hydrodistilled anise EO. (a): microwave power 200W (W1) at 6, 8, 10, 12, 14, 16 and 18 min. (b) : microwave power 300W (W2) at 4, 6, 8 and 10 min. (c) microwave power 400W (W3) at 4, 6, 8 and 10 min. control (HD): conventional hydrodistillation

extraction. However, the prolonged pretreatment time resulted in a significant decrease ($P < 0.05$) in the free scavenging radical activity of anise EO. These results are in agreement with Damyeh et al. [19] who reported that ultrasonic pretreatment prior to hydrodistillation enhances the extraction of the compounds that are responsible for the antioxidant activity of essential oils. Figures 3 and 4 demonstrate the quite correlation between the free radical scavenging activity of the investigated anise EO samples and their content of *trans*-anethole. This finding confirms the hypothesis that *trans*-anethole is responsible for the antioxidant activity of anise EO [9].

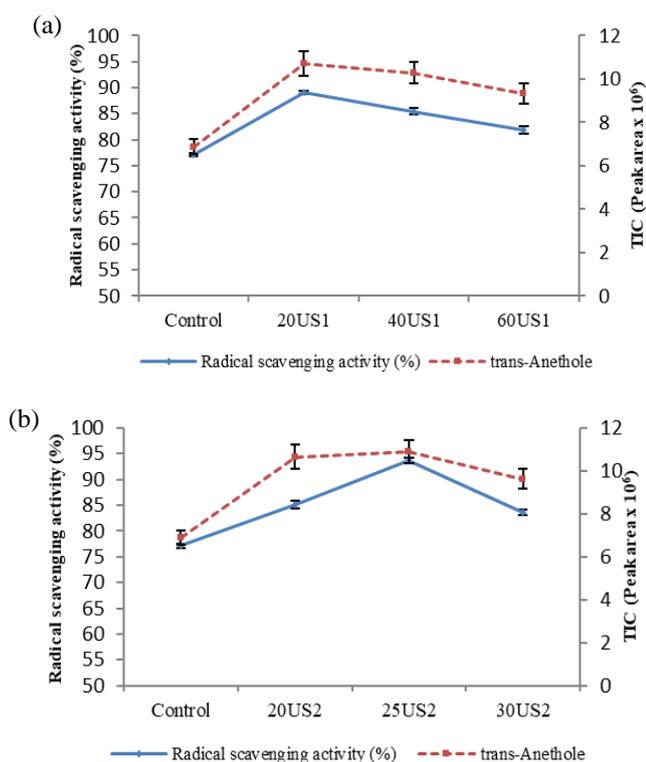


Figure 4. Effect of time and power of ultrasonic pretreatment on the DPPH scavenging activity and *trans*-anethole content of the hydrodistilled anise EO. (a): ultrasonic power 40% of the maximum power (US1) at 20, 40 and 60 min. (b) : ultrasonic power 60% of the maximum power (US2) at 20, 25 and 30 min. control (HD): conventional hydrodistillation

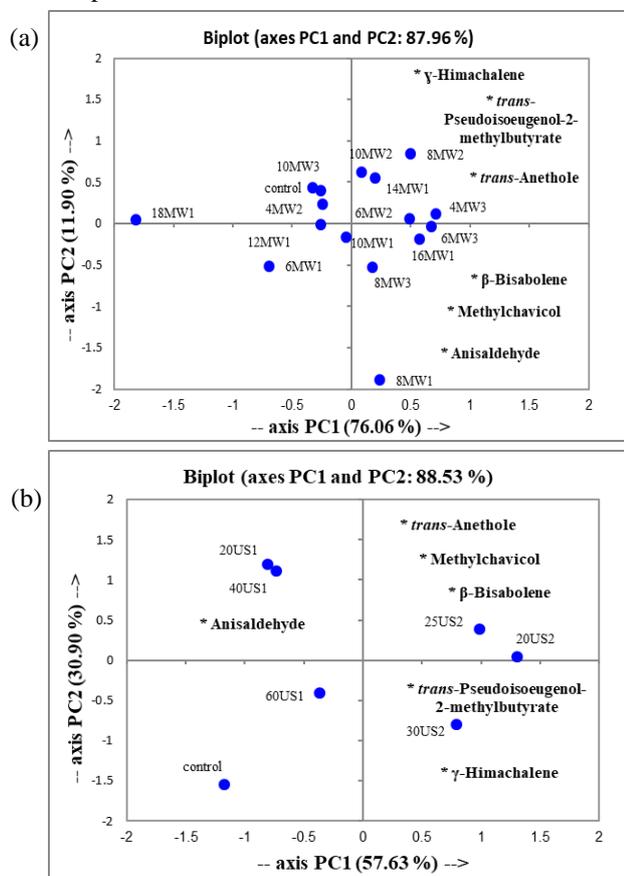
Principal component analysis (PCA)

