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Supercritical Carbon Dioxide Extraction of Essential Oils from *Perovskia atriplicifolia* Benth.

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The supercritical fluid extraction (SFE) of the aerial parts of *Perovskia atriplicifolia* Benth. was studied. The effect of different parameters such as pressure, temperature, modifier identity, and modifier volume on the SFE of the plant was investigated. The extracts were analyzed by GC and GC-MS and compared with the essential oil obtained from *P. atriplicifolia* Benth by steam distillation. The supercritical extracts and the steam-distilled products had very different compositions. The main constituents of the oil obtained by steam distillation were 1,8-cineole, limonene, camphor, β-caryophyllene, α-pinene, camphene, and α-humulene. On the other hand, the major components of SFE extracts were 1,8-cineole, limonene, camphor, β-caryophyllene, γ-cadinene, α-pinene, and α-terpinyl acetate. The results showed that increasing the temperature from 35 to 65 °C (at a constant pressure of 100 atm) drastically reduced the number of extracted components. Also, the number of extracted constituents and the percent of main analytes increased when lower pressures were used. Using different modifiers (e.g., methanol, ethanol, dichloromethane, and hexane) for the extraction of the plant at low pressure (100 atm) and temperature (35 °C) showed that hexane was more selective than the other modifiers.

INTRODUCTION

Perovskia atriplicifolia Benth. grows wild in rocky places of Afghanistan and Pakistan. This plant is used as a cooling medicine in the treatment of fevers. The small lavender flowers have a sweet flavor and can be eaten in salads or used as a garnish. Also, this species is suitable for forming a decorative hedge of moderate height. The oil of *P. atriplicifolia* was found to be rich in β-thujone, sabinene, α-pinene, and 1,8-cineole (*1*). These compounds are important products thanks to their pharmaceutical and nutritional uses (for their antibacterial and flavoring properties). In the essential oil obtained from the glandular trichomes of *P. atriplicifolia* cultivated in Italy, camphor (14.9%) was the main constituent (*2*). Rao (*3*) identified eight components in the oil of *P. atriplicifolia*. Younos et al. (*4*) found that the oil of *P. atriplicifolia* grown in Afghanistan contained α-pinene, β-pinene, camphene, α-terpinene, eucalyptol, camphor, borneol, menthol, bornyl acetate, β-caryophyllene, and cedrol. The essential oil of *P. atriplicifolia* cultivated in Iran was found to contain 1,8-cineole + limonene (40.13%), α-pinene (9.13%), β-pinene (6.59%), camphene

(6.17%), and camphor (5.36%) (*5, 6*). More recently, the essential oil of *P. atriplicifolia* grown wild in Quetta, Pakistan (and the structure of its compounds), has been described (*7*). Of 19 compounds identified in this oil, the monoterpenes, δ-3-carene (22.3%) and 1,8-cineole (27.5%), amounted to up to 50% of the total oil. On the other hand, β-caryophyllene (10.8%) and α-humulene (5.7%) were the dominant sesquiterpenes. Also, Ali et al. (*8*) isolated a new acylated steroid glucoside from *P. atriplicifolia* grown in Baluchistan, Pakistan.

Steam distillation has traditionally been applied for essential oil recovery from plant materials. This technique presents some shortcomings, namely, losses of volatile compounds, low extraction efficiency, and long extraction time. Also, elevated temperatures and water can cause degradation or chemical modifications of essential oils (*9−11*). As an alternative, supercritical fluid extraction (SFE) is a particularly suitable method for the extraction of natural materials. The importance of SFE has increased in food application in recent years (*12−14*). The most popular supercritical fluid is CO₂. Carbon dioxide is an excellent extraction medium for nonpolar species such as alkenes and terpenes. It is also reasonably good for moderately polar species, including polycyclic aromatic hydrocarbons (PAHs), aldehydes, esters, alcohols, and fats, but it is less useful for polar components (*15−19*). The extraction of polar mol-

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Table 1. Experimental Conditions for Supercritical Fluid Extraction of *P. atriplicifolia* Benth. Essential Oils

run	pressure (atm)	temp (°C)	modifier	modifier vol (μL)
1	100	35		
2	200	35		
3	300	35		
4	100	45		
5	100	55		
6	100	65		
7	100	35	MeOH	80
8	100	35	MeOH	400
9	100	35	EtOH	80
10	100	35	EtOH	400
11	100	35	CH_2Cl_2	80
12	100	35	CH_2Cl_2	400
13	100	35	C_6H_{14}	80
14	100	35	C_6H_{14}	400
15	300	45		
16	300	45	MeOH	80

ecules requires addition of a modifier, most commonly methanol or ethanol (*20−22*).

