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Bioactive Extracts from *Swartzia latifolia*

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The aim was to carry out phytochemical screening of the extracts from various parts of the *Swartzia latifolia* plant, followed by the antimicrobial assay on the bacteria *Staphylococcus aureus*, *Streptococcus mutans* and *Escherichia coli* and yeast *Candida albicans*, soon after the antioxidant activity. Therefore, evaluations were started with a phytochemical survey of some parts of the plant, such as leaves, stem, stem bark, root and root bark, which indicated, qualitatively, the presence of tannins, flavonoids, triterpenoids and saponins. The root bark presented better results as the presence of the chemical groups mentioned above, as strong or very strong. In this way, the inhibition halos of the crude ethanolic and hexane extracts of the root bark against the bacteria and yeast were analyzed, where there was no inhibition on *E. coli* and *C. albicans*, but strong inhibition on *S. aureus* at concentrations of 20 and 30 mg L^{-1} in ethanolic (14 mm and 14 mm, respectively) and hexane extracts (19 and 15 mm, respectively) and *S. mutans* in the active ethanolic extract in 20 mg L^{-1} (9 mm) and 30 mg L^{-1} (11 mm), and active in the hexane extract 8 and 11 mm, in the same concentrations. For the antioxidant activity, it was only possible to verify the ethanolic extract of the root bark, which presented 0.04 mg mL^{-1}, while the BHT was 0.20 mg mL^{-1}.

1. Introduction

The use of plants and their products, such as extracts, for medicinal purposes, has long occurred. The recipes that make up the use of traditional medicine have become an inheritance for future societies (Phillipson, 2001). Certainly, a great variety of biodiversity, can bring great possibilities of plants with medicinal effects to the current ills, with that, it is worth to emphasize that Brazil is the holder of greater biodiversity of the Earth (MMA, 2002), of these one can obtain great diversity of edible and / or medicinal plants (Almeida et al., 2009; Bogusz Junior et al., 2012). Thus, the Leguminosae family stands out with 727 genera, where Brazil has 25% of this total, or 188 genera (Santos and Melo Filho, 2013).
One of the species belonging to this family is *Swartzia latifolia*, still little studied, its genus, *Swartzia*, indicates to be a plant with bioactive effects on the mollusk *Biomphalaria glabrata*, fungi and bacteria, insecticide, diarrhea, vermifuge, etc., due to its composition Chemistry that favors such effects as, saponins, flavonoids, diterpenoids, alkaloids, among others. Some populations in South America make use of traditional medicine from parts of the plant (Santos and Melo Filho, 2013). It is noteworthy that the bioactivity and chemical composition studies of the *S. latifolia* species are incipient. Therefore, the objectives of this work are to perform a phytochemical screening and biological effects on gram positive and gram negative bacteria and *Candida albicans* yeast using crude extracts of the *S. latifolia* species obtained in Boa Vista, Roraima, Brazil.

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2. Material and Methods

2.1 Collection and preparation of the sample

The leaves, stem, root, stem bark and root bark of the *S. latifolia* were obtained at the Cauamé Campus of the Federal University of Roraima (UFRR), on the banks of the Cauamé river. This material was taken to the Laboratory of Environmental Chemistry of the Nucleus of Research and Post-Graduation in Sciences and Technology (NPPGCT), Paricarana Campus, UFRR. Samples were duly sanitized, dried at room temperature and weighed. Then the samples were dried greenhouse with air circulation at 40 °C. After drying, it was again weighed, ground in a knife mill and pealed at 20 to 40 mesh, and therefore weighed and stored for subsequent extraction (Santos et al., 2015).

2.2 Obtaining crude hexane and ethanolic extracts of *S. latifolia* and phytochemical screening

To obtain hexane and ethanolic crude extracts, the method described by Matos (1988) was followed, where extraction of each part of the plant with hexane solvent was done, after which the material was filtered and separated from the pie, which was reserved for ethanolic extraction, the material was also filtered and reserved for the pie, this time derived from the ethanolic extraction. After the exhaustive extraction, the extract was obtained by separating it from the solvent with rotoevaporation under reduced pressure and at 45 °C.
In order to obtain qualitative information about the chemical composition of alkaloids, flavonoids, saponins, and triterpenoids (Matos, 1988), the hexane (EH) and ethanolic (EE) extracts were concentrated in *S. latifolia*.

