Extracting Tannins from Jatobá (*Hymenaea courbaril* L.var *stilbocarpa*) Using Supercritical Carbon Dioxide and Water as Modifier

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ABSTRACT

Some of the Brazilian indigenous plants are studied due to their high phenolic compounds. *Hymenaea courbaril* L. var. *stilbocarpa*, commonly known as jatobá, is spread almost in all Brazil and its bark extracts have tannins with high antioxidant activity. In traditional medicine, they are used in treatment of various diseases, such as diarrhea, hypertension, rheumatism, stomach problems, urinary system and inflammatory processes in general. Therefore, several technologies for the extraction of tannins from various plant sources have been proposed in literature. Nonetheless, the use of supercritical fluid extraction (SFE) to obtain tannins from jatobá to the best of our knowledge has not yet been reported. SFE is recognized as a technology that produces extracts of high quality by using CO_2; it has gained popularity for isolating substances with high quality due to its shorter extraction time and reduced solvent consumption. However, due to the apolar property of CO_2 in SFE, a significant amount of polar solvent modifiers has to be added in order to extract tannins due to their high polarity. In this study, it was evaluated the effectiveness of SFE in obtaining extracts rich in tannins. The experiments were carried out using two temperatures (323 and 333 K) and three pressures (15, 25 and 35 MPa) and water as modifier at 10% (v/v). The extracts were evaluated for their global yield (X_0) and tannins content. Higher tannins contents were obtained using more aggressive extraction condition, 15 MPa and 323 K.

***Keywords**: Jatobá bark; tannins; supercritical fluid extraction; modifier.*

INTRODUCTION

Despite being considered one of the countries with the richest biodiversity in the world, Brazil has been poorly exploiting its natural resources [1]. Indeed, plants have been of crucial importance for human healthcare along centuries and their extracts present antioxidant, antiulcer, antimalarial, anticancer and anti-inflammatory activities.

Hymenaea courbaril stilbocarpa ("jatobá") is a Brazilian plant which has been reported due to its great natural antioxidant properties. Jatobá bark provides an extract with high 5-lipoxygenase inhibitory activity; its extract has also been used in the cosmetic industry owing to the polycatechin, which has moisturizing and skin-lightening effects. Flavonoids of varied structures [2] and diterpenes [3] are also common in Hymenaea species. Terpenes and phenols have various biological activities, such as protection against infections and insects [4], antimicrobial activity [5], antifungal, antibacterial and molluscicides properties [6]. Chemical analysis of the extracts also indicates its importance in biologically active compounds including sesquiterpenes and oligosaccharides [3]. Furthermore, they are used as an astringent in affections of the bladder and prostate cancer, bronchitis, coughs and whooping cough [7].

It is known that the high content of phenolic compounds in extracts of jatobá is due to the presence of condensed tannins, also known as poliflavonoids, which are present in higher plants [8]. Tannins are known to possess high antimicrobial and antioxidant activities [9]; acting as an astringent [6].
The important role of antioxidants in human health has been demonstrated, thus increasing the interest in such products and their demand by consumers. Commonly, the extracts are prepared by traditional methods using organic solvents. Such techniques have limitations in obtaining solvent-free extracts and may cause degradation and loss of target components. For this reason, supercritical fluid extraction (SFE) is well known as a clean technology that avoids or minimizes damages to the environment and has high selectivity, extracting the desired compounds by changing the operational conditions. SFE proved to obtain extracts with high quality by using CO_2. However, only CO_2 is not sufficient to obtain the extracts with antioxidant properties (tannins) due to its low polarity. Then, the use of a modifier can viabilize the extraction of tannins via SFE. Hence, this study aimed to evaluate the effectiveness of SFE using CO_2 with water as modifier (10 % v/v) at different conditions of temperature and pressure to achieve extracts rich in tannins compounds from jatobá bark.

