Anti-Proteus Activity of Some South African Medicinal Plants: Their Potential for the Prevention of Rheumatoid Arthritis

I. E. Cock,^{a,b*} S.F. van Vuuren^c

*^aEnvironmental Futures Centre, Nathan Campus, Griffith University, 170 Kessels Rd,
Nathan, Queensland 4111, Australia*

*^bBiomolecular and Physical Sciences, Nathan Campus, Griffith University, 170
Kessels Rd, Nathan, Queensland 4111, Australia*

*^cDepartment of Pharmacy and Pharmacology, Faculty of Health Sciences, University
of the Witwatersrand, Parktown, Gauteng, 2193, South Africa.*

**Author of correspondence: Dr. I. E. Cock:*

E-mail: I.Cock@griffith.edu.au

Tel: +61 7 37357637

ABSTRACT

A wide variety of herbal remedies are used in traditional African medicine to treat rheumatoid arthritis (RA) and inflammation. Thirty four extracts from 13 South African plant species with a history of ethnobotanical usage in the treatment of inflammation were investigated for their ability to control two microbial triggers for RA (*P. mirabilis* and *P. vulgaris*). Twenty nine of the extracts (85.3 %) inhibited the growth of *P. mirabilis* and 23 of them tested (67.7 %) inhibited the growth of *P. vulgaris*. Methanol and water extracts of *Carpobrotus edulis*, *Lippia javanica*, *Pelargonium viridiflorum*, *Ptaeroxylon obliquum*, *Syzygium cordatum* leaf and bark, *Terminalia pruinoides*, *Terminalia sericea*, *Warburgia salutaris* bark and an aqueous extract of *W. salutaris* leaf were effective *Proteus* inhibitors, with MIC values < 2000 µg/ml. The most potent extracts were examined by RP-HPLC and UV-Vis spectroscopy for the presence of resveratrol. Only extracts from *T. pruinoides* and *T. sericea* contained resveratrol, indicating it was not responsible for the anti-*Proteus* properties reported here. All extracts with *Proteus* inhibitory activity were also either non-toxic, or of low toxicity in the *Artemia nauplii* bioassay. The low toxicity of these extracts and their inhibitory bioactivity against *Proteus* spp. indicate their potential for blocking the onset of rheumatoid arthritis.

Keywords: Rheumatoid arthritis, *Proteus mirabilis*, *Proteus vulgaris*, inflammation, South African plants, resveratrol.

INTRODUCTION

Rheumatoid Arthritis (RA) is a chronic inflammatory disorder of the joints which afflicts approximately 0.5 - 1 % of the world's population, with approximately three times as many women affected as men (Lawrence et al. 1998). Onset generally occurs in middle to old age, although younger individuals may also be affected. The disorder is characterized by inflammation of the synovial membranes of the joints, causing the joint to become swollen, hot and painful and reducing its mobility. The disease may produce only mild to moderate symptoms in some individuals. In many sufferers it produces severe, disabling pain and a substantial loss of function and mobility if not adequately treated. There is currently no known cure for RA and current treatment strategies aim to either alleviate the symptoms (particularly pain and swelling) with analgesics and anti-inflammatory agents, and/or to somehow modify the disease

process through the use of disease modifying anti-rheumatic drugs (DMARDs). None of these current treatments is ideal as prolonged usage of these drugs is often accompanied by unwanted side effects and toxicity (Alataha et al. 2003). There is still a need to find and develop safer, more effective drugs for the treatment of RA which will not only alleviate the symptoms, but which may also cure or prevent the disease. A greater understanding of the disease's onset and progression should assist in more relevant drug discovery and development.

The causes of RA are currently not well understood. It is generally accepted to be an autoimmune disorder which may be triggered by specific microbial infections in genetically susceptible individuals (individuals with the MHC class 2 allele HLA-DR4) (Nepom et al. 1989). There has been much conjecture as to the nature of the arthritogenic infection(s) and several bacterial (Ebringer and Rashid. 2006; Auger et al. 1996; Holoshitz et al. 1986) and viral (Alvarez-Lafuente et al. 2005) agents have been proposed. The roles of most of these in the pathogenic mechanisms that lead to RA have not been conclusively demonstrated, many being ruled out as causative agents because of contradictory evidence. Indeed, the only bacteria whose link with RA has not been conclusively disproven is that with the *Proteus* species (especially *Proteus mirabilis*) infections. Many studies have documented the presence of elevated serum levels of antibodies specific to *Proteus mirabilis* in individuals suffering from RA (Rashid et al. 1999; Blankenberg-Sprenkels et al. 1998; Chou et al. 1998; Subair et al. 1995; Wanchu et al. 1997; Senior et al. 1995). Furthermore, *Proteus mirabilis* infections have been consistently reported in urine samples from patients with RA (Senior et al. 1999).

The involvement of *Proteus* species as a RA trigger has also been confirmed by immunological experiments which demonstrated that sera from rabbits immunised with HLA-DR4 positive lymphocytes would bind to *Proteus* but not to any of the other 18 microbial species tested (Ebringer et al. 1985). Furthermore, amino acid sequence homologies have been identified between the 'EQ/KRRRAA' motif present in RA HLA-susceptibility antigens and the 'ESRRAL' amino acid sequence present in *P. mirabilis* haemolysins (Ebringer et al. 1992). A further homology between the 'LRREI' sequence of type XI collagen (present in joint cartilage) and the 'IRRET'

motif present in *Proteus* spp. urease enzyme has also been reported (Wilson et al. 1995).

Proteus mirabilis is present in soil and water and also is part of the normal flora of the human gastrointestinal tract. It is not pathogenic in most individuals. It is only when it enters the urinary tract or the bloodstream that it seems to become pathogenic and stimulates an immune response (Coker et al, 2000). It has been postulated that *Proteus* infections (particularly in the urinary tract) induce the production of anti-ESRAAL and anti-IRRET antibodies and these antibodies are cross-reactive with tissues containing EQ/KRRA or LRREI peptide sequences, particularly hyaline cartilage in the joints of the hands and feet (Ebringer and Rashid. 2006). This antibody binding activates a number of downstream processes including inflammatory cascades involving acute phase proteins, components of the complement cascade, vasoactive amines, as well as cytokines, tumour necrosis factor- α (TNF- α) and various other cytotoxic components (Firestein 2003; Choy and Panayi 2001). Furthermore, this antibody's cross-reactivity activates further cellular processes, including those involving the action of natural killer cells. These events subsequently result in the development of *Proteus* reactive RA. Initial infections often result in mild symptoms. Recurrent infections result in enhanced production of cross-reactive antibodies and intensification of the immunological reactions with resulting tissue damage.

