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# Genotoxicity of polar and apolar extracts obtained from *Qualea multiflora* and *Qualea grandiflora*

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ABSTRACT

*Ethnopharmacological relevance:* The species *Qualea grandiflora* and *Qualea multiflora*, which belong to the Vochysiaceae family, are common in the Brazilian savannah (Cerrado biome), and the local inhabitants use these species to treat external ulcers and gastric diseases and as an anti-inflammatory agent. Studies have demonstrated that these plants contain compounds that exhibit pharmacological activities; however, the risks associated with their consumption are not known.
*Material and methods:* In the present study, the mutagenicity of polar and apolar extracts from *Qualea grandiflora* and *Qualea multiflora* were assessed by employing the Ames assay with and without metabolic activation. Additionally, phytochemical analyses (HPLC–ESI-IT-MS, HPLC–UV-PDA and GC–IT-MS) were performed to identify the chemical constituents present in these species, including the evaluation of physico-chemical properties, such as polarity or apolarity of the organic compounds, which are related to each fraction obtained. These studies provide important information regarding the biochemical behaviour of these compounds.
*Results:* All extracts exhibited mutagenicity, inducing frameshift mutations and base substitutions in DNA. Phytochemical analysis identified terpenes, ellagic acid derivatives and phytosteroids.
*Conclusions:* The mutagenicity observed might be due to the presence of pentacyclic triterpenes and polyphenols, which are able to generate reactive oxygen species (ROS) and result in the potential to cause DNA damage. The genetic risk identified in this present work shows that special attention should be considered for the use of compounds obtained from these plant species in medicinal treatments. Further studies must be conducted to identify safe therapeutic doses.
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## 1. Introduction

The Brazilian Savannah, locally known as Cerrado, contains approximately 2000 species of shrubs/trees and approximately 5000 species of herbaceous species that composes its flora (Borges and Shepherd, 2005). According to Myers et al. (2000), Cerrado is a biodiversity hotspot that contains at least 0.5% of the 300,000 vegetal species known in world.

The genus, *Qualea*, which belong to the Vochysiaceae family, comprises approximately 200 species of tropical trees. In the Brazilian Savannah, the species *Qualea grandiflora* Mart. and *Qualea multiflora* Mart. are very common. Local inhabitants use these species as popular medicinal treatments for external ulcers, gastric diseases and inflammation.

According to Hiruma-Lima et al. (2006), *Qualea grandiflora* shows activity against gastric ulcers. In another work using the same plant, Gaspi et al. (2006) verified analgesic and anticonvulsant properties. Studies with *Qualea multiflora* demonstrated that this species was able to kill both eggs and adult forms of *Schistosoma mansoni* (Souza et al., 1984).

The scientific literature about these plants is very scarce, and the possible risks involved in the consumption of compounds derived from these plants are unknown. Mutational events are involved in several degenerative diseases, such as cancer and arteriosclerosis (De Flora, 1998). The literature describes many plants containing mutagenic compounds, such as furocoumarins (Varanda et al., 2002), tannins, anthraquinones (Ferreira and Vargas, 1999) and flavonoids (Rietjens et al., 2005; Santos et al., 2008); therefore,

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research on the mutagenicity of medicinal plants is important due to the danger posed to human health. The present work aimed to evaluate the mutagenicity of polar and apolar extracts obtained from the bark of *Qualea grandiflora* and *Qualea multiflora* by employing the Ames assay. This study was developed considering the structural properties of the organic compounds present in each extract, such as the type of substituent groups and polarity. The use of three different solvents resulted in the isolation of groups of organic compounds with quite distinct physico-chemical properties, such as polarity and hydrophilicity. Additionally, a phytochemical analysis was performed to identify the compounds present on the plant extracts evaluated.

## 2. Material and methods

### 2.1. Plant material

The barks of *Qualea grandiflora* were collected close to the Pedro Afonso Road, Tocantins State, Brazil, on October 25, 2003 by Cristiano Borges Pereira. Bark samples of *Qualea multiflora* were collected at the Cerrado region of Tocantins State on August 02, 2003 by Dr. Clélia Hiruma-Lima. The identification of both species was performed by Dr. Solange de Fatima Lolis, and the vouchers are kept at the University Tocantins herbarium, which are numbered as 3379 and 4158, respectively.

### 2.2. Preparation of extracts

The air-dried and powdered barks (0.5 kg) of *Qualea grandiflora* and *Qualea multiflora* were extracted successively with chloroform ($CHCl_3$) and methanol (MeOH) at room temperature (one week for each solvent). Solvents were evaporated at 60 °C under reduced pressure. The process performed with the plant material from *Qualea grandiflora* yielded 18.5 g of chloroform extract ($ECHCl_3$) and 54.3 g of methanol extract (EMeOH). The bark of *Qualea multiflora* yielded 16.9 g of the $ECHCl_3$ and 51.1 g of the EMeOH. Comparative thin layer chromatographic analysis showed the presence of polyphenols, terpenes, saponins and tannins in methanol extracts as well as steroids and terpenes in chloroform extracts. The aqueous extracts ($EH_2O$) from these species were obtained by infusion (100 g of powdered bark/1000 mL water). Two hours after this process, the extracts were filtered through a Buchner funnel, concentrated and lyophilised. The presence of tannins in water extract was identified employing the following colour assay with ferric ions. Three drops of ferric chloride (0.5 M) were added to tubes with hydrated samples (2 mg $mL^{-1}$). Tubes were agitated, and colour variation was observed. All samples presented a blue–grey colouration, indicating the presence of hydrolysable tannins.

