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Brazilian green propolis extracts obtained by conventional processes and by processes at high pressure with supercritical carbon dioxide, ethanol and water

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ABSTRACT

Extracts of green propolis were obtained by conventional process at atmospheric pressure and at high pressure using supercritical carbon dioxide ($scCO_2$) as solvent and, ethanol and water and its mixtures as solvents or as CO_2 co-solvents. All extracts were evaluated with regards to the total phenol content, total flavonoids, Artepillin C, p-Coumaric Acid and Kaempferide and also was evaluated the antioxidant activity, and *in vitro* antimicrobial activity. The highest overall extraction yields were 53.5% in the three-step sequential extraction at high pressure and 44.7% at low pressure in a Soxhlet extractor. All extracts showed high antioxidant activity and presented antimicrobial activity. The highest levels of phenols of 222 mg GAE/g extract, of flavonoids of 67.0 mg CE/g extract, of artepillin C of 62.4 mg Artepillin C/g extract and high antioxidant activity were obtained in the ethanolic extract (80%) of the second stage of the two-step sequential process.

1. Introduction

Propolis is a natural, resinous and sticky material, making by bees from material collected of different sources, such as tree buds and exudates found on plant wounds [1,2]. The medical use of propolis extract preparations has led to an interest in its composition, where the flavonoids which are found in different plants, they are the main polyphenols in propolis [3,4]. Many beneficial properties are attributed to propolis including antibacterial, antifungal, antiviral, antitumoral and anesthetic activity and it also possess a very low toxicity [5–8]. One of the major phenolic acids found in green propolis is 3,5-diprenyl-4-hydroxycinnamic acid (DHCA), also known as Artepillin C, which is obtained from Baccharis dracunculifolia, which is the main botanical source of this propolis type [9]. The amount of Artepillin C is associated with different states of maturity of the leaves in the Baccharis extracts [4,10].

Although conventional extraction methods are conducted at atmospheric pressure and have often been used to extract polyphenol compounds, technologies using supercritical CO_2 as solvent have been studied in an attempt to perform the process with better environmental and economic characteristics of the products [1,11,12].

The use of high pressures extraction techniques is an attractive alternative because it allows for fast extraction, so that solvents remain in a liquid state well above their boiling points. These conditions improve analyte solubility and the kinetics of desorption from matrices. Extraction processes employing supercritical carbon dioxide ($scCO_2$) take advantage of the ability of some chemicals to act as excellent solvents at temperatures and pressures above their critical points [13]. The scCO2 is considered a green, non-toxic solvent (GRAS) and also presents itself as nonflammable. In previous studies it was shown that when using a sequential extraction with scCO2 (in first step) and other solvents as water and ethanol on subsequents steps (second and third steps), fractions of extracts with high phenolic compounds content can be obtained [14–17].

The objective of this work was to obtain phenolic-rich green propolis extracts by processes at high and low pressure. Extractions at high pressure were conducted in different steps. In one step using $scCO_2$ with an EtOH-H_2O (80:20, v/v) hydroalcoholic mixture as a co-solvent, in two steps: sequential extraction in a fixed bed using $scCO_2$ followed by a hydroalcoholic EtOH-H_2O (80:20, v/v) extraction, and in three steps: sequential extraction in a fixed bed using $scCO_2$ followed by ethanol (second step) and finally with water (third step). All high pressure

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Nomenclature

SFE	Supercritical fluid extraction
PLE	Pressurized liquid extraction
SVE	Stirred vessel extractor
SOE	Soxhlet extraction
%VM	Volatiles + moisture
ρ_r	Real density
ρ_a	Apparent density
ε	Porosity
TP	Total phenolic
DPPH	1,1-diphenil- 2-picrilhidrazil
GAE	Gallic acid equivalent
TF	Total flavonoids
CE	Catechin equivalent
HPLC	High performance liquid chromatography
AoA	Antioxidant activity
EC_{50}	Effective concentration
AmA	Antimicrobial activity
SE_{80}	Supercritical fluid extraction more co-solvent with EtOH-H_2O (80:20, v/v) stage in one steps
S_1	Supercritical fluid extraction in 1st stage in two sequential step extraction
E_{80}	Pressurized liquid extraction in the 2nd stage with EtOH-H_2O (80:20, v/v) in two sequential step extraction
S_2	Supercritical fluid extraction in 1st stage in three sequential step extraction
E	Pressurized liquid extraction in the 2nd stage with EtOH absolute in three sequential step extraction
W	Pressurized liquid extraction in the 3rd stage with water in three sequential step extraction
DHCA	3,5-diprenyl-4-hydroxycinnamic acid (Artepillin C)
HCA	4-hydroxycinnamic acid (p-Coumaric acid)
MTHF	4-methoxy-3,5,7-trihydroxyflavone (Kaempferide)

