Iranian Journal of Pharmaceutical Sciences
2018: 14 (1): 35-44
www.ijps.ir

Original Article

Effect of Powder Sizes, pH of Water, Ultrasound and Method of Distillation on Extraction of Fennel Essential Oil and Anethole Content

Mahdi Tavakolizadeh [a], Ghazaleh Hadian [a], Mohammad Reza Khoshayand [b], Faraz Mojab[c*]

[a]*Department of Pharmacognosy, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.*
[b]*Department of Drug and Food Control, Pharmaceutical Quality Assurance Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*
[c]*Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences Research Center (PSRC), Shahid Beheshty University of Medical Sciences (SBMU), Tehran, Iran.*

Abstract

The essential oils are frequently used in food processing industry, perfumery, cosmetics, and pharmaceuticals. Most of the oils were obtained by steam distillation and water distillation. It is necessary to specify the best conditions of essential oils production to get much more major compound(s) and higher yield oil. So, fennel oil was selected in this study. The objective of this work was to identify the effect of powder's particle size, pH of water, method of distillation and using ultrasound on extraction of fennel essential oil (and its major constituent, anethole). We used a statistical method called D-optimal Design that appointed pH, particle size, and method for each assay. First, the seeds powder distillated directly. In the second series, the seeds were placed in an ultrasonic apparatus for 30 minutes. The essential oils were subsequently isolated by two methods, hydro-distillation, and steam-distillation in different sizes and pH in Clevenger apparatus. Gas chromatography was used to determination of major component (E-anethole) in fennel essential oils. Finally, optimum conditions according to the statistical results are as follows: Method: steam distillation, and mesh size: 50. So it shows that steam distillation is more efficiently than water distillation.

Keywords: D-optimal Design, fennel, essential oil, anethole, particle size, pH, distillation, ultrasound.

Corresponding Author: Faraz Mojab, Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences Research Center (PSRC), Shahid Beheshty University of Medical Sciences (SBMU), Tehran, Iran.
Tel: +9821-88200061
E-Mail: sfmojab@sbmu.ac.ir
Cite this article as: Tavakolizadeh M, Hadian G, Khoshayand M, Mojab F, Effect of Powder Sizes, pH of Water, Ultrasound and Method of Distillation on Extraction of Fennel Essential Oil and Anethole Content. Iranian Journal of Pharmaceutical Sciences, 2018, 14 (1): 35-44.

1. Introduction

The increased interest in natural products, rather than synthetic agents, has focused attention on plants as a source of flavoring compounds [1]. Essential oils are volatile aromatic compounds that evaporate in air at normal temperature. Essential oil is the basic material of producing perfumes and cosmetics. The essential oil which is derived from spices, such as fennel oil, pepper oil, cinnamon oil, ginger oil, clove oil, and coriander oil is commonly used as a food and beverages flavor [2]. One of the potential plants is *Foeniculumvulgare* Mill. (Fennel), a plant that belongs to Umbelliferae (Apiaceae). The fennel oil is useful in pharmaceutical industry as flavor and carminative [3]. The main component of fennel oil is anethole (55-75%) [4]. The fennel oil quality depends on the amount of anethole content. The fennel oil with higher anethole content has better quality [5].So, it is necessary to specify the best conditions of essential oils to get much more anethole and higher oil yield.

Previous researches have shown that factors such as extraction method, mesh size, and distillation time affect the quality and quantity of essential oil of aromatic plants.

Essential oil of fennel cultivated in Iran was obtained by hydrodistillation and supercritical (CO2) extraction (SFE) methods. The effects of different parameters, such as pressure, temperature, modifier volume, and extraction time, on the supercritical fluid extraction of fennel oil were investigated. The results showed that under pressure of 350 atm, temperature 55ºC, methanol 5% and dynamic extraction time of 45 min, the extraction was more selective for the extraction of (E)-anethol. Sixteen compounds were identified in the hydrodistilled oil. However, by using supercritical carbon dioxide in optimum conditions, only nine components represented more than 99% of the oil. The results showed that in Iranian fennel oil, (E)-anethol is a major component [6].

