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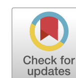
Antimicrobial activity and toxicity of extracts from the bark and leaves of South African indigenous Meliaceae against selected pathogens



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ABSTRACT

Species of the family Meliaceae in traditional medicine are well documented. This study evaluated the antimicrobial efficacy and toxicity of aqueous, methanolic and dichloromethane leaf and bark extracts of South African Meliaceae against selected pathogens. The species sampled represent four of the seven indigenous genera and seven of the 14 indigenous species. Antimicrobial activity was evaluated using the micro-plate dilution assay and the toxicity potential was determined using the brine shrimp lethality assay. About 69% of the extracts investigated showed moderate (0.25— 0.50 mg/ml) activities against the oral pathogens (*Streptococcus mutans* ATCC 25,175 and *Fusobacterium nucleatum* ATCC 25,586) tested. *Pseudomonas aeruginosa* and *S. mutans* was recorded as the most susceptible pathogens to the extracts. The antimicrobial activity of the extracts from *Ekebergia pterophylla, Nymania capensis and Turraea obtusifolia* (here documented for the first time) demonstrated varied activity depending on the pathogen. The aqueous extracts showed no antimicrobial activity with some exceptions against *Streptococcus mutans* (ATCC 25,175), where *Ekebergia capensis* and *Trichilia dregeana* exhibited noteworthy activity (0.13 mg/ml). In the brine shrimp assay, all DCM extracts of the studied parts of the plant species demonstrated minimal to no toxicity levels. The results obtained have lent credence to folkloric usage of some of the South African species of Meliaceae for anti-infective purposes including traditional uses against oral pathogens.

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1. Introduction

Traditional medicinal plant species are rich sources of affordable and novel biologically active compounds which are of great interest, hence studies have been directed toward traditionally used medicinal plants (Hostettmann et al., 2000). Several researchers have reported the antimicrobial efficacy of South African plant species in order to validate the traditional use of medicinal plants in the treatment of infections (Van Vuuren 2008; Van Vuuren and Holl, 2017).

The traditional and contemporary uses of indigenous South African species of the Meliaceae family as medicine are well documented (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Rabe and Van Staden, 1997; Neuwinger, 2000; Kokwaro, 2009; Van Wyk et al., 2009; Komane et al., 2011; Mabona et al., 2013). In addition to medicinal uses, the plants are also used in horticulture (for ornamental purposes and shade), as food, for magical purposes, and for making wooden items and implements. Some uses are recurrent for various species and also for the same species in different parts of Africa. The main recorded medicinal uses of Meliaceae species are as anthelmintics and antimicrobials. They are further used to treat a wide range of medical conditions including cardiovascular and respiratory systems, infections of the urinary and gastrointestinal tract, dermatological conditions and even oral infections (Watt and Breyer-Brandwijk, 1962; Neuwinger, 2000).

Gastrointestinal disorders (GIT) are one of the most frequently recorded infections treated using Meliaceae. Disorders of the GIT are caused by various micro-organisms such as *Pseudomonas aeruginosa, Bacillus cereus, E. coli* and various *Salmonella* and *Shigella* species. Common GIT disorders include dysentery, constipation, vomiting, stomach/abdominal pain, diarrhoea and gastroenteritis (WHO, 2008). They have been reported especially in the developing world where lack of sanitation contribute to morbidity and even mortality (Pawlowski et al., 2009). Contaminated drinking water has claimed millions of lives worldwide, mainly of infants and children due to outbreaks of diarrhoea, dysentery, or cholera (Curtis et al., 2000; Bartlett, 2003). Traditional medicine has been used in developing countries to treat various gastrointestinal problems such as diarrhoea (Lin et al., 2002) and South Africa is no exception. Medicinal plants have been reported for the treatment of various GIT disorders from simple types such as vomiting and peptic ulcers. The traditional treatment of gastrointestinal tract infections using *Ekebergia capensis* Sparrm., *Nymania capensis* (Thunb.) Lindb., *Trichilia dregeana* Sond., *Trichilia emetica* Vahl

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subsp. *emetica, Turraea floribunda* Hochst. and *Turraea obtusifolia* Hochst. have been documented (Van Wyk et al., 1997; Neuwinger, 2000; Kokwaro, 2009;Van Wyk and Gericke, 2018).

Urinary tract infection (UTIs) are clinical conditions ranging from the asymptomatic presence of bacteria, in the urine to severe UTIs with resultant sepsis (Tanagho and Mcaninch, 2004). About 150 million people, mostly women, develop a urinary tract infection yearly (Salvatore et al., 2011; Flores-Mireles et al., 2015). Women are more susceptible to UTIs because of their anatomy and reproductive physiology (Vasudevan, 2014). In childhood, UTIs are common in uncircumcised males less than three months of age and in females less than one year (Bagshaw, and Laupland, 2006). Many bacterial genera are associated with UTIs. Members of the Enterobacteriaceae are the most common organisms isolated from uncomplicated UTIs and *Escherichia coli* has been reportedly known to be the most frequent uropathogen, constituting about 80–85% of UTIs (Amdekar et al., 2011; Chen et al., 2011). *Pseudomonas aeruginosa* and *Enterococcus* species have also been reported to be associated with these infections (Hameed et al., 2017). Medicinal plants play an important role in the treatment of various diseases including UTIs as they act as disinfectants, analgesics, diuretics or as pain relievers. Traditional treatments of UTIs by *N. capensis, T. dregeana, T. emetica, T. floribunda,* and *Turraea nilotica* have been documented (Mabogo, 1990; Pujol, 1990; Bhat and Jacobs, 1995; Hutchings et al., 1996; Neuwinger, 2000; Von Koenen, 2001; Coates-Palgrave, 2002; Van Wyk, 2011).

Oral diseases are major health problems globally (Torwane et al., 2014). High prevalence, severity, and cost of oral healthcare makes it a huge public health problem in South Africa (Singh, 2011). In a review by Van Wyk and Van Wyk (2010), there is an increase in the percentage untreated caries in children between the ages of 12 and 15 and more than 70% of these cases go untreated. Various bacteria are involved in oral infection, including *Streptococcus mutans* and *Fusobacterium nucleatum* (Takarada et al., 2004; Gupti, 2012).

Increased resistance by pathogenic bacteria to currently used antibiotics and other chemotherapeutics, opportunistic infections in immune-compromised individuals, financial considerations and rise in disease incidence particularly in developing countries leads to the need for alternative prevention, treatment options and products for oral diseases that are safe, effective, and economical (Badria and Zidan, 2004; Bidault et al., 2007). Antibiotics used for the treatment of oral diseases have been reported to reduce oral microbiota and have adverse side effects such as vomiting, tooth staining, and diarrhoea (Park et al., 2003). *Trichilia emetica* and *Turraea nilotica* have been reported for the traditional treatment of oral infections (Mathias, 1982; Burkill, 1998; Neuwinger, 2000).

