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Chemical composition of the essential oil and supercritical CO_2 extracts of *Zataria multiflora* Boiss

Homeira Ebrahimzadeh[a], Yadollah Yamini[b,*], Fatemeh Sefidkon[c], Marzieh Chaloosi[d], Seied Mahdi Pourmortazavi[b]

[a]*Department of Chemistry, Shahid Beheshti University, Tehran, Iran*
[b]*Department of Chemistry, Tarbiat Modarres University, Tehran, Iran*
[c]*Research Institute of Forests and Rangelands, PO Box 13185-116, Tehran, Iran*
[d]*Faculty of Chemistry, Teacher Training University, Tehran, Iran*

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Abstract

Essential oil of *Zataria multiflora* Boiss, cultivated in Iran, was isolated by steam distillation and compared with supercritical fluid CO_2 extracts. The oils and extracts were analyzed by capillary gas chromatography, using flame ionization and mass spectrometric detection. Different parameters, such as temperature, pressure, extraction period (dynamic) and modifier (methanol) concentration were employed to maximize the SFE efficiency. The results showed that, under optimum conditions ($P = 30.4$ MPa, $T = 55$ °C, $t_{\text{dynamic}} = 20$ min and $V_{\text{modifier}} = 0$ µl) extraction of *Z. multiflora* Boiss was more efficient. Chemical analysis revealed that components extracted under different SFE conditions possessed widely different percentages of constituents [thymol (14.2–67.6%), λ-terpinene (0.1–19.5%) and ρ-cymene (3.6–12.0%)]. The amounts, of co-extracted cuticular waxes varied too. Oil obtained by steam distillation was also compared to the SFE extracts. The results showed that the major components of *Z. multiflora* Boiss were thymol (44.6%), λ-terpinene (21.5%) and ρ-cymene (13.7%), based on steam distillation.

Keywords: *Zataria multiflora* Boiss; Supercritical; Carbon dioxide; Steam distillation; Essential oil; Thymol; λ-Terpinene; ρ-Cymene

1. Introduction

An essential oil can be defined as the volatile material present in plants (Reverchon, 1997; Spencer, 1992). As a rule, it consists of a complex mixture of terpenes, oxygenated terpenes, sesquiterpenes and oxygenated sesquiterpenes. The essential oil of plants is usually isolated by steam or hydro distillation and solvent extraction (Keita, Vincent, Schmit, & Belanger, 2000; Kerrola, Galambosi, & Kallio, 1994; Lorenzo, Dellacassa, Bonaccorsi, & Mondello, 2001; Luque de Castro & Garcia-Ayuso, 1998). The disadvantages of all these techniques are: low yield, losses of volatile compounds, long extraction times, toxic solvent residues, and degradation of unsaturated compounds, giving undesirable off-flavour compounds (Assis, Silva pinto, & Lancas, 2000; Lehotay, 1997; Oszagyan et al., 1996; Sato, Goto, & Hirose, 1995), due to heat. One of the most common applications of supercritical fluid technology in the natural product industry is patented (Vitzthum & Hubert, 1975). Supercritical fluid extraction has received increasing attention in a variety of fields due to the following features: (a) supercritical fluids provide high solubility and improved mass-transfer rates; (b) operation can be manipulated by changing the pressure or temperature; (c) using SFE, the natural characteristics of the oils are conserved and thermal degradation or hydrolysis and solvent contaminations are avoided; and (d) SFE offers a rapid method for extraction of oils from complex matrices (Anitescu, Doneanu, & Radulescu, 1997; Dugo et al., 1995; Roy, Goto, & Hirose, 1996). Carbon dioxide is widely used as an extraction fluid, because it is chemically inert, non-toxic, inexpensive, colourless, odourless, tasteless, readily available,

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* Corresponding author. Tel.: +98-21-800-6631; fax: +98-21-800-6544.

