PHCOG MAG. ORIGINAL ARTICLE

A Comparison of the Antimicrobial Activity and Toxicity of Six Combretum and Two Terminalia Species from Southern Africa

I. E. Cock[1,2], S.F. Van Vuuren[3]

[1]Environmental Futures Research Institute, and [2]Biomolecular and Physical Sciences, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, Australia, [3]Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, Parktown, South Africa

Submitted: 25-03-2014 Revised: 09-06-2014 Published: 21-01-2015

ABSTRACT

Background: Plants of the family Combretaceae are amongst the most widely used plants for traditional medicinal purposes in southern Africa. In particular, many species of Combretum and Terminalia are used for their antibacterial, antifungal, antiprotozoal, antiviral, antidiarrhoeal, analgesic, antimalarial, antioxidant, anti-inflammatory and anticancer activities, yet their antimicrobial potential has not been rigorously studied and compared. **Materials and Methods:** A survey of antimicrobial activity was undertaken on selected South African Combretum and Terminalia species. Sixteen extracts from 6 Combretum and 2 Terminalia plant species with a history of medicinal usage were investigated by disc diffusion assay against a panel of bacteria and fungi and their MIC values were determined. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** All extracts tested displayed broad spectrum antibacterial activity, inhibiting the growth of 12-16 (75-100%) of the bacteria tested, with Gram-positive and Gram-negative bacteria being approximately equally susceptible. Potent antibacterial activities (generally in the range 200-5000 μg/ml) were evident for all Combretaceae extracts against both Gram-positive and Gram-negative bacteria. Similarly, the extracts also displayed good antifungal activity, inhibiting the growth of 2-3 (66.7-100%) of the fungal species tested, with fungal growth inhibition activities generally in the range 200–4000 μg/ml. In general, the Terminalia extracts had better efficacies than the Combretum extracts. Furthermore, the methanol extracts were generally better antimicrobial agents than the water extracts. All extracts were also shown to be non-toxic in the Artemia nauplii bioassay. **Conclusion:** The lack of toxicity of these extracts and their inhibitory bioactivity against a panel of bacteria and fungi indicate their potential as medicinal agents and partially validate their usage in multiple South African traditional medicinal systems.

Keywords: Antibacterial activity, antifungal activity, combretaceae, combretum, terminalia, toxicity

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.149740

Quick Response Code:

INTRODUCTION

Combretaceae is a large family of trees, shrubs, vines and mangroves which consists of approximately 17 genera and 525 species.[1] The Combretaceae occur mainly in tropical and subtropical regions internationally, with the highest diversity in Asia and Africa.[2] Two of the largest and most useful genera are Combretum, consisting of approximately 250 species of trees, shrubs and lianas, and Terminalia, consisting of approximately 150 species of trees and shrubs.[1] Due to the widespread distribution and biodiversity of the Combretaceae across Southern Africa, they are readily available and easily harvested for medicinal use. Possibly for this reason, they are widely used in diverse traditional healing systems for a wide variety of diseases and afflictions. The leaves and bark are predominantly used for these purposes.[3] Indeed, the fruit of many African species are considered toxic and are not used for internal consumption.

The largest body of literature on the medicinal properties of the Combretaceae has focused on

Address of correspondence:
Dr. I. E. Cock, Environmental Futures Research Institute, Nathan Campus, Griffith University, 170 Kessels Rd, Nathan, Queensland 4111, Australia.
E-mail: I.Cock@griffith.edu.au

the Asian species. There is a wealth of published information into all aspects of the medicinal usage and phytochemistry of Asian Combretaceae. Studies into Combretaceae in other regions of the world has increased substantially in recent years although they are not yet as extensive as studies into Asian species. Whilst a substantial body of literature has now been published on South African Combretum and Terminalia species, most of this has been directed either at establishing and reporting the ethnobotanical usage of the plants in various traditional medicinal systems, or to examine the phytochemistry of the plants. Surprisingly, until recent years, few of the antimicrobial properties of South African Combretaceae had been rigorously studied by comprehensive bioactivity driven approaches. Recently, several studies have begun to focus on examining the anti-infective properties of the South African Combretaceae, both to confirm their efficacy in traditional systems, as well as being a part of deeper studies aimed at bioactivity driven isolations of useful new therapeutic compounds.[4,5]

In Southern Africa, several species of Combretum and Terminalia are used in a variety of traditional medicinal systems and many of these uses have been previously documented [Table 1]. Many species of Combretaceae have been used to treat a diverse range of diseases and ailments ranging from the treatment of abdominal pain, bilharzia, various heart ailments, mental illnesses and stings to the use as wound antiseptics and vermifuges.[3,6-9] Many of the medicinal uses of both Combretum and Terminalia may be related to the antibacterial activities of these plants.

Several recent studies have examined the antibacterial and antifungal activities of selected South African Combretum and Terminalia. Most of these studies report on the bioactivities of either a single species, or in some cases a couple of species, against limited panels of microbes associated with specific diseases. For example, the usage of South African Terminalia in several traditional medicinal systems to treat a variety of STI's has prompted several studies into the efficacy of extracts and/or their phytochemistry.[10,11] Recent studies have also reported on

