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Antimicrobial activity, cytotoxicity and selectivity index of *Banisteriopsis laevifolia* (A. Juss.) B. Gates leaves

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ABSTRACT

Banisteriopsis laevifolia (A. Juss.) B. Gates (Malpighiaceae) is largely distributed in the cerrado Brazilian biome. In this study, phytochemical screening, antimicrobial and antifungal activities of leaves extract and partitions were evaluated. Phytochemical screening tests indicated the presence of flavonoids, terpenoids, phenols, sugar, steroids, triterpenoids, and tannins compounds. No alkaloids or nitrogenated compounds were found. Antioxidant, antimicrobial and antifungal activities were tested. The extract and partitions from the *B. laevifolia* leaves demonstrated relevant scavenging free radical DPPH effect. The crude extract and partitions inhibit bacteria growth with minimum inhibitory concentrations (MIC) below 400 mg L^{-1} for most oral microorganisms tested. Meanwhile, the antifungal activity proved to be very promising for the ethanolic extract and partitions (31–375 μg mL^{-1}) against all yeasts tested. The antimicrobial activities results are very promising since the ethanol extract and the more active *n*-buthanol partition showed great selectivity (0.9–1.2; 0.1–0.7, respectively) against microorganisms and relatively low toxicity to Vero cells. Analysis by UHPLC-ESI–MS^n from the most bioactive fractions (ethyl acetate and *n*-buthanol) permitted to identify ten phenolic compounds reported in the Banisteriopsis family that exert recognized antioxidant, antimicrobial and antifungal activity. Main secondary metabolites found were phenolic acids and flavonoid glycosides, mainly derivatives from quercetin and rutin. The biological activity results and MS analysis for the *B.laevifolia* leaves revealed that they have efficient antimicrobial agents, and contributed to knowledge of the genus.

1. Introduction

The Malpighiaceae family is related to their medicinal potential, including antimicrobial, antifungal, and anticolinisterasic activities (Frias et al., 2012). The Banisteriopsis genus is constituted of 92 liane and shrubs species distributed from Mexico to Argentina, two thirds of which are concentrated in Brazil. The *B. argyrophylla* extracts were described with anti-inflammatory action and are used to treat ovarian hemorrhage, nephritis and blenorrhée diseases (Rodrigues and Carvalho, 2001). The *B. Country Side* has been used as a diuretic and *B. megaphylla* has antipyretic activity and it may be used in lung diseases treatment. Few results are found for the species *B. anisandra*. Recently, ethanol extracts of this species showed antimicrobial activity *in vitro* (Padua et al., 2013).The species *B. anisandraís* leaves and roots which have been used in folk medicine for the fungicides infections topical treatment was target to phytochemical studies. Some fungicides infections classes were found. Alkaloids were not isolated but only phenanthrenoids structures (Freitas et al., 2015). Furthermore, the well-known *B. caapi* species has significant pharmacological and psychoactive activities due to the alkaloids present in its leaves and roots (Wang et al., 2010; Stankovic et al., 2015). Studies on the extracts and partitions effectiveness against oral bacteria have been published for some Brazilian cerrado plant species, such as *Kielmeyera coriacea* Mart. & Zucc (Martins et al., 2013) and *Maclura tinctoria* (L.) D. Don ex Steud

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(Lamounier et al., 2012). In view of the wide medical application of Malpighiacea family and the lack of studies directed specifically to *B. leavifolia* (A. Juss.) B. Gates (known as silver liana, "cipó-prata"), this study investigated the antimicrobial activities of the ethanolic extract and its fractions against oral bacteria and strains, evaluating their cytotoxic effects in Vero cells (ATCC CCL 81) and RAW 264.7 cells (ATCC TIB 71).

2. Material and methods

2.1. Plant material

The *Banisteriopsis leavifolia* (A. Juss.) B. Gates leaves samples were collected in October 2014, in Monte Alegre de Minas, located at the following geographical coordinates obtained by GPS: (18°34′56.85″S; 48°26′06.40″W)-MG, Brazil. The plant material was identified properly by a specialist (Dra. Maria Cândida Mamede, herbarium São Paulo), and a voucher specimen of the plant was stored in the Herbarium of the Universidade Federal de Uberlândia-UFU (Uberlândia-Brazil) under code number 67.075. The appropriate autorization for biological research was achieved at Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq-Brazil), under process number 010300/2014-7. Furthermore, this species is not endangered or threatened with extinction, and only the plants leaves were used, which readily regenerate, preserving thus the species' population.

2.2. Extract preparation

The *B. leavifolia* (220.0 g) fresh leaves were air-dried until the moisture reached 10% (automatic infrared-moisture balance Kett, model FD600, 105 °C, for 15 min), cleaned, cut into small pieces, peeled, and minced. The powdered material was homogenized and extracted with 2L of ethanol (95% purity) by maceration at room temperature for 7 days, in dark conditions. The extract was decanted, filtered and concentrated under reduced pressure at 40 °C vacuum (RV10 basic, IKA, Germany). The lyophilized extract (25.8 g) (Lyophilizer LS 3000, TERRONI, Brazil) were transferred to an amber flask and then stored at -18 ± 5 °C for further analysis. The extraction was carried out at the Núcleo de Pesquisa em Produtos Naturais (NuPPeN), Universidade Federal de Uberlândia-UFU.

