

Antimicrobial Activity and Chemical Investigation of Brazilian *Drosera*

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The antimicrobial activity of three different extracts (hexanic, ethyl acetate, methanol) obtained from Brazilian Drosera species (D. communis, D. montana var. montana, D. brevifolia, D. villosa var. graomogolensis, D. villosa var. villosa, Drosera sp. 1, and Drosera sp. 2) were tested against Staphylococcus aureus (ATCC 25923), Enterococcus faecium (ATCC23212), Pseudomonas aeruginosa (ATCC27853), Escherichia coli (ATCC11229), Salmonella choleraesuis (ATCC10708), Klebsiella pneumoniae (ATCC13883), and Candida albicans (a human isolate). Better antimicrobial activity was observed with D. communis and D. montana var. montana ethyl acetate extracts. Phytochemical analyses from D. communis, D. montana var. montana and D. brevifolia yielded 5-hydroxy-2-methyl-1,4-naphthoquinone (plumbagin); long chain aliphatic hydrocarbons were isolated from D. communis and from D. villosa var. villosa, a mixture of long chain aliphatic alcohols and carboxylic acids, was isolated from D. communis and 3 β -O-acetylaleuritic acid from D. villosa var. villosa.

Key words: *Drosera* species - Droseraceae - antimicrobial activity - plumbagin - aliphatic compounds

There is evidence of the medicinal use of various plants since pre-history (Cowan 1999). The medicinal use of *Drosera* sp. extracts has been mentioned in the literature since the XVI century as an important antitussive for different respiratory diseases, including tuberculosis (Schnell 1984). The antimicrobial activity of extracts of aerial parts of *D. peltata* against oral bacteria has also been investigated (Didry et al. 1998). Tinctures prepared with *D. rotundifolia* leaves may also be used for the treatment of various respiratory diseases (Blumenthal et al. 1998). Since the advent of antibiotics, in the fifties, the use of plant derivatives as antimicrobials has been very limited. However, in recent years, the upsurge of bacterial resistance to antimicrobial drugs has stimulated the search for new drugs of plant origin. The worldwide emergence of *Haemophilus*, *Klebsiella*, *Escherichia coli*, and many other β -lactamase producers became a major therapeutic problem. Methicillin-resistant strains of *Staphylococcus aureus* are widely distributed in hospitals and are increasingly being isolated from community-acquired infectious strains. Vancomycin-resistant *Enterococci* and *Enterobacter* spp. resistant to most of the known drugs including quinolones, macrolides, cell wall inhibitors, have

all increased dramatically over the past decade. Given the evidence for the rapid global spread of resistant clinical isolates and the appearance of drug resistant strains among community acquired infections, the need for discovery or development of new antimicrobial agents active towards these resistant strains is of paramount importance (Bradford 2001). For this reason the search for new antimicrobial drugs among plants became an important alternative. As part of our investigation on the antibacterial properties of plants, a phytochemical study based on the bioactivity screening of five different crude extracts from each of the eight Brazilian *Drosera* species, was undertaken.

MATERIALS AND METHODS

Plant material - The carnivorous plant species were collected in the region of Ponta Grossa, in the state of Paraná, Southern Brazil, and identified by specialists. Voucher specimens of those species with medicinal use were deposited in the Herbarium of the State University of Londrina.

Preparation of the extracts - The *Drosera* species: *D. communis* (2.32 g); *D. montana* var. *montana* (2.33 g); *D. brevifolia* (2.30 g); *D. villosa* var. *graomogolensis* (2.30 g); *D. villosa* var. *villosa* (2.33 g); *D. sp 1* (2.32 g); *D. sp 2* (2.32 g), and *D. capillaris* (2.33 g) were washed under tap water to remove the soil. Each species was extracted with hexane, ethyl acetate, and methanol and the extracts were concentrated in a rotavapor.