Skin diseases are health problems commonly affecting all ages (Marks and Miller, 2013). It presents a major health burden in both developed and underdeveloped countries. Most bacterial infections of the skin are caused by *Staphylococcus aureus* and *Staphylococcus epidermidis* because they are nearly always present on the skin (Alipour and Khanmohammadi, 2011). Due to increased resistance to antibiotics, plant extracts are of interest as antiseptics and antimicrobial agents. Plants and plant extracts have been reported for the treatment of skin disorders for centuries (Alipour and Khanmohammadi, 2011). *Ekebergia capensis, T. dregeana, T. emetica, T. floribunda* and *T. nilotica*. have been reported as being used in traditional treatments for skin diseases (Komane et al., 2011; Van Wyk, 2011; Mabona and Van Vuuren, 2013; Van Wyk and Gericke, 2018).

Due to the reliance of the African population on plants as sources of medicines, there is a need to evaluate the efficacy and safety of traditional remedies. Hence, this study aimed to determine the antimicrobial efficacy and potential toxicity of aqueous, methanolic and dichloromethane leaf and bark extracts of seven South African indigenous Meliaceae plants against selected pathogens.

2. Materials and methods

2.1. Plant collection and identification

Leaves and bark of seven indigenous species of the Meliaceae family were collected mainly from private gardens in autumn. Plants were identified by experienced taxonomists and botanists (Ben-Erik van Wyk and Geoff Nichols) and voucher specimens are housed at the Herbarium of the University of Johannesburg (JRAU). The species, voucher specimens and site of collection were *Ekebergia capensis* Sparrm. [KK 161–17; Umtentweni, KwaZulu-Natal (KZN)]; *Ekebergia pterophylla* (C.DC.) Hofmeyr [KK 158–17; near Izingolweni, KZN]; *Nymania capensis* (Thunb.) Lindb. [IMH & BEVW 29–16; near Vanwyksdorp, Little Karoo]; *Trichilia dregeana* Sond. [KK 155–17; Renishaw Farm, Scottburgh, KZN]; *Trichilia emetica* Vahl [KK 156–17; St Lucia, KZN]; *Turraea floribunda* Hochst. [KK 154–17; Renishaw Farm, Scottburgh, KZN]; *Turraea obtusifolia* Hochst. [KK 162–17; Southport Village, KZN].

2.2. Extract preparation

2.2.1. Preparation of organic (dichloromethane, methanol) solvent extracts

Leaves and barks were air-dried and pulverized and then milled using a Waring commercial blender to yield a fine powder. Extraction for each plant was carried out by immersing the dried, powdered material (60 g) in 500 ml dichloromethane or methanol. Samples were left in a platform shaker incubator (Labcon) for 24 h. Samples were filtered using a Buchner funnel and Whatman No. 1 filter paper. The residues were re-extracted with fresh solvent for another 24 h. The filtrates were left in a fume hood for the solvent to evaporate. The dried organic extracts were stored in sealed, sterile glass bottles at room temperature for further analysis.

2.2.2. Preparation of aqueous extracts

Aqueous extracts were prepared by immersing 60 g of the powdered plant material in sterile distilled water and left in the platform shaker incubator for 24 h at 25 °C. The liquid extracts were filtered and kept at −80 °C prior to lyophilisation. Lyophilisation was achieved using a freeze dryer (Virtis) for a minimum of 48 h. Prior to use, extracts were left overnight under UV light to eliminate microbial contaminants. All dried aqueous extracts were stored in sealed bottles at room temperature until further use. The final weight of extract obtained for each sample was divided by the weight of the plant material used to prepare each extract (biomass) and then multiplied by hundred to calculate the percentage yield of each extract (Table 2).

2.3. Screening of medicinal plants for antimicrobial activity

Samples were prepared by weighing out the crude extracts and adding a calculated volume of solvent for a final starting concentration of 32 mg/ml for the minimum inhibitoryconcentration (MIC) assays. Acetone was used as a solvent of choice for DCM and methanol extracts as it has been reported to have minimal antimicrobial effects (Eloff, 1998) while sterile, distilled water was used for aqueous extracts. Culture and media preparation were performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2012). The choice of test organisms was based on recorded ethnobotanical uses. American Type Culture Collection (ATCC) microbial strains used in this study include *Bacillus cereus* (ATCC 11,175), *Pseudomonas aeruginosa* (ATCC 27,853), *Enterococcus faecalis* (ATCC 29,212), *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 25,923), *Moraxella catarrhalis* (ATCC 23,246), *Streptococcus mutans* (ATCC 25,175) and *Fusobacterium nucleatum* sub *nucleatum* (ATCC 25,586) (Table 1).

Table 1
Micro-organisms used in this study.

Pathogen	Agar plate for purity assessment	Media for MIC Analysis	Incubation Conditions	Infection
Bacillus cereus (ATCC 11,175)	Mueller-Hinton agar	Tryptone Soya broth	37 °C for 18–24 h	Gastrointestinal
Pseudomonas aeruginosa (ATCC 27,853)	Mueller-Hinton agar	Tryptone Soya broth	37 °C for 18–24 h	Gastrointestinal
Enterococcus faecalis (ATCC 29,212)	Mueller-Hinton agar	Tryptone Soya broth	37 °C for 18–24 h	Urinary tract
Escherichia coli (ATCC 8739)	Mueller-Hinton agar	Tryptone Soya broth	37 °C for 18–24 h	Urinary tract
Staphylococcus aureus (ATCC 25,923)	Mueller-Hinton agar	Tryptone Soya broth	37 °C for 18–24 h	Skin and Gastrointestinal
Moraxella catarrhalis (ATCC 23,246)	Mueller-Hinton agar	Tryptone Soya broth	37 °C for 18–24 h	Respiratory
Streptococcus mutans (ATCC 25,175)	Mueller-Hinton agar with 5% blood	Mueller Hinton broth with 5% blood	37°C for 48 h under CO_2 conditions	Dental Caries
Fusobacterium nucleatum sub *nucleatum* (ATCC 25,586)	Blood agar	Mueller Hinton broth with 5% blood	37°C for 4 days under strict anaerobic conditions	Periodontal Diseases

