Extraction of volatile compounds from *Baccharis dracunculifolia* using supercritical carbon dioxide.

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ABSTRACT

Species of the genus *Baccharis* are characterized by the presence of sesquiterpenes, diterpenes, triterpenes and flavonoids. The *Baccharis dracunculifolia* (local name in Brazil: *alecrim-do-campo*) grows naturally in southern and southeastern Brazil, Uruguay, Paraguay, Argentina and Bolivia . Its essential oil has a high value for the fragrance industry. This work has as objective to identify and quantify the most volatile compounds (essential oil) that are obtained from supercritical CO_2 extraction. The volatile fraction was collected in the extraction with supercritical carbon dioxide at 300 bar and 50 °C, using crushed leaves with different diameters and one also with addition of 5% by weight of ethyl acetate and another with 5% by weight of ethanol. Ethyl acetate and ethanol were added to the bed of leaves and not to CO_2. Analyses were performed by gas chromatography coupled to mass spectrometry with capillary column HP - 5MS and identification was performed by comparison of mass spectra with literature data, obtained with the database system GC/MS and retention index relative to a series of alkanes. A total of 22 compounds were identified in the different fractions and the results indicated that the content of monoterpene ranged from 16.5% to 26.5 %, the content of sesquiterpene hydrocarbons ranged from 60.8 % to 74.3% and the content of sesquiterpene alcohols varied from 5.1 % to 10.7 % in the essential oils of this study.

INTRODUCTION

Baccharis dracunculifolia known as “alecrim do campo” belong to the family Asteraceae is found in various parts of South-America with 120 occurring in Brazil. The plant can reach 2–3 m and grows naturally in southern and southeastern Brazil, Uruguay, Paraguay, Argentina and Bolivia [1]. An essential oil is also known as a vassoura oil obtained from the leaves [2,3]. This species is the main plant source of propolis from southeast Brazil (known as green propolis) [4,5], being rich in phenolic derivatives of cinnamic acids. Cassel et al. [2] studied the SFE extraction from *B. dracunculifolia* leaves at 50°C and 100 bar, aimed at extracting the essential oil. Extracts from *B. dracunculifolia* leaves were obtained using SFE at temperatures of 40, 50 and 60 °C and pressure of 200, 300 and 400 bar [6]. The authors analyzed the predominant phenolic compounds in this species, such as 3,5-diprenyl-4-hydroxycinnamic acid (DHCA or artepillin C); 3-prenyl-4-hydroxycinnamic acid (PHCA); 4-hydroxycinnamic acid (p-coumaric acid) and 4-methoxy-3,5,7-trihydroxyflavone (kaempferide). Martinez-Correa et al. [7] performed sequential supercritical extraction at 60°C and 400 bar, and found the profile of the chemical composition of the essential oil from young leaves of *B. dracunculifolia.*

MATERIALS AND METHODS

Raw material characterization

Leaves of *Baccharis dracunculifolia* was kindly provided by Chemical, Biological and Agricultural Pluridisciplinary Research Centre (CPQBA, Campinas, Brazil). The drying of the leaves was performed in CPQBA in dryer with forced air circulation (Fabber, model 170, Piracicaba, Brazil) at 40 °C for 24 hours, then the sample was packed in plastic bags, wrapped in aluminum foil and stored in domestic freezer (model 220, Consul, Brazil) at -10°C. The mean particle diameter was calculated from the fractions of material retained on the following sieves. Tyler meshes: 12 (5.70 %), 16 (30.81 %), 24 (22 %), 32 (17.14 %) 48 (13.84 %) and 100 (10.51 %) using the ASAE procedure [8] employing a vibratory sieve system (Model 1868, Bertel, SP, Brazil). For supercritical extraction, the apparent density of thee particle bed (348.5 kg/m³) was determined according to the method described by Uquiche et al. [9].

Experimental procedure for extraction

Fig. 1 shows a diagrams the extraction process in fixed bed. The apparatus consists of a CO_2 cylinder, thermostatic bath, Bourdon type pressure gauge, heat exchanger (2), high-pressure pump (3), supply tank (4), extractor (7), extract collection flask (9), gas flow meter (11), volume totalizer (12).

