Extraction of Baccharis Oil by Supercritical CO_2

Eduardo Cassel,*,† Caren D. Frizzo,‡ Regina Vanderlinde,‡ Luciana Atti-Serafini,‡ Daniel Lorenzo,§ and Eduardo Dellacassa§

Faculdade de Química, Pontifícia Universidade Católica do Rio Grande do Sul, Ipiranga 6681, 90619-900 Porto Alegre, Brazil, Instituto de Biotecnologia, Universidade de Caxias do Sul, Francisco G. Vargas 1130, 95001-970 Caxias do Sul, Brazil, and Catedra de Farmacognosia y Productos Naturales, Facultad de Quimica, Gal. Flores 2124, 11800 Montevideo, Uruguay

Baccharis dracunculifolia, "Vassoura", is a shrub with spontaneous occurrence in Brazil, Uruguay, Argentina, Paraguay, and Bolivia. The Vassoura oil, valuable in the perfumery industry for its exotic aroma, presents variations in composition depending on the geographical origin and extraction process. The commercial importance is related to the oxygenated fraction content, specifically to (*E*)-nerolidol and spathulenol. In this work we study the dependence of the Vassoura oil composition, obtained by supercritical CO_2 ($SCCO_2$) extraction, with the following parameters: pressure, temperature, $SCCO_2$ flow, equilibrium, and extraction times. The flexibility of the extraction process using $SCCO_2$ allowed one to study the Vassoura oil composition as a function of the extraction parameters. Higher concentrations of (*E*)-nerolidol and spathulenol were obtained in the following experimental conditions: pressure, 10 MPa; temperature, 323.15 K; equilibrium time, 10 min; extraction time, 20 min; $SCCO_2$ flow, 1.0 mL/min. The results were compared with those obtained by hydrodistillation in laboratory conditions. The evaluation of the composition for each extract was performed by gas chromatography and gas chromatography−mass spectrometry.

1. Introduction

Baccharis dracunculifolia (Compositae) is a shrubby plant growing wild in Brazil, from Minas Gerais to Rio Grande do Sul, Uruguay, Paraguay, Argentina,[1] and in the valleys of Bolivia where the plant has application in the folk medicine.[2] The essential oil composition has been studied by several authors.[2−9] The main components are (*E*)-nerolidol, reported for its use in the perfumery industry,[2−9] and spathulenol, reported for its pharmacological activity.[10]

The use of supercritical CO_2 ($SCCO_2$) extraction for essential oils has become an important and discussed application in the supercritical fluid area.[11] The modifications produced as a consequence of the distillation process on thermolabile compounds present in the essential oils can be considered as a problem in the production of natural fragrances. By compariosn, the $SCCO_2$ extraction represents an alternative to resolving this problem by improving the organoleptic properties of the end product.

The aim of this work was to compare the $SCCO_2$ extraction with the hydrodistillation process and to optimize the oxygenated compounds content, specifically (*E*)-nerolidol and spathulenol. The influence of parameters such as temperature, pressure, equilibrium, extraction times, and CO_2 flow on the concentration of these compounds in the essential oil was also studied in this work. The composition of the extracts obtained was analyzed by gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS).

* To whom correspondence should be addressed. E-mail: cassel@eq.pucrs.br. Fax: ++513203612.

† Pontifícia Universidade Católica do Rio Grande do Sul.

‡ Universidade de Caxias do Sul.

§ Catedra de Farmacognosia y Productos Naturales.

2. Material and Methods

2.1. Plant Material. Extractions were carried out using twigs and leaves of *B. dracunculifolia* collected in Campestre da Serra County, RS, Brazil, in June 1999.

2.2. Hydrodistillation. The oils were obtained by hydrodistillation for 1 h on a Clevenger-type apparatus[12] using 150 g of fresh material. The oil samples were dried over anhydrous Na_2SO_4.

2.3. Supercritical Fluid Extraction (SFE). Experiments were conducted using the aerial part of the plant dried at 308.15 K for 120 h and milled to an average particle diameter of 0.2 mm on a cutting mill (Tecnal, Brazil). A 1.5 g amount of sample material was used. The oils were extracted on a Hewlett-Packard 7680T module, fully automated and equipped with a stainless steel thimble (7 mL), an analyte trap for extracts, and an automatic system to collect the samples.

$SCCO_2$ was conducted in two steps. In the first, seven extractions were carried out by keeping the equilibrium and extraction times constant at 30 min, the CO_2 flow at 1.0 mL/min, the temperatures at 313.15, 323.15, and 333.15 K, and the pressures at 9, 10, 11, and 12 MPa. On the basis of these extractions, the optimal experimental conditions were chosen according to the presence of higher contents of oxygenated compounds. In the second step, eight extractions were conducted according to the better condition selected, but other parameters such as the time of equilibrium (0, 10, 20, and 30 min), time of extraction (10, 20, 30, and 40 min), and CO_2 flow (0.5, 1.0, and 2.0 mL/min) were changed.

