

Full Length Research Paper

Screening the root extracts from *Biden pilosa* L. var. *radiata* (Asteraceae) for antimicrobial potentials

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Bidens pilosa L. is a medicinal plant in many regions of the world. The antimicrobial activity of the methanol, acetone and water extracts from the root of the South African ecotype was evaluated using agar dilution method. The extracts showed significant activity against all the bacteria and some fungi species tested in this study. The methanol extract inhibited all Gram-positive and Gram-negative bacteria with minimum inhibitory concentrations ranging from 5 to 10 mg/ml. Although the water extract did not inhibit any of the bacteria, the acetone extract was able to suppress the growth of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Klebsella pneumoniae* in the same concentration range as the methanol extract. All the extracts exhibited 100% inhibition against *Penicillium notatum* at 0.1 mg/ml. In the same pattern, all the extracts showed good activity against *Aspergillus niger* but none could inhibit *Aspergillus flavus*. The best activity was observed in the methanol extract against most bacteria and fungal strains, which suggest the use of organic solvent extraction for good antimicrobial activity. Our results have shown that extracts from the root of this species could inhibit both Gram-positive and Gram-negative bacteria as well as some fungi species. This *in vitro* study on the root therefore support the folkloric use of the whole plant in the treatment of microbial infections but its medicinal use in infections associated with *A. flavus* is not recommended.

Keywords: *Bidens pilosa*, root extract, antimicrobial, medicinal plant.

INTRODUCTION

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Prabuseenivasan et al., 2006). Bacteria for example, have shown a remarkable ability to endure and adapt to their environment including the development of different mechanisms of resistance to most old and new antimicrobial agents (Hersch-Martinez et al., 2005). Bacterial adaptation to antibiotics has been very successful, and over the years the increase in antibiotic resistance has generated a considerable worldwide public health problem (De Esparza et al., 2007). During the past 50 years, there had been a great deal of interest in screening therapeutic agents from plants (Chang et al., 2001). Interest in medicinal plants as a re-emerging health-aid has been fuelled by the rising cost of prescription drugs in the maintenance of personal health

and well-being and the bioprospecting for new plant-derived drugs (Hoareau and Dasilva, 1999; Ojekale et al., 2007).

Bidens pilosa L. is widely used in traditional medicine for anti-influenza, diabetic control and treatment of gastroenteritis (Chang et al., 2001). The plant is an annual, erect, branching herb growing up to 1.5 m tall with quadrangular, minutely hairy stems. The leaves are opposite, toothed; simple and ovate, or compound with three to five or even seven lanceolate leaflets (Morton, 1962; Geissberger and Sequin, 1991). It is widely distributed in the subtropical and tropical regions of the world (Deba et al., 2008). As a leafy vegetable, the species is an excellent source of fibre and certain mineral elements (Odhav et al., 2007). Extensive research in the last few decades have shown that *B. pilosa* possessed anti-hyperglycemic (Ubillas et al., 2000; Hsu et al., 2009), anti-ulcerogenic (Tan et al., 2000), anti-inflammatory (Geissberger and Sequin, 1991; Jager et al., 1996), vasodilative, hypotensive (Dimo et al., 1998; 2001), anti-malarial (Brandao et al., 1997; Andrade-Neto et al., 2004),

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hepato-protective (Chin et al., 1996), antipyretic (Sundararajan et al., 2006), anticancer and antitumor (Steenkamp and Gouws, 2006; Sundararajan et al., 2006; Kviencinski et al., 2008), antioxidant (Abajo et al., 2004; Chiang et al., 2004; Yang et al., 2006), antiviral (Chiang et al., 2003), antifungal (Motsei et al., 2003; Deba et al., 2008) and anti-bacterial (Geissberger and Sequin, 1991; Rabe and van Staden, 1997; Khan et al., 2001; Rojas et al., 2006), activities. Concerning the antimicrobial activity of this species, the available reports in literature focused on either the aerial part or the whole plant. Presently, there is lack of data in available literature references on the antimicrobial activity of the extracts from the subterranean part of *B. pilosa* particularly the South African ecotype. For a suitable comparative antimicrobial activity of different organs of this valuable herb, we proposed the *in vitro* antimicrobial activity study of the root extracts of *B. pilosa*. This study evaluates such activity toward some selected Gram-positive, Gram-negative bacteria and fungal species. In this paper, we present the minimum inhibitory concentrations of the root extracts of *B. pilosa* determined using agar dilution method.

MATERIALS AND METHODS

Plant material and preparation of extracts

Plants were harvested in March 2008 from a single population of *B. pilosa* growing in the medicinal plants garden of the University of Fort Hare Research Farm (33° 11.10'S and 7° 10.60'E; altitude 695 m). The mean annual rainfall of the area is about 700 mm and temperature range of 13 to 25°C. The species was authenticated by Prof. Grierson of the Department of Botany, University of Fort Hare. A voucher specimen (AshMed.2009/1) was prepared and deposited in the Giffen Herbarium of the University of Fort Hare.

