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Effects of ultrasound pre-treatment on quantity and quality of essential oil of tarragon (Artemisia dracunculus L.) leaves

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ABSTRACT

Hydro-distillation with ultrasound was used for extraction of essential oil of tarragon (Artemisia dracunculus L.) leaves. The results of this method were compared with a traditional distillation method considering the extraction kinetics as well as the physicochemical and antioxidant properties of the essential oil. The experiments were ran at three sound power levels (250, 350 and 500 W), three levels of sonication time (20, 30 and 40 min) as well as without sonication as a control in a completely randomized design with three replications. Gas chromatography was used to identify the essential oil compounds. Antioxidant properties were studied using the reducing DPPH radical method. The statistical results showed that the effect of ultrasound power on the essences extracted was not significant. The effect of sonication time on the essential oil content was significant at the 5% level. The highest amount of extracted oil was observed at the power of 500 W for 30 min while the lowest amount was found at 500 W for 40 min. The highest percentage of estragole, which is the most important compound of tarragon was achieved with the proposed method. The highest antioxidant activity was for the extracted essential oil using ultrasound pre-treatment with 350 W power for 30 min and the control samples showed the lowest level.

1. Introduction

The importance of medicinal plants and herbs is verified globally during the past decades. Tarragon (Artemisia dracunculus L.) as a herbaceous plant belongs to Asteraceae family and it is mainly produced in two varieties: French Tarragon and Russian Tarragon (Arabhosseinia et al., 2006). The use of tarragon and its aromatic leaves in seasoning, salads, mustards, vinegar sauces, spices, etc., is the main reason for its cultivation (Sayyah et al., 2004; Arabhosseinia et al., 2006). Tarragon consisted of some active ingredients which treats epilepsy seizure. It also stimulates appetite and increases stomach acid (Aglarova et al., 2007). The most conventional and the simplest method to extract essential oil is hydro-distillation (El Asbahani et al., 2015). There are some disadvantages with this method, including: long extraction time (3–6 h), artifacts and chemical alterations of terpenic molecules by prolonged contact with boiling water (hydrolysis, cyclization), over-heating and loss of some polar molecules in the water extraction (Pingret et al., 2014; El Asbahani et al., 2015; Ribnicky et al., 2006). New extraction techniques called “green techniques” are used by food industry. This leads to the reduction of extraction time, solvent consumption, higher extraction efficiency and better quality of extracted substances (Wang and Weller, 2006; Chemat and Khan, 2011). Ultrasound-assisted extraction is one of these techniques that often improves extraction efficiency and rate, reduces extraction temperature and increases the selection ranges of solvent (El Asbahani et al., 2015). Many papers reported that ultrasound-assisted extraction method increases the efficiency of extraction and extraction time is reduced, such as steroids and triterpenoids from chresta spp (Schinor et al., 2004), ginseng saponins from ginseng roots (Wu et al., 2001), glutinous from sage (Veličković et al., 2006) and polysaccharides from Salvia officinalis L. (Hromadkova et al., 1999).

The ultrasound-assisted extraction method has some advantages but the effects of this method on the extraction yield and kinetics is related to the nature of plants matrix (Wang and Weller, 2006). Following extraction with this method, filtration and separating the extract of the plant material is necessary. Several studies have been done on essential oil extraction with various methods like hydro-distillation (Sefidkon et al., 2007), modern extraction methods such as ultrasound (Tekin et al., 2015), microwave...
2. Materials and methods

2.1. Plant material

Tarragon plants were harvested from a local farm in Varamin, near Tehran, Iran (Fig. 1a). The plants were washed and dried in shade and the dried leaves were separated from the stems (Fig. 1b) and packed in plastic bags. The samples were stored at 4°C prior to the experiments. Before each experiment, the samples were ground into powder for 30 s by using a grinder (Pars Khazar, 320P).

2.2. Essential oil extraction

A Clevenger apparatus was used for hydro-distillation. Twenty grams of dried tarragon leaves was added to a flask and it was mixed with 500 ml of distilled water. The flask was then heated by heating mantle for 2 h counted from the time after condensation of the first drop of vapor in the acquisition column. The amount of oil was measured and afterwards the oil was collected. The extracted oil was stored in glass vials in the refrigerator at 4°C. Experiments were performed in triplicates.

2.3. Ultrasound pre-treatment extraction

A titanium continuous flow ultrasonic cell screwed onto a 20 mm diameter probe connected to an ultrasonic processor (AMMM, MPI, Switzerland) with 1000W power and 20 ± 0.5 kHz frequency is used to generate and transmit ultrasound wave in liquid media. Twenty grams of dried tarragon leaves was added to a beaker and mixed with 500 ml of distilled water. Ultrasound probe with 2 cm depth was placed in a beaker containing distilled water and sample. The samples were exposed to ultrasound at three sound power levels (250, 350 and 500 W), three levels of sonication time (20, 30 and 40 min) with three replications. After sonication beaker’s contents were immediately transferred to Clevenger apparatus and the extraction of these samples was similar to what was explained in Section 2.2.