Based on the evidence linking *Proteus* bacterial infections with pathogenesis, several lines of research are aimed at the treatment and management of RA by targeting the bacteria itself. One strategy is the development of anti-*Proteus* vaccines. Whilst the production of a vaccine may block proliferation of the *Proteus* species, it is also a problematic approach as anti-*Proteus* antibodies could also cross-react with the host's connective tissue in susceptible individuals, thus exacerbating the symptoms of RA. If antibodies lacking cross-reactive epitopes are developed in the future, this approach may be effective as it would enable susceptible individuals to resist some consequences of a *Proteus* infection. However, the development and usage of *Proteus*-sensitive antibiotics may prove a more effective means of blocking RA initiation/progression. Destroying the *Proteus* bacteria would be expected to greatly reduce the impact of the bacteria on inflammation, diminish the production of anti-*Proteus* antibodies and thus decrease disease progression.

Many antibiotics are already known to inhibit *Proteus* growth, however, the development of super-resistant bacterial strains has reduced their value for treating many bacterial infections (Wang et al. 2009; Liaw et al. 2001). The search is ongoing for new antimicrobials, either by (a) the design and synthesis of new agents, or (b) re-searching the repertoire of natural resources for as yet unrecognised or poorly characterised antimicrobial agents. The antiseptic qualities of medicinal plants have been long recognised by many cultures. Recently there has been a revival of interest in herbal medications due to perceptions that there is often a lower incidence of adverse reactions to natural phytochemicals compared to synthetic pharmaceuticals.

In this study, some Southern African plants were identified which have been traditionally used for the treatment of RA as well as other inflammatory disorders (Table 1). Relevant plant species were extracted and the extracts tested against two *Proteus* species (*P. mirabilis* and *P. vulgaris*) to assess their potential to block their proliferation in vivo. Several of these plant species previously identified as having anti-inflammatory properties have also been extensively studied for their antiseptic properties against other bacteria. For example, the antibacterial activity of *Terminalia sericea* against a wide panel of bacteria has been well documented (Steenkamp et al. 2007; Moshi and Mbwambo 2005). Similarly, *Syzygium cordatum* has broad-spectrum antimicrobial activity (Sibandze et al. 2010; Steenkamp et al. 2007). However, we were unable to locate many published studies reporting any activities of these plants against *Proteus* species. Only *Kigelia africana* bark extract (Jeyachandran and Mahesh 2007) and *Agathosma betulina* extracts (Vermaak et al. 2009; Sandasi 2008) have been shown to have inhibitory activity against *P. vulgaris*. No further studies were found examining the ability of the extracts of these plants to inhibit the growth of *P. mirabilis*, which is more commonly associated with RA. Several other plant species examined in this study are closely related taxonomically with species of known anti-*Proteus* activity. *Ballota africana* has not been examined for anti-*Proteus* activity despite its ethnobotanical uses (Table 1), yet the ability of compounds isolated from the related species *Ballota nigra* have been shown to strongly inhibit *P. mirabilis* growth (Didry et al. 1999). Some other species examined in this study (e.g. *Pentanisia prunelloides*, *Ptaeroxylon obliquum*) have not been carefully examined for their antimicrobial properties despite their long history of treatment for rheumatism.

MATERIALS AND METHODS

Ethnobotanical information

Several literature resources (Table 1) were utilised to identify Southern African plants with a history of usage in the treatment of rheumatism and other inflammatory disorders.

Plant collection and extraction

The majority of the plant species tested in this study were collected from the Walter Sisulu Botanical Gardens in Johannesburg, South Africa (SA). *Syzygium cordatum* and *Lippia javanica* were obtained from Maputaland (KwaZulu-Natal, SA) and *Agathosma betulina* was collected from Landmeterskop (Southern Cape, SA). All plants were identified by Andrew Hankey, chief botanist at the Walter Sisulu Botanical Gardens in Johannesburg, SA or respective authorities in the other provinces. Voucher specimens were prepared and are stored at the Department of Pharmacy and Pharmacology, University of Witwatersrand, Johannesburg, SA. All plant materials were air dried and ground into a fine powder.

One gram of each dried plant material was weighed into each of two tubes. Extracts were prepared by adding either 50 ml of AR grade methanol or 50 ml distilled water to the tubes. Plant material was extracted in each solvent for 24 hours at 4 °C with gentle shaking. The extracts were filtered through filter paper (Whatman No. 54). The methanol extracts were subsequently allowed to dry at room temperature. The aqueous extracts were frozen at -70 °C and dried by lyophilisation. These dried extracts were then weighed and redissolved in 10 ml deionised water.

Antibacterial screening

Test microorganisms

Reference strains of *Proteus mirabilis* (ATCC 43071) and *Proteus vulgaris* (ATCC 33420) were obtained from American Tissue Culture Collection (ATCC). Both species were subcultured and maintained in nutrient broth (Oxoid Ltd, UK) at 4 °C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion method (Cock and Mohanty 2011; Cock 2009). Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh nutrient broth until they reached a count of approximately 10^8 cells/ml. One hundred microliters of this microbial suspension was spread onto nutrient agar plates.

The extracts were tested using 5 mm sterilised filter paper discs impregnated with 10 µl of the test sample, then allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4 °C for 2 hours, allowing for prediffusion of the extract into the agar before incubation with the test microbial agents. The plates were incubated at 30 °C for 24 hours, then the diameters of the inhibition zones were measured in millimetres. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed at least in triplicate and mean values were determined. Standard discs of ampicillin (2 µg) and chloramphenicol (10 µg) were obtained from Oxoid Ltd, UK. and served as positive controls for antimicrobial activity. Filter discs impregnated with either 10 µl of distilled water or methanol were used as negative controls.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentrations (MIC) of the plant extracts were determined by the disc diffusion MIC method (Cock and Kukkonen 2011; Vesoul and Cock 2011) across a range of doses. Briefly, the plant extracts were serially diluted in deionised water across a range of concentrations. Discs were impregnated with 10 µl of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was performed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each extract. Linear regression was used to calculate the MIC values.

Toxicity screening

Reference toxin for biological screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Saarchem, South Africa) was prepared as a 1.6 mg/ml solution in distilled water and serially diluted in artificial seawater for use in the *A. franciscana* nauplii bioassay.

***Artemia franciscana* (brine shrimp) nauplii toxicity screening**

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay (Sirdarta and Cock 2010; Sirdarta and Cock 2008; Cock 2008). *A. franciscana* cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Artificial seawater was prepared using Reef Salt, AZOO Co., USA. Seawater solutions at 34 g/l distilled water were prepared prior to use. 2 g of *A. franciscana* cysts were incubated in 1 L synthetic seawater under artificial light at 25 °C, 2000 Lux with continuous aeration. Hatching commenced within 16-18 h of incubation. Newly hatched *A. franciscana* (nauplii) were used within 10 h of hatching. Nauplii were separated from the shells and remaining cysts and concentrated to a suitable density by placing an artificial light at one end of their incubation vessel. The nauplii-rich water closest to the light was removed for viability assays. 400 µl of seawater containing approximately 37 (mean = 37.2, n = 132, SD = 11.6) nauplii were added to wells within a 48 well plate and immediately used for the bioassay. The plant extracts were first diluted to 4 mg/ml in seawater and then used at a 2 mg/ml concentration in the bioassay. A volume of 400 µl of diluted plant extract and the reference toxin were transferred to the wells and incubated at 25 ± 1 °C under artificial light (1000 Lux). A negative control (400 µl seawater) was included in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead brine shrimp counted. The nauplii were considered moribund if no movement of the appendages was observed within 10 sec. After 72 h all nauplii were sacrificed (by the addition of 50 µl glacial acetic acid) and counted to determine the total number per well. The LC₅₀ with 95 % confidence limits for each treatment was calculated using Probit analysis.

