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Comparison of the Chemical Composition of Extracts from *Scutellaria lateriflora* Using Accelerated Solvent Extraction and Supercritical Fluid Extraction versus Standard Hot Water or 70% Ethanol Extraction

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The aqueous extract of American skullcap (*Scutellaria lateriflora* L. (*S. lateriflora*), Lamiaceae) has been traditionally used by North American Indians as a nerve tonic and for its sedative and diuretic properties. Recent reports stated that flavonoids and possibly amino acids are responsible for the anxiolytic activity. As a part of our search for environmentally friendly solvents to extract the active components from medicinal plants, we used *S. lateriflora* in a comparison of accelerated solvent extraction (ASE) using water, and supercritical fluid extraction (SFE) using CO_2 and 10% EtOH as modifier, at different temperatures. Flavonoids and amino acids were quantified by HPLC−UV and HPLC−MS, respectively. The flavonoid content was compared with conventional extraction methods (hot water extraction and 70% ethanol). The use of ASE at 85 °C with water as solvent gave the best results for flavonoid glycosides and amino acids, whereas SFE gave higher yields of flavonoid aglycones. However, the results obtained for total flavonoids were not significatively superior to hot water extraction or 70% aqueous EtOH extract.

KEYWORDS: Scutellaria lateriflora; accelerated solvent extraction; supercritical fluid extraction; hot water extraction; ethanol extraction; flavonoids; amino acids

INTRODUCTION

American skullcap (*Scutellaria lateriflora* L. (*S. lateriflora*), Lamiaceae) is a perennial herb indigenous to North America, growing in wet places from Canada to Florida and westward to British Columbia, Oregon, and New Mexico. It derives its common name from the helmet-shaped upper lid of the seedpods. The aqueous extract of the flowering parts has been traditionally used by Native Americans as a nerve tonic and for its sedative and diuretic properties (*1*, *2*).

Most of the literature on the chemistry of American skullcap has been published only recently. Mono- and diterpenes (*3*, *4*), flavonoids (*5*), and amino acids (*6*) have been reported. As is the case with many herbs, the activity of skullcap is probably due to several compounds acting on different targets. Based on a recent study, flavonoids and amino acids are thought to be responsible for the anxiolytic activity (*6*).

S. lateriflora contains the flavonoid glycosides baicalin, dihydrobaicalin, ikonnikoside I, lateriflorin, scutellarin, and oroxylin A-7-*O*-glucuronide and the aglycones baicalein, oroxylin A, wogonin, and 5,6,7-trihydroxy-2′-methoxyflavone. In *S. lateriflora* baicalin is found in the highest concentration (*5*). While there are few studies on the bioactivity of American skullcap, *Scutellaria baicalensis* (*S. baicalensis*) has been extensively documented. Flavonoids are considered to be the active compounds. Reports published on *S. lateriflora* and *S. baicalensis* show that baicalin, baicalein, oroxylin A, and wogonin act on different brain receptors in vitro (*5*, *7*−*11*). Behavioral studies demonstrated that wogonin, a benzodiazepine receptor ligand, exerted potent anxiolysis in mice without sedative and myorelaxant actions (*12*). A recent report showed that baicalin is transformed to baicalein by intestinal bacteria, absorbed in the intestine and conjugated back to baicalin in the plasma (*13*, *14*). The detection of baicalin in the plasma confirms its bioavailability.

The amino acid GABA (γ-amino butyric acid) has been reported to be partially responsible for the anxiolytic activity of Valerian (*Valeriana officinalis* (*V. officinalis*)). Santos et al. (*15*−*17*) reported that the amount of GABA in an aqueous extract of *V. officinalis* induced the in vitro release of [^{3}*H*]GABA in rat brain synaptosomes and inhibited its reuptake. These results suggest that an in vivo mechanism of GABA release in

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neurons may act in the same manner. However, GABA cannot cross the brain−blood barrier (*18*), but glutamine, another amino acid occurring in large amounts in valerian, can. In the brain, glutamine can be metabolized to GABA by GABAergic neurons. High levels of glutamine in *V. officinalis* may explain the anxiolytic activity observed in vivo. As glutamine is especially high in skullcap (*6*), glutamine may also be partially responsible for the anxiolytic activity of skullcap.