2.4. Extraction kinetics

The kinetics of the essential oil extraction was studied by checking the amount of oil yield during the extraction process. After seeing the first drop of oil, the oil content of acquisition column was measured and read at specific times (2, 4, 6, 8, 10, 12, 15, 20, 40, 60, 80, 100 and 120 min) to evaluate the kinetics of extraction. With converts column height to volume, the amount of oil in the mentioned time was measured in ml.

2.5. Physical properties of tarragon essential oil

The essential oil extracted from tarragon was analyzed for the physical properties. The refractive index were measured by a refractometer (HSR-500, ATAGO, Japan) and as too low amounts of tarragon essential oil were available for determination of specific gravity using the standard method calculated specific gravity was manually done. Experiments were performed in triplicates.

2.6. Antioxidant activity

Antioxidant activity was measured according to DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) radical scavenging method (Samaram et al., 2015). First, standard curve was obtained with absorbance of different concentrations of DPPH at 515 nm using a spectrophotometer (CE 2502, CECIL, England). Then, 3.9 cc of methanolic solution of 25 μg mg of DPPH was mixed with 0.1 cc of methanol and the absorption was measured at 515 nm and related concentration of DPPH was reported as concentration of DPPH at t = 0.

The experiment was repeated with 0.1 cc oil instead of methanol and measured concentration of DPPH was reported as the concentration of DPPH at time t. The remaining DPPH obtained from the following equation (Mirsaeedghazi et al., 2010).

\[
\%DPPH_{t=0} = \frac{[DPPH]_t}{[DPPH]_{t=0}}
\]

The last test was repeated with several concentrations of oil until the remaining DPPH achieve 50%. The oil concentration which can reduce remaining DPPH to 50% was introduced as effective concentration (EC50). Experiments were performed in triplicates.

2.7. Gas chromatography

Gas Chromatography (GC) analyses were carried out on a Shimadzu-17A gas chromatograph (Tokyo, Japan) equipped with a flame ionization detection (FID) system and a CBP-5 capillary fused
silica column (25 m; 0.25 mm I.D.; 0.22 μm film thickness, methyl 5% phenyl polysiloxane). The following temperature program was employed: 40 °C for 1 min then programmed at 5 °C/min to 250 °C, held for 20 min. Other operating conditions were as follows: carrier gas, helium (99.999%); inlet pressure, 74 kPa; linear velocity of 20 cm/s; injector temperature, 220 °C; detector temperature, 250 °C; split ratio, 1:25. Hydrogen gas was generated with hydrogen generator (OPGU–2200 s, Shimadzu-Japan) for FID at a flow of 22 ml min⁻¹.

GC–MS analyses were performed on an Agilent Technologies 6890 GC system coupled with a 5973 network mass selective detector and equipped with a HP1–MS capillary fused silica column (30m; 0.25 mm I.D.; 0.25 μm film thicknesses, methyl polysiloxane). The temperature program initiated at 40 °C, for 1 min, then raised at 3 °C/min to 250 °C, held for 20 min. Other operating conditions were as follows: carrier gas, helium (99.999%); flow rate, 1 ml min⁻¹; detector temperature, 250 °C; split ratio, 1:50. Mass spectra were taken at 70 eV. Mass range was from 20 to 500 amu. An Enhanced ChemStation G1701 DA version D.00.01.27 was used for the data collection and processing.

The injections into GC and GC–MS were carried out using a 1-μL micro-syringe model Hamilton 7001. Centrifuges were performed by Hermle Z 200 A centrifuge instrument (Wehingen-Germany).

