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Optimization and Comparison of Ultrasound-Assisted Extraction of Estragole from Tarragon Leaves with Hydro-Distillation Method

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A comparative study of ultrasound-assisted extraction (UAE) and hydro-distillation was performed for fast extraction of estragole from tarragon (*Artemisia dracunculus* L.) dried leaves. Several influential parameters of the UAE procedure in the extraction of estragole (type of solvent, extraction cycles, solvent to material ratio, irradiation time and particle size) were investigated and optimized. It was found that UAE offers a more rapid extraction of estragole than hydrodistillation. The optimum parameters were solvent to material ratio of 8:1 v/m, 96% (w/w) ethanol in water as extraction solvent, particle size of 1.18 mm, irradiation time of 5 min, output power of 63 W, 9 pulses, and ultrasonic frequency of 20 kHz. The recovery of estragole by UAE under optimal conditions was 44.4% based on dry extract. The benefit of ultrasound was to decrease the extraction time (5 min) relative to the classical hydrodistillation method (3 h). The experimental results also indicated that ultrasound-assisted extraction is a simple, rapid and effective method for extraction of the volatile oil components of tarragon.

Keywords: *Artemisia dracunculus* L., Tarragon, Estragole, Ultrasound-assisted extraction, Essential oil, Hydrodistillation

INTRODUCTION

The genus Artemisia with approximately 800 species is industrially important due to its antibacterial, antifungal and insecticidal activities, and as a flavoring and preservative agent in food products [1,2]. *Artemisia dracunculus* L. or tarragon is one of the well-known, valuable and native medicinal plants in Iran, traditionally used as a flavoring agent or for therapeutic effects such as treatment of epilepsy and diabetes in Iranian traditional medicine [3-6]. There are many reports of this herb about its anti-inflammatory, general tonic, wound healing, headache relieving, anti-ulcer and digestion improving properties [7,8]. Biological characteristics and useful features of tarragon were represented recently in a review [9]. Tarragon essential oil has antispasmodic, anti-inflammatory, neuromuscular and carminative effects and is employed as antifungal, antibacterial and antitumor agent [10-14]. In addition, it is advised in the cases of digestion difficulty, intestinal bloating, nausea, wind colic and flatulence [15-17]. Recently, tarragon essential oil has been utilized for reducing the oxidation rate of soybean oil under accelerated conditions at 60ºC (oven test) [18]. The dried aerial parts of *A. dracunculus* L. possess anticonvulsant and sedative functions owing to the existence of monoterpenes in their essential oil [19].

In most of the numerous research on the composition of essential oil of tarragon, estragole (methyl chavicol or 1-allyl-4-methoxybenzene) was the major component. Chemical structure of estragole is shown in Fig. 1. Estragole is exploited in manufacture of perfumes and flavors, and also found in other plants; for example, fennel, anise and basil and obtained from these plants by hydro-distillation process [8,20]. This procedure consumes a lot of time and energy.

Nowadays, ultrasound-assisted extraction (UAE) is considered as an efficient method for extracting natural compounds from herbs because of allowing the penetration

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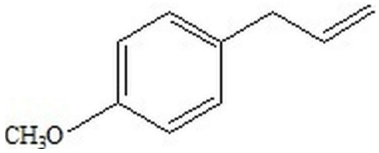

Fig. 1. Chemical structure of estragole (methyl chavicol, 1-allyl-4-methoxybenzene).

of solvents into cellular materials and increasing mass transfer that results in the enhancement of extraction. The application of ultrasound-assisted extraction is recommended for thermolabile compounds, like essential oils from aromatic plants such as citrus flowers [21], artemisia [22], lavender [23] or from waste solid residues of Salvia sp. [24]. UAE not only improved yields but as the method is fast and run at low temperature, the final product usually showed less thermal degradation than hydro-distillation method. Moreover, several studies have been run out on extraction of the main aroma compounds from spices. For example, the vanillin was extracted from vanilla pods [25] carvone from caraway seeds [26]. The use of ultrasound for the extraction of essential oil could diminish the danger of thermal degradation as demonstrated during the extraction of essential oils from fresh garlic (*Allium sativum*) cloves [27]. The choice of a suitable solvent in combination with ultrasound irradiation influences mass transport processes and subsequently efficiency of the extraction. The most widely used solvent to extract edible oils from plant sources is *n*-hexane, however, recently, the use of alternative solvents such as alcohols (isopropanol or ethanol) has increased due to environmental, health and safety concerns [28,29].