Reverse phase high performance liquid chromatography (RP-HPLC)

Gradient RP-HPLC was performed at a flow rate of 1 mg/ml using a Dionex Ultimate 3000 pump and gradient system, an Ultimate 3000 variable wavelength programmable photodiode array detector (Dionex) and a Phenomenex reverse phase C₁₈ column (150 mm x 4.6 mm). The HPLC system was equipped with Chromeleon software (Dionex). The gradient was constructed as follows: 5 min elution with 5 % acetonitrile, followed by a 30 min gradient to 65 % acetonitrile. The system was then re-equilibrated to 5 % acetonitrile for subsequent chromatograms. The column temperature was maintained at 20 °C throughout. Plant extract samples (10 µl) were

injected manually. Resveratrol (Sigma) was dissolved in ethanol (AR grade), diluted in deionized water and 10 µl aliquots of various concentrations were injected as a standard to calibrate the HPLC column. 10 µl of deionised water was used as a negative control to produce the baseline curve and zero the equipment prior to each experiment.

Statistical analysis

Data are expressed as the mean $\pm$ SEM of at least three independent experiments.

RESULTS

Liquid extraction yields

Extraction of 1 g of the dried plant materials with methanol and water yielded dried plant extracts ranging from 6 mg (*W. salutaris* bark water extract) to 284 mg (*P. obliquum* leaf methanolic extract) (Table 2). No general trend was evident with regards to which solvent was more efficient at extracting material from the plant samples. Methanol extracted a greater amount of material for seven of the sixteen plant samples tested (43.8 %), whereas water extracted the greater amount of material for eight of the sixteen plant samples (50 %). One source provided equal masses of extract with both water and methanol. No obvious correlation was noted between the plant part tested and the amount of extracted material. For two plant species (*Syzygium cordatum* and *Warburgia salutaris*), both bark and leaf materials were extracted. Approximately 5 times as much material was extracted from *Syzygium cordatum* bark than from the leaves by methanol. Similarly, water extracted twice as much material from the bark than from the leaves. In contrast, much more material was extracted from *Warburgia salutaris* leaves than from the bark by both methanol and water (approximately 8 fold and 2 fold higher respectively). With another plant (*Tulbaghia violaceae*), leaf and root materials were extracted and tested. Methanol extracted significantly more material from *Tulbaghia violaceae* leaves than from the roots. However, water extracted much more material from *Tulbaghia violaceae* roots than from the leaves. All dried extracts were resuspended in 10 ml of deionised water, yielding the concentrations shown in Table 2.

Antibacterial activity

Decoctions and tinctures are the main forms in which these plants are traditionally used in ethnobotanical medicinal systems. Therefore methanolic and aqueous extracts were tested undiluted to provide an approximate measure of the efficacy of the form in which the traditional medications would be used. Aliquots (10 μ l) of each extract were screened against *P. mirabilis* (Figure 1a) and *P. vulgaris* (Figure 1b). *P. mirabilis* was susceptible to inhibition by a wider range of plant extracts than was *P. vulgaris*. Indeed, the only plant material for which neither the methanol or water extracts inhibited *P. mirabilis* growth was the *T. violaceae* root extracts. *P. mirabilis* was inhibited by 29 of the 34 (85.3 %) extracts tested compared to 23 of the 34 (67.7 %) extracts tested for *P. vulgaris*. *P. vulgaris* was susceptible to neither the methanol nor the water extracts of *S. cordatum* bark and leaf extracts, *K. africana* leaf extracts, *P. fasciculata* leaf extracts and *T. violaceae* root extracts.

Zones of inhibition >10 mm were considered to indicate significant antibiotic activity (Figure 1). The most effective inhibitors of *P. mirabilis* growth were the water extracts of *Terminalia sericea* and *Terminalia pruinoides* based on the zones of inhibition (17.3 ± 0.6 mm and 15.7 ± 0.6 mm respectively). Indeed, these extracts displayed more potent *P. mirabilis* growth inhibition than either of the control antibiotics (ampicillin and chloramphenicol). The *T. sericea* and *T. pruinoides* methanol extracts also strongly inhibited *P. mirabilis* growth, albeit with smaller zones of inhibition (14.7 ± 0.6 mm and 11.0 ± 1.0 mm respectively) than seen with the water extracts. The *S. cordatum* bark methanol (13.0 ± 1.0 mm) and water (13.7 ± 0.6 mm) extracts, *S. cordatum* leaf methanol (10.0 ± 1.0 mm) and water (13.0 ± 0 mm) extracts, *C. edulis* leaf methanol (11.0 ± 1.0 mm) and water (11.7 ± 0.6 mm) extracts, *K. africana* water extract (10.7 ± 0.6 mm), *L. javanica* methanol extract (11.7 ± 0.6 mm) and the *P. fasciculata* leaf methanol (10.7 ± 0.3 mm) and water (11.3 ± 0.6 mm) extracts all displayed strong inhibitory activity against *P. mirabilis* and thus were considered the most promising.

The *T. sericea* and *T. pruinoides* methanol extracts (15.7 ± 0.6 mm and 12.7 ± 0.6 mm respectively) and water extracts (15.0 ± 0 mm and 14.7 ± 0.6 mm respectively) were similarly effective against *P. vulgaris*. The *S. cordatum* bark methanol (13.3 ± 0.6 mm) and water (12.3 ± 0.6 mm) extracts, *S. cordatum* leaf methanol (10.7 ± 0.6 mm) and water (12.7 ± 0.6 mm) extracts, and the *L. javannica* methanol extract (15.3 ± 0.6

mm) also displayed strong inhibitory activity (> 10 mm) against *P. vulgaris* and thus were considered the most promising.

