Phytochemical screening, antibacterial and antifungal activity of *Garuleum woodii* Schinz. root extracts against human pathogenic microbes

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The antimicrobial potential of *Garuleum woodii* root extracts were investigated using 96 wells microplate and agar dilution methods, against selected human pathogenic bacteria and fungi strains. The qualitative phytochemical screening of the extracts revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids and glycosides. The extracts exhibited varied degrees of inhibitions, but the water extract was the most potent against both Gram negative and Gram positive bacteria strains with minimum inhibition concentration (MIC) ranging from 0.09 to 3.13 mg/ml for all the bacteria, except *Escherichia coli* that was inhibited at 12.50 mg/ml. Similarly, the hexane, ethanol and acetone extracts suppressed the growth of *Candida rugosa*, *Cryptococcus neoformans* and *Candida albicans* as well as *Trichophyton mucoides* at MIC ranging from 0.50 to 1.00 mg/ml. The water extract also inhibited these fungi at 10.00 mg/ml. The minimum fungicidal concentration (MFC) of all the extracts was 5.00 to 10.00 mg/ml. The present study has shown that the root extracts of *G. woodii* possessed strong antimicrobial activity against these nosocomial human pathogens, which is an indication of its broad spectrum therapeutic potential. The water extract was the most potent against bacteria, while ethanol extract was effective against fungal infections; therefore, the use of infusion and decoction from the root is encouraged. The overall antimicrobial potency of this species makes it a candidate for antibiotic bioprospecting, particularly for South and southern Africa.

**Key words:** *Garuleum woodii* root extracts, nosocomial, pathogen, broad spectrum, antibiotic bioprospecting.

**INTRODUCTION**

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases (Prabuseenivasan et al., 2006; Ashafa and Afolayan, 2009a). Bacteria for example, have shown a remarkable ability to endure and adapt to their environment by developing 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). One of such plants is *Garuleum woodii*.

*Garuleum woodii* Schinz. is an aromatic plant belonging to the Asteraceae family that is known for several medicinal uses. *G. woodii*, named after John Medley Wood (1827-1915), is a small well branched shrublet or suffrutex growing up to 0.6 m tall. This species is found growing on rocky slopes and cliffs at 1800 - 2000 m or...
higher. It is ecologically distributed around Lesotho, Free State, KwaZulu-Natal (KZN) and Mpumalanga Provinces of South Africa. The stems and leaves are rough with sticky hairs. The leaves are pinnate and glandular on long stalk, and the flower head is showy with pink, blue or white rays (Pooley, 2003). Eight Garuleum species are found in Southern Africa growing in mountains and open fields (Leistner, 2000). Of these species, only G. woodii and Garuleum pinnatifidum are found in the Free State in South Africa. In the folkloric medicine of South Africa, Garuleum species are used in the management of human and livestock diseases (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 2008). These attributes make plants in this genus ethnobotanically important, although in South Africa, only Garuleum bipinnatum has been validated scientifically. It therefore becomes imperative that other Garuleum species be scientifically investigated for their medicinal potentials.

Concerning the antimicrobial activity of these species, there is lack of data in available literature on the antimicrobial activity of the extracts from the subterranean part on G. woodii, particularly from the Eastern Free State, South Africa. This necessitated the study of the in vitro antimicrobial activity of the root extracts of G. woodii against some selected Gram positive, Gram-negative bacteria and fungal species. In this paper, we presented the minimum inhibitory concentrations of the root extracts of G. woodii determined using 96 wells microdilution and agar dilution methods.

**MATERIALS AND METHODS**

**Plant collection**

The plant material was collected in February 2012 from a single population of G. woodii Schinz. growing on the Platberg Mountain (28° 16.2.3 S and 29° 12.38.5 S; altitude 2370 m) in Harrismith, Free State, during one of the series of student’s vegetation studies excursion. The annual rainfall of the areas is about 750 to 1300 mm and the temperature ranges from 17 to 22°C. The species was authenticated by Dr. Erwin Sieben of the Department of Plant Sciences, University of the Free State, Qwaqwa Campus. Herbarium voucher specimen (AshMed/01/2012/QwaHb) was prepared and deposited at the University herbarium. The shoot and root were separated and dried in the oven at 40°C to a constant weight and then pulverized using Waring Commercial Laboratory (Labcon PTY, Durban, South Africa) electric blender.

