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Antimicrobial Activity of *Schistostephium heptalobium* Leaf Extracts

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ABSTRACT

Schistostephium heptalobium belongs to the family Asteraceae, which is a source of many aromatic and medicinal plants. The species *S. heptalobium* is used by healers and herbalists in the Eastern Cape Province of South Africa to treat various medical ailments. Three crude extracts of leaves were obtained by using methanol, acetone and water. Five Gram-positive species (*Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus kristinae* and *Streptococcus pyogenes*) and five Gram-negative species (*Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were used to determine the antibacterial activity of these three crude extracts. All three extracts exhibited positive results for all Gram-positive bacteria while both the acetone and methanol extracts were strongly effective against most Gram-negative bacteria tested. Five fungal species, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium notatum*, *Mucor heamalis* and *Fusarium oxysporum* were used for an antimycotic assay, all of which were inhibited, showing that this plant can be used as a complementary source for traditional medicines.

Keywords: antibacterial, antifungal, traditional medicine

INTRODUCTION

The use of natural or traditional medicine was suppressed and associated with heathen practices and witchcraft during the South African colonial era (Jager *et al*. 1996; Rabe and van Staden 1997; Kelmanson *et al*. 2000). Hence, the reputation of the use of such medicine by indigenous people was undermined. Over the past century, however, research has changed the direction and focus of natural and traditional use of these plants by indigenous people (Fennell *et al*. 2006). As a result, pharmaceutical companies and related industries have currently directed their business towards the use of plants, plant extract or plant-derived phyto-chemicals to treat diseases or are used in nutrition, perfumery and aromatheraptetic industries (Tan *et al*. 2002; Auge *et al*. 2003; Nguefack *et al*. 2004).

The genus *Schistostephium* belongs to the Asteraceae family, which is regarded as one of the largest families of flowering plants known for its economic importance, as a source of food, with many of its members having herbal qualities and being highly aromatic (Wagner *et al*. 1990; Rabe and van Staden 1997; Webber *et al*. 1999; Dalton and Cupp 2000; reviewed extensively in Teixeira da Silva 2003, 2004; Teixeira da Silva *et al*. 2005). This genus *Schistostephium* is characterized by three species, *S. heptalobium*, *S. flabelliform*, and *S. hippilifolium*, all of which are used for the treatment of influenza, coughs, cold and flu by traditional healers of the Eastern Cape Province in South Africa (Webber *et al*. 1999).

S. heptalobium is a shrub which grows up to 1.2 m tall. It is characterized by bright yellow flowers with an aromatic smell and a bitter taste.

The antimicrobial activity of crude methanol (99.5%, Merck Chemical Ltd., South Africa), acetone (99.5%, Merck) and water (distilled water) extracts of this plant was tested against Gram-positive and Gram-negative bacteria while additional studies were done to ascertain their antifungal activity.

MATERIALS AND METHODS

Plant material

During the summer months (September-December), plant shoots (leaves and stems) at their vegetative stage were collected from a farm in Fort Beaufort in the Eastern Cape. The plant was identified by the curator of the Schonland Herbarium at Rhodes University, Grahamstown. A voucher specimen (Mayekiso 13) was deposited at the Griffen Herbarium, University of Fort Hare, Eastern Cape.

Preparation of extract

S. heptalobum leaves were oven-dried at 60°C overnight. Dried leaves (1000 g) were separated equally and homogenized in acetone (99.5%, Merck), methanol (99.5%, Merck) and water on an orbital shaker for 24 h. The extracts were filtered through Whatman № 1 filter paper using a Buchner funnel, and each filtrate was concentrated to dryness under reduced pressure at 40°C using a rotary evaporator (BiBBY STERILIN Ltd., BiBBY RE 100, UK). Each extract was resuspended in the respective solvent to yield a 50 mg/ml stock solution.

Antibacterial assay

The three extracts were individually tested against 10 laboratory isolates of bacterial species provided by the Department of Microbiology and Biochemistry, Rhodes University: Five Gram-positive species (*Bacillus cereus*, *Staphylococcus epidermidis*, *S. aureus*, *Micrococcus kristinae* and *Streptococcus pyogenes*) and five Gram-negative species (*Escherichia coli*, *Salmonella pooni*, *Serratia marcescens*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*). Each microorganism was maintained on nutrient agar plates and was recovered for testing by growth in nutrient broth for 24 h. Before use, each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan and Meyer 1997).

