Antibacterial, antifungal and antitubercular activity of (the roots of) *Pelargonium reniforme* (CURT) and *Pelargonium sidoides* (DC) (Geraniaceae) root extracts

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**Abstract**

Root extracts of *Pelargonium reniforme* CURT and *Pelargonium sidoides* DC were evaluated for antibacterial and antifungal assays using the agar dilution while antitubercular assays were done using the BACTEC method at concentrations ranging from $5 \times 10^3$ to 500.0 mg/L. The ethanol and acetone extracts of the roots of *P. sidoides* inhibited the growth of *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* at a concentration of $5 \times 10^3$ mg/L. Both acetone and ethanol extracts of *P. reniforme* and only the ethanol extract of *P. sidoides* inhibited the growth of *Aspergillus niger* and *Fusarium oxysporum* significantly at a concentration of $5 \times 10^3$ mg/L. Growth of *Rhizopus stolonifer* was suppressed by the ethanol extract of *P. reniforme* and *P. sidoides* at $5 \times 10^3$ and $1 \times 10^3$ mg/L, respectively. Acetone, chloroform and ethanol extracts of *P. reniforme* showed activity against *M. tuberculosis* exhibiting a minimum inhibitory concentration of $5 \times 10^3$ mg/L.

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1. Introduction

The importance of *Pelargonium* species (Geraniaceae) is well documented (Watt and Breyer-Brandwijk, 1962; Hutchings, 1996). The genus *Pelargonium* comprises more than 250 natural species of perennial small shrubs, which are limited in their geographical distribution. About 80% of *Pelargonium* species are confined to the southern parts of Africa, while others occur in Australia, New Zealand and the Far East. These species usually grow in short grassland and sometimes with shrubs and trees on stony soil varying from sand to clay-loam, shale or basalt. The plants are evergreen when cultivated, but die back in nature during droughts and winter (May to August) (Van der Walt and Vorster, 1985).

*Pelargonium reniforme* CURT and *Pelargonium sidoides* DC are highly valued by traditional healers for their curative properties and they are well known to generations of Khoi/San and Xhosa (South African tribes) traditional healers (Wagner and Bladt, 1975). The Xhosa and the Zulu tribes of South Africa use these species to treat coughs, diarrhoea and tuberculosis (Watt and Breyer-Brandwijk, 1962). The medicinally active ingredients are found in the bitter tasting roots of the plants (Helmstadter, 1996). A commonly used medicine produced in Germany, named, ‘Umckaloabo’ originates from the roots of *P. sidoides* and *P. reniforme* (Helmstadter, 1996; Kayser et al., 1998). This herbal medicine is extensively used in Germany for bronchitis, antibacterial and antifungal infections. Although this herbal medicine (Umckaloabo®) is successfully employed in modern phytotherapy in Europe to cure infectious diseases of the respiratory tract, the scientific basis of its remedial effect is still unclear (Kayser and Kolodziej, 1995).

Bacteria, which are associated with either primary or secondary infections of bronchitis, are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. *H. influenzae*, a Gram-negative bacterium, is an obligate human parasite that is passed from person to person by way of the respiratory route. *M. catarrhalis*, a Gram-negative bacterium, causes bronchitis and pneumonia in children and adults. *S. pneumoniae*, a Gram-positive bacterium, infects the upper respiratory tract and can cause pneumonia, also it can infect the...
lining of the brain-spinal cord (meningitis), bones (osteomyelitis), joints (arthritis), ears (otitis media) and sinuses (sinusitis and bronchitis) (Benjamin et al., 1991).

Apergillus niger, Fusarium oxysporum and Rhizopus stolonifer are some of the fungal pathogens that can affect and bronchitis) (MacSween and Whatley, 1992). F. oxysporum is responsible for fusariosis, skin infection, respiratory tract infections (tuberculosis and bronchitis) and arthritis and produces a 76% mortality rate in hospitalised immunocompromised patients (Monier et al., 1994). R. stolonifer causes mucorosis disease and it has been reported that exposure to large numbers of Rhizopus spores can cause respiratory complications (Alexopoulos et al., 1996).

