

Full Length Research Paper

Comparative extraction of volatile oil components from *Pimpinella affinis ledeb* using supercritical CO₂ and steam distillation

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The isolation of volatile components from *Pimpinella affinis ledeb* was carried out using supercritical extraction and steam distillation method. The oils were analyzed by GC-FID and GC/MS. The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). The major constituent detected was *trans- α -Bergamotene*. By using of Taguchi design the effect of operational parameters such as; temperature and pressure were investigated in supercritical fluid extraction (SFE). As a comparison, conventional steam distillation was conducted and the influences of temperature and extraction time were studied as the important parameters for improvement of oil extraction and its major component. Results show that the maximum yield of SFE and steam distillation was 5.20%, 0.82%, respectively based on dry weight of plant materials (w/w).

Keywords: *pimpinella affinis ledeb*, steam distillation, *trans- α -Bergamotene*, essential oil, supercritical fluid extraction.

INTRODUCTION

Pimpinella has about 150 species in the world which mostly are yearlings. *Pimpinella affinis* [syn. *P. reuteriana* Boiss., *P. griffithiana* Boiss., *P. ambigua* W. D. Koch ex Wolff, *P. multiradiata* (Boiss.) Korov., *P. korovinii* R. Kamelin] presents in different regions of Iran, Iraq, Syria and Israel. It is a biennial aromatic plant, 20–110 cm in height, with white umbel inflorescences and ellipsoid fruits. It grows wild in the center and north of Iran and is more abundant in Chalous and Khojir (Askari et al., 2006; Mozaffarian, 1996).

Commonly, isolation of essential oils from plants was performed by solvent extraction, steam distillation, or hydrodistillation. In extraction of essential oils with organic solvents, evaporation of the solvent is usually required and operating conditions employed for evaporation could cause product degradation (Morales et al., 1998). Steam distillation and hydrodistillation are used as alternative to organic solvents, however, these

methods have some disadvantages such as requiring a long extraction time and the processes lead to thermal degradation. In recent years, there has been increased interest in supercritical fluid technology for the applications in food processing industry, and extraction with supercritical fluid has become an interesting alternative to the conventional extraction procedures (Assis et al., 2000; Khajeh et al., 2005).

Most advantages of SFE are: (a) it is an environmentally friendly process; (b) supercritical fluids provide high solubility and improved mass-transfer rates; (c) the properties of a supercritical fluid can be altered by varying the pressure and temperature, allowing selective extraction. Thus SFE has found applications in the extraction of flavors and fragrances from their natural sources and as a result, many studies have been published using this environmentally friendly extraction method. The extraction of ginger oil (Balachandran et al., 2006), coriander seed (Illes et al., 2000), *Baccharis* oil (Cassel et al., 2000), bioactive compounds from sunflower leaves (Casas et al., 2008), chamomile (Povh et al., 2001), *Hyssopus officinalis*

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(Schulz and Stahl-Biskup, 1991) are just a few of numerous studies reported in the literature with regard to use of SFE for the extraction of spices and medicinal herbs.

There are some reports on essential oil analysis of other *Pimpinella* species in the literature. The main compounds of the stem plus leaf oils of *P. affinis*, *P. puberula* and *P. tragioides* were found to be limonene, pregeijerene and *trans- α -Bergamotene*; The main components of the aerial parts oil of *P. aromatica* were methyl chavicol and *trans*-anethol. Methyl chavicol was also the major compound in the fruit oil of *P. aromatica*. The main compounds of fruit and leaf oils of *P. cumbrae* were α -bisabolol and δ -3-carene. The major compound of *P. anisum* was *trans*-anethol and that of *P. aurea* were β -bisabolene and viridiflorol. Limonene and Methyl eugenol were the major constituent of the *P. barbata* oil. The main constituents of oils of *P. eriocarpa* were limonene, Elemicine and pregeijerene. β -caryophyllene, β -pinene and germacrene D were the main compounds in *P. kotschyana* and *P. tragioides* oils, respectively [12-15].