3. Results and discussion

3.1. Extraction yield

Clevenger apparatus as the conventional method were compared with ultrasound pre-treatment followed with Clevenger by same solvent. The yields of the extracts are shown in Table 1. The application of ultrasonic pre-treatment did not have any effect on extraction yield. Results revealed that the yield of different extraction treatments varied from 1.87% (in power: 500 W and sonication time: 40 min) to 2.18% (in power: 500 W and sonication time: 30 min), while the efficiency of the control sample was equal to 2.08%. No significant effect of sonication on the amount of distilled oil was observed. In certain internal and external structures of the plant, essential oils are stored with its droplets. They have such a thin skin that might be easily destroyed (by sonication in the case of external structures) but for internal structures, the milling degree of plant material affects the gained yield (El Asbahanani et al., 2015). The effect of preliminary maceration on the efficiency of essential oil was examined for the distilled oil of peppermint, marjoram and chamomile (Kowalski et al., 2015). They reported that when oil distillation from marjoram and peppermint is subjected to prior ultrasound assisted maceration, the amount of distilled oil will be higher compared to the control sample. However, no significant effect of sonication was observed in the amount of oil extracted from chamomile. Based on their report, the contribution of sonication might be weaker on the inner cell walls (Zhang et al., 2015). In addition, the extraction yield of polysaccharides increases pattern up to 25 min but decreases beyond this time (Maran et al., 2008). Results showed negative effects of 40 min sonication against on 20 and 30 min sonication. This phenomenon could be due to temperature changes during the long sonication time which destroy the oils. Therefore, after the maximum extraction yield was achieved, longer time of ultrasonic extraction was not necessary. In extraction of pectin from grapefruit peel, more cavitation bubble will create at higher temperatures (Bagherian et al., 2011). In turn, burst of these bubbles increases shear stress and degradation pectins.

It can be concluded that there is always an optimum sonication time to achieve the best yield. A duration of 25 min for sonication is an ideal time for extraction yield, since the extraction yield shows an increasing pattern up to 25 min but decreases beyond this time (Maran et al., 2015). In addition the extraction yield of polysaccharides increases rapidly during the initial 60 min and the optimal extraction result is gained, while after 60 min, the increase of extraction yield is slow (Chen et al., 2012).

3.2. Effect of ultrasonic power on extraction

In order to evaluate the effect of power on extraction efficiency of tarragon essential oil, extractions were performed with three power levels including: 250, 350 and 500 W. The effect of ultrasound showed an increase of efficiency by increasing of power from 250 to 350 W and then decreased by increasing power from 350 to 500 W. However, statistical studies showed that the effect of ultrasound power on the essences extracted was not significant at the 5% level.

During cavitation, too high power would lead to an increase in the bubble numbers in the solvent, which may lower the efficiency of ultrasound energy transferred into the medium (Filgueiras et al., 2000). It was suggested that the ultrasonic intensity affect collapse pressures and local temperatures and the number of free radicals produced (Zhang et al., 2015). So, it creates an environment which plays an important role in the stability of targeted compounds. At higher ultrasonic intensities, the cavitation bubble collapse will be more severe, as a result of greater ultrasonic intensity. Bigger bubble would be formed and higher shear forces would be stimulated. On the other hand, when there is an increase in the power, the process of pulsation and the burst of bubbles will be more rapid and the number of bubbles will increase, so more free radicals will concentrate in the aqueous solution ingredients. These generated radicals will react with solution ingredients, thus, degradation rate will increase as power increases.

3.3. Effect of ultrasonic duration on extraction

Sonication time is an important factor affecting the extraction efficiency (Xu et al., 2015). It is necessary to determine the optimum sonication time to avoid excessive use of ultrasound energy. According to statistical studies and the significant effect of sonication time, Duncan test was used to compare the results. The results showed that 40 min sonication is significantly different at 5% level with 20 and 30 min to extract the tarragon essential oil. At all three levels of power, efficiency increases to 30 min to reach maximum efficiency, this is due to disruption of biological cell walls to greater availability of essential oils and facilitate the release of contents. Ultrasonic wave accelerates cell walls disruption. This phenomenon creates a larger contact area between solvent and material and more oil will appear on the surface of UAE than conventional extraction processes. However, as the distance increases, this effect would be weaker on the inner cell walls (Zhang et al., 2008). Results showed negative effects of 40 min sonication against on 20 and 30 min sonication. This phenomenon could be due to temperature changes during the long sonication time which destroy the oils. Therefore, after the maximum extraction yield was achieved, longer time of ultrasonic extraction was not necessary. In extraction of pectin from grapefruit peel, more cavitation bubble will create at higher temperatures (Bagherian et al., 2011). In turn, burst of these bubbles increases shear stress and degradation pectins.

Table 1

Yields of essential oil extraction in percentage at different conditions.

<table>
<thead>
<tr>
<th>Sonication Power, (W)</th>
<th>Sonication time, (min)</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>2.11 ± 0.02 a</td>
<td>2.11 ± 0.02 a</td>
<td>2.01 ± 0.013 a</td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>2.11 ± 0.06 a</td>
<td>2.15 ± 0.027 a</td>
<td>1.94 ± 0.4 a</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>2.04 ± 0.06 a</td>
<td>2.18 ± 0.034 a</td>
<td>1.8 ± 0.013 a</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.08 ± 0.013 a</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation values (n = 3).
The same letters are not significantly different (p < 0.05).
The kinetics of essential oil extraction of Tarragon at different pretreated conditions, a) 20 min sonication b) 30 min sonication and c) 40 min sonication.