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

Plant species	Common name	Part used medicinally	Part used in this study	Medicinal use	References
Combretum collinum	Bushwillow (English), boswilg (Afrikaans),	Roots, bark, leaves	Leaves	Used to treat coughs, colds, infertility, venereal diseases, diarrhoea and dysentry, sores and wounds	3, 6, 9, 13
Combretum erythrophloeum	Unknown	Roots, bark, leaves	Leaves	Used to treat coughs, colds, infertility, venereal diseases, diarrhoea and dysentry, sores and wounds	3, 6, 9, 13
Combretum erythrophyllum	River bushwillow (English), riviervaderlandswilg (Afrikaans), modubunoka (Pedi), uMdubu (Zulu)	Roots, bark, leaves. Fruit are also occasionally used to cure hiccups but are generally considered toxic	Leaves	Used to treat coughs, colds, infertility, venereal diseases, diarrhoea and dysentry, sores and wounds	3, 6, 9, 13
Combretum hereroense	Russet bushwillow, mouse-eared Combretum (English), kierieklapper (Afrikaans), Ithetshane (Ndebele), murovamhuru (Shona)	Roots, bark, leaves	Leaves	Used to treat coughs, colds, infertility, venereal diseases, diarrhoea and dysentry, sores and wounds	3, 6, 9, 13
Combretum microphyllium	River flame-creeper, burning bush (English), vlamklimop (Afrikaans), ganwa-musero (Shona)	Leaves	Leaves	The ethnobotany of this plant is unknown although reports have shown it to have good antibacterial, antifungal and antioxidant activities	4, 5, 12
Combretum molle	Velvet bushwillow (English), basterrooibos (Afrikaans), umbondo (Ndebele), umBondwe (Zulu)	Roots, bark, leaves	Leaves	Used to treat coughs, colds, infertility, venereal diseases, diarrhoea and dysentry, sores and wounds	3, 6, 9, 13
Terminalia pruinoides	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	4, 14
Terminalia sericea	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	3, 6, 9, 14

the antifungal activity of large panels of South African Combretum and Terminalia species as a means of comparing their efficacies.[4,5,12] There is a need for a similar approach to determine and compare the antibacterial activity of these plants.

This study aimed to examine the antimicrobial activity of several Combretum and Terminalia species against a broad panel of bacterial and fungal species. A selection of southern African Combretaceae that were traditionally used in a variety of medicinal systems was identified [Table 1]. Whilst several studies directly comparing the antifungal activity of a broad panel of Combretaceae has previously been published,[5,12] we were unable to find a similar study directly comparing the antibacterial activity of extensive panels of Combretum and Terminalia species. Our study aimed to take a similar approach to these antifungal screening studies to directly compare the antimicrobial activity of the selected Combretaceae species, and thus not only to confirm the validity of their usage in traditional medicinal systems, but also to benchmark and compare their efficacies against a panel of medicinally important bacteria and fungi.

MATERIALS AND METHODS

Plant collection and extraction

All of the plant species tested in this study were collected from the Walter Sisulu Botanical Gardens in Johannesburg, South Africa. All plants were identified by Andrew Hankey, chief botanist at the Walter Sisulu Botanical Gardens or one of the other resident botanists. Voucher specimens were prepared and are stored at the Department of Pharmacy and Pharmacology, University of Witwatersrand, South Africa. All plant materials were air dried in the shade and ground into a fine powder. One gram of each dried plant material was weighed into two tubes for each plant. Extracts were prepared by adding 50 ml of AR grade methanol or distilled water to the tubes. Plant material was extracted in each solvent for 24 h 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 lyophilization. The resultant dry extracts were weighed and redissolved in 10 ml deionized water.

Antibacterial screening

Test microorganisms

Reference strains of *Alicaligenes faecalis* (ATCC 8750), *Aeromonas hydrophilia* (ATCC 7965), *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *Citrobacter freundi* (ATCC 44864), *Escherichia coli* (ATCC 8739), *Klebsiella pneumonia* (ATCC 13883), *Proteus mirabilis* (ATCC 43071), *Proteus vulgaris* (ATCC 33420), *Pseudomonas aeruginosa* (ATCC 27858), *Pseudomonas fluorescens* (ATCC 13525), *Salmomnella typhimurin* (ATCC 14028), *Serratia marcescens* (ATCC 13880), *Shigella sonnei* (ATCC 9290), *Stapyylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 2223) were obtained from American Tissue Culture Collection (ATCC) and subcultured and maintained in nutrient broth at 4°C. *Aspergillus niger* (ATCC 16404), *Candida albicans* (ATCC 10231) and *Rhizopus stolonifer* (ATCC 6227a) were subcultured and maintained in Sabouraud broth at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion method.[14-16] 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 microbial suspension was spread onto nutrient agar plates.

The extracts were tested using 5 mm sterilised filter paper discs. Discs were impregnated with 10 µl of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4°C for 2 h before incubation with the test microbial agents. Plates inoculated with *Alcaligenes faecalis*, *Aeromonas hydrophilia*, *Bacillus cereus*, *Bacillus subtilis*, *Candida albicans*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens*, *Rhizopus stolonifer, Salmonella typhimurium* and *Serratia marcescens* were incubated at 30°C for 24 h, and then the diameters of the inhibition zones were measured in millimetres. Plates inoculated with *Escherichia coli*, *Shigella sonnei*, *Staphylococcus aureus* and *Staphylococcus epidermidis* were incubated at 37°C for 24 h, then the diameters of the inhibition zones were measured. Plates inoculated with *Aspergillius niger* were grown at room temperature for 48 h, and then the diameters of the inhibition zones were measured. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate and mean values were determined. Standard discs of ampicillin (2 µg) and chloramphenicol (10 µg) were obtained from Oxoid Ltd. and served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of distilled water were used as negative controls.

Minimum inhibitory concentration determination

The minimum inhibitory concentrations (MIC) of the plant extracts were determined by the disc diffusion MIC method[17,18] across a range of doses. 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, Saarchen, South Africa) was prepared as a 1.6 mg/ml solution in distilled water and was serially diluted in synthetic seawater for use in the *A. franciscana* nauplii bioassay. The stock was diluted in artificial seawater for use in the bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using a modified *Artemia franciscana* nauplii lethality assay.[19-21] Briefly, *A. franciscana* cysts were obtained from North American Brine Shrimp, LLC, USA (harvested from the Great Salt Lake, Utah). Synthetic 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 were concentrated to a suitable density by placing an artificial light at one end of their incubation vessel and the nauplii rich water closest to the light was removed for biological assays. 400 µl of seawater containing approximately 43 (mean = 42.7, n = 42, SD = 10.4) nauplii were added to wells of a 48 well plate and immediately used for bioassay. The plant extracts were diluted to 4 mg/ml in seawater for toxicity testing, resulting in a 2 mg/ml concentration in the bioassay. 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 run in at least triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered moribund if no movement of the appendages was observed within 10 s. After 48 h all nauplii were sacrificed and counted to determine the total number per well. The LC_{50} with 95% confidence limits for each treatment was calculated using Probit analysis.

Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments.

RESULTS

Liquid extraction yields

Extraction of 1 g of dried plant material with water and methanol yielded dried plant extracts ranging from 50 mg (*C. erythrophyllum* leaf methanolic extract) to 220 mg (*T. sericea* 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 4 of the 8 plant samples tested (50.0%), whereas water extracted the greater amount of material for 3 of the 8 plant samples (37.5%). One plant (*T. sericea*) extracted approximately equal masses in both water (210 mg) and methanol (220 mg). The dried extracts were resuspended in 10 ml of deionized water resulting in the extract concentrations shown in Table 2.

Antimicrobial activity

As decoctions and tinctures are the main forms in which these plants are traditionally administered in ethnobotanical medicinal systems, the zones of inhibition of the 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 µl) of each extract were tested in the disc diffusion assay against panels of Gram positive [Figure 1] and Gram negative bacteria [Figure 2], as well as fungi [Figure 3]. The methanol and water extracts of all Combretaceae species generally displayed good inhibitory activity against the Gram positive bacteria. All species inhibited

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)
Combretum collinum	Leaves	Methanol	126	12.6
	Leaves	Water	70	7
Combretum erythrophloeum	Leaves	Methanol	122	12.2
	Leaves	Water	65	6.5
Combretum erythrophyllum	Leaves	Methanol	50	5
	Leaves	Water	75	7.5
Combretum hereroense	Leaves	Methanol	203	20.3
	Leaves	Water	138	13.8
Combretum microphyllium	Leaves	Methanol	136	13.6
	Leaves	Water	153	15.3
Combretum molle	Leaves	Methanol	133	13.3
	Leaves	Water	71	7.1
Terminalia pruinoides	Leaves	Methanol	76	7.6
	Leaves	Water	137	13.7
Terminalia sericea	Leaves	Methanol	220	22
	Leaves	Water	210	21

the growth of *B. cereus*, *B. subtilis* and *S. aureus* although the Terminalia species appeared to have a greater efficacy than the Combretum species (as determined by zones of inhibition). Only *S. epidermidis* was resistant to any of the extracts tested, still being capable of growth in the presence of *C. erythrophloeum* water extract, *C. erythrophyllum* water extract, *C. hereroense* water extract and *C. microphyllum* water extract. In general, the methanol extracts of all Combretum species tested were better inhibitors of Gram positive bacterial growth (as determined by zones of inhibition) than were the water extracts. Interestingly, the opposite trend was apparent for both Terminalia species, with the water extracts more effectively inhibiting bacterial growth. This is likely to reflect the very high contents of highly water soluble tannins present in all Terminalia, and the known strong antibacterial activity of these tannins.[12,13,22-24]

Gram negative bacterial growth was also inhibited by a broad range of Combretaceae extracts. Indeed, only *P. aeruginosa* was resistant to any of the extracts tested, still being capable of growth in the presence of *C. erthrophyllum* and *C. microphillum* water extracts, as well as both the methanolic and water extracts of *T. sericea*. All Gram negative bacteria were highly susceptible to the plant extracts, with large zones of inhibition (>10 mm) evident for all species. Unlike the Gram positive bacteria, the Combretum water extracts generally proved to be more effective growth inhibitors of Gram negative bacterial growth than the methanolic extracts (as determined by the number of extracts inhibiting bacterial growth and the size of the zone of inhibition) with some exceptions (*C. hereroense* in general, and other Combretum extracts against individual bacterial species). Similar to the Gram positive bacteria, the aqueous Terminalia extracts again displayed greater antibacterial efficacy against the Gram negative bacteria than the methanolic extracts. The Terminalia extracts were generally more effective against the Gram negative bacteria (as determined by inhibition zones) than were the Combretum extracts.

Fungal growth was also highly susceptible to the Combretaceae plant extracts. In general, the methanolic extracts displayed greater had greater efficacy than the water extracts. *A. niger* was more strongly inhibited by the methanolic extracts than the water extracts for 6 (*C. collinum*, *C. erythrophloeum, C. hereroense*, *C. microphyllum, C. molle, T. sericea*) of the 8 plants screened (75.0%). In contrast, the water extracts of only 1 (12.5%) plant species (*C. erythrophyllum*) inhibited *A. niger* growth more strongly than the corresponding methanolic extract, and extracts of a single species (*T. pruinoides*) did not inhibit *A. niger* growth at all. Similarly, *C. albicans* was more susceptible to the methanolic extracts than the water extracts. Six (75.0%) of the methanolic extracts (*C. collinum*, *C. erythrophloeum, C. hereroense*, *C. microphyllum, C. molle, T. pruinoides*) more strongly inhibited *C. albicans* growth than did the water extracts. The water extracts of 1 (12.5%) plant species (*C. erythrophyllum*) inhibited *C. albicans* growth more strongly than the methanolic extracts, and neither extract of a single species (*T. sericea*) inhibited *C. albicans* growth. *R. stolonifer* was also more strongly inhibited by the water extracts than the methanolic extracts. Four (50.0%) of the aqueous Combretaceae extracts (*C. erythrophloeum*, *C. erythrophyllum, T. pruinoides, T. sericea*) more strongly inhibited *R. stolonifer* growth than did the corresponding methanolic extracts, whilst the methanolic extracts were the strongest for 3 Combretaceae species (*C. collinum*, *C. microphyllum*, *C. molle*). Both extracts of a single species (*C. hereroense*) inhibited *R. stolonifer* growth with approximately equal efficacies. With the exception of *C. albicans*, the *T. sericea* extracts had the greatest fungal activity (determined by zones of inhibition).

