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Effect of Different Extraction Processes on the Recovery of Extracts from Achyrocline satureioides D.C.: An Evaluation of Antioxidant Activity

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Abstract: In the present work, the global yield, composition, and antioxidant activities of the extracts from Achyrocline satureioides obtained by different separation techniques were evaluated. Centrifugation, hydrodistillation, low pressure solvent extraction (LPSE), Soxhlet, supercritical fluid extraction, and ultrasound processes were used. The total phenolic compounds and quercetin present in the extracts were quantified. The antioxidant activity of the extracts was evaluated by the coupled reaction of β-carotene/linolenic acid. Higher global yields were obtained by LPSE and Soxhlet. The analysis indicated the presence of flavonoids in all extracts. The antioxidant activities of the extracts were larger than that of the reference substance, β-carotene.

Keywords: Achyrocline satureioides, antioxidant activity, centrifugation, hydrodistillation, low pressure solvent extraction, quercetin, Soxhlet, supercritical fluid extraction, ultrasound extraction

INTRODUCTION

The plants are an important source of natural bioactive products and many of these are used to form the base of a variety of drugs. In order to develop new

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medicines several areas such as botany, chemistry, and technology are involved. Several researches have investigated species from tropical areas and observed the importance of the biodiversity in the development of new medicine.

Achyrocline satureioides D.C., known as “macela” or marcela” is an aromatic shrub native to South America, which is widely used in folk medicine (1). It had been used in the treatment of breathing infections and colds in Argentinean communities (2). Pharmacological investigations revealed important anti-inflammatory, analgesic, antispasmodic, and sedative activities (3). Hepatoprotective and digestive effect were also demonstrated in the aqueous extract of A. satureioides (4). Recently, the anti-HIV activity of this specie was observed (5).

A. flaccida and A. alata, belonging to the Achyrocline genera, had also demonstrated biological properties as antioxidant and anti-inflammatory activities (6). The therapeutic action of Achyrocline species is mainly due the presence of flavonoids (7). Flavonoids, the largest of the polyphenolic group, are substances that are present in several parts of the plants such as flowers, leaves, fruits, and seeds (8). Studies on flavonoids have shown a wide range of biological effects as antioxidant, anti-inflammatory, antiviral, anti-allergic, and protective action on nervous and cardiac system (9–11). In foods and plants, flavonoids provide color, texture, and taste (12). According to Holiman et al. (13), the average intake of these compounds in The Netherlands is 23 mg/day, of which quercetin contributed 16 mg/day, where the major sources of flavonols and flavones are tea (49% of total intake), onions (29%), and apples (7%). The major dietary sources of flavonols and flavones can vary between populations. In Japan the major source of flavonols and flavones is tea, while in the United States, Finland, Greece, and former Yugoslavia onions and apples are the predominant sources (13).

Phytochemical analysis on A. satureioides revealed the presence of flavonoids with therapeutics properties as quercetin 3-methyl-ether (anti-inflammatory and antispasmodic activities (3)); luteonin (anti-inflammatory and cytoprotective activities (3, 8)) and mainly quercetin (anti-inflammatory, antispasmodic, antiulcer, gastroprotective, antioxidant, cytoprotective, anti-hypertensive, antiarrhythmic, antiallergic, antitumoral, antimicrobial, anti-lipase activities, and inhibiter of HIV1 virus reproduction (3, 8, 14–17)). Beside these compounds, others were identified in the ethanolic extracts of aerial parts from A. satureioides as galangin, galangin 3-methyl ether, caffeic acid and two esters of calleryanin with caffeic acid and protocatechuic acid extracted (18).

Although there are several works about the properties of A. satureioides extracts, up to now a comparison of the chemical composition of extracts obtained by different separation techniques has not been performed. The recovery of compounds like flavonoids can be carried out by a variety of separation methods, for instance organic solvent extraction (1, 7), and supercritical fluid extraction (SFE) (19, 20). However, there is no universal extraction process that can be used to obtain all kind of compounds. Each technique has its specificity. For instance, the low pressure solvent extraction (LPSE) process is dependent on the type of solvent used; centrifugation and ultrasound are dependent on the type of solvent as well as of the velocity (rotation) and the time of ultrasound applied, respectively. On the other hand, supercritical fluid extraction is mainly influenced by the temperature and the pressure used. The best choice for a process will be the one that promotes the success in the extraction of the target compounds. In order to make possible the use of A. satureioides in an industrial procedure to extract bioactive compounds, it is necessary at first to evaluate the separation techniques applied to this specific raw material with respect to the global yield, the composition, and the recovery of target compounds. In this way, the purpose of this work was to determine the global yields, to quantify the amount of total phenolics, the content of quercetin, and to evaluate the antioxidant activity of A. satureioides extracts from leaves and thin branches obtained by different separation processes.