MATERIALS AND METHODS

The bark of "jatobá" was purchased from Ervas-Brasil Produtos Naturais (São Paulo, Brazil). The raw material was comminuted in a knife mill (Marconi, model MA 340, Piracicaba, Brazil), packed in plastic bags and stored in a domestic freezer (Double Action, Metalfrio, São Paulo, Brazil) at 258 K.
SFEs were carried out using a system equipped with a 415 mL extraction vessel (3.4×10^{-2} m of diameter and 37.5×10^{-2} m of length, internal dimensions) for the extraction with CO_2 + H_2O. The raw material was placed inside a nylon cell presenting approximately the same measures of the vessel, which in turn was placed inside the extractor vessel. The experiments were carried out in two temperatures (323 and 333 K) and three pressures (15, 25 and 35 MPa). Carbon dioxide (99.5% purity, Gama Gases Especiais, São Paulo, Brazil) was admitted into the system keeping the relation S/F (ratio between the mass of solvent (S) and the mass of solid (F)) constant and equal to 50; water was used as a modifier at 10% (v/v) for a total of six different extraction conditions. The extraction time was 77±2 minutes.
The global yield (X_0) was calculated as the percentage (%) of mass extract ($m_{extract}$) out of the total initial mass of raw material (m_{RM}) loaded in the extraction cell, as shown in Equation 1:

$$\% X_0 = \frac{m_{extract}}{m_{RM}} \times 100 \quad (1)$$

The determination of tannins content in Jatobá bark extracts was based on the method described by Martins [10] and Farmacopéia Brasileira IV [11], and validated by Bott [12]. The method is based on precipitation of tannins with casein and at the blue coloration due to the reduction of Folin-Denis reagent by polyphenols. The calibration curve was built with pyrogallol as reference compound at different concentrations: 0.5; 1.0; 1.5; 2.0; 2.5; 3.0 and 3.5 μg/mL. The final extract dilution was 0.1 mg/mL.
For total polyphenols determination, an aliquot of the sample solution (0.5 mL) was added to the Folin-Denis (0.5 mL) and sodium carbonate (4 mL). The absorbance was measured at 730 nm on a spectrophotometer, just 2 min after the addition of sodium carbonate.

For non-tannin fraction determination, a suspension consisting of 20 mL of the diluted extract with 0.3 g of casein was stirred for 60 min. The suspension was filtered after casein precipitation. An aliquot of filtrate solution (0.5 mL) was added to the Folin-Denis (0.5 mL) and sodium carbonate (4 mL). The reading was performed as described in the determination of total polyphenols.
The total tannin content was calculated as the difference between the total polyphenols content (without casein) and the fraction of non-tannin (polyphenols do not precipitate with casein) according to the equations below:

$$A^{1\%} = \frac{A_3 \times 10}{C} \quad (2)$$

$$PT = \frac{FD \times A_1}{(p-m) \times A^{1\%}} \quad (3)$$

$$FNT = \frac{FD \times A_2}{(p-m) \times A^{1\%}} \quad (4)$$

$$TT = PT - FNT \quad (5)$$

Where: $A^{1\%}$ is the specific absorbance of reference solution; A_3 is the absorbance measured for the reference compound; C is the concentration (mg/mL); PT is the total polyphenols (%, w/w); FD is the dilution factor; FNT is the non-tannin fraction; A_2 is the non-tannin fraction absorbance and; TT is the total tannins (%).

RESULTS AND DISCUSSION

In Figure 1 it is possible to visualize the behavior of the global yield with operational conditions (temperature and pressure) using CO_2 + H_2O.

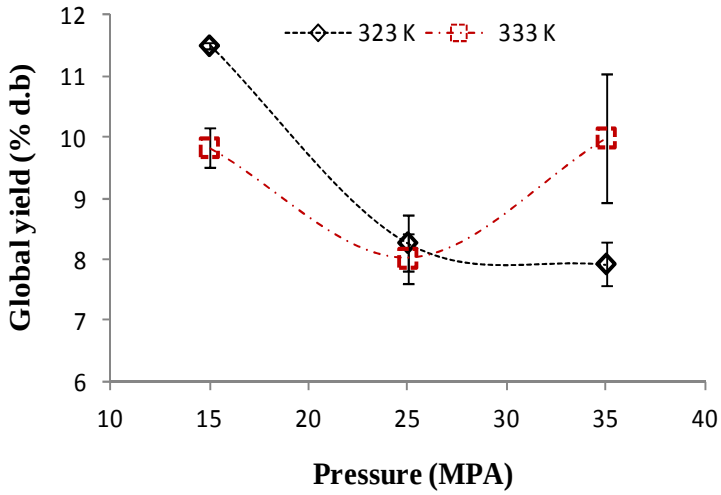

Figure1. Global yield (X_0) determined for jatobá extracts using CO_2 + H_2O.