Previously, researchers have reported antimicrobial activity of extracts of Pelargonium and their constituents against a few bacterial (Staphylococcus aureus, S. pneumoniae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and H. influenzae), and fungal (Microsporum canis, M. gypseum, A. fumigatus, Mucor racemosus, R. nigricans) pathogens as well as opportunistic yeasts such as Candida albicans, C. glabrata, C. krusei and Cryptococcus neoformans (Kolodziej, 2000; Kolodziej et al., 2003; Latte and Kolodziej, 2000). Plant extracts of P. reniforme and P. sidoides have not been tested against the fungal pathogens, A. niger, F. oxysporum, R. stolonifer and the Gram-negative bacteria M. catarrhalis, which are indirectly responsible for secondary infections in cases of bronchitis and tuberculosis. In the present study, we have investigated their antimicrobial activity against the bacteria and fungi mainly responsible for bronchitis. We have also confirmed the findings of other researchers on the antibacterial activity of these species against S. pneumoniae and H. influenzae.

Tuberculosis (TB) kills approximately 2 million people each year, the global epidemic is growing and becoming more problematic. The breakdown in health services, the spread of HIV/AIDS and the emergence of multidrug-resistant strains of Mycobacterium tuberculosis (MDR) TB are contributing to the worsening impact of this disease. It is estimated that between 2002 and 2020, approximately a billion people will be newly infected, more than 150 million people will get sick, and 36 million will die of TB. The current threat in TB treatment lies in the emergence of strains resistant to two of the best antitubercular drugs, isoniazid (INH) and rifampicin (RIF). The current TB-treatment comprises of 3–4 drugs for a period of 6–9 months (Bloom, 2002). Novel drugs are required which can shorten this long-treatment period and target multidrug resistant strains of TB. Previous studies have investigated the anti-TB and antimycobacterial activity of the two Pelargonium species. Kolodziej (2000) and Kolodziej et al. (2003) tested acetone extract of both plant species against M. tuberculosis using Alamar blue assay and acetone extracts of P. sidoides using the BACTEC radiometric system, respectively. Seider and Taylor (2004) investigated the two plant species against rapidly growing mycobacteria (M. aurum and M. fortuitum, M. phlei, M. abscessus and M. smegmatis). This is the first report on antibacterial activity of these plants extracted using various solvents such as chloroform, acetone and ethanol against M. tuberculosis using BACTEC radiometric method.

2. Materials and methods

2.1. Plant material

Roots of P. reniforme and P. sidoides were collected from Qwaqwa, a region in the Free State province of South Africa. Voucher specimens of P. reniforme (P 092558) and P. sidoides (P 092559) were deposited and identified at the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria, South Africa.

2.2. Preparation of extracts

Air-dried and powdered roots of P. reniforme and P. sidoides (300 g each) were extracted three times with 1 L of acetone, chloroform and ethanol separately for 2 h at room temperature. The extracts were filtered through Whatman No. 1 filter paper and concentrated with a rotary vacuum evaporator (Büchi Laboratoris, Technik AG, Germany) to dryness at reduced pressure. For antibacterial and antifungal assays, acetone and ethanol extracts were dissolved in acetone to a concentration of $5 \times 10^4$ and $1 \times 10^5$ mg/L, respectively. For the antibacterial assay, all (3) extracts were dissolved in dimethyl sulphoxide (DMSO) to a concentration of $5 \times 10^5$ mg/L.

2.3. Microorganisms and in vitro antimicrobial assays

2.3.1. Bacteria

The bacteria used in this investigation H. influenzae (UPM 2), M. catarrhalis (UPM 4), and S. pneumoniae (UPM 9) were clinical isolates which were obtained from the Department of Pathology, University of Pretoria, South Africa. Cultures were maintained on Colombia agar (Oxoid, Basingstoke, UK) slants supplemented with 5% horse blood to form chocolate agar. For assays, organisms were subcultured once and incubated at 37 °C on Mueller-Hinton (MH), (BIOLAB, Merck, South Africa) agar for 24 h.

