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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 5×10^3 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 5×10^3 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 5×10^3 mg/L. Growth of *Rhizopus stolonifer* was suppressed by the ethanol extract of *P. reniforme* and *P. sidoides* at 5×10^3 and 1×10^3 mg/L, respectively. Acetone, chloroform and ethanol extracts of *P. reniforme* showed activity against *M. tuberculosis* exhibiting a minimum inhibitory concentration of 5×10^3 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

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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 the respiratory tract. *A. niger*, is a causative agent of pulmonary diseases including aspergillosis, bronchial asthma and acute allergic alveolitis. The fungus colonizes old tuberculosis or bronchiostatic cavities, in which it forms a large colony (aspergilloma); or it may actually invade the lung tissue to produce haemorrhagic and necrotizing pneumonia (MacSween and Whaley, 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 *Pelargoniums* 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; Latté 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 antitubercular 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 Laboratories, 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 5×10^4 and 1×10^5 mg/L, respectively. For the antitubercular assay, all (3) extracts were dissolved in dimethyl sulphoxide (DMSO) to a concentration of 5×10^5 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. Antibacterial 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 (5×10^3 , 1×10^3 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 obtain a concentration of 5×10^5 mg/L and stored at 4 °C until used. Subsequent dilutions were made in DMSO and added to

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.

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 1×10^4 to 1×10^5 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 Δ GI. 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 Δ GI 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

5×10^3 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 7.5×10^3 and 5×10^3 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 (5×10^3 mg/L) tested, similar to the findings of Magama et al., 2002, when testing *Euclea crispata*. 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 5×10^3 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 8×10^3 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 1×10^3 and 500.0 mg/L, respec-

