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Screening for Anti-infective Properties of Selected Medicinal Plants from Botswana

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ABSTRACT

Thirty nine ethanol extracts from 26 plants widely distributed in Botswana were screened for antimicrobial activities against three Gram-positive, two Gram-negative bacterial strains, two *Candida* species and one *Mycobacterium* species. Screening was carried out at an initial concentration of 500 µg/disc using the disc agar diffusion method. Most of the plant species gave rise to antifungal activities (20/24), some of them specifically, such as *Acrotome inflata*, *Bridelia mollis*, *Dichrostachys cinerea*, *Dicerocaryum eriocarpum*, *Dicoma capensis*, *Gomphrena celosioides*, *Tagetes minuta* and *Waltheria indica*. Minimal inhibitory concentrations (MICs) of extracts with inhibitory activities against *Candida albicans*, a major opportunistic infective agent in immunocompromised patients, varied between 0.039 and 2.5 mg/ml. Extracts of 10 plant species inhibited the growth of *Mycobacterium aurum*, a non-pathogenic model organism with similar drug susceptibility as *Mycobacterium tuberculosis*. MIC values of anti-mycobacterial extracts ranged from 0.039 to 2.5 mg/ml. Interestingly, *Ocimum canum* leaf extracts and *Elephantorrhiza burkei* root extracts displayed the lowest MICs against both HIV/AIDS opportunistic pathogens with values of 0.039 mg/ml against *Mycobacterium aurum*, and MIC values of 0.039 and 0.078 mg/ml against *Candida albicans*, respectively. Extracts of two plant species, *Elephantorrhiza elephantina* and *Persiceria limbata*, exhibited antimicrobial properties against all eight microorganisms tested and only these two extracts were active against the Gram-negative *Escherichia coli* strain. Antimicrobial ethanol extracts with the lowest MIC values did not show acute *in-vitro* cytotoxicity up to a concentration of 1000 µg/ml using human embryonic kidney cells (HEK293). The findings confirm some anti-infective and wound healing ethnomedical uses in Botswana and show the potential to develop antimicrobial preparations from community natural resources.

Keywords: antifungal, antibacterial, *Mycobacterium aurum*, *Candida albicans*, *Elephantorrhiza burkei*, *Ocimum canum*

INTRODUCTION

The emergence of drug-resistant pathogens is becoming a major global health problem. Drug resistances occur particularly in relation to hospital-acquired infections in industrialised countries and often as a consequence of uncompleted treatment regimes in developing countries (Fischbach and Walsh 2009). Most significantly, HIV/AIDS has reconfigured the pattern of antimicrobial infections. Opportunistic infections, such as tuberculosis and emerging multidrug (MDR) and extensively drug resistant (XDR) mycobacterial strains have become a major public health threat particularly in countries with high HIV/AIDS prevalence and limited health care capacity (Dorman and Chaisson 2007). The treatment of mycoses has been reported to be lagging even more behind bacterial chemotherapy and fewer antifungal than antibacterial substances are available (Cruz *et al*. 2007). *Candida albicans* is an opportunistic fungus causing oral candidiasis commonly characterised by the development of oral thrush. Up to 90% of HIV-infected individuals suffer from at least one episode during the course of the disease (Runyoro 2006). The transmission and spread of drug-resistant pathogens is further promoted through the increased mobility of people world-wide. Therefore, new prototype antimicrobial agents are perpetually needed to address this situation. Paradoxically, contrary to the urgent need for the development of new antimicrobials, the discovery pipelines of major pharmaceutical industries are nearly empty (Fischbach and Walsh 2009; Devasahayam *et al.* 2010) and international public health organisations have called for new, multi-pronged efforts to develop next-generation drugs (NIAID-NIH 2009; Devasahayam *et al.* 2010). One promising approach is to develop new antimicrobials from natural products. Historically, the majority of new drugs have been generated from natural products and 52% of all new chemical entities in the time period between 1981 and 2006 are biological or natural products, natural product derivatives or natural product mimics (Newman and Cragg 2007; Li and Vederas 2009).