2.3.2. Antimicrobial assay

For the antibacterial assay, the minimum inhibitory concentrations (MIC which is defined as the lowest concentration of the extract that inhibits more than 99% of the bacterial population) of the acetone and ethanol extracts were determined by incorporating various amounts ($5 \times 10^3$, $1 \times 10^3$ and $500.0$ mg/L) of the extracts into chocolate agar in sterile bottles and placed in a water bath (50 °C) to prevent solidification, then withdrawn into Petri dishes and left to solidify for 4 h. The bacterial colonies were transferred into the sterile screw-capped
round tubes with glass beads to which 5 ml of the saline (0.9% w/v NaCl) was added for achieving McFarland No. 1 turbidity standard (10^8 CFU/ml). A hundred microlitres of each suspension was smeared on Petri dishes containing the extracts and the chocolate agar. The plates (three replications) were incubated at 37 °C for 24 h and antimicrobial activity was evaluated thereafter. Streptomycin sulphate (Sigma Chemical Co., South Africa) was added to the chocolate agar plates (final concentrations of 500.0, 10.0 and 50.0 mg/L) and served as a positive control. Three Petri dishes containing only 200 μl acetone mixed with chocolate agar served as negative controls. The highest concentration of acetone (4%) did not affect the growth of any of the organisms.

2.3.3. Fungi

The fungal pathogens used in the study, *A. niger* (UPFC 13), *F. oxysporum* (UPFC 97) and *R. stolonifer* (UPFC 312) were from culture collection at the Department of Microbiology and Plant Pathology, University of Pretoria, South Africa. Each fungus was maintained on Potato Dextrose Agar (PDA), (BIOLAB, Merck, South Africa) for 7 days at ±25 °C.

2.3.4. Antifungal assay

For the antifungal assay, the required amount of acetone and ethanol extracts were added to sterile PDA in 5 ml Petri dishes before congealing to yield final concentrations of 5 × 10^3, 1 × 10^3 and 500.0 mg/L. PDA plates with acetone alone inoculated with fungi served as growth controls. Once the agar had solidified, a 5-mm plug of a 7-day-old fungal culture was placed in the centre of the Petri dish containing the extract-amended and unamended PDA plates. The plates were sealed with parafilm and placed in a 25 °C incubator. Fungal growth was measured on two diametric lines after 3, 6 and 9 days of growth. Each treatment was replicated three times and results expressed as the mean of three replicates. The results of 6 days growth was statistically analysed using analysis of variance (ANOVA) and comparison of means by Duncan’s Multiple Range Test. The antifungal agent amphotericin B (Fluka, Germany) added to the agar plates (final concentration of 0.5, 1.0 and 2.0 mg/L) served as a positive control. The highest concentration of acetone (4%) did not affect any of the organisms.

2.4. M. tuberculosis

A drug-susceptible strain of *M. tuberculosis*, H37Rv obtained from American Type, MD, USA Culture Collection (ATCC, 27294), was used (to investigate the activity of the plant extracts).

2.4.1. Antitubercular assay

The radiometric respiratory technique using the BACTEC system was used for susceptibility testing against *M. tuberculosis* as described previously (Lall and Meyer, 2001; Lall et al., 2003). Solutions of all the extracts were prepared in DMSO to achieve the desired final concentrations of 5000.0, 2500.0, 1000.0 and 500.0 mg/L together with PANTA (Becton Dickinson and Company, Ferndale, South Africa), an antimicrobial supplement.

Control experiments showed that a final concentration of DMSO (1%) in the medium had no adverse effect on the growth of *M. tuberculosis*. Streptomycin, isoniazid, rifampicin and ethambutanol (Sigma Chemical Co., South Africa), were used as positive drug controls. A homogenous culture (0.1 ml) of *M. tuberculosis*, yielding 1 × 10^5 to 1 × 10^6 colony-forming units per millilitre (CFU ml⁻¹), was inoculated in the vials containing the extracts as well as in the control vials (Heifets et al., 1985). Three extract-free vials were used as controls (medium +1% DMSO): two vials (V1) were inoculated in the same way as the vials containing the extracts, and one (V2) was inoculated with a 1:100 dilution of the inoculum (1:100 control) to produce an initial concentration representing 1% of the bacterial population (1 × 10^2 to 1 × 10^3 CFU ml⁻¹). The MIC was defined as the lowest concentration of the extract that inhibited >99% of the bacterial population.