2.3. Ethanol extract liquid-liquid partition

A sample (22.0 g) of this extract was re-dissolved in 300 mL of MeOH-H_2O (9:1) and partitioned with equal volumes (600 mL; 3×200 mL) of a solvents sequence (hexane, dichloromethane, ethyl acetate and *n*-buthanol). The hexane (496.8 mg), dichloromethane (2.6 mg), ethyl acetate (6.0 mg) and *n*-buthanol (6.0 mg) partitions were concentrated under reduced pressure, at 40 °C using rotavapor (RV10 basic, IKA, Germany), and lyophilized (Liophilizer LS 3000, TERRONI, Brazil).

2.4. Phytochemical analysis

Qualitative phytochemical tests with extract and partitions of *B. leavifolia* leaves were analyzed and the methods were described by Wagner and Bladt (1996). It was used a 1000 μg mL^{-1} solution in methanol of the each sample for the screening. Chemical constituents of the extracts and partitions were analyzed by thin layer chromatography (TLC) using aluminum-backed TLC plates (CCM Alugram®, Xtra SIL G/UV). The TLC plates were developed with one of the two eluent systems, i.e., Mobile phase 1 (ethyl acetate: formic acid: acetic acid: water; 10.0:1.1:1.1:2.6; polar/acidic); and Mobile phase 2 (chloroform: methanol: ammonium hydroxide, 9.0:1.0:0.25; polar/basic). Development of the chromatograms was done in a closed tank in which the atmosphere had been saturated with the eluent vapour by lining the tank with filter paper wetted with the eluent. The phytoconstituents, namely alkaloids, amino acids, terpenoids, saponins, triterpenes, steroids, flavonoids, tannins and phenolic compounds were screened.

2.4.1. Tin Layer Cromathography (TLC) analysis of the extracts and partitions

Visible bands were marked under daylight and ultraviolet light (254 and 360 nm, Spectroline Model ENF-240/FE, UV lamp, N.Y. USA; Fluorescence Analysis Cabinet Model CN-10/FE), before spraying with freshly prepared solutions: *p*-anisaldehyde (H_3CCO_2H:MeOH:H_2SO_4; 0.5:10.0:85.0:5.0); NP/PEG (10.0:8.0); Liebermann-Buchard (Anydride acetic acid:H_2SO_4:EtOH; 5.0:5.0:50.0); sol. $FeCl_3$ (10%); Dragendorf (sol. A: sol. B; 1:1) spray reagents. The plates were carefully heated at 105 °C, 5–10 min, for optimal color development.

2.5. Antioxidant activity measurement

All Ultraviolet-Vis absorptions measurements were performed on a spectrophotometer UV–vis U-300 (Hitachi, Kyoto, Japan) equipped with a water thermostatic cell holder. Glass cells with a 1 cm optical path were used. The methodology for determining the antioxidant activity is already properly established (Morais et al., 2009).

2.5.1. Total phenolic content

The total phenolic content was determined using the modified Folin-Ciocalteau reagent method (Singleton and Rossi, 1965; Sousa et al., 2007) with modifications (Morais et al., 2009). A mass of 12.5 mg of the test sample (ethanol extract or partitions) was transferred quantitatively into a volumetric flask and the final volume (25.0 mL) was completed with methanol. An aliquot of 0.5mLwas transferred to a test tube. Then, 2.5 mL of a solution of Folin-Ciocalteu reactive (10% v v^{-1}) and 2.0 mL of Na_2CO_3 solution (7.5% v v^{-1}) freshly prepared were added. This mixture was kept in a water bath, at a temperature of 50 °C, for 5 min. Next, the absorbance of the cooled mixture was recorded, at 760 nm, in a UV–vis U-2000 (Hitachi, Kyoto, Japan) spectrophotometer. Gallic acid was used for the standard curve construction at several concentrations (10.0, 20.0, 40.0, 60.0, and 80.0 μg mL^{-1}). The total phenolic content was determined by interpoling the samples absorbance values against the calibration curve constructed. The final results were expressed as gallic acid equivalents per gram of dry extract (mg GAE g^{-1}) (Table 2). The analysis was performed in triplicate.

2.5.2. Total proanthocyanidins content

The total proanthocyanidins content was determined by the vanillin method with modifications (Godefroot et al., 1981; Morais et al., 2009). A mass of 12.5 mg of the ethanol extract or partitions was dissolved in a 25.0 mL volumetric flask and the final volume completed with methanol. An aliquot of 0.5 mL from the solution was transferred to a test tube and 3.0 mL of a freshly prepared solution of vanillin (10.0 mg mL^{-1}) in sulphuric acid (70% v v^{-1}) were added. The resulting mixture was kept in a water bath, at 50 °C, for 15 min. The sample was cooled and the absorbance was recorded at 500 nm in a UV–vis U-200 (Hitachi, Kyoto, Japan) spectrophotometer. The tannins content was determined by interpolating the samples absorbance values against a standard curve constructed from catechin standards (5.0, 10.0, 15.0, 20.0, and 30.0 μg mL^{-1}). The final results were expressed as catechin equivalents per gram of dry extract (mg CE g^{-1}) (Table 2). The analysis was performed in triplicate.