Medicinal plants are a major source of secondary metabolites which are potentially useful in combating infections. Consequently, according to the World Health Organisation (WHO) about 80% of the world population depend on medicinal plants for their health needs (Kasilo 2000). In developing countries these readily available and culturally important traditional medicines form the basis of an accessible and affordable health-care regime and are an important source of livelihood for indigenous and rural populations (Dahlberg and Trygger 2009). Traditional medicinal plants have an advantage over other natural product sources because patient treatment observations accumulated over many generations in communities can be correlated with laboratory findings to assess efficacy and safety. However, this approach, termed reverse pharmacology, has only recently attracted attention (Vaidya 2006; Padwardhan and Mashelkar 2009). Systematic attempts to utilise natural medicinal resources to the benefit of developing countries have been made only very unevenly. As the example of Devil's claw (*Harpagophytum procumbens*) showed, it is industrialised countries which import raw materials from developing countries and resell them as highly priced processed medicines to the very countries of origin (Stewart

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and Cole 2005). Lastly, there remains a concern about the absence of scientific evidence for the efficacy of traditional medicines. It is for these reasons that medicinal plants have become the focus of research to validate their traditional uses through the determination of their actual pharmacological effects (de Lima *et al.* 2006). Against this background we performed a screening to identify anti-infective properties against a battery of microorganisms including Gram-positive and Gram-negative bacteria, two *Candida* species and *Mycobacterium aurum*.

MATERIALS AND METHODS

Plant materials

Very few comprehensive studies on ethnomedical uses and biological activities of higher plants in Botswana exist. In order to contribute to a better understanding of potentially useful medicinal properties of plants a variety of samples were collected during June 2008 from two villages, Mmankgodi and Kolobeng, located in Kweneng District, Botswana. Plants were selected based on their relative prominent distribution in the study area of the Mmankgodi community, on some information on ethnomedical uses in Botswana gathered from the literature and informal interviews with community members. Plant species were taxonomically authenticated by M. Muzila (University of Botswana Herbarium), processed as voucher specimen according to good botanical practice and deposited at the University of Botswana Herbarium. Information about the use of collected medicinal plants was gathered from the literature focusing on plant uses in Botswana and from informal interviews with members of the Mmankgodi community.

Preparation of extracts

Plant samples were dried at room temperature and ground to a fine powder using a grinding machine (Grinder A10, IKA Labortechnik, Germany). One gram of the pulverised material was extracted with 10 ml of absolute ethanol for 10 minutes at room temperature using a Vortex® mixer (E10 Vortex 2 Genie, LASEC, South Africa). The suspension was centrifuged for 10 min at room temperature and 10 000 rpm (Biofuge stratus, Heraeus, Germany) and the supernatant was transferred into a pre-weighed glass vial. The concentrated extract was dried at room temperature under a stream of air and weighed. A stock solution of 10 mg/ml in absolute ethanol was prepared and sterile-filtered using a 0.2 µM filter (Sterile Millex-FG, Millipore, Bedford, MA 01730). For this initial screening we chose ethanol as extraction medium because its polarity is closest to water, which is the main solvent for traditional medicinal preparations, but lesser prone to antimicrobial contamination during extract preparation.

Microorganisms and growth conditions

Bacillus subtilis (ATCC 6633), *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 11229), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 9144), *Candida albicans* (ATCC 10231), *Candida mycoderma* (a kind donation from the National Health Laboratory, Gaborone, Botswana) and *Mycobacterium aurum A+* (a generous gift from Prof. Pete Smith, Department of Pharamacology, University of Cape Town, Medical School, Cape Town, South Africa) were used as test organisms. Bacteria were grown in Nutrient Broth (HiMedia, South Africa) at 37°C for 24 hrs and *Candida* species in Sabouraud Dextrose Broth (HiMedia) at 25°C for 24 hrs. *Mycobacterium aurum* A+ was cultivated in Middlebrook 7H9 medium (Difco Laboratories, Detroit, USA) supplemented with 10% OADC (oleic acid/albumin/dextrose/catalase, Difco Laboratories, Detroit, USA). Microorganisms were maintained on respective agar plates at 4°C or stored as glycerol cultures at -80°C for future use.

Antimicrobial susceptibility assays

Microorganisms were resuscitated from the respective plates [Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), Middlebrook 7H10 slant agar (MBA)] and grown in respective broth media [Nutrient Broth (NB), Sabouraud Dextrose Broth (SD) and Middlebrook 7H9 with 10% OADC (MB)]. Bacterial densities were determined by plating serial dilutions of the overnight culture (10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}) onto NA, SDA and MBA agar plates. After incubation at 37°C for 24 hrs colonies were counted and colony-forming units (cfu) per ml were determined.