*Mycobacterium* growing in 7H12 medium containing 14C-labelled substrate (palmitic acid) use the substrate and produced 14CO₂. The amount of 14CO₂ detected (reflecting the rate and amount of growth occurring in the sealed vial) is expressed in terms of the growth index (GI) (Middlebrook et al., 1977). Inoculated bottles were incubated at 37 °C and each bottle was assayed everyday to measure GI, at about the same hour(s) until cumulative results were interpretable. The difference in the GI values of the last two days is designated as ΔGI. The GI readings of the vials containing the test extracts were compared with the control vials (V2). Readings were taken until the control vials, containing a hundred times lower dilution of the inoculum than the test vials, reached a GI of 30 or more. If the ΔGI values of the vials containing the test extracts were less than the control vials, the population was reported to be susceptible to the compound. Each test was replicated three times.

Whenever results suggested contamination (e.g. large, rapid increase in GI), bottles were inspected and the organisms were stained by Ziehl-Neelsen stain to determine whether the visible microbial growth was a mycobacterial organism (Kleeberg et al., 1980). With this stain, the bacilli appear as brilliantly stained red rods against a deep sky-blue background. Organisms often have a beaded appearance because of their polyphosphate content and unstained vacuoles (Joklik et al., 1968).

Since anecdotal evidence suggests the use of a combination of ethanol extracts of two *Pelargonium* species (1:1) combined ethanol and acetone root extracts from both species were screened for antitubercular activity.

3. Results and discussion

It was found from the antibacterial assay that the ethanol and acetone extracts of *P. sidoides* and its combination (1:1 to investigate additive effect) with *P. reniforme* was active at

BACTEC 12B vials containing 4 ml of 7H12 medium broth to achieve the desired final concentrations of 5000.0, 2500.0, 1000.0 and 500.0 mg/L together with PANTA (Becton Dickinson and Company, Ferndale, South Africa), an antimicrobial supplement.
5 × 10³ mg/L against *H. influenzae*, *M. catarrhalis* and *S. pneumoniae*. Complete inhibition activity of three bacteria on exposure to Streptomycin sulphate was observed at 10.0 mg/L. Kayser and Kolodziej (1997), found moderate activity of *P. sidoides* against *S. pneumoniae* and *H. influenzae* at concentrations of 7.5 × 10² and 5 × 10³ mg/L, respectively, by ethanol (70%) root extracts. There have been few reports of these bacterial organisms being susceptible to other plant extracts. Christoph et al. (2001) found antibacterial activity of Australian tea tree oil from *Melaleuca alternifolia* (Cheel) and niaouli oil isolated from *M. quinquenervia* at 0.01 (%v v⁻¹) against *M. catarrhalis*. We found that acetone extracts of *P. reniforme* were not active against these bacteria at the highest concentration (5 × 10³ mg/L) tested, similar to the findings of Magama et al., 2002, when testing *Euclea crispa*. Essential oils of *P. graveolens* were found to be inactive against *Moraxella* sp. (Lis-Balchin et al., 1998b). Gram-negative bacteria have been found to be less susceptible to plant extracts in earlier studies done by other researchers (Kuhnt et al., 1994; Afolayan and Meyer, 1995). Essential oils from leaves of scented *Pelargonium* species such as *P. graveolens*, *P. tomentosum*, *P. odoratissimum*, *P. denticulatum* and *P. ficifolium* have been found to possess good antibacterial activity against Gram-positive bacteria such as *S. aureus*, *Proteus vulgaris*, *Bacillus cereus*, and *S. epidermidis* (Lis-Balchin et al., 1998a, 2003).

