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Antibacterial Activity of the Crude Ethanol Extract from *Jacaranda decurrens* Leaves

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SUMMARY. This study evaluated the antimicrobial activity of the leaves crude ethanol extract of *Jacaranda decurrens* Cham. (Bignoniaceae). The crude ethanol extract was obtained from the material collected in Senador Canedo and Mossâmedes, Goiás, Brazil, pulverized and submitted to phytochemical screening. The antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria using the well diffusion test and the agar dilution method for determining the minimum inhibitory concentration (MIC). The phytochemical screening showed the presence of flavonoid heterosides and coumarins. The crude ethanol extract demonstrated antimicrobial activity against all microorganisms tested. The MIC of *J. decurrens* for the Gram-positive bacteria varied from 2.18 mg/mL to 8.75 mg/mL. The MIC for the Gram-negative bacteria was 17.5 mg/mL except for *Pseudomonas aeruginosa* (MIC = 8.75 mg/mL) and *Serratia marcescens* (MIC = 35 mg/mL). This was the first report of antimicrobial activity of *J. decurrens*.

INTRODUCTION

Resistance to antibiotics has been described as a major threat to public health [1]. Bacterial infections remain major causes of morbidity and mortality in hospitals around the world [2]. However, in recent years, research of new substances with possible antimicrobial activity has intensified. A large number of plants have been investigated for the purpose of detecting chemical compounds with activity on bacteria and fungi [3,4].

Some studies with medicinal plants has showed that species in Bignoniaceae family has antimicrobial activity. Suffredini *et al.* [5] evaluated 16 extracts of Bignoniaceae species present in the Amazon and Atlantic forests through the microdilution test and observed that 10 species inhibited the growth of *Staphylococcus aureus* and 1 species inhibited the growth of *Enterococcus faecalis*. Haque *et al.* [6] observed that the chloroform and n-hexane extracts of the *Stereospermum chelonoides* DC. (Bignoniaceae) stem bark had antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungus. *Candida albicans* and *Staphylococcus aureus* were the most sensitives to ethanol extract of the *Kingelia africana* (Lam.) Benth. – Bignoniaceae [7] stem bark.

Jacaranda decurrens Cham. (Carobinha) is one of the species belonging to Bignoniaceae family and fairly common in Brazilian Savannah. It is a sessile subshrub with composed and recomposed leaves, to 50 cm tall, flowers deep lilac and green fruits [8]. The leaves and roots are used as teas or "garrafadas" (popular formulation) for treatment of gynecological infections and depurative of blood, being marketed at free fairs, municipal markets and bunker installed on public places [9].

Some studies have been done with this species. Varanda *et al.* [10] isolated ursolic acid and evaluated the toxic activity on the insect herbivore *Shizaphis graminum*. In low concentrations ursolic acid caused deleterious effects

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on insects. Decrease in the reproductive rate of *Shizaphis graminum* was also observed. Fenner *et al.* [11] in an ethnobotanic bibliographic study about medicinal plants used by Brazilian population in the treatment of signs and symptoms related to fungus infection found that this species is also used for treatment of skin disease. In this same study they found that 16 other species of Bignoniaceae family are also used by popular medicine for the treatment of infectious diseases.

Plants that have established therapeutic use by the people have been special object of study because determine whether the traditional uses are consistent with the laboratory tests. No mention of antimicrobial activity of *J. decurrens* leaves was founding 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 *J. decurrens* leaves on sporulated and non-sporulated Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Botanical material

The botanical material constituted of *Jacaranda decurrens* Cham. leaves was collected in the municipality of Senador Canedo, Goiás, Brazil (16° 45´ 24.6´´ S and 49º 15´ 54.8´´ W, at an altitude of 747 m), in May 2006 and Mossâmedes (Serra Dourada), Goiás, Brazil (16° 07´ 36´´ S and 50º 12´ 54" W, at an altitude of 615 m), in June 1999. The botanical material was identified by Prof. Dr. José Realino de Paula of the Federal University of Goiás. A vocher specimen was deposited at the herbarium of that institution under registration number UFG – 27805. The leaves were oven dried with air circulation at 40 °C and then pulverized by blade mill.

