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ORIGINAL ARTICLE

Optimization of ultrasonic assisted extraction of fatty acids from *Borago Officinalis* L. flower by central composite design

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Abstract In the present study, the ultrasonic assisted extraction (UAE) of essential oils and fatty acids from *Borago officinalis* L. flower was developed by using *n*-hexane as extracting solvent. The obtained extracts were compared by hydrodistillation. Four parameters such as temperature, time, power of ultrasonic, and the ratio of extracting solvent volume to the weight of the plant were optimized using a central composite design after a full factorial design. Based on direct observation and analysis, the highest yields for UAE were obtained at a temperature of 48 °C, an extraction time of 30 min, minimum power of ultrasonic and in the ratio of extracting solvent volume to weight of plant 36:1 mL/g. The chemical compositions of the UAE extract were identified by GC–MS after derivation. The extraction yield base on ultrasonic assisted extraction varied in the range of 0.12–1.04% (w/w).

1. Introduction

Borago officinalis L. is an annual herb (De Haro-Bailón and Del Rio, 1998). All of its derivations consist of seeds, flowers, and leaves that have used for medicinal and culinary purposes. They can be used for many diseases such as rheumatoid arthritis (it has an anti-inflammatory effect), asthma, premenstrual, and multiple sclerosis (Namal Senanayake and Shahidi, 2002; Kim et al., 2010). Borage is one of the best sources of cis, cis, cis-6,9,12-octadecatrienoic acid, gamma-linolenic acid (GLA), (De Haro-Bailón and Del Rio, 1998; Kim et al., 2010; Wettasinghe and Shahidi, 1999; Venskutonis et al., 2008; Kotnik et al., 2006; Soto et al., 2008; Mhamdi et al., 2010; Jen Lin and Wan Chen, 2008). In the human body, GLA synthesizes from cis, cis-9,12-octadecadienoic acid, linoleic acid, but many factors such as stress, alcohol consumption, and diabetes can

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affect the amount of GLA produced. Under this condition, complementary drugs which contain GLA are necessary (De Haro-Bailón and Del Rio, 1998). Borage also contains beneficial components such as hexadecanoeic acid (palmitic acid), oleic acid, linoleic acid, octadecanoic acid (stearic acid) and also monoterpene hydrocarbons (Venskutonis et al., 2008; Mhamdi et al., 2010).

Choosing an extraction method depends on many factors such as effectiveness, capital cost, operating cost, simplicity of operation, and waste production. In the past, many extraction methods such as hot expelling, cold pressing, Soxhlet or extraction by organic solvents were used for extraction from Borage. However, these techniques are time-consuming and require large volumes of expensive organic solvents. By using a new extraction techniques (such as ultrasonic assisted extraction) in order to reduce the amount of solvent required, extraction time is also reduced. Therefore, UAE can also reduce the cost of sample preparation (Shen and Shao, 2005). Cavitation bubbles produced during the UAE process may attain significant internal temperature and pressure, so cell fragmentation of plant tissues is happened. Therefore by increasing of surface areas, the rate of mass transfer is dramatically increased, so that the extraction efficiency is improved (Bossio et al., 2008; Toma et al., 2001). Due to these excellent characteristics, the UAE became one of the best techniques for the extraction of plant tissues (Banjoo and Nelson, 2005; Zhang et al., 2009). This technique can adapt for use on a small or large scale. Two common devices in the application of ultrasound are: ultrasonic bath and probe units. The former is suitable to sample preparation for analytical purpose and the latter is efficient in large-scale extraction (Zhang et al., 2009; Wang et al., 2008).

In this approach, an experimental-design method based on a full factorial and central composite design has been developed to optimize the effectiveness of the UAE of essential oils and fatty acids from *Borago officinalis* L. In this paper, full factorial design 2^n, was used to determine the main factors. The selected factors in this approach were: temperature, time, power of ultrasonic bath, and the volume of extraction solvent used for certain amount of plants. The central composite design (CCD) and response surface methodology were applied to find the optimum levels of significant factors. STATGRAPHICS, statistical and graphical analysis software, was used for the experimental table generation and analysis of the results.

This work investigated the effects of the mentioned experimental parameters on the ultrasonic assisted extraction yield and selectively of *Borago officinalis* L. essential oils and fatty acids. To the best of our knowledge, no report has yet appeared on the UAE of the *Borago officinalis* L. flower.

2. Experimental

2.1. Reagents

HPLC grade hexane, methanol and sulfuric acid were purchased from Merck (Darmstadt, Germany). Nitrogen (99.99% purity), was obtained from Sabalan Co. (Tehran, Iran).