The relative level of antibacterial activity was further quantified by determining the MIC values for each extract against the bacterial species which were shown to be susceptible by disc diffusion assays. In general, the MIC values against *P. mirabilis* were substantially lower than against *P. vulgaris*, with one notable exception (*P. viridiflorum* leaf methanol extract). The *T. sericea* leaf methanol and water extracts, *T. pruinoidea* leaf methanol and water extracts, *S. cordatum* bark methanol and water extracts, *S. cordatum* leaf methanol and water extracts, *C. edulis* leaf methanol and water extracts and the *L. javanica* leaf methanol extract were again the most effective antibacterial agents against both bacterial species, with MIC values generally below 1000 µg/ml. *S. cordatum* leaf water extract was the most effective anti-*P. mirabilis* agent (as determined by MIC), with a MIC of 49 µg/ml. Although still an effective growth inhibitor of *P. vulgaris*, this extract displayed a higher MIC value of 658 µg/ml.

Quantification of toxicity

The plant extracts were serially diluted in artificial seawater for toxicity testing in the *Artemia* nauplii lethality bioassay. For comparison, the reference toxin potassium dichromate was also tested in the bioassay. Figures 2a and 2b show the % mortality induced in the *Artemia* nauplii following 24 and 48 hours of exposure respectively. Potassium dichromate (reference toxin) rapidly induced mortality within the first 3 hours of exposure (results not shown). After 24 hours exposure, potassium dichromate had killed all of the *Artemia* nauplii. At 24 hours, the only extracts to induce mortality significantly above that of the seawater control were the *P. fasciculata* leaf water extract, *T. violaceae* root water extract and the *T. violaceae* leaf water extract. Although the levels of mortality had increased for most extracts after 48 h exposure (notably for the *L. javanica* leaf methanolic extract and the *W. salutaris* bark methanolic extract), the % mortality was still below 50 % and thus these extracts were considered of low toxicity.

To compare the toxicity of those plant extracts showing significant mortality with other toxins, the LC₅₀ values of the extracts was determined by testing across the concentration range 2000 to 125 µg/ml in the *Artemia nauplii* bioassay. For comparison, potassium dichromate was tested across the same concentration range. Table 4 shows the LC₅₀ values of the extracts which had shown toxicity towards *A. franciscana*. Whilst the *P. fasciculata* leaf water extract initially appeared toxic in the extract mortality screening study (Figure 2), determination of its LC₅₀ demonstrates that it is non-toxic as LC₅₀ values of ≥ 1000 µg/ml have previously been defined as non-toxic in the *Artemia nauplii* bioassay (Meyer et al. 1982). The *T. violaceae* root and leaf water extracts displayed 24 hour LC₅₀ values of approximately 800 µg/ml which is considered to be low toxicity. At 48 hours, both the *T. violaceae* root and leaf water extracts showed slightly higher (although still low) toxicity, both with 48 hour LC₅₀ values of approximately 700 µg/ml. In contrast, potassium dichromate (the positive control) displayed substantially higher toxicity at 24 hours (24 h LC₅₀ 92 µg/ml) and 48 hours (48 h LC₅₀ 85 µg/ml).

RP-HPLC analysis of extracts displaying antimicrobial activity

The *Proteus* growth inhibition and MIC studies indicated that the *L. javanica* leaf, *S. cordatum* bark, *S. cordatum* leaf, *T. pruinoidea* leaf and *T. sericea* leaf extracts were the most promising extracts for further phytochemical characterisation. These were examined for the presence of the phytochemical trans-3,4,5-trihydroxy-trans-stilbene (resveratrol). Resveratrol is a potent phenolic anti-inflammatory antioxidant produced as a phytoalexin (plant antibiotic) by many diverse plant species including grapes, berries, peanuts as well as some species of pine trees, *Acacias* spp., *Terminalias* spp. (including *T. sericea*), orchids and lilies (Kopp, 1998; Polunin et al, 2002; Joseph et al, 2007). The methanol extracts of the plant species displaying anti-*Proteus* activity were further analysed by RP-HPLC chromatography to determine whether they contain resveratrol. However, resveratrol itself did not show anti-*Proteus* activity at levels up to 1000 µg/ml.

Of the plant extracts tested, only the *T. pruinoides* (Figure 3d) and *T. sericea* (Figure 3e) extracts displayed peaks at the same elution volume as pure resveratrol (Figure 3f). The UV/Vis spectra showed that these peaks had spectral properties consistent with that of pure resveratrol (Figure 4g), with absorption maxima at approximately 216 nm and 303 nm. However, both the *T. pruinoides* and the *T. sericea* extracts contained only low levels of resveratrol. None of the other plant extracts gave chromatograms or had UV/Vis spectral properties indicative of resveratrol.

DISCUSSION

Plant remedies are becoming increasingly sought after in the treatment of a myriad of diseases and disorders due both to their perception of greater safety than many synthetic drugs, and the failure of current drug regimes to effectively treat many diseases. This is especially true for rheumatoid arthritis. The current treatments utilising DMARDs to alleviate the symptoms of RA and/or alter the disease progression are not entirely effective and have been associated with numerous adverse effects (Aletaha et al. 2003). Furthermore, many of the current treatments are aimed at treating the symptoms of RA without addressing the underlying causes and pathogenic mechanisms. A better understanding of the mechanisms for initiation and progression of RA is important for developing of new drugs to target specific processes and thus more effectively treat RA.

Although the etiology of RA is not completely understood, the role of *Proteus* spp. (especially *P. mirabilis*) infection as a trigger of RA is now widely accepted due to a wealth of supporting evidence (as reviewed by Ebringer and Rashid 2006). A treatment regime targeting the microbial trigger of RA is an attractive prospect as it would be expected to block/decrease production of self-reactive antibodies linked to disease onset and progression. The results presented here demonstrate the ability of a variety of plants which are used traditionally to treat RA and inflammatory complaints in Southern Africa to inhibit *P. mirabilis* and *P. vulgaris* growth when tested in the form that they would have been used in traditional medicine i.e. decoctions and

tinctures. Further quantification by MIC determination demonstrated the efficacy of these extracts as inhibitors of the possible microbial trigger of RA. *L. javanica* leaf, *S. cordatum* bark and leaf, *T. pruinoides* leaf and *T. sericea* leaf extracts were of particular interest, with MIC's as low as 49 µg/ml for some extracts. Indeed, these plant extracts proved to be substantially stronger than the control antibiotics (ampicillin and chloramphenicol) and are thus worthy of further phytochemical and mechanistic studies. The current study was limited to the inhibitory activity towards a microbial trigger of RA. Many of the plant extracts studied here may also have effects on other inflammatory processes (e.g. cytokine release) and therefore may help control RA by pluripotent/multifaceted mechanisms.