The MICs were determined using the micro-plate dilution assay with tetrazolium violet reduction as an indicator for growth (Eloff, 1998; CLSI, 2012). Sterilized Mueller Hinton broth (100 μl) was aseptically added to each well of a 96 well micro-titre plate. Then, 100 μl of the respective plant extract (32 mg/ml) was added to the first row. Ciprofloxacin (0.01 mg/ml) was used as the positive control while acetone and water were used as negative controls. The mixture of the extract and broth in the first row was serially diluted down the column (16 to 0.0625 mg/ml). To each well of the micro-titre plate, 100 μl of a 0.5 McFarland's standardized culture suspension (1×10^8 colony forming units -CFU/ml) was added. The plates were then sealed with a sterile adhesive sealing film (AEC Amersham) to prevent evaporation of the test sample, and then incubated at 37 °C for 24 h while the oral pathogens were incubated in a CO_2 enriched environment (Table 1). After incubation, 40 μl of 0.4 mg/ml *p*-iodonitrotetrazolium (INT) was added to each well, the plates were left at room temperature for 1–6 h before reading. In the case of *Streptococcus mutans* and *Fusobacterium nucleatum,* the plates reincubated anaerobically before reading. Development of the pink/red colouration indicated growth of the bacterial cells while clear wells confirmed inhibition. Each strain responds to INT in a different manner. Hence the use of a culture control having only pathogen and INT which was used as a reference point. When the control column turned pink, the remainder of the plate could be read. The culture control also serves as a reference point of culture viability. The experiment was carried out in duplicate and on consecutive days for reproducibility. The lowest concentration at which the plant extract inhibited bacterial growth was considered the MIC.

2.4. Toxicity testing

The brine shrimp lethality assay (BSLA) was used for the toxicity screening in this study (Sarah et al., 2017). Tropic Marine® Sea Salt (16 g) was dissolved in 1 ml of deionised water to make artificial seawater. A sealed bottomless plastic bottle was inversely placed in a glass beaker. The saltwater and brine shrimp (*Artemia franciscana*) eggs (Ocean Nutrition™) (0.5 g) were then added. The saltwater was aerated with a rotary pump (Kiho) and a constant source of light for warmth (220–240 V) to ensure a high hatch rate. Brine shrimp eggs were incubated at 25 °C for 18–24 h. After incubation, the saltwater with brine shrimp was transferred to a shallow plastic container, at an angle, and a light source was placed over for approximately 30 min. This was done so that the brine shrimp move towards the light to increase the sample size of brine shrimp during collection. After 30 min, 48-well micro-titre plates were prepared by adding 400 μl saltwater containing 38–68 live brine shrimp to each well. Plant sample (400 μl of 1 mg/ml) was then added to the wells. Potassium dichromate (1.6 mg/ml) was used as the positive control while saltwater (32 mg/ml) was used as the negative control. Experiments were performed in triplicate. Dead brine shrimp were counted after 24 and 48 h by viewing plates under a light microscope (Olympus) at 40 × magnification. After counting at 48 h a lethal dose of acetic acid (Saarchem; 100% (v/v); 50 μl) was added to each well, and after 30 min, a final count was undertaken, and percentage mortality was evaluated. A mortality percentage of 50% and above was considered toxic (Bussmann et al., 2011).

2.5. Statistical analysis

Data were expressed as means of two replicates and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan multiple test using SPSS. Values were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Percentage yield of different solvent extracts

Highest yield was recorded for methanol, followed by aqueous extracts for both leaf and bark of each sample (Table 2). This result infers that methanol which is both polar and non-polar extracted both the polar and non-polar component in the plant material, water which is a polar solvent was able to extract the polar component while DCM which is fairly polar and strongly non-polar extracted the non-polar component of the plant materials hence the increase in yield from Aqueous to DCM to Methanol.

Table 2
Percentage yield of different solvent extracts of seven indigenous species of South African Meliaceae.

Species	Plant part	Extract yield (% dry wt)		
		Aqueous	Dichloromethane	Methanol
Ekebergia capensis	Leaf	8.3	3.3	9.3
	Bark	5.0	3.3	6.7
Ekebergia pterophylla	Leaf	13.0	8.3	19.7
	Bark	6.7	5.0	8.3
Nymania capensis	Leaf	11.0	3.3	18.3
	Bark	6.7	6.7	10
Trichilia dregeana	Leaf	3.3	3.3	4.7
	Bark	3.3	1.7	3.3
Trichilia emetica	Leaf	3.3	1.7	6.0
	Bark	3.3	1.7	3.3
Turraea floribunda	Leaf	6.2	3.3	9.0
	Bark	3.3	1.7	5.0
Turraea obtusifolia	Leaf	6.3	3.3	10.3
	Bark	5.0	3.3	6.7

3.2. Antimicrobial activity

The antimicrobial activity of plant extracts (bark and leaf of each plant) were studied against eight pathogens (Table 1). MIC average results of the DCM, methanol and aqueous extracts are represented in Table 3. MIC values (≤0.16 mg/ml) considered to be noteworthy are marked in bold while MIC values (> 0.16 -≤1.00 mg/ml) considered to be moderate are in italics as shown in Table 3 (Van Vuuren and Holl, 2017). An MIC value of ≥ 8.00 mg/ml was given to plant extracts where no end point was observed as this was the highest concentration tested. The MIC values varied between test pathogens for the positive control (ciprofloxacin), with a range of 0.08–0.63 ug /ml, hence showing that the conventional antimicrobial was responsive. On the other hand, the negative controls (acetone and water) did not inhibit the growth of any of the test pathogens. The DCM leaf extracts of all samples showed best activity against all pathogens tested with noteworthy (MIC value of 0.13 mg/ml) activity against *P. aeruginosa* and mostly moderate activity (MIC value range of 0.25–0.50 mg/ml) against the two oral pathogens tested (*S. mutans* and *F. nucleatum*). Most of the DCM leaf extracts had moderate activity against *B. cereus*. The DCM bark extracts also showed noteworthy (MIC value of 0.13 mg/ml) activity against *P. aeruginosa* and moderate activity against *S. mutans* and *F. nucleatum.* The methanol leaf extracts had moderate activity (MIC value range of 0.25 - 0.50 mg/ml) against *S. mutans, P. aeruginosa*, and *F. nucleatum*. The methanol bark extracts had moderate activity against *S. mutans* and *P. aeruginosa*. The aqueous extracts showed no activity (MIC value ≥ 8.00) against all pathogens tested except against *S. mutans,* where *E. capensis* and *E. pterophylla* had noteworthy (0.13 mg/ml) activity (Table 3). Bagla et al. (2012), reported the antimicrobial activity of DCM leaf extracts of *E. capensis* against *S. aureus* (0.13 mg/ml), *E. coli* (0.16 mg/ml), *E. faecalis* (0.32 mg/ml), and *P. aeruginosa* (0.32 mg/ml). Mabona et al. (2013), also reported MIC of 0.50 mg/ml of DCM leaf extracts of *E. capensis* against *S. aureus* and MIC of 1 mg/ml against *P. aeruginosa*. Both studies reported negligible activity for the aqueous leaf extracts of *E. capensis*. These are congruent with the current study except that this study recorded weak activity against *E. faecalis* (Table 3). The DCM bark extract of *E. capensis* had noteworthy activity (Table 3) against *P. aeruginosa* (0.13 mg/ml), and moderate activity against *B. cereus* (0.50 mg/ml) and *S. mutans* (0.50 mg/ml) in this study. The activity of DCM extracts in this study (Table 3) agrees with the report of Mabona et al. (2013), where MIC values of 0.38–2 mg/ml were reported for the DCM extract of *E. capensis* against different strains of *S. aureus*. The methanol leaf extract of *E. capensis*had moderate antimicrobial activity with MIC of 0.50 mg/ml against the two oral pathogens (*S. mutans* and *F. nucleatum*) and were moderately active against *B. cereus, E. coli, M. catarrhalis* and *P. aeruginosa* at an MIC value of 1.00 mg/ml. (Table 3). Bagla et al. (2012), recorded noteworthy activity (MIC of 0.16 mg/ml) for the methanol leaf extract of *E. capensis* against *S. aureus, E. coli, E. faecalis* and *P. aeruginosa.* This activity is not supported in this study as the MIC value of 1.00 mg/ml was recorded against *E. coli* and *P. aeruginosa,* and an MIC of 2.00 mg/ml was recorded against *S. aureus* and *E. faecalis* (Table 3).This variation could be due to plant locality and the different strains of pathogens used in each study. The methanol bark extract had moderate activity (MIC of 0.50 mg/ml) against *B. cereus, M. catarrhalis,* and *S. mutans*, and an MIC value of 1.00 mg/ml against *E. faecalis, S. aureus, E. coli*, *P. aeruginosa* and *F. nucleatum* (Table 3). Rabe and Van Staden (1997) and Ngeny et al. (2013) reported weak activity (MIC of 2.00–4.00 mg/ml) against *S. aureus, E. coli,* and *P. aeruginosa. Ekebergia capensis* has been reported to be used in the treatment of various diseases of the gastrointestinal tract, the urinary tract and the skin (Van Wyk et al., 1997; Neuwinger, 2000; Kokwaro, 2009; Van Wyk and Gericke, 2018). Hence, this study confirms some traditional uses of *E. capensis*. Moreover, this study reports for the first time the antimicrobial activity of *Ekebergia* species against oral pathogens.