2.3 Phytochemical Prospecting of Ethanol Extracts

Concentrated ethanolic extracts were carefully screened for identification of chemical constituents, such as acetogenins, alkaloids, steroids, phenols, tannins, flavonoids, saponins and triterpenoids, qualitatively.
According to Matos (1988) seven portions of 3 to 4 mL were separated into test tubes with their respective numbers and two 10 mL portions in identified beakers, one of which was measured. The beakers were placed in a water bath until dry, then stored in a desiccator for later use.
The portions separated in test tubes were concentrated in a water bath until half volume was obtained. Shortly after the acid was acidified to pH 4 and then filtered. The solution, the insoluble residue and the test tubes were reserved for further testing (Matos, 1988).

Test for Alkaloids
One-third of the aqueous solution obtained was obtained Phytochemical Prospecting with Ethanol Extracts, added NH_4OH to pH 11, and the organic bases were extracted with three successive 30, 20 and 10 mL portions of the ether-chloroform (3+1) mixture, in a separatory funnel (the aqueous phase was reserved for further testing). The ether-chloroform solution was removed, treated with anhydrous Na_2SO_4 to remove excess water. The filtrate was separated into two portions. One was dried for chromate plates and the organic bases were again extracted from the other with three small successive portions of dilute HCl.

Test for Phenols and Tannins
Tube number 1 was taken and three drops of $FeCl_3$ alcohol solution was added. It was stirred well and any variation of its color or formation of abundant, dark precipitate was observed. It was compared to a blank test, ie using only water and ferric chloride.

Test for Flavonoids
A test tube with the previously prepared portion was used, basified to pH 11, which should have a red-orange color to indicate the presence of flavonoid constituent (Matos, 1988).
And another test to confirm is to add to the tube number 7 a few centigrams of granulated or tape magnesium and 0.5 ml of concentrated HCl. The reaction was terminated at the end of the effervescence and observed by comparison of color change of the reaction mixture in tubes 5 and 7 (acidified) (Matos, 1988).
Characteristic for identification of flavonoids is the appearance or intensification of red color (Matos, 1988).

Test for Saponins
The chloroform insoluble residue was separated in the previous step (triterpenoid test), which was redissolved with 5 to 10 mL of distilled water and filtered in a test tube. The tube with the solution was stirred strongly for 2 to 3 minutes and observed foamed (Matos, 1988).
This next operation was carried out to confirm the presence of saponins, with the addition of 2 mL of concentrated HCl to the contents of the previously prepared test tube, which should remain in a water bath,

immersed for one hour and therefore must be cooled, at room temperature, and neutralized and stirred. In this procedure to confirm saponins should present precipitate and no foaming (Matos, 1988).

Test for Steroids and Triterpenoids (Lieberman-Burchard)

The dried residue of the beaker was removed 2 to 3 times with 1 to 2 mL of chloroform, the residue should be thoroughly triturated with the solvent. Then it was filtered with chloroform solution dropwise into a small glass funnel closed with a small portion of cotton, covered with small amounts of anhydrous Na_2SO_4 in a suitably dried test tube. After 1 mL of acetic anhydride is added, shaken gently and then add 3 drops of concentrated H_2SO_4, which should be gently shaken (Matos, 1988).

Test for Acetogenins and Alkaloids

The hexane and ethanolic extracts were submitted to TLC analysis (Thin Layer Chromatography). The plates were sprayed with Kedde reagent, which indicates sesquiterpene lactone, γ-lactone α, β-unsaturated, usually present in acetogenins and sprayed with Dragendorff reagent, which indicates the presence of alkaloids (Lima et al., 2012).

Fractionation of the Crude Extracts

For Sarker, Latif and Gray (2006) the crude extracts obtained have a great mixture of compounds that, in order to separate them, is important to fractionate them, to separate the technique of separation by different polarities in chromatographic column (CC). Matos (1988) indicates the use of a chromatographic column, with hexane and ethanolic extracts, silica gel and elution with hexane, dichloromethane, ethyl acetate and methanol, in increasing polarity gradient.

Thin Layer Chromatography

For the realization of the method, glass plates were coated with a suspension of Merck silica gel 60 G in distilled water (1:2): 0.25 mm thickness for analytical TLC activated at 100 ºC in an oven.

The fractions were concentrated in a rotoevaporator, which enabled them to be obtained. Comparative analyzes were performed by TLC using different eluent systems, with UV light and sulfuric vanillin, thus bringing together several fractions.