The aim of this work was the investigation of the effects of different parameters such as pressure, temperature, modifier [such as *n*-hexane, dichloromethane, methanol, and ethanol] and its concentration on the composition of *P. atriplicifolia* Benth. essential oil obtained by supercritical fluid carbon dioxide extraction, qualitatively. The essential oil obtained by steam distillation was used for comparison. To the best of our knowledge, no report has yet appeared on the SFE of *P. atriplicifolia* Benth.

EXPERIMENTAL PROCEDURES

Materials. Plant material was collected at flowering stage from the National Garden in Tehran (in September 2000). A herbarium specimen (no. 75598) has been deposited at the herbarium of the Research Institute of Forests and Rangelands (TARI).

The HPLC grade solvents were all purchased from Aldrich (Tehran, Iran). Pure carbon dioxide (Sabalan, Tehran, 99.99%) was used for all supercritical fluid extractions.

Apparatus and Procedures. All SFE experiments were performed on a Suprex MPS/225 (Pittsburgh, PA) in the SFE mode. Extractions were performed with an 8 mL volume extraction vessel at three different pressures (100, 200, and 300 atm) and four different temperatures (35, 45, 55, and 65 °C) for a duration of 15 min static followed by 20 min dynamic. A Duraflow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes. The supercritical CO_2 flow rate through the Duraflow restrictor was ~0.4−0.5 mL/min (compressed).

Exactly 1.0 g of the shade-dried aerial parts of the plant, mostly leaves and flowers, was mixed with 2 mm diameter glass beads and then charged into extraction vessel. This procedure was used to maximize the contact surface between the plant material and the supercritical carbon dioxide under various conditions of pressure, temperature, modifier, and modifier volume. **Table 1** shows the experimental conditions for each of the SFE runs. The extracted analytes were collected in 3 mL of dichloromethane in a 5 mL volumetric flask (for preventing the loss of solvent due to bubbling). The final volume of the extract was adjusted to 5.0 mL of dichloromethane at the end of the extraction. To obtain better collection efficiency, the 5.0 mL flask was placed in an ice bath during the dynamic extraction stage. For all of the modifier studies, the modifiers were spiked directly into the extraction vessel prior to the extraction.

A simple laboratory quickfit apparatus, which contained a 2000 mL steam generator flask, a distilling flask, a condenser, and a receiving vessel, was used to perform the steam distillation. An 80 g shade-dried aerial part of the plant was subjected to steam distillation in the assembly described above. The steam generator flask was filled with 1000 mL of distilled water and heated with a heating mantle. As water was vaporized, the steam passed through the distillation flask containing the plant. The vapor passed through the cooled tube, where it condensed. The volatile components were collected into the receiving flask (500 mL) during 1.5 h of steam distillation.

GC and GC-MS Analyses. The gas chromatographic analyses were performed by a Shimadzu 9A gas chromatograph equipped with a flame ionization detector. A fused-silica column (60 m × 0.25 mm i.d., film thickness = 0.25 μm) coated with a DB-1 (J&W Scientific, Folsom, CA) stationary phase was used. Helium was the carrier gas at a linear velocity of 30 cm/s. The column temperature program was initial temperature of 50 °C, ramped at 4 °C/min to 250 °C. The injector and detector temperatures were 250 and 265 °C, respectively.

The SFE samples (1 μL) were injected (without any further dilution) using the split mode with a split ratio of 1/60. Steam-distilled volatile oils were diluted 30 times with dichloromethane, and 1 μL of diluted solution was injected into the GC-MS. The analysis was carried out on a Varian 3400 equipped with a DB-1 column with the same characteristics as the one used in the GC. The transfer line was 260 °C, the ionization energy 70 eV, the scan time 1 s, and the mass range 40−300 amu. The percentage of compounds was calculated by the area normalization method without considering response factors. The components of extracts and volatile oils were identified by comparison of their retention time and mass spectra with those of Sigma Chemical Co. standard compounds and those published in the previously published literature by different authors (*1−4, 23*).