Fennel seeds, growing wild in Montenegro, were extracted with supercritical CO2 (SC-CO2) at a flow rate of 0.2 kg CO2/h under varying extraction conditions in order to determine yield, composition, and organoleptic characteristics of extract. The extracts obtained were compared to fennel seed oil isolated by hydrodistillation. In the SC-CO2, extracts as well in the hydrodistilled oil, the major compound was trans-anethole (68.6–75.0%) and (62.0%), respectively. With pressure varying from 80 to 150 bar and temperature varying from 40 to 57 °C, it was found that for the selected herb material, the optimal conditions of SC-CO2 extraction (high percentage of trans-anethole) are pressure: 100 bar; temperature: 40 °C; and extraction time: 120 min. Organoleptic tests confirmed that hydrodistilled oil possessed a less intense fennel seed aroma than extracts obtained by SC-CO2 [7].

The influence of distillation conditions on the essential oil composition of fennel has been studied previously. For instance hydrodistillation was conducted in four different process time (15, 30, 60 and 150 minutes) and the steam distillation was conducted at 150 minutes. The oils were analyzed by GC and GC/MS. 91.37 to 97.57% of the oil consisted of these compounds. *E*-anethole, estragole, and fenchone were the most frequent compounds. The highest percent of anethole was at 30 minute [8].

Desirable percentage and compounds of the essential oil could be achieved from *Pimpinella affinis* by selection of the condition of essential oil extraction. Three methods of oil extraction including hydrodistillation, water-steamdistillation, and direct steam distillation were applied based upon Taguchi statistical method in three levels to determine the quantitative and qualitative effects of the parameters namely method, time, and mesh on essential oil. Mean comparisons of essential oil yield showed that more content of limonene, trans-α-bergamotene, and geijerene was obtained by mesh size 20 in hydro-distillation and steam distillation methods. Mesh size 40 and time 2.5 h gave the best result for elemicin and germacrene B in steam distillation method. The maximum and minimum essential oil yield was respectively obtained by mesh size 20 and 14 in water-steam distillation method [9].

In another study on other herbs, the effect of three distillation methods including hydrodistillation, water-steam distillation, and direct steam distillation on the essential oil content and composition of *Thymus vulgaris* were studied. Among distillation methods, the highest oil yield was obtained by direct steam distillation (1.20%) [10].

The ultrasonically assisted extraction is a newer technique used in extraction technology for oils and other plant components [11-12].

Probably ultrasound with the destruction of plant tissues facilitates the release of essential oils. We want to know that if this method has any effect on the amount of essential oil of fennel and its active ingredient. Also, the essential oils manufacturers may use different types of water (minerals, springs, wells, plumbing, distillates, etc.) to extract the essential oils, which may have different pH and affect the essential oils, and also may change and transform them. In this study, the effect of different pH (acidic and basic) on the amount of essential oil and its active ingredient will be investigated. On the other hand, it may already have been known that the crushing and cutting of the plant's tissues by increasing the particle surface can facilitate the penetration of boiling water and steam into the tissue, increasing the amount of essential oil. In this manuscript, different sizes of particles of fennel seeds are prepared and this will be checked and assured. Pharmacopoeia essential oils (other than essential oils of citrus) should be prepared by steam distillation method. In this paper, the difference between this method and water distillation on fennel seeds and its effect on the amount of active ingredient will be investigated.

The objective of this work was to identify the effect of powder’s particle size, pH of water, method of distillation and using ultrasound on extraction of fennel essential oil (and especially, major component anethole).The probably effects of pH of water used in distillation methods, on essential oil yield and major component content is studied, too.

2. Materials and Methods

The study was carried out in the School of Pharmacy; SBMU. We used a statistical method called D-optimal Design that appointed pH, particle size, and method for each assay (Table 1). The dried fennel seeds were purchased (herbal market, Tehran, June 2015) and identified. Then, it milled and passed from different meshes. Two series of experiments were conducted. First, the seeds powder distilled directly. In the second series the seeds were placed in an ultrasonic apparatus (Chtomtech UC-10200 BDT) for 30 minutes [13]. The essential oils were subsequently isolated by two methods, hydrodistillation in Clevenger-type apparatus and steam distillation, in different sizes (25, 30, 40, 50) and pH. Some solutions with different pH (5.5, 5.8, 7, 7.4 and 8.5) were prepared (14). Then, all of the solvents were confirmed by pH-meter. A total of 50 g of dry seeds were used in each distillation (for 3 hrs).