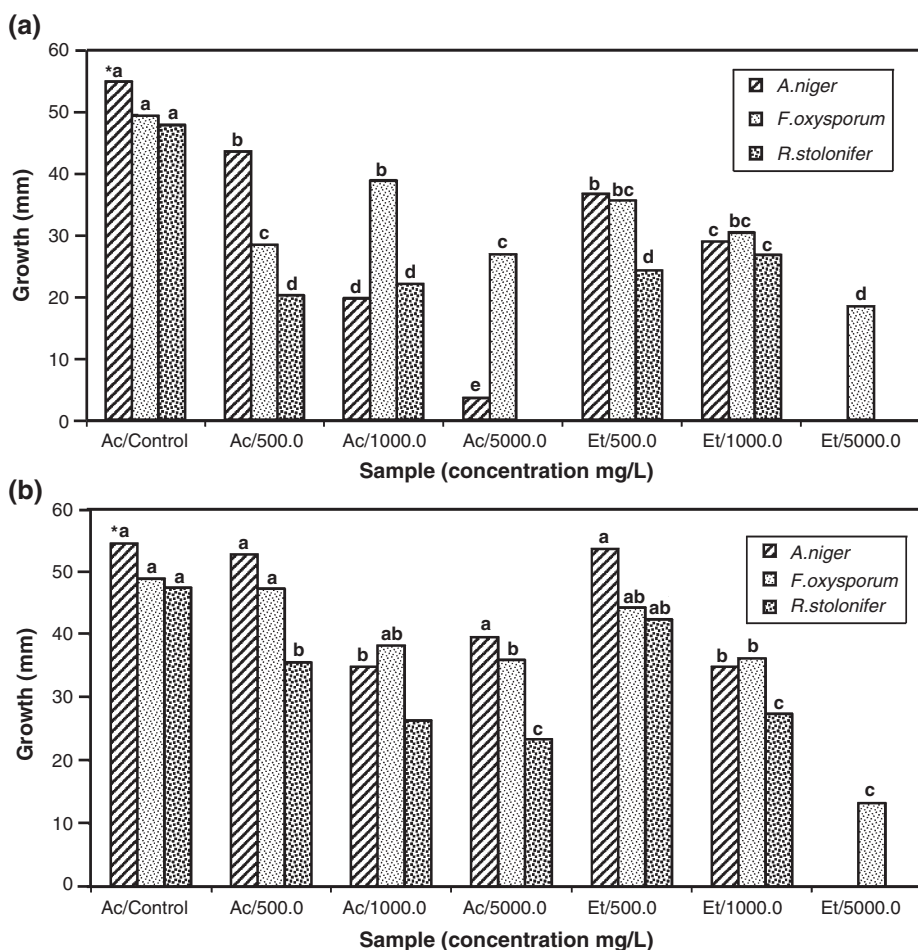


Fig. 1. Antifungal activity of (a) *P. reniforme* acetone and ethanol extract and (b) *P. sidoides* acetone and ethanol extract. Results are expressed as a mean of three replicates and are significantly different. *Values of the bars within the sample concentration not followed by the same letter are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

Table 1

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

Plant species	Sensitive strain	
	MIC ^a (mg/L)	ΔGI ^b values (mg/L)
<i>Pelargonium reniforme</i> (acetone)	5 × 10 ³	1.5 ± 0.7 (S ^c)
<i>P. reniforme</i> (chloroform)	5 × 10 ³	0.5 ± 0.7 (S)
<i>P. reniforme</i> (ethanol)	5 × 10 ³	2.5 ± 0.7 (S)
<i>P. reniforme</i> + <i>P. sidoides</i> (acetone)	5 × 10 ³	−1.0 ± 2.8 (S)
<i>P. reniforme</i> + <i>P. sidoides</i> (chloroform)	5 × 10 ³	1.0 ± 0.0 (S)
<i>P. reniforme</i> + <i>P. sidoides</i> (ethanol)	5 × 10 ³	1.5 ± 0.7 (S)
<i>P. sidoides</i> (acetone)	na ^d	35.5 ± 6.3 (R ^e)
<i>P. sidoides</i> (ethanol)	na	276.0 ± 9.89 (R)
<i>P. sidoides</i> (chloroform)	na	18.5 ± 4.94 (R)
Streptomycin	4.0	−5.0 ± 0.0 (S)
Ethambutol	6.0	0.33 ± 0.0 (S)
Rifampicin	0.2	0.0 ± 0.0 (S)
Isoniazid	2.0	4.0 ± 0.0 (S)

^a Minimum inhibitory concentration.

^b ΔGI value (mean ± SD) of the control vial was 20 ± 1.4 for the sensitive strain.

^c Susceptible.

^d Not active at highest concentration tested.

^e Resistant at the highest concentration tested.

tively. Chamundeewari et al., 2004 found an MIC of 2.5 × 10³ mg/L when an ethanol root extract of *Trewia polycarpa* was tested against *A. niger*.

The antitubercular assay of extracts was interpreted on day five or six when the control vials (V2) reached a GI value of 30 or more (Table 1). Acetone, chloroform and ethanol extracts from the roots of *P. reniforme* showed inhibitory activity at 5 × 10³ mg/L against the drug-sensitive strain of *M. tuberculosis* but the combination of root extracts from both *Pelargonium* species did not show any additive effects. Activities of the standard antituberculosis drugs (streptomycin, ethambutol, rifampicin and isoniazid), used as positive controls, were much stronger than those of the extracts. Our results are in agreement with previous reports on antitubercular activity of water extracts of *Thymus vulgaris*, *Nidorella anomala*, *Cryptocarya latifolia* and acetone extract of *Rapanea melanophloeos* where MICs were also found to be 5 × 10³ mg/L against *M. tuberculosis* (Lall and Meyer, 1999). Extracts of *P. sidoides* were not active against *M. tuberculosis* similar to the results obtained by Fabry et al., 1998 where methanol extracts of *Entada abyssinica*, *Terminalia spinosa*, *Harrisonia abyssinica*, *Ximenia caffra*, *Azadirachta indica* and *Spilanthes mauritiana* were inactive against *M. tuberculosis* at concentrations ranging from 2 × 10³ to 500.0 mg/L. The bacteria and fungi inhibited in this study have been associated with infections of the respiratory tract, which the local inhabitants of South Africa treat using roots of *P. sidoides* and *P. reniforme*. The antimicrobial activity of extracts tested in this study still remains very weak. The fact that extracts inhibited the growth of the bacteria mentioned does not directly provide a direct rationale for the use of the extract in cases of tuberculosis. The reputed benefit of the two *Pelargonium* species in respiratory tract infections may be due in part to a stimulation of the immune system.

