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RESEARCH ARTICLE

Antimicrobial activity of the crude ethanol extract from *Hyptidendron canum* leaves

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Abstract

This study proposed to evaluate the antimicrobial activity of the crude ethanol extract of *Hyptidendron canum* (Pohl ex Benth.) Harley (Lamiaceae) leaves. The crude ethanol extract was obtained from the dried, pulverized leaves. The *H. canum* leaf powder was submitted to phytochemical screening. The antimicrobial activity was evaluated against Gram-positive and -negative bacteria and *Candida albicans* using the well diffusion test and the agar dilution method for determining the minimum inhibitory concentration (MIC). Phytochemical screening showed the presence of flavonoid and saponinic heterosides. The extract demonstrated antimicrobial activity against all microorganisms tested. The MIC for non-sporulated Gram-positive bacteria varied from 4.37 to 17.5 mg/mL. The sporulated Gram-positive bacteria tested had their growth inhibited by *H. canum* (MIC from 4.37 to 35 mg/mL). The MIC of *H. canum* for most of the Gram-negative bacteria was 70 mg/mL, except for *Pseudomonas aeruginosa* (MIC = 17.5 mg/mL), at which concentration *C. albicans* was also inhibited. This is the first report of phytochemical screening and antimicrobial activity of *H. canum*.

Keywords: *Flavonoids; lamiaceae; MIC; saponins*

Introduction

The Lamiaceae (Labiatae) family is composed of usually aromatic herbs, bushes, and occasionally trees, with generally quadrangular branches. There are approximately 300 genera and 7500 species. In the Brazilian savannah there are about 26 genera and 350 species, of which *Hyptidendron* Harley is a native genus (Souza & Lorenzi, 2005). Within this genus is *Hyptidendron canum* (Pohl ex Benth.) Harley (syn: *Hyptis cana* Pohl ex Benth.) (Paula et al., 2000), initially classified as *Hyptis cana* by George Bentham, 1833, part of the *Hyptis* genus, but later reclassified by Raymond M. Harley, 1988, to a new *Hyptidendron* genus, based on the macro-morphological and anatomical characteristics and on the number of chromosomes (Harley, 1988).

H. canum is found mostly in the central plateau of Brazil, covering the states of Goiás, Mato Grosso, and part of Minas Gerais (Fernandes & Lee, 1998). It is an 8 m tall tree with fissured suberous bark and young shoots with new leaves emerging from the base. The new leaves are light green on the upper side and a lighter, grayish-green shade on the underside; the chalice is grayish-green with shades of dark purple; and the corolla is purple, with an 8–10 mm long tube, and a 4 mm long, slightly rimmed nucleus (Epling, 1949). The seeds have rough, dark brown, opaque tegument and whitish basal hilum (Vuaden et al., 2005).

Species of the Lamiaceae family are used in Brazilian popular medicine as antiseptics (*Lavandula angustifolia* Mill., *Lavandula officinalis* Chaix, *Marrubium vulgare* L., *Mentha piperita* L., *Thymus vulgaris* L.) and as a fungicide (*Ocimum gratissimum* L.) (Fenner et al., 2006;

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Silva et al., 2005). The Mumbuca, Jalapão-Tocantins, community use it as an antibiotic (*Hyptis crenata* L.) and a cardioprotector (*Hyptis* sp), popularly known as "Pau Vitória" (Coelho et al., 2005), and it is used by the population of Itaberaba-Bahia for flu treatment (*Plectranthus amboinicus* (Lour.) Spreng, *Rosmarinus officinalis* L., and *Mentha pulegium* L.) (Alcântara Jr et al., 2005). In Mexico there are reports of the use of *Hyptis suaveolens* (L.) Poit. as an antiseptic, and in various African countries there are reports of the use of *Hyptis spicigera* Urb. for insect control (Falcão & Menezes, 2003).

H. canum is traditionally used in the form of infused or decocted leaf or root tea (Brandão, 1991), as an antimalarian, anti-inflammatory, anti-ulcerative, and antihepatotoxic plant (Ferri & Ferreira, 1992).

