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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 500W 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 (Arabhosseini et al., 2007). 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; Arabhosseini et al., 2006). Tarragon consisted of some active ingredients which treats epilepsy seizure. It also stimulates appetite and increases stomach acid (Aglarova et al., 2008; Smigielski et al., 2014). The tarragon essential oil include: estragole, a-pinene, β-pinene, sabinene, limonene, (Z)-β-ocimen, (E)-β-ocimen and limonene (Duke, 2002).

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 *Salviaofficinalis* 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

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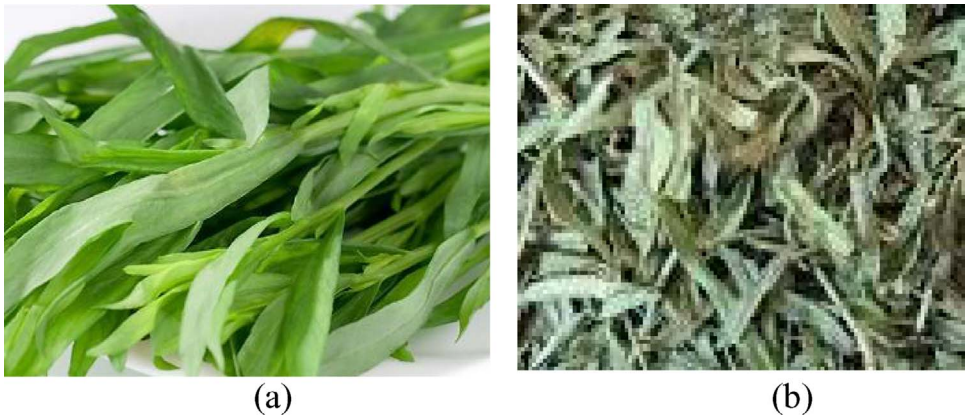

Fig. 1. French tarragon, used for this research. a) fresh leaves and b) dried leaves.

(Bendahou et al., 2008) and supercritical fluid (Santoyo et al., 2005), however limited information was found on the use of ultrasound and distillation in order to understand what physicochemical effects has on oil extraction yield and its kinetics.

The privilege of reducing extraction time can overcome the side effects of distillation. It is worth noting that other processes such as filtration and separation of extracts gained from plant material are necessary for this method to be effective.

The objective of this research was evaluating the effect of ultrasound pre-treatment on tarragon leaves essential oil yield, extraction kinetics, physical properties, antioxidant activity and the ingredients of the extracted oil.

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 shadow 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) with1000W 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.

A water bath (memmert WNB 14, Germany) was used to control the temperature during extraction.

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 convers 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 $\frac{mg}{lit}$ 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).

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

The last test was repeated with several concentrations of oil until the reminding 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