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Antimicrobial and cytotoxic activities of medicinal plants of the Brazilian cerrado, using Brazilian cachaça as extractor liquid

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ABSTRACT

Ethnopharmacological importance: Many species of plants in the Brazilian cerrado (savanna) are widely used in ethnomedicine. However, the safety and effectiveness of medicinal plants used in communities with little or no access to manufactured drugs should be evaluated.
Aim of the study: Evaluate the antimicrobial and cytotoxic activities of extracts from eight plant species, obtained using Brazilian cachaça as the extractor liquid.
Materials and methods: The extracts were tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Candida parapsilosis*, promastigote forms of *Leishmania amazonensis*, and poliovirus. In addition, cytotoxic activity was assayed in Vero cells and in human erythrocytes.
Results: The plant species *Curatella americana*, *Sclerolobium aureum*, and *Plathymenia reticulata* showed the best activity against yeasts, especially the crude extract of *C. americana* and its ethyl-acetate fraction. *Kielmeyera lathrophyton* showed a minimum inhibitory concentration of 250 μg/ml against *S. aureus*, and was inactive against Gram-negative bacteria. The extract obtained from *Annona coriacea* showed the best activity against the promastigote forms of *Leishmania amazonensis* (IC_{50} = 175 μg/ml). Only *C. americana* showed potential for antipoliovirus activity. The concentrations of the crude extracts that showed toxicity to VERO cells had CC_{50} between 31 and 470 μg/ml, and the lyophilized Brazilian cachaça showed a CC_{50} of 307 μg/ml. None of the extracts showed toxicity against human erythrocytes.
Conclusions: Among the plant species studied, *C. americana* proved to be effective against microorganisms, especially as an antifungal. The results will help in the search for alternative drugs to be used in pharmacotherapy, and will contribute to establish safe and effective use of phytomedicines in the treatment of infectious diseases.

1. Introduction

There are an estimated 160,000 species of plants, animals and fungi in the "cerrado biome" (savanna), which throughout their history have been undergoing complex interactions (Dias, 1992). The cerrado covers approximately 2 million km^2, 23% of the area of Brazil (Ratter et al., 1997). Cerrado plants are of great therapeutic value for a high percentage of the population, especially people living far from urban areas. Numerous scientific studies have confirmed the biological activities of extracts or compounds isolated from cerrado plants (Ríos and Recio, 2005; Braga et al., 2007; Gomig et al., 2008; Hiruma-Lima et al., 2009; Reichling et al., 2009; Domínguez-Carmona et al., 2010). New antimicrobial agents (Nakamura et al., 2004; Magassouba et al., 2007; Duarte et al., 2007; Singh et al., 2008; Ntutela et al., 2009; Gordon and Wareham, 2010) used to treat or control infectious diseases are being sought in order to decrease side effects, lower drug costs, and broaden the spectrum of activity against resistant microorganisms and opportunistic microbes affecting immunocompromised patients (Maregesi et al., 2008). A Brazilian brandy called "cachaça" obtained by sugar-cane distillation is commonly used in home remedies made from cerrado

Abbreviations: AQF, aqueous fraction; ATCC, American type culture collection; CC_{50}, 50% cytotoxic concentration; CE, crude extract; CFU, colony-forming units; CPE, cytopathic effect; EAF, ethyl-acetate fraction; EC_{50}, 50% effective concentration; FBS, fetal bovine serum; HTO, Tocantins Herbarium; MIC, minimal inhibitory concentration; OD, optical density; SI, selectivity index; SSG, saline solution containing 1% glucose and buffered to pH 7.25; $TCID_{50}$, 50% tissue culture infective dose.

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Table 1
Selected plant species from Brazilian cerrado for the evaluation of antimicrobial activity, and cytotoxicity.

