

Full Length Research Paper

Antimicrobial activity of *Gunnera perpensa* and *Heteromorpha arborescens* var. *abyssinica*

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Methanol and water extracts from *Gunnera perpensa* rhizomes and *Heteromorpha arborescens* roots were investigated for their *in vitro* antimicrobial activities. These plants are implicated in the treatment of dysmenorrhoea in the Eastern Cape, South Africa. Ten bacterial strains (5 Gram positive and 5 Gram negative) and four fungal strains were selected for the antimicrobial assays. Both plant species were active against the Gram positive bacteria, with *G. perpensa* being more effective than *H. arborescens*. The antifungal assays showed all extracts significantly inhibiting the growth of four strains and *Candida albicans* not inhibited by all. The LC₅₀ values ranged from 0.07 to 3.81 for all the extracts. Albeit the fact that most of the fungal species used in the investigation are not directly implicated as human pathogens, it was important to assess the extracts activity against them. These may give rise to fungicides that can be employed in therapy.

Key words: *Gunnera perpensa*, *Heteromorpha arborescens*, antimicrobial activity, bacteria and fungi.

INTRODUCTION

There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years; with tuberculosis being the most recent having multiple drug resistant strains (MDR) and the recently discovered extremely drug resistant (XDR) *Mycobacterium tuberculosis* (Jones et al., 2008). Despite several available antimycotic drugs, the treatment of immunocompromised patients is still limited due to a number of factors. This is largely due to the use of broad-spectrum commercial antimicrobial drugs, low drug potency, poor solubility of drugs, emergence of resistance strains and drug toxicity (Aliero and Afolayan, 2006; Aliero et al., 2006; McCutchen et al., 1994; Li et al., 1995; Nwosu and Okafor, 1995).

With regards to fungal infections the same can be said, as an increase of AIDS-related opportunistic fungal pathogens with *Candida albicans* being the first to be identified and the emergence of resistance strains (Fan-Harvard et al., 1991; Silva et al., 2001; Afolayan et al., 2002). This is also in some cases further compounded by the side effects and complications that arise with the re-

peated use of antibiotics (Aliero and Afolayan, 2006; Marchese and Shito, 2001; Poole, 2001). Hence, the screening of medicinal plant extracts and other by-products for antimicrobial activity has become a cost effective option with enormous potential for novel antibiotic prototypes (Maurer-Grimes et al., 1996; Rabe and Van Staden, 1997; Afolayan, 2003).

Heteromorpha arborescens has many and varied uses in traditional medicine (Hutchings et al., 1996; Van Wyk and Gericke, 2000; Lundgaard et al., 2007). It is of importance to investigate its use as an antimicrobial remedy.

Gunnera perpensa on the other hand has been studied extensively mostly for its analgesic and anti-inflammatory activities (Drewes et al., 2005; Khan et al., 2004; Hutchings et al., 1996; Van Wyk et al., 1997), some work however, particularly on the stems and leaves have shown its potential as a wound healing remedy (Grierson and Afolayan, 1999b), hence, the importance to access the rhizomes for antimicrobial activity.

MATERIALS AND METHODS

Plant material

The plant material was collected from the wild; *G. perpensa* in the Port Saint Johns (31°34'03.87"S 29°35'08.34"E 69 m above sea

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Table 1. Anti-bacterial activity of *Gunnera perpensa*.

Bacteria –species	Gram +/-	MIC (mg/ml)	
		Methanol	Water
<i>Staphylococcus aureus</i>	+	1.0	1.0
<i>Staphylococcus epidermidis</i>	+	1.0	1.0
<i>Bacillus cereus</i>	+	0.5	1.0
<i>Micrococcus kristinae</i>	+	0.5	0.5
<i>Streptococcus faecalis</i>	+	1.0	7.0
<i>Escherichia coli</i>	-	0.5	na
<i>Pseudomonas aeruginosa</i>	-	1.0	5.0
<i>Shigella flexneri</i>	-	0.1	5.0
<i>Klebsiella pneumoniae</i>	-	1.0	1.0
<i>Serratia marcescens</i>	-	1.0	1.0

Minimum inhibitory concentration (mg/ml); na, not active.

level) area, while *H. arborescens* was collected from Flagstaff (31°06'21.58"S 29°29'23.82"E 862 m above sea level), both in the Eastern Cape Province of South Africa. Proper identification of *G. perpensa* was done at the Kei Herbarium of Walter Sisulu University, while *H. arborescens* was done at the Kwa Zulu Natal Herbarium in Durban.

