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Chemical Composition, Antioxidant and Biological Activity of Leaves *Passiflora foetida*

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The aim of this study was to determine the chemical composition (IR), characterize minerals (ICP-OES), antioxidant activity, total phenols, inhibition of acetylcholinesterase (AChE) and antimicrobial activity of leaf extract of wild passion fruit (*Passiflora foetida* L.). The results obtained by Infrared of the hexane extract indicate the presence of aliphatic compounds such as fatty acids and esters, long chain alcohols and steroids. The major minerals were: phosphorus (1093.00 mg L^{-1}), magnesium (418.77 mg L^{-1}), sulfur (282.56 mg L^{-1}) and potassium (157.46 mg L^{-1}). The total phenolic content was 16.78 mg of gallic acid equivalent 100 g^{-1} extract. The hexane extract showed inhibition of 17.97% for antioxidant activity. The activity on AChE was 96.46% inhibition. As for microorganisms, the extract showed inhibition on Gram-positive bacteria *Bacillus cereus* (77.93%), *Staphylococcus aureus* (88.25%), Gram-negative *Citrobacter freundii* (82.74%), *Salmonella typhimurium* (83.21%) and yeast *C. albicans* (53.29%).

1. Introduction

Studies have been directed to the species belonging to the genus *Passiflora*, these being popularly known as passion fruit (*Passiflora foetida* L.) of the Passifloraceae family. This species can be found in riverbeds, roadside forests, forests and coastal vegetation. In Brazil they are used in the form of lotions against skin diseases. The main phytochemicals of this plant are alkaloids, phenols, flavonoids and cyanogenic compounds (Dhawan et al., 2004), fatty acids (Hasan, Dhawan and Sharma, 1980). The Passifloraceae family is the group of Angiosperms and has tropical distribution, including about 20 genera and 600 species. *Passiflora* is the genus of the family in Brazilian flora and can be found mainly on forest edges all over the country. In Brazil there are five genera and about 120 species (Paula et al., 2015; Lorenzi and Matos, 2008).
With respect to the species P. foetida, study results have anti-inflammatory activities, analgesic (Sasikala et al., 2011), antidiarrheal (Asadujjaman et al., 2014), antiepileptic (Pavan et al., 2009), anticancer (Balasubramaniam et al., 2010), antidepressant (Santos et al., 2011), antihyperglycemic (Asir et al., 2014; Siriwardhene et al., 2013), antihypertensive (Ranganatha et al., 2013), antihistamine (Chivapat et al., 2011), anti-inflammatory (Fernandes et al., 2013), antimicrobial and antibacterial (Mohanasundari et al., 2007; Baby et al., 2010), cytotoxic (Jusavil et al., 2015), estrogenic (Michel et al., 2012) and hepatoprotective (Anandan et

al., 2009). Objective to determine the chemical composition, to evaluate its antioxidant, antiacetylcholinesterase and antimicrobial activity, through *in vitro* assays, in Roraima state, Brazil.

Of the above objectives is of great importance today, since it is estimated that by 2050 there will be about 115 million people in the world with Alzheimer's Disease (AD). Symptoms of AD include a regression of various physiological functions, causing difficulties in language, memory, emotional or personality behavior, and cognitive abilities. AD and other brain problems may be associated with the release of acetylcholine. Choline and acetyl coenzyme A produce acetylcholine, which is then released into the synaptic cleft and interacts with its receptors, nicotinic or muscarinic. Acetylcholine degrades by the action of acetylcholinesterase (AChE), whose reaction products are choline and acetate (Čolović et al., 2013; WHO, 2012).

2. Materials and Method

2.1 Collection, preparation of sample and obtaining crude oil

The leaves of passion fruit (*P. foetida*) were collected at the Paricarana campus of the Federal University of Roraima (UFRR) and taken to the Environmental Chemistry Laboratory (Research Center, Post-Graduate Course in Science and Technology-NPPGCT-UFRR). The leaves were collected, oven dried at 50 ^{o}C for 72 h and ground in a blender, the granulometry was standardized at 20-40 mesh. 150 g of the ground sample was used to obtain the extract using a Soxhlet extractor as solvent the hexane for 5 h. The crude extract was concentrated in rotoevaporator.

