Obtaining phenolic compounds from Barbatimão (*Stryphnodendron adstringens* (Mart.) Coville) bark by Pressurized Liquid extraction

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1. Introduction

Barbatimão (*Stryphnodendron adstringens* (Mart.) Coville) is a Brazilian medicinal plant known for its wide pharmacological application, such as antimicrobial, anti-inflammatory, antioxidant activities, among others[1]. Several studies have correlated the medicinal properties of this species, mainly the extracts of its bark, with its phenolic composition, with emphasis on the high content of tannins (9 - 39%), being composed mainly of condensed tannins, also known as proanthocyanidins[2]. However, studies applying emerging extraction techniques to obtain phenolic compounds rich extract are scarce, in which only the application of ultrasound-assisted extraction (UAE)[3,1] and microwave-assisted extraction (MAE)[4] have been found. Moreover, it is known that pressurized liquid extraction (PLE) is a technique that has shown promising results in terms of obtaining phenolic compounds[5]. Within this context, the objective of this work was to study the influence of PLE temperature (60, 80, and 100 °C) to obtain a phenolic-rich extract from Barbatimão bark.

2. Materials and Methods

2.1. Chemicals and sample preparation

Ethyl alcohol P.A. and distilled water were used as the extraction solvents. Folin-Denis (Sinergia, Campinas, SP, Brazil), sodium carbonate anhydrous (Sinergia, Campinas, SP, Brazil) and Tannic acid (Synth, Diadema, SP, Brazil) were used to evaluate total phenolic content. Barbatimão barks were donated by the company Kampo de Ervas (Ribeirão Preto, SP, Brazil). The material was supplied dry with a moisture content of 13.155 ± 0.072%. The dry barks were crushed and ground in a knife mill (Marconi, Piracicaba, SP, Brazil). The mean particle size, real density and bulk density of the sample was 0.50 ± 0.02 mm, 1465.4 ± 1.0 kg/m^3, and 541.6 ± 12.7 kg/m^3, respectively.

2.2. Pressurized liquid extraction and extract evaluation

The extractions were performed applying the dynamic method using a mixture of ethanol and water (50%, w/w). Approximately 3.0 g of dried sample was used, forming a fixed bed inside a 42.95 cm^3 stainless steel column. The extractions were performed varying the temperatures (60, 80, and 100 ºC). Pressure was kept constant at 10.0 ± 0.5 MPa and the solvent flow rate at 3.7 mL/min, in order to keep the mass flow rate constant (3.4 g/min). The extraction time was 60 min and was defined from preliminary tests, in which it had been verified that all the easily accessible solute was extracted. The extracts were collected in glass flasks and stored under freezing (−18 °C) in the absence of light, for further analyses. The global extraction yield (X_0) was calculated by the ratio between the dry extract mass (m_{ext}) and the dry sample mass (F) (Equation 1).

$$X_0\ (\%, w/w) = (m_{ext}/F) \times 100 \quad (1)$$

The TPC was determined according to the Folin-Denis method[6] with some modifications. Absorbance was read at 730 nm in spectrophotometer (Thermo Fisher Scientific, Waltham, MA, EUA). The results were expressed in mg of equivalent tannic acid (ETA) per g of dry RM (raw material).

2.3. Statistical analysis

The results were statistically evaluated by one-way analysis of variance (ANOVA) with significant differences at the level of 5% analyzed by Tukey's test.

3. Results and discussion

Figure 1 presents the PLE kinetics of global yield and TPC performed at 60, 80 and 100 °C. Regardless of the time, the highest X_0 were obtained at 100 °C. During the first 20 min, the X_0 at 60 and 80 ºC did not show significant differences but were significantly lower than from 100 ºC; after, although some significant differences, the X_0 was very similar between the three temperatures, reaching 43.35 ± 2.09, 45.99 ± 1.23, and 46.49 ± 0.40 % (w/w) at 60 min. Moreover, at 30 min of extraction, approximately 99% of the global yield was recovered at 100 °C. It is known that the increase in the solvent temperature promotes a decrease in surface tension and viscosity while diffusivity and extract solubility increase. In this regard, the increase in the solute solubility and the low interaction between solute and matrix facilitates the solute diffusion to the matrix surface. Thus, mass transfer rate increases leading to higher global yields[7]. The temperature significantly affects the phenolic extraction process from *barbatimão* bark by PLE during the 60 min. The accumulated TPC obtained was 434.45 ± 24.98, 472.20 ± 17.96, and 491.34 ± 78.80 mg ETA/g dry RM respectively for 60, 80, and 100 °C. Hence, in 30 min of extraction largest amount of TPC was recovered, approximately 93, 95, and 97% for 60, 80, and 100 °C, respectively.

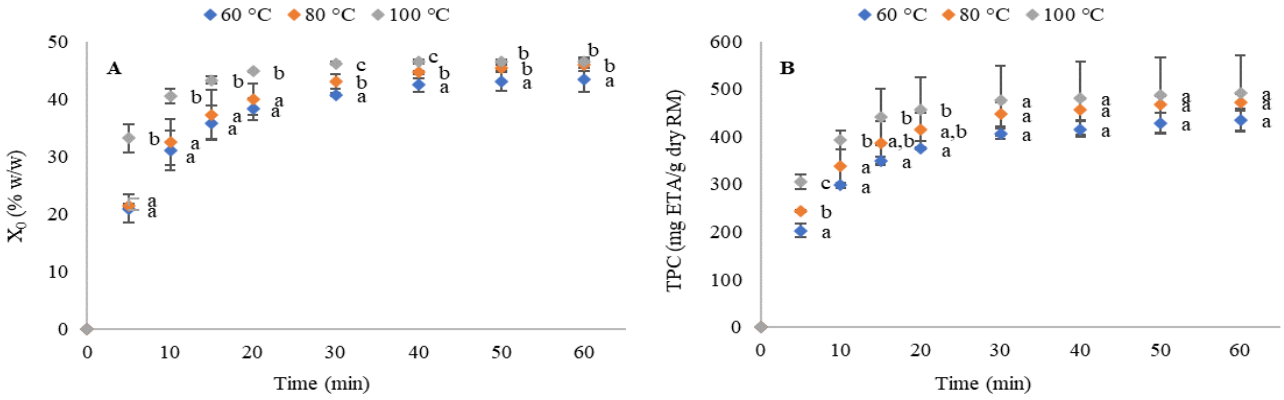

Figure 1. PLE curves from *barbatimão* bark at different temperatures: (A) X_0 and (B) TPC. Different lowercase letters indicate significant difference between temperatures at the same extraction time ($P < 0.05$).

4. Conclusions

Pressurized liquid extraction showed an environmentally friendly process for recovering phenolic compounds from barbatimão bark. The results indicate that temperature positively influenced the global yield and TPC recovery. Specifically, in 30 min of extraction at 100 °C, it was possible to recover about 99% of the global yield and 97% of phenolic compounds. Finally, the PLE application to recover phenolic compounds was not decisive. Further extract's bioactivity evaluation is necessary to understand the process behavior and support the optimized conditions.

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