Water is nontoxic, nonflammable, and readily available, rendering it an environmentally friendly solvent. Moreover, its physicochemical properties change dramatically at elevated temperatures and pressures. In certain processes, water can replace organic solvents for food and pharmaceutical manufacturing, reducing costs and eliminating the environmental problems associated with production and disposal of organic solvents. Specifically, subcritical water extraction uses hot water under pressures sufficiently elevated to maintain it in a liquid state. This technique was used to selectively extract different classes of compounds (*19*). With hot liquid water, polar compounds can be extracted at lower temperature, while lipophilic compounds can be extracted at higher temperatures. In certain cases, to avoid the use of non-GRAS (generally recognized as safe) solvents, supercritical fluid extraction (SFE) can be used, using carbon dioxide (CO_2) (SFE-CO_2) as the entrainer. Carbon dioxide has the advantages of being chemically inert, having low toxicity, and not contributing to pollution (*20*). When the SFE-CO_2 extraction is complete, the CO_2 returns to its gaseous form at ambient conditions, resulting in a solvent-free extract and circumventing the need for a complex separation step. Previous results on *S. baicalensis* showed that SFE-CO_2 extraction with modifier was suitable to extract flavonoids (*20*).

The aim of this study is to evaluate different environmentally friendly solvents to extract the actives from *Scutellaria lateriflora* and to compare these extracts to the standard extraction techniques (hot water and 70% ethanol extraction).

MATERIAL AND METHODS

Plant Material. Dried skullcap was supplied by Blessed Herbs (Oakham, MA, Lot No. L4519CO). The dry biomass was stored at −20 °C before being ground to a particle size of 0.4 mm as determined by ASAE S319.1 (*21*).

Standards. The amino acids were purchased from Sigma Chemical Co. (St. Louis, MO). Baicalein (97.5% purity) and baicalin (95.5% purity) were obtained from Indofine Chemical Co. (Somerville, NJ). Scutellarin (94.4% purity) was purchased from Herbstandard, Inc. (Chesterfield, MO) and wogonin (99.9% purity) was obtained from ChromaDex Inc. (Laguna Hills, CA). Lateriflorin (86.0% purity), ikonnikoside I (99.0% purity), and dihydrobaicalin (92.0% purity) were isolated as described previously (*5*). Oroxylin A was calculated as baicalein, while oroxylin A-7-*O*-glucuronide was calculated as baicalin. The purity of isolated compounds was measured by HPLC−UV.

Accelerated Solvent Extraction. Accelerated solvent extractions (ASEs) were performed using a Dionex model ASE 200 equipped with a solvent controller (Dionex Corp, Sunnyvale, CA). To prepare a single extraction cartridge, about 30 g of 30−40 mesh sand (SX0075-3, EM Sciences, Gibbstown, NJ) was mixed with 0.5 g of dry ground herb. The extractions were conducted at a pressure of 10 MPa for a total of 30 min with three 10 min cycles and with 90 s nitrogen purges. All extractions were collected in water. The temperatures examined were 85, 100, 150, 170, and 190 °C. Extractions were performed in triplicate. All extracts were freeze-dried using a Labconco Freezone 18 instrument (Kansas City, MO) and stored at −20 °C.

Supercritical Fluid Extraction. Supercritical fluid extractions (SFE-CO_2) were performed using an ISCO SFX 3560 (Lincoln, NE) two-pump system. About 10 g of 30−40 mesh sand (SX0075-3, EM Sciences) was mixed with 1 g of dry ground herb and placed in the SFE cartridge. The SFE process consisted of a static extraction time of 5 min followed by dynamic extraction for 30 min. The flow rate was 1.5 mL/min of CO_2 (Airgas UN1013, Radnar, PA). The restrictor temperature and extraction pressure were set at 60 °C and 20 MPa, respectively. SFE-CO_2 extractions were conducted at temperatures of 40, 60, and 80 °C. Having the restrictor set at 60 °C allowed for extractions to be carried out within ±20 °C of the restrictor temperature. Extracts were collected in 10 mL of ethanol. All conditions were performed in triplicate. SFE-CO_2 extractions were also performed with 10% (v/v) ethanol modifier, with all other operating conditions remaining identical.

Hot Water Extraction. The extraction was done using 5 g of plant material in 100 mL of water, at 85 °C for 30 min on a platform shaker (New Brunswick Co., Edison, NJ). Extracts were then filtered and freeze-dried using a Labconco Freezone 6 instrument (Kansas City, MO). The extracts were analyzed directly after they were freeze-dried.