2.2 Determination of functional groups by Infrared spectroscopy (IR)

The samples were analyzed in the Infrared region using the IRPrestige-21 Shimadzu Spectrophotometer from the Post-Graduation Program in Chemistry of the UFRR, using KBr pellets. For this analysis the following conditions were listed: measurement mode: transmittance; number of scans: 16; resolution: 4 cm^{-1}; wavelength range: 4000-400 cm^{-1}.

2.3 Determination of minerals by ICP-OES

The Inductively Coupled Plasma/Atomic Emission Spectrometry (ICP-OES) is a technique widely used in research laboratories. ICP-OES is a multielement analysis technique that operates over a wide linear range of concentrations (4 to 5 orders of magnitude), with fast analysis and low cost. Besides these advantages, another important factor is that the technique allows the direct analysis of liquid samples, in case of pneumatic nebulization. The samples were digested using concentrated nitric acid and 30% hydrogen peroxide under microwave oven heating. The equipment used was ICP-OES of the brand Spectro, model Arcos, the procedure was carried out at the Analytical Center of the Institute of Chemistry of the University of São Paulo (USP). Power applied: 1400 W; Radiofrequency of the RF generator: 27.12 MHz; Plasma gas flow rate: 12 L min^{-1}; Aux gas flow: 1 L min^{-1}; Nebulization gas flow rate: 0.85 L min^{-1}; Sample introduction rate: 0.85 L min^{-1}.

2.4 Antioxidant activity

The free radical scavenger activity DPPH (2,2-diphenyl-1-picryl-hydrazyl) was determined according to Miranda and Fraga (2006). DPPH is a stable violet staining radical which in the presence of an antioxidant activity (AA) is reduced to a yellowish coloration. Initially stock solutions were prepared from the concentration samples 3.81 g mL^{-1} and 2.4 mg DPPH standard in 100 mL of 50% ethanol as solvent. In each tube 600 μL of the sample or standard and 5 mL of the DPPH solution were added. The reaction was incubated for 60 min in the absence of light. After the incubation, the absorbance at 515 nm was read by UV spectrophotometry (Shimadzu UV-1800). The analyses were performed in triplicate. The calibration curve was made by preparing diluted standards from the 60 mM stock concentration in the range of 10-50 mM and at the same time the blank was made with ethanol. The sequestering activity of the DPPH free radical was expressed as % AA.

2.5 Total phenols

The content of total phenolic compounds was determined by the Folin-Ciocalteau spectrophotometric method according to the methodology proposed by Singleton et al. (1999) with modifications. Gallic acid was used as reference standard. This method involves the reduction of the reactant by the phenolic compounds of the samples with formation of a blue complex. 0.1525 g of the extract was weighed and diluted in 40 mL of ethanol. An aliquot of 0.1 mL of the extract (1:3 dilutions) was transferred to a 10 mL test tube and 3 mL of distilled water followed by 0.25 mL of the Folin-Ciocalteau reagent. The reaction was allowed to proceed for 3 min then 2 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) was added. A blank test was conducted under the same conditions, so that 0.1 mL of distilled water was used in place of the sample. The samples were protected from light and heated to 37 °C bath for 30 min. The absorbance was at 765 nm. The quantification of total phenols in the extracts was performed in triplicate. Gallic acid at the concentrations of 2, 4, 8, 10, 12

and 14 mg L^{-1} was used as standard to construct a calibration curve. From the equation of the line obtained in the graph curve of the gallic acid standard, the total phenolic content was calculated, expressing the results in mg of gallic acid 100 g^{-1} of the sample.

