

CHAPTER 3

**ANTIMICROBIAL ACTIVITY OF *HIPPOBROMUS*
PAUCIFLORUS: A MEDICINAL PLANT USED FOR THE
TREATMENT OF EYE INFECTIONS IN THE EASTERN
CAPE, SOUTH AFRICA**

CHAPTER 3

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RESEARCH ARTICLE

Antimicrobial activity of *Hippobromus pauciflorus*: A medicinal plant used for the treatment of eye infections in the Eastern Cape, South Africa

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Abstract

Hippobromus pauciflorus (L.f.) Radlk (Sapindaceae) is used in traditional medicine to treat various human and animal diseases including eye infections, dysentery and diarrhea in the Eastern Cape Province of South Africa. The antimicrobial activity of the acetone, methanol and water extracts from the leaves, stem bark and roots of this herb was investigated against ten bacterial and four fungal species using the dilution method on solid agar medium. The acetone extracts from the stem bark and roots were active against Gram-positive and Gram-negative bacteria with MIC ranging between 0.1 and 10.0 mg/mL, whereas the acetone extract of the leaves inhibited the growth of Gram-positive bacterial strains at 1.0 to 5.0 mg/mL. The methanol extracts of the three plant parts were the most active and showed activity against all the bacterial isolates with MIC values ranging between 0.5 to 10 mg/mL. The water extracts of the leaf showed activity against Gram-positive bacteria at 1.0 to 5.0 mg/mL. The methanol extracts were particularly inhibitory to the growth of the fungi with inhibition percentages ranging from 78.70 to 100% on *Aspergillus niger* and *Penicillium notatum* at 10 mg/mL. The acetone extracts were active against *A. niger* (51.76%) and *P. notatum* (77.22%). The water extract of the bark significantly inhibited the growth of *P. notatum* (81.02%).

Keywords: *Hippobromus pauciflorus*; antimicrobial; antibacterial; antifungal

Introduction

Infectious diseases constitute one of the main problems that modern medicine has faced over the last 30 years. Prominent among these diseases are eye infections, caused by exposure to bacterial, fungal, viral and other microbial agents. Symptoms of eye infections include redness, swelling of the eyes, itching, increased tear production and photophobia. Also it has been shown that up to 90% of all HIV/AIDS patients contract fungal infections at some point during the course of the disease (Diamond, 1991) and that 10–20% die as a direct consequence of fungal infection (Drouhent & Dupont, 1989). Although fungal-related diseases may not be as common as bacterial infections, they are often difficult to eradicate, especially in immunosuppressive situations (Bryce, 1992). Bacterial infections are prevalent in

developing countries due to factors such as inadequate sanitation, poor hygiene and overcrowded living conditions (Rasoanaivo & Ratsimamanga-Urveg, 1993). Despite the high proportion of efficient antibiotics available nowadays, the emergence of resistant micro-organisms has lowered their potency (Bacq-Calberg et al., 1999). In addition, certain antibiotics have undesirable side effects while the emergence of previously uncommon infections is also a serious medical problem (Marchese & Shito, 2001). This has led scientists to search for new antimicrobial substances from various sources including medicinal plants. Traditionally, plants have been used as sources of medicine in virtually all cultures (Baquar, 1995). During the last decade, the use of medicinal plants has expanded globally and is gaining popularity. Such plants have continued to be used for primary healthcare not only in poor developing

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countries, but also in countries where conventional medicine is predominant in the national healthcare system (Lanfranco, 1999). The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Maurer-Grimes et al., 1996; Rabe & van Staden, 1997; Afolayan, 2003).

Hippobromus pauciflorus (L.f.) Radlk (Sapindaceae), locally known as "ulathile" in the Eastern Cape province of South Africa, is a resinous tree that grows up to 5 m in height. It is widely distributed in riverine thickets, along stream banks and at the margins of evergreen forests of South Africa. The leaves are simple and are arranged in alternate fashion. Several medicinal uses of the plant are reported. For example, the leaves of *H. pauciflorus* are used by traditional healers for the treatment of malaria (Clarkson et al., 2004); according to ethnomedical information from the indigenous people of the Eastern Cape, the leaves are crushed and squeezed into infected eyes. The root is regarded by the Zulus as a love charm and is also used to manage dysentery and diarrhea; extracts from the plant leaves are used for the treatment of livestock diseases and conjunctivitis in the Eastern Cape (Masika & Afolayan, 2003). Despite the reported medicinal uses of this plant, its antimicrobial activity has not been reported in scientific literature. The aim of this study was to investigate the antimicrobial activity of *H. pauciflorus* by preliminary bioassay screening of its extracts against 10 selected bacterial and four fungal strains. Among these organisms are *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*, *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*, all of which have been implicated in eye infections (Cuong & Michael, 2002; Hirotsoshi et al., 2006; Fabiana et al., 2004). According to Mathekga and Meyer (1998), *in vitro* antimicrobial screening methods could provide the preliminary observations necessary to select among crude extracts, those with potentially useful properties for further chemical and pharmacological investigations.