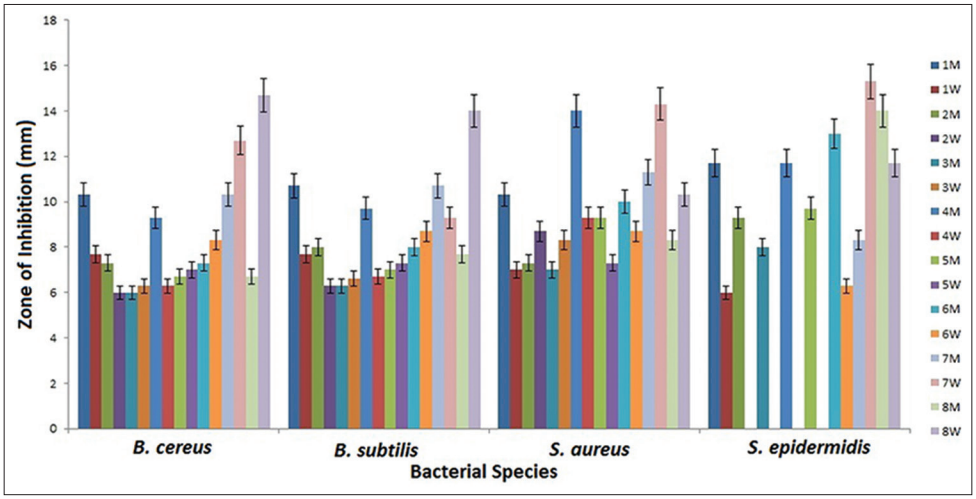

Figure 1: Antibacterial activity of plant water (W) and methanolic (M) extracts measured as zones of inhibition (mm) against Gram positive bacteria. 1 = C. collinum leaf; 2 = C. erythrophloeum leaf; 3 = C. erythrophyllum leaf; 4 = C. hereroense leaf; 5 = C. microphillum leaf; 6 = C. molle leaf; 7 = T. pruinoides leaf; 8 = T. sericea leaf. Results are expressed as mean ± SEM

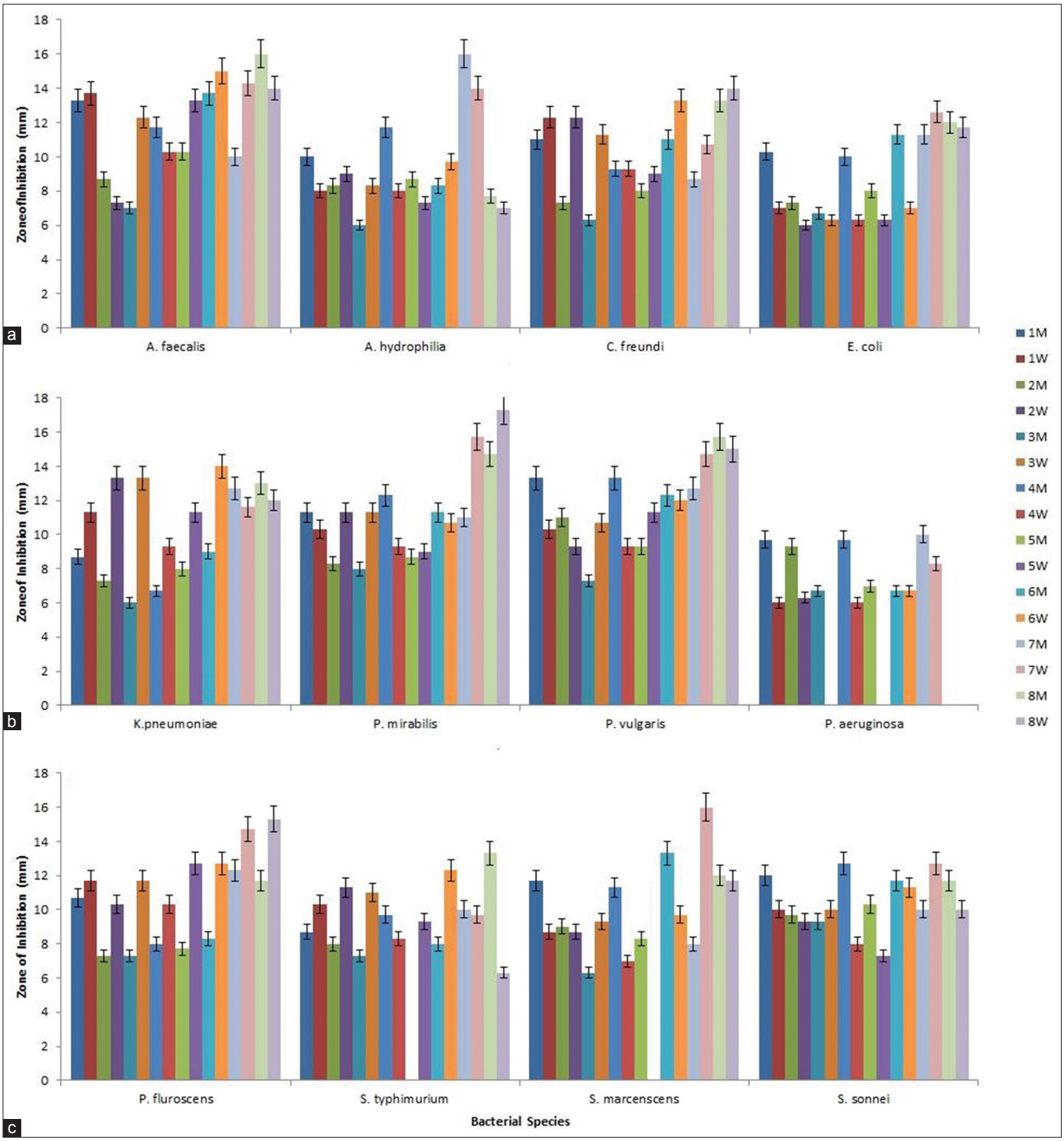

Figure 2: Antibacterial activity of plant water (W) and methanolic (M) extracts measured as zones of inhibition (mm) against the Gram negative bacteria (a) A. faecalis, A. hydrophillia, C, freundi, E. coli, (b) K. pneumonia, P. mirabilis, P. vulgaris, P. aeruginosa, (c) P. fluorescens, S. typhimurium, S. marcescens, S. sonnei. 1 = C. collinum leaf; 2 = C. erythrophloeum leaf; 3 = C. erythrophyllum leaf; 4 = C. hereroense leaf; 5 = C. microphillum leaf; 6 = C. molle leaf; 7 = T. pruinoides leaf; 8 = T. sericea leaf. Results are expressed as mean ± SEM

The *C. collinum* and *C. molle* methanolic extracts also displayed good efficacy against all fungal species tested.