**Preparation of extracts**

In brief, 40 g each of the powdered root material was extracted in acetone, hexane, ethanol and distilled water, respectively, with shaking on Labcon Platform shaker (Laboratory Consumables, PTY, Durban, South Africa) for 24 h. All extracts were filtered using Whatman No. 1 filter paper. The filtrates from acetone, ethanol and hexane were concentrated under reduced pressure 40°C using (Cole Parmer SB 1100, Shanghai, China) rotary evaporator. Acetone, ethanol and hexane used were of high analytical grade (Merck Chemicals (PTY), Wadeville, South Africa). The water extract was freeze-dried using Virtis Bench Top (SP Scientific Series, USA) freeze dryer. The yields were 2.7, 3.4, 3.6 and 4.2 g for acetone, hexane, ethanol and water, respectively. The hexane extract was dissolved in ethanol and individual extract was reconstituted in their respective solvent to give a stock solution of 50 mg/ml (Taylor et al., 1996).

**Phytochemical screening**

Phytochemical constituents of G. woodii root was determined in the aqueous extract and powdered plant material by adopting standard methods as described by Harborne (1973), Trease and Evans (1989), Sofowora (1993) and Edeoga et al. (2005).

**Test for alkaloids**

Briefly, 0.5 g of the powdered root material was stirred in 5 ml of 1% aqueous hydrochloric acid, heated on a water bath and filtered. Then, 1 ml of the filtrate was treated with few drops of Mayer’s reagent and a second portion was treated same way only with Dragendorff’s reagent. Turbidity of precipitation with either of those reagents was taken as preliminary evidence for the presence of alkaloids in the extract.

**Test for tannins**

In the test for tannins, 0.5 g of dried powdered sample was boiled in 20 ml of water in a test tube and filtered. Few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration as indication of tannins.

**Test for phlobatannins**

In brief, aqueous extract was boiled with 1% aqueous hydrochloric acid and observed for deposition of red precipitate as indication of phlobatannins.

**Test for saponins**

Approximately 2 g of powdered material was boiled in 20 ml of distilled water in a water bath and filtered. Next, 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously again and then observed for the formation of emulsion as indication of saponin.

**Test for flavonoids**

A portion of the powdered material was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Development of yellow colouration is an indication of the presence of flavonoids.

**Test for steroids**

In this test, 2 ml of acetic anhydride was added to 0.5 g of ethanolic extract with 2 ml concentrated H2SO4. The colour change from violet to blue or green is indication of steroids.
Test for terpenoids (Salkowski's test)
In brief, 5 ml of extract was mixed with 2 ml chloroform and 3 ml H$_2$SO$_4$ was carefully added to form a layer. A reddish brown colouration of the interface was indication of terpenoids.

Test for cardiac glycosides (Keller-Kiliani test)
In this test, 5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H$_2$SO$_4$. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout the thin layer.

Choice of solvents
The choices of extracting solvents (acetone, water and ethanol) were based on the fact that: acetone is capable of extracting antimicrobial compounds from plant materials (Ellof, 1998); water is the conventional solvent used in traditional medicine practice all over the world and ethanol/alcohol is the alternative solvent used in many regions to extract active ingredients from plant materials for home use.

Test organisms
Four Gram-positive bacteria namely; Staphylococcus aureus (ATCC6538) Staphylococcus aureus (OK2a) Staphylococcus aureus (OK2b) Bacillus pumilus (ATCC14884) and six (6) Gram-negative bacteria, Escherichia coli (ATCC8739), Shigella flexneri (KZN), Shigella sonnei (ATCC29930), Proteus vulgaris (CSIR0030), Enterobacter faecalis (KZN) and Acinetobacter calcoaceticus antratus (CSIR) 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. 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 15.0 and 20.0 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 x 105 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 for 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).

Antifungal activity assay
Antimycotic activity of G. woodii root extracts was investigated using four fungal species (Candida rugosa, Cryptococcus neoformans, Candida albicans and Trichophyton mucoides). All fungal cultures were maintained on potato dextrose agar (PDA) (Biolab, Johannesburg, South Africa) and were recovered for testing by subculturing on nutrient broth for 24 h 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, 10.0 and 15.0 mg/ml and poured into Petri dishes. Blank plates containing only PDA or PDA solvent served as controls. Organisms were streaked in radial pattern on the actively growing portions of the mother fungal plates and incubated at 25°C for 48 h. The concentration of extract with no observable fungal growth was taken as the minimum inhibitory concentration.