### 2.3. Phytochemical analysis

All procedures described for the phytochemical analysis were based on previous studies with the species *Qualea parviflora*, performed in our laboratory and published (Nasser et al., 2006, 2008; Mazzolin et al., 2010). Data collected in these previous works were important parameters to the conclusions obtained and are presented herein.

#### 2.3.1. Methanol extract

Chromatographic profiles of the EMeOH from *Qualea grandiflora* and *Qualea multiflora* were obtained by HPLC–ESI-IT-MS (high-performance liquid chromatography coupled to electrospray ionization ion trap multiple mass spectrometry) and HPLC–UV-PDA (high performance liquid chromatography with diode array detector). To perform HPLC–ESI-IT-MS, aliquots (10 mg) of EMeOH (*Qualea grandiflora* and *Qualea multiflora*, independently) were diluted in water (4.0 mL) and filtered in Sep-Pak cartridges (Sigma, RP 18). The cartridges were washed with MeOH (4.0 mL). Methanolic fraction was filtered in Millex (0.45 μm) and analysed using HPLC–ESI-IT-MS (Hypersil BDS C18, 250 × 2.1 mm d.i. × 5 μm). The mobile phase used was either water (eluent A) or acetonitrile (eluent B), and both contained 0.5% of acetic acid. The gradient program was as follows: 10% B (5 min), 10–66% B (35 min), 66–95% B (10 min) and 95% B isocratic (5 min). The total time of analysis was 55 min with a 200 μL/min flow.

To HPLC–UV-PDA (RP-18, 250 × 4.60 mm i.d. × 5 μm) aliquots, 10 mg of EMeOH (*Qualea grandiflora* and *Qualea multiflora*, independently) was diluted in water (4.0 mL) and filtered in Sep-Pak cartridges (Sigma, RP 18). The cartridges were washed with MeOH (4.0 mL). Methanolic fraction was filtered in Millex (0.45 μm). The mobile phase used was either water (eluent A) or acetonitrile (eluent B), and both contained 0.05% of TFA. The gradient program was as follows: 16–40% B (30 min), 40–70% B (5 min), 70–100% B (5 min) and 100% B isocratic (5 min). The total time of analysis was 45 min with a 1.0 mL/min flow.

#### 2.3.2. Chloroform extract

GC–IT-MS (gas chromatography–ion trap-mass spectrometry) analysis was carried out with $ECHCl_3$ of *Qualea grandiflora* and *Qualea multiflora*, independently. Aliquots of extracts (10 mg) were diluted in dichloromethane (4 mL) and filtered in Sep-Pak cartridges (Sigma, Silica). Cartridges were washed with MeOH (4 mL), and fractions were filtered in Millex (0.45 μm) and analysed using GC–IT-MS (LM-5, 15 m × 0.2 mm; 0.2 μm). The temperature in the column was optimised, and the program used was as follows: 150–300 °C (8 °C/min) remaining 10 min at this temperature range (temperatures of the injector and detector were maintained at 250 °C and 310 °C, respectively). Injection volume was 1 μL, and the injection mode was splitless/split (0.12 s followed by split 1:20). The total analysis time was 30 min.

### 2.4. Ames mutagenicity assay

The *Salmonella* mutagenicity assay was performed by pre-incubating the test extracts for 20–30 min with *Salmonella typhimurium* strains TA100, TA98, TA97a and TA102 with and without metabolic activation (Maron and Ames, 1983). S9-mix was freshly prepared before each test from an Aroclor-1254-induced rat liver fraction was obtained (lyophilised) from Moltox–Molecular Toxicology Inc. *Salmonella typhimurium* strains were kindly provided by Dr. B. Ames of the University of California at Berkeley.

Five different doses of the different extracts from the bark of *Qualea grandiflora* and *Qualea multiflora* were evaluated in this assay. All the extracts were diluted in dimethylsulfoxide (DMSO). The EMeOH of *Qualea grandiflora* was tested at concentrations of 2.26, 5.52, 9.05, 13.57 and 18.10 mg/plate, and the $ECHCl_3$ at concentrations of 1.92, 3.85, 7.70, 11.55 and 15.40 mg/plate. The methanol extract of *Qualea multiflora* was evaluated at concentrations of 2.5, 5.0, 10.0, 15.0 and 20.0 mg/plate, and the chloroform extract of this species at concentrations of 2.07, 4.15, 8.30, 12.45 and 16.60 mg/plate. The $EH_2O$ from both species were assessed in doses of 1.25, 2.5, 5.0, 7.5 and 10.0 mg/plate.

The concentrations selected were based on the bacterial toxicity in preliminary tests. 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 his+ revertants or as an alteration in the auxotrophic background (i.e., background lawn).

The various concentrations of each extract or fraction to be tested were added to 500 μL of buffer (pH 7.4) and 100 μL of bacterial culture and then incubated at 37 °C for 20–30 min. After this

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