Herbal remedies have long been used to alleviate rheumatic complaints in all traditional medicine systems. In many cases, the major anti-inflammatory components of these plants are available in a pure form and already available as anti-inflammatory agents. These compounds include a variety of polyphenolic compounds, of which 3,5,4'-trihydroxy-trans-stilbene (resveratrol) has received much recent attention due to its presence in many plants used in the treatment of inflammation, and due to its potent anti-rheumatoid activity (Elmali et al. 2007; Wang et al. 2006). Most studies examining the role of resveratrol in the treatment of RA focus on its ability to act as a potent specific inhibitor of NF-κB activating as an inducer for two major inflammatory cytokines, TNF-α and IL-1β (Elmali et al. 2007). Thus, resveratrol treatment is known to block cytokine production and inflammation via its inhibition of NF-κB activation. It is possible that resveratrol may also have other anti-rheumatic effects as it also has known antibacterial activity against a variety of bacterial species (Paulo et al. 2010). Of the plant extracts examined in this study, only the Terminalia species (*T. pruinoides* and *T. sericea*) contained resveratrol, and then only at comparatively low levels.

However, resveratrol did not inhibit the growth of either *P. mirabilis* or *P. vulgaris* in our test systems, even at concentrations up to 1000 µg/ml. Furthermore, we were unable to find any reports of its *in vitro* anti-Proteus growth inhibition. Resveratrol is

reported to inhibit *P. mirabilis* swarming and virulence factor expression *in vivo*, so it may affect *P. mirabilis* colonisation and infection within the urinary tract (Wang et al. 2006) but through mechanisms other than growth inhibition or bactericidal mechanisms. Any extracts inhibiting *Proteus* spp. growth that also contained resveratrol could express anti-RA activity via several mechanisms (growth inhibition, colonisation blocking, cytokine production inhibition) and might be particularly interesting for the treatment of RA. Further studies are required to determine if the levels of resveratrol present in the *Terminalia* spp. examined in this study would be sufficient to inhibit swarming and cytokine production.

The absence of resveratrol in the other plant species which demonstrated anti-*Proteus* activity confirms that the growth inhibitory activity seen for these extracts was induced by different phytochemical components. Compounds from other phytochemical classes including alkaloids, anthraquinones, flavonoids, polyphenolics, phytosterols, saponins, tannins and terpenes have also been linked with anti-bacterial activity in different plant species and thus may be responsible (at least in part) for the anti-*Proteus* activities reported here. The terpenes isomenthone and diosphenol as found in *A. betulina* are well known for their antimicrobial and urinary tract disinfectant properties (van Wyk et al. 2009). *S. cordatum* extracts have been shown to contain pentacyclic triterpenoids, phytosterols, proanthocyanidins and tannins (van Wyk et al. 2009), any of which could be responsible for antimicrobial and anti-inflammatory properties. *Terminalia* spp. and *W. salutaris* are also high in sesquiterpenoids (including polygoidal) and tannins which also have reported antibacterial properties (van Wyk et al. 2009). Similarly, the volatile oils from *L. javanica* and *Pellargonium* spp. are rich in monoterpenoids with strong antibacterial and anti-inflammatory properties (van Wyk et al. 2009). Furthermore, the oils from *Pellargonium* spp. have also been reported to be rich in sesquiterpenes and alkaloids,. The terpenoid and saponin extracts of *P. viridiflorum* are thought to have both antimicrobial and anti-inflammatory activity (van Wyk et al. 2009). Several *C. edulis* and *K. africana* flavonoids have documented antimicrobial activity (van Wyk et al. 2009). Further bioactivity-guided separation studies are recommended to identify the anti-*Proteus* compounds in these plant extracts.

The findings reported here also demonstrate that none of plant extracts tested displayed significant toxicity in the *Artemia* nauplii bioassay. Indeed, of the extracts tested, only the *P. fasciculata* leaf water extract, and the *T. violaceae* root and leaf water extracts induced mortality significantly above that of the negative control. Determination of the LC₅₀ values indicated that only *T. violaceae* extracts displayed LC₅₀ values below 1000 µg/ml. Therefore all other plant extracts are defined as non-toxic as compounds with an LC₅₀ of greater than 1000 µg/ml towards *Artemia* nauplii have been previously defined as being non-toxic (Meyer et al. 1982). Even the *T. violaceae* extracts which did not show significant anti-Proteus activity (with LC₅₀ values > 700 µg/ml) are considered of only low toxicity. It was therefore determined that all extracts tested here were either non-toxic or of low toxicity.

CONCLUSIONS

The antimicrobial efficacies of this study against the anti-inflammatory marker *Proteus* spp. partially validate the usage of these particular plants in traditional South African medicinal systems to treat RA and other inflammatory conditions. They also indicate that the phytochemistry and pharmacology of these plants are worthy of further study, particularly for any other activities associated with the treatment of RA (e.g. inhibition of cytokine production). Whilst the extracts examined in this report are promising as anti-RA agents, caution is needed before these extracts can be applied to medicinal purposes. In particular, further toxicity studies using human cell lines are needed to verify the suitability of these extracts for further clinical studies.

ACKNOWLEDGEMENTS

The authors are most grateful to Andrew Hankey, chief botanist at the Walter Sisulu Botanical Gardens in Johannesburg, South Africa for providing and identifying the plant materials used in these studies. We also thank Professor Charles de Koning and Myron Johnson of the Dept Chemistry, Witwatersrand University for access to their HPLC and for assistance with its operation. Financial support was provided by the

Department of Pharmacy and Pharmacology, University of the Witwatersrand, South Africa.

REFERENCES

- Alataha D, Kapral T, Smolen JS (2003) Toxicity profiles of traditional disease modifying antirheumatic drugs for rheumatoid arthritis. *ARD* 62:482-486.
- Alvarez-Lafuente R, Fernández-Gutierrez B, de Miguel S et al (2005) Potential relationship between herpes viruses and rheumatoid arthritis: analysis and quantitative real time polymerase chain reaction. *ARD* 64: 1357-1359.
- Auger I, Escola JM, Gorvel JP, Roudier J (1996) HLA-DR4 and HLA-DR10 motifs that carry susceptibility to rheumatoid arthritis bind 70-kD heat shock proteins. *Nat Med* 2:306–310.
- Blankenberg-Sprenkels SD, Fielder M, Feltkamp TW et al (1998) Antibodies to *Klebsiella pneumoniae* in Dutch patients with ankylosing spondylitis and acute anterior uveitis and to *Proteus mirabilis* in rheumatoid arthritis. *J Rheumatol* 25:743–7.
- Chou CT, Uksila J, Toivanen P (1998) Enterobacterial antibodies in Chinese patients with rheumatoid arthritis and ankylosing spondylitis. *Clin Exp Rheumatol* 1998;16:161–4.
- Choy EHS, Panayi GS (2001) Cytokine pathways and joint inflammation in rheumatoid arthritis. *New Engl J Med* 344(12):907-916.
- Cock IE (2009) Antibacterial activity of *Eucalyptus major* and *Eucalyptus baileyana* methanolic extracts *Internet J Microbiol* 6(1).
- Cock IE (2008) Assessment of the toxicity of selected Australian native plant extracts using the *Artemia franciscana* nauplii bioassay. *Internet J Toxicol* 5(2).
- Cock IE, Kukkonen L (2011) An examination of the medicinal potential of *Scaevola spinescens*: toxicity, antibacterial and antiviral activities. *Pharmacog Res* 3(2):85-94.
- Cock IE, Mohanty S (2011) Evaluation of the antibacterial activity and toxicity of *Terminalia ferdinandiana* fruit extracts. *Pharmacog J* 3(20):72-79.
- Coker C, Poore CA, Li X, Mobley HLT (2000) Pathogenesis of *Proteus mirabilis* urinary tract infection. *Microbes Infect* 2:1497-1505.