experiments were performed under the conditions of 50 °C and 250 bar. For comparison, low pressure extractions were conducted at different proportions of EtOH-H_2O in stirred vessel and in soxhlet extractors. The extracts were characterized for total phenolics and total flavonoids, for the compounds 3,5-diprenyl-4-hydroxycinnamic acid (Artepillin C or DHCA), 4-hydroxycinnamic acid (p-Coumaric acid or HCA) and 4-methoxy-3,5,7-trihydroxyflavone (Kaempferide or MTHF) with regards to color, antioxidant activity by DPPH and antimicrobial activity.

2. Material and methods

2.1. Raw material and reagents

Green propolis were acquired in the Brazilian state of Minas Gerais, classified as group 12 by Park et al. [1].

Samples were cold-ground with mechanical stirring and they were packaged in plastic bags and stored in a freezer (model 220, Consul, Brasil) at −18 °C. The methods and equipment used for the characterization of the raw material with respect to volatiles + moisture content, moisture content, real and apparent density (ρ_r, ρ_a) was done as described by Monroy et al. [14], and the porosity (ε) as described by Rahman [18], calculated as $\varepsilon = 1\text{-}(\rho_a/\rho_r)$.

2.2. Atmospheric pressure extraction in a soxhlet and stirred vessel extractors

The extracts from conventional process, at atmospheric pressure, were performed for comparison purpose with the extracts obtained at high pressure extraction. The extractions in stirred vessel (SVE) was performed according to Farias-Campomanes et al. [19] with some modifications, in which 2 g of ground green propolis was placed in 25 mL of EtOH-H_2O mixtures with different proportions of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% (v/v), at 50 °C and 150 rpm for 30 min, as shown in Fig. 1. Soxhlet extraction (SOE) was done using 5 g of ground green propolis and 150 mL of EtOH-H_2O mixtures at 0, 30, 50, 80 and 100% (v/v) for 180 min, as shown in Fig. 1.

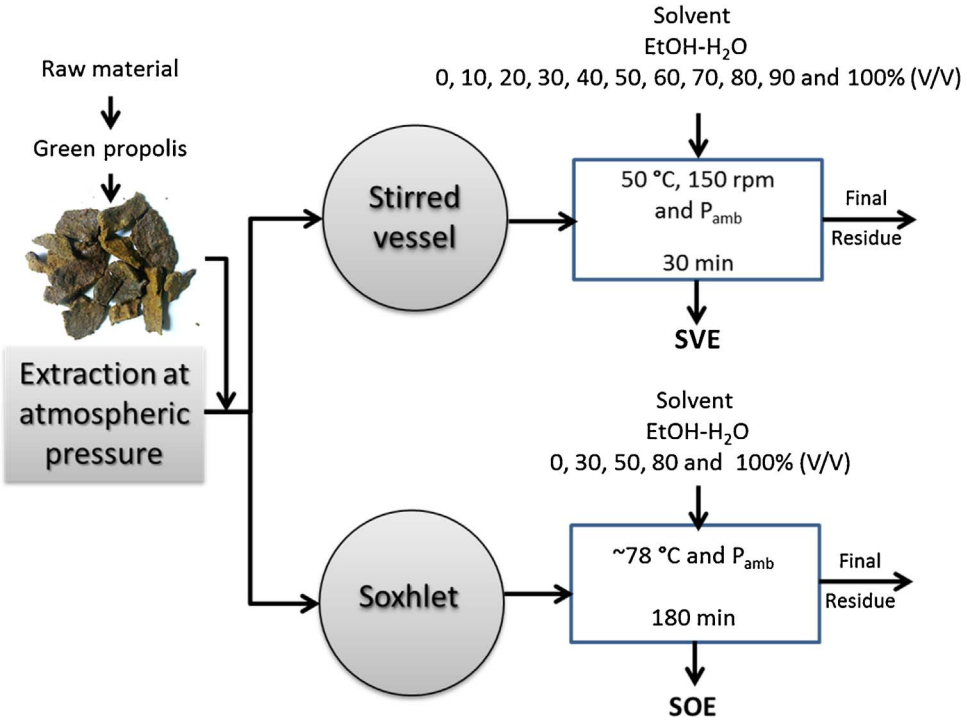

Fig. 1. Schematic diagram of the experimental procedure for extraction at atmospheric pressure.