Ethanolic Extract. The extraction was done using 5 g of plant material in 100 mL of 70% aqueous ethanol, for 24 h at room temperature on a platform shaker. Extracts were then filtered, evaporated and freeze-dried using the Labconco Freezone 6 instrument. The extract was analyzed immediately after being freeze-dried.

HPLC Analysis of Flavonoids. A 10 mg amount of the dried extract was dissolved in 1 mL of 70% EtOH and sonicated in an ultrasonic bath. The analyses were performed on an Agilent HPLC system (series 1100) with quaternary pump, UV−vis detector (photodiode array detector, DAD), and automatic sample injector (Agilent Technologies, Burlington, MA). Separation for flavonoids was achieved on an RP-18 column (Zorbax Eclipse XDB C-18 5μm 250 × 4.6 mm i.d. with Zorbax C-18 guard column (Agilent). Eluents A MeCN (0.05% TFA) and B H_2O (0.05% TFA): 0 min, 14% A; 40 min, 58% A; 42 min., 100% A; 47min, 100% A; 49 min, 14% A; Conditioning: 15 min. The flow rate was 1.0 mL/min. The flavonoids were detected at 280 nm.

Quantification of the Flavonoids. The flavonoids were quantified by an external standardization method. A calibration curve was done with baicalein, baicalin, scutellarin, wogonin, lateriflorin, ikonnikoside I, dihydrobaicalin, and quantified by HPLC−UV at 280 nm. Oroxylin A was calculated as baicalein, while oroxylin A-7-*O*-glucuronide was calculated as baicalin.

HPLC-MS Analysis of Amino Acids. A 10 mg amount of the dried extracts was dissolved in 1 mL of distilled water and then sonicated. The analyses were performed on an Agilent HPLC system (series 1100) with quaternary pump, UV−vis detector (DAD) automatic sample injector, and an Agilent MSD trap with ESI interface (Agilent 1100 series, Agilent). Separation was achieved on an RP-18 column (Synergi Hydro-RP 4 μm, 250 × 2 mm i.d., Phenomenex, Torrance, CA) using a precolumn ODS, 4 × 3 mm i.d. Eluents A H_2O (0.03% HFB) and B MeCN (0.03% HFB): 0 min, 0% B; 5 min, 25% B; 18 min, 100% B; 21 min, 100% B; 21.5 min, 0% B. The flow rate was 0.35 mL/min. The temperature was 22.0 °C. MS conditions: positive ion mode; scan range, 100−230 *m/z*; accumulation time, 100 ms; skim, 1:14.6 V; capillary exit offset, 38.1 V; trap drive, 34.8.

Quantification of the Amino Acids. The amino acids were quantified without derivatization using an external standardization method (calibration curve with identical standard). Peak areas of the $[M + H]^+$ ion were extracted and quantified using the software QuantAnalysis 1.4 (Agilent).

Reagents. HPLC grade acetonitrile (MeCN) was bought from Fisher Chemical Co. (Fairlawn, NJ). Trifluoroacetic acid (TFA) and heptafluorobutyric acid (HFB) were purchased from Sigma.

Data Analysis. An ANOVA analysis followed by a Fisher's PLSD was performed for each experiment using StatView SAS version 5.0.1. All statistical analyses were performed at a level of significance of 0.01.

RESULTS AND DISCUSSION

Flavonoids. The aerial parts of *S. lateriflora* contain principally flavonoid glycosides with baicalin as the major one followed by dihydrobaicalin, lateriflorin, ikonnikoside I, scutellarin and oroxylin A-7-*O*-glucuronide (**Figure 1**). The major