H. canum is a plant that has undergone very little study to date. Phytochemical screening and the antimicrobial activity of the crude extract have not been previously reported. Antimicrobial activity has been detected in other species belonging to its family (Lamiaceae): Wadt et al. (1996) noted that the crude extract of *Leonurus sibiricus* L. (cordão de frade) inhibited *C. albicans*, *S. aureus*, and *P. aeruginosa*. Souza et al. (2002) verified that the ethanol extract from *H. ovalifolia* Benth. (Lamiaceae) leaves inhibited *in vitro Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum*, and *Trichophyton mentagrophytes* at a concentration of 250 μg/mL. *Origanum vulgare* ssp. *hirtum* (Link) Letsw (*Origanum majorana* L.) (Lamiaceae) essential oil inhibited both Gram-positive and Gram-negative microorganisms in *in vitro* studies by Dorman and Deans (2000). The *Hyptis suaveolens* (Lamiaceae) essential oil showed antibacterial activity at a concentration of 5 mg/mL against *S. aureus* UCH 511, *B. cereus*, *E. coli* NCTC 7001, and *P. aeruginosa* UCH 655, and antifungal action against *C. albicans* (Asekun et al., 1999). In the essential oils of rosemary (*Rosmarinus officinalis* L.), which also belongs to the Lamiaceae family, adequate inhibitory activity against *C. albicans*, *Cryptococcus neoformans*, and *Mycobacterium intracellularae* was also found, but no activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *Saccharomyces cerevisiae*, *Aspergillus flavus*, *Aspergillus fumigatus*, and *Trichophyton mentagrophytes* (Porte & Godoy, 2001). The essential oil from *Ocimum selloi* Benth. (Lamiaceae) leaves presented slight *in vitro* antibacterial activity (9.3 mm inhibition halo) against *E. coli* ATCC 25922 and *S. aureus* ATCC 5923 strains, yet did not present inhibition halos for *P. aeruginosa* ATCC 27853 (Farago et al., 2004). *Ocimum gratissimum* (Lamiaceae) *in vitro* antifungal activity was detected against *Microsporum canis*, *Microsporum gypseum*, *Trichophyton rubrum*, *Trichophyton mentagrophytes* dermatophytes (Silva et al., 2005), and against *Cryptococcus neoformans* (Lemos et al., 2005). Other authors have noted bactericidal activity in the aqueous and methanol extracts of the leaves of three plants used as popular medicine in India against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Chatterjee et al., 2007).

No mention of phytochemical screening and antimicrobial activity of *H. canum* was found in the literature studied. The purpose of this study was to evaluate the phytochemical screening of the leaf powder and the antimicrobial activity of the crude ethanol extract from *H. canum* leaves on sporulated and non-sporulated Gram-positive bacteria and gram-negative bacteria, and its antifungal activity against *Candida albicans*.

Materials and methods

Botanical material

The botanical material, consisting of *Hyptidendron canun* (Pohl ex Benth.) Harley leaves, was collected in the municipality of Goiânia, Goiás (16°43′26.1″ S and 49°15′54.8″ W, at an altitude of 885 m), from February to April 2005, and identified by Prof. Dr. José Realino de Paula of the Federal University of Goiás. A voucher specimen was deposited at the herbarium of that institution under registration number UFG/29862.

The leaves were oven dried with air circulation at 40°C and then pulverized by blade mill.

Preparation of the crude ethanol extract

The *H. canum* leaf powder was macerated in ethanol at 95% (v/v) P.A. in a 1:1 proportion at room temperature, undergoing occasional shaking for 72 h, followed by filtration.

The extract obtained was concentrated in a rotavapor at 40°C and the vegetable residue was extracted twice again analogously, thereby obtaining the crude ethanol extract. To perform the antimicrobial tests *in vitro*, the extract was solubilized in dimethylsulfoxide (DMSO) at 1:3 (v/v).

Phytochemical screening

The *H. canum* leaf powder was submitted to phytochemical screening, using quantative analytical techniques for alkaloids, starch, coumarins, anthraquinone heterosides, digitalis heterosides, steroids, triterpenes, flavonoid heterosides, saponinic heterosides, and tannins, the research techniques following methodologies adapted from Costa (2001).

Flavonoid dosage

Total flavonoid dosage was performed in triplicate according to the methodology described in Farmacopéia Brasileira IV (2001).