The calculation of the time started when the first fennel oil dropped into the decanter and it finished when the fennel oil was not dropped anymore [14]. Analytical gas chromatography (GC) was used to determine the essential oil composition.

2.1. Hydrodistillation

Table 1. D-optimal design for appointed pH, particle size and distillation method.

Run	pH	Mesh size	Method
1	5.5	25	water
2	5.5	50	water
3	5.8	40	water
4	7	50	water
5	7.4	25	water
6	7.4	40	water
7	8.5	25	water
8	8.5	50	water
9	-----	25	steam
10	-----	30	steam
11	-----	40	steam
12	-----	50	steam

The essential oil yield was determined by hydrodistillation in Clevenger apparatus. 50 g of seeds was used for essential oil extraction. The extraction was performed at boiling water over three following hours and the oil content was recorded. The process completed by adding anhydrous Na_2SO_4 into fennel oil to absorb any residual water from it.

2.2. Steam distillation Extract

Steam distillation is a distillation process using steam. It flows through a circular pipe which is located below the porous material and the vapor moves upward through the material that is located on the top sieve [12]. 50 g of the seeds was used for essential oil extraction by using this method. Finally, the essential oil content was recorded and the mixture was dried using anhydrous Na_2SO_4.

2.3. Gas Chromatography

The essential oils were analyzed using an Agilent gas chromatograph, Model 7890A, equipped with a flame ionization detector (FID) and an HP-5 capillary column (30m× 320 µm, film thickness 0.25 µm). Injector and detector temperatures were set as 250 °C and 300 °C, respectively. Column oven temperature was programmed from 50 °C to 120 °C at a rate of 3 °C/min; initial and final temperatures were held for 3 and 10 min. Nitrogen was used as carrier gas at a flow rate of 2 mL/min. The samples were solubilized in Hexane (50µlof oil /1000 µl of solvent) and 1 µl of solution was injected, using split mode (split ratio, 1:10). As standard (major component of fennel oil), different dilutions of

E-anethole (No. 8100429, Merck) was injected to GC and its calibration curvedrawn up.

3. Results and Discussion

The volume of fennel oil and the anethole concentration in the first series of tests (without ultrasound) are given in table 2.

Table 3 shows the results in the second series of tests (with ultrasound).

According to figure 1 and 2, in both methods of steam distillation (without and with ultrasound), whatever the plant became smaller, the volume of essential oils and the concentration of anethole was less. So, powdering the seeds should be avoided in this method. It seems that water steams couldn’t pass through the mass of seeds powder and it decreases the yield of essential oils.

Table 2. The results of fennel oil volume and the anethole concentration related to pH, size and distillation methods (without ultrasound).

Run	oil volume (mL)	Anethole (g/mL)
1	0.7	0.062
2	0.9	0.075
3	1.1	0.072
4	1	0.073
5	0.6	0.063
6	0.7	0.084
7	0.9	0.087
8	0.9	0.075
9	0.8	0.084
10	0.6	0.077
11	0.9	0.073
12	0.6	0.075

Table 3. The results of fennel oil volume and the anethole concentration related to pH, size, and distillation methods (with ultrasound).

Run	oil volume (mL)	Anethole (g/mL)
1	0.8	0.089
2	1	0.072
3	1	0.080
4	1.4	0.076
5	0.5	0.076
6	1	0.094
7	0.6	0.080
8	1.5	0.088
9	0.9	0.079
10	0.8	0.086
11	1	0.068
12	1.3	0.057

According to figure 3 and 4, in the water distillation method at the same condition, the volume of essential oils and the concentration of anethole were more. These results are the same with the study done by Habibzadeh et al [9].According to figure 5 and 6, pH has no significant effect on the volume of essential oils and the concentration of anethole.

As a result of comparing the two stages (without and with ultrasound) we find that after the ultrasound, the volume of essential oils and the concentration of anethole approximately 20% increased [11]. Maybe, ultrasound improve penetrate steam or water into herb tissues and oil exit (and anethole). This is the first report of using ultrasound in the extraction of essential oils.