Pimpinella affinis Ledeb is one of the two-year types of this kind in Iran which the attempts were performed to identify the constituents of essential oil of this type from different habitats in past few years. Askari et al. showed that the major component of the extracted essential oil from stems/leaves of this plant was detected an organic material identified as *trans- α -Bergamotene*, which is an aromatic compound. Furthermore, it uses in preparation of many aromatic compounds as raw material in flavor and fragrance industries (Askari et al., 2006).

The main objective of this study was to determine the composition of essential oils from aerial parts of *Pimpinella affinis* leddeb cultivated in Iran and to compare the data obtained from SFE and steam distillation. Also, by using Taguchi design the effect of process parameters on yield of extracts were investigated in two processes.

Experimental

Plant material

The aerial parts of *Pimpinella affinis* leddeb. were collected from Natural Resources Research Station Nowshahr (a city of Mazandaran Province in Iran) in June/July 2010. After sending to the laboratory, fresh plants were dried at room temperature. The stems/leaves were identified and milled to small particles.

Reagents

N-Butanol (99%) was purchased from Merck Co. to be used as the collecting volatile components. Parsa Gas

Co. supplied CO₂ with purity higher than 99.9% to use as the extracting solvent in supercritical extraction.

Steam distillation

Dried plant (50.0 g) were placed in the retort or still. After the steam flow rate reached to the required amount, the retort connected to steam source and steam passed through the plant to vaporize the volatile oils in the plant material. The amount of oil recovered (w/w, dry-basis) was measured gravimetrically.

SFE procedure

A schematic diagram of the experimental apparatus is shown in Figure 1. The extraction vessel was approximately 34 mL (stainless steel, height 0.11 m and internal diameter 0.02 m) placed in an equipped oven to regulate the temperature accurately. The powdered plant material (6.0 g) was mixed with glass beads and charged into the extraction vessel. Extraction process was carried out for 30 min in static mode and then 20 min in dynamic mode. The extracted materials collected in a test tube containing 5.0 ml n-butanol. In order to improve the collection efficiency, the tube was placed in an ice bath during the dynamic extraction stage. The yield of the extracted oil was calculated based on dry weight of plant materials (w/w).

GC and GC/MS analysis

The device used model was gas chromatograph (Thermo-UFM) equipped with FID (hydrogen flame ionization), Ph-5 column (10 m \times 0.1 mm i.d., film thickness 0.4 μ m), carrier gas; helium at a rate of 0.5 ml.min⁻¹. Oven temperature was programmed at 60°C for 3 min, then increased to 210°C at a rate of 3°C min⁻¹ and finally increased to 285°C at a rate of 80°C min⁻¹. Injector and detector temperatures were kept at 280°C. GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m \times 0.25 mm i.d., film thickness 0.25 μ m). oven temperature was programmed at 50-280 °C with rate of 4 °C/min; Injection chamber temperature was set 10 degrees higher than the final column temperature; carrier gas, helium at a linear velocity of 31.5 cm/s; split ratio, 1:60; ionization energy, 70 eV; scan time, 1 s; mass range, 40-300 amu.

Identification of the components

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices,

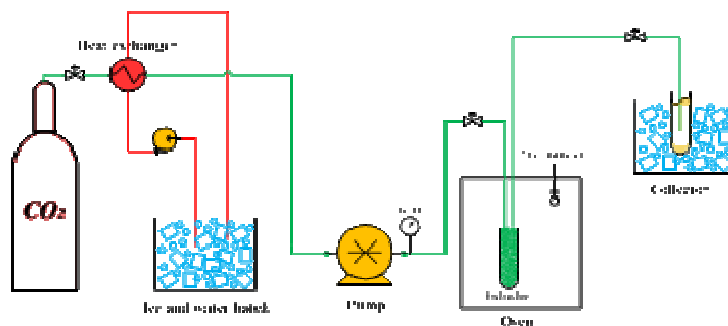


Figure 1. Schematic diagram of SFE apparatus.