- Didry N, Seidel V, Dubreuil L et al (1999) Isolation and antibacterial activity of polypropanoid derivatives from *Ballota nigra*. J Ethnopharmacol 67:197-202.
- Ebringer A, Cunningham P, Ahmadi K, et al (1992) Sequence similarity between HLA-DR1 and DR4 subtypes associated with rheumatoid arthritis and Proteus/Serratia membrane haemolysins. Ann Rheum Dis 51:1245–1246.
- Ebringer A, Ptaszynska T, Corbett M, et al (1985) Antibodies to Proteus in rheumatoid arthritis. Lancet ii: 305–307.
- Ebringer A, Rashid T (2006) Rheumatoid arthritis is an autoimmune disease triggered by Proteus urinary tract infection. Clin Develop Immunol 13(1): 41-48.
- Elmali N, Baysal O, Harma A et al (2007) Effects of resveratrol in inflammatory arthritis. Inflammation 30:1-6.
- Firestein GS (2003) Evolving concepts of rheumatoid arthritis. Nature 423:356-361.
- Holoshitz J, Klajman A, Drucker I, et al (1986) T lymphocytes of rheumatoid arthritis patients show augmented reactivity to a fraction of mycobacteria cross-reactive with cartilage. Lancet 2:305–309.
- Hutchings A, Scott AH, Lewis G, Cunningham A (1996) Zulu medicinal plants: an inventory. University of Natal Press, Pietmaritzburg, South Africa.
- Jeyachandran R, Mahesh A (2007) Antimicrobial evaluation of *Kigelia africana* (Lam). Res J Microbiol 2(8):645-649.
- Joseph CC, Moshi MJ, Innocent E, Nkunya MHH (2007) Isolation of a stilbene glycoside and other constituents of *Terminalia sericea*. Afr J Tradit Complement and Altern Med 4(4):383-386.
- Kopp P (1998) Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of ‘French paradox’? Eur J Endocrinol 138:619–620.
- Lawrence RC, Helmick CH, Arnett FC et al (1998) Estimates of the prevalence of arthritis and selected musculoskeletal disorders in the United States. Arthritis Rheum 41:778–799.
- Liaw SJ, Lai HC, Ho SW et al (2001) Characterisation of p-nitrophenylglycerol-resistant *Proteus mirabilis* super-swarming mutants. J Med Microbiol 50(12):1039-1048.

- Meyer BN, Ferrigni NR, Putnam JE et al (1982) Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med* 45:31-34.
- Moshi MJ, Mbwambo ZH (2005) Some pharmacological properties of extracts of *Terminalia sericea* roots. *J Ethnopharmacol* 97(1):43-47.
- Nepom GT, Byers P, Seyfried C et al (1989) HLA genes associated with rheumatoid arthritis. *Arthritis and Rheum* 32(1):15-21.
- Paulo L, Ferreira S, Gallardo E et al (2010). Antimicrobial activity and effects of resveratrol on human pathogenic bacteria. *World Journal of Microbiol Biotechnol* 26: 1533-1538.
- Pujol J (1990) *Naturafrica – The herbalists handbook*. Jean Pujol Natural healers foundation, Durban South Africa.
- Polunin KE, Schmalz HG, Polunina IA (2002) Organometallic chemistry. Chromium arene complexes in synthesis of trans-resveratrol. *Russ Chem B+ (International Edition)* 51(7):1319-1324.
- Rashid T, Darlington G, Kjeldsen-Kragh J et al (1999) *Proteus* IgG antibodies and C-reactive protein in English, Norwegian and Spanish patients with rheumatoid arthritis. *Clin Rheumatol* 18:190–195.
- Sandasi M (2008) The effect of plant extracts on biofilm formation and development. M Tech Dissertation; Tshwane University of Technology, Tshwane, South Africa.
- Senior BW, Anderson GA, Morley KD, Kerr MA (1999) Evidence that patients with rheumatoid arthritis have asymptomatic ‘non-significant’ *Proteus mirabilis* bacteriuria more frequently than healthy controls. *J Infect* 38:99–106.
- Senior BW, McBride PD, Morley KD, Kerr MA (1995) The detection of raised levels of IgM to *Proteus mirabilis* in sera from patients with rheumatoid arthritis. *J Med Microbiol* 43:176–84.
- Sibandze GF, van Zyl RL, van Vuuren SF (2010) The anti-diarrhoeal properties of *Breonadia salicina*, *Syzygium cordatum* and *Ozoroa sphaerocarpa* when used in combination in Swazi traditional medicine. *J Ethnopharmacol* 132(2):506-511.

- Sirdaarta J, Cock IE (2010) Effect of *Aloe barbadensis* Miller juice on oxidative stress biomarkers in aerobic cells using *Artemia franciscana* as a model. *Phytother Res* 24: 360-364.
- Sirdaarta J, Cock IE (2008) Vitamin E and TroloxTM reduce toxicity of *Aloe barbadensis* Miller juice in *Artemia franciscana* nauplii but individually are toxic at high concentrations. *Internet J Toxicol* 5(1).
- Steenkamp V, Fernandes AC, van Rensburg CEJ (2007) Antibacterial activity of Venda medicinal plants. *Fitoterapia* 78: 561-564.
- Subair H, Tiwana H, Fielder M et al (1995) Elevation in anti-Proteus antibodies in patients with rheumatoid arthritis from Bermuda and England. *J Rheumatol* 22:1825-8.
- Van Wyk BE, van Oudtshoorn B, Gericke N (2009) *Medicinal plants of South Africa*. 2nd Edition, Briza Publications, Pretoria South Africa.
- Vermaak I, Viljoen AM, Hamman JH, van Vuuren SF (2009) The effect of simulated gastrointestinal conditions on the microbial activity and chemical composition of indigenous South African plant extracts. *S Afr J Bot* 75: 594-599.
- Vesoul J, Cock IE (2011) An examination of the medicinal potential of *Pittosporum phylliraedes*: toxicity, antibacterial and antifungal activities. *Pharmacognosy Communications* 1(2):8-17.
- Wanchu A, Deodhar SD, Sharma M et al (1997) Elevated levels of anti-Proteus antibodies in patients with active rheumatoid arthritis. *Indian J Med Res* 105:39-42.
- Wang M, Guo Q, Xu X et al (2009) New plasmid-mediated quinolone resistance gene qnrC, found in a clinical isolate of *Proteus mirabilis*. *Antimicrob Agents Ch* 53(5):1892-1897.
- Wang WB, Lai HC, Hsueh PR, et al (2006) Inhibition of swarming and virulence factor expression in *Proteus mirabilis* by resveratrol. *J Med Microbiol* 55:1313-1321.
- Wilson C, Ebringer A, Ahmadi K, et al (1995) Shared amino acid sequences between major histocompatibility complex class II glycoproteins, type XI collagen and *Proteus mirabilis* in rheumatoid arthritis. *Ann Rheum Dis* 54:216-220.