According to statistical results, we found that a 2 factor interaction model appropriately fit the data. The correlation coefficient (R2) and adjusted R2 of this model are 0.913 and 0.814, respectively,which means that the model can explain more than 91% of the variability in the response and that less than 9% of the variability is due to noise. In addition, the coefficient of variation (C.V. %), an estimate of the standard deviation around the mean associated with the experiment, is 4.32,which indicates the precision

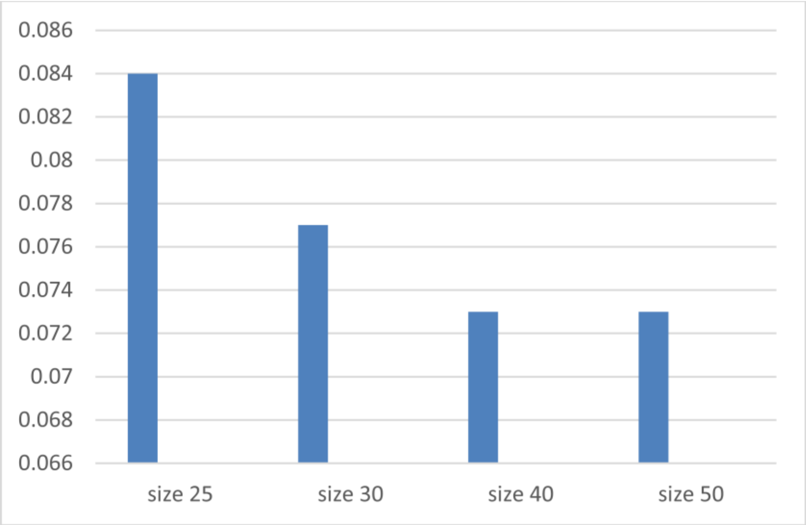

Figure 1. Anethole concentration at different sizes in a steamdistillation method,in a non-ultrasound step.

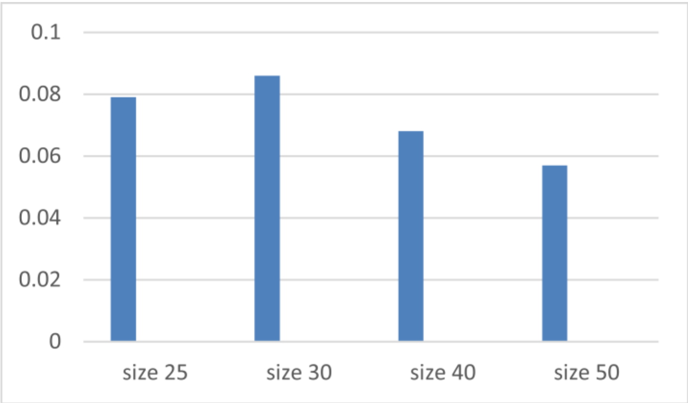

Figure 2. Anethole concentration at different sizes in a steamdistillation method with ultrasound.

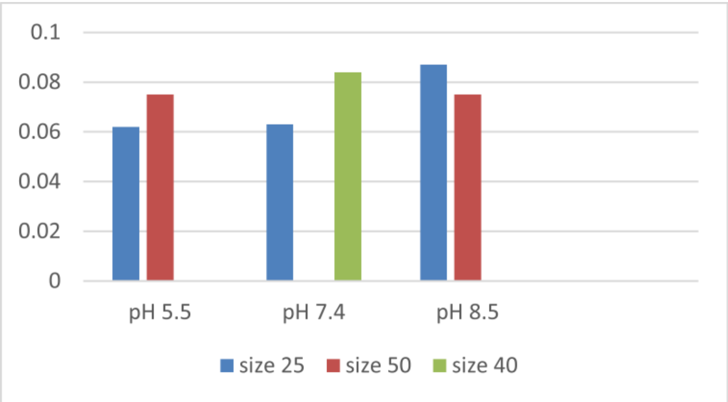

Figure 3. Anethole concentration at different sizes with the same pH in a waterdistillation method,in a non-ultrasound step.

