SEQUENCIAL EXTRACTION OF *ARRABIDAEA CHICA* VERLOT USING SUPERCRITICAL CARBON DIOXIDE, ETHANOL AND WATER AS SOLVENTS: GLOBAL YIELD AND CONCENTRATION OF CARAJURIN.

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Abstract. *Arrabidaea chica* (Humb. & Bonpl.) Verlot., popularly known as Crajirú, rich in phenolics compounds, is a plant family Bignoniaceae largely found in South America, being common in the Amazon. Among the phenolic compounds of interest are highlighted three anthocyanin pigments majority: of type 3-deoxyanthocyanins: pigment (1) 6,7,3',4'-tetrahydroxy-5-methoxy flavone, pigment (2) 6,7,4' - trihydroxy-5-methoxy flavone pigment and (3) carajurin 6,7 - dihydroxy-5, 4'-dimethoxy flavone which is the major pigment. Anthocyanins polar pigments are traditionally extracted by conventional methods using aqueous, methanol and ethanol, slightly acidified. Supercritical fluid extraction (SFE) is considered a green technology innovation, adequate for high valuable products. Supercritical carbon dioxide in combination with ethanol and water allows the extraction of polar components such as anthocyanins. The aim of this study was to analyze the kinetics of extraction sequence using three solvents with different polarities, in terms of global yield of compounds and concentration of carajurin in the extracts of *Arrabidaea chica* using carbon dioxide in a first step, a second step in ethanol slightly acidified and water slightly acidified as a third step, at temperatures 40 °C and 50 °C and pressures 300 bar and 400 bar. The global yield extraction using supercritical carbon dioxide was lower compared to ethanol and water. However the extracts obtained with supercritical carbon dioxide showed greater selectivity extraction carajurin compared with other solvents such as ethanol and water.

Keywords: Carajurin, *Arrabidaea chica*, supercritical fluids extraction

1. Introduction

Arrabidaea chica (Humb. & Bonpl.) Verlot, popularly known as crajiru is a plant family Bignoniaceae, largely found in South America and is common in the Amazon [1]. The staining properties of this plant are due to three anthocyanin pigments majority of type 3-desoxiantocianidinas: pigment (1) 6,7,3',4'-tetrahydroxy-5-methoxy flavilium, pigment (2) 6,7,4'- trihydroxy-5-methoxy flavilium pigment and (3) 6,7-dihydroxy-5, 4'-dimethoxy flavilium, known popularly as carajurina, which is the major pigment [2].

According to Barroso et al.[3], the *Arrabidaea chica* is one of 71 medicinal plants that can be used as herbal medicines by the "Sistema Único de Saúde" of Brazil (SUS) and until 2012 were deposited just over 20 patents, which identifies opportunities for development in research for this species.

Anthocyanins polar pigments are traditionally extracted by conventional methods, such as solid-liquid extraction using solvents such as: water, ethanol and methanol, slightly acidified. Supercritical carbon dioxide can be used in combination with ethanol and water to extract polar compounds, such as anthocyanins [4]. Supercritical fluid extraction (SFE) is considered a green technology innovation, adequate for high valuable products due to the use of low temperatures, efficiency in solvent use and reduced energy consumption.

2. Materials and Methods

2.1. Raw material characterization

Leaves of *Arrabidaea chica* (Humb. & Bonpl.) Verlot was kindly provided by Chemical, Biological and Agricultural Pluridisciplinary Research Centre (CPQBA, Campinas, Brazil) in February of 2012. The drying of the leaves was performed in CPQBA in dryer with forced air circulation (Fabber, model 170, Piracicaba, Brazil) at 40 °C for 24 hours, then the sample was packed in plastic bags, wrapped in aluminum foil and stored in domestic freezer (model 220, Consul, Brazil) at -10°C. The moisture content, 5.3 ± 0.3 %, was determined by Karl Fischer method (Metrohm 701 KF Titrino equipped with 832 KF Oven Thermoprep). The mean particle diameter was calculated from the fractions of material retained on the following sieves. Tyler meshes: 8 (0.02 %), 12 (0.32 %), 16 (14.1 %) 24 (42.65 %) 32 (21.64 %) 48 (14.18 %) and bottom (19.51 %) using the ASAE procedure [5] employing a vibratory sieve system (Model 1868, Bertel, SP, Brazil). Particle density (1.32 g/cm³) was determined by helium gas pycnometry (Model ULTRAPYC 1200e, Quantachrome, Florida, USA). For supercritical extraction, the apparent density of thee particle bed (0.27 g/cm³) was determined according to the method described by Uquiche et al. [6]. Bed porosity (0.797) was calculated from the real density of the sample and the apparent density of the bed according to the method described by Rahman et al. [7].