RESULTS AND DISCUSSION

The principal parameter for determining the solubility of an organic molecule in CO_2 is the density of the CO_2 phase (*24*). The density determines the number of interactions between CO_2 and molecules of the organic compounds. If sufficient interactions occur, the cohesive forces between individual molecules of the organic compound are broken and solubilization will occur. Therefore, the solubilization of organic molecules in CO_2 will be a function of the molecular weight of the organic compound and the level of interaction between CO_2 and organic molecules (*25, 26*).

Results of our experiments showed that the major compounds of *P. atriplicifolia* are α-pinene, camphene, β-pinene, δ-3-carene, 1,8-cineole + limonene, camphor, β-caryophyllene, α-humulene, and T-cadinol. Detailed identification and quantitation of the compounds found in *P. atriplicifolia* volatile oils collected by SFE runs and steam distillation were performed by GC-MS and are reported in **Table 2**.

Effects of Pressure and Temperature. Pressure and temperature are the two most important physical parameters in SFE, and they have both theoretical and practical implications for the extraction process. Together, they define the density of the supercritical fluid. The maximum extraction fluid density is achieved with high pressure at temperatures close to the critical temperature of the fluid. The common belief among analytical chemists is that the maximum solubility of a solute in the supercritical fluid is achieved at the maximum fluid density.

In the first part of this study, the effect of pressure on the composition of extracted essential oils at three different levels (100, 200, and 300 atm) was investigated. The results showed that the concentrations of α-pinene, camphene, β-pinene, δ-3-carene, 1,8-cineole + limonene, and camphor in the essential oil, as main compounds, decreased when higher pressures were used (**Figure 1**). However, increasing pressure enhanced the contents of β-caryophyllene, α-humulene, γ-cadinene, and T-cadinol in the plant oils.

The effect of four different temperatures (35, 45, 55, and 65 °C) at constant pressure (100 atm) on the extraction of essential oils from *P. atriplicifolia* using pure supercritical CO_2 was

Table 2. Composition of *P. atriplicifolia* Oils Obtained by Steam Distillation (SD) and SFE Runs (According to **Table 1**)

no.[a]	compound	RI[b]	SD	run 1	run 2	run 3	run 4	run 5	run 6	run 7	run 8
1	α-pinene	942	7.3	4.3	4.3	3.7	14.1	15.1	14.0	5.7	3.7
2	camphene	953	2.9	2.7	2.4	2.0	5.1	6.8	5.6		
3	β-pinene	976	2.7	1.9	1.8	1.2	4.2	5.1	5.9	5.2	3.3
4	myrcene	985	0.7	0.3	0.3		1.5		1.4		
5	δ-3-carene	1006	5.4	4.9	4.4	3.5	6.8	7.8	7.1	8.8	4.9
6	α-terpinene	1011	0.2								
7	1,8-cineole +	1021	29	32.4	28.0	25.8	26.9	27.2	26	31.6	25.7
8	limonene	1022									
9	(E)-β-ocimene	1025	0.2								
10	γ-terpinene	1047	0.3	0.6							
11	(E)-sabinene hydrate	1052	0.3		0.4						
12	terpinolene	1076	0.6								
13	α-thujone	1084									
14	camphor	1117	14.8	21.5	18.7	17.3	12.0	8.3	11	16.5	22.6
15	borneol	1144	2.2	2.9	3.4	2.5	1.8			2.3	3.0
16	δ-terpineol	1169			0.47						
17	linalyl acetate	1235	0.4	0.2	0.4						
18	bornyl acetate	1264	3.0	3.6	3.6		2.6			2.3	3.1
19	α-terpinenyl acetate	1327	3.1	2.8	4.1	3.5	2.2			2.2	3.3
20	α-copaene	1371	0.4	0.4	0.4	0.4					
21	α-gurjanene	1406	1.0			0.6	1.7	2.2	4.3		
22	β-caryophyllene	1414	8.7	5.5	8.8	9.2	4.4	1.1		5.9	9.2
23	α-humulene	1447	6.7	2.7	5.3	6.8	3.5			5.0	7.3
24	valencene	1487									
25	γ-cadinene	1502	2.0	2.0	2.9	3.0		13.4	11.6		1.9
26	calamenene	1505		0.4							
27	δ-cadinene	1510	1.6		0.3	1.9					
28	caryophyllene oxide	1566	0.8	0.5	1.7	1.2					
29	globulol	1590	0.3		0.5						
30	cubenol	1598	0.6	0.6	1.0	1.0	2.0	2.8	3.9		
31	T-cadinol	1619	3.5	1.8	6.1	8.2					
32	γ-eudesmol	1628	0.4	0.6	1.0	1.6	0.8				
33	α-eudesmol	1636				0.7					
34	unknown	1639–2118	0.9	7.4		7.2	10.4	10.2	9.2	14.5	12