Moderate activity of the DCM leaf extract of *E. pterophylla* was recorded against *B. cereus, P. aeruginosa, S. mutans*, and *F. nucleatum*. The DCM bark extract also had noteworthy activity against *P. aeruginosa* and moderate activity against *B. cereus, S. aureus, M. catarrhalis, S. mutans* and *F. nucleatum* (Table 3). Methanol extracts of *E. pterophylla* (leaf and bark) had weak activity (MIC of 1.00–2.00 mg/ml) against all test pathogens except *P. aeruginosa* and *S. mutans* where moderate activity was noted (Table 3). The aqueous extract had no activity (≥ 8.00) against all test pathogens except *S. mutans* where the aqueous leaf extract of *E. pterophylla* showed noteworthy activity (Table 3). This study recorded the antimicrobial activity of *E. pterophylla* for the first time and its strong activity against oral pathogens was noted.

Nymania capensis is applied traditionally for wound healing (Von Koenen, 2001; Mabona and Van Vuuren, 2013). The leaf infusion is also reported to be used in treating influenza (Arnold et al., 2002). In this study, the DCM leaf extract of *N. capensis* had moderate activity against *B. cereus, S. mutans* and *F. nucleatum* while the DCM bark extract had noteworthy activity (Table 3) against *P. aeruginosa* and moderate activity against *S. mutans* and *F. nucleatum*. Both leaf and bark methanol extracts had weak activity (MIC of 2.00–4.00 mg/ml) against all the pathogens tested except for *P. aeruginosa, S. mutans* and *F. nucleatum* (Table 3). Aqueous extracts had no activity (≥ 8.00 mg/ml) against all pathogens tested except *S. mutans,* where both leaf and bark extracts of *N. capensis* had weak (MIC of 4.00 mg/ml) activity (Table 3). This study supports the traditional uses of *N. capensis* in treating skin diseases and GIT infections. The antimicrobial activity of *N. capensis* is here reported for the first time, including the moderate activity against oral pathogens.

Bark infusions of *T. dregeana* were reported to be drunk or used as an enema for diarrhoea, stomach complaints, kidney pain, bronchial inflammation and for skincare (Arnold and Gulumian, 1984; Mabogo, 1990; Hutchings et al., 1996; Coates-Palgrave, 2002; Van Wyk, 2011). The DCM leaf extract of *T. dregeana* had moderate activity against *B. cereus, S. mutans,* and *F. nucleatum* (Table 3). The bark extract showed moderate activity against *B. cereus* and *F. nucleatum* (Table 3). In a study by Mthethwa (2009), methanol extracts of *T. dregeana* had weak activity (> 6.25 mg/ml) against *E. coli*, and *S. aureus.* This is congruent with this study, except that the methanol leaf extract had moderate to weak activity against *E. coli* and *S. aureus* respectively (Table 3). Methanol leaf extract had moderate activity against *M. catarrhalis, P. aeruginosa* and *F. nucleatum,* with *S. mutans* having the highest antimicrobial activity (Table 3). In line with other studies (Eldeen et al., 2005; Mthethwa, 2009; Naidoo et al., 2013), aqueous extracts of *T. dregeana* had no antimicrobial activity except against *S. mutans* where moderate activities were observed for both aqueous leaf and bark extract of *T. dregeana* (Table 3). Also, moderate inhibition against oral pathogens was observed.

The DCM leaf extract of *T. emetica* showed noteworthy activity against *P. aeruginosa* and moderate activity against *S. aureus, S. mutans* and *F. nucleatum*. The DCM bark extract of *T. emetica* had moderate activity only against *F. nucleatum* (Table 3). The methanol extract showed moderate activity against *B. cereus, E. faecalis* and *S. mutans* (Table 3). This study is in line with previous antimicrobial reports on *T. emetica* (Germano et al., 2005; Shai et al., 2008; Mabona et al., 2013; Konate' et al., 2015; Van Vuuren et al., 2015; Kouitcheu Mabeku et al., 2017). The activity observed against *S. mutans* and *F. nucleatum* supports the traditional use of *T. emetica* as chewing sticks for dental care (Neuwinger, 2000). This study also supports traditional uses of *T. emetica* in treating various GIT and UTIs (Watt and Breyer-Brandwijk, 1962; Pujol, 1990; Hutchings et al., 1996; Van Wyk et al., 2009; Komane et al., 2011).