E-mail address: yyamini@modares.ac.ir (Y. Yamini).

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non-pollutant, non-flammable, safe, excellent for extraction of non-polar and moderately polar analytes. Selectivity or solvent power is adjustable, ranging from gas-like to liquid-like values. Also, CO_2 evaporates on decompression at the restrictor outlet, which facilitates off-line collection of extracts in a solvent for subsequent analysis or on-line combination with methods such as GC (Modey, Mulholland, & Raynor, 1996). In order to extract polar or ionic compounds, via SFE, organic solvents have been added as modifiers or the compounds are first derivatives to decrease their polarity (Ashraf-Khorassani & Taylor, 1997; Castaneda-Acosta, Cain, Fischer, & Knopf, 1995; Lin, Tsai, & Wen, 1999; Michael & James, 1996). Avishan Shirazi is the Persian name for *Zataria multiflora* Boiss (*Z. multiflora*), belonging to the family Labiatae, and it is native to Iran. *Z. multiflora* is used traditionally in food, especially in yoghurt flavouring. There are also commercial pharmaceuticals with formulae based on *Z. multiflora* essential oil. This oil has been used commonly in medicine for the treatment of respiratory tract as an antiseptic, antitussive and irritable bowel syndrome treatment (Aynehchi, 1991). Also, the extracts of aerial parts of *Z. multiflora* showed anti-inflammatory effects against acute and chronic inflammations in mice and rats (Hosseinzadeh, Ramezani, & Salmani, 2000). The aim of the present work is the investigation of the effects of different parameters, such as pressure, temperature, modifier volume and dynamic extraction time, on the supercritical fluid carbon dioxide extraction of *Z. multiflora*. The essential oil obtained by steam distillation was used for comparison. Steam distillation of *Z. multiflora* has already been reported (Shafiee & Javidnia, 1997). However, to the best of our knowledge no report has yet appeared on the SFE of *Z. multiflora*.

2. Material and methods

2.1. Plant material

The plant materials (cultivate) were collected from the research station of Alborz, near Tehran, Iran, in June 2001. The dried plant was stored in the herbarium of the research Institute of Forests and Rangelands (TARI). Immediately prior to SFE, the sample was ground in a blender to produce a fine powder. The average particle size was 0.4 mm.

2.2. Steam distillation

The oil of the plant (93 g) was obtained by a steam distillation method in a Clavenger-type apparatus. The extraction yield was 3% (w/w), based on the dry weight.

2.3. Reagents

HPLC grade dichloromethane and methanol were purchased from Aldrich. Carbon dioxide (99.99% purity), contained in a cylinder with an eductor tube, was obtained from Sabalan Co. (Tehran, Iran).

2.4. Supercritical fluid extraction (SFE)

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode was used for all the extractions. The extraction vessel was an 8 ml stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 10.1, 20.3 and 30.4 MPa and temperatures of 35, 45 and 55 °C for durations of 15 min static, followed by 10, 20 or 30 min dynamic. A Duraflow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes. In order to prevent sample plugging, the restrict point was warmed electrically. The supercritical CO_2 flow rate through the Duraflow restrictor was approximately 0.3–0.4 ml/min (compressed). The extract was collected in a 2 ml volumetric flask containing dichloromethane. Plant powder (2.5 g) was mixed with 2 mm diameter glass beads, and then charged into the 8 ml extraction vessel. The plant was then extracted with supercritical CO_2 under various conditions in accordance with the Taguchi method (Roy, 1990). Table 1 shows the experimental conditions for each of the SFE runs. The extracted analytes were collected in dichloromethane in a 2.0 ml volumetric flask. The final volume of the extract was adjusted to 2.0 ml with dichloromethane at the end of the extraction. In order to improve the collection efficiency, the 2.0 ml volumetric flask was placed in an ice bath during the dynamic extraction stage. For all the modifier studies, methanol was spiked directly into the extraction vessel with charged sample prior to the extraction.