2.6 Inhibition of acetylcholinesterase (AChE)

Aliquots of a working solution (25 µL) (sample in DMSO 10 mg mL^{-1}) were added to microplate wells and positive and negative controls were also prepared. To the first five wells of a column (positive control) 25 µL of an eserine solution prepared at 10 mg mL^{-1} (31 mM; 2.7 mM in the whole reaction mixture 275 µL) in Tris/HCl at pH 8.0) was added. Then, 25 µL of acetylthiocholine iodide (ATChI, Sigma A5751) 15 mM; the reaction mixture, 125 µL of 5',5-dithio-bis (2-nitrobenzoate) (DTNB, Sigma D8130) (3 mM) and 50 µL of Tris/HCl (50 mM, pH 8) containing 0.1% (m/v) bovine serum albumin was added to each well. Absorbance was measured at 405 nm every 1 min for 8 times. Then 25 µL (0.226 U mL^{-1}) of Electric eel AChE (type VI-S) provided by Sigma (C3389-500UN) in Tris/HCl was added to each well. Absorbance was measured at 405 nm by 10 times (Frank and Gupta, 2005; Ellman et al., 1961).

2.7 Antimicrobial activity

Two Gram-negative bacteria were used: *Citrobacter freundii* (ATCC 8090), *Salmonella typhimurium* (ATCC 13311), two Gram-positive bacteria: *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923) and a fungus (yeast) *Candida albicans* (ATCC 18804). Ampicillin (antibiotic) was used as a control at a concentration of 12.5 mg mL^{-1}. The procedures established by Zacchino and Gupta (2007) were used in this bioassay. The procedures for Minimum Inhibitory Concentration (MIC) described below were used in the assay. Concentrations assayed were 250, 125, 62.5, 31.25, 15.6, 7.81; 3.90 and 1.95 µg mL^{-1}. Samples were weighed and dissolved in DMSO to 50 mg mL^{-1}. Forty µL of this solution was added to a flask containing 960 µL of BHI (Brain Heart Infusion) broth (working solution). A pre-inoculum was prepared in which the bacteria and the yeast, stored under refrigeration, were transferred with a platinum loop to test tubes containing 3 mL of freshly made BHI broth. The tubes were incubated at 37 °C for 18 h. Then, the pre-inoculum (500 µL) was transferred to tubes containing 4.5 mL of sterile distilled water. The tubes were homogenized and the concentration adjusted to 0.5 of McFarland turbidity standard (108 CFU mL^{-1}), thereby obtaining the inocula used in the bioassays. Assays were performed in 96-microwell plates in duplicate. One hundred µL of BHI broth was added to each well. In the first well 100 µL of working solution was also added. The solution was homogenized and 100 µL transferred to the next well and so on until the last well, from where 100 µL was discarded. Then, 100 µL of microorganism inocula was added to wells. Eight different concentrations of each sample were tested. A positive control devoid of the working solution allowed us to examine microorganism growth. A negative control, which lacked the inoculum permitted us to discount the color coming from the working solution. A control plate containing 100 µL of BHI culture medium and 100 µL of sterile distilled water were added to the experiment as a control of BHI broth sterility. Microorganism growth was measured in ELISA plate reader (492 nm) immediately after ending the experiment (0 h). They were incubated at 37 °C and read again after 24 h of experiments, ending the test.

3. Results and Discussion

3.1 Identification of functional groups by IR

Analyzing of the spectrum of the hexane extract it was possible to assign absorptions at 2,923.23 and 2,851.83 cm^{-1} corresponding to the axial deformations of CH of CH_2 and CH_3 groups which are common in various classes of aliphatic compounds such as fatty acids and esters, alcohols (Socrates, 1979; Silverstein et al., 1991). In addition to confirmation signals of CH_2 (1,462.8 cm^{-1}) and CH_3 (1,376 cm^{-1}) in the IR spectrum, the axial strain deformation vibration of CO between 1,242.9-1,024.8 cm^{-1} suggests the presence of the alcohol and phenol group in the molecule. The absorption at 835.56 and 720.45 cm^{-1} are the angular deformation vibrations outside the C-H plane characteristic of the alkenes. The absorption in the 609.62 cm^{-1} region is from axial deformation attributed to the C-S bond. Absorption in the 1,737.8 cm^{-1} region is a function of axial deformation of C=O of esters. Moreover, the absorption band in the 3,436.4 cm^{-1} comes from the axial deformation of O-H in intermolecular hydrogen bonding (Silverstein et al., 1991) evidencing the presence of fatty acids.