MATERIAL AND METHODS

Raw Material Preparation

Leaves and thin branches from A. satureioides were collected in the Experimental Field of the Chemical, Biological, and Agricultural Pluridisciplinary Research Center (CPQBA/UNICAMP), in Campinas (São Paulo, Brazil). The raw material was dried at ambient conditions under the shadow, subsequently triturated in a knife mill (Tecnal, model TE-631, São Paulo, Brazil), and vacuum packed in plastic bags and stored in a domestic freezer (Metalfrio, double action, São Paulo, Brazil) at −15°C. The size distribution of the particles was determined using a mechanical agitator (Abrosinox, Granutest, Santo Amaro, Brazil) with the rheostat set at 10 for 10 minutes. Sieves of 24, 32, and 48 mesh (Tyler series) were used. The characterization of the size distribution of the particles is necessary for the standardization of the fixed bed used in the SFE and LPSE processes.

Experimental Separation Procedures

A. Centrifugation: In this process, $(1.04 \pm 0.04) \times 10^{-3}$ kg of A. satureioides and 32.7 ± 0.2 mL of ethanol (99.5%, ECIBRA, lot 16021) were used. The solution was centrifuged at 25°C in a centrifuge machine (Jouan, modelo BR4i, St. Herblain, France) at 7500 rpm for 5 min, according to Pesek et al. (21). After the extraction, the mixture of extract + solvent + raw material was separated by vacuum filtration. The solvent was evaporated using a rotovap (Laborota, model 4001, Viertrieb, Germany), with vacuum control (Heidolph Instruments GMBH, Viertrieb, Germany), at 40°C.

B. Hydrodistillation: Approximately $(25.0 \pm 0.1) \times 10^{-3}$ kg of A. satureioides were hydrodistilled using a Clevenger type apparatus; the solid to solvent ratio was 1:10 (w/v). The extraction was carried in 3 hours, using the methodology described by AOAC 962.17 (22).

C. Low Pressure Solvent Extraction (LPSE): The LPSE was conducted at ambient temperature (25°C) using a glass column (length of 30×10^{-2} m and internal diameter 2.8×10^{-2} m). The bed was formed inside a nylon basket (80 Mesh). Ethanol (99.9%, Merck k331878827, chromatographic grade) remained in contact with $(2.93 \pm 0.04) \times 10^{-3}$ kg of raw material for 3 hours; a pump (Cole Parmer Instrument Co, Chicago, USA) maintained the solvent recirculation. The solid to solvent ratio was 1:10 (w/v). After the extraction, the solvent was evaporated using a rotovap, with vacuum control, at 40°C.

D. Soxhlet: Approximately $(9.95 \pm 0.04) \times 10^{-3}$ kg of A. satureioides and 300 mL of ethanol (99.5%, ECIBRA, lot 16.021) were used. The extraction was done in a Soxhlet apparatus for 3 hours and after that the solvent was evaporated using a rotovap, with vacuum control, at 40°C.

E. Supercritical Fluid Extraction: The experimental runs were performed using a SFE unit described by Rodrigues et al. (23) containing a fixed bed extraction cell of maximum allowable pressure of 400 bar. The data were taken at 30°C and pressures of 150, 200, 250 bar, and total solvent flow rate of $(4.8 \pm 0.5) \times 10^{-5}$ kg/s, using the methodology described by Pereira et al. (24), for exhaustive extraction (in this case with 90 min of duration). The bed was formed inside the extraction cell using $(3.04 \pm 0.08) \times 10^{-3}$ kg of A. satureioides; glass beads (10 Mesh) were used to fill the empty space of the extraction cell.