Preparation of the crude ethanol extract

The *Jacaranda decurrens* leaf powder was macerated in ethanol 95% (V/V) P.A. in a 1:3 proportion at room temperature, undergoing mechanical shaking for 4 h, followed by filtration [12]. 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 test *in vitro,* the extract was solubilised in DMSO at 1:3 (p/V).

Phytochemical screening

The *Jacaranda decurrens* leaf powder was submitted to phytochemical screening according to alkaloids, starch, coumarins, triterpenes, anthraquinone heterosides, and digitalic heterosides, steroids, flavonoid heterosides, saponinic heteroside and tannins research techniques, following methodologies adapted from Costa [13].

Total flavonoid dosage

Total flavonoid dosage was performed in triplicate according to the methodology described in Farmacopéia Brasileira IV [14].

Antimicrobial activity evaluation

Microorganisms

The antimicrobial activity evaluation was performed with the following bacteria: *Staphylococcus aureus* 4081, *Micrococcus luteus* ATCC 9341, *Micrococcus roseus* 1740, *Bacillus cereus* 14576, *Bacillus stearothermophylus* 1262, *Bacillus subtilis* ATCC 6633, *Enterobacter cloaceae* HMA/FTA 502, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 8739 and 11229, *Pseudomonas aeruginosa* ATCC 9027 and *Serratia marcescens* ATCC 14756. To determine the minimal inhibitory concentration (MIC) the microorganisms mentioned above were used. 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 screening was performed according to NCCLS [15] with modifications. The inoculum was prepared from cultures in tilted incubated agar 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

The Petri dishes for the diffusion test were prepared in two stages. A basic layer containing 20 mL of Müller Hinton agar which after solidification received a second layer containing 100 µL of microbial suspension 10.0 mL of Müller 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 extract diluted 1:3 (p/V) in DMSO were inoculated, while the control plate was inoculated with DMSO. This stage was performed in triplicate. The plates containing Gram-positive bacteria received a disc of (Oxoid® 10 µg) penicillin, while the Gram-negative bacteria received a disc of (Oxoid® 15 µg) eritromicine.

The plates were pre-incubated at room tem-

perature 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 milimetric ruler. This qualitative screening was performed to verify antimicrobial activity in the extract analyzed.

Determination of minimal inhibitory concentration (MIC)

The ethanol extract of *J. decurrens* leaves was weighed (1.400 mg) and diluted in 2 mL of DMSO in a test tube (C1). 1.0 mL of DMSO was added to the 2nd and 3rd tubes (C2 and C3). In the sequence of tubes C4 to C8 was added sterile distilled water. A 1.0 mL aliquot was removed from test tube 1 and added to test tube 2 and so on successively, making a dilution series up to C8. Subsequently 19 mL of Müeller Hinton agar liquefied at 50 °C was added to each of the test tubes, homogenized and poured rapidly onto sterilized Petri dishes.

After dilution the concentration of crude ethanol extract varied from 0.27 mg/mL to 35.0 mg/mL. Control plates containing DMSO were also prepared. The sterility test was also performed incubating all the plates in a drying oven at room temperature for 24 h. The microbial inocules were later transferred to a Steers' inoculator [16] and placed on the Müeller 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 MIC.

RESULTS AND DISCUSSION

In this study the choice of microorganisms has been done taking into account their morphological characteristics. The crude ethanol extract from *Jacaranda decurrens* leaves inhibited the development of Gram-positive bacteria with a MIC varying from 2.18 mg/mL to 8.75 mg/mL. The sporulated Gram-positive bacteria *Bacillus cereus* 14576, *Bacillus stearothermophylus* 1262, *Bacillus subtilis* ATCC 6633 were inhibited by the extract respectively with following MIC: 2.18 mg/mL, 8.75 mg/mL and 4.37 mg/mL (Table 1).