The relative level of antimicrobial activity was further evaluated by determining the MIC values [Table 3] for each extract against the bacterial and fungal species which were shown to be susceptible by disc diffusion assays. Most of the extracts were effective at inhibiting microbial growth at low concentrations, with MIC values against the bacterial and fungal species that they inhibited often <1000 μg/mL (<10 μg impregnated in the disc), indicating the potent antimicrobial activity of these

Table 3: Minimum inhibitory concentrations (µg/ml) of plant water and methanolic extracts against susceptible bacteria

Plant species	Part used	Solvent	Gram negative rods												Gram positive rods		Gram positive cocci		Fungi		
			A. faecalis	*A. hydrophilia*	*C. freundi*	*E. coli*	*K.pneumoniae*	*P. mirabilis*	*P. vulgaris*	*P. aeruginosa*	*P. fluroscens*	*S. typhimurium*	*S. marcenscens*	*S. sonnei*	*B. cereus*	*B. subtilis*	*S. aureus*	*S. epidermidis*	*A. niger*	*C. albicans*	*R. stolonifer*
Combretum collinum	Leaf	Methanol	302	434	516	428	1236	355	240	555	454	226	575	268	959	1874	330	317	621	337	495
		Water	427	315	401	1584	292	243	567	2016	413	336	255	431	235	-	1452	2873	1944	467	141
Combretum erythrophloeum	Leaf	Methanol	536	342	2254	5341	3050	527	351	501	6100	2238	435	505	3050	-	469	501	4278	1878	306
		Water	1738	685	706	2466	430	514	354	1873	431	696	406	366	4664	-	130	-	4869	2768	423
Combretum erythrophyllum	Leaf	Methanol	2500	2500	1187	1924	1463	696	463	1239	693	551	758	410	3875	-	875	552	3875	3875	1892
		Water	701	717	536	2235	361	432	561	-	697	477	674	431	4327	-	1794	-	2055	1589	359
Combretum hereroense	Leaf	Methanol	573	491	337	434	9150	289	240	450	1225	837	605	371	446	876	465	286	5075	4486	395
		Water	496	645	408	3446	506	655	408	4371	597	726	2270	287	4895	1440	348	-	5567	5210	287
Combretum microphillum	Leaf	Methanol	440	436	494	460	1225	383	330	1648	417	873	454	599	3450	-	501	500	3900	1008	494
		Water	423	453	294	1480	205	315	508	-	521	315	-	1369	427	-	1570	-	4083	1432	1673
Combretum molle	Leaf	Methanol	293	337	503	366	831	340	570	1870	355	287	374	296	3650	-	652	371	126	172	259
		Water	344	478	385	1437	544	474	363	2629	277	603	483	565	348	324	1658	2366	1240	568	531
T. pruinoides	Leaf	Methanol	264	514	281	278	432	313	926	360	788	463	571	464	637	314	395	261	-	623	367
		Water	212	365	363	624	531	224	379	512	463	396	383	393	607	287	333	290	378	478	392
T. sericea	Leaf	Methanol	276	293	693	396	254	417	508	-	547	993	410	262	712	578	240	657	418	-	215
		Water	354	437	383	276	318	103	520	-	683	1250	2030	353	31	124	770	462	538	-	235

Numbers indicate the mean MIC values of at least triplicate determinations. - indicates no growth inhibition

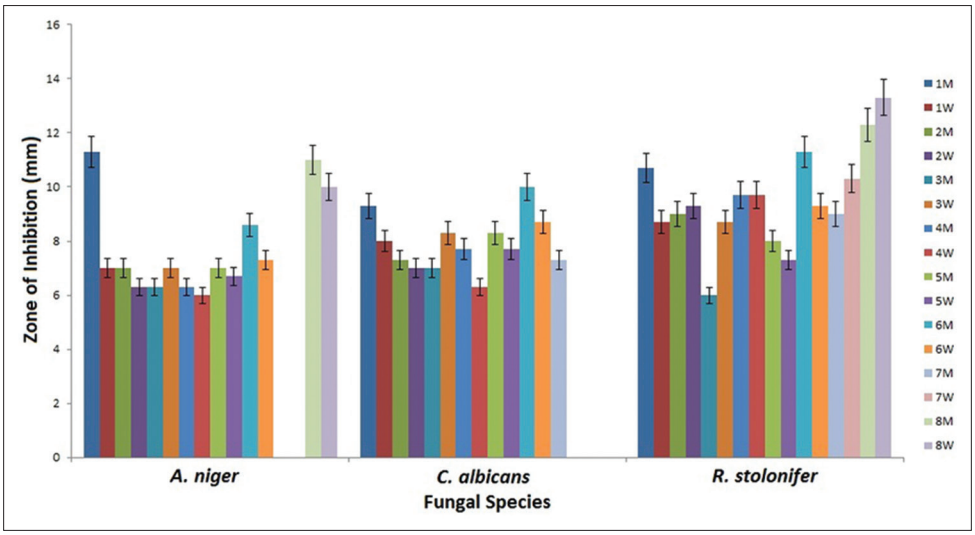

Figure 3: Antifungal activity of plant water (W) and methanolic (M) extracts measured as zones of inhibition (mm) against Gram positive bacteria. 1 = C. collinum leaf; 2 = C. erythrophloeum leaf; 3 = C. erythrophyllum leaf; 4 = C. hereroense leaf; 5 = C. microphillum leaf; 6 = C. molle leaf; 7 = T. pruinoides leaf; 8 = T. sericea leaf. Results are expressed as mean ± SEM

extracts. These MIC's compare favourably with the dosages of the pure standards ampicillin, chloramphenicol and nystatin which were tested using per 2 µg, 10 µg and 100 µg per disc respectively. The *T. pruinoides* and *T. sericea* leaf extracts were particularly effective, generally having MIC values for the species they inhibited <500 µg/mL (<5 µg impregnated in the disc). Indeed, the *T. sericea* water extract was a particularly good inhibitor of *B. cereus*, displaying an MIC value of 31 µg/mL (approximately 0.3 µg extract impregnated in the disc).