The roots were separated, carefully rinsed under running tap, dried in the oven at 40°C to a constant weight before it was pulverized. Thirty gram each of the powdered material was extracted in acetone and methanol. The water extract was prepared by boiling equal weight (30 g) of the dry root material in water for 15 min in a flask. This was allowed to cool and transferred to an orbital shaker for 12 h. All extracts were filtered using Whatman No 1 filter paper. The filtrates from acetone and methanol were concentrated under reduced pressure 40°C using (Laborota 4000-efficient, Heidolph, Germany) rotary evaporator. The water extract was freeze dried using "Virtis BenchTop 'K' Series, USA" freeze dryer. The yields were 2.2, 0.7 and 1.6 g for methanol, acetone and water respectively. Individual extract was reconstituted in their respective solvent to give a stock solution of 50 mg/ml (Taylor et al., 1996). This was diluted to the required concentrations of 0.1, 0.5, 1.0, 5.0, and 10 mg/ml for the bioassay analysis.

Antibacterial activity assay

Five Gram-positive bacteria namely; *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Micrococcus Kristinae*, *Streptococcus faecalis*, and five Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Klebsella pneumoniae* and *Serratia marcescens* were all laboratory isolates. They were obtained from the Department of Biochemistry

and Microbiology, University of Fort Hare, South Africa. The organisms were maintained on nutrient agar plates and were revived for bioassay by subculturing in fresh nutrient broth (Biolab, Johannesburg, South Africa) for 24 h before being used.

The method described by Meyer and Afolayan, (1995) was adopted with slight modifications. Briefly; nutrient agar (Biolab, Johannesburg, South Africa) was prepared by autoclaving and allowed to cool to 55°C before the addition of the extracts. The agar medium containing the extracts at final concentrations of 0.1, 0.5, 1.0, 5.0 and 10 mg/ml were poured into petri dishes, swirled gently until the agar began to set, and left over night for solvent evaporation. Agar plates containing 1% of the respective solvent served as controls. Organisms were streaked in radial pattern on the agar plates. The inoculum size of each test strain was standardized at 5×10^5 cfu/ml using McFarland Nephelometer standard according to the National Committee for Clinical Laboratory Standards. The plates were incubated under aerobic conditions at 37°C and examined after 24 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 (Mathekga et al., 2000). Chloramphenicol and streptomycin (standard antibiotics) were used as positive controls in the experiment.

Antifungal assay

Antimycotic activity of *B. pilosa* was investigated using four fungal species (*Aspergillus niger*, *Aspergillus flavus*, *Penicillium notatum* and *Candida albicans*). All fungal cultures were maintained on potato dextrose agar (PDA) (Biolab, Johannesburg, South Africa) 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 the molten agar at 45°C to final concentrations of 0.1, 0.5, 1.0, 5.0 and 10.0 mg/ml and poured into Petri dishes. Blank plates containing only PDA or PDA with the respective solvent served as controls. The prepared plates containing the extracts 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 3 and 5 days as required for fungal species. The diameter of fungal growth was measured and expressed as percentage growth inhibition of three replicates (Ashafa et al., 2008). Due to the nature of *C. albicans*, the organism was streaked radially like the bacteria.

Statistical analysis

Significant differences within the means of treatments and controls were measured and calculated using the LSD statistical test. LC₅₀ (the concentration at which 50% of growth was obtained) was calculated by extrapolation.

RESULTS AND DISCUSSION

Antibacterial activity

The minimum inhibitory concentrations (MICs) of acetone, methanol and water extracts from the roots of *B. pilosa* are represented in Table 1. The methanol extract inhibited both the Gram-positive and Gram-negative bacteria strains with MIC ranging from 5.0 to 10.0 mg/ml. The acetone extract suppressed the growth of Gram-positive bacteria *S. aureus* and *S. epidermidis* and

Table 1. Antibacterial activity of the extracts from the roots of *Bidens pilosa*.

Bacteria	Gram (±)	Minimum inhibitory concentration (MIC) mg/ml				
		Methanol	Acetone	Hot water	Cloramphenicol (µg/ml)	Streptomycin (µg/ml)
<i>Staphylococcus aureus</i>	+	5.0	10.0	Na	<2	<2
<i>Staphylococcus epidermidis</i>	+	5.0	5.0	Na	<2	<2
<i>Bacillus cereus</i>	+	10.0	Na	Na	<2	<2
<i>Micrococcus kristinae</i>	+	10.0	Na	Na	<0.5	<2
<i>Streptococcus faecalis</i>	+	10.0	Na	Na	<2	<4
<i>Escherichia coli</i>	-	5.0	5.0	Na	<2	<2
<i>Pseudomonas aeruginosa</i>	-	10.0	10.0	Na	<10	<4
<i>Shigella flexneri</i>	-	10.0	Na	Na	<2	<2
<i>Klebsella pneumoniae</i>	-	5.0	10.0	Na	<2	<2
<i>Serratia marcescens</i>	-	10.0	Na	Na	<2	<2

Na = not active at 10 mg/ml, which was the highest concentration tested.