no.[a]	compound	RI[b]	run 9	run 10	run 11	run 12	run 13	run 14	run 15	run16
1	α-pinene	942	15.6	12	7.0	6.2	13.9	5.8	4.7	4.3
2	camphene	953	6.1	4.9					2.0	1.8
3	β-pinene	976	5.7	4.0	5.9	5.2	3.9	5.4	1.5	1.3
4	myrcene	985	1.4	1.3			13.0			
5	δ-3-carene	1006	8.6	7.3	10.3	8.5	7.6	8.9	1.8	1.7
6	α-terpinene	1011								
7	1,8-cineole +	1021	30.2	27.9	34.6	37.3	4.9	31.5	16.8	17.1
8	limonene	1022								
9	(E)-β-ocimene	1025	0.4	0.6						
10	γ-terpinene	1047							0.3	0.2
11	(E)-sabinene hydrate	1052								
12	terpinolene	1076								
13	α-thujone	1084								0.4
14	camphor	1117	10.5	15.2	14.7	17.8	4.2	12.2	13.3	13.8
15	borneol	1144	1.2	1.8		2.1	6.5		3.1	3.2
16	δ-terpineol	1169							0.3	0.4
17	linalyl acetate	1235							0.3	0.3
18	bornyl acetate	1264	1.1	2.3			4.9		3.4	3.4
19	α-terpinenyl acetate	1327		0.6			8.2	19.9	3.7	3.6
20	α-copaene	1371							0.3	0.3
21	α-gurjanene	1406							0.9	1.0
22	β-caryophyllene	1414	3.2	0.7	6.1	4.6	4.9		9.0	10.1
23	α-humulene	1447	2.3	4.7	4.1	3.4	4.7		7.0	8.0
24	valencene	1487								0.8
25	γ-cadinene	1502	1.0	0.9					1.9	3.4
26	calamenene	1505							0.3	0.6
27	δ-cadinene	1510	0.4	1.5						3.5
28	caryophyllene oxide	1566	0.7	1.1					1.4	1.5
29	globulol	1590							0.5	0.7
30	cubenol	1598		0.6					1.0	1.2
31	T-cadinol	1619							6.8	8.6
32	γ-eudesmol	1628	0.8	1.8					0.9	0.4
33	α-eudesmol	1636							1.3	0.6
34	unknown	1639–2118	10.8	10.8	24.3	14.9	23.3	22.1	26.5	7.8

[a] Compounds are listed in the order of elution time from a DB-1 column. [b] Retention indices.

investigated. Increasing the temperature from 35 to 55 °C at constant pressure drastically increased the concentrations of α-pinene, camphene, β-pinene, and δ-3-carene in essential oil, but increasing the temperature from 55 to 65 °C reduced their