Test organisms were streaked in a radial pattern on sterile nutrient agar (Agar-extract plates were prepared by autoclaving the

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nutrient agar (Biolab) and allowed to cool at about 60°C before the addition of the extracts. The agar medium containing the extracts at final concentrations of 0.1, 0.5, 1.0 and 5.0 mg/ml was poured into the Petri dishes. The plates were then swirled carefully until the agar began to set and left overnight for the solvent to evaporate. Agar plates containing 0.5 ml of acetone, methanol or water were used as controls respectively and incubated in the dark at 37°C for 24 h.

Each treatment was performed in triplicate and complete inhibition of bacterial growth was required for an extract to be declared bioactive. Chloramphenicol and streptomycin were used as standard controls in the experiment (Collins *et al*. 1989; Grierson and Afolayan 1999).

Antifungal assay

Five species of fungi were used for the antimycotic investigation (*Aspergillus flavus*, *A. niger*, *Penicillium notatum*, *Mucor heamalis* and *Fusarium oxysporum*). The cultures were maintained on potato dextrose agar (PDA, Meck-biolab Diagnostics, South Africa) and were recovered for testing by subculturing on fresh PDA for 3 days. PDA plates were prepared by autoclaving before the addition of the three filtered extracts which were mixed with molten agar at 45°C to final concentrations of 0.1, 0.1, 1.0 and 5.0 mg/ml, poured into Petri dishes (Gammaster LTD, South Africa) and left overnight for the solvent to evaporate. PDA with the respective solvents served as controls. The prepared plates containing the extracts were inoculated with plugs obtained from the actively growing margins of recovered fungal cultures and were incubated in the dark at 25°C for 5 days.

The diameter of fungal growth was measured and expressed as the percentage growth inhibition of three replicates. Significance differences within the means of the treatments and the controls were calculated using the LSD statistical test for fungal growth. LC_{50} (the concentration at which there was 50% inhibition of the growth of the test fungi) was calculated by extrapolation (Afolayan and Meyer 1997).

RESULTS

Antibacterial testing

The Minimum Inhibitory Concentration (MIC) values of the acetone, methanol and water extracts from the leaves of *S. heptalobum* against the tested bacterial species are presented in **Table 1**. Methanol and acetone extracts inhibited the growth of Gram-positive bacteria at 5 mg/ml, which was the highest concentration tested in the study. Acetone and methanol extracts of the leaves showed activity against all Gram-positive and Gram-negative bacteria at an MIC level of 5 mg/ml except for *S. marcescens* and *K. pneumoniae* where no activity was observed at any MIC level used in this study. The water extract of the leaves did not show any activity against any bacterial species.

Antifungal testing

The results of the antifungal assay of *S. heptalobum* extracts are presented in **Table 2**. All the extracts showed activity against the tested organisms at a concentration of 5 mg/ml. The acetone extract inhibited the growth of all fungal species with percentage inhibitory ranging from 61.2% *A. flavus* to 100% in *M. heamalis*. The acetone extract of the leaves completely inhibited the growth of *M. heamalis*.

Table 1 Antibacterial activity of leaf extracts of *Schistostephium heptalobium*.

Bacteria species	Gram +/−	MIC (mg/ml)[a]				
		Acetone	Methanol	Water	Chlor[c]	Strept[d]
Bacillus cereus	+	5	5	na[b]	<2	<2
Staphylococcus epidermidis	+	5	5	na	<2	<2
Staphylococcus aureus	+	5	5	na	<2	<2
Micrococcus kristinae	+	5	5	na	<0.2	<2
Streptococcus pyrogenes	+	5	5	na	<2	<2
Escherichia coli	−	5	5	na	<2	<2
Salmonella pooni	−	5	5	na	<2	<2
Serratia marcescens	−	na	na	na	<2	<2
Pseudomonas aeruginosa	−	5	5	na	<20	<5
Klebsiella pneumoniae	−	na	na	na	<2	<2

Minimum inhibition concentration (mg/ml); n[b], na, not active.
Chlor[c] = chloramphenicol in µg/ml; Strept[d] = streptomycin sulphate in µg/ml.

Table 2 Antifungal activity of the leaf extract of *S. heptalobium*.