2.2. Plant material

Borago officinalis L. flowers were collected from Damash (Iran) in July 2010. Flowers were dried in a dark place at room temperature. Before extraction, they were kept in an oven at 30 °C, for 24 h. Then, the sample was ground in a blender to produce a fine powder. The average particle size was 0.4 mm.

2.3. Hydrodistillation

The flowers of the plant (40 g of shade dried flowers of plant) were submitted to hydrodistillation for 4.5–5.0 h, using a simple Clevenger-type apparatus which contained a 1000 mL flask, a condenser and measuring tube with stopcock. A return tube for the aqueous part of the distillate connects the bottom of the measuring tube and the vertical tube. The yield of the oil was 0.05% (w/w) based on dry plant weight.

2.4. Ultrasonic assisted extraction

A Power Sonic 505 HWASHIN Technology Korea, clean bath with constant frequency (40 kHz) was used for all the extractions. At first, plant powder (exactly 0.5 g) was charged into a suitable glass vessel. Then, the exact volume of hexane was added as an extracting solvent. For optimization of experimental parameters of UAE, a central composite design (CCD) after a full factorial design (2^4) was used. Table 1 shows the experimental condition of CCD for each run. After each run, with filtering of the solid material, 1 mL of the extract solution was poured into a sample vial for the methylation of fatty acids. The rest of solution was poured into a 50 mL beaker and the bubbling of the solution was done by nitrogen gas. Then, the weight of the extracted oil was measured, and the extraction yield was calculated (Table 1).

2.5. Derivation process

Esterification of extracted fatty acids is necessary prior to GC–MS analysis. For this purpose, 1 mL of extract solution was poured in a round bottomed flask. Ten milliliter of methanol with 2% sulfuric acid was added. The solution was refluxed for an hour at 65 °C and then it was poured to an extracting funnel. Ten milliliter of hexane and 10 mL distilled water were

Table 1 UAE central composite design condition and extraction yield for *Borage officinalis* L.

Factors run	Temperature (°C)	Ratio between extracting solvent and plant weight (mL g^{-1})	Extraction yield (%)
1	37.5	30	0.81
2	50	30	0.99
3	25	20	0.12
4	37.5	30	0.80
5	50	40	1.03
6	37.5	20	0.51
7	37.5	30	0.84
8	37.5	40	0.87
9	25	30	0.38
10	50	20	0.63
11	25	40	0.71
12	37.5	30	0.82

added to the solution. The organic layer consists of analytes and hexane was separated and used for GC–MS analysis.

2.6. GC–MS analysis

The GC–MS analysis was carried out on a TRACE MS (Thermo Quest-Finnigan, Italy) and equipped with a 60 m DB-5 column (5% biphenyl + 95% methylpolysiloxane) fused silica capillary column, 0.25 mm i.d. and 0.25 μm film thicknesses. The transfer line temperature was 250 °C. The ionization energy was 70 eV with a scan time of 0.4 s and mass range of 40–460 amu. Helium (purity 99.999%) was used as a carrier gas with a linear velocity of 1.1 mL/min. The components of the extract were identified by comparing their mass spectra with those in the NIST and Wiley mass spectra libraries. The data obtained were also confirmed by comparing their retention indices, either with those of authentic compounds or with data published in the literature (McLafetry and Stauffer, 1989; Sandra and Bicchi, 1987).

2.7. Optimization strategy of UAE

Since various parameters potentially affect the extraction process, these parameters have to be optimized in order to quantitatively extract the analytes of interest. Different parameters can affect the extraction yield such as temperature (A), power of ultrasonic (B), time(C) and the ratio of volume of extracting solvent to weight of the plant (D) were examined. In this study, for the estimation and optimization of effective parameters on extraction of *Borago officinalis* L. by UAE, full factorial design was applied for screening of the variables. After choosing the significant variables, in order to investigate the interaction between variables, a central composite design (CCD) was performed and a response surface equation was derived. The experimental design matrix and data analyses were performed using the STATGRAPHICS plus 5.1 software.

3. Result and discussion

3.1. Optimization of UAE

In order to select the variables that have main effect on the UAE, full factorial design at two selected levels for each parameter was used as a screening method. The total design matrix showed 20 runs (2^4 + 4 center points) to be carried out randomly to eliminate the effects of extraneous or nuisance variables. The ANOVA results were evaluated to determine the main effects. The results of 20 experiments, using a full factorial design for estimating the effects of the above factors, at two selected levels for each parameters, show that only temperature (A) and the ratio of volume of extracting solvent to the weight of the plant (D) are the most effective parameters. The analysis of the results is visualized using standardized main effect Pareto charts (P = 95%) as shown in Fig. 1. The results illustrated in Fig. 1 also confirm that only the factors of A and D are the most effective factors. The parameter is considered as a significant parameter when its value is higher than $\pm t$. All the other variables and their interactions are not significant factors in the studied range. Therefore, the central composite design for these two factors (A and D) was applied to determine the optimum condition of UAE.