Comparing the two isotherms it is evident an inversion pressure around 25 MPa. This characteristic indicates that at pressure above this point (35 MPa), the influence of the vapor

pressure of the extracted compounds presents predominant influence over solvent density. Thus, at 35 MPa, the global yield increased (10 %) with temperature due to the increase in the solute vapor pressure. At constant temperature of 323 K, the increase in pressure caused a decrease in yield. The yield at this temperature at pressures of 25 and 35 MPa showed modest difference, 8.2 % and 7.9 %, respectively; the highest yield was found at 15 MPa (11.5 %). This behavior can be due to matrix modifications and higher desorption of less polar compounds in the presence of water. Moreover, at this same pressure (15 MPa), the increase in temperature caused decreased solubility in the solvent, leading to a lower yield at 333 K (9.8 %). At 333K there was a reduction in the recovery of the extract when the pressure was increased from 15 MPa (9.8 %) to 25 MPa (8.0 %), and further increasing to 35 MPa. One hypothesis is that one class of compounds extracted at 15 MPa with CO_2 + H_2O may have had its solubility decreased with increasing pressure to 25 MPa, and another class of compounds had its solubility increased at higher pressure. Therefore, the highest extract recovery was obtained at 15 MPa and 323 K.

The tannins content of the extracts was evaluated and can be seen in Figure 2.

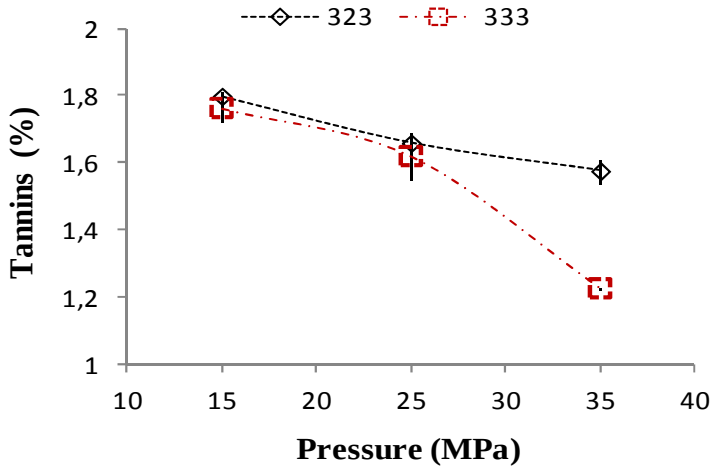

Figure2. Total tannins content for jatobá extracts using CO_2 + H_2O.

According to the results, higher levels of tannins in the extracts were obtained at 15 MPa, 1.8 % and 1.76 % at 323 and 333 K, respectively. This same behavior was observed for the extracts at 25 MPa at the same temperatures, with levels of 1.66 % and 1.62 % at 323 and 333 K, respectively. At 35 MPa, major differences can be noted in tannins levels at the two temperatures, 1.57% and 1.22% at 323 and 333 K, respectively. The higher tannins content was obtained for the condition that showed the highest recovery of total extract (X_0), 15 MPa and 323 K. Although high temperatures are necessary to overcome the solute-matrix interactions [13], the tannins are unstable at high temperatures and can easily undergo oxidation reactions and lose its antioxidant activity [14]. In this context, the increase in temperature was evident only in the condition of 35 MPa, and may have caused the degradation of some of these compounds. Also, some studies show the efficiency of tannin extraction in different solvents. Water and water-based solvents are preferably used in the extraction of the tannins and polyphenols [15], [16] and showed to be the extraction solution with the highest amount of tannins [17], [18].

CONCLUSION

Evaluating the global yield and the tannins content at each temperature and pressure it is possible to conclude that the extraction condition at 323 K and 15 MPa obtained higher amount of extract and had the highest content of tannins compounds.

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