The aim of this study was to employ UAE to extract estragole from tarragon in a short time. Various parameters such as kind of extraction solvent, ratio of solvent/solid (herb), and ultrasound power and pulse number were explored and compared with conventional hydro-distillation technique.

EXPERIMENTAL

Chemicals and Plant Material

Fresh leaves of *Artemisia dracunculus* L. were collected in June 2011 in Shahriar (Tehran, Iran), and dried at room temperature (25-30 ºC) until constant weight. Subsequently, The dry tarragon leaves were grounded in laboratoy with a blade mixer then sieved with stainless steel seives to classify the particle size (0.15-1.18 mm) and kept in labled capped plastic inside refrigerator at 4 ºC until use. Ethanol, acetone, *n*-hexane, ethyl acetate and dichloromethane were purchased from Merck (Darmstadt, Germany).

Hydrodistillation

The ground herb material (40 g with particle size of 0.6 mm) was submitted to hydro-distillation process *via* applying a Clevenger-type apparatus, and distillated with 600 ml water for 3 h. The essential oil was gathered, dried under anhydrous sodium sulphate, and stored at 4 ºC until gas chromatographic (GC) and GC-mass spectrometric (GC-MS) analyses.

Ultrasound-Assisted Extraction

An ultrasonic probe, Sonopuls ultrasonic homogenizer model HD2070, with MS73 probe, 20 kHz working frequency, 70 W output amplitude, setting displayed in % on the scale of 10-100% from Bandelin (Berlin, Germany) was utilized for extraction experiments. The probe was placed 1 cm from the top surface of the extraction cell, and then operated at 80% output amplitude with 3 pulses.

Exactly 0.8 g of the weighed plant powder (particle size, 0.6 mm) was transferred into a 15 ml tube containing 8 ml of extraction solvent. After soaking the plant sample for one night in the solvent, it was sonicated with the probe at room temperature. The impacts of extraction conditions, including liquid/solid ratio, ultrasound time, ultrasound power, duty cycles, particle size of plant powder and type of solvent (*i.e.*, acetone, *n*-hexane, ethyl acetate, dichloromethane, and 50, 70 and 96% (v/v) of ethanol in water), on the extraction efficiency of estragole were evaluated.

Similarly, another investigation was carried out in the absence of ultrasound irradiation. All of the experiments were duplicates. The temperature at the end of the extraction was about 30 ºC. After extraction and filtration, 50 µl of internal standard (5 mg ml^{-1} of thymol in methanol) was added to 1 ml of the supernatant taken for GC analysis. For ethanolic extracts, following sonication and filtration, 1 ml of distilled water was moved into 1 ml of supernatant.

Then the solution was Vortexed for 1 min and extracted with 1 ml of *n*-hexane. After separating the upper layer, the extraction was repeated one more time. Eventually, all the organic layers were combined and to 1 ml of this solution, the same as other samples, 50 µl of the internal standard was added.

GC and GC-MS Analyses

The essential oil and ultrasound extracts were analyzed by GC-FID and GC-MS. All of the separations and analyses were accomplished through a Thermoquest 2000 GC chromatograph with a fused silica capillary column (DB1; 30 m × 0.25 mm, i.d.; 0.25 µm film thickness) followed by a FID. Nitrogen was exploited as a carrier gas with a flow rate of 1.3 ml min^{-1}. The column temperature was programmed from 50 ºC to 70 ºC with a ramp of 5 ºC min^{-1}, then increasing to 75 ºC (2 ºC min^{-1}), 100 ºC (10 ºC min^{-1}) and finally 260 ºC (30 ºC min^{-1}). A 1 µl of each extract was injected into GC split/splitless injection port under split conditions with a split ratio of 1:5. The injector and detector temperatures were 250 ºC and 300 ºC, respectively. For GC analysis of the essential oil, an aliquot of 1 mg of the essential oil was diluted with 1 ml of *n*-hexane and afterwards, 50 µl of internal standard was transferred into it. One µl of this solution was injected into GC. Thymol, which was not present in the extracts, was applied as the internal standard. Mean values expressed as relative peak areas (estragole area/internal standard area) were used for comparison of different extraction approaches.