The extractor (7) with 2 cm inner diameter was packed manually with approximately 7 g of dried and milled leaves, forming a bed of particles, and the remaining volume of the extractor was filled with glass beads of 6 mesh. The thermostatic bath was set at 50 °C and the pressure was adjusted by pumping CO_2 until the preset pressure of 300 bar. The static period of 20 minutes was established as the time to stabilize the system. The supercritical extraction started by allowing CO2 to pass through the bed at flow rate of 4.0×10^{-5} kg/s. The gaseous CO_2 which left the collector was conducted into a trap (10) in order to capture the lighter components that could be dragged by it. This trap was prepared by packing the adsorbent Porapack Q - between glass wool in a glass tube of 6 mm diameter and 100 mm length. Finally, CO_2 was led to a gas flow meter (11) (Cole Parmer Model 32908-69 Instrument Company) for controlling the CO2 flow, and to a volume totalizer (12) for measuring the volume of carbon dioxide used in the extraction.

The pipe line was washed with ethanol and with the help of a peristaltic pump (8) for recovering the extract deposited in the same along the extraction. This material was added to vial the extraction and then dried in vacuum oven to evaporate the solvent. The global extraction yield (X_0) was calculated from the mass extracted (extraction + cleaning) and the initial mass added into the extractor (dry basis). The extract obtained in the collector (9) was called heavy extract and extract more volatile captured in the trap (10) was named light weight extract (essential oil).

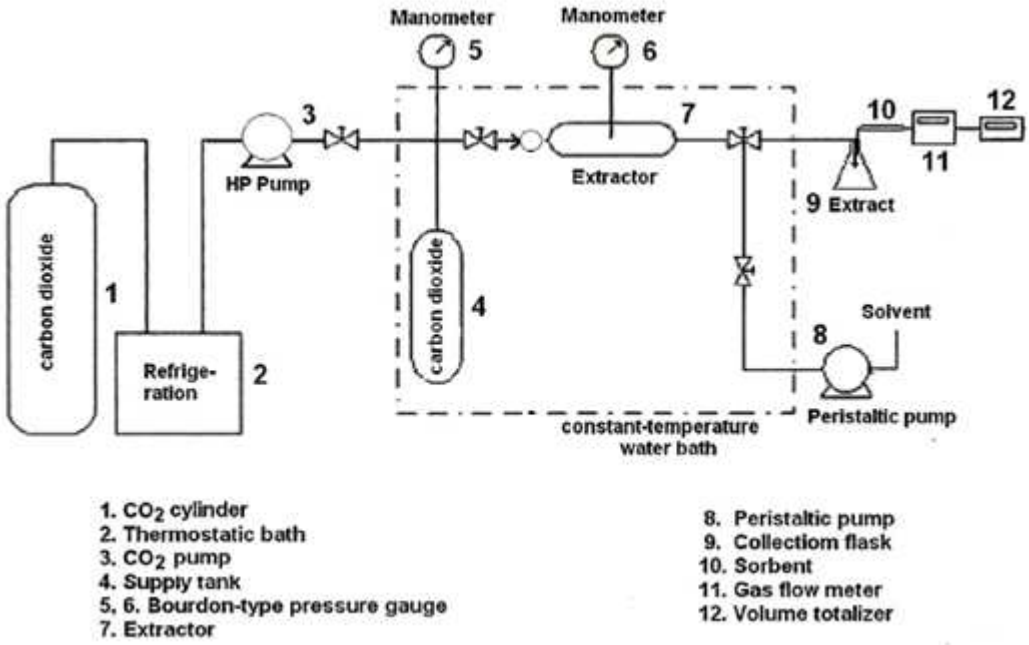

Figure 1: Experimental extraction unit

Other two extractions were performed by adding co-solvents matrix leaves a 5 % mass ethanol and another 5 % (mass) of ethyl acetate at a temperature of 50 ° C and a pressure of 300 bar.

Gas chromatography-mass spectrometry (GC-MS)

The volatiles captured on Porapak-Q column were eluted in 1 mL of ethyl acetate and analyzed to identify the CPQBA. Analyses were performed by gas chromatography coupled to mass spectrometry (GC 6890N, Agilent 5975) with a capillary column HP-5MS (30m x 0.25 mm x 0.25 mM) and helium carrier gas 1mL/min. The programming of heating the column was: 55 ° C - 120 ° C at 20 ° C / min; 120 ° C - 150 ° C at 1.5 ° C/min ; 150 ° C - 250 ° C at 20 ° C / min to 250 ° C (10 min) [10].
The temperatures of injector and detector were 220 ° C and 250 ° C respectively. The identification of compounds was performed by comparing the mass spectra obtained with literature data [11] with the database of the GC / MS system - Wiley library and NIST, and the retention index on a series of n-alkanes (C9-C20).

RESULTS

Effect of particle diameter on the global extraction yield

Figure 1 shows extraction curves obtained with SFE 300 bar and 50 °C from samples of pounded leaves with different particle sizes.