2.2. Experimental procedure for fractionated high pressure

The fractionated extracts were in three steps at temperatures of 40 °C and 50 °C and pressures of 300 bar and 400 bar. In the first step, supercritical carbon dioxide was used with a flow of 1.65 g/min (L/min) which was read at the output of the system under conditions of temperature and local pressure of 0.93 bar at 25 °C (ρ= 1.65 g / L), the second and third steps were performed with ethanol (ρ = 785 g/L, 25 °C) acidified with 0.3 % citric acid and water (ρ = 1000 g/L, 25 ° C) acidified with 0.3 % citric acid with flow of 0.39 g/min (0.5 mL/min) and 0.5g/min (0.5 mL / min), respectively. Figure 1 schematizes the experimental conditions and the same colors and hatch in this diagram represent the same conditions of temperature and pressure.

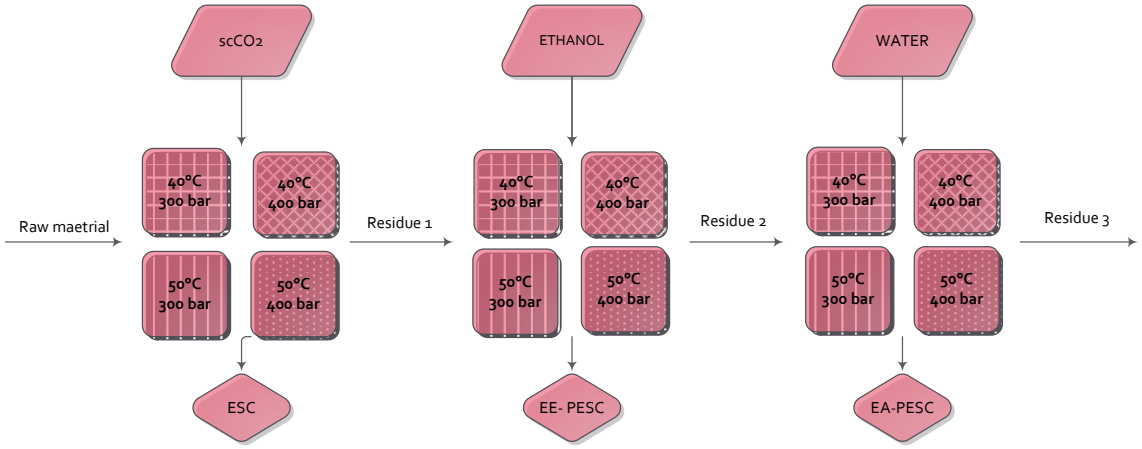


Figure 1. Scheme of the sequential extraction process in fixed bed in three different steps.

2.3. Extract composition

Determination of total phenolic compounds (TPC) The determination of total phenolic compounds was performed by the Folin-Ciocalteu second procedure SINGLETON et al. [8] and results were expressed as gallic acid equivalents (GAE)/g. The absorbance was determined at 750 nm wavelength in a spectrophotometer (UV-VIS Lambda 40, Perkin Elmer, USA) and results were calculated using a pre-prepared calibration curve of gallic acid. Assays were performed in triplicate.

Determination of total flavonoid content (TF) For the quantification of total flavonoids was used the method developed by ZHISHEN et al. [9] and results were expressed in mg catechin equivalents (mg CE)/g. The absorbance was determined at 510 nm wavelength in a spectrophotometer (UV-VIS Lambda 40, Perkin Elmer, USA) and results were calculated using a pre-prepared calibration curve of gallic acid. Assays were performed in triplicate.

Quantitative analysis of carajurin by high performance liquid chromatography (HPLC) The carajurin analysis was conducted by HPLC-DAD [(Shimatzu SCL-10A system; LC-10AT; FCV-10AL, CTO-10AS with UV detector at Shimatzu diode array (model SPD-M10A) and C-18 column (Phenomenex Gemine (250mm x 4.6 mm id 3 mm))]. Operating conditions for analysis were the same described by Devia et al. [10].