F. Ultrasound: Approximately $(1.0 \pm 0.1) \times 10^{-3}$ kg of A. satureioides and 30 mL of ethanol (99.5%, ECIBRA, lot 16021) were used for the ultrasound extraction. The mixture was placed in a tube and inserted in an ultrasound apparatus (Unique, model Max Clean 1400, Indaiatuba, Brazil) for 30 min, at ambient temperature. After the extraction, the mixture of extract + solvent + raw material was separated by vacuum filtration. The solvent was evaporated using a rotovap, with vacuum control, at 40°C.

The global yields were calculated as the ratio of mass of extract to the mass of A. satureioides (dry basis – d.b.).

Analysis of the Extracts

Thin Layer Chromatography

The extracts were analyzed by thin layer chromatography (TLC) using silica plates (60-PF254, Merck, 20 × 20 cm, 0.25 mm of height, lot 940378601). The extracts were eluted using the following solvent mixtures:

1. n-hexane (P.A., Merck, lot K33 039974 412): ethyl acetate (P.A., Merck, lot K32 703923 346) at 85:15, and
2. chloroform (P.A., Merck, lot 31503045 301); acetone (P.A., Synth, lot 76928): formic acid (P.A. Vetec, 044755) at 75:16.5:8.5.

acetone (P.A., Synth, lot 76928): formic acid (P.A. Vetec, 044755) at 75:16.5:8.5, according to the literature (25). The plates were revealed using anisaldehyde solution (100 mL of glacial acetic acid, 2 mL of sulfuric acid and 1 mL of anisaldehyde) followed by heating at 100°C, and two solutions to identify the presence of flavonoids: (flav_1) boric acid (99.5%, Synth, lot 35851)/oxalic acid (99.5%, Synth, lot 34230) solution; (flav_2) 2-aminoethyl diphenylborinate (CAS 524-95-8 Sigma 123 k2512) followed by UV-365 visualization (UVP Upland, model cc10, Upland, USA). Quercetin (PVP, lot 0464) was used as standard.

Quantification of Total Phenolics and Quercetin

The total phenolics were determined using the methodology described by Singleton and Rossi (26) with modifications. About 1 mL of the extract and 1 mL of Folin-Ciocalteau (Merck, lot OC274942) reagent were mixed. After 3 min, 1 mL of Na_2CO_3 saturated solution (approximately 35%) was added and the volume was completed to 10 mL with distilled water. The mixture was kept in a place protected from light for 90 min. The absorbance was read at 725 nm, using gallic acid (Sigma, lot 023K01171) as standard (27–30).

The quantification of quercetin in the extracts was conducted as follows: the extracts were diluted in methanol (P.A., Merck, lot K 28030409 029) in a concentration up to 0.07 mg extract/mL methanol. The solution absorbance was read in a spectrophotometer (Hitachi, U-3010, Tokyo, Japan) at 371 nm. The quantification was obtained by comparison with the quercetin standard curve.

Antioxidant Activity

Antioxidant activities of all extracts were determined using the methodology of Hammerschmidt and Pratt (31) with modifications (32). The reaction substrate was obtained using 10 mg of β-carotene (99%, Acros, lot 7235-40-7), 10 mL of chloroform (Merck, P.A., lot K31503045 301), 60 mg of linolenic acid

(99%, Sigma, lot 054K1214) and 200 mg of Tween 40 (Synth, P.A., lot 032K0104). The solution was concentrated in a rotovap system (Laborota, model 4001, Vertrieb, Germany) at 50°C and afterwards diluted with 50 mL of bi-distilled water. For the oxidation reaction, to 1 mL of substrate was added 2 mL of bi-distilled water and 0.05 mL of the sample diluted in ethanol (99.5%, Ecibra, lot 16.269) (20 mg of extract/1 mL of ethanol). Then, the mixture was set into a water bath (Tecnal, TE 159, Piracicaba, Brazil) at 40°C and the reaction product was monitored using a spectrophotometer (Hitachi, U-3010, Tokyo, Japan) by reading the absorbance at 470 nm after 2 hours of reaction. The antioxidant activities (AAs) were calculated by Equation (1):

$$\%AA = 100 \times \left[1 - \frac{(Abs_e^o - Abs_e^t)}{(Abs_c^o - Abs_c^t)}\right] \tag{1}$$

where Abs^0 and Abs^t are the absorbance at 470 nm read at the beginning of the reaction and at the time t, respectively; the subscript *e* and *c* indicates extract and control samples.