Antimicrobial activity evaluation

Microorganisms

Antimicrobial activity evaluation was performed with the following bacteria: *Staphylococcus aureus* 481, *Micrococcus roseus* ATCC 1740, *Micrococcus luteus* ATCC 9341, *Bacillus cereus* ATCC 14576, *Bacillus stearothermophylus* ATCC 1262, *Bacillus subtilis (atrophaeus)* 6633, *Enterobacter cloacae* HMA/FTA 502, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* 8739, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 9027, and *Serratia marcescens* ATCC 14756, and against the *Candida albicans* NTC 2010 fungus. To determine the minimal inhibitory concentration (MIC), as well as the microorganisms mentioned above, the following bacteria were used: *S. aureus* (ATCC 6538, ATCC 25923), *S. aureus* (897, 912, 915, 934, 937), *Staphylococcus epidermides* ATCC 12228, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853. The microorganisms belong to the bacterial strain collection of the Bacteriology Laboratory, Department of Microbiology, Tropical Pathology and Public Health Institute (IPTSP), Federal University of Goiás. The antimicrobial activity of the extracts was determined according to the NCCLS (2003) recommendation, with modifications. The inoculum was prepared from cultures in ASI agar incubated at 37°C for 24 h in a 2 mL saline solution, until turvation equivalent to half the MacFarland 1.0 scale was reached.

Well diffusion test

Petri dishes for the diffusion test were prepared in two stages: a basic layer containing 20 mL of Mueller Hinton agar, which after solidification received a second layer containing 100 μL of microbial suspension in 10.0 mL of Mueller Hinton agar liquefied at 50°C. The dishes were kept on a flat surface until agar solidification. Later, 5.0 mm diameter orifices were made on the plate in a circular pattern at equidistant points, where 10 μL of crude ethanol extract diluted 1:3 (v/v) in DMSO was inoculated, while the control plate was inoculated with DMSO. This stage was performed in triplicate. The plates containing Gram-positive bacteria received a disk of (Oxoid® 10 μg) penicillin, while the Gram-negative bacteria received a disk with 15 μg erythromycin (Oxoid®).

The plates were pre-incubated at room temperature for 2 h for diffusion of the extract. They were then incubated at 37°C for 24 h, after which the inhibition halo was measured with a millimetric ruler. This qualitative screening was performed to verify antimicrobial activity in the extract analyzed.

Determination of minimal inhibitory concentration (MIC)

The ethanol extract of *H. canum* leaves was weighed (2800 mg) and diluted in 3 mL of DMSO in a test tube (H1); 1.5 mL of DMSO was added to second and third test tubes (H2 and H3); and 1.5 mL of sterile distilled water was added to a sequence of five more test tubes (H4–H8).

A 1.5 mL aliquot was removed from test tube 1 and added to test tube 2, and so on successively, making a dilution series up to H8. Subsequently, 18.5 mL of Mueller Hinton agar liquefied at 50°C was added to each of these tubes, then the contents were homogenized, and poured rapidly onto sterilized Petri dishes. After dilution the concentrations of crude ethanol extract varied from 0.55 to 70 mg/mL. Control plates containing DMSO were also prepared. A sterility test was also performed, for which all plates were incubated in a drying oven at room temperature for 24 h. The microbial inocula were later transferred to a Steers' inoculator (Steers et al., 1959) and placed on Mueller Hinton agar plates containing the different concentrations of the crude ethanol extract. The plates were incubated at 37°C for 24 h. The lowest concentration able to inhibit microbial development was considered to be the MIC.

Results

Phytochemical screening

Phytochemical screening performed on *H. canum* leaf powder evidenced the presence of flavonoid and saponinic heterosides. In this sample, alkaloids, starch, coumarins, anthraquinone heterosides, digitalis heterosides, steroids, triterpenes, and tannins were not detected.

The total flavonoid percentage in the sample of *H. canum* leaves was 0.95%.

Antimicrobial activity evaluation of the crude ethanol extract

The crude ethanol extract from *H. canum* leaves inhibited the development of *S. aureus* 481, *M. roseus* ATCC 1740, *M. luteus* ATCC 9341, all sporulated Gram-positive bacteria, and all Gram-negative bacteria tested. There were also traces of inhibition of *C. albicans* NTC 2010 in the well diffusion test (Table 1).