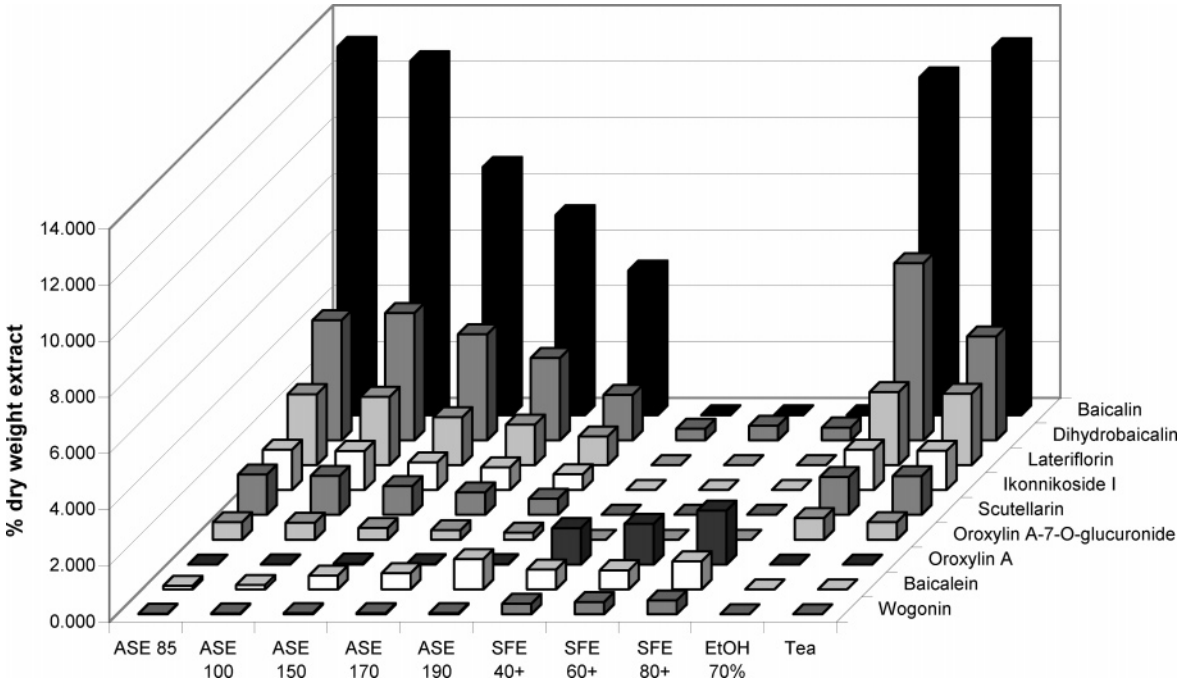

Figure 1. Bar diagram representing the percentage of flavonoids expressed in terms of dry weight of extract.

Table 1. Percentage of Total Flavonoids in the Extract

treatment	total flavonoids[a] (% extract)	std dev
ASE 85 °C	23.616a	1.814
ASE 100 °C	23.191a	0.948
ASE 150 °C	17.339b	0.749
ASE 170 °C	14.104c	0.675
ASE 190 °C	10.340d	0.944
SFE 40 °C + EtOH	2.791e	0.741
SFE 60 °C + EtOH	2.063e	1.870
SFE 80 °C + EtOH	3.878e	0.502
hot water extraction	22.784a	1.760
70% EtOH	24.583a	1.185

[a] Means not followed by the same letter are significantly different ($p < 0.01$).

flavonoid aglycone is oroxylin A followed by baicalein and wogonin. When extracted using ASE at 85 °C with water, the recoveries of baicalin, baicalein, wogonin, dihydrobaicalin, lateriflorin, ikonnikoside I, scutellarin, and oroxylin A-7-*O*-glucuronide were 13.1. 0.13, 0.02, 4.27, 2.53, 1.44, 1.44, and 0.62%, respectively.

Figure 1 shows that the use of the ASE with water extracted more flavonoid glycosides (baicalin, dihydrobaicalin, lateriflorin, ikonnikoside I, scutellarin, and oroxylin A-7-*O*-glucuronide) than SFE-CO_2 with 10% (v/v) ethanol. On the other hand, SFE-CO_2 with 10% (v/v) ethanol generally extracted more aglycones, such as wogonin and oroxylin A, than the ASE, hot water extraction, or ethanol extract. However, the aglycone, baicalein, displayed similar recoveries, whether using the ASE (150−190 °C) or SFE-CO_2.

As shown in **Table 1**, the highest percentages of total flavonoids obtained by combining aglycones and flavonoid glycosides, were reported using hot water extraction, 70% ethanol extraction, and the ASE at temperatures of 85 and 100 °C. Extracting with the ASE at temperatures of 150 or 170 °C resulted in significantly lower recovery. The lowest total flavonoid recovery was obtained with either the ASE at 190 °C or with the use of SFE-CO_2 at all tested temperatures with 10% (v/v) ethanol. With respect to the flavonoid glycosides (**Table 2**), scutellarin, ikonnikoside I, baicalin, lateriflorin, dihydrobaicalin, and oroxylin A-7-*O*-glucuronide, using the ASE 85 °C resulted in the highest recovery. The use of the ASE at 190 °C was best for the extraction of baicalein. This supports the results that show that high temperatures reduce the polarity of water, increasing its ability to solvate nonpolar compounds (*19*). However, there was no influence of temperature on the extraction of wogonin and oroxylin A. The ASE were performed using water as a solvent under a pressure of 10 MPa. This pressure setting was chosen on the basis of similar conditions used by Ju and Howard (*22*) for the extraction of anthocyanins from grape skins. In the grape skin system, the optimum temperatures, using water, for the recovery of anthocyanins were between 80 and 100 °C. As a whole, Ju and Howard (*22*) surveyed temperatures from 20 to 140 °C, using mixtures of organic solvents and water. Because we were solely interested in water, we opted to use higher temperatures. According to the results, we should investigate lower temperature as the extraction of flavonoid glycosides was inversely proportional to the temperature.