2.5. GC and GC/MS analysis

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a FID and a DB-1 fused silica column (60 m×0.25 mm i.d., film thickness 0.25 μm). Oven temperature was programmed at 50 °C for 5 min, then increased to 250 °C at a rate of 4 °C/min. Injector and detector temperatures were 250 and 265 °C, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The SFE samples (1 μl) were injected (without any further dilution) using the split mode with a split ratio of 1/60. Steam-distilled extracts were diluted 45 times and 1 μl of diluted solution was injected into the GC with the same split ratio.

The GC/MS analysis was carried out on a Varian 3400 equipped with a DB-1 column with the same characteristics as the one used in GC. The transfer line

Table 1
SFE experimental conditions for *Zataria multiflora* Boiss extraction

Run	Pressure (MPa)	Temperature (°C)	Dynamic time (min)	Modifier volume (µl)
1	10.1	35	10	–
2	10.1	45	20	80
3	10.1	55	30	400
4	20.3	35	20	400
5	20.3	45	30	–
6	20.3	55	10	80
7	30.4	35	30	80
8	30.4	45	10	400
9	30.4	55	20	–

temperature was 260 °C. The ionization energy was 70 eV with a scan time of 1 s and mass range of 40–300 amu. The percentages of compounds were calculated by the area normalization method, without considering response factors. The components of oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds. Data obtained were confirmed by comparison of their retention indices, either with those of authentic compounds or with the data published in the literature (Sandra & Bicchi, 1987).

3. Results and discussion

3.1. General

A static–dynamic approach was tested for all extractions of *Z. multiflora* and the results were compared with essential oil composition obtained through steam distillation. A static extraction period was employed in order to increase the sample-extractant contact duration. This was followed by a dynamic extraction period in which extractant passed continuously through the extraction chamber, thus, displacing the equilibrium towards quantitativeness.

3.2. Optimization of the experimental conditions

Several parameters have to be optimized in order to extract the analytes of interest quantitatively, in a short period of time. Among them the pressure and temperature of the fluid, the nature and concentrations of the modifier and the extraction times are generally considered as the most important factors. The optimization of the method can be carried out step-by-step or by using an experimental design. Table 1 shows different conditions of experiments carried out with SFE for extractions of *Z. multiflora* according to the Taguchi experimental design (Roy, 1990). By considering total GC peak area under different conditions (Table 2), the optimum values found for different variables are: $P = 30.4$ MPa, $T = 55\,^{\circ}\mathrm{C}$, $t_{\mathrm{dynamic}} = 20$ min and $V_{\mathrm{modifier}} = 0$ µl. In these conditions the extraction efficiency is similar to that of steam distillation. For the complete recovery of the main components of the plant, higher pressures are necessary. This is because raising the extraction pressure, at constant temperature, leads to higher fluid density, which increases the solubility of the analytes. To obtain quantitative recovery of analytes, they must be efficiently partitioned from the sample matrix into the supercritical fluid. Raising the extraction temperature to 55 °C made the partitioning process easier by increasing the vapour pressure of the main components. The influence of the dynamic extraction time on the composition of the extracts was studied. Extraction was

Table 2
Zataria multiflora Boiss oils obtained by SFE and steam distillation (the compounds are listed in order of elution time from a DB-1 column)[a]