The fractions collected should be monitored by TLC, and when necessary, will be developed with 1% sulfuric vanillin under heating. It is based on the similarity between the retention factors (rf), so the fractions can be pooled and grouped (Matos, 1988).

2.4 Antioxidant activity of ethanolic extract of *S. latifolia*

The antioxidant potential was evaluated by the DPPH (2,2-diphenyl-1-picrylhydracil) method, in which the activity is measured according to the radical sequestration, in this way the purple to pale yellow discoloration occurs during the reaction, qualitative observation. In order to evaluate quantitatively, 1.2 mL of ethanolic DPPH solution (300 µmol) was mixed with 2.8 mL of each dilution of the stock solution (1 mg mL^{-1}) of the ethanolic extract, whose concentrations 0.0175; 0.0263; 0.0437 and 0.0630 mg mL^{-1} as the positive control, the standard BHT (2,6-di-tert-butyl-4-methylphenol) was used in the same procedures. A negative control was made with 2.8 mL of ethanol and 1.2 mL of DPPH, plus a blank, for each of the samples, containing 2.8 mL of extract and 1.2 mL of ethanol. After the abovementioned procedures, the samples were allowed to react for 30 min in a light-protected environment and then absorbance was analyzed in UV-VIS spectrophotometer at 515 nm, whose data were converted to a percentage of antioxidant activity. The tests were performed in triplicate and the Efficient Concentration (EC_{50}) values (Brand-Wiliams et al., 1995).

The percentage of antioxidant activity (% AA) was determined from the absorbance values of all concentrations of the samples, according to the equation:

$$\%AA = \frac{(|Sample| - AbsWhite).100}{(|Control| - |\quad|)}$$

Where: Abs_{Sample} = Sample Absorbance, $Abs_{control}$ = Control Absorbance and Abs_{White} = White Absorbance

2.5 Bioassays of *S. latifolia* antimicrobial activity on bacteria and yeasts

The antimicrobial activity of *S. latifolia* was performed Bauer et al. (1966), which consists of an agar diffusion method with application of extract (10 mg mL^{-1}) to the culture medium in which an inhibition zone can result and this can be measured. According to Alves et al. (2000) the evaluation of antimicrobial activity is measured the diameter of inhibition with a millimeter ruler, where: inactive, halo <9 mm; partially active, halo = 9-12 mm; active, halo = 13-18 mm; and very active, halo> 18 mm. The microorganisms used in the bioassay were: gram

positive bacteria (*Streptococcus mutans* (CBAM 241) and *Staphylococcus aureus* (ATCC 25923)) and gram negative (*Escherichia coli* (CBAM 001)) and yeast *Candida albicans* (ATCC 10231).

3. Results and Discussion

3.1 Chemical composition of *S. latifolia*

Phytochemical screening in raw extracts of leaves, stem, root, stem bark and root bark provided qualitative information on the chemical profile present in *S. latifolia* (Table 1)

Table 1: Qualitative chemical composition in parts of S. latifolia

	Leave	Stem	Root	Stem bark	Root bark
Phenols	O	O	O	O	O
Tannins	+	+	+++	+++	+++
Flavonoids	+++	+++	+++	+++	+++
Steroids	O	O	-	O	O
Triterpenoids	O	+++	-	++	++++
Saponins	+++	+++	-	+	+++

Legend: O = Absent; - = Not tested; + = Present (+ Very Little, ++ Little, +++ Strong, ++++ Very Strong)

As observed in Table 1, phenols and steroids are not present in *S. latifolia*, as well as the absence of triterpenoids in leaves. However, other chemicals of medicinal interest were present, such as tannins, flavonoids, saponins and triterpenoids in three parts of the plant. The root bark was the part of the plant where it presented in greater quantity of these chemical components, whose presence was strong and very strong. Thus, for this reason, we chose to work with the root bark for antioxidant activity and bioassays on gram positive and gram negative bacteria, as well as yeast.

Some of the substances identified in *S. latifolia* were also identified in other species of the genus *Swartzia*: flavonoids, triterpenoids and saponins, but tannins were not mentioned. These substances certainly confer on the medicinal potential genus on fungi, bacteria, larvicide, molluscicide and antimalarial (Santos and Melo Filho, 2013).