RESULTS AND DISCUSSION

Extraction Processes

Table 1 shows the global yield of A. satureioides extracts obtained by different separation processes. According to Table 1, the higher global yields were obtained in LPSE (11 ± 3%) followed by the Soxhlet (6.9 ± 0.7%) and ultrasound (6.6 ± 0.8%) processes. However, these techniques are not selective. The low global yield obtained by hydrodistillation is due to the fact that this methodology extracts only volatile oil or essential oil. The global yields of the extracts obtained by SFE were larger than that of the hydrodistillation (0.035%). However, this process is generally selective, so, it is important to know the composition of these extracts.

In relation to the SFE results, the analysis of variance (ANOVA) indicated that the differences in yield for the experiments done at 150 and 200 bar were not significant ($p_{value} = 0.639$). Nevertheless, as the pressure increased from 200 to 250 bar a significant increase in the global yield was observed ($p_{value} = 0.002$). In this region, it can be observed that raising the pressure from 200 to 250 bar (corresponding to an increase in carbon dioxide density from 891.4 to 923.3 kg/m^3) resulted in an increase in 65% in the global yield.

Analysis of the Extracts

Figure 1 shows the TLC plate obtained using the mixture n-hexane:ethyl acetate (85:15) as eluent, revealed with anisaldehyde, flav_1 and flav_2. It

Table 1. Global yield of *A. satureioides* extracts obtained by different separation processes and quantity of total phenolics and quercetin present in each extract, and antioxidant activity (after 2 hours of reaction) of extracts

Separation process	Global yield (% b.s.)	Total Phenolics × 10^3 (kg/kg extract)	Quercetin × 10^3 (kg/kg extract)	Quercetin/total phenolics (%)	Antioxidant activity (%)
Centrifugation	2.7 ± 0.4	130 ± 2	48 ± 2	36.9	—
Hydrodistillation	0.035 ± 0.003	n.a.	n.a.	n.a.	—
LPSE	11 ± 3	49.30 ± 0.05	31.60 ± 0.02	64.1	81 ± 1
Soxhlet	6.9 ± 0.7	55 ± 1	38.8 ± 0.6	70.5	92 ± 3
SFE – 150 bar/30°C	1.159 ± 0.008	56 ± 3	12.72 ± 0.05	22.7	81.63 ± 0.01
SFE – 200 bar/30°C	1.1 ± 0.1	47.9 ± 0.5	16.62 ± 0.01	34.7	82 ± 1
SFE – 250 bar/30°C	1.84 ± 0.09	47 ± 1	13.27± 0.01	28.2	91 ± 3
Ultrasound	6.6 ± 0.8	73.742 ± 0.004	23.448 ± 0.004	31.8	85 ± 2

n.a.= not analyzed.

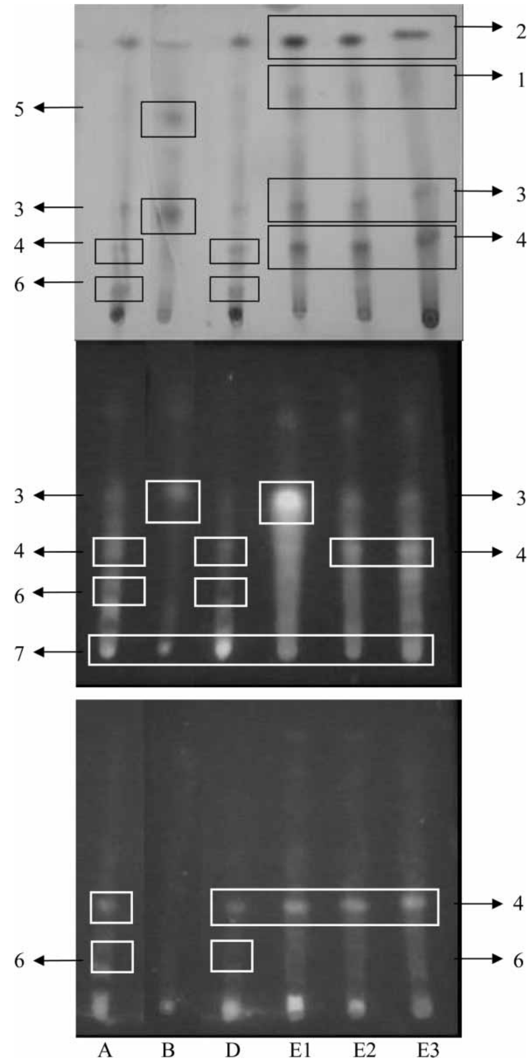

Figure 1. TLC analysis of the *A. satureioides* extracts using anisaldehyde, flav_1 and flav_2 from top to bottom, respectively: (A) centrifugation (B) hydrodistillation, (D) Soxhlet (E1) SFE at 150 bar/30°C, (E2) SFE at 200 bar/30°C, (E3) SFE at 250 bar/30°C.