The acetone and ethanol root extracts of *P. reniforme* and ethanol root extract of *P. sidoides* showed activity against the fungal pathogens at a concentration of 5 × 10³ mg/L (Fig. 1a–b). Activity of amphotericin B was observed on each fungi at 0.5 mg/L. Previous in vitro antifungal assays Latté and Kolodziej (2000) had revealed that the aqueous acetone extracts of the roots of *P. reniforme* were less potent exhibiting a MIC of 8 × 10³ mg/L against the filamentous fungi (*Aspergillus fumigatus*, *Rhizopus nigricans*, *Penicillium italicum*) and opportunistic yeasts tested. Lis-Balchin and Deans (1996) assessed the methanolic extracts of representative species and cultivars of *Pelargonium* for activity against 25 different species of bacteria and *A. niger*. All samples were active against at least 18 bacterial species and some were active against all 25 species, although there was very poor antifungal action. Other plant extracts have been found to be antifungal against the fungi tested in this study. Chandrasekaran and Venkatesalu (2004), investigated the water and methanol extracts of *Syzygium jambolanum* for antifungal activity against *A. niger* and *R. stolonifer* and the highest zones of inhibition were recorded at 1 × 10³ and 500.0 mg/L, respec-

![Fig. 1. Antifungal activity of (a) *P. reniforme* acetone and ethanol extract and (b) *P. sidoides* acetone and ethanol extract. Results are expressed as a mean of three replicates and are significantly different. *Values of the bars within the sample concentration not followed by the same letter are significantly different, *P*<0.01. Ac=acetone; Et=ethanol.](image-url)
Table 1

Antitubercular activity of *Pelargonium* root extracts against the drug sensitive strain of *Mycobacterium tuberculosis* (H37Rv) determined by the BACTEC radiometric method

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Sensitive strain</th>
<th>MIC&lt;sup&gt;a&lt;/sup&gt;(mg/L)</th>
<th>AGI&lt;sup&gt;b&lt;/sup&gt; values (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pelargonium reniforme</em> (acetone)</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.5±0.7 (S’)</td>
<td></td>
</tr>
<tr>
<td><em>P. reniforme</em> (chloroform)</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.5±0.7 (S)</td>
<td></td>
</tr>
<tr>
<td><em>P. reniforme</em> (ethanol)</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.5±0.7 (S)</td>
<td></td>
</tr>
<tr>
<td><em>P. reniforme</em>+<em>P. sidoides</em> (acetone)</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>–1.0±2.8 (S)</td>
<td></td>
</tr>
<tr>
<td><em>P. reniforme</em>+<em>P. sidoides</em> (chloroform)</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.0±0.0 (S)</td>
<td></td>
</tr>
<tr>
<td><em>P. reniforme</em>+<em>P. sidoides</em> (ethanol)</td>
<td>5 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.5±0.7 (S)</td>
<td></td>
</tr>
<tr>
<td><em>P. sidoides</em> (acetone)</td>
<td>na&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35.5±6.3 (R’&lt;sup&gt;d&lt;/sup&gt;)</td>
<td></td>
</tr>
<tr>
<td><em>P. sidoides</em> (ethanol)</td>
<td>na</td>
<td>276.0±9.89 (R)</td>
<td></td>
</tr>
<tr>
<td><em>P. sidoides</em> (chloroform)</td>
<td>na</td>
<td>18.5±4.94 (R)</td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>4.0</td>
<td>–5.0±0.0 (S)</td>
<td></td>
</tr>
<tr>
<td>Ethambutol</td>
<td>6.0</td>
<td>0.33±0.0 (S)</td>
<td></td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.2</td>
<td>0.0±0.0 (S)</td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td>2.0</td>
<td>4.0±0.0 (S)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Minimum inhibitory concentration.
<sup>b</sup> AGI value (mean±SD) of the control vial was 20±1.4 for the sensitive strain.
<sup>c</sup> Susceptible.
<sup>d</sup> Not active at highest concentration tested.
<sup>e</sup> Resistant at the highest concentration tested.

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References


