Study of the use of cosolvent to obtain extracts from *Achyrocline satureioides* and its potential in perfumery industry

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ABSTRACT

Currently, the worldwide increasing demand for natural products has stimulated the sustainable use of the extensive Latin America native flora, which is extremely rich in genetic and olfactory diversity. *Achyrocline satureioides*, also known as *marcela*, is a medium-sized annual or biannual aromatic herb that produces small flowers. It is widely used in Brazil, Argentina, Uruguay and Paraguay due to its medicinal properties. Previous studies indicate that the extracts of marcela obtained with supercritical carbon dioxide (CO_2) have potential applications in perfumery industry, thus meeting an international demand of the industries for new aromatic notes. This study goal was to evaluate the influence of the use of cosolvents in supercritical extraction (SFE) of *marcela* and to explore their aromatic potential through olfactometric evaluation. The SFE operational conditions were 90 bar and 50 ºC for all extractions. Samples of 70 g of a homogenized mix of twigs and flowers, previously dried and milled were used. Pure CO_2, CO_2+ethanol 2% (w/w) and CO_2+water 2% (w/w) were evaluated as solvents. The volatile compounds of the extracts were extracted by SPME and characterized by gas chromatography coupled with mass spectrometry. The model based on concept of broken and intact cells was used for the mathematical modeling of the extraction curves. The mass transfer parameters of each extraction condition were successfully obtained.

Keywords: *Achyrocline satureioides*, SFE, Mathematical modeling, cosolvent, aromatic potential

INTRODUCTION

Achyrocline satureioides (Lam.) DC., also known as *marcela*, *macela*, *macela-da-terra*, *camomila-nacional*, and others belong to the *Asteraceae* family [1]. Marcela is a perennial herbaceous plant, erect or of decumbent branches, very branched, of 60-120 cm of height, native of fields and open areas of the south of Brazil. This aromatic herb is an annual or biannual species of medium size that produces small flowers that have a particular smell and bitter and aromatic taste.

Marcela has its origin in South America and it is used in Brazil, Argentina, Uruguay and Paraguay due to its choleretic, antispasmodic and hepatoprotective properties [2]. In addition to these medicinal uses, previous studies show that the extracts of marcela obtained with supercritical CO_2 have potential use in perfumery industry, thus meeting the international demand of the industries for new aromatic notes [3].

Supercritical fluid extraction (SFE) is a recognized process, known by its low waste production and potential of extraction of fragrant compounds of high added value.

The use of marcela extracts for perfumery applications was already investigated with the use of carbon dioxide (CO_2) as solvent in SFE process [3]. Thus, aiming at furthering this research, the goal of this is to investigate the influence of cosolvents in SFE of marcela and explore its aromatic potential through the olfatometric evaluation. The antioxidant activity of the extracts was also investigated using the DPPH method. In addition, the mathematical model based on the concept of intact and broken cells proposed by Sovová [4] was fitted to the experimental extraction data.

MATERIALS AND METHODS

The plant material used in the experiments was purchased from a supplier in the city of Santo Antônio da Patrulha in the state of Rio Grande do Sul. Dry aerial parts of *A. satureioides* consisted of inflorescences and branches were used as raw material. The plant was conditioned in plastic bags at room temperature in the Unit Operations Lab of the Polytechnic School of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

The samples were milled in an industrial blender (SKYMSEN) and the average particle diameter was evaluated with the used of sieves (ENDECOTTS).To determine the specific mass, the actual volume of the plant was measured using the gas pycnometry technique [5], using a multipicnometer (QUANTACHROME). The gas used was nitrogen and the mass of the sample used was 3.22 g. From the pressure drop established in the experiment, it is possible to determine the volume occupied by the sample applying Equation 1.

$$V_S = V_C - V_R(\left(\frac{P_1}{P_2}\right) - 1) \tag{1}$$

Where, V_S is the volume of the sample, V_C is the volume of the cell, V_R is a reference volume of the equipment, P_1 is the initial pressure and P_2 is the final pressure. The specific mass is then determined by the ratio of the mass of the sample to its volume (V_S). The moisture of the samples was determined using a thermogravimetric scale (BEL ENGINEERING) at 60ºC.

The marcela extracts were obtained using a supercritical extraction pilot unit previously described in the literature [6]. The temperature and pressure operating conditions were, respectively, 323.15 K and 90 bar, similar to those used by Barroso [3] in the investigation of extracts obtained without the use of cosolvents and their potential in perfumery. Three solvent conditions were investigate: pure CO_2, CO_2+ethanol (2% w/w) and CO_2+water (2% w/w), all at 2.77×10^{-4} kg/s flow of CO_2.