Table 1: The ethnobotanical usage and common names of the plant species tested in this study.

Plant Species	Common Name	Part Used Medicinally	Part Used in This Study	Medicinal Use	References
<i>Agathosma betulina</i>	Buchu (English, Khoi), boegoe (Afrikaans), ibuchu (Xhosa).	Leaves	Leaves	For relief of the symptoms of rheumatism, as an antibiotic for cleaning wounds, treating bruises, as a diuretic. Used for the treatment of stomach complaints, kidney and urinary tract diseases.	Watt and Breyer-Brandwijk 1962; van Wyk et al. 2009.
<i>Ballota africana</i>	Kattekruid (Afrikaans)	Leaves	Leaves	For the treatment of arthritis, colds, influenza, measles, fever, asthma, bronchitis, heart and liver disorders.	Watt and Breyer-Brandwijk 1962; van Wyk et al. 2009.
<i>Carpobrotus edulis</i>	Sour fig (English), suurvy, perdevy and vyerank (Afrikaans), gaukum (Khoi)	Leaf juice or leaf pulp	Leaves	Used to treat inflammation, dysentery, digestive troubles, tuberculosis, toothache, earache. The juice is used as an antiseptic for wounds and burns and vaginal thrush.	Watt and Breyer-Brandwijk 1962; van Wyk et al. 2009.

<i>Kigelia africana</i>	Sausage tree (English), worsboom (Afrikaans), modukguhlu (Sotho), muvevha (Venda), umfongothi (Zulu)	Fruit, bark	Leaves	Used for the treatment of rheumatism, dysentry, syphilis, ulcers and sores.	Watt and Breyer- Brandwijk 1962; Hutchings et al. 1996; van Wyk et al. 2009.
<i>Lippia javanica</i>	Fever tea (English), koorsbossie (Afrikaans), mumara (Shona), musukudu, bokhukhwane (Tswana), inzinziniba (Xhosa), umsuzwane (Zulu)	Leaves	Leaves	Used for the treatment of respiratory disorders and inflammation	van Wyk et al. 2009.
<i>Pelargonium fasciculata</i>	Unknown	Leaves, tubers	Leaves	Unknown, although several related species were used to treat diarrhoea, dysentery and inflammation.	van Wyk et al. 2009.
<i>Pittosporum viridiflorum</i>	Cheesewood (English), kasuur (Afrikaans), kgalagangwe (Sotho), umkhwenkhwe (Zhosa), umfusamvu (Zulu)	Bark, leaves	Leaves	Used to treat pain, fever, inflammation	Watt and Breyer- Brandwijk 1962; Hutchings et al. 1996; van Wyk et al. 2009.

<i>Ptaeroxylon obliquum</i>	Sneezewood (English), nieshout (Afrikaans), umthathe (Xhosa, Zulu)	Wood, bark	Leaves	Used to treat arthritis and rheumatism, relief of headache.	Watt and Breyer-Brandwijk 1962; Pujol 1990; Hutchings et al. 1996; van Wyk et al. 2009.
<i>Syzygium cordatum</i>	Waterberry (English), waterbessie (Afrikaans), montlho (Sotho), umdoni (Xhosa, Zulu)	Bark, leaves, roots	Bark, leaves	Used to treat respiratory complaints, asthma, rheumatism, diarrhoea.	Watt and Breyer-Brandwijk 1962; Hutchings et al. 1996; van Wyk et al. 2009.
<i>Terminalia pruinoides</i>	Purple pod cluster leaf (English), sterkbos (Afrikaans), ivikane (Ndebele), muchanana (Shona)	Roots, bark, leaves	Leaves	Used to treat fungal disorders. Used in Somalia to relieve postnatal abdominal pains	Masoko et al. 2005; Orwa et al. 2009.
<i>Terminalia sericea</i>	Silver cluster leaf (English), vaalboom (Afrikaans), mangwe (Ndebele), moxonono (Sotho), mususu (Shona, Venda), amangwe (Zulu)	Roots, bark, leaves	Leaves	Used in the treatment of stomach disorders and diarrhoea, pneumonia, inflammation and diabetes	Watt and Breyer-Brandwijk 1962; Pujol 1990; Hutchings et al. 1996; van Wyk et al. 2009.

<i>Tulbaghia violaceae</i>	Wild garlic (English), wilde knoffel (Afrikaans), isihaqa (Zulu)	Leaves, roots (bulbs)	Leaves, roots (bulbs)	Usually used to treat colds and fever, also used to treat asthma, inflammation, tuberculosis, oesophagus cancer.	Watt and Breyer-Brandwijk 1962; Hutchings et al. 1996; van Wyk et al. 2009.
<i>Warburgia salutaris</i>	Pepper-bark tree (English), peperbasboom (Afrikaans), shibaha (Tsonga), mulanga (Venda), isibhaha (Zulu)	Bark, root bark	Bark, leaves	Treatment of rheumatism, coughs and colds, influenza, malaria, venereal disease, headache, toothache.	Watt and Breyer-Brandwijk 1962; Hutchings et al. 1996; van Wyk et al. 2009.

Table 2: The mass of dried material extracted with water and methanol and the concentration after resuspension in deionised water.

Plant species	Plant part extracted	Solvent	Dried extract (mg)	Resuspended extract concentration (mg/ml)
<i>Agathosma betulina</i>	leaves	methanol	160	16.0
		water	210	21.0
<i>Ballota africana</i>	leaves	methanol	120	12.0
		water	90	9.0
<i>Carpobrotus edulis</i>	leaves	methanol	273	27.3
		water	248	24.8
<i>Kigelia africana</i>	leaves	methanol	40	4.0
		water	40	4.0
<i>Lippia javanica</i>	leaves	methanol	122	12.2
		water	46	4.6
<i>Pelargonium fasciculata</i>	leaves	methanol	96	9.6
		water	151	15.1
<i>Pittosporum viridiflorum</i>	leaves	methanol	212	21.2
		water	270	27.0
<i>Ptaeroxylon obliquum</i>	leaves	methanol	284	28.4
		water	250	25.0
<i>Syzygium cordatum</i>	bark	methanol	270	27.0
		water	220	22.0
<i>Syzygium cordatum</i>	leaves	methanol	50	5.0
		water	110	11.0
<i>Terminalia pruinoides</i>	leaves	methanol	76	7.6
		water	137	13.7
<i>Terminalia sericea</i>	leaves	methanol	220	22.0
		water	210	21.0
<i>Tulbaghia violacea</i>	roots	methanol	20	2.0

		water	140	14.0
<i>Tulbaghia violacea</i>	leaves	methanol	150	15.0
		water	50	5.0
<i>Warburgia salutaris</i>	bark	methanol	11	1.1
		water	6	0.6
<i>Warburgia salutaris</i>	leaves	methanol	82	8.2
		water	101	10.1

Table 3: Minimum inhibitory concentrations ($\mu\text{g/ml}$) of plant water and methanolic extracts against susceptible bacteria.