The minimal inhibitory concentration of the extract for most of the non-sporulated Gram-positive bacteria was 4.37 mg/mL, except for *S. aureus* 481, *S. aureus* 897, and *S. epidermides* ATCC 12228, which presented an MIC of 17.5 mg/ mL (Table 1). All the sporulated Gram-positive bacteria tested were inhibited by *H. canum* with MIC varying from 4.37 to 35 mg/mL (Table 1).

The *H. canum* MIC for most of the Gram-negative bacteria was 70 mg/mL, except *P. aerugionosa* ATCC 9027, where MIC was 17.5 mg/mL. Inhibition of *C. albicans*

Table 1. Crude ethanol extract of *H. canum* leaves inhibition halo average (mm) using diffusion test in agar and minimal inhibitory concentration (MIC) in mg/mL.

	Crude ethanol extract of *Hyptidendron canum* leaves		
Microorganism	Inhibition zone (mm)	MIC (mg/mL)	Control Inhibition zone
Gram-positive bacteria			**Penicillin**
Staphylococcus aureus ATCC 6538	—	4.37	—
Staphylococcus aureus ATCC 25923	—	4.37	—
Staphylococcus aureus 481	19	17.5	44
Staphylococcus aureus 897	—	17.5	—
Staphylococcus aureus 912	—	4.37	—
Staphylococcus aureus 915	—	4.37	—
Staphylococcus aureu 934	—	4.37	—
Staphylococcus aureus 937	—	4.37	—
Staphylococcus epidermides ATCC 12228	—	17.5	—
Micrococcus roseus ATCC 1740	18	4.37	20
Micrococcus luteus ATCC 9341	10	4.37	77
Sporulated Gram-positive bacteria			
Bacillus cereus ATCC 14576	11	4.37	12
Bacillus stearothermophylus ATCC 1262	11	35.0	53
Bacillus subtilis (atrophaeus) 6633	13	4.37	37
Gram-negative bacteria			**Erythromycin**
Enterobacter cloacae HMA/FTA 502	8	70.0	N
Enterobacter aerogenes ATCC 13048	12	70.0	N
Escherichia coli 8739	15	70.0	11
Escherichia coli ATCC 11229	11	70.0	18
Escherichia coli ATCC 25922	—	70.0	—
Pseudomonas aeruginosa ATCC 9027	10	17.5	10
Pseudomonas aeruginosa ATCC 27853	—	70.0	—
Serratia marcescens ATCC 14756	10	70.0	15
Fungus			
Candida albicans NTC 2010	T	17.5	—

N, no formation of inhibition halo; T, traces of inhibition; —, not tested.

NTC 2010 was noted, with minimal inhibitory concentration of 17.5 mg/ mL (Table 1).

Discussion

Phytochemical screening of the *H. canum* leaf powder identified flavonoid and saponinic heterosides. This is the first report on phytochemical screening of this species. According to Simões et al. (2004), saponinic heterosides present mucolitic, expectorant, diuretic, antiseptic, laxative, antimicrobial, and anti-inflammatory activity and increased membrane permeability. Some flavonoids present antitumoral action as well as being able to act as antiviral, antihemorrhagic, hormonal, anti-inflammatory, antimicrobial, and antioxidizing agents. Isobe et al. (2006) verified potent antimicrobial activity in the two isolated flavonoids of *Hyptis fasciculata* Benth. (Lamiaceae) cirsilineol and cirsimaritin against *Helicobacter pylori.*

Under test conditions, the crude ethanol extract of *H. canum* exhibited good antibacterial activity against Gram-positive and sporulated Gram-positive bacteria. Yet activity against *Candida albicans* NTC 2010 and against Gram-negative bacteria was also noted. No mention of antimicrobial activity of *H. canum* was found in the literature studied.

Results suggest that the crude ethanol extract from *H. canum* leaves presents antimicrobial action which can be compared to a few other species of its family and may be due to the presence of flavonoids and/or saponins in its chemical constitution. Yet *in vivo* research on this plant is necessary to determine its antimicrobial action mechanism, active principle toxicity, side effects, and pharmacokinetic properties. This study presents the first description of phytochemical screening of the leaf powder and the antimicrobial activity of the crude ethanol extract of *H. canum* leaves collected in Goiânia, Brazil, and suggests the possibility of finding new vegetable drugs for the treatment of fungal and bacterial infections.

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