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Alibertia edulis (L.C. Rich.) A.C. Rich – A potent diuretic arising from Brazilian indigenous species

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ABSTRACT

Ethnopharmacological relevance: Although *Alibertia edulis* (L.C. Rich.) A.C. Rich decoction is used in Brazilian folk medicine due to its possible antihypertensive effect, this species has never been critically investigated as a hypotensive drug. So, the aim of this study was to evaluate the possible hypotensive and antihypertensive effects of the oral administration of *Alibertia edulis* aqueous extract (AEAE) in normotensive and hypertensive rats, and evaluate its inter-relation with a possible diuretic activity.

Material and methods: Different doses of AEAE (20, 65 and 200 mg/kg) were tested on the mean arterial pressure (MAP) of normotensive Wistar rats and after induction of renovascular hypertension (two-kidney, one-clip Goldblatt model). In addition, the diuretic effects of AEAE were compared with hydrochlorothiazide (HCTZ) in an acute and repeated-dose treatment for 7 days. Volume, sodium, potassium, chloride, calcium contents, pH and density were estimated in urine samples collected after 8 or 24 h. Plasma sodium, potassium, total protein, urea, creatinine, AST and ALT concentrations were measured in samples collected at the end of the experimental period (seventh day). Finally, the antioxidant activity of the AEAE was assessed using the DPPH radical scavenging and ferric ions reducing power assay.

Results: The intraduodenal administration of the HCTZ and AEAE significantly reduced, in a dose-dependent manner, the MAP in both normotensive and hypertensive rats. Otherwise, the heart rate was not affected by any treatment. Acute and prolonged oral administration of AEAE (200 mg/kg) and HCTZ caused a significant increase in volume and urinary concentrations of sodium, potassium and chloride. Moreover, urinary calcium concentration was significantly increased after administration of AEAE (200 mg/kg). Finally, AEAE was able to present important in vitro antioxidant properties.

Conclusion: The results obtained have shown that AEAE presents potent diuretic activity and significant hypotensive and antihypertensive effect. In addition, this study may confirm part of the pharmacological activity popularly attributed to this species and opens perspective for the future use in various renal and cardiovascular diseases.

Abbreviations: 2K1C, Two-kidney-one-clip; AEAE, Alibertia edulis aqueous extract; AF, Acetate fraction; ALT, Alanine transaminase; ANOVA, Analysis of variance; AqF, Aqueous fraction; AST, Aspartate transaminase; BF, n-buthanol fraction; BW, Body weight; Ca^{+2}, Calcium; Cl^{-}, Chloride; DPPH, 2,2-diphenyl-1-picryl-hydrazil; EL, Excretion load; Fe, Ferro; FRAP, Ferric reducing antioxidant power; GAE, Gallic acid equivalentes; g/ml, grams/milliliters; h, hours; H_2O, Water; H_2SO_4, Sulfuric acid; HCTZ, Hydrochlorothiazide; HOAc, Acetic acid; HPLC-PDA, High-Performance Liquid Chromatography Photo-Diode Array; i.v., intravenous; IC_{50}, Half maximal inhibitory concentration; IU, International units; K^{+}:, Potassium; MAP, Mean arterial pressure; MeOH, Methanol; mEq/l, miliequivalent/liters; mEq/min/100 g, miliequivalent/minutes/100 g of body weight (BW); mg/kg, milligrams/kilograms; mg, milligrams; min, minutes; ml/min, milliliters/minutes; ml, milliliters; mm Hg, millimeters of mercury; Na^{+}, Sodium; NaCl, Sodium chloride; nm, nanometers; NMR, Nuclear magnetic resonance; °C, Degree Celsius; pH, Hydrogen potential; RE, Rutin equivalentes; rpm, rotation per minute; SBP, Systolic blood pressure; SEM, Standard error of the mean; TAA, Total antioxidant activity; TLC, Thin layer chromatography; TPTZ, 2,4,6-Tris(2-piridil)-s-triazina; Ux, Electrolytes concentration; µl/100 g, microliters/100 g of body weight (BW); µM, micrometer; v/v/v, volume/volume/volume; V, Urinary flow; w/v, weight/volume

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1. Introduction

In Brazil, the state of Mato Grosso do Sul has one of the richest biodiversity in Latin America (Cordeiro et al., 2014). The state has at least two major biomes, Cerrado and south Pantanal (Myers et al., 2000; Ribas and Schoereder, 2006). In addition to the rich and still preserved vegetation, the state has different indigenous communities that keep important popular knowledge about the therapeutic activities of various natural products (Bueno et al., 2005). In the region known as Grande Dourados, located in the southern state, there are more than 20 thousand Indians of different ethnic groups, including Guarani-Kaiowá, Kadiwéu, and Terena (Sacchi et al., 2013). It is in this place of intense cultural effervescence that much ethnopharmacological knowledge has been transmitted from generation to generation for hundreds of years, showing great potential for ethnopharmacological validation of the popular culture of these people (Leonti, 2011).

In this region, where the predominant native vegetation is Cerrado, the use of several species of the family Rubiaceae, including *Alibertia, Psychotria*, *Palicourea* and *Tocoyena* is observed, with the largest number of species with several pharmaceutical and economic interests (Martins and Nunez, 2015). Among species widely used in the region, *Alibertia edulis* (L.C. Rich.) A.C. Rich., popularly known as "marmelada-bola" and "marmelo-do-cerrado" (Brochini et al., 1994; Persson, 2000) stands out. For many years, this species has been popularly used for the treatment of hypertension throughout the state of Mato Grosso do Sul, especially in the region of Grande Dourados (Sangalli et al., 2002; Bueno et al., 2005).