The triterpenoids can have many benefits to human health, the literature highlights its bioactive potential against cancer, anti-inflammatory, anti-proliferative, among others (Pattolla and Rao, 2012). According to Güçlü-Ustündağ and Mazza (2007), saponins have anti-cancer and anti-cholesterol activity, but other applications are observed, such as in the pharmaceutical, cosmetics and food industries. As in the aforementioned compounds, flavonoids have biological properties on plants as protection from ultraviolet radiation and phytopathogens (Ferreyra et al., 2012). Tannins have high antimicrobial potential, but other properties are reported by Chung et al. (1998), as an aid in blood coagulation, reduction in blood pressure, anticancer and antimutagenic activities, among many others.

3.2 Antioxidant activity of *S. latifolia*

After the phytochemical screening the antioxidant activity was evaluated by the DPPH method, in which initially, and in a qualitative way, the discoloration of the sample with the reagent was observed, leaving a purple to pale yellow coloration and soon thereafter was quantified by UV -Vis, values found for the lowest inhibitory concentration (EC_{50}) of the crude ethanolic extract of the root bark of 0.04 mg mL^{-1} and 0.20 mg mL^{-1} for BHT (standard) (Table 2).

Table 2: Efficient concentration of the crude ethanolic extract of S. latifolia root bark

Sample	CE_{50} (mg mL^{-1})
Crude ethanolic extract	0.04
BHT (standard)	0.20

The crude ethanol extract of *S. latifolia* showed significant antioxidant activity when compared to the BHT standard. Thus, the crude ethanolic extract of the root bark presents in its composition substances whose action inhibits the radical DPPH. According to Azevedo et al. (2014) phenolic compounds such as flavonoids

and tannins may have high antioxidant potential, according to the authors, this potential may have positive effects on several diseases derived from oxidative stress, such as cancer, for example.

3.3 Biological assay of extracts of *S. latifolia* root bark

The crude ethanolic and hexanic extracts of the *S. latifolia* root bark showed growth inhibition for the gram positive *S. aureus* and *S. mutans* strains, being active for the ethanolic extract (EE) and very active for the hexanic extract), The latter having the best response to the 20 mg L^{-1} concentration. In the case of the antibacterial activity of the ethanolic and hexanic extracts against *S. mutans*, they are active for the two concentrations having a similar behavior for the concentrations of 20 mg L^{-1} and 30 mg L^{-1}. The results are presented in Table 3, where the gram negative bacterial strain of *E. coli* and *C. albicans* yeast are very resistant against the extracts tested.

Table 3: Measurements, in millimeters, of the inhibition halos caused by the crude ethanolic (EE) and hexanic S. latifolia extract, 24 and 48 hours after incubation

Extract / concentration	Bacteria			Yeast
	S. aureus	*S. mutans*	*E. coli*	*C. albicans*
EE [20 mg L^{-1}]	14 mm (A)	9 mm (PA)	I	I
EE [30 mg L^{-1}]	14 mm (A)	11 mm (PA)	I	I
EH [20 mg L^{-1}]	19 mm (VA)	8 mm (PA)	I	I
EH [30 mg L^{-1}]	15 mm (A)	11 mm (PA)	I	I

Legend: I (inactive): <9 mm; PA (partially active): 9-12 mm; A (active): 13-18 mm; VA (very active): > 18 m; EE = ethanolic extract; EH = hexane extract.

It is worth noting that there are no data in the literature regarding the inhibition of fungi and bacteria by crude ethanolic and hexanic extracts of *S. latifolia*, in order to compare biological assays. However, it is observed in the literature that species of the genus *Swartzia* have an effective biological potential (Santos and Melo Filho, 2013).

Thus, many of the compounds analyzed in phytochemical screening may be associated with the inhibition of S. aureus and *S. mutans* bacteria as in the case of tannins, saponins, flavonoids and triterpenoids (Miranda et al., 2013; Thimothe et al., 2007).

Chung et al. (1998) reports the antimicrobial potential of tannins, that is, such compounds may inhibit the growth of many species of fungi, yeasts, and even viruses.

4. Conclusions

The phytochemical study of the ethanolic extract of the root bark was more significant, indicating, therefore, the presence of different groups of substances, such as flavonoids, triterpenoids, saponins and tannins. The results of the phytochemical screening test are in line with those found in the literature compared to genus. The crude ethanolic extract of the root bark has chemical compounds that contribute to an antioxidant action against the radical DPPH. Finally, the potential of gram-positive bacteria from the ethanolic and hexane crude extracts, but inactive on gram-negative bacteria and yeast.

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