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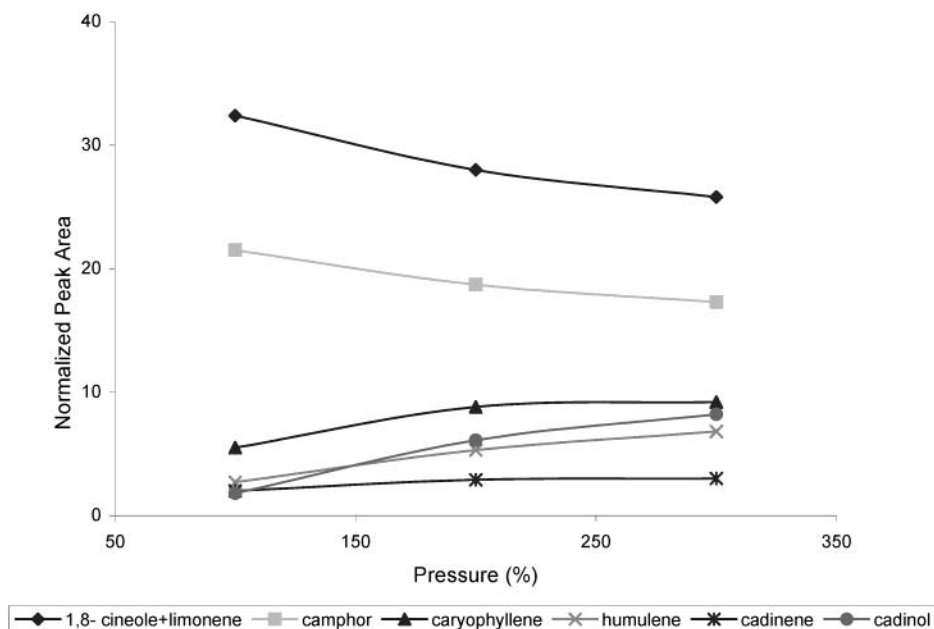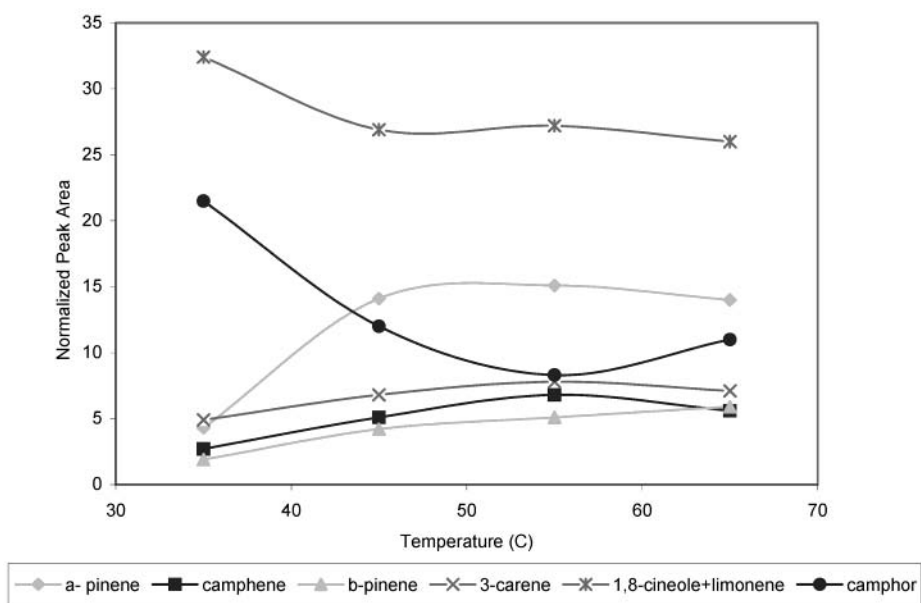*J. Agric. Food Chem.,* Vol. 51, No. 18, 2003   **5417**

Figure 1. Graph representing the percentage of normalized peak area (percent) for main compounds of *P. atriplicifolia* Benth. eesential oil versus pressure of supercritical CO_2 (at constant temperature of 35 °C).

Figure 2. Graph representing the percentage of normalized peak area (percent) for main compounds of *P. atriplicifolia* Benth. eesential oil versus temperature of supercritical CO_2 (at constant pressure of 100 atm).

content in the oil composition. However, this order was reversed for camphor (**Figure 2**). By considering the total GC peak area of the main components (such as 1,8-cineloe + limonene and camphor) under different conditions of pressure and temperature, the optimum values found for these variables were $P = 100$ atm and $T = 35$ °C.

Effects of Modifier. Due to the limited solubility of polar organic compounds in supercritical carbon dioxide, quantitative extraction of these compounds with pure supercritical CO_2 is not possible. The addition of a polar modifier to supercritical carbon dioxide has been shown to offer tremendous increases in the extraction efficency of polar organic compounds, but due to different interactions of the modifier with sample matrices, choosing a specific modifier for a class of compounds without experiment is not possible (27–29).