GC-MS analysis was conducted on a Hewlett-Packard HP 6890N GC instrument (Palo Alta, California, USA) coupled with a HP MSD 5973N quadrupole mass spectrometer. The extracts and the essential oil were separated on a HP-5MS capillary column (30 m × 0.25 mm, i.d.; 0.25 µm film thickness). A 0.5 µl of the sample (distillate or extract) was injected with a split ratio of 1:20. The column temperature was programmed from an initial temperature of 50 ºC to 100 ºC at 2 ºC min^{-1}, subsequently to 200 ºC at 10 ºC min^{-1}, and finally from 200 ºC to 260 ºC at a rate of 20 ºC min^{-1}. The injector and ion source temperatures were 260 ºC and 250 ºC, respectively. Helium was employed as the carrier gas with a flow rate of 1.1 ml min^{-1}. The ionizing energy was 70 eV. All data were collected *via* achieving full-scan mass spectra within the scan range of 40-550 amu. GC chromatograms of estragole in the essential oil and the extracts are illustrated in Fig. 2.

RESULTS AND DISCUSSION

Essential Oil Acquired by Hydrodistillation

The identified compounds in tarragon essential oil, their retention indices and percentage compositions, and the concentrations of the most significant compounds are summarized in Table 1. The individual peaks were recognized through comparison of their retention indices relative to (C6-C25) *n*-alkanes with those of authentic samples and the literature [30] as well as comparing their mass spectra to the Wiley 7 mass spectral libraries (New York, NY, USA). Volatile compounds were characterized by GC-MS. As it is seen in Table 1, the main component is estragole (78.93%).

Optimization of Ultrasound-Assisted Extraction Conditions

Effect of solvent on extraction of estragole. The choice of extracting solvent has a substantial influence on extraction yield. As estragole is slightly polar, the selection of a set of solvents, for instance *n*-hexane, dichloromethane, ethyl acetate, acetone, and 50, 70 and 96% (v/v) ethanol in water was done according to their polarities. Other experimental parameters were set as follows: ratio of liquid to solid, 8:1 (ml g^{-1}); extraction time, 5 min at ambient temperature; ultrasonic power, 80% with 3 cycles; and particle size of plant powder, 0.6 mm. The frequency of ultrasound was fixed at 20 kHz. As observed in Fig. 3, the area ratio of estragole ranges from 0.12% to 0.84%. The results indicated that 96% ethanol was the best alternative for extraction of estragole from tarragon. Therefore, the next experiments were run using 96% ethanol.

Effect of solvent/solid ratio. Figure 4 depicts that the highest extraction yield is attained when the solvent/solid ratio is diminished from 20:1 to 8:1. The phenomenon of lower extraction yield at higher solvent/solid ratios was also observed by Vongsangnak, who found that a larger solvent volume does not lead to a higher saponin yield from the cultured cells of *Panax notoginseng* by microwave extraction [31].