Concentrations (mg/ml)	*A. flavus*	*A. niger*	*F. oxysporum*	*P. notatum*	*M. heamalis*
Acetone extract					
5.0	61.25 c	70.14 e	63.82 c	84.41 d	100.00 e
1.0	44.63 cb	45.89 d	35.85 b	44.71 c	67.50 d
0.5	41.31 b	35.28 c	32.11 b	31.94 cb	50.00 c
0.1	34.03 b	29.18 b	21.60 ab	19.19 b	22.22 b
Control	0.00 a	0.00a	0.00 a	0.00 a	0.00 a
LC_{50} mg/ml	2.29	1.68	3.02	1.53	0.50
Methanol extract					
5.0	65.06 d	64.09 e	82.18 c	63.50 d	88.61 e
1.0	44.97 dc	46.68 d	73.87 c	50.37 cd	49.44 d
0.5	33.47 bc	36.82 c	29.12 b	39.04 c	39.00 c
0.1	15.58 ab	22.34 b	10.91 ab	19.19 b	18.05 b
Control	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
LC_{50} mg/ml	2.00	1.76	0.73	0.98	1.06
Water extract					
5.0	65.07 b	70.69 c	79.80 c	78.73 c	75.00 e
1.0	43.86 b	44.69 b	37.04 b	36.80 b	61.67 d
0.5	18.91 a	13.09 a	26.35 b	28.24 b	45.83 c
0.1	5.05 a	8.80 a	19.03 ab	15.32 ab	8.33 b
Control	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
LC_{50} mg/ml	2.16	1.82	2.21	2.26	0.63

Values (mm) are means of percentage growth inhibition of three replicates: values within a column followed by same superscript of the same species are not significantly different at $p \leq 0.05$ according to the LSD test. LCD_{50} values is in mg/ml.

Similarly, the growth of microorganisms was inhibited by the methanol extract at 5 mg/ml, which was the highest concentration used. The methanol extract inhibited growth of *M. heamalis* (88.6%) and *P. notatum* (63.5%), whereas the water extract also showed appreciable results against *F. oxysporum* (79.8%) and *A. flavus* (65.1%). All the extracts showed positive activity against all fungal species tested (**Table 2**).

DISCUSSION

The antibacterial activity of various plant species has been performed to determine their MIC (reviewed in Benkeblia 2007). MIC determines the lowest concentration at which the extract effectively kills or inhibits microbial growth, most of which are implicated in food poisoning while some are responsible for abscesses (Fraser 1986; Gramum and Lund 1997; Mhinana *et al*. 2007). Many bacterial species are associated with a range of clinical conditions, food spoilage and incidents of food-borne diseases such as gastroenteritis (Salkinoja-Salonen *et al*. 1999; Mhinana *et al*. 2007).

The use of these bacteria and fungi in this study was based on their importance in human veterinary use.

Extract from the leaves of *S. heptalobium* inhibited bacteria and fungi (**Tables 1** and **2**, respectively), with greatest activity against most bacteria except for *Serratia marcescens* and *Klebsiella pneumoniae*, thereby suggesting a broad spectrum antibacterial property. The water extract did not show any antibacterial activity whereas only the methanol and acetone extracts did. This may be due to the low concentration of extract used (5.0 mg/ml). Most often traditional healers prepare extracts using water and it is unlikely for water to extract all compounds that are responsible for antibacterial activity in the acetone and methanol extracts. This could be due to a difference in polarity of the medium in which the extractions were done. Deep-seated compound sometimes require less polar substances in order to be extracted. The less polar compounds like acetone and methanol can breakdown or weaken the cell wall and increase the release of compounds within the cell. Water on the other hand cannot breakdown the cell wall.

The traditional healers using water will not be able to extract all compounds easily. However, through communication with some traditional healers it was noted that some healers use hot water or boiling water to extract the compounds from the plant. Boiling water assists in the extraction of compounds by rupturing the cell wall and thereby removing the contents from within the cells (Ali *et al*. 1999; Femenia *et al*. 1999). In this case of *S. heptalobium* a larger quantity of the leaves of a plant is soaked in hot water thereby extracting active compounds that could be used to cure various ailments. Furthermore, other traditional healers sieve plant portions or allow the extract to settle at the bottom of a container which they then use.

Since this study deals with the antimicrobial activity of the crude extracts of *S. heptalobium*, it is believed that the crude extract contains various compounds which could be of important medicinal value. This study can also open up other avenues of investigation like isolation of compounds. However the current study was meant to verify the use of *S. heptalobium* by traditional healers. As *S. heptalobium* is often used as a traditional herb for cures against various ailments. These results would therefore assist in complimenting the knowledge of traditional healers and scientifically justify their uses. This would aid in the use of many plant species as alternative cures to current contemporary medicines.

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