Materials and methods

Plant material

The plant material was collected in August 2007 from Sikhuthwana village near Alice in the Eastern Cape province of South Africa. The plant was identified at the Department of Botany, University of Fort Hare, by D.S. Grierson, and a voucher specimen (SC Pendota med. 2007/1) was deposited at the Griffen Herbarium. Plants were separated into roots, stem bark and leaves and were dried at room temperature (30°C).

Extract preparation

The dried leaves, stem bark and roots of the plant samples were pulverized. Powdered plant material (40 g each) was separately extracted in acetone, methanol and water for 48 h on an orbital shaker (Stuart Scientific Orbital Shaker, Greater Manchester UK). The extracts were filtered through Whatman No. 1 filter paper. The acetone and methanol extracts were evaporated to dryness under reduced pressure at 40°C using a rotary evaporator (Laborota 4000-efficient, Heidolph, Germany), while the water extracts were freeze-dried using a Savant Refrigerated Vapor Trap (RVT4104, Farmingdale, NY, USA). Individual extracts were re-dissolved in their respective solvents to give 50 mg/mL stock solution (Taylor et al., 1996). This was then diluted to the required concentrations of 0.1, 0.5, 1, 5, 7, and 10 mg/mL for the bioassay.

Antibacterial assay

The bacterial cultures used in this study were obtained as laboratory isolates from the Department of Biochemistry and Microbiology, University of Fort Hare. They consisted of five Gram-positive (*S. aureus*, *S. epidermidis*, *Bacillus cereus*, *Micrococcus kristinae*, and *S. faecalis*) and five Gram-negative (*E. coli*, *P. aeruginosa*, *Shigella flexneri*, *Klebsella pneumoniae* and *S. marcescens*) species. Each bacterial species was maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth (Biolab No.2, Wadeville, Gauteng, South Africa) for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan & Meyer, 1997; Grierson & Afolayan, 1999). The bacteria were streaked in a radial pattern on the agar plates (Meyer & Afolayan, 1995). Plates were incubated at 37°C and examined after 24 and 48 h. Each treatment was performed in triplicate, and complete suppression of growth at a specific concentration of an extract was required for it to be declared active (Sindambiwe et al., 1999; Mathekga et al., 2000). Each extract was tested at 0.1, 0.5, 1, 5, 7 and 10 mg/mL. Blank plates containing only nutrient agar and another set containing 2% acetone or methanol served as controls. Acetone and methanol have been reported to be non-toxic to the organisms at 2% (Meyer & Afolayan, 1995; Mathekga & Meyer, 1998).

Antifungal assay

Antimycotic activity of *H. pauciflorus* was investigated using four fungal species (*A. niger*, *A. flavus*, *P. notatum* and *C. albicans*). All fungal cultures were maintained on potato dextrose agar (PDA) (Biolab) and were recovered for testing by subculturing on PDA for 3 days at 25°C prior to bioassay. PDA plates were prepared by autoclaving before the addition of the extracts. Each

extract was vortexed with molten agar at 45°C to final concentrations of 0.1, 0.5, 1, 5, and 10 mg/mL and poured into Petri dishes. Plates containing only PDA or PDA with the respective solvent served as controls. The prepared plates were inoculated with plugs (5 mm in diameter) obtained from the actively growing portions of the mother fungal plates and incubated at 25°C for 5 days. The diameter of fungal growth was measured and expressed as percentage growth inhibition of three replicates (Barreto et al., 1997; Quiroga et al., 2001; Lewu et al., 2006; Koduru et al., 2006). Due to the nature of *C. albicans*, the organism was streaked radially like the bacteria. Significant differences between the means of treatments and controls were measured and calculated using the LSD statistical test (Steel & Torrie, 1960). LC_{50} (the concentration at which 50% of growth inhibition was obtained) was calculated by extrapolation.