The amount of extracted estragole was decreased at

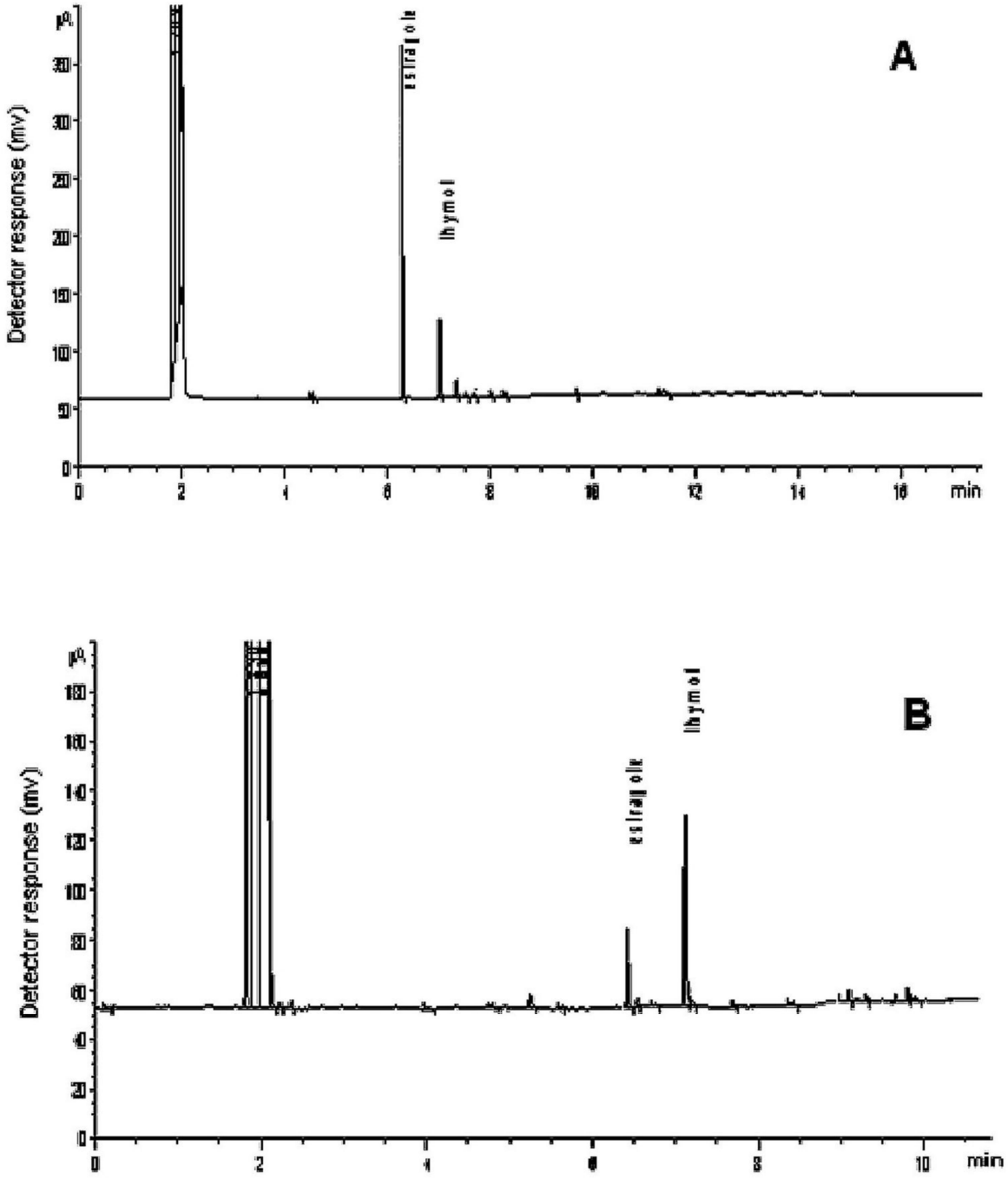

Fig. 2. GC analysis of aromatic components in tarragon essential oil (A), and in ultrasonic extracts (B).

lower ratios of solvent/solid than 8:1 which may be due to the fact that when the solvent is saturated with the extracted compounds, there is not enough concentration gradient thus, the material transfer from the matrix and subsequently, the extraction process would be decreased considerably. Hence, the ratio of 8:1 was selected for further research.

Table 1. Chemical Composition of Tarragon Essential Oil Obtained by Hydro-Distillation

NO.	Compound	Retention index[b]	Area (%)
1	α-pinene	938	2.50
2	Limonene	1032	4.10
3	β-Phellandrene	1032	3.70
4	(Z)-β-Ocimene	1037	0.10
5	(E)-β-ocimene	1050	0.33
6	Myrtenol	1197	0.14
7	Estragole	1199	78.93
8	Anisaldehyde	1250	1.02
9	Bornyl acetate	1289	0.90
10	Eugenol	1359	0.52
11	Methyl cinammate	1379	0.5
12	Methyl eugenol	1407	1.34
13	(E)-β-Ionone	1489	0.41
14	Anisyl propanoate	1512	0.58
15	(E)-ortho-3-Methoxy cinammat aldehyde	1529	0.97
16	Germacrene D	1482	0.09
17	Spathulenol	1578	0.21
	Monoterpene hydrocarbons	-	10.73
	Oxygenated monoterpenes	-	83.35
	Sesquiterpene hydrocarbons	-	0.09
	Oxygenated sesquiterpenes	-	0.21
	Other compounds	-	1.96
	Total	-	96.34
	Yield (%)		0.65%

[b]Retention indices relative to C6-C25 n-alkanes calculated on non-polar HP5MS capillary column.