3. Results and Discussion

3.1. Kinetics of extraction

Four kinetics curves of extraction were constructed with three different solvents ($scCO_2$, ethanol and water) under the conditions of 40 ° C and 300 bar, 40 ° C and 400 bar, 50 ° C and 300 bar and 50 ° C and bar 400 (Figure 2). The curves show the cumulative yield of dry extract (discounting the mass of citric acid incorporated) as a function of S/F (mass of solvent used in the extraction / initial mass of raw material), indicating that the ease or difficulty with which solutes are extracted.

The four kinetics curves of extraction showed similar profiles. In the first stage of extraction with $scCO_2$ yields ranged from 1.2 to 1.9 %. In aqueous extraction and subsequent ethanol, extraction yields ranged from 11.7 % to 13 % and between 23 and 26 %, respectively. The yield cumulative total of three stages, the four operating conditions of temperature and pressure, ranged between 37 % and 39 % and did not differ among themselves as to the global yield.

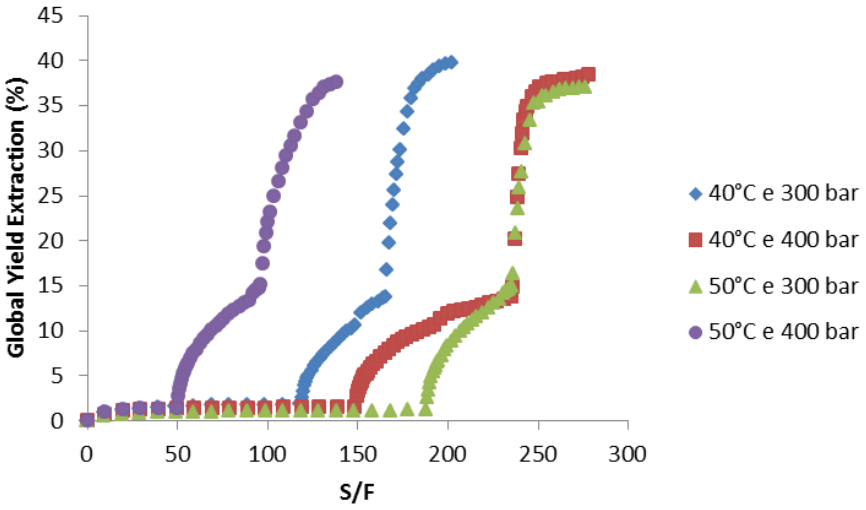


Figure 2. Kinetics of extraction under four conditions of operation using $scCO_2$, ethanol and water as solvents.

During the collection of fractions of extracts for construction of the extraction curve, there was a blackout in the third stage of the samples with water. Therefore was performed a modification in the process and the third stage is now done by conventional aqueous extraction from residue 2, the same operating temperature and ambient pressure, so getting the third extract (EA/PESC).

3.2. Total compounds phenolics (TP) and total flavonoids (TF)

Figure 3 represents the phenolic concentration compounds in the extracts obtained from sequential extraction in three steps. It is observed that all extracts obtained with $scCO_2$ showed statistically significant differences, moreover there was a decrease of phenolic compounds with increased temperature and pressure,

with the highest concentration (69.3 mg GAE/g extract) obtained at the operating condition 40 ° C and 300 bar.

In relation to the total flavonoid content (Figure 4), the extracts obtained with $scCO_2$ showed higher concentration of total flavonoids compared to other solvents. The extracts obtained with $scCO_2$ the condition of 40 ° C and 300 bar and 50 ° C and 300 bar showed no significant differences, which demonstrates that the temperature didn't influence the concentration of total flavonoids in these extracts. The ethanol extracts showed statistically significant differences. The aqueous extracts obtained at 50 °C and 300 bar and 50 °C and 400 bar didn't show statistically significant differences. In general, the concentration of flavonoids in the extract decreased with increasing pressure and temperature.