As expected, SFE-CO_2 with modifier did not extract flavone glycosides with the exception of a small amount of dihydrobaicalin, but allowed for similar recovery of the aglycone baicalein as with the ASE set at 190 °C. SFE-CO_2 with modifier at all temperatures was significantly better than ASE for the extraction of wogonin and oroxylin A. In the case of wogonin there was no significant difference between the different temperature treatments. In the case of oroxylin A, a temperature of 80 °C gave significantly higher yields. Previous results showed that SFE-CO_2 with EtOH as modifier can be used to extract glycosylated flavonoid, baicalin and aglycones, baicalein and wogonin, from *S. baicalensis* (*20*). They found that the optimal conditions were CO_2−MeOH−H_2O (20:2.1:0.9) at 50 °C and 20 MPa using a ISCO SFX 2-10 extractor. The SFE-CO_2 work done in our study was with 10% (v/v) EtOH at a pressure of 20 MPa and at extraction temperatures of 40, 60, and 80 °C, which were within ±20 °C of the set restrictor temperature. The ethanol was preferred to methanol for toxicity reasons.

As flavonoid glycosides are contained in higher proportion and as baicalin is transformed to baicalein by intestinal bacteria,

Table 2. Percentage of Flavonoids Expressed in Dry Weight of Extract

	ASE					SFE			hot water	
treatment	85 °C	100 °C	150 °C	170 °C	190 °C	40 °C + EtOH	60 °C + EtOH	80 °C + EtOH	extraction	70% EtOH
baicalin (SD)	13.141 (1.314)	12.628 (0.506)	8.860 (0.472)	7.134 (0.253)	5.160 (0.557)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	13.087 (0.546)	12.048 (0.509)
dihydrobaicalin (SD)	4.279 (0.744)	4.534 (0.010)	3.778 (0.203)	2.939 (0.267)	1.621 (0.371)	0.406 (0.167)	0.518 (0.264)	0.454 (0.018)	3.705 (1.090)	6.312 (0.651)
lateriflorin (SD)	2.530 (0.135)	2.437 (0.112)	1.707 (0.056)	1.442 (0.052)	1.025 (0.111)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	2.542 (0.114)	2.599 (0.113)
ikonnikoside I (SD)	1.438 (0.073)	1.392 (0.070)	0.980 (0.023)	0.806 (0.020)	0.562 (0.066)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.401 (0.030)	2.542 (0.114)
scutellarin (SD)	1.439 (0.089)	1.388 (0.079)	1.025 (0.029)	0.801 (0.017)	0.578 (0.063)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	1.381 (0.046)	1.347 (0.058)
oroxylin A 7-*O*-glucuronide (SD)	0.629 (0.023)	0.606 (0.023)	0.420 (0.010)	0.340 (0.007)	0.255 (0.028)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.625 (0.043)	0.776 (0.079)
oroxylin A	0.002 (0.004)	0.013 (0.005)	0.034 (0.030)	0.020 (0.017)	0.015 (0.013)	1.301 (0.207)	1.451 (0.356)	1.926 (0.325)	0.000 (0.000)	0.000 (0.000)
baicalein	0.134 (0.066)	0.160 (0.057)	0.489 (0.209)	0.578 (0.146)	1.081 (0.263)	0.708 (0.293)	0.685 (0.054)	1.003 (0.102)	1.381 (0.046)	1.347 (0.058)
wogonin	0.024 (0.000)	0.033 (0.001)	0.044 (0.002)	0.043 (0.012)	0.044 (0.014)	0.376 (0.085)	0.441 (0.010)	0.495 (0.084)	0.004 (0.001)	0.006 (0.001)