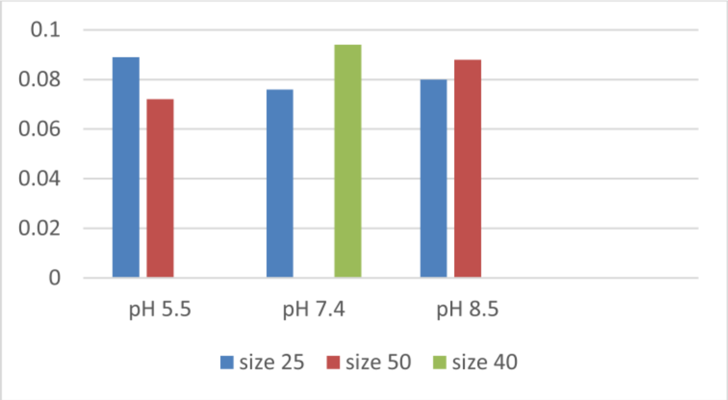

Figure 4. Anethole concentration at different sizes with the same pH in a water distillation method,with ultrasound.

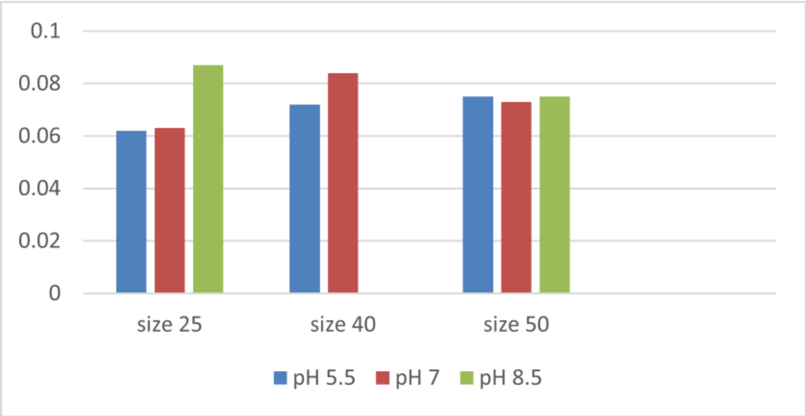

Figure 5. Anethole concentration at different pH in a waterdistillation method,in a non-ultrasound step.

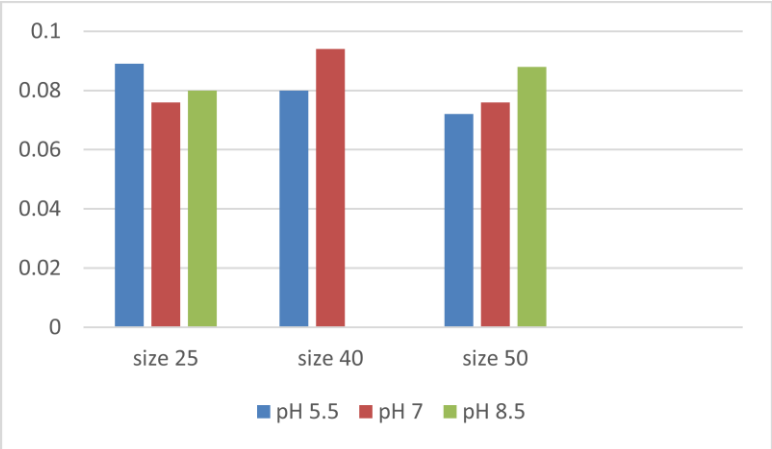

Figure 6. Anethole concentration at different pH in a waterdistillation method,with ultrasound.

reliability of the model. Thus it can be concluded that the 2 factor intraction model can be used to determination of optimal factors.

In figure 7, the response surface shows a curvature along the particle size and pH on Anethole concentration.