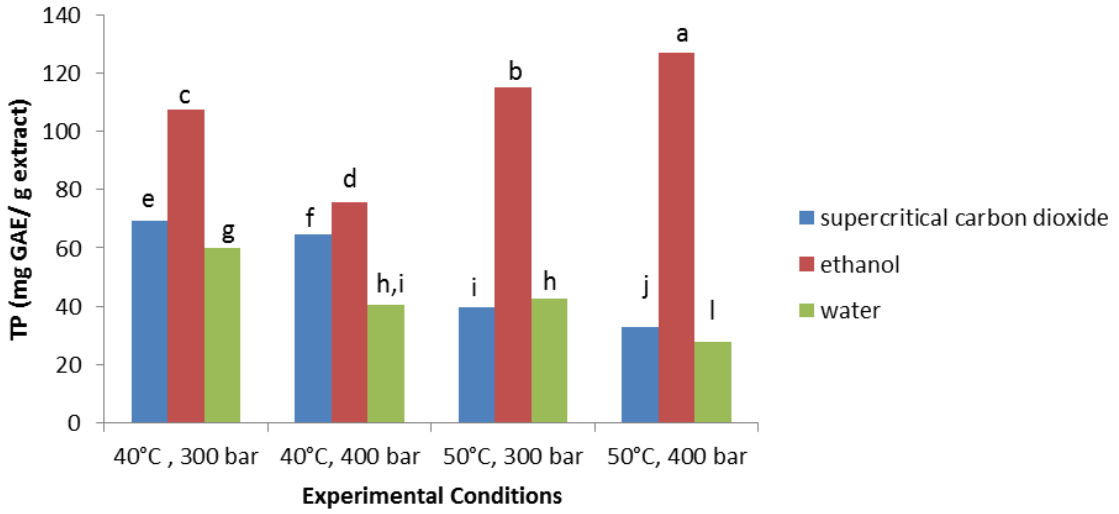


Figure 3. Total phenolic compounds (TP) for the different extracts of *A. chica* obtained in fixed bed in three steps. Different letters represent significant differences ($p < 0.05$).

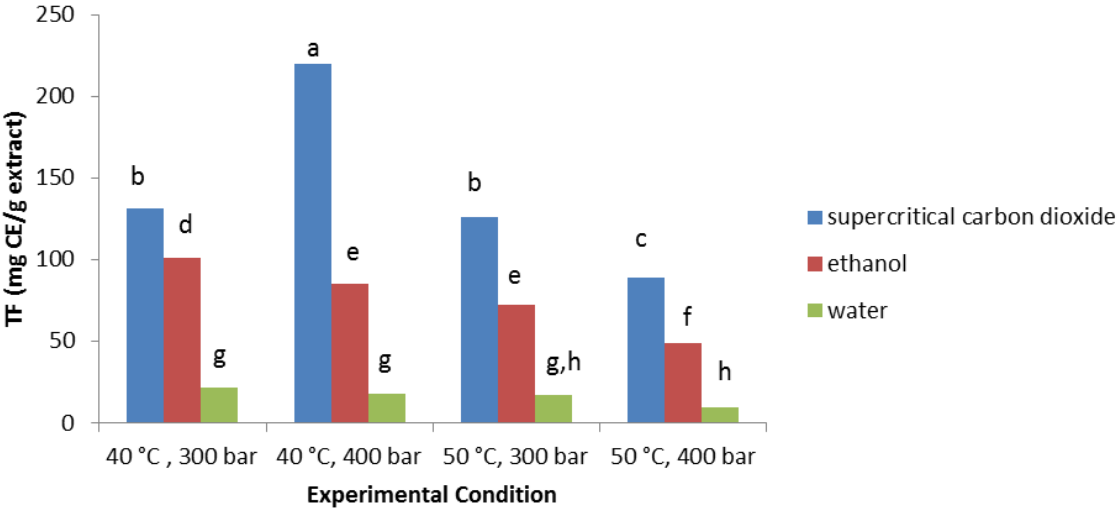


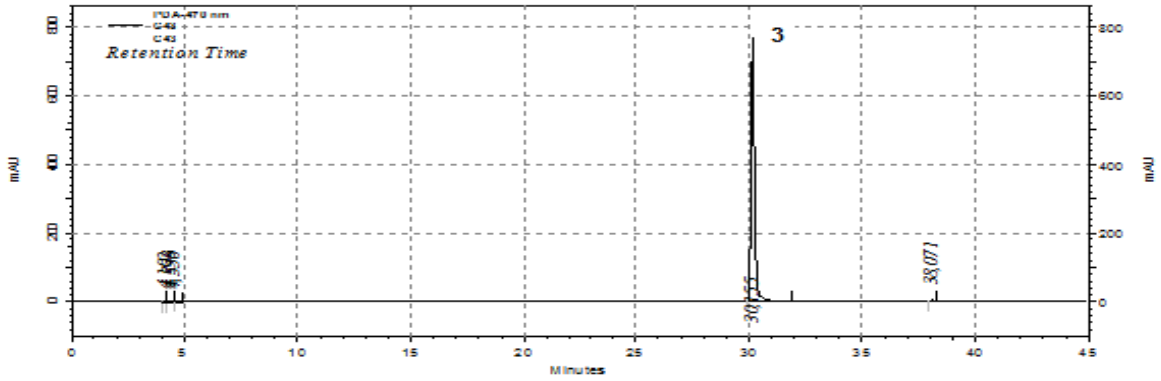
Figure 4. Total flavonoid (TF) for the different extracts of *A. chica* obtained in fixed bed in three steps. Different letters represent significant differences ($p < 0.05$).

