

Antimicrobial activity of the essential oil of *Bowdichia virgilioides* Kunt.

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RESUMO: “Atividade antimicrobiana do óleo essencial de *Bowdichia virgilioides* Kunt.”. O óleo essencial das folhas de *Bowdichia virgilioides* Kunt. (Fabaceae) foi testado para a verificação da sua atividade antimicrobiana contra dezoito microorganismos patogênicos, usando o método de difusão em meio sólido. Foi observada atividade contra *Candida albicans*, *Candida guilliermondii*, *Candida stellatoidea*, *Micrococcus luteus* e *Trichophyton rubrum*.

Unitermos: *Bowdichia virgilioides*, Fabaceae, atividade antimicrobiana, óleo essencial.

ABSTRACT: The essential oil of *Bowdichia virgilioides* Kunt. (Fabaceae) leaves was tested for their antimicrobial activity against eighteen pathogenic microorganisms using standard gel diffusion method. It showed activity against *Candida albicans*, *Candida guilliermondii*, *Candida stellatoidea*, *Micrococcus luteus* and *Trichophyton rubrum*.

Keywords: *Bowdichia virgilioides*, Fabaceae, antimicrobial activity, essential oil.

INTRODUCTION

Bowdichia virgilioides Kunt. (Fabaceae) is a medium size tree found in the tropical forests of South America. In Northeastern Brazil it is popularly known as “sucupira”, and its bark is used for healing of wounds, as anti-ulcer and anti-diabetic (Bacchi, 1986; Oliveira; Saito, 1987-1989; Macedo; Ferreira, 2004) while the seeds are used in the treatment of rheumatism, arthritis, and skin diseases (Cruz, 1965). The importance of this plant promoted its inclusion in the first Brazilian Pharmacopoeia (Brandão et al., 2006). Various bioactivities, including antimalarial (Deharo et al., 2001), hypoglycemic (Barbosa-Filho et al., 2005) and inhibitor of the enzyme acetylcholinesterase (Barbosa-Filho et al., 2006), of crude extracts from this plant were reported. Previous chemical investigation resulted in the isolation of flavonoids (Velozeo et al., 1999a; Velozeo et al., 1999b; Arriaga et al., 2000; Juck et al., 2006), benzofuranoids (Melo et al., 2001), essential oil (Arriaga et al., 1998), triterpenoids (Torrenergria et al., 1985; Marinho et al., 1994; Melo et al., 2001) and alkaloids (Torrenergria et al., 1985; Torrenergria et al., 1989; Marinho et al., 1994; Barbosa-Filho et al., 2004). This work describes the antimicrobial activity of the essential oil of *Bowdichia virgilioides*.

MATERIAL AND METHODS

Botanical material

The plant was collected in December 2004, near the city of Santa Rita, State of Paraíba, Brazil, a coastal area around the Atlantic Forest. The voucher samples (*Agra et Góis 6243*) were deposited in the Herbarium Prof. Lauro Pires Xavier (JPB) and in the reference collection of the Laboratório de Tecnologia Farmacêutica from Universidade Federal da Paraíba, Brazil.

Extraction of the essential oil

Fresh leaves of *Bowdichia virgilioides* (1000 g) were cut into pieces, and subjected to steam distillation in a Clevenger-type apparatus (Matos et al., 1999). The essential oil obtained (0.15% w/w) had yellow color and characteristic odor and was dried over anhydrous sodium sulfate and filtered. The oil was kept in amber bottle flask and maintained in temperature lower than 4 °C.

Microorganisms

For the bioassays 4 bacteria and 14 fungi were used: *Staphylococcus aureus* (ATCC 25923),

Table 1. Halos diameter average (mm) of the evaluation of the MIC of the essential oil of *Bowdichia virgilioides* against bacteria and fungi, in solid medium.

Microorganisms	Essential oil (%)						Control		
	100	32	16	8	4	2	Microorganisms	Ketoconazole 50µg/mL	Chloramphenicol 30µg/mL
<i>Staphylococcus aureus</i> ATCC 25923	0	0	0	0	0	0	+	-	22
<i>Staphylococcus epidermidis</i> ATCC 12228	0	0	0	0	0	0	+	-	16
<i>Micrococcus luteus</i> ATCC 9341	10	8	0	0	0	0	+	-	20
<i>Pseudomonas aeruginosa</i> ATCC 27853	0	0	0	0	0	0	+	-	10
<i>Candida albicans</i> ATCC 90028	10	8	0	0	0	0	+	10	-
<i>Candida guilliermondii</i> LM 28	12	10	0	0	0	0	+	12	-
<i>Candida krusei</i> LM 07	0	0	0	0	0	0	+	0	-
<i>Candida stellatoidea</i> LM 96	10	8	0	0	0	0	+	14	-
<i>Candida parapsilosis</i> LM 1E	0	0	0	0	0	0	+	10	-
<i>Candida tropicalis</i> LM 25	0	0	0	0	0	0	+	12	-
<i>Trichosporon inkin</i> LM 267	0	0	0	0	0	0	+	0	-
<i>Cryptococcus neoformans</i> M 570	0	0	0	0	0	0	+	8	-
<i>Tricophyton rubrum</i> LM 105	10	7	0	0	0	0	+	10	-
<i>Tricophyton mentagrophytes</i> LM 103	0	0	0	0	0	0	+	12	-
<i>Penicillium</i> FCF 281	0	0	0	0	0	0	+	0	-
<i>Fusarium</i> LM-10a	0	0	0	0	0	0	+	0	-
<i>Aspergillus flavus</i> LM-136	0	0	0	0	0	0	+	10	-
<i>Rhizopus</i> LM 03	0	0	0	0	0	0	+	10	-