Gram-negative bacteria *E. coli*, *P. aeruginosa* and *K. pneumoniae* with MIC ranging from 5.0 to 10.0 mg/ml. According to previous reports, the leaf extracts of *B. pilosa* were active against Gram-positive bacteria but not active against the Gram-negative strains (Rabe and Van Staden, 1997), while the whole plant extracts inhibited both Gram-positive and Gram-negative bacteria (Khan et al., 2001). The results from our study showed that extracts from the roots of *B. pilosa* suppressed the growth of both Gram-positive and Gram-negative bacteria. The water extract was not active against any of the bacteria strains at 10 mg/ml, a situation which is not surprising as such have been reported for water extract from other plant species (Ashafa et al., 2008). Although, all the extracts inhibited most of the microorganisms at concentrations higher than that of standard antibiotics streptomycin and chloramphenicol, yet, the methanol extract inhibited *P. aeruginosa*, a notable antibiotic resistant bacterium at a concentration that compares favourably with streptomycin. Generally, the methanol extract was more active than the acetone and water extracts which is an indication that the solvent (methanol) was able to extract the compounds responsible for the antibacterial activity of the herb. The root is the site for the synthesis of many of the compounds found in the shoot of most plants. Since the crude root extracts of *B. pilosa* had good antibacterial activity, it is therefore possible that the antibacterial compounds in this plant are also contained in the root.

Antifungal activity

The antifungal activity of the root extracts of *B. pilosa* was evaluated using agar dilution method and the results are presented in Table 2. The acetone, methanol and water extracts exhibited low activity against *A. flavus* but suppressed the growth of *P. notatum* 100% at 0.1 mg/ml.

Also, all the extracts exhibited good activity against *A. niger* but none could inhibit *C. albicans* except the methanol extract at 10 mg/ml. Motsei et al. (2003) gave a similar report on the ethanolic extract of the leaves against three species of *C. albicans*. Previous studies have shown that *B. pilosa* contains compounds like flavonoids, phenylacetylenes, alkaloids, sterols, triterpenoids and tannins (Brandao et al., 1997; Khan et al., 2001) that are responsible for the antimicrobial activity of this species.

The results obtained from the study conducted by Rabe and Van Staden (1997), showed that the leaf extracts from *B. pilosa* inhibited Gram-positive bacteria but could not inhibit Gram-negative strains *E. coli* and *K. pneumoniae*. The fractionated whole plant extracts from this species were reported to inhibit these two organisms (Khan et al., 2001). The results from this present study have shown that the root extracts from *B. pilosa* inhibited both the *E. coli* and *K. pneumoniae* at minimum inhibitory concentration of 5 mg/ml, which is an indication that the compounds responsible for this activity could be present in the root. This Gram-specific inhibition between the leaves and roots of this species could in part explain why the whole plant is employed in the treatment of microbial infections (Khan et al., 2001). The plants (*B. pilosa*) obtained from the Papua area of New Guinea had no activity against *A. niger* and *C. albicans* (Khan et al., 2001) but the South African (Eastern Cape) ecotype exhibited very good activity against *A. niger* and moderate activity against *Candida albicans*. This in vitro study has shown that extracts from the root of *B. pilosa* were effective against an array of pathogenic microorganisms. However, the extracts were ineffective against *A. flavus*; therefore, the medicinal use of this species in infections associated with this fungus is not recommended.

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Table 2. Antifungal activity of extracts from the root of *Bidens pilosa*.

Concentration (mg/ml)	<i>A. niger</i>	<i>A. flavus</i>	<i>P. notatum</i>
Acetone			
10	100 ^f	45.83 ^b	100 ^b
5	91.67 ^e	0.00 ^a	100 ^b
1	74.44 ^d	0.00 ^a	100 ^b
0.5	68.89 ^c	0.00 ^a	100 ^b
0.1	46.94 ^b	0.00 ^a	100 ^b
Control	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	0.14	10.91	0.05
Methanol			
10	93.33 ^c	76.02 ^b	100 ^b
5	90.28 ^c	0.00 ^a	100 ^b
1	88.06 ^c	0.00 ^a	100 ^b
0.5	80.83 ^b	0.00 ^a	100 ^b
0.1	76.94 ^b	0.00 ^a	100 ^b
Control	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	0.06	6.58	0.05
Water			
10	0.00 ^a	0.00 ^a	100 ^b
5	0.00 ^a	0.00 ^a	100 ^b
1	36.39 ^b	0.00 ^a	100 ^b
0.5	49.72 ^c	0.00 ^a	100 ^b
0.1	67.22 ^d	0.00 ^a	100 ^b
Control	0.00 ^a	0.00 ^a	0.00 ^a
LC ₅₀	0.07	0.00	0.05

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.

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