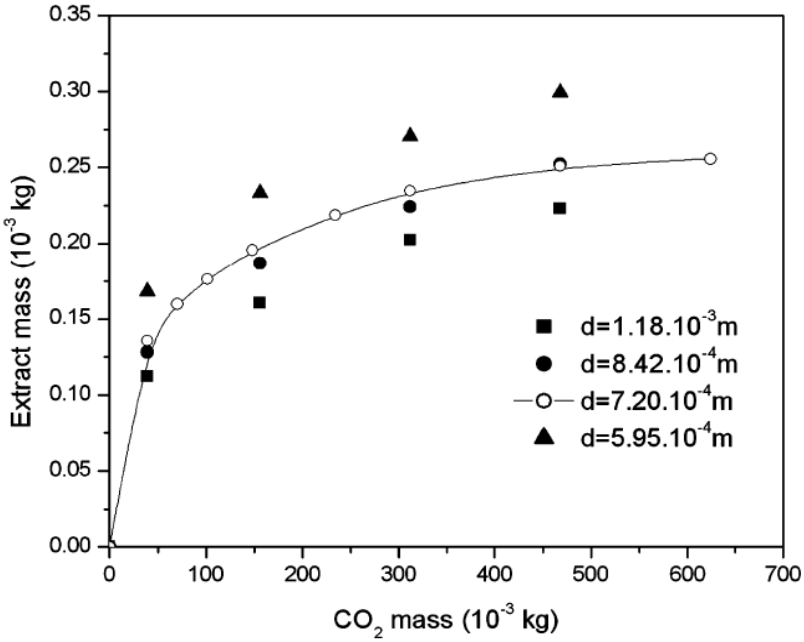

Figure 1. Extraction curve for *B. dracunculifolia* leaves obtained at 300 bar and 50°C [6]

The maximum global extraction yield (4.27%) was obtained from the pounded material with the smallest average particle diameter of 5.95×10^{-4} m and a minimum (3.18%) using the highest average diameter of 1.18×10^{-3} m . It was found that the global yield increased with decrease in the average particle diameter due to the greater amount of material released by the rupture of higher surface fraction of particles, and by reducing the internal resistance to mass transfer by diffusion.

The global extraction yield of SFE (50 ° C and 300 bar) using cosolvents, 5% (w/w) of ethyl acetate and 5% (w/w) of ethanol in leaf matrix was 3.69 % and 3.09%, respectively. These values were not higher than, but close to the value obtained by SFE (3.77%) without co-solvent in the same operating condition.

Rendimento da fração volátil capturada em Porapack- Q

Table 1 shows the global yields of the light fraction of the extracts obtained by supercritical fluid extraction and hydrodistillation, studied by Cassel et al. [2]. The global yield of the extract captured on the adsorbent was about half the global yield obtained by hydrodistillation and SFE with carbon dioxide, shown in the literature. It is quite possible that the heavy fraction obtained in this work contains part of the essential oil.

Table 1: Global yield (wt%) of the volatile fraction captured in traps with polymer Porapack-Q.

Process	A (%)	B (%)	Reference
SFE (50°C – 300 bar) $d = 1.18.10^{-3}$ m	0.17	3.18	*
SFE (50°C – 300 bar) $d = 0.84.10^{-3}$ m	0.16	3.60	*
SFE (50°C – 300 bar) $d = 0.59.10^{-3}$ m	0.18	4.27	*
SFE (50°C – 300 bar) $d = 0.72.10^{-3}$ m (a)	0.18	3.69	*
SFE (50°C – 300 bar) $d = 0.72.10^{-3}$ m (b)	0.18	3.09	*
Hydrodistillation	0.36	--	[2]
SFE (50°C -100 bar)	0.38	--	[2]

* This work.
(a) SFE + 5% ethyl acetate
(b) SFE + 5% etanol
A = fraction + volatile essential oil
B= essential oil

Quantitative Analysis of volatiles by GC-MS

Table 2. shows the comparison of the chemical composition of the oil retained in the Porapak-Q adsorbent obtained by SFE with literature.