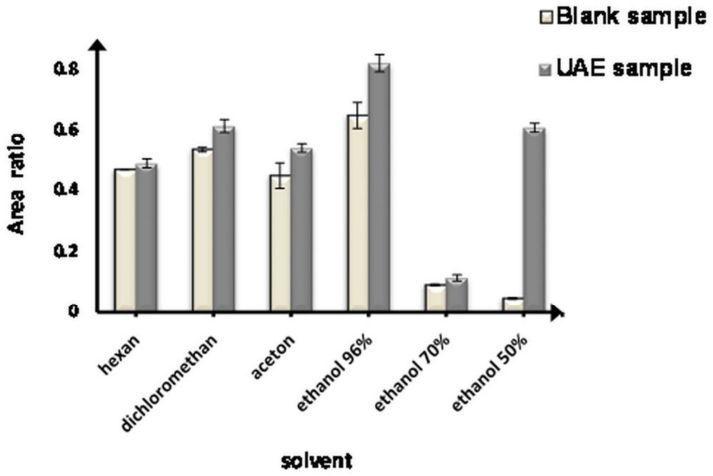

Fig. 3. Effect of solvent on extraction yield of estragole under output power of 56 W, 3 pulses, solid/liquid ratio of 1:8 and plant particle size of 0.6 mm, using 96% ethanol at ambient temperature for 5 min.

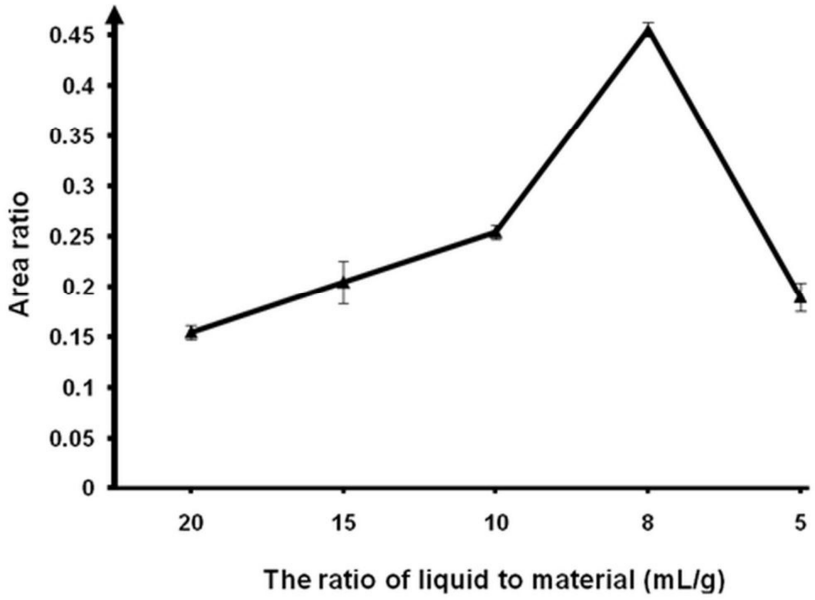

Fig. 4. Effect of liquid/solid ratio (ml g^{-1}) on estragole extraction efficiency by UAE under output power of 56 W, 3 pulses and plant particle size of 0.6 mm, using 96% ethanol at ambient temperature for 5 min.

Effect of ultrasound power on extraction efficiency of estragole. Once the liquid/solid ratio (ml g^{-1}) was adjusted to 8:1, the samples were extracted for 5 min with different extraction powers at a constant pulse number of 3. The obtained results demonstrated that the extraction yield improved by raising the ultrasound power. Increasing the power generated more bubbles that collapsed and disrupted the cell walls, leading to more penetration of the solvent into the cells, more release of the components from the cells into the solvent and an enhancement in the mass transfer procedure. However, at greater ultrasonic powers the compounds would be decomposed; so 90% output power (63 W) was sufficient to reach the most desirable extraction of estragole.

Effects of ultrasound exposure mode and pulse number. After scrutinizing the impact of ultrasound pulse number on the extraction efficiency, it was established that the efficiency improved obviously with increasing the duty cycles to 9. On the other hand, by utilizing a continuous irradiation the ultrasound probe might be damaged and also electric energy consumption would be enhanced. Consequently, pulse mode could be exploited to safely gain better yields. These results were consistent with what previously reported by Sun [32] on improving the extraction yield of all-trans-β-carotene from citrus peels by UAE, and by Herrera [33] on the noticeable influence of duty cycle upon the ultrasound extraction of phenolic compounds from strawberries.

Effect of ultrasound time on extraction of estragole. The impact of ultrasound time on the extraction efficiencies of the oils was examined with ultrasound power of 90%, 9 cycles, and solvent/solid ratio of 8:1. Figure 5 shows that the amount of estragole increases appreciably by enhancing the extraction time from 0 to 5 min due to mass transfer of estragole from the cellular material to the solvent through diffusion and osmosis. Similar results were achieved in the extraction of all-trans-β-carotene from citrus peels [32]. Thus, 5 min was chosen as the optimum ultrasound time for the next studies.