Susceptibility testing - Tests were performed by adding 0.5 ml of each plant extract to 15 ml of melted and cooled Muller Hinton Agar (MHA) and poured into Petri dishes (pour plate technique). Young bacterial cultures (log phase) in Muller Hinton Broth, at 36°C, were streaked

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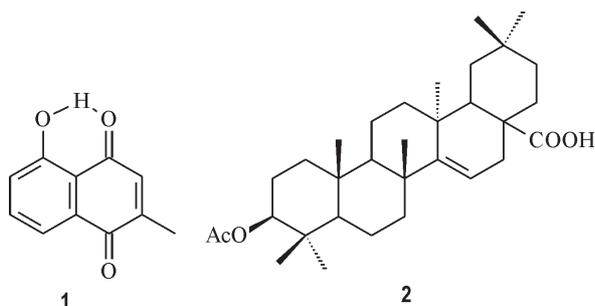
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with a standardized loop (10 µl) on MHA with incorporated plant extracts and incubated over-night at 36°C. Growth inhibition was observed after 18 h compared to bacterial growth in MHA without plant extracts.

Phytochemical analysis - The organic extracts of *D. communis* (methylene chloride), *D. montana* var. *montana* (ethyl acetate and methanol), *D. brevifolia* (hexane), and *D. villosa* var. *villosa* (ethyl acetate) were submitted to silica gel column chromatography and eluted with solvents of increasing polarity. The isolated compounds had their structures elucidated by one and two dimensional ¹H and ¹³C NMR, IR, GC, and GC-MS. The compounds isolated and/or identified were: 5-hydroxy-2-methyl-1,4-naphthoquinone (1, plumbagin) from *D. communis*, *D. montana* var. *montana*, and *D. brevifolia*; long chain aliphatic hydrocarbons in *D. communis* and *D. villosa* var. *villosa*; a mixture of long chain aliphatic alcohols and carboxylic acids in *D. communis*; and 3β-*O*-acetylaleuritic acid (2) in *D. villosa* var. *villosa*, this last previously described as an active agent against *S. aureus* (Peres et al. 1997). The structures of 1 and 2 were confirmed by comparison of the ¹³C NMR spectral data with values described in the literature for plumbagin (Sankaram et al. 1986) and 3β-*O*-acetylaleuritic acid (Ahmad & Atta-ur-Rahman 1994).

RESULTS AND DISCUSSION

Best results were obtained with ethyl acetate extracts from *D. montana* var. *montana* and *D. communis*, as shown in the Table. With the exception of *K. pneumoniae* (sensitive), the other gram-negative bacteria were resistant to all extracts (*P. aeruginosa*, *E. coli*, *S. choleraesuis*). Among gram-positive bacteria, *S. aureus* was susceptible at least to one and up to three extracts (hexane, ethyl acetate, and methanol). *C. albicans*, a yeast, was also susceptible to at least one extract of the species. We consider these as preliminary results and a larger number of bacterial isolates must be tested, with different concentrations of plant extracts in order to establish their real antimicrobial activity. Another feature to be determined is the relation between extract concentration and bacteriostatic or bactericidal effect. The isolation of the naphthoquinone plumbagin (1), from *D. communis*, *D. montana* var. *montana*, and *D. brevifolia*, together with the non detection of this naphthoquinone in *D. villosa* var. *villosa*, are in agreement with the tests accomplished in this study and with the studies on the antimicrobial activ-



Plumbagin and 3β-*O*-acetylaleuritic acid, isolated from *Drosera* spp.

TABLE
Antimicrobial activity of *Drosera* species extracts

	<i>D. communis</i>			<i>D. montana</i>			<i>D. brevifolia</i>			<i>D. villosa</i> ^a			<i>D. villosa</i> ^b			<i>Drosera</i> sp. 1			<i>Drosera</i> sp. 2			<i>D. cappilaris</i>			
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
<i>Staphylococcus aureus</i>	+	+	+	+	-	-	+	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-
<i>Enterococcus faecium</i>	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Candida albicans</i>	+	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-

a: var. *grammogolenis*; b: var. *villosa*; + inhibited growth; - growth not affected; extracts - A: hexane; B: ethyl acetate; C: methanolic

ity of hydroxy-naphthoquinones described in the literature (Didry et al. 1998). The antimicrobial activity of *D. villosa* var. *villosa* towards *S. aureus* is consistent with the presence of 3 β -*O*-acetylaleuritolic acid as described in the literature (Peres et al. 1997).

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