The extracts were characterized by gas chromatographic coupled with mass spectrometry, olfactometric analysis and antioxidant activity. The sampling of the volatile compounds was performed through solid phase micro extraction (SPME) in headspace. The extracts were dissolved in 2 mL of methanol in a 20 mL vial. The samples were heated at 50 °C during a total of 45 min, 30 min of equilibrium and 15 min of fiber exposure. The SPME fiber (50/30 mm DVB/Carboxen/PDMS) was conditioned at 270 °C for 30 min before use and then exposed to the headspace. SPME by headspace is widely used in the determination of volatile compounds, being used almost exclusively in food taste and aroma analysis [7].

GC–MS analysis was performed in a Agilent 7890A coupled to a mass spectrometer (Agilent 5975C. The analytical column was a silica HP-5MS silica capillary column with a stationary phase of 5% phenyl and 95% methyl silox (30m x 250μm i.d., 0.25μm phase thickness). The conditions of the chromatograph used for analysis were as follows: column temperature in the oven, 60 °C for 4 minutes, increasing at 5 °C/min to 180 °C, 20 °C/min to 250°C, and 250 °C for 5 min; injector temperature, 250 °C; injection mode, splitless; injection with SPME fiber; the carrier gas was helium with flow rate 0.8 mL/min; the interface temperature, 280 °C; MS EI mode; EI voltage, 70 eV; range of acquisition masses, m/9z 40-450. The compounds were identified by comparing their retention times in the column used, determined from a series of n-alkanes (C8-C20), with those of pure standards or reported in the literature [8]. A comparison of the mass spectra was also performed to confirm the identity of the compounds.

Olfactometry analysis [9] was performed by a team of seven trained assessors that evaluated the extracts of interest as to their applicability in the perfumery, indicating their intensity according to a provided odor scale. The odor scale used was as follows: 1 = no value; 2 = little value; 3 = interesting; 4 = very interesting; 5 = exceptional. Regarding the qualitative attributes, the extracts were evaluated for the presence of odor. Notes equal to or greater than 4 indicate that the product analyzed has high potential for use in perfumery.

The antioxidant activity was investigated using the DPPH method, which is based on the capture of the DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical by antioxidants, producing a decrease in absorbance at 515 nm [10]. From an initial solution of DPPH (60 μM), DPPH solutions in methanol were prepared by varying the concentration of 10 μM to 50 μM DPPH. DPPH is reduced, changing from violet to light yellow when reacting with an antioxidant component. The reaction time between marcela extracts and DPPH was 60 min. The absorbance was read at 515 nm in a spectrophotometer (BIOSPECTRO SP-220, Brazil). The calibration curve of the DPPH ($R^2 = 0.9988$) was obtained according to a methodology already described in the literature [11]. Based on these data, it was possible to determine the antioxidant activity ($g_{extract}/g_{DPPH}$) and IC_{50} ($g_{extrato}/L_{DPPH}$), which is the amount of extract necessary to reduce the initial concentration of DPPH by 50%.

For the mathematical modeling of the process, the model based on the concept of intact and broken cells proposed by Sovová [4] was used. This model describes the process considering a cylindrical extractor vessel where the solvent flows in the axial direction with a certain superficial velocity (u) through a bed containing the raw material to be extracted. The size of the particles and their initial concentration of solute are considered homogeneous. Another hypothesis is that the pressure and temperature in the bed are constant. The author presented the solution depending on the period of extraction according to Eq. (2).

$$e = \begin{cases} q y_r [1 - \exp(-Z)], & q < q_m \\ y_r [q - q_m \exp(z_w - Z)], & q_m \leq q \leq q_n \\ x_0 - \frac{y_r}{W} \ln\left\{1 + \left[\exp\left(W \frac{x_0}{y_r} - 1\right) \exp[W(q_m - q)] \frac{x_k}{x_0}\right]\right\}, & q \geq q_n \end{cases} \tag{2}$$

Where

$$Z = \frac{k_f a_0 \rho}{\dot{q}(1 - \varepsilon)\rho_s} \tag{3}$$

$$W = \frac{k_s a_0}{\dot{q}(1-\varepsilon)} \tag{4}$$

And for a spherical particle the specific area is expressed by:

$$a_0 = \frac{6(1-\varepsilon)}{d} \tag{5}$$

In the above equations ρ is the density of the fluid phase, ρ_s is the density of solid phase, ε the bed porosity, t is the time of extraction, y_r is the solubility, Z and W are parameters for the fast and slow period, respectively, and are directly proportional to the mass transfer coefficients of each phase, respectively ; k_f and k_s are the mass transfer coefficients of fluid and solid phases. The specific amount of solvent is defined as q and the subscripts m and n correspond to the beginning of extraction inside of particles and the ending of the extraction of solute within easy reach, respectively, while q represents the rate of solvent mass ratio. The term z_w corresponds to the boundary coordinate between fast and slow extraction; u is the superficial velocity of the solvent. The prediction of these parameters allows simulation of the extraction yield, altering other experimental conditions such as extractor volume, bed porosity, particle size, among others.