3.2 Quantitative analysis of carajurin by high performance liquid chromatography (HPLC)

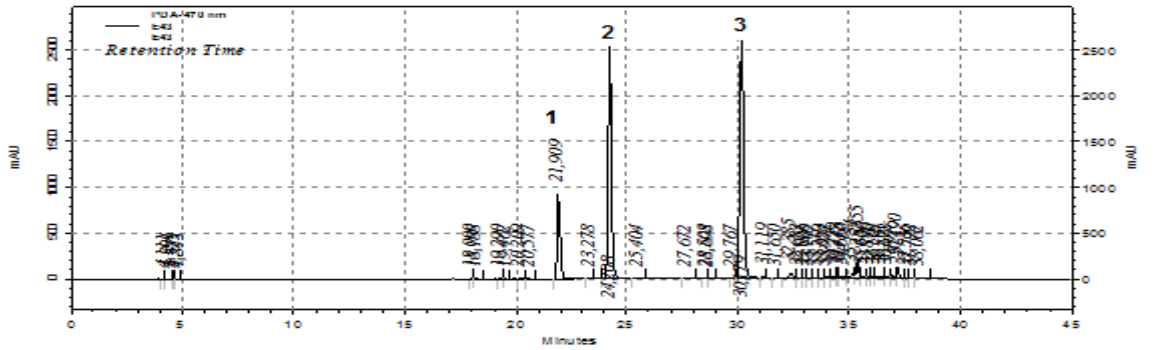
Figure 5 was observed from the chromatograms of the extracts obtained by $scCO_2$, ethanol, water under at 40 °C and 300 bar and qualitatively show a difference in composition between extracts. The extracts were

more selective with $scCO_2$ because extracted a smaller amount of different compounds compared with aqueous and ethanol extracts. The extracts obtained in other conditions: 40 °C and 400 bar, 50 °C and 300 bar, 50 °C and 400 bar showed chromatographic profile similar to that shown in Figure 5.

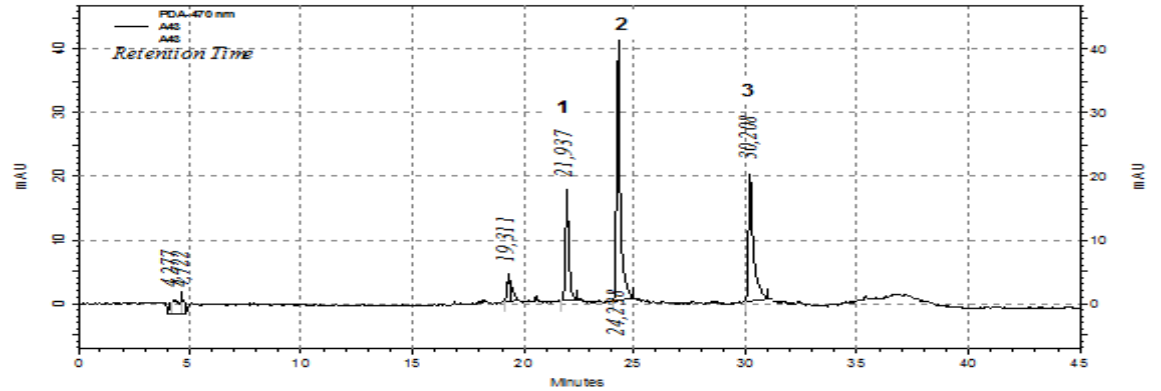
Figure 6 compare the concentrations of the different carajurin extracts of *A. chica*. Generally the ethanolic extracts showed a higher concentration of carajurin when compared with other solvents. Furthermore, it was observed that the extraction with $scCO_2$ prior improves concentration in ethanol extracts of sequential processes in the three steps. However, as shown by the chromatograms, the supercritical extracts showed only carajurin, since the supercritical CO_2 was more selective, and this can be explained by the polarity of carajurin [smaller quantity of OH groups compared to pigments (1) 6,7,3',4'-tetrahydroxy-5-methoxy flavilium pigment and (2) 6,7,4' - trihydroxy-5-methoxy flavilium] (Figure 7).