can be observed that the extracts obtained by the Soxhlet and centrifugation processes presented basically the same composition. In the SFE extract there were compounds that were not detected in the other processes (square 1) while some of the compounds obtained in the other process appeared in a higher amount in the SFE extracts (square 2, 3, and 4). On the other hand, hydrodistillation extracted a compound, characterized by a pink color (square 5) that was not obtained in the other extracts.

According to Fig. 1, in which the plates were revealed with a specific reagent to identify the flavonoids (flav_1 and flav_2), the presence of the flavonoids in the extracts of A. satureioides (square 3, 4, 6, and 7) were detected. However, it is possible that some of the compounds present in the extracts have natural fluorescence. So, in order to confirm the presence of flavonoids, quercetin was used as standard (square 8), as shown in Fig. 2. In this, the TLC analysis was made using the mixture of chloroform: acetone: formic acid (75:16.5:8.5) as eluent, revealed with flav_2.

As observed in Fig. 2, the extracts obtained by Soxhlet, LPSE, centrifugation, and ultrasound present similar bands; in other words, it presented basically the same composition. The results still showed the presence of quercetin in these extracts.

The extracts obtained by SFE apparently had no quercetin, or the amount was so small that it was not possible to identify it by the TLC analysis. It is probably due to the polarity of supercritical CO_2 ($SCCO_2$). Polar compounds such as quercetin are preferentiality not extracted by $SCCO_2$ that is an apolar solvent at the SFE conditions used in the present work. However, quercetin would be extracted if a co-solvent was added in the $SCCO_2$.

Table 1 shows the amount of total phenolics and quercetin present in A. satureioides extracts. The total phenolics and quercetin varied from (47 to 130) $\times 10^{-3}$ kg/kg and (12.72 to 48) $\times 10^{-3}$ kg/kg, respectively. The largest ratios of the content of quercetin to total phenolics were obtained by Soxhlet (70.5%) and LPSE (64.1%). The extracts obtained by centrifugation contained the highest amount of total phenolics (130×10^{-3} kg/kg),

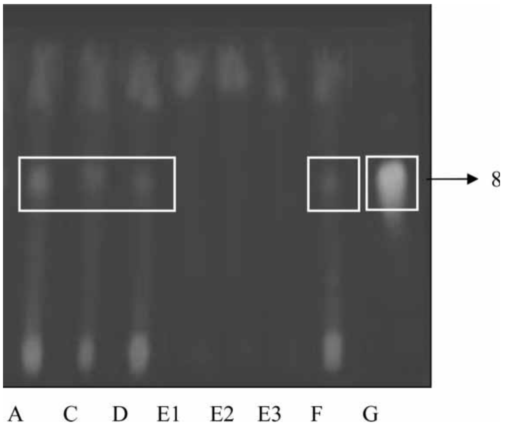

Figure 2. TLC analysis of the *A. satureioides* extracts using flav_2: (A) centrifugation, (C) LPSE, (D) Soxhlet, (E1) SFE at 150 bar/30°C, (E2) SFE at 200 bar/30°C, (E3) SFE at 250 bar/30°C, (F) ultrasound, (G) Standard (quercetin).

however about 36.9% of it is quercetin. In contrast, extracts obtained by Soxhlet had 38.8×10^{-3} kg/kg of total phenolics with quercetin being 70.5%.