Extraction preparation

Rhizome and root samples of *G. perpensa* and *H. arborescens* respectively were chopped, air dried and ground to powder. Two 50 g portions of each dried plant were shaken separately in methanol and water for 24 h on an orbital shaker. The extracts were filtered using a Buchner funnel and Whatman No. 1 filter paper, with the methanol filtrates concentrated to dryness under reduced pressure at a maximum of 40°C using a rotating evaporator. Aqueous filtrates were freeze-dried. Each extract was re-suspended in the respective solvent of extraction to yield a 50 mg/ml stock solution (Taylor et al., 1996; Koduru et al., 2006).

Antibacterial testing

Ten bacterial strains were used in this study, they consisted of five Gram-positive and five Gram-negative strains (Table 1). Bacterial species were maintained on nutrient agar plates and recovered for testing by sub-culturing in nutrient broth (bio. Lab. No.2) for 24 h. Before use each bacterial culture was diluted 1:100 with fresh sterile nutrient broth (Afolayan and Meyer, 1997; Grierson and Afolayan, 1999a; Aliero et al., 2006).

Test organisms were streaked in a radial array on the agar plates (Meyer and Afolayan, 1995). Plates were incubated at 37°C for 24 to 48 h then examined; each treatment was performed in triplicate. In the declaration of an extract being bioactive, complete suppression of growth by a specific concentration was to be observed (Sidambiwe et al., 1999; Mathekga et al., 2000). Concentrations of extracts at 0.1, 0.5, 1.0, 5.0, 7.0 and 10.0 mg/ml were tested against the organisms. Blank plates containing only nutrient agar or nutrient agar with the respective solvents were used as controls.

Antifungal testing

Four species of fungi (Table 3) were employed in the anti-mycotic investigation. Adopting the method employed by (Afolayan and

Table 2. Anti-bacterial activity of *Heteromorpha arborescens*.

Bacterial - species	Gram +/-	MIC (mg/ml)	
		Methanol	Water
<i>Staphylococcus aureus</i>	+	7.0	0.1
<i>Staphylococcus epidermidis</i>	+	10	0.1
<i>Bacillus cereus</i>	+	0.1	na
<i>Micrococcus kristinae</i>	+	0.1	10
<i>Streptococcus faecalis</i>	+	10	na
<i>Escherichia coli</i>	-	10	na
<i>Pseudomonas aeruginosa</i>	-	7.0	na
<i>Shigella flexneri</i>	-	10	na
<i>Klebsiella pneumoniae</i>	-	10	na
<i>Serratia marcescens</i>	-	7.0	na

Minimum inhibitory concentration (mg/ml); na, not active.

Meyer, 1997; Erasto et al., 2006; Aliero et al., 2006) Potato Dextrose Agar (PDA) was prepared and autoclaved before the addition of the extracts. Each extract was mixed with molten agar at 45°C to final concentrations of 0.1, 0.5, 1.0, 5.0, 7.0 and 10.0 mg/ml, then poured into Petri dishes. Each plate was swirled carefully until the agar was evenly distributed, and left overnight for residual solvent to evaporate. Plates containing PDA with the respective solvents served as controls.

The prepared plates were inoculated with plugs obtained from the actively growing margin of fungi plates and incubated at 25°C for 5 days. The diameter of the fungal growth was measured and expressed as percentage growth inhibition of three replicates (Afolayan and Meyer, 1997; Barreto et al., 1997; Quiroga et al., 2001; Koduru et al., 2006; Aliero et al., 2006).

Significant differences within the means of the treatments and controls were calculated using the LSD statistical test at 5% probability (Steel and Torrie, 1960). LC₅₀ (Concentration at which there was 50% inhibition) was calculated by extrapolation.

RESULTS AND DISCUSSION

Antibacterial property

Minimal inhibitory concentration (MIC) values of methanol and aqueous extracts of *G. perpensa* are given on Table 1, while those of *H. arborescens* are given on Table 2. *G. perpensa* extracts from both methanol and water were effective against all Gram positive bacterial strains tested. The methanol extract had MIC values of 0.5 and 1.0 mg/ml, while the aqueous extract was most effective on four strains and fairly weak against *Streptococcus faecalis* with a MIC value of 7.0 mg/ml. This further strengthens the views expressed by (Drewes et al., 2005) on using *G. perpensa* in combating infections caused by *Streptococcus epidermidis* and *Streptococcus aureus* though in that study stems and leaves were used. It is also worth noting its inhibitory activity against *Bacillus cereus*, which is a human pathogen difficult to treat using conventional antibiotics (Mathekga et al., 2000).