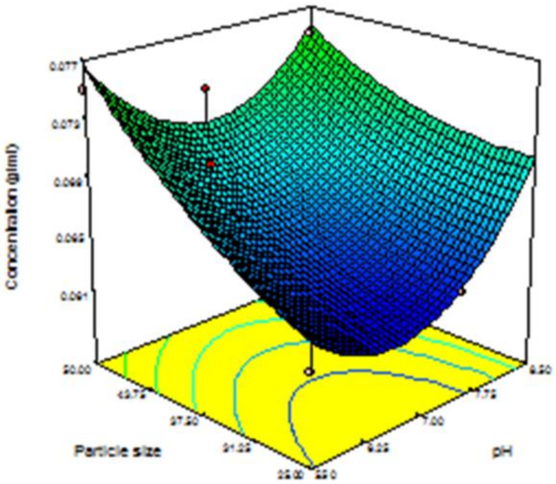

Figure 7. Response surface plot indicating the effect of particle size and pH on Anethole concentration

4. Conclusion

As final results, optimum conditions according to the statistical results are as follows: Method: steam distillation, and mesh size: 50. So, it shows that steam distillation is more efficient than water distillation. These results are identical with those of study by Nikkhah et al [10].

Acknowledgements

The authors wish to thank Ms. F. Tavakkoli, Ms. S. Saremi, and Mr. V. Qeshlaqi (our Laboratory) for kindly help for the research.

References

[1] Stefanini MB, Ming LC, Marques MOM, Facanali R, Meireles MAA, Moura LS, Marchese JA, Sousa LA. Essential oil constituents of different organs of fennel. *Rev. Bras. Pl. Med., Botucatu* (2006) 8: 193-198.

[2] Guenther E. *Essential Oils*, Diterjemahkanoleh Ketaren S. Atsiri M. Jilid I. Jakarta: Universitas Indonesia Press (1987)

[3]Tyler VE, Brady LR, Robbers JE. *Pharmacognosy*.9th ed., Lea and Febiger, Philadelphia (1988) 131.

[4]Evans WC. *Trease and Evans Pharmacognosy*.16th ed., Saunders, Edinburgh (2009) 276.

[5] Iran Ministry of Health (IMH).*Iranian Herbal Pharmacopeia*. IMH, Tehran (2002) 325-333.

[6] Yamini Y, Sefidkon F and Pourmortazavi SM. Comparison of essential oil composition of Iranian fennel (*Foeniculum vulgare*) obtained by supercritical carbon dioxide extraction and hydrodistillation methods. *Flavour Fragr. J.* 2002; 17: 345–348.

[7] Damjanovic B, Lepojevic Z, Zivkovic V, Tolic A. Extraction of fennel (*Foeniculum vulgare* Mill.) seeds with supercritical CO2: Comparison with hydrodistillation. *Food Chem.* 92 (2005) 143–149.

[8] Jamshidi AH, Shams Ardakani MR, Hadjiakhondi A, AbdiK.The influence of distillation conditions on

the essential oil composition of fennel (*Foeniculum vulgare* Mill.). *J. Med. Plants* (2004) 3 (11): 68-72.

[9] Habibzadeh M, Sefidkon F and Fatemi Sh. The effects of different mesh sizes, methods and periods of distillation on essential oil content and composition of *Pimpinellaaffinis*Ledeb. *Iran. J. Med. Aromatic Plants* (2012) 28 (2): 224-234.

[10] Nikkhah F, Sefidkon F and SharifiAshoorabadiE.The effect of distillation methods and plant growth stages on the essential oil content and composition of *Thymus vulgaris* L. *Iran. J. Med. Aromatic Plants* (2009) 25(3): 309-320.

[11] Vinatoru M, Toma M, Radu O, Filip PI, LazurcaD, T.J. Mason, The use of ultrasound for the extraction of bioactive principles from plant materials. *Ultrasonics Sono*chemistry (1997) 135- 139.

[12] *United States Pharmacopeia* (USP). 36th ed., The United States Pharmacopeia Convention, Rockville (2013) 1209-10.

[13] Vilkhu K, Mawson R, Simons L, Bates D. Applications and opportunities for ultrasound assisted extraction in the food industry-A review. *Innovative Food Sci. Emerging Technol.* 9 (2008) 161–169.

[14] Damayanti A and Setyawan E. Essential oil extraction of fennel seed using steam distillation. *Int. J. Sci. Eng.* (2012) 3(2): 12-14.

ONLINE SUBMISSION

www.ijps.ir