Kuglerova et al. (2011) reported the antimicrobial activity of ethanol extract of *Turraea floribunda* with MIC values of 0.256 mg/ml and 0.5 mg/ml. Traditionally, the bark of *T. floribunda* is boiled in water and drunk to treat urinary tract infections or used as an emetic or

Table 3
Minimum inhibitory concentration (mg/ml) of extracts from seven indigenous species of South African Meliaceae against selected pathogens.

Plant samples	Extracts	Mean MIC value (mg/ml) $n = 2$							
		Bacillus cereus ATCC 11,175	*Enterococcus faecalis* ATCC 29,212	*Staphylococcus aureus* ATCC 25,923	*Escherichia coli* ATCC 8739	*Moraxella catarrhalis* ATCC 23,246	*Pseudomonas aeruginosa* ATCC 27,853	*Streptococcus mutans* ATCC 25,175	*Fusobacterium nucleatum* ATCC 25,586
Ekebergia capensis leaf	DCM*	*0.50*^{b,c}	2.00^{e}	1.00^{c}	1.00^{b}	1.00^{b,c}	*0.19*^{b}	*0.25*^{c}	*0.50*^{c}
	Methanol	1.00^{d}	2.00^{e}	2.00^{e}	1.00^{b}	1.00^{a,b}	1.00^{f}	*0.50*^{d}	*0.50*^{c}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	**0.13**^{a,b}	>8.00 g
Ekebergia capensis bark	DCM	*0.50*^{b,c}	4.00^{f}	1.00^{c}	2.00^{c}	2.00^{d}	**0.13**^{a,b}	*0.50*^{d}	1.00^{d}
	Methanol	*0.50*^{b,c}	1.00^{b,c}	1.00^{c}	1.00^{b}	*0.50*^{a,b}	1.00^{f}	*0.50*^{d}	1.00^{d}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	*0.50*^{d}	>8.00 g
Ekebergia pterophylla leaf	DCM	*0.50*^{b,c}	4.00^{f}	2.00^{e}	4.00^{e}	2.00^{d}	*0.50*^{e}	*0.25*^{c}	*0.50*^{c}
	Methanol	2.00^{f}	2.00^{e}	2.00^{e}	1.00^{b}	2.00^{d}	2.00 g	*0.25*^{c}	1.00^{d}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	**0.13**^{a,b}	>8.00
Ekebergia pterophylla bark	DCM	*0.25*^{a,b}	1.00^{b,c}	*0.50*^{b}	2.00^{c}	*0.50*^{a,b}	**0.13**^{a,b}	*0.25*^{c}	*0.50*^{c}
	Methanol	2.00^{f}	2.00^{e}	1.00^{c}	1.00^{b}	1.00^{a,b}	*0.25*^{c}	*0.25*^{c}	1.00^{d}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	1.00^{e}	>8.00 g
Nymania capensis leaf	DCM	*0.50*^{b,c}	2.00^{e}	1.00^{c}	>8.00^{f}	1.50^{c,d}	1.00^{f}	*0.25*^{c}	*0.25*^{b}
	Methanol	2.00^{f}	2.00^{e}	2.00^{e}	8.00^{f}	2.00^{d}	*0.50*^{e}	*0.25*^{c}	1.00^{d}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	4.00 g	>8.00 g
Nymania capensis bark	DCM	1.00^{d}	>8.00 g	2.00^{e}	>8.00^{f}	1.50^{c,d}	**0.13**^{a,b}	*0.50*^{d}	*0.50*^{c}
	Methanol	2.00^{f}	2.00^{e}	2.00^{e}	4.00^{e}	2.00^{d}	*0.50*^{e}	*0.25*^{c}	*0.50*^{c}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	4.00 g	>8.00 g
Trichilia dregeana leaf	DCM	*0.50*^{b,c}	4.00^{f}	2.00^{e}	>8.00^{f}	2.00^{d}	1.00^{f}	*0.50*^{d}	*0.50*^{c}
	Methanol	8.00^{d}	1.50^{d}	2.00^{e}	1.00^{b}	*0.50*^{a,b}	*0.50*^{e}	*0.25*^{c}	*0.50*^{c}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	*0.50*^{d}	>8.00 g
Trichilia dregeana bark	DCM	*0.50*^{b,c}	2.00^{e}	1.00^{c}	2.00^{c}	1.00^{a,b}	>8.00^{h}	1.00^{e}	*0.50*^{c}
	Methanol	1.00 g	4.00^{f}	8.00 g	4.00^{e}	2.00^{d}	1.00^{f}	1.00^{e}	2.00^{e}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	*0.50*^{d}	>8.00 g
Trichilia emetica leaf	DCM	1.00^{d}	2.00^{e}	*0.50*^{b}	>8.00^{f}	1.00^{a,b}	**0.13**^{a,b}	*0.50*^{d}	*0.50*^{c}
	Methanol	1.00^{e}	*0.50*^{a}	2.00^{e}	1.00^{b}	1.50^{c,d}	1.00^{f}	*0.50*^{d}	4.00^{f}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	*0.25*^{c}	>8.00 g
Trichilia emetica bark	DCM	2.00^{f}	>8.00 g	2.00^{e}	>8.00^{f}	2.00^{d}	>8.00^{h}	1.00^{e}	*0.25*^{b}
	Methanol	*0.50*^{b,c}	2.00^{c,d}	4.00^{f}	2.00^{c}	2.00^{d}	1.00^{f}	*0.50*^{d}	1.00^{d}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	8.00^{h}	>8.00 g
Turraea floribunda leaf	DCM	*0.75*^{c,d}	2.00^{e}	1.00^{c}	4.00^{e}	1.00^{a,b}	**0.13**^{a,b}	*0.25*^{c}	*0.50*^{c}
	Methanol	2.00^{f}	2.00^{e}	2.00^{e}	4.00^{e}	2.00^{d}	*0.50*^{e}	*0.25*^{c}	*0.50*^{c}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	2.00^{f}	>8.00 g
Turraea floribunda bark	DCM	1.00^{d}	4.00^{f}	2.00^{e}	>8.00^{f}	2.00^{d}	**0.13**^{a,b}	*0.50*^{d}	*0.25*^{b}
	Methanol	2.00^{f}	2.00^{e}	2.00^{e}	2.00^{c}	2.00^{d}	*0.50*^{e}	*0.25*^{c}	4.00^{f}
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00^{f}	>8.00^{f}	>8.00^{h}	2.00^{f}	>8.00 g
Turraea obtusifolia leaf	DCM	*0.75*^{c,d}	2.00^{e}	2.00^{e}	4.00^{e}	1.00^{a,b}	**0.13**^{a,b}	*0.25*^{c}	*0.25*^{b}
	Methanol	2.00^{f}	2.00^{e}	2.00^{e}	2.00^{c}	2.00^{d}	1.00^{f}	*0.25*^{c}	1.00^{d}