Fig. 2. The kinetics of essential oil extraction of Tarragon at different pretreated conditions, a) 20 min sonication b) 30 min sonication and c) 40 min sonication.

Period from 0 to 20 min and slow period from 20 to 120 min. The most effective of ultrasound waves were during the first 20 min. In this case ultrasound pre-treatment kinetics is higher than Clevenger and after that is lower. The reason for this initial high rate might be that the oils, concentrated in the external region of particle are more accessible than in the internal part in which the plant tissues are more intact. The extraction from external part is attributed to external mass transfer. The mode of such mass transfer is convective because fluid motion is formed due to ultrasonic cavitation. Next, oils extracted from the inner part of root particles must diffuse in the pores of root materials. Consequently, the extraction rate will be much slower.

In a research, the ultrasound substances extraction, extractable from sage was studied and the results showed that the samples and solvent were affected by ultrasonic waves for 5, 10, 20, 40 and 80 min. In the case of both plant materials and independency of solvent polarity, two periods of extraction were easily observed: first washing, means that a rapid increase in the concentration of essential oil at the early beginning of process and second, slow extraction (approximately after the first 10 min) which means that (a slow increase in the concentration with the progress of extraction). Through the optimum time for ultrasonic extraction of about 20 min, maximum concentration of essential oil will be reached in liquid extracts (Veličković et al., 2006).

3.5. Physical properties

The tarragon essential oil was analyzed for physical properties by using standard procedures shown in Table 2. No significant difference was found between the physical constants of essential oils obtained by ultrasound pre-treatment and hydro-distillation methods. In a research, various methods of extraction was studied and no significant effect was observed on physical properties (specific gravity, refractive index, optical rotation, and solubility in 95% ethanol) of orange peel essential oil (Ferhat et al., 2006).

3.6. Antioxidant activity

Antioxidant activity was measured for both conventional and ultrasound pre-treatment extractions, which was carried out at optimum conditions. Results showed that the control sample has the lowest antioxidant activity and the sonicated sample (30 min, power 350 W) has the highest antioxidant activity (Table 3). Also, extraction method showed a significant effect on antioxidant activity of essential oils. Antioxidant activity decreased after sonication at 500 W due to the destructive effect of ultrasonic treatment at high intensity. Ultrasound treatment of samples at 250 W showed that the low intensity of ultrasound can increase antioxidant activity compared to control one; however its increase was lower than other ultrasound treated samples. Similar results are reported in the study of antioxidant activity of papaya seed oil (Samaram et al., 2015).

3.7. Gas chromatography

GC and GC-MS was evaluated for both conventional and ultrasound pre-treatment extraction carried out in the optimized conditions. Table 4 shows the compounds found. Sabine, β-pinene, limonene, (Z)-β-ocimen, (E)-β-ocimen, estragole, geranyl acetate, methyleugenol were the major essential oil compounds based on the highest percentage of extracted oil at different conditions. The analysis of oil extracts showed that estragole was the main ingredient of tarragon oil and according to the results, its value was 76.67% in control sample and 83.06% in the sample treated by 500 W for 30 min. Comparing the composition of essential oils extracted by various methods showed that despite the presence of similar compounds in the oil, its percent was different. For example geranyl acetate was in the range of 0.5-0.71% and sabine in 0.55-1.09%. Among the major compounds of each sample the most compound distillation method caused were sabine (1.09%), β-pinene (0.06%), limonene (2.86%), (Z)-β-ocimen (8.73%),...
(E)-β-ocimen (7.90%). Ultrasound pre-treatment in extracting estragole, geranyl acetate, methylcyclohexenone were effective. Estragole is one of the phenylpropanoidcompound and (E)-β-ocimen is one of monoterpenes compounds. Ultrasound pre-treatment caused the highest percentage of estragole, while the highest percentage of (E)-β-ocimen was extracted in Clevenger method, estragole enhance phenylpropanoid properties and (E)-β-ocimenenhance the monoterpenes properties. Thus, according to the use of essential oil a proper method could be selected.

4. Conclusion

The effect of ultrasound pre-treatment on essential oil of Tarragon leaves was studied. No significant effect of sonication power on the extracted essences was observed while the effect of the sonication time on the essential oil content was significant at 5% level. The extraction kinetics of the sonicated samples showed that the first 20 min could be enough to get the majority of the oil. The physical properties were similar to the essential oil obtained from ultrasound-pre-treatment and control samples. The highest percentage of estragole, which is the major compound of tarragon was achieved with the proposed method.

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