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. Figure 4a and 4b show the % mortality induced in the Artemia nauplii following 24 and 48 h of exposure, respectively. Potassium dichromate (reference toxin) was rapid in its induction of mortality, inducing the onset of mortality within the first 3 h of exposure (results not shown). By 24 h of exposure, potassium dichromate had induced 100% mortality in the Artemia nauplii. In contrast, none of the extracts induced mortality significantly above that of the seawater control, indicating that none of the extracts were toxic. By 48 h exposure, the Artemia nauplii mortalities induced by all of the extracts were still similar to those of the seawater control, indicating that none of the extracts were toxic.

DISCUSSION

The family Combretaceae have proven to be a promising source of antimicrobial growth inhibitors and bactericidal/fungicidal agents. Traditional healers in all regions of the world use species of Combretaceae for a wide range of medicinal purposes including the treatment of abdominal pain and other abdominal disorders, constipation, coughs and colds, conjunctivitis, constipation, coronary diseases, diarrhoea, dysentery, earache, fever, headache, infertility, inflammation and swelling, jaundice, leprosy, parasites, pneumonia, sexually transmitted infections (STI's), sore throats, toothache and ulcers.[22] Some of these conditions are caused by bacteria and fungi. Thus, many of the therapeutic properties of the Combretaceae may be attributable to their antimicrobial activity. The genuses Combretum and Terminalia in particular have a long history of usage in the treatment of a variety of infective conditions. As several Combretum and Terminalia species are common and widely distributed throughout southern and tropical regions of Africa, they are easily collected and readily available for use by traditional healers.

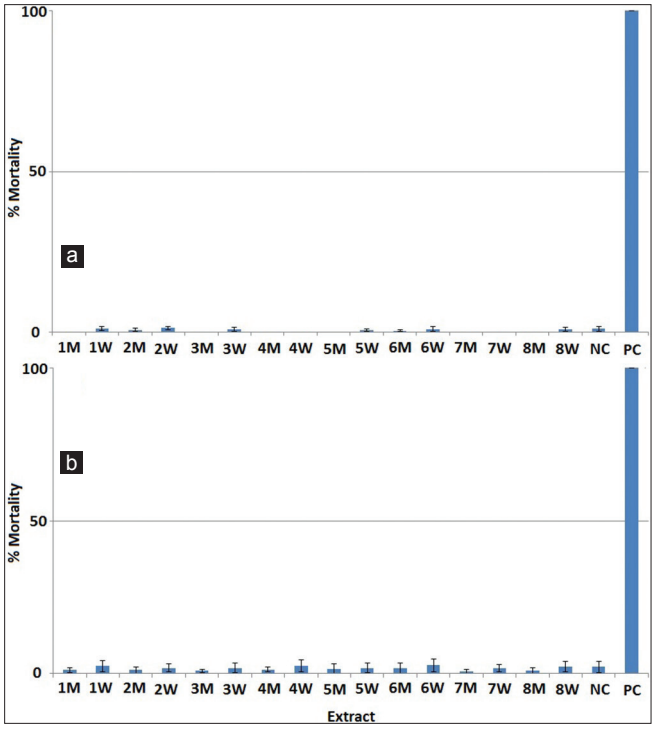

Figure 4: The lethality of methanolic (M) and water (W) plant extracts (2000 µg/mL) and potassium dichromate control (1000 µg/mL) toward Artemia nauplii after (a) 24 h and (b) 48 h exposure. 1 = C. collinum leaf; 2 = C. erythrophloeum leaf; 3 = C. erythrophyllum leaf; 4 = C. hereroense leaf; 5 = C. microphillum leaf; 6 = C. molle leaf; 7 = T. pruinoides leaf water extract; 8 = T. sericea leaf. Results are expressed as mean ± SEM

Potent bacterial growth inhibition effects against both Gram-positive and Gram-negative bacteria were evident for all Combretum extracts tested in this study. These results confirm the potential of the Combretum spp. for the control and treatment of multiple bacterial diseases and conditions and partially validate their usage in traditional medicinal systems. Both Gram-positive and Gram-negative bacteria had similar susceptibilities towards the Combretum species examined in this study. Indeed, good efficacies (generally in the range 200-5000 µg/ml) are reported for all Combretum extracts against both Gram-positive and Gram-negative bacteria. In contrast, previous studies with plant species of other families generally report a greater susceptibility of Gram-positive bacteria towards solvent extracts for South American,[25] Australian[26-30] and other African[31] plant extracts, although examples of plants having a greater effect on Gram-negative bacteria have also been reported.[17,18]

All Combretum species tested in our study displayed good antifungal activity, with all extracts inhibiting the growth of all the fungal species tested. The Combretum extracts were potent antifungal agents, with MIC values generally in the range of 200-4000 µg/ml, and as low as 126 µg/ml (*C. molle* inhibition of *A. niger* growth). In agreement with our studies, previous publications have reported on the antifungal activity of South African Combretum species, with a similar efficacy (250-4000 µg/ml).[32] In contrast, a recent study has also reported substantially more potent antifungal activity of leaf extracts of 24 South African Combretum species against a panel of pathogenic fungi, with MIC values for some species as low as 20 µg/ml.[5]

Similarly, many Terminalia species have a history of usage to treat medical conditions related to microbial infections and numerous recent investigations have reported on their antibacterial properties. Indian and Southern Asian Terminalia species have been particularly well studied. In particular, *T. arjuna*,[33] *T. bellirica*, *T. catappa*[33] and *T. chebula*[34] have been reported to have antibacterial activity against a wide panel of microbes. Recent studies have also

reported on the antimicrobial activity of Australian[15,35] and other African Terminalia.[13,36]

The potent bacterial growth inhibition effects demonstrated in our study indicate the potential of *T. pruinoides* and *T. sericea* in the control and treatment of various bacterial diseases and conditions. Indeed, extracts of both species had potent antibacterial activity towards both Gram-positive and Gram-negative bacteria, often with greater efficacy than the antibiotic controls (as determined by zones of inhibition and by MIC). In common with the Combretum antimicrobial screenings, the Gram-positive and Gram-negative bacteria tested in this report also demonstrated similar susceptibilities towards both of the South African Terminalia extracts.