Plant name	Family	Voucher	Plant parts	Ethnomedical uses
Annona coriacea Mart.	Annonaceae	HTO6880	Leaves	Chronic diarrhea (Rodrigues e Carvalho, 2001), antimalarial (Mesquita et al., 2007). Rheumatism (Cruz, 1995); anti-helmintic (Santos and Sant'Ana, 2000, 2001). Leishmaniasis (Akendengue et al., 1999)
Curatella americana L.	Dilleniaceae	HTO2234	Bark	anti-inflammatory, anti-ulcer and anti-hypertensive (Corrêa, 1984; Souza e Felfili, 2006, Guerrero et al., 2002); cold, healing wounds (Souza and Felfili, 2006); Skin diseases and ulcers (Costa et al., 2008).
Himatanthus obovatus (M. Arg.) Wood	Apocynaceae	HTO9600	Leaves	cancer, herpes, verminosis (Mesquita et al., 2005)
Kielmeyera lathrophyton Saddi	Guttiferae	HTO9601	Bark	Against schistosomiasis, leishmaniasis, malaria, fungal and bacterial infections (Alves et al., 2000)
Plathymenia reticulata Bth	Mimosaceae	HTO3327	Bark	Anti-inflammatory (Fernandes, 2002)
Pterodon emarginatus Vogel	Fabaceae	HTO9602	Bark	Anti-rheumatic, analgesic and antiinflammatory properties (Coelho et al., 2005) Rheumatism, in throat problems, as cleanser and tonic (Carvalho et al., 1999)
Qualea grandiflora Mart.	Volchysiaceae	HTO9603	Leaves	Against diarrheal with blood, the intestinal colic and against amoeba (Rodrigues and Carvalho, 2001). Treat signs and symptoms of gastric disorders (Hiruma-Lima et al., 2006); skin diseases and inflammatory processes (Costa et al., 2008)
Sclerolobium aureum (Tul.) Benth.	Caesalpinaceae	HTO9604	Bark	[a]Treat scabies and antimalarial (Munoz et al., 2000)

[a] Information associated to genus.

plants (Lorenzi and Matos, 2002). In this region, medicinal plants are an important social and cultural component, and sometimes are the only alternatives available to treat health problems for this population. The popular use of the plants selected for this study has already previously been reported in the literature (Table 1); however, evidence of the antimicrobial effects as well the side effects of many of them have not been investigated. In the present study, we evaluated the antibacterial, antifungal, antileishmanial, antiviral, and cytotoxic activities for eight cerrado plant species. The extracts were produced in a similar manner to the popular mode of preparation, which can provide information on the actual performance of the popular use, as well as insights for future studies of these species.

2. Material and methods

2.1. Plant collection

The samples were air-dried in the shade, to reduce deterioration of the plant drug material. The plants were identified by Dr. Solange F. Lolis at the Tocantins Herbarium, Federal University of Tocantins. Voucher specimens were deposited in the same herbarium (Table 1).

2.2. Preparation of crude plant extracts

After drying, the plant material was powdered (Tigre ASN5) and the crude extracts (CE, 10% w/v) were obtained by turbo-extraction (Ultraturrax® UTC-115-KT, Ika Works, Wilmington, NC, USA) for 15 min at $t < 40\,^{\circ}C$ using Brazilian cachaça as the extractor liquid. The cachaça was produced in conformity with the applicable regulation (Federal Decree No. 2314/1997). The turbo-extraction process occurs through a high-speed mixer (5000–20,000 rpm), which by shear force, disintegrates a high proportion of the plant cells, facilitating the solubilization of substances in the extractor liquid. Standardized methods were employed to determine the pH and alcohol content described in the Brazilian Pharmacopoeia, 4th edition (1988). Then, the extracts were filtered first through cotton and then through filter paper (0.0237 g/cm^2) under reduced pressure, concentrated in a rotary vaporizer under reduced pressure, and freeze-dried. The liquid–liquid partition of the CE with biological activity was accomplished by diluting the CE (3 g) in water (30 ml) and 10 portions of 30 ml ethyl acetate. The aqueous (AQF) and ethyl-acetate (EAF) fractions were concentrated and freeze-dried.

2.3. Microorganisms

Extracts were tested against the bacteria *Bacillus subtilis* ATCC 6623, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853; the fungi *Candida albicans* ATCC 10231 and *Candida parapsilosis* ATCC 22019; and the promastigote forms of *Leishmania amazonensis* MHOM/BR/75/Josefa strain.

2.4. Antibacterial susceptibility test

The minimal inhibitory concentrations (MICs) of all extracts and reference antibiotics (tetracycline, vancomycin, and penicillin, from Sigma Chemical Co., St. Louis, MO, USA) were determined by microdilution techniques in Mueller-Hinton broth (Merck) following the protocol established by the CLSI (2009) for bacteria. Inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard [10^8 colony-forming units (CFU)/ml] and diluted 1:10 for the broth microdilution procedure. Microtiter plates were incubated at 37 °C and the MICs were recorded after 24 h of incubation. The MIC was defined as the lowest concentration of compounds at which the microorganism tested did not demonstrate visible growth.

2.5. Antifungal susceptibility test

The MICs of the crude extracts were determined against the yeasts by broth microdilution techniques, as described by the Clinical and Laboratory Standards Institute (2008). MICs were determined in RPMI 1640 (Gibco, Invitrogen Co., New York, USA) with MOPS, pH 7.0. The starting inoculum was 1.0×10^6 CFU/ml. Microtiter trays were incubated at 37 °C in a dark humid chamber, and MICs were recorded after 48 h of incubation. The susceptibility endpoints were defined as the lowest concentration of antifungal agent that resulted in total inhibition of visual growth. Nystatin (Sigma) was used as the control.