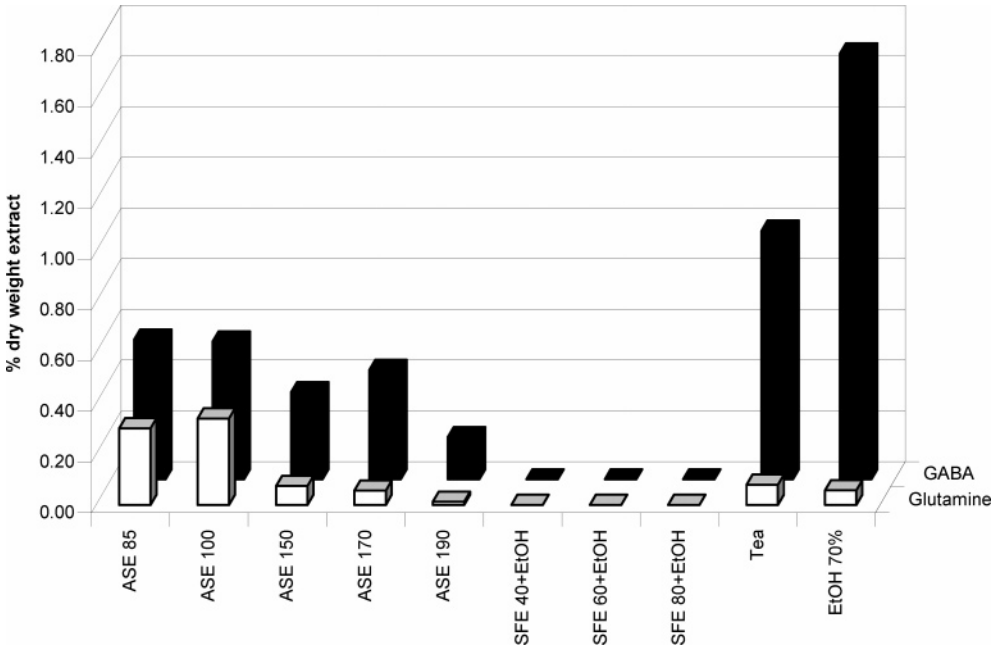

Figure 2. Bar diagram representing the percentage of GABA and glutamine expressed in dry weight of extract.

absorbed in the intestine, and conjugated back to baicalin in the plasma (*13*, *14*), it is more important to focus on increasing the extraction of flavonoid glycosides. In sum, these results show that the use of ASE is useful for the extraction of the flavonoid glycosides, whereas SFE-CO_2 with 10% (v/v) ethanol is, in general, better for the extraction of aglycones. However, the use of a water−ethanol mixture in the ASE and/or lower temperature may effectively increase the recovery of flavonoid glycosides (*14*).

Amino Acids. In *S. lateriflora*, GABA is the major amino acid (around 0.55%), followed by glutamine (0.34%) (**Figure 2**). As tryptophan, phenylalanine, proline, glutamic acid, arginine, asparagine, aspartic acid, tyrosine, isoleucine, leucine, and valine were lower than 0.1% of the dry extract, we just showed the results for the major amino acids with an effect on the brain receptors.

ASE at lower temperature was the best technique to extract glutamine. There was no significant difference in the recovery when using ASE extraction temperatures between 85 and 100 °C. For GABA, the ethanolic extract (70%) allowed the highest recovery. A hot water extraction extract and the ASE 85 and 100 °C showed the second best recovery yields. As glutamine passes the blood−brain barrier and not GABA, the results obtained for glutamine are more important from an activity point of view. Among the conditions tested in this work, the use of the ASE at temperatures between 85 and 100 °C shows the most promise for glutamine.

For tryptophan, phenylalanine, proline, glutamic acid, asparagine, tyrosine, isoleucine, leucine, and valine, an increase in the temperature in the ASE resulted in a decrease in the amino acid content. Only tryptophan was extracted with the SFE technique using ethanol as modifier.

The use of the ASE, using water at 85 °C as solvent is a suitable technique for the extraction of polyphenolics from *S. lateriflora*, giving yields of total flavonoids similar to the conventional extraction techniques, but with a higher concentration of glutamine and a lower amount of GABA. Comparison of the hot water extraction, the ethanolic extract, and the ASE shows that there is no advantage to use of ASE with water for the extraction of the flavonoid glycosides. However, extraction with SFE-CO_2 resulted in higher aglycone yields. In further experiments, an improvement of the experimental conditions of ASE and SFE-CO_2 such as the use of lower temperatures or higher temperatures with lower pressure could be explored.

ABBREVIATIONS USED

ASE, Accelerated solvent extraction; SFE, Supercritical fluid extraction.

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