The minimal inhibitory concentration of the extract for most of the Gram-negative bacteria was 17.5 mg/mL, except for *P. aeruginosa*

Microorganisms	Inhibition halo (mm)					
	J. decurrens **Senador Canedo**	***J. decurrens*** **Serra Dourada**	**DMSO**	**Erythromycin**	**Penicilin**	**MIC (mg/mL) (*J. decurrens* Serra Dourada)**
Non esporulated Gram-positive bacteria						
Staphylococcus aureus 481	19.6	20	NI	ND	44	4.37
Micrococcus roseus 1740	18.6	17	NI	ND	20.3	4.37
Micrococcus luteus 9341	11.0	9.5	NI	ND	78.6	2.18
Esporulated Gram-positive bacteria						
Bacillus cereus 14576	11.6	10.3	NI	ND	12	2.18
Bacillus stearothermophylus 1262	9.5	9.5	NI	ND	53.5	8.75
Bacillus subtilis 6633	19	17.6	NI	ND	37.3	4.37
Gram-negative bacteria						
Enterobacter cloaceae HMA/FTA502	12.25	11.7	NI	8.0	ND	17.5
Enterobacter aerogenes 13048	12.6	14.3	NI	0	ND	17.5
Escherichia coli 8739	15.3	15	NI	11	ND	17.5
Escherichia coli 11229	13	12	NI	18.3	ND	17.5
Pseudomonas aeruginosa 9027	14.3	12.6	NI	10.3	ND	8.75
Serratia marcescens 14756	10.6	10.6	NI	14.6	ND	35

Table 1. Inhibition halo averages (mm) using the agar diffusion test and minimal inhibitory concentration (MIC) in mg/mL of crude ethanol extract of *J. decurrens* leaves and inhibition halo average (mm) of control (penicillin and erythromycin disks). ND =- not done; NI = not inhibited.

ATCC 9027, which presented a MIC of 8.75 mg/mL and for *Serratia marcescens* ATCC 14756 which presented a MIC of 35 mg/mL.

Under test conditions the crude ethanol extract of *J. decurrens* exhibited good antibacterial activity against Gram-positive bacteria (MIC = 2.18 mg/mL). 66.66% of Gram-negative bacteria were inhibited with a concentration of 17.5 mg/mL. The microorganisms more sensitive were *Micrococcus luteus* ATCC 9341 and *Bacillus cereus* 14576 (MIC = 2.18 mg/mL).

The inhibition growth of the bacteria *Pseudomonas aeruginosa* has been relevant in this study. Despite improvements in antibiotic therapy *Pseudomonas aeruginosa* is intrinsically resistant a great number antimicrobial agents, frequently a multi classes of antimicrobial agents [25]. Furthermore it is a leading cause of nosocomial pneumonia in Brazilian hospitals [26]. In the United States, *Pseudomonas aeruginosa* ranked first among all nosocomial pathogens related to pneumonia in intensive care units reported to the national Nosocomial Infection Surveillance System [27].

Phytochemical screening performed on *J. decurrens* leaf powder evidenced the presence of anthocyanidines and anthocyanines, phenolic compounds, flavonoid heterosides, triterpene heterosides, steroids, saponins, starch, cumarines and resins. The total flavonoid percentage in the sample of *J. decurrens* leaves from Serra Dourada was 0.535%. The results obtained from phytochemical screening are similar to those obtained with other species of the Bignoniaceae family: Pauletti *et al.* [17] found triterpenes in *Arrabia samydoides* (Cham.) Sandwith. Flavonoids were found in: *Catalpa bignoides* Wlat. [18], *Godmania aeculifolia* (H. B. K.) Standl. [19], *Tecomella undulata* (SM.) Seen. [20], *Arrabidaea chica* f. *cuprea* (Cham.) Sandwith. [21].

Fukai *et al.* [22] showed antimicrobial activity of 19 types of flavonoids on methicilin-resistant *Staphylococcus aureus*. Tereschuk *et al.* [23] observed that *Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus epidermidis* had inhibited the development by flavonoids isolated from the leaves of *Tagetes minuta* L. Ng *et al.* [24], confirmed the inhibition growth of 4 strains of *Staphylococcus aureus* by coumarin cniforin in that concentration of 25 mg/mL isolated from fruits of *Cnidium monnieri* (L.) Cusson. According to studies conducted by Basile *et al.* [3], the coumarins and flavonoids are two classes of compounds with antimicrobial activity recognized.

CONCLUSIONS

Results suggest that the crude ethanol extract from *J. decurrens* leaves presents antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria, and may be due to the presence of coumarins and/or flavonoids in its chemical constitution. This study presents the first description of the antimicrobial activity of the crude ethanol extract of *J. decurrens* leaves.

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