Received 02/20/05. Accepted 08/15/05

Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
15(3): 209-211, Jul./Set. 2005

Artigo

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

D.C. Michelin[1], S.M. Iha[1], D. Rinaldo[2], M. Sannomiya[2], L.C. Santos[2], W. Vilegas[2], H.R.N. Salgado[1*]

[1]Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas, UNESP, 14801-902, Araraquara, SP, Brasil,
[2]Departamento de Química Orgânica, Instituto de Química de Araraquara, UNESP, 14800-900, Araraquara, SP, Brasil

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*-α-L-rhamnoside, quercetin-3-*O*-α-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), *Sthaphylococcus 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 $CHCl_3$ 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 $ECHCl_3$ (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

* *E-mail: salgadoh@fcfar.unesp.br, Tel. + 55-16-33016967*

Table 1. Phytochemical screening of *D. elliptica*

Test	Leaves		Barks	
	$ECHCl_3$	EMeOH	$ECHCl_3$	EMeOH
Catechins	-	+	-	+
Tannins	-	+	-	+
Gallic acid	-	+	-	+
Flavonoids	-	+	-	-
Saponnins	-	-	-	-
Terpenes	+	+	+	-

$ECHCl_3$ = chloroformic extract; EMeOH = methanolic extract; absent = (-); present = (+)

Table 2. Antimicrobial activities of the methanolic extracts of *D. elliptica.*

Microorganisms	Extracts (mg/mL)						Positive controls (mg/disc)	
	EMeOH leaves			EMeOH barks			Cipro.	Keto.
	50	75	100	50	75	100	5	40
S. epidermidis	-	-	-	-	-	-	25	NT
B. subtilis	12	13	13	9	9	10	20	NT
B. cereus	11	11	12	9	9	9	20	NT
E. faecalis	9	10	12	-	-	-	22	NT
Shigella spp	11	12	12	8	8	9	22	NT
P. mirabilis	-	-	-	-	-	-	22	NT
Salmonella spp	12	14	14	-	-	-	22	NT
C. albicans	11	12	13	8	9	9	NT	16

Diameter of zone (mm), (-) negative; NT: not tested ; Cipro.: ciprofloxacin; Keto.: ketoconazole

Table 3. Minimum inhibitory concentration (MIC) exhibited by the *D. elliptica* MeOH extracts

Microorganisms	MIC (mg/mL)	
	EMeOH leaves	EMeOH Barks
S. epidermidis	NT	NT
B. subtilis	1.25	1.25
B. cereus	NT	NT
E. faecalis	5.0	NT
Shigella spp	1.25	2.5
P. mirabilis	NT	NT
Salmonella spp	5.0	NT
C. albicans	NT	5.0

NT: not tested

the reaction with iron salts.

Iodine vapor and solution of $CeSO_4$ were also used (saponnins and terpenes) as well as anisaldehyde/ sulfuric acid solution for the detection of flavonoids, terpenes, saponnins, gallic acid and catechins (Wagner et al., 1984).

The compounds classes found in the $ECHCl_3$ 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 $CHCl_3$) 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 ºC. After 6 h of growth, each microorganism culture, at a concentration of 10^6 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 $ECHCl_3$ 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.

ACKNOWLEDGEMENTS

PADC-FCF, FAPESP and CNPq - Brazil

REFERENCES

Bauer AW, Kirby MDK, Sherries JC, Truck M 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol 45*: 493-496.

Bylka W, Matlawska I, Pilewski NA 2004. Natural flavonoids as antimicrobial agents. *J Am Nutraceutical Assoc 7*: 24-31.

Gurni AA, Kubitzki K 1981. Flavonoid chemistry and systematics of the Dilleniaceae. *Biochem Syst Ecol 9*: 109-114.

Harborne JB, Williams CA 2000. Advances in flavonoids research since 1992. *Phytochemistry 55*: 481-504.

http://www.propp.ufu.br/revistaeletronica/b/ocorrencia.pdf, acessado em outubro de 2003.

Matheucci LG 1996. *Estudo farmacognóstico e farmacológico de Davilla rugosa Poiret.* São Paulo, 75p. Dissertação de Mestrado - Faculdade de Ciências Farmacêuticas, Universidade de São Paulo.

Nascimento GGF, Locatelli J, Freitas PC, Silva GL 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Braz J Microbiol 31*: 247-256.

NCCLS (National Committee for Clinical Laboratory Standards) *Performance Standards for Antimicrobial Disc Susceptibility Tests 2003*. Approved Standard M2-A7, Wayne, Pennsylvania.

Rodrigues VEG, Carvalho DA 2001. Levantamento etnobotânico de plantas medicinais no domínio do cerrado na região do Alto Rio Grande - Minas Gerais. *Ciênc Agrotec 25*: 102-123.

Scalbert A 1991. Antimicrobial properties of tannins. *Phytochemistry 30*: 3875-3883.

Schneider G 1990. *Arzneidrogen. Ein kompendium für pharmazeuten, biologen und chemiker*. Mannheim: Wissenschaftsverlag.

Simões CMO, Schenkel EP, Gosmann G, Mello JCP, Mentz LA, Petrovick PR 2001. *Farmacognosia, da planta ao medicamento*. 3. ed, Porto Alegre / Florianópolis: Ed. Universidade/ UFRGS, Ed. da UFSC.

Veluri R, Weir TL, Bais HP, Stermitz FR, Vivanco JM 2004. Phytotoxic and antimicrobial activities of catechin derivatives. *J Agric Food Chem 52*: 1077-1082.

Wagner HM, Bladt S, Zgainki EM 1984. *Plant drug analysis*. Berlin: Springer.