(a) $scCO_2$



(b) ethanol



(c) residual aqueous

Figure 5. Chromatogram of the extract of *A. chica* at 40 ° C and 300 bar in three steps (a) $scCO_2$ (b) ethanol (c) residual aqueous. Column Gemini C18 35 ° C, λ = 470 nm, flow 0.5 mL / min. Peak 1: 6,7,3 ', 4'-tetrahydroxy-5-metoxiflavilium; Peak 2: 6,7-trihidoxi-5-metoxiflavilium; Peak 3: carajurin (6,7-dihydroxy-5, 4'-dimetoxiflavilium) .

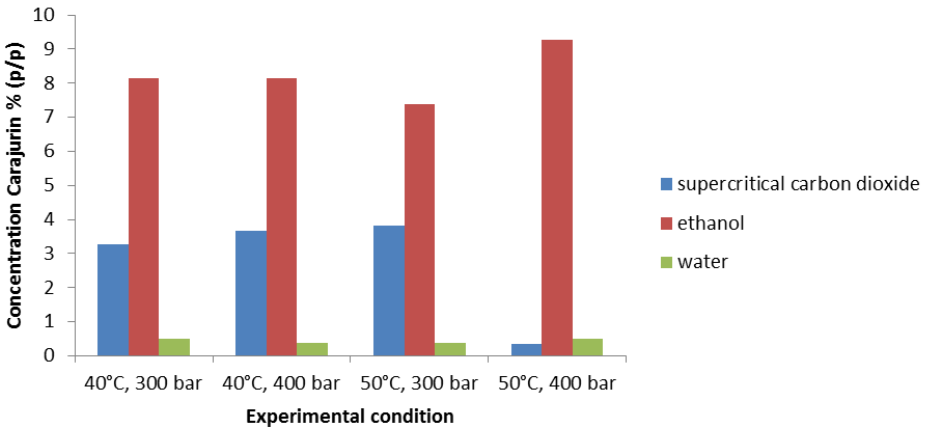


Figure 6. Concentration of carajurin for the different extracts of *A. chica* obtained in fixed bed in three steps.

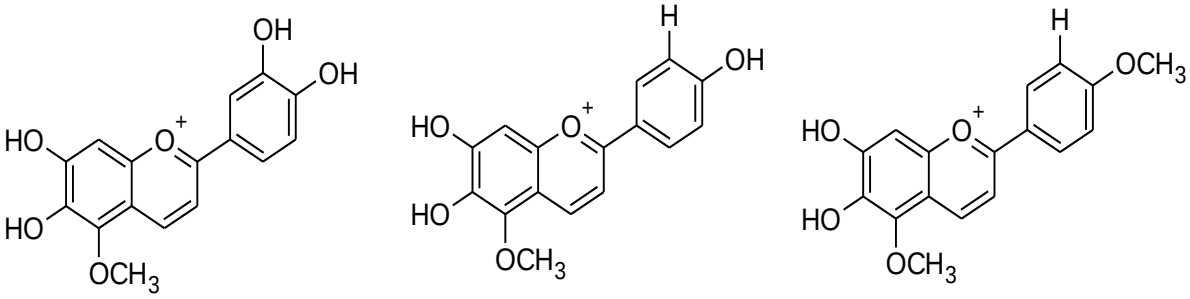


Figure 7. Molecular structure (a) 6,7,3 ', 4'-tetrahydroxy-5-methoxy flavilium (b) 6,7,4' - trihydroxy-5-methoxy flavilium, (c) carajurin (6,7-dihydroxy -5.4 '-dimethoxy flavilium)

4. Conclusion

In the first step using $scCO_2$ as solvent, and in the second step using ethanol as solvent, there was an increase in extraction yield as increased temperature and pressure, but no statistically significant. In the third step, using water as the solvent, the extracts did not show statistically significant differences, so that conditions of temperature and pressure studied in this work had small influence on the overall yield obtained in the different extracts. However, the highest global yield was achieved with the experimental condition of 50 °C and 300 bar.

The highest concentration of total phenolic compounds was obtained using the ethanol extract under the condition of 50 °C and 400 bar. In relation to the total flavonoid concentration, the highest concentration was obtained in the condition 40 ° C and 400 bar.

The ethanol extracts showed a higher concentration of carajurin when compared with other solvents.

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