Table 1. Values of the variables in DOE (design of experiments)

Process	Factors	Low level	High level
		(-1)	(+1)
Steam distillation	Extraction time (min), X_1	60	90
	Temperature (K), X_2	362	372
SFE	Temperature (K), X_1	318	328
	Pressure (MPa), X_2	15	25

Table 2. Taguchi design with corresponding response for SFE (using coded variables)

Standard order	Experimental parameters		Volatile components yield
	No.	X_1	X_2
1	-1	-1	2.70
2	-1	+1	4.30
3	+1	-1	3.40
4	+1	+1	5.20

either with those of authentic compounds or with data published in the literature. The retention indices were calculated for all volatile constituents using a homologous series of *n*-alkanes (McLafferty et al., 1997; Sandra and Bicchi, 1987; Adams, 2002).

Experimental design

Taguchi design was used to determine the effects of temperature and pressure on the yield of SFE and extraction time and temperature on the yield of steam distillation. In design of experiments, the variable parameters, denoted by X_i , were coded as -1 and +1, for the low and high level, respectively (Table 1).

RESULTS AND DISCUSSION

Two sets of experiments were carried out randomly, and the weight of isolated oil was determined on the base of initial weight of dried plant as the response. The

results of SFE and steam distillation methods are presented in Tables 2 and 3, respectively. As shown by these tables the total yield in SFE and steam distillation at different operating conditions vary in the range of 2.70-5.20 wt.% and 0.39-0.82 wt.%, respectively. According to these results, it is found that efficacy of oil isolation by SFE is very higher than steam distillation method.

Separation and identification of the components in the oil and extracts was performed by gas chromatography connected to the mass spectrometer (GC/MS). Table 4 shows the identified compounds obtained by SFE and steam distillation. It was concluded that the extracted compounds in each process was different from each other. However *trans- α -Bergamotene* was the major constituent of oil which was obtained by the two methods. Three other components in essential oil of SFE that showed a higher percentage than the other components were (Z)- β - farnesene (3.24%), Bicyclogermacrene (2.65%) and (Z)- γ -Bisabolene (1.78%) which are used in flavour and fragrance

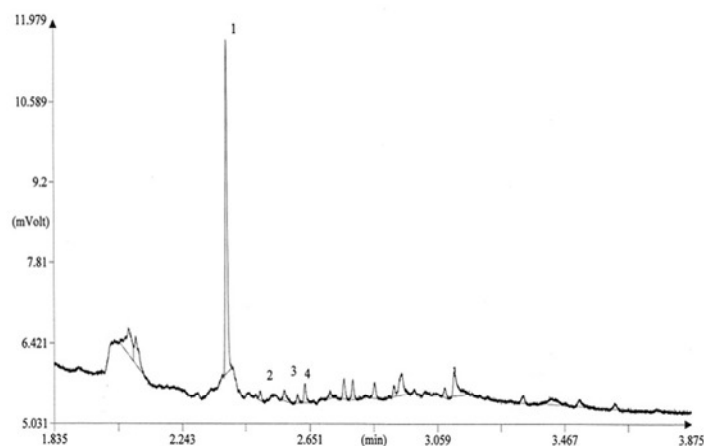
Table 3. Taguchi design with corresponding responses for steam distillation (using coded variables)

Standard order	Experimental parameters		Volatile components yield
No.	X ₁	X ₂	Y, wt. %
1	-1	-1	0.56
2	-1	+1	0.39
3	+1	-1	0.82
4	+1	+1	0.61

Table 4. *Pimpinella affinis ledeb* oils and extracts obtained by SFE and steam distillation.

No.	Compound	R.I. ^a	SFE (%)	Steam distillation (%)
1	α - gurjarene	1407	-	0.14
2	(E)- caryophyllene	1420	1.57	-
3	<i>trans</i> - α -Bergamotene	1432	87.76	91.44
4	(Z)- β - farnesene	1440	3.24	0.57
5	Germacrene D	1482	-	0.39
6	Valencene	1493	1.46	0.31
7	Bicyclogermacrene	1497	2.65	0.43
8	β - bisabolene	1503	-	0.26
9	(Z)- γ -Bisabolene	1512	1.78	-
10	Elemicin	1554	0.71	-
11	Germacrene B	1558	0.33	-
12	α -Bisabolol	1684	0.50	-
13	(E)- coniferyl alcohol	1732	-	6.46

The compositions is average of all experiment in each extraction procedures.
^a retention indices on Ph-5 column; ^b Percent of component based on the area normalization (P %).