Numerous other African Terminalia species have also been reported to have potent antibacterial activity. One study of the South East African species *T. stenostachya* and *T. spinosa* reported strong antibacterial activity against a broad spectrum of medicinally important bacteria including several *Mycobacterium* species, *Streptococcus faecalis*, *Staphylococcus aureus*, *Vibrio cholera*, *Bacillus anthracis*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Escherichia coli*.[36] *Terminalia brownii* also has a history of usage in traditional eastern and central African medicinal systems, including usage for the treatment of diverse medicinal conditions including diarrhoea and gonorrhoea.[37] A recent report has confirmed the antibacterial activity of *T. brownii* against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Bacillus anthracis*.[37] The West African species *Terminalia avicennioides*, which is widely used in Nigerian traditional medicine, has been reported to have good antimicrobial activity towards a methicillin resistant strain of *Staphylococcus aureus*.[38]

The antifungal activity of Southern African Terminalia species have also been previously studied and potent antifungal activity has been reported. One study into the antifungal activity of several South African Terminalia species reported good antifungal activity for *T. pruinoides*, *T. brachystemma*, *T. sericea*, *T. gazensis*, *T. mollis* and *T. sambesciaca* against the fungal species *Candida albicans*, *Cryptococcus neoformans*, *Aspergillus fumigates*, *Microsporum canis* and *Sporothrix schenkii*.[4] Indeed, particularly low MIC values between 0.2-0.8 mg/ml were reported in this study, indicating the efficacy of Terminalia extracts. This was a particularly interesting study as the Cryptococcus and Candida strains used were multi-antibiotic resistant strains. As fungal infections cause significant mortality and morbidity in Africa (especially in immune-compromised individuals), this finding could potentially result in the development of life saving treatments. Whilst this study did not identify the bioactive compounds, it did demonstrate that tannins were unlikely to be responsible due to the non-polar nature of the antifungal components. Interestingly, little data is available on the antifungal activities of Terminalia species from other world regions and much work is needed in this area.

The individual extract components responsible for the antimicrobial potential of the plant extracts were not identified in the current study. However, both the Combretum and the Terminalia are characterized by their high tannin and antioxidant contents.[13,22] It is generally believed that tannins have non-selective biological activities and their structural complexities make them poor candidates for drug design. This perception may be responsible for their disproportionately low degree of directed bioactivity studies, given their wide ranging usage in various traditional medicinal systems. Most interest in the Combretaceae has been for their pharmacognostic and nutraceutical value and they have often been overlooked as potentials for drug discovery. High antioxidant activities have been reported for many Combretaceae.[5,39] Recent studies have documented the exceptionally high antioxidant content of the Australian species *T. ferdinandiana*.[39,40] High antioxidant contents have also been reported for Asian;[41,42] and African Combretaceae.[5] It has previously been postulated that the exceptionally high antioxidant content of the Combretaceae may be responsible for many of the therapeutic effects of these plants.[35] The phytochemistry of the extracts investigated in the current study was not examined. Further studies are required to identify which phytochemical(s) is/are responsible for the recorded bioactivities of these extracts.

The findings reported here also demonstrate that none of the South African Combretaceae extracts displayed significant toxicity toward *Artemia franciscana*. Previously, compounds with an LC_{50} of greater than 1000 μg/mL towards *Artemia* nauplii have been defined as being non-toxic.[43] None of the extracts tested induced elevated mortality (compared to the seawater control) at 2000 μg/ml and are thus considered to be non-toxic. Whilst the Artemia nauplii assay is considered to have good correlation to toxicity toward human cells, caution is needed before these compounds 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 these purposes.

CONCLUSIONS

The results of this study demonstrate the antimicrobial potential of several South African Combretaceae species. Whilst both the Combretum and Terminalia species

displayed broad spectrum antimicrobial activity and good efficacy, the Terminalia species were generally more potent.

ACKNOWLEDGEMENTS

The authors are 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. Financial support for this work was provided by the Department of Pharmacy and Pharmacology, University of the Witwatersrand, South Africa.