RESULTS

The average particle diameter value was considered 0.195 mm, the specific mass 734.5 kg/m^3 and humidity was 4.30%. The accumulated mass obtained using CO_2, CO_2+ethanol and CO_2+water are presented in Table 1.

Table 1 - Extraction of *A. satureioides*.

	CO_2	CO_2+ethanol	CO_2+water
Mass of Extract (g)	2.23	0.31	0.11

The extraction with pure CO_2 as solvent, the mass of extract was superior to those obtained for the extractions with the addition of cosolvent. The lowest mass obtained was using water as the cosolvent. Comparing the water and ethanol cosolvents, the water has a higher dielectric constant compared to other cosolvents generally used in the supercritical extraction, thus presenting greater polarity, which may have caused a decrease in the overall mass of the extracts.

GC-MS analysis allowed the identification of the volatile compounds present in the extracts obtained from *A. satureioides*. In the extract sample obtained with CO_2 without the use of cosolvents 21 compounds were identified; in the sample with extract obtained from CO_2+ethanol, 19 compounds were identified; and in the sample with extract obtained from CO_2+water, 13 compounds were identified. Table 2 shows the percentage composition of the compounds in each extract.

Table 2 - Composition of volatile from *A. satureioides* extracts.

	CO_2	**CO_2+ethanol**	**CO_2+water**
Compound	**Area (%)**		
Copaene <α->	**6.377**	2.398	-
Gurjurene <α->	0.096	-	-
Caryophyllene <E->	**32.027**	**22.064**	**9.356**
Aromadendrene	-	2.306	-
Humulene <α->	5.935	**8.718**	4.039
Caryophyllene<(E)->	1.14	1.900	-
Cadiana-1(6),4-diene <trans->	0.123	0.359	-
Muurulene <γ->	2.562	3.497	1.633
Amorphene <α->	0.251	0.356	-
Selinene <β->	0.973	3.658	-
Guaiene <cis-β->	-	0.137	-
Viridiflorene	2.168	6.180	-
Muurolene <α->	3.95	5.274	2.446
Cadinene <γ->	4.159	-	4.001
Cadinene <δ->	**16.478**	**24.190**	**16.689**
Zonarene	1.141	-	-
Cadina-1,4-diene <trans->	1.312	1.300	-
Cadinene <α->	1.526	-	0.289
Calacorene <α->	1.72	2.893	0.795
Caryophyllenyl alcohol	-	0.318	-
Caryolan-8-ol	0.31	-	-
Caryophyllene oxide	2.554	1.981	**8.283**
Cubenol<1-epi->	0.897	0.598	2.545
Cadinol <epi-α->	-	-	4.149
Cadinol <α->	-	0.100	0.555
Cadalene	0.382	-	0.311
Total identified (%)	98.758	99.767	83.112

In extracts obtained without cosolvent, the major component is Caryophyllene <E-> (32.027%), followed by Cadinene <δ-> (16.478%) and Copaene <α-> (6.377%). In the extracts obtained with the use of ethanol as cosolvent, the major component is Cadinene <δ-> (24,190%), followed by Caryophyllene <E-> (22,064%) and Humulene <α-> (8.718%). In the extracts obtained with the use of water as cosolvent, the major compound is Cadinene <δ-> (16.689%), followed by Caryophyllene <E-> (9.356%) and Caryophyllene oxide (8.283%).

The descriptive olfactometric analysis of the aromatic properties of the marcela extracts was based on the detection and description of their sensorial aspects. The results of the olfactometric analysis are shown in Table 3.

Table 3 - Olfactometry analysis of extracts of *A. satureiodes*.

	CO_2	CO_2+ethanol	CO_2+water
	aroma intensity		
spice / aromatic herb / sweet / herbal	4	-	-
spice / aromatic herb / sweet / herbal	-	3	-
spice / aromatic herb / sweet / wood	-	-	3

All samples presented potential use in perfumery, but the extract obtained without the use of cosolvents was classified as a high interest for perfumery due to its grade 4 out of 5. The odor of spice, aromatic and sweet herbs was detected in all extracts, but with different intensity. In addition, CO_2 and CO_2+ethanol extracts show herbal odor in different intensity, while the CO_2+water extract has a wood odor. A comparison of odors resulting from olfactometric analysis can be done with the odor of the major compounds of each extraction as they influence the overall odor of the extract. The characteristic odor of these compounds is shown in Table 4.