Comparing the results, the SFE process extracted a small amount of total phenolics. It was expected due to the polarity of $SCCO_2$. However, the ratio of quercetin to total phenolics (22.7, 34.7, and 28.2%, for SFE at 150, 200, and 250, respectively) was similar to that of the ultrasound (31.8%) and the centrifugation (36.9%) processes. Although the SFE resulted in smaller global yield, the process was more selective for quercetin, if compared with ultrasound and the centrifugation processes. On the other hand, the amounts of quercetin (12.72, 16.62, and 13.27×10^{-3} kg/kg, for SFE at 150, 200, and 250 respectively) obtained by SFE were the smallest, confirming the TLC results (Fig. 2). The extraction of quercetin by SFE can be improved. Literature shows that the addition of a co-solvent to $SCCO_2$ increases the solvent power in extracting polar compounds (33). Chafer et al. (34) determined the solubility of quercetin in $SCCO_2$ plus ethanol as co-solvent. They verified that the solubility of quercetin increased with the amount of ethanol added, and with the increase of the pressure the solvent power of CO_2 became greater and then more solutes were transferred to the supercritical phase.

The solubility of quercetin in $SCCO_2$ was also measured by Matsuyama et al. (35). They developed a mathematical expression that considers the solubility of quercetin as a function of the $SCCO_2$ density. According to it, the solubility of quercetin in $SCCO_2$ increases with temperature and pressure. However, in our experiments the results at 30°C did not show the same effect. Thus, it is possible that other factors could be affecting the results. Matsuyama et al. (35) verified that the solubility of flavonoids is strongly dependent on the operating pressure at pressures near to the critical condition of CO_2, and good experimental data were not obtained.

Analyzing all the experimental results, it can be observed that there is no apparent relation between global yield, total phenolics, and quercetin extracted by the different processes.

The literature reports that in plants quercetin occurs mainly in leaves and in the other parts as aglycones and glycosides, in which one or more sugar groups is bound to phenolic groups by a glycosidic bond (36). For instance, the aglycone quercetin can be linked to rhamnose forming the quercetin or linked to rutinose forming the rutin as the 3-O-glycoside (37). According to Wach et al. (36), in order to determine the total flavonoid aglycones, a hydrolysis procedure is required to break the glycosidic bonds. They determined the amount of quercetin in some foods (apple, onion) and herbs (Hypericum perforatum and Sambucus nigra). These herbs, H. perforatum and S. nigra, have commonly been used as herbal treatment for various health problems and the study of these plants showed that their leaves contained a significant amount of quercetin, mainly present as glycosides. S. nigra, in addition to high concentrations of anthocyanins, also contains a complex mixture of other flavonoids. Wach et al. (36) also showed that the aglycone form of quercetin was accountable for only about 5% of the total flavonoids content. Thus, the amount of quercetin present in A. satureioides could be higher if the quercetin presents as glycosides was considered.

Studies revealed that the aerial parts of A. satureioides contain a higher concentration of β-caryophyllene and α-pinene in its essential oil. In order to understand the effect of temperature and pressure on the SFE process of the A. satureioides extracts, the solubility of β-caryophyllene and α-pinene in $SCCO_2$ were evaluated. For the system β-caryophyllene-CO_2 the data were determined using the Peng-Robinson equation of state (PR-EOS). The data were fitted using the software Edeflash v.1, developed in the LASEFI group (FEA/UNICAMP), using 0.2802 as binary interaction parameter (k_{ij}) (38). The critical properties and acentric factor were estimated by Somayajulu's group contribution method (39).

Figure 3 shows the solubility of β-caryophyllene in $SCCO_2$. Above 150 bar, the solubility of β-caryophyllene increases with temperature and decreases with pressure. On the other hand, according to Francisco and Sivik (40), the solubility of α-pinene in $SCCO_2$ decreases with temperature and increases with pressure. In this study, the authors showed that at pressures between 150 and 250 bar the solubility of α-pinene in $SCCO_2$ at 40°C (approximately 0.110 and 0.146 g/L CO_2, respectively) was higher than at 60°C (approximately 0.067 and 0.094 g/L CO_2, respectively). Thus, the opposite behavior is observed in the solubility of β-caryophyllene and α-pinene in $SCCO_2$.