3.2 Determination of antioxidant activity by DPPH

In order to evaluate the ability of the constituents of *P. foetida* hexane extract to capture free radicals, solutions of this extract with DPPH were analyzed. The results were expressed as percent inhibition of oxidation, ie, the percentage of antioxidant activity corresponds to the amount of DPPH consumed by the antioxidant. According to Alves et al. (2007), the consumption of DPPH by the sample is directly proportional

to its antioxidant activity. From the result obtained with the hexane extract of *P. foetida* the linear regression analysis was determined, with the equation of the line $y = 0.0099x + 0.0077$ with correlation coefficient r equal to 0.9966. The concentration may range from 0 to 60 mg L^{-1}. The result obtained in the determination of the antioxidant activity of the sample was 17.97% inhibition of the antioxidant activity and according to the calibration curve of the DPPH the concentration value is between 10 to 20 mg L^{-1}, a low antioxidant capacity.

3.3 Determination of total phenol compounds

The phenol content is used extensively in the determination of several extracts and evaluated by the Folin-Ciocalteu reagent. From the reaction between the gallic acid and the Folin-Ciocalteu reagent (Da Silva et al., 2013) the linear regression analysis was determined, with the equation of the line $y = 0.00193x + 0.0459$ with correlation coefficient r equal to 0.9943. The concentration range was from 2 to 16 mg L^{-1}. The total phenol content of the extract was expressed as mg of gallic acid equivalents per 100 g of the extract (mg EAG 100 g^{-1}). The total phenol content found in the crude hexane extract was 16.78 ± 0.26 mg EAG 100 g^{-1}. For the interpretation by result Rufino et al. (2010) state that values up to 100 mg EAG 100 g^{-1} show low concentration of total phenols, values between 100-500 mg EAG 100 g^{-1} indicate medical concentration of phenols and values above 500 mg EAG 100 g^{-1} indicates, therefore, a high concentration of total phenols. Thus, *P. foetida* can be considered as a low total phenol extract, the result was below 100 mg EAG 100 g^{-1}.

3.4 Identification and quantification of minerals by ICP-OES

In the result of the semi quantitative scanning of minerals, elements with concentrations lower than 1 mg L^{-1} were observed, such as Ag, B, Ba, Cd, Co, Cr, Li, Ni, Pb and Ti. Elements with concentrations between 1 and 100 mg L^{-1} are Al, Cu, Fe, Mn, Si, Sr and Zn. The elements with concentrations above 100 mg L^{-1} were: K (157.46 mg L^{-1}), Mg (418.77 mg L^{-1}), P (1093.00 mg L^{-1}), S (282.56 mg L^{-1}). K is important for hypertensive patients and when associated with Na, regulates the functioning of the muscular system and the heart rate (Franco, 1998). Mg and Ca act in the formation of bones, teeth and tissue to support growth, maintenance of body functions and reproduction for humans (Battestin et al., 2002). Quantified major minerals are considered to be macronutrients being indispensable at high concentrations (Battestin et al., 2002) and essential for the nutrition and functioning of the organism (Saidelles et al., 2010).

3.5 Acetylcholinesterase inhibitory activity

The extract of *P. foetida* inhibited the enzyme AChE at a concentration of 10 mg mL^{-1} in 96.46%, while the standard inhibitors, such as eserine and galantamine, inhibited 91.93% and 94.36%, respectively, in the same concentration of the extract. Vinutha et al. (2007) in their AChE inhibition study, used a parameter to classify the extracts analyzed in: potent inhibitors (> 50% inhibition), moderate inhibitors (30-50% inhibition) and weak inhibitors (<30% inhibition). According to this research, the hexane extract of *P. foetida* can be considered a potent inhibitor of AChE. In addition, it is worth emphasizing in this result that the inhibition of the extract was superior to those obtained for the standard inhibitors used in this assay. According to Trevisan and Macedo (2003), extracts with inhibition greater than or equal to 50% may be candidates for fractionation and isolation.