Results and discussion

Antibacterial activity

The minimal inhibitory concentration (MIC) values of acetone, methanol and water extracts from the leaves, stem bark and roots of *H. pauciflorus* against the tested bacteria are shown in Table 1. Methanol extracts from all the plant parts inhibited the growth of both Gram-positive and Gram-negative bacteria at MIC ranging between 0.1 and 10 mg/mL. There was, however, more inhibition of the Gram-positive strains. The acetone extract of the roots suppressed the growth of all test organisms, with inhibition range of 0.5 to 10 mg/mL, while those of the leaves showed activity against Gram-positive bacteria at concentration between 1 to 5 mg/mL. There was no activity against the Gram-negative bacteria at the highest concentration tested with the exception of *S. flexneri*. The water extract of the leaves

was active only against Gram-positive with MIC ranging between 0.5 and 5 mg/mL, while extracts from stem bark and roots were not active against any of the organisms except *S. epidermidis* and *B. cereus*. Generally acetone and methanol extracts showed broad spectra of activity against the tested organisms. The action of *H. pauciflorus* against *S. aureus*, *S. epidermidis* and *P. aeruginosa* is noteworthy. Infections caused by *P. aeruginosa* are among the most difficult to treat with conventional antibiotics (Levison & Jawetz, 1992), while *S. aureus* is the leading cause of eye infections such as bacterial keratitis, conjunctivitis and dacryoadenitis (Hirotohi et al., 2006). Recent reports have highlighted the increasing fluoroquinolone resistance in *S. aureus* isolated from ocular as well as non-ocular infections (Goldstein et al., 1999; Kowalski et al., 2001; Marangon et al., 2004; Morrissey et al., 2004). The susceptibility of *S. epidermidis* to the extract of this plant may be a pointer to its potential as a drug that can be used to manage diseases including eye infections arising from this organism. *S. epidermidis* is one of the most important pathogens involved in nosocomial bloodstream infections, cardiovascular disorders as well as infections of the eye, ear, nose and throat (Cuong & Michael 2002). In this study, the acetone and methanol extracts were more active than the water extracts. Traditionally, however, plant extracts are prepared with water as infusions, decoction and poultices; therefore it would seem unlikely that traditional healers are able to extract those compounds which are responsible for activity in the acetone and methanol extracts.

Antifungal activity

The results of the antifungal assays of *H. pauciflorus* are presented in Table 2. The majority of the extracts showed antimycotic activity against the tested organisms at concentration of 5 mg/mL or lower. Only the methanol extract of the stem bark showed complete inhibition

Table 1. Antibacterial activity of the extracts from the leaves, and stem bark and roots of *H. pauciflorus*.

Bacteria	Gram+/-	MIC (mg/mL)								
		Acetone			Methanol			Water		
		Leaf	Bark	Root	Leaf	Bark	Root	Leaf	Bark	Root
<i>Staphylococcus aureus</i>	+	1.0	0.5	5.0	1.0	1.0	5.0	5.0	na	na
<i>Staphylococcus epidermidis</i>	+	1.0	0.1	0.5	0.5	0.5	0.5	0.5	0.5	na
<i>Bacillus cereus</i>	+	1.0	0.1	0.5	0.5	0.5	1.0	1.0	7.0	na
<i>Micrococcus kristinae</i>	+	5.0	0.1	0.5	1.0	0.1	0.5	5.0	na	na
<i>Streptococcus faecalis</i>	+	5.0	1.0	5.0	7.0	5.0	5.0	5.0	na	na
<i>Echerichia coli</i>	-	na	10.0	10.0	10.0	10.0	10.0	na	na	na
<i>Pseudomonas aeruginosa</i>	-	na	5.0	5.0	5.0	5.0	5.0	na	na	na
<i>Shigella flexneri</i>	-	10.0	5.0	5.0	10.0	7.0	7.0	na	na	na
<i>Klebsella pneumoniae</i>	-	na	na	10.0	10.0	10.0	10.0	na	na	na
<i>Serratia marcescens</i>	-	na	10.0	10.0	10.0	7.0	7.0	na	na	na

MIC, minimum inhibitory concentration; na, not active at 10 mg/mL, which was highest concentration tested.

Table 2. Antifungal activity of extracts from the leaves, stem bark and roots of *H. pauciflorus*.