In this research, the effect of different modifiers at a constant pressure (100 atm) and temperature (35 °C) on the extraction efficiency was also evaluated. In all extractions for this part of the study, different volumes of modifier were added directly onto the extraction vessel prior to the extraction. After 15 min of static extraction, each vessel was flushed with supercritical CO_2 for 20 min. **Table 2** lists the percent of each compound with different volumes of modifier spiked onto the plant materials. **Figure 3** shows the effect of different volumes of modifiers on the main contents of *P. atriplicifolia* essential oil. Runs 7 and 8 show the effect of methanol (80 and 400 μL) on the composition of *P. atriplicifolia* essential oil. Methanol decreases the number of extracted compounds in comparison with the extraction by pure supercritical CO_2 from 22 compounds to 11 compounds (runs 1 and 7). Also, using 1%
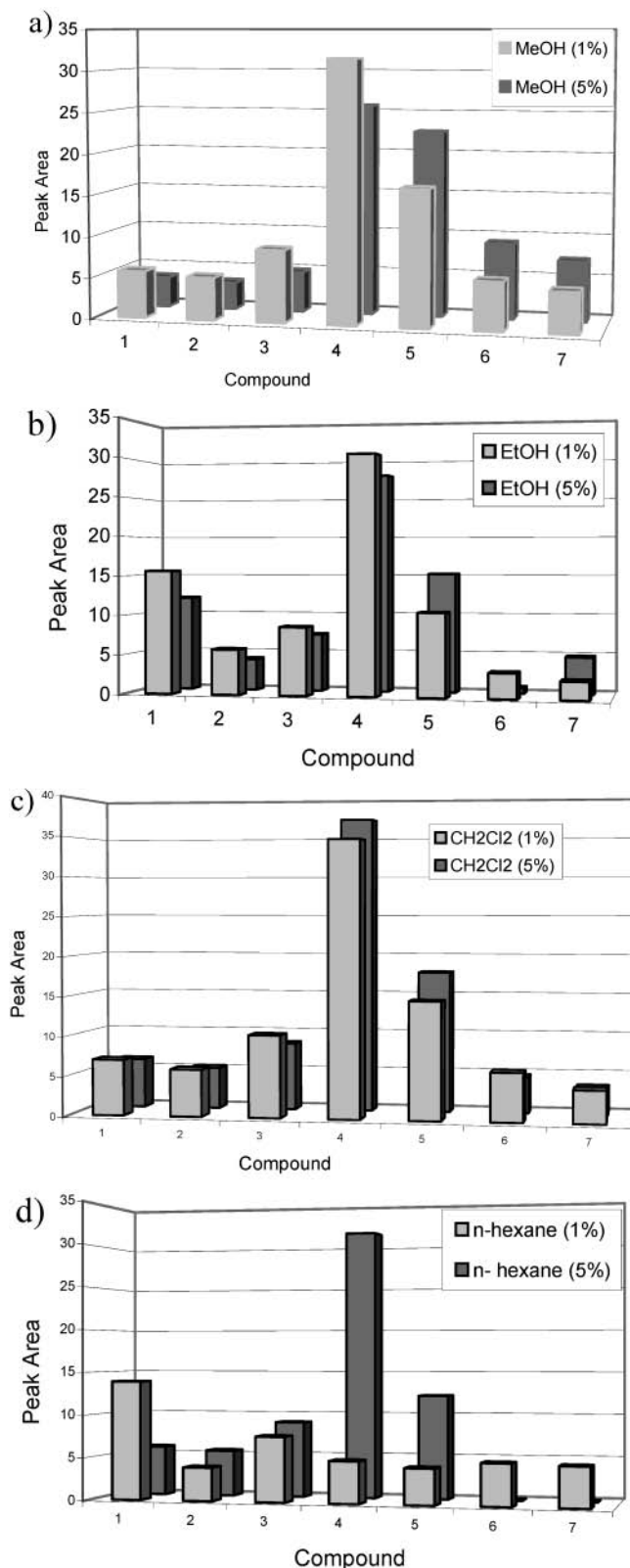
Figure 3. Effects of different volume of modifiers [(a) methanol, (b) ethanol, (c) dichloromethane, and (d) *n*-hexane] on the main contents of *P. atriplicifolia* Benth. essential oil; these main compounds are (1) α-pinene, (2) δ-3-carene, (3) 1,8-cineole + limonene, (4) camphor, (5) β-caryophyllene, and (6) α-humulene. (The extraction pressure was 100 atm and extraction temperature was 35 °C.)