3.6 Evaluation of antimicrobial potential

The biological activity of *P. foetida* leaf extract against pathogenic microorganisms is shown below (Table 2).
When analyzing the percentages of inhibition of the five microorganisms tested, we obtained inhibitions above 50%. It may be noted that the highest concentrations were those which had a greater inhibition ranging from 75-88%. In the Gram-positive group, *S. aureus* presented the highest inhibition in comparison with the five microorganisms tested, and the value was 88.25% at the concentration of 250 μg mL^{-1}, also showing satisfactory sensitivity of the samples at concentrations 125, 62.5 and 31.25 μg mL^{-1}. The bacterium *B. cereus* showed inhibition of 77.93% in the concentration of 250 μg mL^{-1} and also demonstrated satisfactory sensitivity in the concentrations 125, 62.5, 31.25 and 15.62 μg mL^{-1}. For the Gram-negative group, *S. Typhimurium* showed 83.21% inhibition at 62.5 μg mL^{-1} concentration and did not reach satisfactory inhibition at concentrations only 15.62 and 1.95 μg mL^{-1}. The bacterium *C. Freudii* presented inhibition of 82.74% in the concentration of 62.5 μg mL^{-1} and also demonstrated satisfactory sensitivity in the concentrations 125, 62.5, 31.25 and 7.81 μg mL^{-1}. The antibiotic ampicillin, a positive control, inhibited *B. cereus* (95.3%) and *S. aureus* (99.1%) gram-positive bacteria and, in gram-negative bacteria *C. freudii* (96.5%) and *S. typhimurium* (100.0%). The antifungal miconazole, positive control, showed 93.7% inhibition on *C. albicans*. The extract at a concentration of 250 μg mL^{-1} inhibited *C. albicans* in 53.29%.
Other studies using the same species show that the methanolic extract of dry powdered roots showed significant antibacterial activity against *Staphylococcus epidermidis*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* microorganisms when compared to standard antibiotics (Baby et al., 2010). And the dried leaves and nuts extracted with ethanol and acetone were tested against four

bacteria *P. putida*, *Vibrio cholerae*, *Shigella flexneri* and *Streptococcus pyogenes* and the results of the leaf extract showed a remarkable activity against all the pathogenic bacteria (Mohanasundari et al., 2007).

Table 2: Antimicrobial activity of the leaves extract from of P. foetida

Concentration ($\mu g\ mL^{-1}$)	% Inhibition				
	Bacteria Gram-positive		Bacteria Gram-negative		Fungus (Yeast)
	B. cereus	***S. aureus***	***C. freudii***	***S. typhimurium***	***C. albicans***
250	77.93	88.25	63.13	75.82	53.29
125	75.26	77.63	72.35	76.63	18.69
62.5	75.96	68.28	82.74	83.21	10.89
31.25	65.30	53.06	75.95	66.02	0.0
15.62	55.60	37.10	49.56	21.16	0.0
7.81	41.71	18.46	64.47	63.20	0.0
3.90	30.67	1.57	25.60	59.03	2.44
1.95	14.87	0.0	0.0	48.42	0.0

4. Conclusion

IR spectroscopy showed absorption of bands characteristic of classes of aliphatic compounds such as fatty acids and esters, long chain alcohols and steroids, presence of the alcohol and phenol group in the molecule, alkenes, carbon-sulfur bonds and intermolecular hydrogen. The major and quantified minerals were P (1093.00 mg L^{-1}), then Mg (418.77 mg L^{-1}), S (282.56 mg L^{-1}) and K (157.46 mg L^{-1}). Macronutrients are considered indispensable for the nutrition and functioning of the organism. The hexane extract of this species has low total phenol content and a positive correlation with the antioxidant activity. The study of inhibition of AChE (96.46%) of the *P. foetida* species was carried out for the first time, possibly containing potent substances that have the capacity to inhibit this enzyme. It showed inhibition of gram (+) and (-) bacteria in the concentrations 250 to 31.25 $\mu g\ mL^{-1}$ of *B. cereus* (77.93%), *C. freundii* (82.74%), *S. aureus* (88.25%) and *S. typhimurium* (83.21%) and fungus *C. albicans* (53.29%), in the concentration 250 $\mu g\ mL^{-1}$.

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