(*continued on next page*)

Table 3 (*Continued*)

Plant samples	Extracts	Mean MIC value (mg/ml) $n = 2$							
		Bacillus cereus ATCC 11,175	*Enterococcus faecalis* ATCC 29,212	*Staphylococcus aureus* ATCC 25,923	*Escherichia coli* ATCC 8739	*Moraxella catarrhalis* ATCC 23,246	*Pseudomonas aeruginosa* ATCC 27,853	*Streptococcus mutans* ATCC 25,175	*Fusobacterium nucleatum* ATCC 25,586
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00[f]	>8.00[f]	>8.00[h]	2.00[f]	>8.00 g
Turraea obtusifolia bark	DCM	1.00[d]	8.00 g	2.00[e]	>8.00[f]	2.00[d]	*0.38*[d]	*0.25*[c]	*0.50*[c]
	Methanol	2.00[f]	2.00[e]	2.00[e]	2.00[d]	3.00[e]	1.00[f]	*0.50*[d]	1.00[d]
	Aqueous	>8.00 g	>8.00 g	>8.00 g	>8.00[f]	>8.00[f]	>8.00[h]	4.00 g	>8.00 g
Acetone		>8.00 g	>8.00 g	>8.00 g	>8.00[f]	>8.00[f]	>8.00[h]	>8.00[h]	>8.00 g
Ciprofloxacin (ug/ml)		0.08[a]	0.63[a,b]	0.16[a]	0.31[a]	0.31[a]	0.08[a]	0.08[a]	0.16[a]

*DCM = Dichloromethane.

Values are means of two determinations and values bearing different superscripts down the columns are significantly different at $P < 0.05$.

purgative (Watt and Breyer-Brandwijk 1962; Bryant 1966; Bhat and Jacobs, 1995; Kokwaro, 2009). Although water is used for extraction traditionally, this study reports no antimicrobial activity of the aqueous extracts of *T. floribunda* against all test pathogens except for *S. mutans* (MIC of 2.00 mg/ml), where weak activity was observed (Table 3). Both DCM leaf and bark extract of *T. floribunda* had noteworthy activity against *P. aeruginosa* and moderate activity against *S. mutans* and *F. nucleatum* (Table 3). *Turraea obtusifolia* was reported to be taken with porridge as an enema, traditionally for treating stomach and intestinal complaints (Bryant 1966; Hutchings et al., 1996). In this study, the DCM leaf extract of *T. obtusifolia* had noteworthy activity against *P. aeruginosa* and moderate activity against *S. mutans* and *F. nucleatum*. These activities support the traditional uses of *T. obtusifolia* and apparently represent the first record of antimicrobial activity in this species.

Very poor activity was observed for most of the aqueous extracts against all the test pathogens except *S. mutans,* where 57.14% of the leaf extracts exhibited noteworthy to moderate (MIC value range of 0.13–0.50 mg/ml) activity (Table 3). A few (28.57%) of the aqueous bark extracts also showed moderate activity against *S. mutans* (Table 3). This seems significant as aqueous extracts also exhibited a level of activity against this oral pathogen (*S. mutans*).

3.3. Toxicity

All the plants and plant parts tested in this study were deemed safe to use at a concentration of 1 mg/ml using the brine shrimp lethality assay. Table 4 shows the average percentage of mortalities ($n = 3$) of brine shrimp exposed to each plant extract. All percentage mortality rates observed were below 50%, hence all the plant extracts investigated were considered non-toxic according to Bussmann et al. (2011).

Trichilia dregeana (leaf and bark) and *T. floribunda* were found to have the least effect on the brine shrimp with zero percentage mortality recorded at both 24 and 48 h (Table 4). *Trichilia dregeana* and *T. floribunda* have been reported to be of importance in traditional medicine (Van Wyk, 2011; Van Wyk and Gericke, 2018) but this study records for the first time the apparent absence of toxicity at 1 mg/ml.

Previous studies (Ngeny, 2012; Bagla et al., 2012; Irungu et al., 2014) reported the toxicity of *E. capensis* using African monkey kidney (Vero cells), renal cell line (CRFK cells), bovine dermis cell, liver carcinoma cell line (Hep2 cells), and mouse breast cancer cells (4T1). Ngeny (2012) reported relatively low toxicity for the leaf extract and constituents using Vero cells, 4T1 and Hep2 cells. In this study, <10% mortality of the brine shrimp at both 24 and 48 h was observed (Table 4), thus confirming low toxicity. The leaf extract of *E. pterophylla* had zero percentage mortality at 24 h and <10% at 48 h. There seem to be no earlier reports on the toxicity of *E. pterophylla*. In previous studies, no toxicity was observed for different plant parts of *T. emetica* (Germano et al., 2005; Traore et al., 2007; Bero et al., 2009). These results are supported by our study, as no mortality of the brine shrimp was observed for *T. emetica* bark at 24 h and <10% mortality of the brine shrimp by the leaf both at 24 and 48 h (Table 4). This study reported no toxicity (percentage mortality <10) for the leaf and bark of *T. obtusifolia* at 24 and 48 h (Table 4). None of the plants are listed in standard reviews of the poisonous plants of South Africa (*e.g.*, Van Wyk et al., 2002).

In this study, we noted the DCM extracts showed the best antimicrobial activity compared to other extracts studied which could suggest that the active components in these plants are more non-polar than polar.

4. Conclusion

The data presented in this study have lent credence to folkloric usage of the evaluated South African species of the Meliaceae family

Table 4
Percentage mortality of brine shrimp after exposure to DCM extracts (1 mg/ml) from seven indigenous species of South African Meliaceae.

Plant names and parts used	Percentage mortality ($n = 3$)	
	24 h	48 h
Ekebergia capensis leaf	1.56	1.56
Ekebergia capensis bark	1.72	1.72
Ekebergia pterophylla leaf	0.00	6.79
Ekebergia pterophylla bark	1.59	1.63
Nymania capensis leaf	3.33	18.94
Nymania capensis bark	2.63	23.92
Trichilia dregeana leaf	0.00	0.00
Trichilia dregeana bark	0.00	0.00
Trichilia emetica leaf	2.38	2.38
Trichilia emetica bark	0.00	1.61
Turraea floribunda leaf	1.67	1.67
Turraea floribunda bark	0.00	0.00
Turraea obtusifolia leaf	2.22	2.22
Turraea obtusifolia bark	2.50	2.50
Potassium dichromate (1.6 mg/ml)	61.39	100
Dimethyl sulfoxide (DMSO)	2.94	2.94

as antimicrobial agents. Our data also suggest the active component of the studied species are more polar than non-polar. Of importance is the moderate to noteworthy inhibition observed for selected extracts against oral pathogens. Based on the brine shrimp toxicity test, this study suggests a low risk for the indigenous applications of these plant extracts and their uses in the local treatment of oral infection. Nevertheless, further research is essential to isolate and identify the active constituents responsible for these activities.Cell line studies and *in vivo* toxicological assessments of the test extracts are also needed to explore the full range of toxicity.