Determination of minimum fungicidal concentration (MFC)
The contents of the tubes that showed no visible fungal growth or turbidity in the minimum inhibitory concentration experiment were cultured into freshly prepared potato dextrose agar plate to assay for the fungicidal effect of the extracts. The plates containing the test organisms were incubated at 25°C. The minimum fungicidal concentration was regarded as the lowest concentration that did not yield any fungal growth on the solid medium used (Irkin and Korikuoglu, 2007)

RESULTS
Phytochemical screening
The qualitative phytochemical screening of G. woodii extract revealed the presence of alkaloids, tannins, saponins, flavonoids, terpenoids and glycosides. Phlobatannins, steroids and anthraquinone were not detected (Table 1).
Table 1. Qualitative phytochemical screening of the root extracts of *G. woodii* Schinz.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Compound</th>
<th>Acetone extract</th>
<th>Ethanol extract</th>
<th>Water extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phlobatannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Anthraquinone</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Positive (+) sign indicates the presence, while negative sign (-) indicates the absence of the compound tested.

Table 2. Minimum inhibitory concentrations (MIC) of the root extracts from *G. woodii* against human pathogenic bacteria.

<table>
<thead>
<tr>
<th>S/No.</th>
<th>Bacteria strain</th>
<th>Gram +/-</th>
<th>Extract (mg/ml)</th>
<th>Acetone</th>
<th>Hexane</th>
<th>Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em> (ATCC 8739)</td>
<td>-</td>
<td>6.25</td>
<td>1.56</td>
<td>3.13</td>
<td>12.50</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td><em>Proteus vulgaris</em> (CSIR 0030)</td>
<td>-</td>
<td>12.50</td>
<td>0.78</td>
<td>1.56</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><em>Enterobacter faecalis</em> (KZN)</td>
<td>-</td>
<td>3.13</td>
<td>1.56</td>
<td>3.13</td>
<td>1.56</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td><em>Shigella flexneri</em> (KZN)</td>
<td>-</td>
<td>15.00</td>
<td>1.56</td>
<td>3.13</td>
<td>3.13</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td><em>Shigella sonnei</em> (ATCC 29930)</td>
<td>-</td>
<td>15.00</td>
<td>1.56</td>
<td>3.13</td>
<td>3.13</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td><em>Acinetobacter calcoaceticus anitratus</em> (CSIR)</td>
<td>-</td>
<td>15.00</td>
<td>1.56</td>
<td>3.13</td>
<td>3.13</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td><em>Bacillus pumilus</em> (ATCC 14884)</td>
<td>+</td>
<td>1.56</td>
<td>1.56</td>
<td>3.13</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td><em>Staphylococcus aureus</em> (OK2a)</td>
<td>+</td>
<td>0.09</td>
<td>1.56</td>
<td>3.13</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td><em>Staphylococcus aureus</em> (ATCC 6538)</td>
<td>+</td>
<td>0.09</td>
<td>1.56</td>
<td>3.13</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td><em>Staphylococcus aureus</em> (OK2b)</td>
<td>+</td>
<td>0.09</td>
<td>1.56</td>
<td>3.13</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

**Antibacterial activity**

The results of minimum inhibitory concentrations of *G. woodii* extracts are presented in Table 2. The acetone extract was more active against Gram positive bacteria, inhibiting all *S. aureus* strains at 0.09 mg/ml except *B. pumilis* that was inhibited at 1.56 mg/ml. The extract was able to suppress the growth of Gram negative bacteria at 3.13, 6.25, 12.50 and 15.00 mg/ml, respectively. The hexane and ethanol extracts were actively more consistent against both Gram positive and Gram negative bacterial strains with MIC ranging from 0.78 to 3.13 mg/ml. However, the water extract was the most active of all the extracts showing MIC range from 0.09 to 3.13 mg/ml.

Generally all the extracts exhibited strong antibacterial activity against all the tested bacteria in the study. Of interest however, is the effectiveness of the water extract against both Gram positive and Gram negative bacteria.

**Antifungal activity**

The hexane and ethanol extracts were able to inhibit all fungal strains at 0.50 mg/ml. While acetone extract inhibited all fungi tested at 1.00 mg/ml, the water extract did the same by inhibiting all fungi at 10.00 mg/ml. The results obtained from the MFC assays showed that acetone, hexane and ethanol extracts were fungicidal against all fungi tested at 5.00 mg/ml, while the water extract consistently killed the tested fungi strains at 10.00 mg/ml (Tables 3 and 4).

