Antimicrobial activity of *Davilla elliptica* St. Hill (Dilleniaceae)

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ABSTRACT: *Davilla elliptica* St. Hill ("lixinha"), family Dilleniaceae, is commonly used in the Brazilian folk medicine as purgative and stimulant. This work evaluated the antimicrobial activity of the methanol and chloroform extracts of the leaves and barks of *D. elliptica* using the disc-diffusion method. The results obtained showed that the methanolic extracts of the leaves and barks presented antimicrobial activity against the tested microorganisms.

Keywords: *Davilla elliptica*, Dilleniaceae, antimicrobial activity.

INTRODUCTION

*Davilla elliptica* St. Hill (Dilleniaceae) is a native species from Brazil, which is popularly known as ‘lixinha’ (http://www.propp.ufu.br/revistaeletronica/b/ocorrencia.pdf, 2003). There are properties attributed to *D. elliptica* with indications such as purgative and stimulant (Rodrigues; Carvalho, 2001). Phytochemical investigations of different parts of *Davilla* species have revealed the presence of α-tocoferol, and the flavonoids myricetin, quercetin, myricetin-3-O-D-L-rhamnoside, quercetin-3-O-D-L-rhamnoside, kaempferol (Gurni; Kubitzki, 1981), as well as saponins and mucilage (Matheucci, 1996).

Despite the popular use of *D. elliptica* as a medicinal plant, there are no data about the antimicrobial effect of leaf extracts. Thus, the interest in this plant is justifiable because of its potential medicinal value. The present study has the aim of evaluating the antimicrobial activity of *D. elliptica* extracts obtained from the leaves and barks using the disc-diffusion method. It was also made a phytochemical screening of the chloroform and methanol extracts of the leaves and barks of *D. elliptica* by TLC on Si gel.

MATERIAL AND METHODS

Microorganisms

Eight microbial species taken from international collections were analyzed. The bacteria *Bacillus subtilis* (ATCC 9372), *Bacillus cereus* (ATCC 14579), Shigella spp (IAL 1578), *Staphylococcus epidermidis* (ATCC 12226), *Proteus mirabilis* (CDC 305), *Salmonella* spp (ATCC 19196), *Enterococcus faecalis* (ATCC 29212), and the yeast *Candida albicans* (ATCC 10231).

Plant material

Plant samples were collected in Porto Nacional, State of Tocantins, Brazil, in August 2002. The plant was identified and authenticated by Dra. Solange Lolis of the Universidade de Tocantins. A voucher specimen (No. 4583) was deposited at the Herbarium of the Universidade de Tocantins (HTO), campus of Porto Nacional.

Extract preparation

The air-dried and powdered leaves (2.0 kg) and barks (2.0 kg) of *D. elliptica* were extracted separately and exhaustively with CHCl₃ and MeOH successively at room temperature (48 h for each solvent). Solvents were evaporated at 60 °C under reduced pressure affording the extracts coded as ECHCl₃ (55.8 g of the barks and 103.5 g of the leaves) and EMeOH (289.8 g of the barks and 373.8 g of the leaves).

Phytochemical screening

The chromatographic analyses were made by TLC on Si gel eluted with different solvent systems: hexane/ethyl acetate (85:15, v:v), chloroform/methanol/n-propanol/water (5:6:1:4, v:v:v:v) and chloroform/methanol (85:15, v:v).

The flavonoids were identified by their intense coloration in ultraviolet light (254 nm) when revealed with the NP/PEG (diphenylaminoborate/polyethyleneglycol) reagent (Wagner et al., 1984) eluted with chloroform/methanol (85:15, v:v). Authentic standards (Sigma) of the existing flavonoids in our laboratory (quercetin, myricetin and kaempferol) were also used.

The tests for tannins were made according to the proceedings described by Simões et al. (2001) by means of the reaction with the gelatin and Schneider (1990) in...
Iodine vapor and solution of CeSO₄ were also used (saponins and terpenes) as well as anisaldehyde/sulfuric acid solution for the detection of flavonoids, terpenes, saponins, gallic acid and catechins (Wagner et al., 1984).

The compounds classes found in the ECHCl₃ and EMeOH leaves and barks of D. elliptica are indicated in Table 1.

**Disc diffusion method**

The dried plant extracts of leaves and barks were dissolved in the same solvent (MeOH and CHCl₃) to a final concentration of 30 mg/mL. Then they were sterilized by filtration through 0.45 µm Millipore filters. Antimicrobial tests were carried out by the disc diffusion method (Bauer et al., 1966).

The microorganism cultures were grown in Brain Heart Infusion liquid medium at 37 °C. After 6 h of growth, each microorganism culture, at a concentration of 10⁶ cells/mL, was inoculated on the surface of Mueller-
Hinton agar plates (100 µL). Subsequently, filter papers discs (6 mm in diameter) saturated with extracts (20 µL) were placed on the surface of each inoculated plate, in Brain Heart Infusion solid medium. The plates were incubated at 35 ºC for 24 h for bacteria and for 48 h for C. albicans. After this period, the zones of growth inhibition around the discs were measured. Overall, cultured microorganisms with halos equal to or greater than 7 mm were considered susceptible to the tested extract.

The negative control was the solvent used and the positive control was ciprofloxacin (5 µg/disc) for bacteria and ketoconazole (40 µg/disc) for C. albicans. All determinations were made in duplicate.

**Minimum inhibitory concentration**

The minimum inhibitory concentration (MIC) was determined by the dilution method according to National Committee for Clinical Laboratory Standards (NCCLS, 2003). The bacteria were grown in nutrient broth (Brain Heart Infusion liquid medium) for 6 h. After that, 20 µL of 10^6 cells/mL were inoculated in tubes with nutrient broth supplemented with eight different concentrations (25, 50, 100, 200, 400, 500, 600 and 800 µL) of the extracts. After 24 h at 37 ºC, the MIC of each sample was measured by the optical density in the spectrophotometer (620 nm), by comparison of the sample readout with the non inoculated nutrient broth (Nascimento et al., 2000). All determinations were made in duplicate.

**RESULTS AND DISCUSSION**

A total of 8 microorganisms, which consisted of 7 bacteria and 1 yeast, were tested and the results are summarized in Tables 2 and 3. The ECHCl3 extract of D. elliptica leaves and barks did not show any activity and the results are not shown.

As can be observed in Table 2, the EMeOH of D. elliptica leaves and barks possessed the antimicrobial activity against the microorganisms tested. In the assays against the microorganisms by the agar diffusion method (Table 2), the mean zones of inhibition obtained were between 8 to 14 mm.

Both the EMeOH of D. elliptica leaves and barks were active against B. subtilis, B. cereus, Shigella spp and C. albicans. However, in the extracts of the leaves the observed activity was higher. We also observed the antibacterial activity of the EMeOH leaves against E. faecalis and Salmonella spp (Table 2).

The EMeOH of D. elliptica leaves showed activity against six different species of microorganisms, while the EMeOH of D. elliptica barks showed activity against four different species of microorganisms (Table 2).

The MIC values obtained were ranged between 1.25 to 5.0 mg/mL (Table 3). The best results were observed for the EMeOH leaves and barks against B. subtilis and EMeOH leaves against Shigella spp with all of them showing MIC at 1.25 mg/mL (Table 3).

Tannins, gallic acid, some catechins and flavonoids can show antimicrobial activity (Scalbert, 1991; Veluri et al., 2004; Bylka et al., 2004; Harborne et al., 2000). Therefore, the presence of such compounds classes in the EMeOH leaves and barks of the D. elliptica might be responsible for the antimicrobial activity.

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**REFERENCES**