Liquid CO_2 (99.9% pure; White Martins, Brazil) was used for extractions. Extracts were retained by an ODS (hypersil octadecylsilica) trap at 268.15 K during the process and then eluted with 1.0 mL/min of *n*-hexane (Merck, Germany), collected in vials, and kept at 269.15 K until analysis.

10.1021/ie000241f CCC: $19.00
Published on Web 11/14/2000

Table 1. Relative Percentage Composition of Vassoura Extracts

compound	313.15 K 12 MPa	323.15 K 9 MPa	323.15 K 10 MPa	323.15 K 11 MPa	323.15 K 12 MPa	333.15 K 9 MPa	333.15 K 12 MPa	hydrodistillation
α-pinene								5.3
β-pinene								28.2
myrcene								1.9
limonene								10.6
(*E*)-β-ocimene								0.9
α-copaene								0.7
α-gurjunene								traces
β-caryophyllene	3.5	8.3	5.1	4.7	4.2	5.5	4.1	0.6
aromadendrene	1.0	2.0	1.2	1.1	1.2	1.4	1.2	0.1
α-humulene	1.7	2.4	1.5	1.4	1.3	1.7	1.3	0.2
γ-muurolene	1.4	2.8	1.8	1.6	1.7	2.1	1.7	–
germacrene D	5.8	13.7	8.8	8.2	7.1	9.3	6.5	0.9
α-muurolene	1.0	2.0	1.2	1.2	1.1	1.5	1.1	0.1
bicyclogermacrene	6.7	14.6	9.9	9.1	8.3	13.9	7.6	1.6
γ-cadinene								1.4
δ-cadinene	3.5	6.7	4.5	4.2	4.3	5.1	4.4	0.9
α-curcumene	0.5		0.7	0.6		0.8		
(*E*)-calamenene								0.8
caryophyllene oxide	2.3	3.1	3.0	2.6	2.6	1.9	2.7	
(*E*)-nerolidol	27.3	35.9	35.1	32.6	31.8	24.2	34.0	13.5
globulol			0.6					3.1
viridiflorol	0.8		0.9	0.9	0.9		0.9	3.1
spathulenol	11.8	8.5	12.6	13.4	13.8	6.8	14.5	9.8
guaiol								1.1
(*E*)-cadinol								1.2
α-muurolol								0.7
α-cadinol								1.6
α-bisabolol								1.8
hydrocarbons	25.2	52.5	34.8	32.1	29.4	41.4	27.9	53.4
oxygenated compounds	42.2	47.5	52.2	49.5	49.0	32.9	52.1	35.9

Quantification was conducted using a suitable internal standard[14] added to the extracts obtained at the optimal process conditions: 3-octanol (97% pure; Aldrich, Milwaukee, WI) at a concentration of 0.5 mg/mL. Extractions and analyses were performed in triplicate.

2.4. Analytical. The oils were analyzed by GC and GC−MS. The compounds were identified by comparison of the spectral data with those of the library[13] and by their retention times.

Quantitative analyses were performed on a Hewlett-Packard 6890 GC equipped with a flame ionization detector (FID) and a HP−Innowax fused silica capillary column (30 m × 0.32 mm i.d., 0.25 μm film thickness): temperature program, 40 °C (8 min); 40−180 °C (3 °C/min); 180−230 °C (20 °C/min); 230 °C (20 min); injector temperature, 250 °C; detector temperature, 250 °C; injection, split; split ratio, 1:25; carrier gas, H_2 (32 kPa).

Qualitative analyses were performed on a Hewlett-Packard 6890/5973 GC−MS equipped with an HP−Innowax fused silica capillary column (30 m × 0.25 mm i.d, 0.25 μm film thickness): the temperature program was the same as above; interface temperature, 280 °C; injection, split; split ratio, 1:100; carrier gas, He (56 kPa, 36 cm/s); electronic impact, 70 eV; mass range, 40−350; solvent cut, 4 min.

3. Results and Discussion

The hydrodistillation process has been traditionally used in the extraction of essential oils on a laboratory scale. In this work, we intend to compare the efficiency of this process with its relationship to the volatile composition of extracts from *B. dracunculifolia* obtained by $SCCO_2$ extraction. The compositions of the oils obtained by both processes can be observed in Table 1.

The results are presented in the Table 1, where the CO_2 flow, equilibrium, and extraction times were constants and two temperature and pressure conditions were chosen: 323.15 K and 10 MPa; 333.15 K and 12 MPa. Because of the results obtained, the conditions selected for the study of the parameters that influence the SFE process were as follows: temperature, 323.15 K; pressure, 10 MPa.