REFERENCES

1. Maurin O, Chase MW, Jordaan M, Van Der Bank M. Phylogenetic relationships of Combretaceae inferred from nuclear and plastid DNA sequence data: Implications for generic classification. Bot J Linn Soc 2010;162:453-76.
2. Mabberley DJ. The plant-book: A portable dictionary of the vascular plants. 3rd ed. Cambridge: Cambridge University Press; 2008.
3. Van Wyk BE, van Oudtshoorn B, Gericke N. Medicinal Plants of South Africa. Pretoria: Briza Publications; 2009.
4. Masoko P, Eloff JN. Bioautography indicates the multiplicity of antifungal compounds from twenty-four South African Combretum species (Combretaceae). Afr J Biotechnol 2006;5:1625-47.
5. Masoko P, Eloff JN. Screening of twenty-four South African Combretum and six Terminalia species (Combretaceae) for antioxidant activities. Afr J Tradit Complement Altern Med 2007;4:231-9.
6. Watt JM, Breyer-Brandwijk MG. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd ed. London: Livingstone; 1962.
7. Kokwaro O. Medicinal Plants of East Africa. Nairobi: East African Literature; 1976.
8. Gelfand M, Mavi S, Drummond RB, Ndemera B. The Traditional Medical Practitioner in Zimbabwe. Harare: Mambo Press; 1985.
9. Hutchings A, Scott AH, Lewis G, Cunningham AB. Zulu Medicinal Plants: An Inventory. Pietermaritzburg: University of Natal Press; 1996.
10. Van Vuuren SF, Naidoo D. An antimicrobial investigation of plants used traditionally in southern Africa to treat sexually transmitted infections. Jurnal Ethnopharmacol 2010;130:552-8.
11. Tshikalange TE, Meyer JJM, Hussein AA. Antimicrobial activity, toxicity and the isolation of a bioactive compound from plants used to treat sexually transmitted diseases. J Ethnopharmacol 2005;96:515-9.
12. Masoko P, Picard J, Eloff JN. Antifungal activity of twenty-four South African Combretum species (Combretaceae). S Afr J Bot 2007;73:173-83.
13. Eloff JN, Katerere DR, McGaw LJ. The biological activity and chemistry of the southern African Combretaceae. J Ethnopharmacol 2008;119:686-99.
14. Cock I, van Vuuren SF. Anti-Proteus activity of some South African medicinal plants: Their potential for the prevention of rheumatoid arthritis. Inflammopharmacology 2014;22:23-36.
15. Cock IE, Mohanty S. Evaluation of the antibacterial activity and toxicity of *Terminalia ferdinandiana* fruit extracts. Pharmacogn J 2011;3:72-9.
16. Cock IE. Antibacterial activity of *Eucalyptus major* and *Eucalyptus baileyana* methanolic extracts. Internet J Microbiol 2009;6.
17. Vesoul J, Cock IE. An examination of the medicinal potential of *Pittosporum phylliraedes*: Toxicity, antibacterial and antifungal activities. Pharmacogn Commun 2011;1:8-17.
18. Cock IE, Kukkonen L. An examination of the medicinal potential of *Scaevola spinescens*: Toxicity, antibacterial and antiviral activities. Pharmacogn Res 2011;3:85-94.
19. Sirdaarta J, Cock IE. Effect of *Aloe barbadensis* Miller juice on oxidative stress biomarkers in aerobic cells using *Artemia franciscana* as a model. Phytother Res 2010;24:360-4.
20. Sirdaarta J, Cock IE. Vitamin E and Trolox™ reduce toxicity of *Aloe barbadensis* Miller juice in *Artemia franciscana* nauplii but individually are toxic at high concentrations. Internet J Toxicol 2008;5.
21. Cock IE. Assessment of the toxicity of selected Australian native plant extracts using the *Artemia franciscana* nauplii bioassay. Internet J Toxicol 2007;5.
22. Cock IE. The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). Prog Drug Res 2014. [In press].
23. Chang CL, Lin CS. Phytochemical composition, antioxidant activity, and neuroprotective effect of *Terminalia chebula* Retzius extracts. Evid Based Complement Alternat Med 2012;2012:125247.
24. Jain S, Yadav PP, Gill V, Vasudeva N, Singla N. *Terminalia arjuna* a sacred medicinal plant; phytochemical and pharmacological profile. Phytochem Rev 2009;8:491-502.
25. Paz EA, Cerdeiras MP, Fernandez J, Ferreira F, Moyna P, Soubes M, *et al*. Screening of Uruguayan medicinal plants for antimicrobial activity. J Ethnopharmacol 1995;45:67-70.
26. Cock IE. Antimicrobial activity of *Backhousia citriodora* (lemon myrtle) methanolic extracts. Pharmacogn Commun 2013;3:58-63.
27. Cock IE. Antimicrobial activity of *Callistemon citrinus* and *Callistemon salignus* methanolic extracts. Pharmacogn Commun 2012;2:50-7.
28. Cock IE. Antimicrobial activity of *Acacia aulacocarpa* and *Acacia complanta* methanolic extracts. Pharmacogn Commun 2012;2:66-71.
29. Vesoul J, Cock IE. The potential of Bunya nut extracts as antibacterial functional food agents. Pharmacogn Commun 2012;2:72-9.
30. Cock IE. Antibacterial activity of selected Australian native plant extracts. Internet J Microbiol 2007;4.
31. Kudi AC, Umoh JU, Eduvie LO, Gefu J. Screening of some Nigerian medicinal plants for antibacterial activity. J Ethnopharmacol 1999;67:225-8.
32. Baba-Moussa F, Akpagana K, Bouchet P. Antifungal activities of seven West African Combretaceae used in traditional medicine. J Ethnopharmacol 1999;66:335-8.
33. Shinde SL, Junne SB, Wadje SS, Baig MM. The diversity of antibacterial compounds of Terminalia species (Combretaceae). Pak J Biol Sci 2009;12:1483-6.
34. Das B. Materia Medica of Ayurveda. New Delhi, Jain Publishers; 1991. p. 8.
35. Mohanty S, Cock IE. The chemotherapeutic potential of *Terminalia ferdinandiana*: Phytochemistry and bioactivity. Pharmacogn Rev 2012;6:29-36.
36. Mbwambo ZH, Erasto P, Nondo RO, Innocent E, Kidukuli AW. Antibacterial and cytotoxic activities of *Terminalia stenostachya* and *Terminalia spinosa*. Tanzan J Health Res 2011;13:1-8.
37. Fyhrquist P, Mwasumbi L, Haeggstrom CA, Vuorela H, Hiltunen R,

Vuorela P. Ethnobotanical and antimicrobial investigation on some species of Terminalia and Combretum (Combretaceae) growing in Tanzania. J Ethnopharmacol 2002;79:169-77.

38. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. BMC Complement Altern Med 2005;5:6.
39. Netzel M, Netzel G, Tian Q, Schwartz S, Konczak I. Native Australian fruits: A novel source of antioxidants for food. Innov Food Sci Emerg Technol 2007;8:339-46.
40. Konczak I, Zabaras D, Dunstan M, Aguas. Antioxidant capacity and hydrophilic phytochemicals in commercially grown Australian fruits. Food Chem 2010;123:1048-54.
41. Hazra B, Sarkar R, Biswas S, Mandal N. Comparative study of the antioxidant and reactive oxygen species scavenging properties in the extracts of the fruits of *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis*. BMC Complement Altern Med 2010;10:20.
42. Jaiwal BV, Shaikh FK, Waghire HB, Sarwade BP. Comparative anti-oxidative potential of barks phenolics of genus *Terminalia*. Int J Sci Pharm Educ Res 2012;1:6-10.
43. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982;45:31-4.

Cite this article as: Cock I E, Van Vuuren S F. A Comparison of the Antimicrobial Activity and Toxicity of Six Combretum and Two Terminalia Species from Southern Africa. Phcog Mag 2015;11:208-18.

Source of Support: Nil, **Conflict of Interest:** None declared.