Declaration of Competing Interest

None.

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References

Alipour, M., Khanmohammadi, O., 2011. Antibacterial activity of plant extracts against oral and skin pathogens. Afr. J. Microbiol. Res. 5, 2909–2911.
Amdekar, S., Singh, V., Singh, D.D., 2011. Probiotic therapy: immunomodulating approach toward urinary tract infection. Curr. Microbiol. 63, 484–490.
Arnold, H.J., Gulumian, M., 1984. Pharmacopoeia of traditional medicine in Venda. J. Ethnopharmacol. 12, 35–74.
Arnold, T.H., Prentice, C.A., Hawker, L.C., Snyman, E.E., Tomalin, M., Crouch, N.R., Pottas-Bircher, C., 2002. Medicinal and Magical Plants of Southern Africa: an Annotated Checklist. National Botanical Institute, Pretoria.
Badria, F.A., Zidan, O.A., 2004. Natural products for dental caries prevention. J. Med. Food 7, 381–384.
Bagla, V.P., McGaw, L.J., Eloff, J.N., 2012. The antiviral activity of six South African plants traditionally used against infections in ethno veterinary medicine. Vet. Microbiol. 155, 198–206.
Bagshaw, S.M., Laupland, K.B., 2006. Epidemiology of intensive care unit-acquired urinary tract infections. Curr. Opin. Infect. Dis. 19, 67–71.
Bartlett, S., 2003. Water, sanitation and urban children: the need to go beyond "improved" provision. Environ. Urban. 15, 57–70.
Bero, J., Ganfon, H., Jonville, M.C., Frédérich, M., Gbaguidi, F., DeMol, P., Moudachirou, M., Quetin-Leclercq, J., 2009. *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J. Ethnopharmacol. 122, 439–444.
Bhat, R.B., Jacobs, T.V., 1995. Traditional herbal medicine in Transkei. J. Ethnopharmacol. 48, 7–12.
Bidault, P., Chandad, F., Grenier, D., 2007. Risk of bacterial resistance associated with systemic antibiotic therapy in periodontology. J. Can. Dent. Assoc. 73, 721–725.
Bryant, A.T., 1966. Zulu Medicine and Medicine-men. C. Struik, Cape Town.
Burkill, H.M., 1998. The Useful Plants of West Tropical Africa, second ed. Royal Botanic Gardens, Kew, United Kingdom.
Bussmann, R.W., Malca, G., Glenn, A., Sharon, D., Nilsen, B., Parris, B., Dubose, D., Ruiz, D., Saleda, J., Martinez, M., Carillo, L., 2011. Toxicity of medicinal plants used in traditional medicine in Northern Peru. J. Ethnopharmacol. 137, 121–140.
Chen, Y.H., Hsueh, P.R., Badal, R.E., Hawser, S.P., Hoban, D.J., Bouchillon, S.K., Ni, Y., Paterson, D.L., 2011. Antimicrobial susceptibility profiles of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections in the Asia Pacific region according to currently established susceptibility interpretive criteria. J. Infect. 62, 280–291.
Clinical and Laboratory Standards Institute, 2012. Performance standards for antimicrobial susceptibility testing; Approved standard, eight ed. CLSI Document M11-A8, 2012. CLSI, Wayne, PA.
Coates-Palgrave, K., 2002. Trees of Southern Africa, 3rd Edition Struik, Cape Town.
Curtis, V., Cairncross, S., Yonli, R., 2000. Domestic hygiene and diarrhoea – pinpointing the problem. Trop. Med. Int. Health 5, 22–32.
Eldeen, I.M.S., Elgorashi, E.E., Van Staden, J., 2005. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. J. Ethnopharmacol. 102, 457–464.
Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64, 711–713.
Flores-Mireles, A.L., Walker, J.N., Caparon, M., Hultgren, S.J., 2015. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat. Rev. Microbiol. 13, 269–284.
Germano, M.P., D'Angelo, V., Sanogo, R., Catania, S., Alma, R., De Pasquale, R., Bisignano, G., 2005. Hepatoprotective and antibacterial effects of extracts from *Trichilia emetica* Vahl (Meliaceae). J. Ethnopharmacol. 96, 227–232.
Gupti, S., 2012. The Short Textbook of Medical Microbiology for Medical Students. Jaypee Brothers Medical Publishers, New Delhi.
Hameed, I.H., Al-Rubaye, A.F., Kadhim, M.J., 2017. Antimicrobial activity of medicinal plants and urinary tract infections. Int. J. Pharm. Clin. Res. 9, 44–50.
Hostettmann, K., Marston, A., Ndjoko, K., Wolfender, J.L., 2000. The potential of African plants as a source of drugs. Curr. Org. Chem 4, 973–1010.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants: an inventory. University of Natal Press, Durban.
Irungu, B., Orwa, J., Gruhonjic, A., Fitzpatrick, P., Landberg, G., Kimani, F., Midiwo, J., Erdélyi, M., Yenesew, A., 2014. Constituents of the roots and leaves of *Ekebergia capensis* and their potential antiplasmodial and cytotoxic activities. Molecules 19, 14235–14246.
Kokwaro, J.O., 2009. Medicinal Plants of East Africa. University of Nairobi Press, Nairobi.
Komane, B.M., Olivier, E.I., Viljoen, A.M., 2011. *Trichilia emetica* (Meliaceae) – a review of traditional uses, biological activities and phytochemistry. Phytochem. Lett. 4, 1–9.
Konate', K., Yomalan, K., Sytar, O., Brestic, M., 2015. Antidiarrhoeal and antimicrobial profiles extracts of the leaves from *Trichilia emetica* Vahl. (Meliaceae). Asian Pac. J. Trop. Biomed. 5, 242–248.
Kouitcheu Mabeku, L.B., Eyoum Bille, B., Tchouangueu, T.F., Nguepi, E., Leundji, H., 2017. Treatment of *Helicobacter pylori* infected mice with *Bryophyllum pinnatum*, a medicinal plant with antioxidant and antimicrobial properties, reduces bacterial load. Pharm. Biol. 55, 603–610.
Kuglerova, M., Tesarova, H., Grade, J.T., Halamova, K., Wanyana-Maganyi, O., Van Damme, P., Kokoska, L., 2011. Antimicrobial and antioxidative effects of Ugandan medicinal barks. Afr. J. Biotechnol. 10, 3628–3632.
Lin, J., Puckree, T., Mvelase, T.P., 2002. Anti-diarrheal evaluation of some medicinal plants used by Zulu traditional healers. J. Ethnopharmacol. 79, 53–56.
Mabogo, D.E.N., 1990. The Ethnobotany of the VhaVenda. University of Pretoria, Pretoria.
Mabona, U., Van Vuuren, S.F., 2013. Southern African medicinal plants used to treat skin diseases. S. Afr. J. Bot. 87, 175–193.
Mabona, U., Viljoen, A., Shikanga, E., Marston, A., Van Vuuren, S., 2013. Antimicrobial activity of Southern African medicinal plants with dermatological relevance: from an ethnopharmacological screening approach, to combination studies and the isolation of a bioactive compound. J. Ethnopharmacol. 148, 45–55.
Marks, J.G., Miller, J.J., 2013. Lookingbill and Marks' Principles of Dermatology, sixth ed. Elsevier Inc..
Mathias, M.E., 1982. Some medicinal plants of the Hehe (Southern Highlands Pprovince, Tanzania). Taxon 31, 488–494.
Mthethwa, N.S., 2009. Antimicrobial Activity Testing of Traditionally Used Plants for Treating Wounds and Sores at Ongoye area KwaZulu-Natal, South Africa. University of Zululand, KwaZulu-Natal.
Naidoo, D., Van Vuuren, S.F., Van Zyl, R.L., De Wet, H., 2013. Plants traditionally used individually and in combination to treat sexually transmitted infections in Northern Maputaland, South Africa: antimicrobial activity and cytotoxicity. J. Ethnopharmacol. 149, 656–667.
Neuwinger, H.D., 2000. African Traditional Medicine: a Dictionary of Plant Use and Applications. Medpharm Scientific Publications, Stuttgart, Germany.
Ngeny, L.C., 2012. Evaluation of Antimicrobial Activity and Toxicity of Crude Extracts from Selected Kenyan Medicinal Plants. Jomo Kenyatta University of Agriculture and Technology, Kenya.
Ngeny, L.C., Magiri, E., Mutai, C., Mwikwabe, N., Bii, C., 2013. Antimicrobial properties and toxicity of *Hagenia abyssinica* (Bruce) JF Gmel, *Fuerstia africana* TCE Fries, *Asparagus racemosus* (Willd.) and *Ekebergia capensis* Sparrm. Afr. J. Pharmacol. Therap. 2, 76–82.
Pawlowski, S.W., Warren, C.A., Guerrant, R., 2009. Diagnosis and treatment of acute or persistent diarrhoea. Gastroenterology 136, 1874–1886.