No.	Compound	R.I.[b]	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	S.D.
1	α-Thujene	933	–	–	0.70	1.27	–	0.33	–	0.25	0.19	0.68
2	α-Pinene	962	–	1.85	0.11	–	–	1.42	1.10	1.22	1.08	1.23
3	Myrcene	981	0.89	1.39	1.11	5.44	–	0.74	0.57	0.71	0.62	1.93
4	α-Terpinene	1009	1.45	1.48	0.09	–	–	0.57	0.47	0.42	0.74	1.84
5	ρ-Cymene	1013	5.94	9.30	12.0	9.44	3.63	6.63	4.54	6.91	4.50	13.7
6	λ-Terpinene	1051	14.0	19.5	0.05	–	9.31	8.01	6.96	10.45	7.62	21.6
7	Trans-sabinene hydrate	1054	–	1.80	25.0	0.93	1.48	1.72	1.35	1.57	1.19	1.48
8	Linalool	1083	–	1.45	1.26	–	1.68	1.49	1.17	1.38	1.38	1.37
9	Borneol	1161	0.72	–	0.35	–	–	0.01	0.23	0.23	0.76	0.24
10	Thymol	1274	14.22	48.6	38.5	64.7	67.6	65.0	65.2	60.2	67.0	44.6
11	Carvacrol	1280	–	3.25	3.05	4.15	4.28	3.68	3.71	3.38	4.30	2.35
12	α-Terpinenyl acetat	1332	–	–	–	–	–	–	–	0.24	0.24	0.33
13	β-Caryophyllene	1417	–	2.35	1.89	1.74	2.12	2.55	1.98	2.17	2.40	2.20
14	Trace components	920–1417	7.98	4.30	9.74	7.54	6.09	5.95	8.55	8.43	6.27	5.53
15	Heavy components	>1670	54.8	3.54	2.16	4.16	3.80	1.91	4.16	2.6	1.7	0.90
	Total area	–	1.68	11.7	25.8	12.2	8.20	26.9	48.7	50.0	262	300

[a] Chromatographic areas.

[b] Retention indices

performed with supercritical carbon dioxide at the extraction step of 15 min, followed by 10, 20 and 30 min of dynamic extractions. Results showed that increased dynamic extraction time to 20 min enhanced the extraction of most components. In the present work the modifier does not influence the extraction efficiency of the main components.

Steam distillation of *Z. multiflora* showed a relatively simple GC/MS chromatographic pattern. *Z. multiflora* oil constituents obtained by steam distillation and SFE extracts are presented in Table 2. The major compounds, based on steam distillation are: thymol (44.6%), λ-terpinene (21.5%), ρ-cymene (13.7%), carvacrol (2.35%), and β-caryophyllene (2.20%). It is noteworthy that the extracts obtained by SFE under different conditions had compositions similar to that of the oil obtained by steam distillation. However, the quantitative compositions of the two products are quite different. Indeed, at higher pressures and temperatures (30.4 MPa and 55 °C), thymol and carvacrol are more soluble in supercritical carbon dioxide. Thus, for these components, the selectivity of extraction is increased in comparison with steam distillation (Table 2). Thymol and carvacrol are two major constituents in most essential oils, including oils used in a variety of drugs. As shown in Table 2, SFE offers a rapid method for extraction of oil. Depending on the extraction conditions (Runs 5, 6, 7 and 9), SFE may show a high selectivity for thymol. In all obtained extracts, λ-terpinene and carvacrol appear to be the major components. These extracts are richer in thymol. However, the recovery of thymol in SFE is better than that by steam distillation. In run 1 *Z. multiflora* major components are not present; instead, sesquiterpene compounds are found. The major disadvantage of the oil obtained by SFE is the presence of co-extracted cuticular waxes.

4. Conclusion

The supercritical fluid extraction of *Z. multiflora* was studied, and the results were compared with essential oil composition obtained by steam distillation. The SFE method offers many important advantages over steam distillation. SFE requires shorter extraction times (30 min vs. 1 h for steam distillation). Energy cost is rather higher for performing steam distillation than that for achieving SFE conditions. The possibility of manipulating the composition of the oil, by changing the parameters of the extraction (pressure, temperature, modifier volume and dynamic extraction time) is more attainable in SFE. Although the compositions of the oils obtained by SFE and steam distillation are not qualitatively different, they differ quantitatively. We obtained a higher selectivity at high pressure in SFE than that by the steam distillation method.

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