To clarify the effect of MW-HD (Figure 5 a) and US-HD (Figure 5 b) extraction techniques on the composition of the extracted anise essential oil compared to the conventional hydrodistillation HD, the GC-MS data of the extracted essential oils were subjected to PCA. As shown in Figure 5 a, the two first principal components PC1 and PC2 accounted for 87.96% of the total variance of which PC1 explained 76.06% and PC2 explained 11.90%

variation. All identified compounds were located in the positive PC1 axis. As shown in Table 1 these compounds were present in all investigated samples. As shown in Figure 5 a, PC2 separated the volatile compounds into two groups. The major volatile compounds; *trans*-anethole, *trans*-pseudoisoeugenol-2-methylbutyrate and γ -himachalene, were located in the positive PC2 axis, whereas the compounds represented in low values (β -bisabolene, methyl chavicol and anise aldehyde) were located in negative PC2 axis. Samples 4MW3, 6MW3, 16MW1, 6MW2 and 8MW2 were located in the positive PC1 axis. These samples were characterized by high concentrations of the major identified compounds (Table 1); *trans*-anethole, *trans*-pseudoisoeugenol-2-methylbutyrate and γ -himachalene. Control, 10MW3 and 4MW2 samples were located in the negative PC1 and positive PC2 axis. The significant correlation between these samples is related to their similar composition of the volatile compounds, they have low load of the major identified compounds (Table 1); *trans*-anethole, *trans*-pseudoisoeugenol-2-methylbutyrate and γ -himachalene. Control, 10MW3 and 4MW2 samples were located in the negative PC1 and positive PC2 axis. The significant correlation between these samples is related to their similar composition of the volatile compounds, they have low load of the major identified compounds. Sample 8 MW1 showed high distance from the samples located in the positive PC1 axis, it was characterized by high content (0.09×10^6) of methyl chavicol. Sample 18 MW1 is present at extreme distance from the other samples, it was characterized by very low contents of all identified compounds (Table 1).

As shown in Figure 5 b, the two first principal components accounted for 88.53% of total variance of which PC1 explained 57.63% and PC2 explained 30.90% variations. Compounds; *trans*-anethole, methyl chavicol and β -bisabolene have positive loading on PC1, whereas anise aldehyde was located in negative PC1 axis. PC2 classified the volatile compounds into two groups, *trans*-anethole, methyl chavicol and β -bisabolene were located in the positive PC2 axis whereas *trans*-pseudoisoeugenol-2-methylbutyrate and γ -himachalene were located in the negative PC2 axis. A significant correlation was found between 25US2 and 20US2, these samples were characterized by a high content of *trans*-anethole, methyl chavicol and β -bisabolene (Table 2). Sample 30US2 was characterized by a high content of *trans*-pseudoisoeugenol-2-methylbutyrate and γ -himachalene. The significant correlation between 20US1 and 40US1, which are located in the negative PC1 and positive PC2, may be correlated to their

high content of anise aldehyde. Samples 60US1 and control were located in the negative PC1 and PC2 axis, each of them has a specific composition which is significantly different from the other investigated samples.



*Identified volatile compounds. • Extracted samples
Figure 5. Principal Component Analysis (PCA) based on the chemical composition of the essential oil extracted by MW-HD, US-HD and HD extraction. (a): microwave power 200W (W1) at 6, 8, 10, 12, 14, 16 and 18 min., microwave power 300W (W2) at 4, 6, 8 and 10 min., microwave power 400W (W3) at 4, 6, 8 and 10 min.; (b): ultrasonic power 40% of the maximum power (US1) at 20, 40 and 60 min. , ultrasonic power 60% of the maximum power (US2) at 20, 25 and 30 min. control (HD): conventional hydrodistillation.

Conclusion

The results of the present study revealed the efficiency of MW-HD and US-HD extraction techniques, under the optimal working parameters (time and power), in improving the yield, quality and antioxidant activity of anise essential oil compared to the conventional hydrodistillation (HD). However, US-HD extraction showed more promising results than MW-HD. The highest yield of the EO was obtained by US-HD extraction at 60% of the maximum ultrasonic power for 30 min. Whereas, the

optimum conditions for extracting EO having high quality and antioxidant activity were 60% of the maximum ultrasonic power for 25 min.

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