Park, K.M., You, J.S., Lee, H.Y., Baek, N.I., Hwang, J.K., 2003. Kuwanon G: an antibacterial agent from the root bark of *Morus alba* against oral pathogens. J. Ethnopharmacol. 84, 181–185.
Pujol, J., 1990. Nature Africa, The Herbalist Handbook. Jean Pujol Natural Healers' Foundation, Durban.
Rabe, T., Van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56, 81–87.
Salvatore, S., Salvatore, S., Cattoni, E., Siesto, G., Serati, M., Sorice, P., Torella, M., 2011. Urinary tract infections in women. Eur. J. Obstetr. Gynaecol. Reprod. Biol. 156, 131–136.
Sarah, Q.S., Anny, F.C., Misbahuddin, M., 2017. Brine shrimp lethality assay. Bangladesh J. Pharmacol. 12, 186–189.
Shai, L.J., McGaw, L.J., Masoko, P., Eloff, J.N., 2008. Antifungal and antibacterial activity of seven traditionally used South African plant species active against *Candida albicans*. S. Afr. J. Bot. 74, 677–684.
Singh, S., 2011. Dental caries rates in South Africa: implications for oral health planning. S. Afr. J. Epidemiol. Infect. 26, 259–261.
Takarada, K., Kimizuka, R., Takahashi, N., Honma, K., Okuda, K., Kato, T., 2004. A comparison of the antibacterial efficacies of essential oils against oral pathogens. Oral Microbiol. Immunol. 19, 61–64.
Tanagho, E.A., Mcaninch, J.W., 2004. Smith's general urology. Bacterial Infections of the Genitourinary Tract. McGraw-Hill, New York, USA.
Torwane, N.A., Hongal, S., Goel, P., Chandrashekar, B.R., 2014. Role of Ayurveda in management of oral health. Pharmacognosy Rev. 8, 16–21.
Traore, M., Zhai, L., Chen, M., Olsen, C.E., Odile, N., Pierre, G.I., Bosco, O.J., Robert, G.T., Christensen, S.B., 2007. Cytotoxic kurubasch aldehyde from *Trichilia emetica*. Nat. Prod. Res. 21, 13–17.
Van Vuuren, S.F., 2008. Antimicrobial activity of South African medicinal plants. J. Ethnopharmacol. 119, 462–472.
Van Vuuren, S.F., Nkwanyana, M.N., De Wet, H., 2015. Antimicrobial evaluation of plants used for the treatment of diarrhoea in a rural community in Northern Maputaland, KwaZulu-Natal, South Africa. BMC Complement. Altern. Med. 15, 1–8.
Van Vuuren, S., Holl, D., 2017. Antimicrobial natural product research: a review from a South African perspective for the years 2009–2016. J. Ethnopharmacol. 208, 236–252.
Van Wyk, B.-.E., Van Oudtshoorn, B., Gericke, N., 1997. Medicinal Plants of South Africa. Briza Publications, Pretoria.
Van Wyk, B.-.E., Heerden, F.R., Van Oudtshoorn, B., 2002. Poisonous Plants of South Africa. Briza Publications, Pretoria.
Van Wyk, B.-.E., Van Oudtshoorn, B., Gericke, N., 2009. Medicinal Plants of South Africa, second ed. Briza Publications, Pretoria.
Van Wyk, C., Van Wyk, P.J., 2010. Trends in dental caries prevalence, severity and unmet treatment need levels in South Africa between 1983 and 2002. S. Afr. Dent. J. 65, 310–314.
Van Wyk, B.-.E., 2011. The potential of South African plants in the development of new medicinal products. S. Afr. J. Bot. 77, 812–829.
Van Wyk, B.-.E., Gericke, N., 2018. People's Plants: a Guide to Useful Plants of Southern Africa, second ed. Briza Publications, Pretoria.
Vasudevan, R., 2014. Urinary tract infection: an overview of the infection and the associated risk factors. J. Microbiol. Exp. 1, 42–54.
Von Koenen, E.V., 2001. Medicinal, Poisonous, and Edible Plants in Namibia. Klaus Hess Publishers, Namibia.
Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, second ed. Livingstone, London.
WHO, 2008. Traditional Medicine Fact Sheet 134 (WWW Document). World Health Organization. http://www.who.int/mediacentre/factsheets/fs134/en/.