The inhibitory activity of both extracts and especially the aqueous extract against *Pseudomonas aeruginosa* (Gram negative) is of interest. As infections such as mas-

Table 3. Anti-fungal activity of methanol extracts of *G. perpensa* and *Heteromorpha arborescens*.

Concentrations (mg/ml)	Growth inhibition (%)							
	<i>Gunnera perpensa</i>				<i>Heteromorpha arborescens</i>			
	<i>A. flavas</i>	<i>A. niger</i>	<i>P. notatum</i>	<i>C. albicans</i>	<i>A. flavas</i>	<i>A. niger</i>	<i>P. notatum</i>	<i>C. albicans</i>
10.0	82.87 ^f	83.33 ^d	100 ^e	0.00	87.31 ^f	100 ^c	86.94 ^g	0.00
7.0	75.83 ^e	77.50 ^c	100 ^e	0.00	81.39 ^e	100 ^c	74.72 ^f	0.00
5.0	71.39 ^d	71.11 ^b	100 ^e	0.00	76.94 ^d	100 ^c	66.67 ^e	0.00
1.0	68.06 ^c	0.00 ^a	82.78 ^d	0.00	75.56 ^d	100 ^c	49.72 ^d	0.00
0.5	66.39 ^c	0.00 ^a	73.06 ^c	0.00	66.11 ^c	73.06 ^b	36.94 ^b	0.00
0.1	50.56 ^b	0.00 ^a	66.94 ^b	0.00	60.00 ^b	73.89 ^b	25.83 ^b	0.00
Control	0.00 ^a	0.00 ^a	0.00 ^a	0.00	0.00 ^a	0.00 ^a	0.00 ^a	0.00
LC ₅₀	0.10	3.81	0.07	0.00	0.08	0.07	1.07	0.00

Values are means of percentage growth inhibition of three replicates; values within a column followed by the same superscript of the same species are not significantly different at $p < 0.05$ according to the LSD test. LC₅₀ values were calculated by extrapolation.

Table 4. Anti-fungal activity of aqueous extracts of *G. perpensa* and *Heteromorpha arborescens*.

Concentrations (mg/ml)	Growth inhibition (%)							
	<i>Gunnera perpensa</i>				<i>Heteromorpha arborescens</i>			
	<i>A. flavas</i>	<i>A. niger</i>	<i>P. notatum</i>	<i>C. albicans</i>	<i>A. flavas</i>	<i>A. niger</i>	<i>P. notatum</i>	<i>C. albicans</i>
10.0	82.59 ^e	0.00	95.74 ^e	0.00	57.41 ^c	79.35 ^f	85.93 ^f	0.00
7.0	76.94 ^d	0.00	91.94 ^e	0.00	36.39 ^b	64.72 ^e	76.94 ^e	0.00
5.0	76.39 ^d	0.00	88.33 ^d	0.00	0.00 ^a	61.67 ^d	73.06 ^e	0.00
1.0	64.17 ^c	0.00	80.56 ^c	0.00	0.00 ^a	51.94 ^c	62.22 ^d	0.00
0.5	57.22 ^b	0.00	72.78 ^b	0.00	0.00 ^a	40.28 ^b	52.78 ^c	0.00
0.1	0.00 ^a	0.00	0.00 ^a	0.00	0.00 ^a	0.00 ^a	47.22 ^b	0.00
Control	0.00 ^a	0.00	0.00 ^a	0.00	0.00 ^a	0.00 ^a	0.00 ^a	0.00
LC ₅₀	0.45	0.00	0.37	0.00	3.17	0.92	0.30	0.00

Values are means of percentage growth inhibition of three replicates; values within a column followed by the same superscript of the same species are not significantly different at $p < 0.05$ according to the LSD test. LC₅₀ values were calculated by extrapolation.

titis are often difficult to combat (Salie et al., 1996), this result may explain why herbalists may still be using infusions prepared in water due to their efficacy against some of these microbes. The results obtained are still in full agreement with similar work done on *G. perpensa* (Buwa and van Staden, 2006) and the view that methanol extracts of the plant's rhizome are more effective than the aqueous extract in this particular study (Eloff, 1998; Lin et al., 1999; Karaman et al., 2003; Steenkamp et al., 2004). However, these results were contradictory to the study done by McGaw et al. (2000) with regards to activity against *Escherichia coli*.