Conc (mg/mL)	Growth inhibition (%)								
	Leaf			Stem bark			Root		
	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>
Acetone									
10	51.76 ^d	83.98 ^c	72.22 ^c	37.50 ^b	61.11 ^d	77.22 ^f	43.33 ^c	56.30 ^d	72.69 ^c
5	48.89 ^d	74.72 ^d	59.44 ^b	0.00 ^a	45.83 ^c	63.89 ^e	28.06 ^b	43.89 ^c	55.56 ^d
1	43.61 ^d	73.33 ^d	0.00 ^a	0.00 ^a	29.72 ^b	56.11 ^d	0.00 ^a	26.94 ^b	35.00 ^c
0.5	33.89 ^c	61.67 ^c	0.00 ^a	0.00 ^a	0.00 ^a	45.56 ^c	0.00 ^a	0.00 ^a	24.72 ^b
0.1	14.44 ^a	50.28 ^b	0.00 ^a	0.00 ^a	0.00 ^a	22.78 ^b	0.00 ^a	0.00 ^a	0.00 ^a
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	6.93	0.10	4.21	>10	6.36	0.71	>10	8.93	3.92
Methanol									
10	78.70 ^a	77.04 ^e	70.37 ^c	45.46 ^c	61.67 ^d	100.00 ^f	78.33 ^f	65.83 ^d	70.83 ^d
5	72.22 ^c	72.22 ^e	61.39 ^b	30.56 ^b	46.39 ^c	75.83 ^e	69.17 ^c	44.72 ^c	57.22 ^c
1	71.11 ^b	62.50 ^d	0.00 ^a	0.00 ^a	31.67 ^b	64.17 ^d	58.33 ^d	25.83 ^b	29.72 ^b
0.5	69.44 ^b	38.06 ^c	0.00 ^a	0.00 ^a	0.00 ^a	55.00 ^c	46.11 ^c	0.00 ^a	0.00 ^a
0.1	68.33 ^b	26.94 ^b	0.00 ^a	0.00 ^a	0.00 ^a	46.39 ^b	30.00 ^b	0.00 ^a	0.00 ^a
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	0.83	0.74	4.07	>10	6.18	0.27	0.66	8.02	3.95
Water									
10	62.50 ^f	72.78 ^f	0.00 ^a	64.81 ^a	62.87 ^d	81.02 ^f	62.59 ^d	63.06 ^c	66.02 ^c
5	51.39 ^c	62.50 ^c	0.00 ^a	47.50 ^c	48.06	71.67 ^c	49.72 ^c	32.22 ^b	58.33 ^d
1	41.39 ^d	58.61 ^d	0.00 ^a	30.00 ^b	31.39 ^b	64.72 ^d	26.94 ^b	0.00 ^a	42.22 ^c
0.5	34.72 ^c	40.00 ^c	0.00 ^a	0.00 ^a	0.00 ^a	45.83 ^c	0.00 ^a	0.00 ^a	21.94 ^b
0.1	30.28	35.00 ^b	0.00 ^a	0.00 ^a	0.00 ^a	30.56 ^b	0.00 ^a	0.00 ^a	0.00 ^a
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	4.44	0.77	>10	5.72	5.65	0.61	5.11	7.88	2.93

Values are means of percentage growth inhibition of three replicates. Values within a column followed by the same superscript are not significantly different at $p < 0.05$. LC₅₀ values in mg/mL were calculated by extrapolation.

(100%) against *P. notatum* at 10 mg/mL, which was the highest concentration tested in this study. Table 2 does not include the column for *C. albicans*. This is because acetone methanol and water extracts from the leaves, stem bark and roots of *H. pauciflorus* did not show any activity against *C. albicans* in this study. Extracts from the leaves showed more inhibitory activity against the fungi species than the stem bark and root extracts (Table 2). The water extract of the leaves did not show any activity against *P. notatum*. The susceptibility of *A. flavus* to the extracts of *H. pauciflorus* is noteworthy, as this fungus causes a broad spectrum of diseases in humans, ranging from hypersensitivity reactions to invasive infections associated with angioinvasion (Hedayati et al., 2007). It is also the second leading cause of aspergillosis (Denning, 1998; Morgan et al., 2005). *A. niger* was reported to be resistant to dichloromethane, aqueous and methanol extracts of 14 plants used in traditional medicines in Paraguay (Portillo et al., 2001). Generally, the ability of the extracts of this plant to inhibit the growth of several bacteria and fungi species makes it a candidate in bioprospecting for antibiotic drugs. Work is in progress on the isolation, purification and structural elucidation of the bioactive compounds in this plant in order to further validate the claims for its

use in traditional medicine by the people of the Eastern Cape in South Africa.

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