**Figure 2.** GC chromatogram of supercritical CO₂ extract belonging to run 4 [temperature = 328 k, pressure = 25 MPa] 1- *trans*- α -Bergamotene; 2- bicyclogermacrene; 3- elemicin; 4- germacrene.

industry. In essential oils of steam distillation apart from *trans*- α -Bergamotene which was the main compound, (E)-coniferyl alcohol (6.46%) showed higher percentage from other compounds. The GC/MS chromatograms for

supercritical CO₂ extraction (run 4) and steam distillation (run 4) that are illustrated in Figures 2 and 3, respectively.

Analysis of variance (ANOVA) with confidence level of

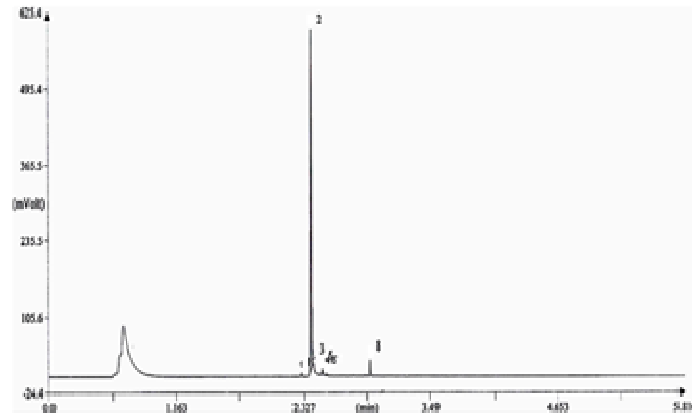


Figure 3. GC chromatogram for steam distillation belonging to run 4 [time= 90 min, temperature = 372 k,] 1: α – gurjarene; 2: trans- α -Bergamotene; 3: β - farnesene; 4: germacrene D; 5: valencene; 6: β - bisabolene; 7: bicylogermacrene; 8: E-coniferyl alcohol

Table 5. ANOVA for oils and extracts in SFE and steam distillation (SD).

Source	Degree of freedom	Mean square(MSS)	F-Value	P-Value
SFE:				
X ₁	1	0.64000	64.00	0.079
X ₂	1	2.89000	289.00	0.037
SD:				
X ₁	1	0.057600	144.00	0.053
X ₂	1	0.036100	90.25	0.067

* significant parameters (P-Value < 0.05)