Effect of particle size on extraction of estragole. In earlier investigations, particle size was recognized as one of the key factors that can affect the efficiency of terpenoid extraction from herbs [34, 35], hence, particle sizes of 0.15, 0.3, 0.6 and 1.18 mm were selected in this work. The results confirmed that by decreasing the particle diameter, the amount of estragole in the extracts declined. Zhao reported

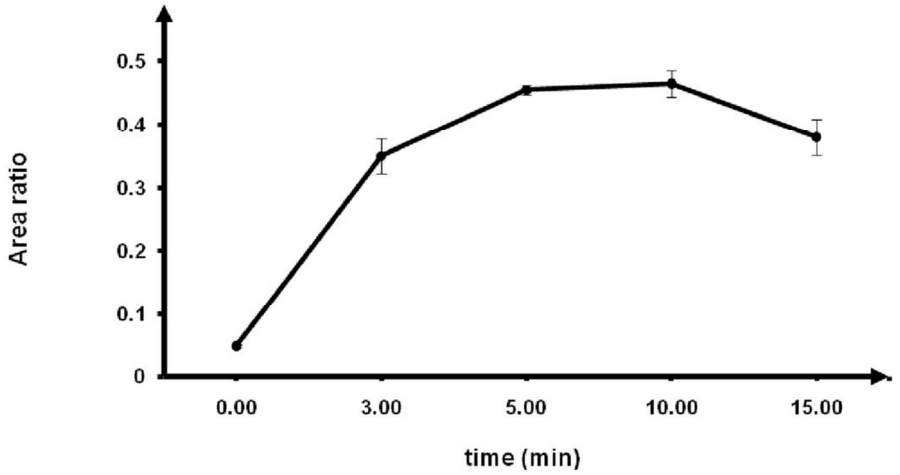

Fig. 5. Effect of ultrasound time on extraction efficiency of estragole under output power of 63 W, 9 pulses, solid/liquid ratio of 1:8 and plant particle size of 0.6 mm, using 96% ethanol at ambient temperature.

Table 2. Optimization of Ultrasound-Assisted Extraction of Estragole from Tarragon Extract

Variable	Tested range	Optimum value
Irradiation amplitude output (%)	0-70 w	63
Pulse	3-9	9
Irradiation time (min)	0-15	5
Ethanol (%)	50-96	96
Temperature (ºC)	Ambient temperature	Ambient temperature
Extracting volume (ml)	10	10
Liquid: solid ratio(mlg^{-1})	5-20	8
Probe position	1 cm	1 cm

that with reducing the particle size, diffusion will no longer be a significant step in the extraction of such small particles; thereby the extraction yield will not improve anymore [36]. Nevertheless, small particles stay at the surface of solvent throughout the extraction; this may cause their limited exposure to ultrasonic waves. Meanwhile, the essential oil may be lost from the surface of small particles during milling. Accordingly, the optimal size was set at 1.18 mm.

Comparison between Ultrasound-Assisted Extraction and Hydro-Distillation

Table 2 represents the optimum parameters acquired by UAE. Following a 5 min extraction by USE, using 10 ml of 96% ethanol and solvent/solid ratio of 8:1, the recovery was nearly 44.4%, and after 3 h by hydro-distillation was 85.3%. As a result, The application of ultrasound for the extraction of essential oil from dried tarragon leaves, offers important

advantages over traditional method, namely: short extraction time (5 min against 180 min for hydrodistillation), low cost (as low amount of nan-toxic solvent) compared with hydrodistillation which used a large quantity of water and energy as well as released carbon dioxide in the atmosphere. Morovere, using heat and water in hydrodistillation accelerated many reactions, specially hydrolysis, trans-esterification or oxidation, and hence some of essential oil compounds were degraded.

CONCLUSIONS

Ultrasound-assisted extraction method with a probe horn at 20 kHz was applied to the extraction of estragole from the dry leaves of *Artemisia dracunculus* L. Ultrasonic wave technique was a powerful tool which efficiently improved the extraction performance of estragole at a short time using a food grade solvent (96% ethanol). Influences of other experimental parameters on the extraction yield of estragole were evaluated, and the optimal conditions were liquid/solid ratio of 8:1 (ml g^{-1}), particle size of 1.18 mm, and US irradiation time of 5 min (output power, 63 W; 9 pulses). Under the optimum conditions, the extraction yield of estragole was 44.4%. The attained results were much favorable for fast extraction of estragole with a rapid, inexpensive and simple method without using high amount of energy relative to conventional method (hydro-distillation).

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