In addition, the solubility of the mixture of monoterpenes including α-pinene was lower than that of single components (40). Francisco and Sivik (40) also verified that the solubility of eucalyptus oil was lower than that observed for pure components and the respective mixtures. These results demonstrate that the solubility of the same compound can be modified in the presence of other compounds. Then, the complexity of the extracts and the existence of interactions solute-solute, solute-matrix, and

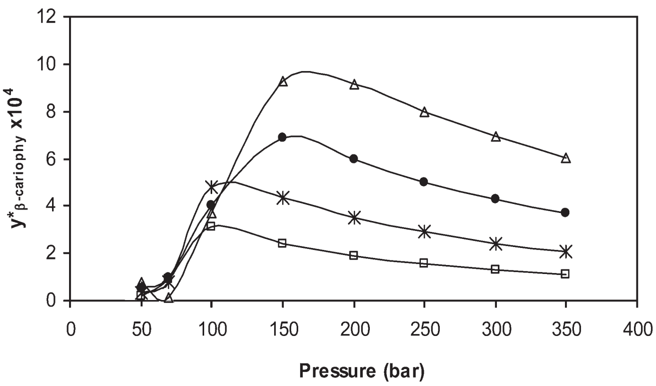

Figure 3. Solubility of β-caryophyllene ($y^*_{\beta\text{-caryophy}}$) in $SCCO_2$ at temperatures of: 30°C (□), 40°C (*), 50°C (●), 60°C (△), calculated using PR-EOS.

solute-solvent had direct influence on the solubility of compounds like quercetin, in our study.

Then, the reason for the variation observed in the experimental results for SFE global yields and composition can be due to the influence of solubility of other compounds like β-caryophyllene and α-pinene that demonstrate inverse behavior in $SCCO_2$, and interactions solute-solute, solute-matrix, solute-solvent.

Antioxidant Activity of the Extracts

Table 1 shows the antioxidant activity (AA) of A. satureioides extracts after 2 hours of reaction. All the extracts had AA larger than that of β-carotene (control). The analysis of variance (ANOVA) indicated that the differences were statistically significant among the AA of the extracts ($p_{value} = 0.005$).

The higher AAs were observed in the extract obtained by Soxhlet (92 ± 3%) and SFE_250 bar/30°C (91 ± 3%). There was no statistically significant difference between the AA of these extracts ($p_{value} = 0.863$).

Analyzing Table 1, it can be observed that the AA of the extracts can not be explained by the content of total phenolics and quercetin in the extract. The Soxhlet extract, that had higher AA, contains 38.8 mg/mg of quercetin. On the other hand, the SFE_250 bar/30°C extract, that had also higher AA, contains 13.27 mg/mg of quercetin.

The AA can be attributed to some compound not identified or to some group of compounds that was extracted by the different separation processes in a dissimilar amount. Another possibility is the synergism among the compounds, increasing the AA of some extracts.

CONCLUSIONS

In the present work, the global yield, composition and the antioxidant activities of the extracts from A. satureioides leaves and thin branches obtained by different separation techniques were evaluated. Higher global yields were obtained by LPSE (11 ± 3%) and Soxhlet (6.9 ± 0.7%). The TLC analysis indicated the presence of flavonoids in all extracts. The extract obtained by centrifugation contain a higher amount of quercetin ($48 \pm 2 \times 10^{-3}$ kg/kg extract) and the Soxhlet extract was shown to have the highest ratio of quercetin to the total phenolic fraction (70.5%). The antioxidant activities of the A. satureioides extracts were larger than that of β-carotene; however, it was not possible to identify the compound or group of compounds responsible for it. The solubility of β-caryophyllene in $SCCO_2$ was estimated, and the existence of interactions can affect the solubility of this compound and others in $SCCO_2$. According to literature, the solubility of quercetin in $SCCO_2$ increases with pressure; nonetheless, this effect was not observed in our study probably due to the effect of compounds interactions. Although in our study the effect of temperature was not evaluated, it is known that at higher pressure the solubility of substances with low volatility, as quercetin, increase with temperature. Thus, it is expected that at higher temperatures, SFE extracts will be richer in quercetin.

The fact that all of the extracts had antioxidant activities indicates that, beside quercetin, other compounds can have an important effect in the antioxidant activity. An effective analysis of these extracts with other techniques such as ^{1}H NMR and ^{13}C NMR can identify the other bioactive compounds or group of bioactive compounds.

This study contributed to show that the A. satureioides extracts obtained by different separation processes had antioxidant activity. In all of them, the presence of phenolic compounds was verified, thus indicating the possibility of its use in the formulations of foods and drugs.

ABBREVIATIONS

SFE	supercritical fluid extraction
LPSE	low pressure solvent extraction
TLC	thin layer chromatography
AA	antioxidant activity
flav_1 or flav_2	reagents used in TLC analysis to reveal the flavonoids
$SCCO_2$	supercritical carbon dioxide

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