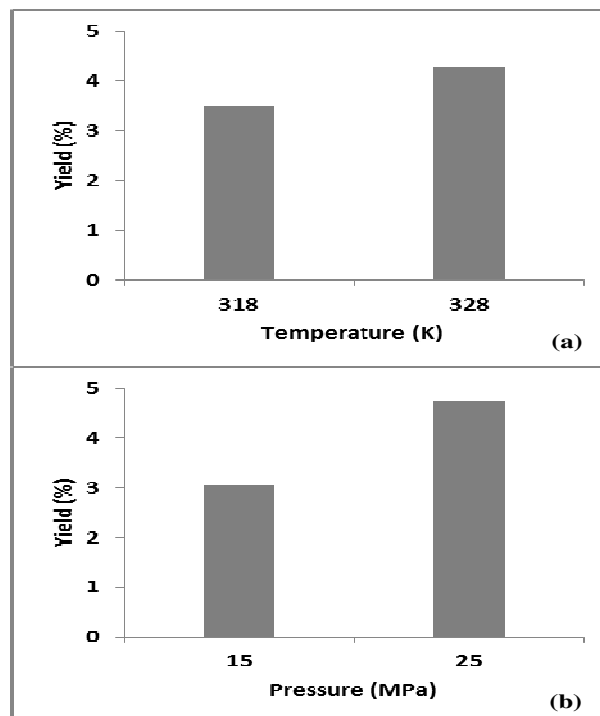


Figure 4. main-effect plot of parameters on extraction yield of SFE

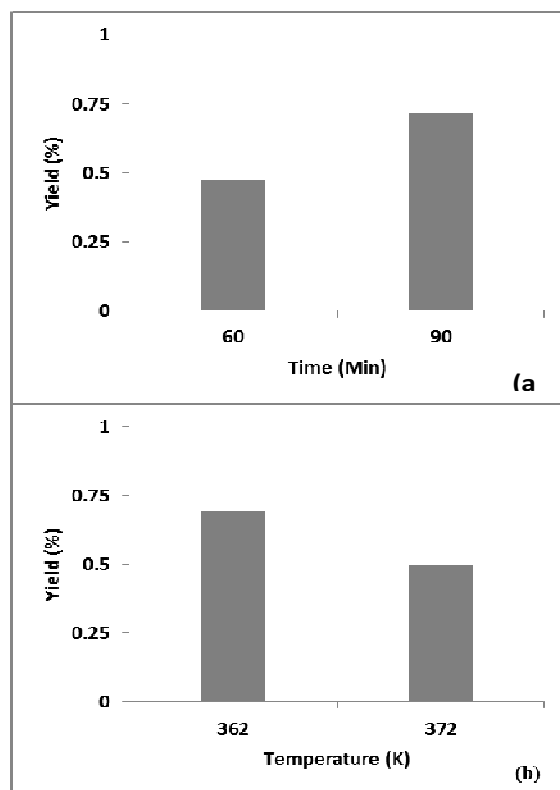


Figure 5. main-effect plot of parameters on extraction yield of steam distillation.

95% or significant area of 5%, was performed on the obtained results by using MINITAB 16 statistical software and significant effects of each parameter was analyzed. The summary ANOVA is shown in Table 5. In these results, those effects with calculated P-values less than 0.05 would be significant and if ($0.05 < P < 0.1$) the effect is possible significance. In Figures 4 and 5, the main effects of each parameter in SFE and steam distillation were shown, respectively.

As expected, the extraction yield significantly increased with pressure (Figure 4b), due to increase in the solubility of oil compounds in supercritical CO₂. This was attributed to the enhancement of CO₂ density at higher pressures (Machmudah et al., 2008).

The temperature showed two opposite effects in SFE process because it affects on both CO₂ density and volatility of the analytes in the solid matrix. By increasing the temperature, the volatilities of the analytes increase whereas the supercritical CO₂ density decreases (Martín et al., 2007). In this particular study, the positive effect of increasing volatility was more than the negative effect of CO₂ density reduction. Therefore, by increasing the temperature from 318 K to 328 K at the constant pressure, the extracted oil increased (Figure 4a).

Figure 5a shows that time has the positive effect and by passing the time, extraction yield increase during steam distillation. As it could be seen in Figure 5b,

temperature has the negative effect on the yield. Increasing the temperature has an influence on degradation of organic compounds. Therefore, a reduction is occurred in the yield of product.

CONCLUSIONS

Pimpinella affinis ledeb cultivated in Iran was processed to extract the oil and extracts by SFE and steam distillation. The effect of pressure and temperature in SFE and the effect of extraction time and temperature were evaluated in steam distillation. Results revealed that temperature and pressure had the positive effect on the extracted yield of SFE whereas in steam distillation, temperature has the negative effect on yield. Increasing temperature causes degradation of organic compounds in steam distillation. Therefore, a reduction occurred in the yield of product. But, the extraction time had positive effect on extraction yield. It was observed that in SFE, maximum yield was 5.2 wt.% at 25 MPa, 328 K during only 20 min dynamic conditions, whereas in steam distillation, the maximum yield was achieved 0.82 wt.% at atmospheric pressure and 362 K, during 90 min. So, SFE offers an important advantage over steam distillation for isolation of oil and *trans- α -Bergamotene* from *Pimpinella affinis ledeb* by a clean solvent, a safe process at low temperature preventing

degradation of *trans- α -Bergamotene* and within lower extraction time.

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