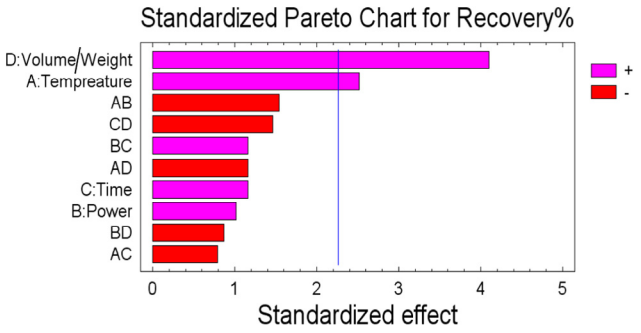


Figure 1 Pareto chart (P = 0.05) of full factorial design which represent the estimated effects of parameters and interactions on extraction yield.

The central composite design has been carried out on 12 randomized runs (2^2 + (2 × 2) + 4 center points.) The result of this design permitted the response to be modeled by fitting a second order polynomial, which can be expressed through the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{12} X_1 X_2 + \beta_{22} X_2^2$$

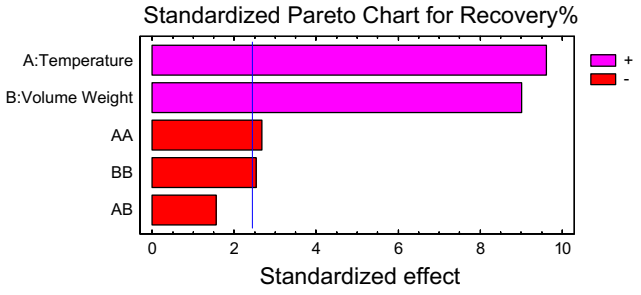


Figure 2 Pareto chart (P = 0.05) of central composite design which represent the positive significant effect of two main factors (temperature and ratio of volume of extracting solvent to the weight of plant upon the extraction).

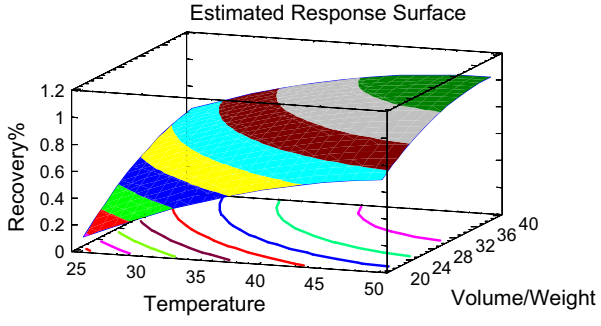


Figure 3 Estimated response surface by plotting extraction yield (%) versa temperature and the ratio between extracting solvent and plant weight.

Table 2 Composition of *Borage officinalis* L. extract obtained by ultrasonic assisted extraction.

No.	Compound	RI[a]	R2	R3	R4	R5	R6	R8	R9	R10	R11
1	α-Pinene	941	1.17	0.05	5.54	17.75	0.06	1.11	7.41	3.12	0.96
2	Camphore	1158	0.02	2.71	1.37	4.07	0.03	0.09	0.11	0.07	1.25
3	Borneol	1179	0.63	12.38	6.99	13.80	0.34	0.07	0.01	0.68	0.09
4	Carvacrol	1299	0.04	0.01	0.02	0.03	0.03	0.07	7.24	0.02	86.18
5	Caryophyllene	1143	0.07	0.01	0.09	0.01	0.03	0.10	0.01	0.01	0.01
6	γ-Candinene	1536	0.30	0.62	0.72	0.49	1.00	0.50	0.00	0.23	0.27
7	Candinol	1663	26.46	5.87	6.54	5.47	17.33	27.73	6.67	3.67	0.01
8	Hexadecanoeic acid methyl ester	1919	40.09	39.57	37.58	31.67	35.82	42.76	30.29	43.01	5.83
9	9,12-Octadecadienoic acid methyl ester	2201	13.76	30.40	28.09	20.39	30.16	22.74	28.19	38.31	3.89
10	6,9,12-Octadecatrienic acid methyl ester	2205	3.97	8.13	6.70	5.04	9.03	0.23	8.19	9.06	0.94
11	Octadecanoic acid methyl ester	2222	9.16	0.24	1.38	1.27	2.89	4.38	9.00	0.39	0.09
	Other compounds		4.34	0.01	4.98	0.01	3.28	0.40	2.88	1.43	0.48

[a] Kovat's retention indices on DB-5 column.