Species	Plant Part	Solvent	<i>P. mirabilis</i>	<i>P. vulgaris</i>
<i>Agathosma betulina</i>	leaves	methanol	878	-
		water	-	-
<i>Ballota africana</i>	leaves	methanol	4278	-
		water	-	-
<i>Carpobrotus edulis</i>	leaves	methanol	205	670
		water	561	1246
<i>Kigelia africana</i>	leaves	methanol	2483	-
		water	285	-
<i>Lippia javanica</i>	leaves	methanol	313	926
		water	1873	1768
<i>Pelargonium fasciculata</i>	leaves	methanol	806	-
		water	1843	-
<i>Pittosporum viridiflorum</i>	leaves	methanol	765	497
		water	1025	876
<i>Ptaeroxylon obliquum</i>	leaves	methanol	239	511
		water	487	727
<i>Syzygium cordatum</i>	bark	methanol	696	751
		water	932	1325
<i>Syzygium cordatum</i>	leaves	methanol	474	641
		water	49	658

<i>Terminalia pruinoides</i>	leaves	methanol	313	926
		water	224	379
<i>Terminalia sericea</i>	leaves	methanol	417	508
		water	103	520
<i>Tulbaghia violaceae</i>	roots	methanol	-	-
		water	-	-
<i>Tulbaghia violaceae</i>	leaves	methanol	-	-
		water	125	-
<i>Warburgia salutaris</i>	bark	methanol	623	714
		water	450	566
<i>Warburgia salutaris</i>	leaves	methanol	465	-
		water	688	1203

Numbers indicate the mean MIC values of at least triplicate determinations.

– indicates no growth inhibition at the highest concentration tested.

Figure 1: Antibacterial activity of plant water and methanolic extracts measured as zones of inhibition (mm) against (a) *Proteus mirabilis* and (b) *Proteus vulgaris*. Inhibition zones are represented as the means of at least triplicate experiments $\pm$ SEM. 1 = *A. betulina* leaf methanolic extract; 2 = *A. betulina* leaf water extract; 3 = *B. africana* leaf methanolic extract; 4 = *B. africana* leaf water extract; 5 = *C. edulis* leaf methanolic extract; 6 = *C. edulis* leaf water extract; 7 = *K. africana* leaf methanolic extract; 8 = *K. africana* leaf methanolic extract; 9 = *L. javanica* leaf methanolic extract; 10 = *L. javanica* leaf water extract; 11 = *P. fasciculata* leaf methanolic extract; 12 = *P. fasciculata* leaf water extract; 13 = *P. prunelloides* leaf methanolic extract; 14 = *P. prunelloides* leaf water extract; 15 = *P. viridiflorum* leaf methanolic extract; 16 = *P. viridiflorum* leaf water extract; 17 = *P. obliquum* leaf methanolic extract; 18 = *P. obliquum* leaf water extract; 19 = *S. cordatum* bark methanolic extract; 20 = *S. cordatum* bark water extract; 21 = *S. cordatum* leaf methanolic extract; 22 = *S. cordatum* leaf water extract; 23 = *T.*

pruinoides leaf methanolic extract; 24 = *T. pruinoides* leaf water extract; 25 = *T. sericea* leaf methanolic extract; 26 = *T. sericea* leaf water extract; 27 = *T. violaceae* root methanolic extract; 28 = *T. violaceae* root water extract; 29 = *T. violaceae* leaf methanolic extract; 30 = *T. violaceae* leaf water extract; 31 = *W. salutaris* bark methanolic extract; 32 = *W. salutaris* bark water extract; 33 = *W. salutaris* leaf methanolic extract; 34 = *W. salutaris* leaf water extract; 35 = ampicillin (2 µg) control; 36 = chloramphenicol (10 µg) control.

Figure 2: The lethality of plant extracts (2000 $\mu\text{g/mL}$) and potassium dichromate control (1000 $\mu\text{g/mL}$) towards *Artemia nauplii* after (a) 24 hours and (b) 48 hours exposure. All bioassays were performed in triplicate and the means $\pm$ SEM are shown here. 1 = *A. betulina* leaf methanolic extract; 2 = *A. betulina* leaf water extract; 3 = *B. africana* leaf methanolic extract; 4 = *B. africana* leaf water extract; 5 = *C. edulis* leaf methanolic extract; 6 = *C. edulis* leaf water extract; 7 = *K. africana* leaf methanolic extract; 8 = *K. africana* leaf methanolic extract; 9 = *L. javanica* leaf methanolic extract; 10 = *L. javanica* leaf water extract; 11 = *P. fasciculata* leaf methanolic extract; 12 = *P. fasciculata* leaf water extract; 13 = *P. viridiflorum* leaf methanolic extract; 14 = *P. viridiflorum* leaf water extract; 15 = *P. obliquum* leaf methanolic extract; 16 = *P. obliquum* leaf water extract; 17 = *S. cordatum* bark methanolic extract; 18 = *S. cordatum* bark water extract; 19 = *S. cordatum* leaf methanolic extract; 20 = *S. cordatum* leaf water extract; 21 = *T. pruinoides* leaf methanolic extract; 22 = *T. pruinoides* leaf water extract; 23 = *T. sericea* leaf methanolic extract; 24 = *T. sericea* leaf water extract; 25 = *T. violaceae* root methanolic extract; 26 = *T. violaceae* root water extract; 27 = *T. violaceae* leaf methanolic extract; 28 = *T. violaceae* leaf water extract; 29 = *W. salutaris* bark methanolic extract; 30 = *W. salutaris* bark water extract; 31 = *W. salutaris* leaf methanolic extract; 32 = *W. salutaris* leaf water extract; 33 = potassium dichromate control; 34 = deionised water control.

Figure 3: RP-HPLC chromatogram of 10 μ l injections of the methanolic extracts of (a) *L. javanica* leaf, (b) *S. cordatum* bark, (c) *S. cordatum* leaf, (d) *T. pruinoides* leaf, (e) *T. sericea* leaf, (f) resveratrol standard and (g) a representative resveratrol UV/Vis spectrum. Extracts were dried and resuspended in deionised water. Absorbance was recorded at 305 nm and the arrows indicate the resveratrol peaks in the relevant chromatograms. Chromatography conditions were as described in the methods section.