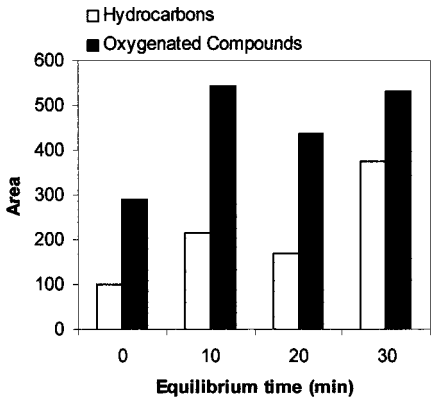

Figure 1. Amount of Vassoura extract in relation to the equilibrium time (area−value obtained by integration on GC−FID analysis) at $T = 323.15$ K and $P = 10$ MPa.

Figure 1 shows the influence of the equilibrium time on the essential oil composition. It was observed that the composition of the oil was similar in the range of 10−20 min, allowing one to choose the minor time as suitable for the extraction process. Moreover, the amount of essential oil extracted was higher at a time of 10 min than at a time of 20 min.

When the extraction time is considered, it is possible to observe in Figure 2 that at 20 min the process showed the highest oxygenated/hydrocarbon compounds relationship, as expected, even if the amount extracted at 30 min was higher.

The third parameter studied, for the conditions of temperature and pressure selected, was the CO_2 flow used in the SFEs. Figure 3 shows that 1.0 mL/min as the CO_2 flow was suitable for the extraction. The results obtained at a flow of 2.0 mL/min justified the behavior due to the conveyance of the oil extracted by CO_2. This

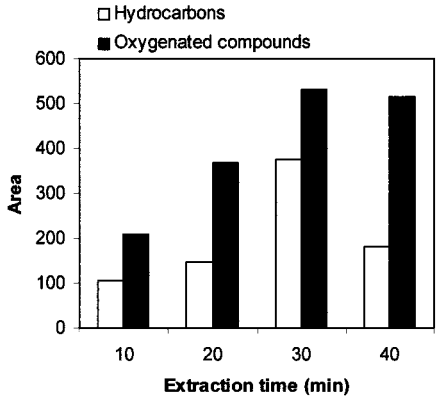

Figure 2. Amount of Vassoura extract in relation to the extraction time (area−value obtained by integration on GC−FID analysis) at $T = 323.15$ K and $P = 10$ MPa.

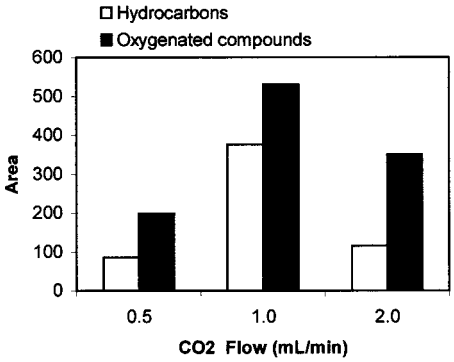

Figure 3. Amount of Vassoura extract in relation to the CO_2 flow (area−value obtained by integration on GC−FID analysis) at $T = 323.15$ K and $P = 10$ MPa.

same fact was observed in Figure 2 for the condition at 40 min, when hydrocarbons were dragged by $SCCO_2$.

The nonoxygenated monoterpenes, present in the hydrodistilled oil in high contents (β-pinene, 28.2%; limonene 10.6%), were not detected in the $SCCO_2$ extracts. However, low contents of this monoterpene fraction have been reported by some authors[15−17] for the same experimental conditions of pressure as those used in this work. Probably all of the components are present in the $SCCO_2$ extracts but at lower contents than those obtained in the hydrodistillation, as a consequence of the smaller quantities of plant material used in the $SCCO_2$ extraction and the corresponding amounts of extracts.[11] This feature can be considered as a commercial advantage for this process[16] because of the quality of the aromatic profile of the extracts.

Earlier reports[15] showed higher contents of oxygenated compounds for the $SCCO_2$ extracts as was verified in this work, where the total contents of these compounds reached 52.2% for the optimal experimental conditions selected (323.15 K and 10 MPa). The hydrodistilled oil showed, by comparison, lower contents of oxygenated compounds (35.9%).

The total yields obtained by hydrodistillation and SFE were 0.36% and 0.38%, respectively. The contents of (*E*)-nerolidol and spathulenol obtained by $SCCO_2$, 47.5% and 16.4%, were higher than those obtained in the hydrodistilled oil, 13.5 and 9.8%.

