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Mutagenicity and chemopreventive activities of *Astronium* species assessed by Ames test

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ABSTRACT

In the neotropical savannah, *Astronium* species are used in popular medicine to treat allergies, inflammation, diarrhea and ulcers. Given that natural products are promising starting points for the discovery of novel potentially therapeutic agents, the aim of the present study was to investigate the mutagenic and antimutagenic activities of hydroalcoholic extracts of *Astronium* spp. The mutagenicity was determined by the Ames test on *Salmonella typhimurium* strains TA98, TA97a, TA100 and TA102. The antimutagenicity was tested against the direct-acting and indirect-acting mutagens. The results showed that none of the extracts induce any increase in the number of revertants, demonstrating the absence of mutagenic activity. On the other hand, the results on the antimutagenic potential showed a moderate inhibitory effect against NPD and a strong protective effect against B[*a*]P and AFB_1. This study highlights the importance of screening species of *Astronium* for new medicinal compounds. The promising results obtained open up new avenues for further study and provide a better understanding the mechanisms by which these species act in protecting DNA from damage. However, further pharmacological and toxicological investigations of crude extracts of *Astronium* spp., as well as of its secondary metabolites, are necessary to determine the mechanism(s) of action to guarantee their safer and more effective application to human health.

1. Introduction

The biodiversity of the Brazilian flora is very broad, and several native Brazilian medical plant species have a long tradition of use, with great phytotherapeutic potential. The Brazilian cerrado (neotropical savannah) is one of the major biogeographic regions (biomes) of the world, with more than 7000 native species of vascular plants. Many of these plants are commonly used as natural remedies by people living in the cerrado area (Rocha et al., 2011).

Owing to the rich plant diversity found in the cerrado, it is may be useful to explore the presence in cerrado plants of various biological activities; however, this biome has been poorly researched to assess the efficacy and therapeutic effects of crude extracts or isolated compounds (Souza et al., 2012).

In the cerrado, the species *Astronium fraxinifolium*, *A. graveolens* and *A. urundeuva* are very common, known locally as "gonça lo-alves", "aroeira" and "guaritá", respectively, and accepted in popular medicine as a treatment for allergies, inflammation, diarrhea and ulcers (Viana et al., 1997; Silva et al., 2011). Relatively few studies exist concerning the chemical composition of *Astronium* species. Chen et al. (1984) described the presence of (*E*)-*β*-ocimene as the major volatile in *A. graveolens*, and more recently two works, independently, described the composition of *A. urundeuva* and *A. fraxinifolium* essential oils, the previous being mainly Δ^3-carene and the later a complex mixture of *β*-ocimenes, bicyclogermacrene, limonene and α-terpinolene (Maia et al., 2002; Montanari et al., 2012). Viana et al. (2003) isolated three dimeric chalcones from ethyl acetate extract of *A. urundeuva* barks, and Silva et al. (2011) characterized the gallotanin composition of *A. urundeuva* and *A. graveolens* by mass spectrometry.

According to the literature, the species *A. urundeuva* has been studied the most, showing antiulcer (Souza et al., 2007; Carlini

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et al., 2010), anti-inflammatory (Viana et al., 2003; Souza et al., 2007), antibacterial, antifungal (Sá et al., 2009), neuroprotective (Nobre Junior et al., 2007) activities and effects on gastrointestinal transit (Menezes and Rao, 1988) and in colitis (Rao et al., 1986).

With respect to other species, Montanari et al. (2012) describe the resultant antibacterial activity of essential oils from *A. fraxinifolium*, *A. urundeuva* and others three Anacardiaceae species against Gram-positive and Gram-negative bacteria. Recently, Hernández et al. (2014) determined the free radical-scavenging and the antiangiogenic properties of *A. graveolens* leaf extract and observed that 1,2,3,4,6-Penta-O-galloyl-D-glucopyranose is the most active compound of the extract. Lima et al. (2014) investigated leishmanicidal activity of *A. fraxinifolium* and *Plectranthus amboinicus* against *Leishmania (Viannia) braziliensis* and *in vivo* studies showed a high efficacy when the infected animals were intralesionally treated with leaf ethanolic extract from *A. fraxinifolium*.

Regarding the wide use of the genus *Astronium* in folk medicine, the research on various pharmacological activities with positive results and the risk of using medicinal extracts without detailed study, the aim of the present study was to determine potential mutagenic and/or antimutagenic effects of hydroalcoholic extracts of leaves and stalks of *A. graveolens* and *A. urundeuva*, and leaves and bark of *A. fraxinifolium*, by the Ames test. The mutagenic activities of extracts were assayed in the *S. typhimurium* test-strains TA98 and TA97a (detect frameshift mutations), TA100 (detect base-pair-substitution mutations) and TA102 (normally used to detect mutagens that cause oxidative damage and base-pair-substitution mutations) and the protective (antimutagenic) effects were measured against the mutagenicity of direct and indirect-acting mutagens, namely 4-nitro-*o*-phenylenediamine (NPD), mitomycin C (MMC), benzo[*a*]pyrene (B[*a*]P) and aflatoxin B_1 (AFB_1).