Table 2: Relative percentage composition of the essential oil of *B. dracunculifolia* obtained by different extraction methods

t_R (min)	Area (%)					RI	Compounds	MM
	SFE	ET	EA	[2]*	[7]*			
5.34	1.41	1.65	1.98	-	-	931	α-pinene (a,c)	136
6.35	0.45	-	0.49	-	-	971	sabinene (a,c)	136
6.45	6.54	6.67	9.35	-	-	975	β-pinene (a,c)	136
6.80	1.34	0.59	0.11	-	-	989	β-myrcene (a,b,c)	136
8.01	14.28	7.59	13.6	-	0.51	1027	limonene (a,b,c)	136
8.73	-	-	-	-	-	1049	Contaminat	
8.92	-	-	-	-	-	1054	Contaminant	
9.10	-	-	-	-	-	1060	Contaminant	
20.64	-	4.64	0.85	-	0.71	1348	α-cubebene (a,b)	204
21.71	-	-	-	-	0.49	1374	α-copaene	204
22.10	0.68	-	0.76	-	-	1383	β-bourbonene (a,b,c)	204
22.34	0.73	-	0.76	-	0.22	1389	β-cubebene (a,b,c)	204
22.43	1.01	1.15	1.12	-	-	1391	β-elemene (a,b,c)	204
23.09	-	-	-	-	1.1	1408	α-gurjunene	204
23.54	14.26	15.61	16.4	5.1	7.71	1418	*trans*-caryophyllene (a,b,c)	204
24.28	-	-	0.80	1.2	2.7	1437	aromadendrene (a,b,c)	204
24.87	2.04	2.41	2.33	1.5	-	1431	α-humulene (a,b)	204

Table 2: continuation

t_R (min)	Area (%)					RI	Compounds	MM
	SFE	ET	EA	[2]*	[7]*			
26.05	21.88	26.05	21.4	8.8	-	1481	germacrene D (a,b)	204
26.09	-	-	1.49	-	0.62	1482	allo-aromadendrene (a,b,c)	204
26.67	18.31	22.7	20.9	9.9	8.22	1496	biciclogermacrene (a,b)	204
26.94	0.51	-	-	-	-	1503	germacrene A	204
27.29	-	-	-	-	-	1506	γ-cadinene	204
27.68	1.42	1.81	1.64	4.5	-	1522	δ-cadinene (a,b,c)	204
29.29	5.71	4.91	3.17	35.1	6.5	1564	nerolidol (a,b,c)	222
29.67	1.06	-	-	-	-	1574	germacrene D-4-ol (a,b)	222
29.72	1.62	2.90	1.95	12.6	4.55	1575	spatulenol (a,b,c)	220
29.94	-	-	-	3.0	-	1581	caryophyllene oxide	220
35.00	1.59	-	-	-	-	1719	farnesol (E,E) (a,b,c)	220
35.76	0.74	1.27	-	-	-	1740	farnesol (E,Z) (a,b,c)	220

TR: retention time RI: retention index
SFE: SFE (50°C -300 bar)
ET: SFE (50°C -300 bar) + 5 % ethanol
EC: SFE (50°C -300 bar) + 5% ethyl acetate
[2]*: SFE (50°C -100 bar) (Cassel et al. 2000)
[7]*: SFE (60°C -400 bar) (Martinez-Correa et al. 2012)
a: database Wiley library GC/MS
b: retention index from the series of n-alkanes capillary column HP-5MS
c: compared with the mass spectral fragmentation of literature

According to analysis by GC/MS results indicated that the content of monoterpene ranged from 16.5 to 26.5%, the content of sesquiterpene hydrocarbons ranged from 60.8 to 74.3% and the content of sesquiterpene alcohols ranged from 5.1 to 10.7% in the essential oils studied (SFE, ET, EA).

The oil obtained from *B. dracunculifolia* by Cassel et al. [2] differed from the other oil samples for not having monoterpenes in its composition, as shown in Table 2. Based on the operating principle of Porapak, the use of this adsorbent is a way to avoid the loss of monoterpene hydrocarbons during supercritical extraction.

The major compounds of the volatile captured in SFE (relative area) were the sesquiterpene hydrocarbons: germacrene D (21.4 to 26.1%), bicyclogermacrene (18.3 to 20.9%), trans-caryophyllene (14.3 to 16.4%) and monoterpenes: limonene (7.6 to 14.3%) and β -pinene (6.5 to 9.4%).

The average global yield of volatiles was 0.17%, being less than 0.38% obtained by Cassel et al [2] in supercritical extraction (Table 2). This low global yield may be associated with the retention of the volatiles in the heavy fraction of the extract (essential oil). This argument can be used again to explain the low amount of (E) nerolidol and spathulenol (oxygen compounds most commercially important in the oil [12]) present in the supercritical extracts of this work in relation to the data previously mentioned (Table 2).

CONCLUSION

A total of 22 compounds were identified in the different fractions. The global yield of volatiles trapped in polymer Porapak-Q was 0.17%. However, it is likely that the extract represented by the heavy fraction (essential oil) this work also contained part of these volatile.

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