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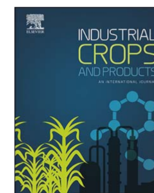

Ultrasound-assisted extraction of phenolic compounds from Macela (*Achyrolcine satureioides*) extracts

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ABSTRACT

Achyrocline satureioides or Macela, a native plant from South America, has been studied due to its potential antioxidant activity. This study sought to optimize the conditions for extraction of the bioactive compounds from Macela. The solvents 50% (v/v) acetone and 70% (v/v) ethanol were selected from preliminary assays, for a comparison study. The application of ultrasound for the extractions increased the amount of total phenolic compounds by 6.1-fold and the antioxidant activity by 3.4-fold compared with conventional extraction. Principal component analysis revealed that both solvents did not present statistical differences in the extractions, although the liquid chromatography analysis showed that relative to ethanol, the acetone extracts had a higher amount of quercetin, the major flavonoid present in Macela plants. Therefore, the best ultrasound-assisted extraction condition for both solvents studied was a temperature of 25 °C and a 1:40 solute:solvent ratio (w/v), independent of the extraction time (10, 50 or 90 min).

1. Introduction

Achyrocline satureioides (Lam.) D.C. is a medium-sized aromatic annual herb native to South America. In Brazil, it is commonly known as Marcela or Macela and is primarily used in popular medicine. Nonetheless, the commercial plants are harvested in their natural habitat, which confers to the plants some phytochemical and edaphic differences related to their place of origin (Ferraro et al., 2008).

The infusions of the Macela flowers are frequently used in folk medicine for gastrointestinal inflammation and spasms, respiratory problems, gallbladder and liver disorders and viral infections, due to their anti-atherosclerotic, anti-inflammatory, analgesic, antispasmodic, hepatoprotective and sedative activities (Ferraro et al., 2008; Kadarian et al., 2002; Simões et al., 1988). These pharmacological effects are associated with the chemical constituents of the plant and are directly correlated with the presence of the flavonoids, secondary metabolites with antioxidant activity. Thus, the antioxidant activity should be well established (Ferraro et al., 2008; Simões et al., 1988). Among the flavonoids found in *A. satureioides,* quercetin and luteolin are extensively studied for anticancer therapy, followed by 3-*O*-methylquercetin (Carini et al., 2014). Macela was recently included in the Brazilian Pharmacopoeia, as well as within MERCOSUR, for specific use as a food product (Retta et al., 2012). Consequently, Macela extracts could be used to maintain the oxidative stability of food products during storage (Campagnol et al., 2011) and applied in nutraceutical formulations.

The type of extraction to be used is an important consideration in the extraction of bioactive compounds. Direct extraction using solvents is the most common technique to obtain extracts with high antioxidant activity. The main factor to be considered in the solvent choice is the polarity of the targeted compound. Besides, the molecular affinity between the solvent and solute, mass transfer, use of co-solvent, environmental safety, human toxicity and financial feasibility, should also be taken into account in the selection of the solvent for bioactive compound extraction (Azmir et al., 2013). Moreover, ultrasound technology can reduce the extraction time and solvent consumption, while maximizing the recovery of bioactive compounds from plants (Corbin et al., 2015). Acoustic cavitations produced by ultrasound lead to high shear forces in the matrix and can disrupt cell walls, enabling solvent penetration into the plant material and allowing the intracellular content to be released. Additionally, the implosion of cavitation bubbles in a liquid medium leads to macro-turbulences and micro-mixing. Diverse mechanisms, such as fragmentation, erosion, capillarity, detexturation and sonoporation, are involved in cavitation. All of these mechanisms, individually or combined, act in the ultrasound extraction process and collaborate in bioactive extraction (Chemat et al., 2017; Corbin et al., 2015). Previously, Guss et al. (2017) studied the preventive action of Macela extracts obtained by ultrasonic-assisted extraction, against contrast-induced nephropathy in mice, using 100% ethanol as solvent.

In this context, the present work investigated conventional extraction combined with ultrasonication for maximizing the recovery of

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polyphenols from *A. satureioides*. Two suitable solvents were selected from ethanol, acetone, methanol and combinations of these solvents with water, in addition to water alone. Extractions were conducted using ultrasound (40 kHz [± 6%] and 100 W) with the two selected solvents (70% (v/v) ethanol and 50% (v/v) acetone) for three different conditions of time (10, 50 and 90 min), temperature (25, 37.5 and 50 °C) and solute:solvent (w/v) ratio (1:20, 1:40 and 1:60). The effect of the extraction methods and conditions on the polyphenol composition was investigated by principal component analysis (PCA).

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu phenol reagent (2N), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), 2,20-diphenyl-1-picrylhydrazyl (DPPH*), 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS*+) and potassium persulfate were purchased from Sigma Chemical Co (St. Louis, MO, USA). Ethanol (99.8%) and methanol (99.8%) were from Panreac (Barcelona, Spain). Acetone (99.6%) was from Neon (Suzano, Brazil).