H. arborescens showed a higher activity than *G. perpensa*. Methanol extracts were fairly effective while the aqueous extracts were more effective with lower MIC values of 0.1 mg/ml against *Staphylococcus aureus* and *S. epidermidis*. Interestingly for the other three gram positive bacterial strains, the methanol extract was very effective while the aqueous extract was active against *Micrococcus kristinae* at 10.0 mg/ml.

Methanol extracts of *G. perpensa* were active against all the bacteria used in this study while the aqueous extract was not active against *E. coli*. *Shigella flexneri*, a diarrhoea causing pathogen was susceptible to the methanol extract at 0.1 mg/ml. However, both extracts had an MIC value of 1.0 mg/ml against *Klebsiella pneumoniae* and *Serratia marscens*.

Contrary to *G. perpensa*, *H. arborescens* was fairly weak against the gram-negative bacteria, with MIC values of 7.0 and 10.0 mg/ml while the aqueous extracts were not active as shown on Table 2.

Antifungal property

The results of the antifungal assays of both *G. perpensa* and *H. arborescens* are shown on Tables 3 and 4. Extracts from both plants did not show any percentage inhibition against *C. albicans* but *G. perpensa* was effective against *Penicillium notatum*, *Aspergillus flavas* and

Aspergillus niger with LC₅₀ values ranging from 0.07 to 3.81. *A. niger* was reported to be resistant to dichloromethane, aqueous and methanolic extracts of 14 plants used for traditional medicine in Paraguay (Portillo et al., 2001). In this investigation methanol extracts significantly suppressed the growth of *A. niger* at concentrations above 1.0 mg/ml, increasing from 71 to 83.3% at 5 to 10 mg/ml respectively. This was similar to the methanol extracts used by Aliero et al. (2006). Similar results were also reported by Novarro et al. (2003) for methanol extract from *Sedum oxypetalum* (Crassulaceae) which inhibited the growth of *A. niger* significantly. The ability of the extracts to inhibit *A. niger* is of paramount importance, as the fungus has been implicated in cases of immunocompromised patients that frequently develop opportunistic and superficial mycosis (Ngane et al., 2000; Portillo et al., 2001). No growth inhibition percentage was observed against *C. albicans*, while there was some activity against *A. flavus* and *P. notatum*. A 50.56% growth inhibition of *A. flavus* was exhibited from the lowest concentration of 0.1 mg/ml while at the highest concentration of 10.0 mg/ml there was 82.87% growth inhibition. The best result was obtained against *P. notatum* where inhibition rose from 66% at 0.1 mg/ml to 82.78% at 1.0 mg/ml then 100% growth inhibition was at 5.0 mg/ml and above (Table 3).

Water extract of *G. perpersa* showed no percentage growth inhibition for both *A. niger* and *C. albicans* at all concentrations. At 0.5 mg/ml, the aqueous extract showed a 57.22% growth inhibition of *A. flavus* while 82% was the highest growth inhibition observed at 10.0 mg/ml. As for *P. notatum*, a 74.78% growth inhibition was exhibited at 0.5 mg/ml and the highest inhibition percentage of 95.74 was achieved at 10.0 mg/ml (Table 4).

On the other hand *H. arborescens* was more effective than *G. perpersa* against *A. flavus*, *A. niger* and *P. notatum* with LC₅₀ values ranging from 0.07 to 1.07. However, it was also ineffective against *C. albicans* as no percentage growth inhibition was observed. The methanol extract was most effective against *A. niger* with a 100% growth inhibition observed at 1.0 mg/ml and above. While against *P. notatum* and *A. flavus* growth inhibitions ranged from 25.83% at 1.0 mg/ml to 86.94% at 10.0 mg/ml and 60% at 0.1 mg/ml to 87.31% at 10.0 mg/ml respectively (Table 3). On the other hand aqueous extracts were less effective against *A. flavus*. A growth inhibition of 36.39% was observed at 7.0 mg/ml and 57.41% at 10.0 mg/ml. A higher activity was observed against *A. niger* and *P. notatum* with growth inhibition ranging from 40.28% to 85.93% at concentrations of between 0.1 and 10.0 mg/ml (Table 4).

Thus it can be said that aqueous extracts of both plant species were less effective than the methanol extracts with that of *G. perpersa* not inhibiting both *C. albicans* and *A. niger*. Aqueous extract of *H. arborescens* was able to inhibit the 3 other species except *C. albicans* with LC₅₀ values ranging from 0.30 to 3.81.

Although most of the fungal species used in the inves-

tigation are not directly implicated as human pathogens, it was important to assess the extracts' activity against them. These may give rise to fungicides that can be employed in therapy.

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