Staphylococcus epidermidis (ATCC 12228), *Micrococcus luteus* (ATCC 9341), *Pseudomonas aeruginosa* (ATCC 27853), *Candida albicans* (ATCC 90028), *Candida guilliermondii* (LM 28), *Candida krusei* (LM 07), *Candida stellatoidea* (LM 96), *Candida parapsilosis* (LM 1E), *Candida tropicalis* (LM 25), *Trichosporon inkin* (LM 267), *Cryptococcus neoformans* (LM 570), *Tricophyton rubrum* (LM 105), *Tricophyton mentagrophytes* (LM 103), *Penicillium* (FCF 281), *Fusarium* (LM 10a), *Aspergillus flavus* (LM 136) and *Rhizopus* (LM 03).

Culture media

The assays for the antimicrobial activities were carried out in Muller-Hinton Agar (Merck) and Sabouraud Dextrose Agar (DIFCO Laboratories) for bacteria and fungi, respectively.

Essays of antimicrobial activities

The essential oil was tested "in natura" (100%) and in dilutions from 32 until 2%, according to Allegrini et al. (1973). For this purpose, 1.2 mL of the oil, 0.04 mL of Tween 80 (Sigma Chemical) and sterile distilled water enough to complete 5 mL were placed in sterile glass tubes, 70 x 10 mm (32% dilution). The resultant emulsion

was homogenized in agitator Vortex (FANEM) for five minutes. The seriate dilutions were made in proportion of two. Beginning at the first tube, 2.5 mL were transferred to a second one, which contained 2.5 mL of sterile distilled water following by agitation and homogenization. This process was successively repeated until the sixth dilution, corresponding to 2%. The tests performed to evaluate the antibacterial and antifungal activities of the volatile oil were carried out by the method of diffusion in solid media (Cleeland et al., 1991; Bawer et al., 1996; Hadacek; Greger, 2000). 1 mL of previously prepared suspension of each microorganism was deposited in dischargeable and sterile Petri dishes (15 x 90 mm), to which were previously added 20 mL of ASD with slow homogenization. Cavities were made with sterile glass cannulas with 6 mm in diameter in the solid culture media and were inoculated with 50 µL of each dilution of the tested oil. The controls were made for each microorganism with the standard antimicrobial chloramphenicol at 30 µg/mL for bacteria, and ketoconazole at 50 µg/mL for fungi. The assay system was incubated at 37 °C, during 24 – 48 hours for bacteria and leveduriforms fungi; and at room temperature in a period of 10 – 14 days for the filamentous fungi. Each assay was carried out in duplicate and the results were expressed by arithmetic media of the halos of inhibition obtained. The biological activity of

the oil was considered positive when the media of the inhibition of the halos were equal or superior to 10 mm in diameter.

RESULTS AND DISCUSSION

In the previous works, the oil of *Bowdichia virgilioides* fruits was reported to contain farnesol, geraniol and caryophyllene (Jorge-Neto, 1970). The volatile constituents from roots of the plant were reported (Arriaga et al., 1998). Antimicrobial activity from the essential oil of seeds was evaluated and it showed activity against Gram-positive *Bacillus subtilis*, *Bacillus vulgaris*, *Enterococcus faecalis* and *Staphylococcus aureus* and had low activity in vitro against Gram-negative *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Escherichia coli* (Feitosa et al., 2004). To our knowledge, there are no published reports on the chemical composition and antimicrobial activity of the essential oil from *Bowdichia virgilioides* leaves.

As part of this systematic research, the essential oils constituents of leaves were extracted by hydrodistillation in a Clevenger-type apparatus. The obtained crude essential oil was then investigated. Therefore, we focused our study on the antimicrobial property of the essential oil.

Composition and biological activity of the essential oils can vary with the climate, geographical area, seasons, soil conditions, crop period and extraction technique (Carvalho-Filho et al., 2006). The antimicrobial activity was tested in vitro by using standard gel diffusion method with the microorganisms as seen in Table 1.

CONCLUSION

In summary, the study of volatile constituents showed that the oil inhibited the growth of only one strain of the bacteria, when tested with the oil at the concentration of 100%. Among all the fungi strains tested, only four were sensitive to the essential oil of *B. virgilioides*. The oil was active against *Micrococcus luteus*, *Candida albicans*, *Candida guilliermondii*, *Candida stellatoidea* and *Trichophyton rubrum*, being *C. guilliermondii* the most sensitive microorganism. These results led to the conclusion that the essential oil tested has a weak activity against the tested microorganisms. Further investigations looking at determination of new bioactive constituents should be carried out.

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