Table 4 - Aroma of the major compounds.

Compound	Aroma
Copaene <α->	wood, spices
Caryophyllene <E->	wood, spice
Cadinene <δ->	thyme, remedy, wood
Humulene <α->	wood
Caryophyllene oxide	herb, sweet, spices

The antioxidant activity (AA) of the extracts of *A. satureioides* results are presented in Table 5.

Table 5 - Antioxidant activity in marcela extracts.

	CO_2	CO_2+ethanol	CO_2+water
IC_{50} (g/L)	9.6656	2.1254	2.773

Considering that the lower the value of IC_{50} the higher the AA of the analyzed extract, it can be observed that extracts obtained with the use of cosolvents could obtain a higher AA. The lowest AA is presented when there is no use of cosolvents in the supercritical extraction, while the highest AA occurs with the use of CO_2+ethanol.

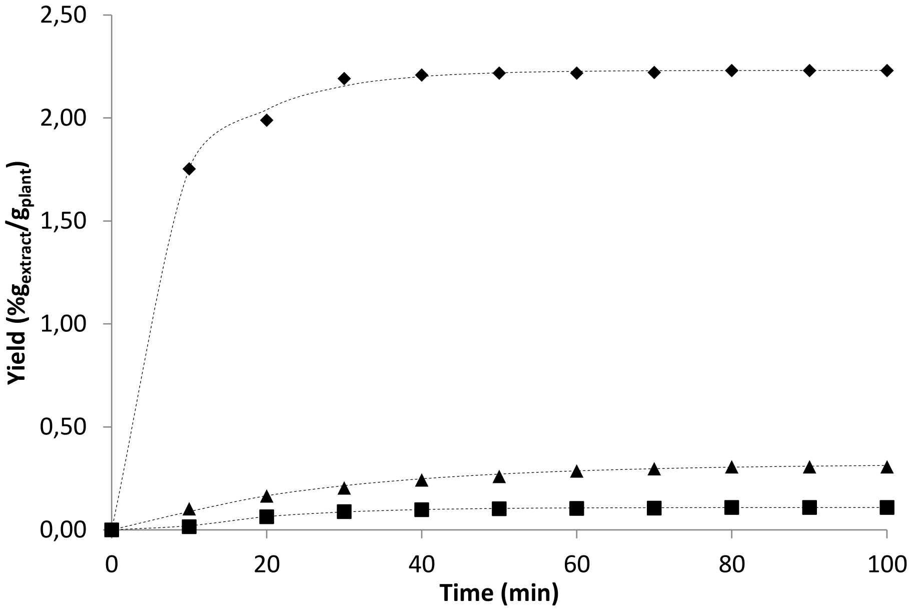
The yield *vs* time curves were obtained for each extraction condition and are presented in Figure 1 along with the mathematical model curves. The parameters estimated are shown in Table 6.

Table 6 - Estimated parameters for each extraction.

		CO_2	CO_2+ethanol	CO_2+water
R^2		0.9991	0.9953	0.9987
xk		0.0080349	0.0018538	0.0010853
yr		0.12309	0.021422	0.00032891
Z		0.11959	0.017547	0.0010218
W		0.38688	0.16082	0.35301
kf	$m\ s^{-1}$	2.73E-09	3.22E-10	2.33E-11
ks	$m\ s^{-1}$	3.43E-09	1.43E-09	3.13E-09

Observing that the correlation all coefficient R^2 are greater than 0.9950 for all the extraction conditions, it can be affirmed that the curve modeled by the equations proposed by Sovová in the chosen mathematical model [4] describes satisfactorily the experimental data of the curve of supercritical extraction of crude extract expressed in $\%g_{extract}/g_{plant}$, as a function of time in minutes.

Figure 1 - Yield curve for the supercritical extraction of *A. satureioides.*



Mathematical model (----) and experimental data: pure CO_2 (◆), CO_2+ethanol (▲) and CO_2+ water (■).

CONCLUSION

This evaluated the influence of the use of cosolvents on the SFE of *A. satureioides* and explored its aromatic potential through olfactometric analysis. The chromatographic analysis showed that the use of cosolvents in obtaining extracts of marcela influences its final composition. The olfactory analysis indicated that the extracts obtained without cosolvent have a very interesting odor, and the extracts obtained with cosolvent also presented interesting odor at a lower intensity, according to the odor scale used. The antioxidant activity was also dependent on the use of cosolvents: the extracts obtained with cosolvents had a better antioxidant activity compared to the extract obtained without cosolvents. The mathematical model based on the concept of intact and broken cells fitted well the experimental data, thus, it can be concluded that the chose mathematical model [4] was adequate to simulate the supercritical extraction process of *A. satureioides*.

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