2. Material and methods

2.1. Chemicals and culture media

Dimethylsulfoxide (DMSO), nicotinamide adenine dinucleotide phosphate sodium salt (NADP), D-glucose-6-phosphate disodium salt, magnesium chloride, L-histidine monohydrate, D-biotin, NPD, sodium azide (SA), MMC, 2-anthramine (2-AA), 2-aminofluorene (2-AF), B[*a*]P and AFB_1 were purchased from Sigma Chemical (St. Louis, MO, USA). Oxoid Nutrient Broth No. 2 (Oxoid, England) and Difco Bacto Agar (Franklin Lakes, NJ, USA) were used as bacterial media. D-glucose, magnesium sulfate, citric acid monohydrate, anhydrous dibasic potassium phosphate, sodium ammonium phosphate, monobasic sodium phosphate, dibasic sodium phosphate and sodium chloride were purchased from Merck (Whitehouse Station, NJ, USA).

2.2. Plant material and extraction

A. graveolens Jacq. (voucher 148133) was collected in the Bosque dos Jequitibas, Campinas, São Paulo State, Brazil, in October 2007, by Dr. E. Ramos. *A. urundeuva* (Allemao) Engl. (syn: *Myracrodruon urundeuva* Allemao) (voucher 1444) was obtained in Votuporanga, São Paulo State, Brazil, in November 2007, by J.Y. Tamashiro. Plants were identified by Dr. J.Y. Tamashiro, and voucher specimens were deposited at the Herbarium of State University of Campinas (HUEC) in Campinas, São Paulo State, Brazil. *A. fraxinifolium* Schott. (voucher 333) was collected in Porto Nacional, Tocantins State, Brazil, in December 2007, by Dr. Cristiano B. Pereira. The plant was identified by Dr. Eduardo R. dos Santos and a voucher specimen was deposited at the Herbarium of Foundation University of Tocantins (HUTO) in Palmas, Tocantins State, Brazil.

Parts of the plants were separated, dried at 40 °C to constant mass and then pulverized and stored in the dark, under cool, dry conditions, until used. Hydroalcoholic extracts were prepared in 7:3 (v/v) EtOH/H_2O, in a stainless steel percolator (20 L). The plant powder was soaked in the solvent for 2 h before packing the percolator, with a solvent:plant powder ratio of 5:1 (w/w). The percolation was performed at a moderate flow rate of 2 mL/min/kg. The solvent was eliminated from the extract in a rotary evaporator (Heidolph Laborota 4001), equipped with low-pressure pump control, with a Heidolph Rotavac Control valve. The residues were suspended in MeOH/H_2O (8:2, v/v), then partitioned with *n*-hexane. The dried extracts were powdered and stored in amber bottles at 4 °C. Hydroalcoholic extracts of leaves and stalk of *A. graveolens* and *A. urundeuva*, and of leaves and bark of *A. fraxinifolium*, were produced. An aliquot of each hydroalcoholic extract was analyzed by High Performance Liquid Chromatography coupled to a Photodiode Array Detector (HPLC–PAD).

2.3. Chromatographic profiling and identification of metabolite classes

Astronium extracts were analyzed by HPLC–PAD system (Jasco, Tokyo, Japan) equipped with a PU-2089 quaternary pump further coupled to a MD-2010 Photodiode array detector (PAD). Separation was performed on a reversed phase Synergi Hydro C18 column (250 mm × 4.6 mm, 4 μm; Phenomenex) equipped with guard column (4.0 mm × 2.0 mm, 4 μm; Phenomenex) with aqueous 0.5% trifluoroacetic acid (TFA) and acetonitrile 0.5% TFA as elution buffers A and B respectively. The following gradient was applied at a constant flow of 1 mL/min: 0 min 1% B, 50 min 30% B, 60 min 100% B, and 65 min 100% B. EZChrom Elite Data System software (Chromatec, Idstein, Germany) was used for system operation and data processing. Metabolites were identified by UV spectral analysis and Co. injection of standards.

2.4. Metabolic activation system (S9 mixture)

The S9 fraction, used for metabolic conversion of carcinogens to their active mutagenic forms, was prepared from livers of Sprague–Dawley rats treated with the polychlorinated biphenyl mixture Aroclor 1254 (500 mg/kg) and purchased from Molecular Toxicology (Boone, NC, USA). The metabolic activation system consisted of 4% S9 fraction, 1% 0.4 M $MgCl_2$, 1% 1.65 M KCl, 0.5% 1 M D-glucose-6-phosphate disodium, 4% 0.1 M NADP, 50% 0.2 M phosphate buffer and 39.5% sterile distilled water. The activation system was prepared according to Maron and Ames (1983).

2.5. Mutagenicity assay

Mutagenic activity was estimated by the *Salmonella*/microsome assay, with the *Salmonella typhimurium* tester strains TA98, TA100, TA97a and TA102, kindly provided by Dr. B.N. Ames (Berkeley, CA, USA), with (+S9) and without (−S9) metabolization by the pre-incubation method (Maron and Ames, 1983).

The strains were taken from frozen cultures and grown overnight for 12–14 h in Oxoid Nutrient Broth No. 2. The metabolic activation mixture (S9) was freshly prepared before each test. For comparison of activity among the *Astronium* extracts, five different doses of each of extracts were assayed. All of them were diluted in DMSO. The concentrations of *Astronium* spp. extracts were based on a preliminary toxicity test. In all subsequent assays, the upper limit of the dose range tested was either the highest non-toxic dose or the lowest toxic dose determined in this preliminary assay. Toxicity was apparent either as a reduction in the number of