In the above equation, Y is the response function, x_1 and x_2 are the independent variables, β_0 is an intercept, β_1, β_2, β_{11}, β_{12} and β_{22} are the regression coefficients.

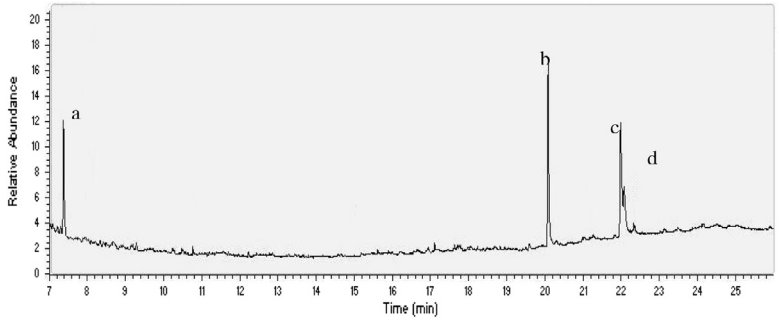
The data obtained were evaluated by the ANOVA test and the effects of variables on the extraction efficiency are shown by using Pareto chart in Fig. 2. As Fig. 2 shows, the entire two factors of temperature and the ratio of volume of extracting solvent to the weight of the plant have positive significant effect upon the extraction. As it was shown in Fig. 2, temperature has the largest influence on the extraction yield. The extraction yields were increased with an increase in the temperature, because raising the extraction temperature leads to a rising in the effective collision. The increase of the oil diffusion coefficient and the raising solubility of the oil in the extracting solvent at higher temperatures caused the increase of the oil mass transfer from the plants into the solution.

Fig. 2 shows that the solvent to plant ratio is a second important factor that has positive significant effect on extraction yields. The oil extraction yield increased with increasing the solvent to plant ratio due to a higher driving force in the more diluted solution. Therefore, the salvations of analytes were increased in the more diluted solution, as the extraction yields increased.

The estimated response surface for the temperature and the solvent to plant ratio versus the extraction yield and related counters is shown in Fig. 3. After analyzing the results, the following conditions were selected to evaluate the performance of the extraction procedure: temperature of 48 °C and solvent to plant ratio of 36:1 mL/g.

3.2. GC–MS analysis

As mentioned in Section 2.5, the fatty acid (FA) determination included extraction of lipid samples with organic solvents, followed by transformation of the isolated lipid to fatty acid methyl esters (FAME) and quantification of FAME by GC–MS. Table 2 shows their peak area ratios obtained by hydrodistillation and different condition of UAE. As shown in Table 2, there were many useful compounds that were extracted from Borage flowers; these include α-pinene, camphor, hexadecanoic acid, 9,12-octadecadienoic acid, cis, cis, cis-6,9,12 octadecatrienoic acid, cis, cis-9,12-octadecadienoic acid, octadecanoic acid. Fig.4 shows GC–MS chromatogram of the extracting compounds obtained by the optimum condition of UAE (temperature of 48 °C and the ratio of extracting solvent volume to weight of plant 36:1 mL/g). The major components of oil were based on hydrodistillation, except aliphatic alkanes, which belong to terpenes were α-cadinene (14.6%), α-thujene (13.6%),



(a) α – Pinene
(b) Hexadecanoeic acid methyl ester
(c) 9,12- Octadecadienoic acid methyl ester
(d) 6,9,12- Octadecatrienoic acid methyl ester
(e) Octadecanoic acid methyl ester

Figure 4 GC–MS chromatogram of UAE extract in optimum condition.

(E,E)-2,4 decadienal (10.2%), α-pinene (8.2%), ledene (7.7%) and viridiflorol (7.2%). As it can be concluded from Table 2, the obtained components by hydrodistillation and UAE were very different. The major components of UAE were fatty acids (58.37%), while the terpenes (51.3%) were mainly obtained by hydrodistillation (Fasih Ramandi et al., 2011).

4. Conclusion

In this study, the effect of different parameters such as temperature, time, power of ultrasonic and the ratio of extracting solvent volume to the weight of the plant on the ultrasonic assisted extraction of *Borago officinalis* L. was investigated.

The condition of extraction was optimized using the chemometric method. At a temperature of 48 °C, extraction time of 30 min, power of ultrasonic minimum and the ratio of extracting solvent volume to the weight of plant 36:1 mL/g; the highest oil extraction yield of 1.04% were obtained.

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