**DISCUSSION**

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries of the world and are good source of many potent and powerful drugs (Mahesh and Satish, 2008). In Africa, the use of plant derived remedies in traditional health care practices is common and widespread (Rukangira, 2012) even before the introduction of synthetic antibiotics and other modern drugs (Olajuyigbe and Afolayan, 2012). The problems of microbial drug resistance, increase of opportunistic infections and the toxicity effect of continued use of several antimicrobial agents have
The results from our study have shown that root extracts of *G. woodii* possessed strong antimicrobial activity against the tested bacteria and fungi. Some of the bacteria tested in the present study are known to cause abdominal cramps, diarrhoea, skin and mucosa infection, as well as urinary tract and gastrointestinal infections. Particularly, *A. calcoaceticus* has been reported as the causative agent of meningitis, fulminating septicaemia, pulmonary and ophthalmic infections, chronic synovitis, skin diseases, and wound infections (Pal and Kale, 1981). Likewise, staphylococci are known to cause food poisoning resulting in nausea, vomiting, diarrhoea and dehydration. They are also known as causative agents of impetigo, cellulitis, scalded skin syndrome and mastitis in breast feeding mothers. The results from our study have shown that root extracts from *G. woodii* suppressed the growth of these two bacteria strains at relatively low concentrations. Interestingly, the water extract inhibited *A. calcoaceticus* and the three strains of *Staphylococcus* at MIC of 0.09 to 1.56 mg/ml. Although the different extracts exhibited varied degree of inhibition, previous reports have attributed this to the type of extracting solvents and the plant material involved (Olajuyigbe and Afolayan, 2011). The use of alcohol as extractant is often encouraged to extract antimicrobial compounds from plant materials (Solewa et al., 2010; Sati and Joshi, 2011; Olajuyigbe and Afolayan, 2012). In the present study, it is likely that the antibacterial compounds in *G. woodii* are more soluble in water. Hence, the water extract is the most active against both Gram negative and Gram positive bacteria strains. *Candida* spp. are the most common cause of human fungal infections, representing nearly 96% of all opportunistic mycoses (Pfaller and Diekema, 2007; Pfaller et al., 2007). Up to 2% of intensive care unit (ICU) patients suffer from invasive candidiasis, the incidence of which shows an alarming increase during recent years (Eggimann et al., 2003; Manolakaki et al., 2010). Overall, *Candida* spp. are the 4th most common cause of hospital acquired bloodstream infections in the United States and 7th in Europe, and approximately 10% of all ICU-acquired bloodstream infections are caused by *Candida* spp. (Pfaller et al., 1998; Wisplinghoff et al., 2004; Marchetti et al., 2004). The results from our study have shown that hexane and ethanol extracts suppressed the growth of *C. rugosa*, *C. neoformans* and *C. albicans* as well as *T. mucoides* at 0.50 mg/ml. The water extract was also able to inhibit and kill these organisms at 10.00 mg/ml.

The ability of the root extracts of *G. woodii* to inhibit and kill the bacteria and fungi tested in this study at low concentrations is an indication of its broad spectrum antimicrobial and great therapeutic potential of this species. Although the varied sensitivity may be attributed to the different resistance levels between the microbial strains (Ahmad and Aqil, 2007; Olajuyigbe and Afolayan, 2012), the observed inconsistency in the sensitivity amongst Gram negative and Gram positive bacteria along with the fungal isolates could be ascribed to the anatomical or structural differences between these microorganisms (Olajuyigbe and Afolayan, 2011). Since the plant is growing in the rural area of the eastern Free State occupied by poor people, the use of infusion and decoction is encouraged. Moreover, since most of these

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**Table 3. Minimum inhibitory concentrations (MIC) of the root extracts from *G. woodii* against non-filamentous human pathogenic fungi.**

<table>
<thead>
<tr>
<th>S/N</th>
<th>Fungi strains</th>
<th>Extracts (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
</tr>
<tr>
<td>1</td>
<td><em>Candida rugosa</em></td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td><em>Candida neoformans</em></td>
<td>1.00</td>
</tr>
<tr>
<td>3</td>
<td><em>Candida albicans</em></td>
<td>1.00</td>
</tr>
<tr>
<td>4</td>
<td><em>Trichophyton mucoides</em></td>
<td>1.00</td>
</tr>
</tbody>
</table>

**Table 4. Minimum fungicidal concentrations (MFC) of the root extracts from *G. woodii* against non-filamentous human pathogenic fungi.**

<table>
<thead>
<tr>
<th>S/N</th>
<th>Fungi strains</th>
<th>Extracts (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Acetone</td>
</tr>
<tr>
<td>1</td>
<td><em>C. rugosa</em></td>
<td>5.00</td>
</tr>
<tr>
<td>2</td>
<td><em>C. neoformans</em></td>
<td>5.00</td>
</tr>
<tr>
<td>3</td>
<td><em>C. albicans</em></td>
<td>5.00</td>
</tr>
<tr>
<td>4</td>
<td><em>T. mucoides</em></td>
<td>5.00</td>
</tr>
</tbody>
</table>
people cannot afford the high cost of good alcohol, the best results can be achieved with water as extracting solvent. Further studies are needed in order to properly explore the medicinal potentials of G. woodii Schinz.

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