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References

- Afolayan, A.J., Meyer, J.J.M., 1995. Antibacterial activity of *Helichrysum aureonitens* (Asteraceae). *Journal of Ethnopharmacology* 47, 109–111.
- Alexopoulos, C.J., Mims, C.W., Blackwell, M., 1996. *Introductory Mycology*, 4th edn. John Wiley and Sons, New York, USA, pp. 868–869.
- Benjamin, D.C., Kadner, R.J., Volk, W.A., 1991. *Essential of Medical Microbiology*. (4th edn) J.B. Lippincott Company, Philadelphia, pp. 41–65.
- Bloom, B.R., 2002. Tuberculosis—the global view. *Journal of Medicine* 346, 1434–1435.
- Chamundeewari, D., Vasantha, J., Gopalakrishnan, S., 2004. Antibacterial and antifungal activities of *Trewia polycarpa* roots. *Fitoterapia* 75, 85–88.
- Chandrasekaran, M., Venkatesalu, V., 2004. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *Journal of Ethnopharmacology* 91, 105–108.
- Christoph, F., Kaulfers, P.M., Stahl-Biskup, E., 2001. In vitro evaluation of the antibacterial activity of beta-triketones admixed to Melaleuca oils. *Planta Medica* 67, 768–771.
- Fabry, W., Okemo, P.O., Ansorg, R., 1998. Antibacterial activity of East African medicinal plants. *Journal of Ethnopharmacology* 60, 79–84.
- Heifets, L.B., Iseman, M.D., Cook, J.L., Lindholm-Levy, P.J., Drupa, I., 1985. Determination of in vitro susceptibility of *Mycobacterium tuberculosis* to cephalosporins by radiometric and conventional methods. *Antimicrobial Agents and Chemotherapy* 27, 11–15.
- Helmstadter, A., 1996. Umckaloabo—Late vindication of a secret remedy. *Pharmaceutical Historian* 26, 2–4.
- Hutchings, A., 1996. *Zulu Medicinal Plants*. University of Natal Press, Pietermaritzburg.
- Joklik, W.K., Willet, H.P., Amos, D.B., 1968. *Mycobacteriaceae*. *Zinsser Microbiology*, 17th edn. Appleton-century-crafts, New York, pp. 651–664.
- Kayser, O., Kolodziej, H., 1995. Highly oxygenated coumarins from *Pelargonium sidoides*. *Phytochemistry* 39, 1181–1185.
- Kayser, O., Kolodziej, H., 1997. Antibacterial activity of extracts and constituents of *Pelargonium reniforme* and *Pelargonium sidoides*. *Planta Medica* 63, 508–510.
- Kayser, O., Latté, K.P., Kolodziej, H., Hammerschmidt, F.J., 1998. Composition of the essential oils from *Pelargonium sidoides* DC and *Pelargonium reniforme* CURT. *Flavour and Fragrance Journal* 13, 209–212.
- Kolodziej, H., 2000. Traditionally used *Pelargonium* species: chemistry and biological activity of umckaloabo extracts and their constituents. *Current Topics in Phytochemistry* 3, 77–93.
- Kolodziej, H., Kayser, O., Radtke, O., Kiderlen, A., Koch, E., 2003. Pharmacological profile of extracts of *Pelargonium sidoides* and their constituents. *Phytomedicine* 10 (Supplement IV), 18–24.
- Kleeberg, H.H., Koornhof, H.J., Palmhart, H., 1980. *Laboratory Manual of Tuberculosis Methods*. Tuberculosis Research Institute, MRC, Pretoria, South Africa.
- Kuhnt, M., Probestle, A., Rimpler, H., Bauer, R., Heinrich, M., 1994. Biological and Pharmacological activities and further constituents of *Hyptis verticillata*. *Planta Medica* 61, 227–232.
- Lall, N., Meyer, J.J.M., 1999. In vitro inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. *Journal of Ethnopharmacology* 66, 347–354.
- Lall, N., Meyer, J.J.M., 2001. Inhibition of drug-sensitive and drug-resistant strains of *Mycobacterium tuberculosis* by diospyrin, isolated from *Euclea natalensis*. *Journal of Ethnopharmacology* 78, 213–216.
- Lall, N., Das Sarma, M., Hazra, B., Meyer, J.J.M., 2003. Antimycobacterial activity of diospyrin derivatives and a structural analogue of diospyrin