methanol as modifier increases the contents of α-pinene, β-pinene, β-caryophyllene, δ-3-carene, and α-humulene and decrease the percentages of 1,8-cineole + limonene and camphor

in the extracted essential oil. On the other hand, methanol increased the percent of coextracted compounds with large retention number (unknown compounds) in the oil composition. Ethanol was also tested for the extraction of essential oil from *P. atriplicifolia*. Results showed that the addition of ethanol enhanced the concentrations of α-pinene, camphene, β-pinene, myrcene, and δ-3-carene and decreased the contents of 1,8-cineole + limonene, camphor, and β-caryophyllene in the plant oil. However, these results showed that the addition of 1% ethanol, as modifier, was more effective than the addition of 5% ethanol. Dichloromethane as modifier decreased the number of extracted compounds from 22 with pure supercritical CO_2 to 8 and 9 compounds in the presence of 80 and 400 μL of dichloromethane, respectively. Dichloromethane also increased the concentrations of α-pinene, β-pinene, δ-3-carene, 1,8-cineole + limonene, and α-humulene and reduced the concentration of camphor in the *P. atriplicifplia* essential oil composition. However, dichloromethane increased the content of coextracted compounds with large retention indices (unknown compounds) in the essential oil composition. Furthermore, it was found that dichloromethane was a selective modifier for the extraction of 1,8-cineole + limonene and δ-3-carene from *P. atriplicifolia*. By using hexane as modifier, the number of identified compounds decreased from 22 to 12 and 7 compounds in runs 13 and 14 (hexane volumes were 80 and 400 μL, respectively). In the presence of hexane as modifier the percent of α-pinene, β-pinene, δ-3-carene, and α-terpenyl acetate increased and the percent of 1,8-cineole + limonene and camphor decreased in comparison with extraction by pure carbon dioxide. On the other hand, hexane was a very efficient modifier for the extraction of α-terpenyl acetate (run 14). In addition to these experiments, methanol was also used for the extraction of essential oil from *P. atriplicifolia* at a pressure of 300 atm and a temperature of 45 °C. Runs 15 and 16 compare the essential oil obtained from pure supercritical fluid carbon dioxide and modified supercritical CO_2 with 80 μL of methanol in this condition. As shown in **Table 2**, using modifier in this condition increased the concentrations of 1,8-cineole + limonene, camphor, β-caryophyllene, and T-cadinol as the main components of *P. atriplicifolia* oil and decreased the percentage of coextracted compounds with large retention numbers (unknown compounds) in the oil composition.

Comparison of Methods. The SFE method was compared with the traditional steam distillation procedure. **Table 2** reports the averaged determinations of SFE and steam distillation methods. This results show that different compounds can be extracted from *P. atriplicifolia* by using SFE and that different concentrations of extracted compounds were obtained by SFE and classical extraction procedure.

As already mentioned in the literature (*20, 21*), SFE is more selective than steam distillation for essential oil compounds. This better selectivity could explain why by using 400 μL of hexane as modifier in a supercritical CO_2 extraction (run 14) only 7 components can be extracted, when, in the essential oil obtained by steam distillation, 28 compounds were determined. Furthermore, SFE is less tedious and minimizes the risk of compound degradation due to heat. In addition, SFE presents some advantages over steam distillation such as extraction rate (35 min for SFE and 1.5 h for steam distillation), cleanliness, and the possibility of manipulating the composition of the extract.

Conclusion. The SFE method was compared with the steam distillation method in the extraction of the essential oil of *P. atriplicifolia* Benth. The effect of different pressures, temper-

atures, modifiers, and modifier concentrations on the efficiency of the extraction of essential oil from *P. atriplicifolia* was investigated. The results showed that increasing the pressure from 100 to 300 atm at a constant temperature of 35 °C reduced the concentration of the main compounds such as α-pinene, δ-3-carene, 1,8-cineole + limonene, and camphor in the essential oil. Also, results showed that at constant pressure (100 atm), increasing the temperature from 35 to 55 °C reduced the percent of the main compounds in the essential oil composition. Results indicated that the type of modifier could have a major effect on the quality of *P. atriplicifolia* Benth. essential oil. Of all the modifiers used in this study, hexane provided the highest SFE selectivity when used for the extraction of essential oil of *P. atriplicifolia*.

Finally, SFE shows various results in comparison with the conventional steam distillation procedure. Furthermore, SFE gives a better selectivity for compounds of interest; changing extraction variables is less tedious, and it has a shorter extraction time.

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