Despite its widespread use, the different preparations obtained from *Alibertia edulis* lacks detailed ethnopharmacological studies that validate its popular use. It is worth mentioning that the popular widespread use does not guarantee efficacy and safety of a natural product, and only detailed studies can point out possible risks of their pharmacological activity, such as electrolyte disturbances, arrhythmias, and other indicators of toxicity.

Few pharmacological studies have been conducted for this species, most of them focused on its antitumor activities (Gupta et al., 1996). A phytochemical investigation with the stem from *Alibertia edulis* led to the isolation and identification of an iridoid ester and a saponin, which showed moderate inhibitory activity against *Candida albicans* and *Candida krusei* (Da Silva et al., 2008). Moreover, it was also possible to identify the presence of oleanane (Brochini, 1994), tannins and some alkaloids (Soto-Sobenis et al., 2001).

Thus, considering the available ethnobotanical information and extensive popular use of this species in the treatment of hypertension, the aim of this study was to evaluate the possible hypotensive and antihypertensive activities of the oral administration of *Alibertia edulis* aqueous extract in normotensive and hypertensive rats and evaluate its inter-relation with a possible diuretic activity.

2. Materials and methods

2.1. Drugs and spectral measurements

Hydrochlorothiazide (HCTZ), Gallic Acid, Rutin, 2,2-diphenyl-1-picrylhydrazil (DPPH), TPTZ (2,4,6-Tripyridyl-s-Triazine) were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Folin–Ciocalteu was obtained from Merck (Darmstadt, Germany); spectroscopy- grade acetonitrile and methanol were purchased from Merck (Darmstadt. Germany); water was purified using a Milli-Q system (Millipore). Antioxidant activity assays were recorded in MeOH using 700S Femto UV Spectrophotometer at 517 nm wavelength. Preparative Thin Layer Chromatography (TLC) was carried out on silica 60 F254 TLC plates (Merck). Sephadex LH-20 (Sigma-Aldrich) was used for column chromatography.

2.2. Phytochemical study

2.2.1. Plant material

Alibertia edulis leaves were collected in November/December 2013 from the local vegetation of the Federal University of Grande Dourados (UFGD) (Dourados, Brazil) at 458 m above sea level (S22°11′43.7 - W54°56′08.5). A voucher specimen was authenticated by Dr. Zefa Valdevina Pereira under number 4649 and deposited in the herbarium of UFGD. *Alibertia edulis* leaves were air-dried in an oven at 40 °C for 10 days (58.1% humidity) and then the dry plant was cut and ground into a powder using mechanical milling.

2.2.2. Alibertia edulis aqueous extract (AEAE) preparation and fractionation

Dry leaves were extracted by decoction (1:10 w/v at temperature 97 °C by 15 min) in a similar manner to that used popularly in Brazil (Bueno et al., 2005). The extract was filtered and lyophilized, yielding 36.9%. Further liquid/liquid extractions on the AEAE were carried out using ethyl acetate (AF) and n-buthanol (BF). Fractions were obtained after decantation and evaporated under vacuum at approximately 40 °C (AF and BF) or lyophilized (aqueous fraction - AqF) yielding 12.4% of AF, 15.8% of BF and 65.7% of AqF. The AEAE and the three fractions were analyzed by TLC using n-buthanol/acetic acid/water (65:25:15 v/v/v) and BF was selected in order to be fractioned. A BF (103.0 mg) sample was chromatographed on a Sephadex LH-20 column (50.0 cm x 2.0 cm) with MeOH as eluent in order to give seven fractions: AE-1 (25.2 mg), AE-2 (5.5 mg), AE-3 (22.4 mg), AE-4 (12.5 mg), AE-5 (8.9 mg) AE-6 (4.5 mg), and AE-7 (5.8 mg). Compounds were visualized under UV254/366 light and by spraying with H2SO4/H2O/HOAc (4:16:80 v/v/v).

2.2.3. Phytochemical analysis

2.2.3.1. Screening. The total phenolic content of AEAE and fractions (AF, BF and AqF) were determined by using the Folin & Ciocalteu's phenol reagent. An aliquot (1 ml) of the extracts was added to 25 ml volumetric flask, containing 9 ml of distilled water. Blank using distilled water was prepared. One milliliter of Folin & Ciocalteu's reagent was added to the mixture and shaken. After 5 min, 10 ml of 7% Na2CO3 solution was added to the mixture. The solution was diluted to volume (25 ml) with distilled water and mixed. After incubation for 120 min at room temperature, the absorbance against prepared blank was determined at 750 nm with an UV–vis Spectrophotometer. Standard curve of Gallic acid solution (25, 100, 300, 400, 500, 600 and 700 μg/ml) was prepared using the similar procedure. Samples were measured in three replicates.

Aluminum chloride colorimetric method was used for determination of total flavonoids. The extract and its fractions (0.5 ml of 1:10 g/ml) in methanol was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm in triplicate. The calibration curve was prepared by preparing quercetin solutions at concentrations 10–100 μg/ml in methanol (Chang et al., 2002).

2.2.3.2. Nuclear Magnetic Resonance (NMR) spectroscopy. NMR spectra was recorded on Agilent 500 DD2 spectrometer equipped with 5 mm 1 H{15N–31 P} PFG high-field inverse detection z-gradient probe. ^{1}H (499.719 MHz) and ^{13}C (125.666 MHz) NMR spectra were recorded in methanol-d_4 at 25 °C. Chemical shifts are given on the δ scale and are referenced to residual methanol (δH 3.30 and δC 49.00).