4. Conclusion

The flexibility in the management of the variables involved in the SFE process allows one to optimize the experimental conditions considering the selectivity of a substance or classes of substances of interest. From the reported experiments it is possible to define, for the selected plant material, the optimal conditions of $SCCO_2$ extraction: temperature, 323.15 K; pressure, 10 MPa; equilibrium time, 10 min; extraction time, 20 min; CO_2 flow, 1.0 mL/min.

The selectivity of $SCCO_2$ allowed one to maximize the concentration of the oxygenated compounds, with this process being more advantageous than the hydrodistillation, as demonstrated for the obtention of (*E*)-nerolidol and spathulenol, when compared with hydrodistillation.

Literature Cited

(1) Barroso, G. M. Compositae-Subtribo Baccharidinae Hoffmann. Estudo das Espécies ocorrentes no Brasil. *Rodriguesia* **1976**, *18*, 3.

(2) Loayza, I.; Abujder, D.; Aranda, R.; Jakupovic, J.; Collin, G.; Deslauriers H.; Jean, F. I. Essential Oils of *Baccharis salicifolia, Baccharis latifolia* and *Baccharis dracunculifolia. Phytochemistry* **1995**, *38*, 381.

(3) Loayza, I.; Collin, G.; Gagnon, M.; Deslauriers, H.; Dellacassa, E. Huiles Essentielles de*Baccharis latifolia, B. salicifolia* de Bolivia et de *B. dracunculifolia* en Provenance d'Uruguay. *Rivista Ital. EPPOS* **1993**, *Numero Speciale*, 728.

(4) Ferracini, V. L.; Paraiba, L. C.; Leitao Filho, H. F.; Silva, A. G.; Nascimento, L. R.; Marsaioli, A. Essential Oil of Seven Brazilian Baccharis Species. *J. Essent. Oil Res.* **1995**, *7*, 355.

(5) Queiroga, C. L.; Fukai, A.; Marsaioli, A. Composition of the Essential Oil of Vassoura. *J. Braz. Chem. Soc.* **1990**, *1*, 105.

(6) Weyerstahl, P.; Christiansen, C.; Marschall, H. Isolation and Synthesis of Isohumbertiol, the First Naturally Occurring Sesquiterpene Alcohol with a Humbertiane Skeleton. *Liebigs Ann. Chem.* **1992**, 1325.

(7) Weyerstahl, P.; Marschall, H.; Schneider, K. Synthesis of *ras-cis*- and *-trans*-dracunculifoliol and Their 10-Epimers. *Liebigs Ann. Chem.* **1995**, 231.

(8) Weyerstahl, P.; Christiansen, C.; Marschall, H. New Sesquiterpene Ethers and Other Constituents Isolated from Brazilian Vassoura Oil. *Liebigs Ann. Chem.* **1995**, 1039.

(9) Weyerstahl, P.; Christiansen, C.; Marschall, H. Constituents of Brazilian Vassoura Oil. *Flavour Fragrance J.* **1996**, *11*, 15.

(10) Fullas, F.; Hussain, R. A.; Chai, H.; Pezzuto, J. M.; Soejarto, D. D.; Kinghorn, A. D. Cytotoxic Constituents of *Baccharis gaudichaudiana. J. Nat. Prod.* **1994**, *56*, 801.

(11) Reverchon, E. Supercritical fluid extraction and fractionation of essential oils and related products. *J. Supercrit. Fluids* **1997,** *10*, 1.

(12) Mechkovski, A.; Akerele, C. O. *Quality Control Methods for Medicinal Plant Materials*; WHO/PHARM/92.559; World Health Organization: Geneva, Switzerland, 1992.

(13) MacLafferty, F. W.; Stenhagen, E.; Abrahamsson, S. *Registry of Mass Spectral Data*; John Wiley & Sons: New York, 1997.

(14) Walker, D. F. G.; Bartle, K. D.; Breen, D. G. P. A.; Clifford, A. A.; Costiou, S. Quantitative Method for the Analysis of Flavour and Fragrance Components from Lavender and Rosemary by Studying the Kinetics of Their Supercritical Fluid Extracts. *Analyst* **1994**, *119*, 2789.

(15) Tateo, F.; Fellin, M. Production of Rosemary Oleoresin using Supercritical Carbon Dioxide. *Perfum. Flav.* **1988**, *13*, 27.

(16) Ondarza, M.; Sanchez, A. Steam distillation and supercritical fluid extraction of some mexican spices. *Chromatographia* **1990**, *30*, 16.

(17) Mendes, R. L.; Coelho, J. P.; Fernandes, H. L.; Marrucho, I. J.; Cabral, J. M. S.; Novais, J. M.; Palavra, A. F. Application of Supercritical CO_2 Extraction to Microalgae and Plants. *J. Chem. Technol. Biotechnol.* **1995**, *62*, 53.

Received for review February 16, 2000
Accepted October 3, 2000

IE000241F