2.2. Preparation of plant material for extraction

The raw material, composed by inflorescences of *A.satureioides,* was donated by Chamel Indústria e Com de Produtos Naturais (Campo Largo, Brazil). According to the supplier, the plant material was collected in Inácio Martins (latitude: 25°34′16“S; longitude: 51°04′44“W) and dried at 50–60 °C for 6–7 h. This material was ground in a laboratory mill (Requipal, MR 320, São Paulo, Brazil) and sieved to obtain particle sizes < 0.6 mm. The pulverized material was vacuum-packaged in polyethylene bags and stored frozen (Brastemp, São Paulo, Brazil) at −10 °C. The physicochemical composition (expressed as wet weight percentage) of the material was 5.71 ± 0.27% protein, 2.4 ± 0.06% ash, 6.48 ± 0.37% fat and 12.21 ± 0.09% moisture.

2.3. Selection of solvents

As shown in Table 1, ethanol, acetone, methanol, and combinations of these solvents with water, in addition to water alone, were used to select the two most suitable solvents. Suitability was based on the antioxidant capacity and total phenolic responses. The extraction process was performed using sonication at 1:20 solid:solvent (w/v) ratio at 25 °C for 30 min.

2.4. Extraction procedures

About 1 g of dry powdered macela and solvent, 50% (v/v) acetone and 70% (v/v) ethanol, respectively, were mixed in 50 mL centrifuge tubes. The volume of solvent added varied to meet the desired w/v ratio. The mixtures were immersed in a 40 kHz (± 6%) and 100 W ultrasonic water bath (Fisher Scientific, FS30D, Mexico) for predetermined time.

Table 1
Solvents used in the preliminarily experiments.

Extracts	Solvent (v/v)
1	Water
2	Ethanol 30%
3	Ethanol 50%
4	Ethanol 70%
5	Pure Ethanol
6	Acetone 30%
7	Acetone 50%
8	Acetone 70%
9	Pure Acetone
10	Methanol 30%
11	Methanol 50%
12	Methanol 70%
13	Pure Methanol

The obtained extracts were filtrated through qualitative paper filter (80 g/m^2; J Prolab, São José dos Pinhais, Brazil). Then the extracts were rotary evaporated under vacuum at 50 °C (MA120, Marconi, Piracicaba, Brazil). The weight of the samples was stabilized in a shelf dryer, to obtain the extract yields. In order to avoid solvent influence and solubilization problems, the concentrated extracts were solubilized in methanol (10 mL), as well as in Zhao et al. (2006) and Babbar et al. (2014), thereby, obtaining an appropriate original dilution factor for measurements. Diluted extracts were stored in amber bottles at 8 ± 1 °C, before analysis.

The extraction process was accomplished at three temperatures (25, 37.5 and 50 °C), three solute:solvent (w/v) ratios (1:20, 1:40 and 1:60) for three extraction times (10, 50 and 90 min).

2.5. Total phenolic content

The Folin-Ciocalteu assay was used to determined the total phenolic content (TPC) of the Macela extracts, according to Singleton and Rossi (1965), with slight modifications. A 20 μL aliquot of the Macela extract solution (appropriately diluted) was mixed with 100 μL of Folin–Ciocalteu reagent (10-fold dilution) and allowed to react at ambient temperature for 3 min in the dark. Then, 200 μL of saturated sodium carbonate solution was added, and the mixture was allowed to stand for 30 min. The absorbance of the reaction mixture was measured at 720 nm, using a UV–vis spectrophotometer (Shimadzu, UV-1800, Tokyo, Japan). Quercetin (0.1–0.9 mg/mL) was used as a reference standard, since it is majority phenolic compound present on *A. Satureioides* (Ferraro et al., 1981; Retta et al., 2012). Therefore, the results were expressed as milligram quercetin equivalents per 100 g Macela powdered.

2.6. Antioxidant activity determinations

The ABTS*+ assay was done as reported by Arnao et al. (2001) and Re et al. (1999), with some modifications. The absorbance of ABTS*+ was taken at 734 nm. Absorbance was read at 6 min after initial mixing.

The free radical scavenging activity was determined using the stable DPPH* radical, as described by Brand-Williams et al. (1995), with some adjustments. DPPH* stock solution (60 μmol/L) was prepared by dissolving DPPH* in methanol. The Macela extract was reacted with DPPH* solution for 30 min in the dark. Absorbance was recorded at 517 nm.

The ferric reducing antioxidant power (FRAP) assay was performed based on a previous method (Benzie and Strain, 1996), with some adaptations. FRAP solution was prepared by combining 300 mM acetate buffer pH 3.6 and 10 mM 2,4,6-tripyridyl-*s*-triazine (TPTZ) solution with 40 mM HCl and 20 mM ferric chloride solution. The Macela extract was allowed to react with the FRAP solution for 30 min.

For all antioxidant activity analyses, the Macela extracts were diluted as needed. All measurements were done in triplicate. Estimation of the Trolox equivalent antioxidant capacity (TEAC), represented as mMol of Trolox equivalents per 100 g of Macela powdered, was done using a concentration-response curve (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 mM Trolox). Results were expressed as mean ± standard deviation.

2.7. Ultra-performance liquid chromatography (UPLC) analysis

UPLC was used for analysis of the phenolic composition of the extracts obtained using, respectively, 50% acetone and 70% ethanol as solvent. This method was based on Barbi et al. (2017) and Pedro et al. (2018) works. The chromatographic separation was achieved on an

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