- against *Mycobacterium tuberculosis* in vitro. *Journal of Antimicrobial Chemotherapy* 51, 435–438.
- Latté, K.P., Kolodziej, H., 2000. Antifungal effects of hydrolysable tannins and related compounds on dermatophytes, mould fungi and yeasts. *Zeitschrift für Naturforschung (C)* 55, 467–472.
- Lis-Balchin, M., Deans, S.G., 1996. Antimicrobial effects of hydrophilic extracts of Pelargonium species (Geraniaceae). *Letters in Applied Microbiology* 23, 205–207.
- Lis-Balchin, M., Buchbauer, G., Ribisch, K., Wenger, M.T., 1998. Comparative antibacterial effects of novel Pelargonium essential oils and solvent extracts. *Letters in Applied Microbiology* 27, 135–141.
- Lis-Balchin, M., Buchbauer, G., Ribisch, K., Hirtenlehner, T., Resch, M., 1998. Antimicrobial activity of Pelargonium essential oils added to quiche filling as a model food system. *Letters in Applied Microbiology* 27, 1207–1210.
- Lis-Balchin, M., Steyrl, H., Krenn, E., 2003. The comparative effect of novel Pelargonium essential oils and their corresponding hydrosols as antimicrobial agents in a model food system. *Phytotherapy Research* 17, 60–65.
- MacSween, R.N.M., Whaley, K., 1992. *Muir's Textbook of Pathology*, 13th edn. Edward Arnold, London, pp. 35–42.
- Magama, S., Pretorius, J.C., Zietsman, P.C., 2002. Antimicrobial properties of extracts from *Euclea crispa* subsp. *crispa* (Ebenaceae) towards human pathogens. *South African Journal of Botany* 69, 193–198.
- Middlebrook, G., Reggiards, Z., Tigertt, W.D., 1977. Automable radiometric detection of growth of *Mycobacterium tuberculosis* in selective media. *American Review of Respiratory Disease* 115, 1067–1069.
- Monier, M.F., Piens, M.A., Rabodonirina, M., 1994. Fusarium infections in immunocompromised patients: case reports and literature review. *European Journal of Clinical Pharmacology, Infectious Diseases* 13, 152–161.
- Seider, V., Taylor, P., 2004. In vitro activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. *International Journal of Antimicrobial Agents* 23, 613–619.
- Van der Walt, J.J., Vorster, P.J., 1985. *Pelargoniums of Southern Africa*, vol. 3. National Botanical Garden, Kirstenbosch, South Africa.
- Wagner, H., Bladt, S., 1975. Coumarins of South African Plant species, *Pelargonium*. *Phytochemistry* 14, 1061–1064.
- Watt, J.M., Breyer-Brandwijk, M.G., 1962. *The Medicinal and Poisonous Plants of Southern Africa*. E.S. Livingstone, Edinburgh.