



Antimicrobial Activity of the Extract of Stem Bark of *Diploptropis Ferruginea* Benth

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ABSTRACT

This is the first report about the antibacterial activity of *Diploptropis ferruginea* Benth. In this study, the ethanol extract of *D. ferruginea* was tested for its antimicrobial activity against strains gram-positive and gram-negative. In order to determine the minimal inhibitory concentration, assays were carried out by micro dilution method. The extract was screened for antimicrobial activity, and it showed antibacterial activity against *Escherichia coli* and *Pseudomonas aeruginosa*.

Key words: Antimicrobial activity, *Diploptropis ferruginea*, *Escherichia coli*, Fabaceae, *Pseudomonas aeruginosa*

INTRODUCTION

Medicinal plant is any vegetal that containing substances, that can be used therapeutically. These plants are widely used by the alternative medicine.^[1] Medicinal plant is any vegetal that containing substances, that can be used therapeutically. These plants are widely used by the alternative medicine. The factor that favors the area of natural products in Brazil

is the ethnobotanic and ethnopharmacological knowledge of the Brazilian population.^[2,3]

The Fabaceae have a cosmopolitan distribution, consisting of 700 genera and more than 17000 species. The genus, *Diploptropis* consists of approximately 22 species, including, *Diploptropis ferruginea* Benth.^[4] *Diploptropis ferruginea* Benth. (Fabaceae) is a tree popularly known in Northeastern Brazil as “sucupira-preta” and “Sicupira.” It is used in folk medicine for the treatment of rheumatism, arthritis, gastric ulcer and diabetes.^[5,6]

Previous pharmacological study, diploptropin a flavonoid isolated from the bark of the cause of *Diploptropis ferruginea* inhibited the phasic contractions induced by both acetylcholine and histamine in guinea-pig ileum in a concentration-dependent manner.^[7] The purpose

Access this article online	
Quick Response Code:	Website: www.jyoungpharm.in
	DOI: 10.4103/0975-1483.90237

of this study was to evaluate the *in vitro* antibacterial activity of extracts of *Diplotropis ferruginea* stem bark using microdilution method the susceptibilities of bacteria strains.

MATERIALS AND METHODS

Plant material

The stem bark of *Diplotropis ferruginea* was collected in the municipality of Caraúbas, State of Rio Grande do Norte, Northeastern Brazil in May 2002 to September 2005. Botanic material was identified by Prof. Maria de Fátima Agra, of the Laboratório de Tecnologia Farmacêutica. A voucher specimen (AGRA and D. ALMEIDA 5559) is deposited at the Herbario Professor Lauro Pires Xavier (JPB) of Federal University of Paraíba.

Extraction and isolation

The dried powdered stem bark of *D. ferruginea* (3 kg) was exhaustively extracted with 95% EtOH at room temperature. The extract was concentrated under vacuum yielding 95 g of the crude product. This was suspended in a MeOH:H₂O (3:7, v/v) mixture and partitioned with hexane, CHCl₃ and EtOAc. The hexane fraction was then subjected to silica gel column chromatography and eluted with hexane, CHCl₃ and MeOH in an increasing polarity gradient to give 152 fractions. The fractions were monitored by TLC and classified into 25 groups. Fraction 97-102 was purified by preparative TLC over silica gel using CHCl₃:MeOH (9:1) to afford flavonoid 1 (61 mg). The dry extract was prepared using nebulized spray dry technique.^[6]

Antibacterial activity

The antibacterial action of *Diplotropis ferruginea* was evaluated using the bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (S1) and the following fungi: *Candida albicans* (LM24), *Candida albicans* (LM514), *Candida albicans* (LM905), *Candida tropicalis* (LM

057), *Candida krusei* (LM 905), *Geotrichum* sp (LM 515), *Tricophyton rubrum* (LM 50), *Tricophyton rubrum* (LM 02), *Tricophyton mentagrophytes* (LM 740), *Penicillium* (FCF 281), *Fusarium* (LM 003), *Aspergillus flavus* (LM 136) and *Fusarium* SP (LM 003). The bacteria were grown in Agar Mueller Hinton (DIFCO Lab. Co). The antimicrobial agent used for control was dimetilsulfóxido (DMSO).

The antibacterial action of extract and flavonoids isolated from *Diplotropis ferruginea* was performed by Broth Microdilution method as described in National Committee for Clinical Laboratory Standards with 100 ml aliquots of diluted EMeOH and standards antimicrobial agents as controls (Peniciline G Benzantine). Bacterial suspensions were standardized with 0.5 ml Mc Farland standard.^[8,9] The minimum bactericidal concentrations were determined by the emergence of a blue color (resazurin) at the wells indicating absence of growth.^[10]

RESULTS AND DISCUSSION

The research of the medicinal plants with antimicrobial activity is increasing, with the objective of finding a drug or phytoconstituent with antimicrobial properties and less collateral effects.^[11] The antibacterial activity of extract and flavonoid isolated from *D. ferruginea* presented excellent results against the pathogenic microorganisms tested [Table 1].

The extracts presented antibacterial activity against clinically relevant pathogens (gram positive and gram negative). *D. ferruginea* stem bark extract was active against *Pseudomonas aeruginosa* (MIC 1000 and 500 µg/ml for both strains), *Escherichia coli* (MIC 128 µg/ml for both strains).

The microorganisms have acquired multi resistance to antibiotics and the clinical treatment is been conducted by this fact. As the time passes the therapeutic properties of determined plants are been more investigated in order to fight infections.^[12] Then, considering the antimicrobial property different plants were studied and used to treat diverse types of infections.^[13] Such data embase the importance of investments in natural products research

Table 1: Antibacterial activity of extract of *Diplotropis ferruginea* Benth

Yeast	Extract of <i>Diplotropis ferruginea</i> (µg/ml)					DPTN	Peniciline G benzantine 50 µg/ml	Control negative
	EHA 15	EHex 15	ECHCl ₃ 15	ED 1000	ED 500			
<i>Pseudomonas auriginosas</i> B6	+	+	+	12	10	+	12	+
<i>Escherichia coli</i> S-1	+	+	+	14	10	+	10	+
<i>Staphylococcus epidermides</i> ATCC 12228	+	+	+	+	+	+	14	+
<i>Staphylococcus aureus</i> ATCC 6538	+	+	+	+	+	+	17	+

+ Growth of bacteria; EHA hidroalcoolic extract; EHex; hexane extract; ECHCl₃; chloroformic extract; ED extract Dry; DPTN 370 Flavonoid

with the objective to discover new drugs and to defeat the microorganisms defenses mechanisms.^[14,15]

This study led to the conclusion that the antimicrobial activity of the nebulized extract dry of *D. ferruginea* was satisfactory against the bacteria *Pseudomonas aeruginosa* and *Escherichia coli*. These results are important as a preparation for further research with this same material. However, more specific studies must be carried out to elucidate the mechanisms involved in these activities.

ACKNOWLEDGMENTS

The authors are thankful to the CNPq and CAPES for financial support.

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How to cite this article: Cerqueira GS, Rocha N, Almeida J, de Freitas A, Lima EO, Filho J, et al. Antimicrobial activity of the extract of stem bark of *Diplotropis ferruginea* benth. *J Young Pharmacists* 2011;3:284-6.

Source of Support: CNPq and CAPES, **Conflict of Interest:** None declared.

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