1. Agar diffusion assay

The agar diffusion assays were carried out as described by Al-Fatimi *et al.* (2007). Briefly, 200 µl of an inoculation culture (1×10^6 cfu/ml) was mixed with 20 ml sterile liquid SDA or NA and poured into Petri-dishes (Falcon) to give a solid plate. An aliquot of 20 µl of plant extracts (500 µg extract dissolved in absolute ethanol) were applied on sterile 6 mm cartridge susceptibility discs (Mast Diagnostics, Mast Group Ltd, Merseyside, UK) inserted into 96-well plates (Costar). After evaporation of the solvent, discs were then deposited on the surface of the inoculated agar plates. Fungazole and ampicillin (Sigma) were used as positive controls in the same concentrations as plant extracts for growth inhibition of *Candida* species and bacteria, respectively, while ethanol served as negative control. Plates with bacteria and fungi, respectively, were incubated for 24 hrs at 37°C. The width of inhibition zones (from edge of susceptibility disc to edge of inhibition zone) was determined from duplicate experiments.

2. Determination of minimal inhibitory concentrations (MICs) and minimal bactericidal/fungicidal concentrations (MBC/MFC)

Minimal inhibitory concentrations (MICs) against *Mycobacterium aurum* and *Candida albicans* were detected using the microplate serial dilution method (Eloff 1998; McGaw *et al.* 2008a) with slight modifications. Briefly, 100 µl of plant extract stock solutions (10 mg/ml in ethanol) was serially diluted resulting in a concentration range of 0.019 to 2.5 mg/ml. The same volume of an actively growing culture of *Candida albicans* (optical density of 0.8 at 600 nm) or *Mycobacterium aurum* (optical density of 0.125 at 550 nm) was added to the different wells and plates were incubated in a humid chamber at 37°C for 24 h (*Candida albicans*) or 24-72 h (*Mycobacterium aurum*), respectively. Susceptibility was detected by adding 40 µl of a 0.2 mg/ml stock solution of the tetrazolium salt indicator MTT (3-4,5-dimethylthiazol- 2-yl-2,5-diphenyl tetrazolium bromide) to each well. Following an incubation period of 1 to 4 hours at 37°C during which MTT is reduced to a blue-purple colour by viable microorganism, MICs were determined as the lowest concentrations at which growth inhibition occurred. To distinguish bacteriostatic/fungistatic from bactericidal/fungicidal effects of plant extracts 100 µl aliquots were taken from each well and spread evenly onto respective agar plates. The concentration at which a reduction of ≥99% colony growth of microorganisms occurred was determined as minimal bactericidal/fungicidal concentration (MBC, MFC). Fungazole and isoniazide (Sigma) were used as positive control at the same concentrations as plant extracts.

Cytotoxicity determination

General cytotoxicity of plant extracts (CC_{50}) were determined by the MTT viability assay as described by Mosmann (1983) with some modifications. Briefly, human embryonic kidney (HEK293) cells (kindly donated by the Botswana Vaccine Institute, Gaborone, Botswana) were adjusted to 1×10^5/ml in Dulbecco's Modified Eagle Medium (DMEM, Highveld Biological, South Africa) without phenol red and supplemented with 10% fetal calf serum (Highveld Biological) and antibiotics penicillin and streptomycin (Sigma). A volume of 100 µl of HEK cell suspension was transferred to wells of a 96-well plate (Costar) and left to adhere overnight in a 37°C/5% CO_2 incubator. Cells were exposed for 48 hrs to serial dilutions of plant extracts in DMEM to an end concentration ranging from 0.0001 to 1000 µg/ml in a 37°C/5% CO_2 incubator. 10 µl of a MTT (Sigma) stock solution of 5 mg/ml in water was added to each well and the plate was incubated for further 4 hrs. Supernatant was removed carefully and MTT crystals were

dissolved by adding 100 µl dimethylsulfoxide (DMSO). Absorbance was measured at 570 nm using a microplate reader (Tecan Sunrise, Waesi Pharmaceuticals, Gaborone, Botswana). Berberine hydrochloride (a kind gift from S. Khalid, Faculty of Pharmacy, University of Science and Technology, Sudan) whose cytotoxic concentration at which 50% of cells have died (CC_{50}) was determined as 2.6 µg/ml was used as positive control. Assays were performed in triplicates and best-fit curves were generated using GraphPad Prism 4.0 software (GraphPad Software, La Jolla, CA, USA).

RESULTS AND DISCUSSION

General antimicrobial profile

A total of 39 ethanol plant extracts from 26 different species belonging to 17 families were studied. Plant species were representing a wide variety of plant families with species belonging to the family of Fabaceae most prominently present (5/26) (**Table 1**). Ethnomedical uses mentioned most frequently were the treatment of sores/wounds/abscesses followed by plant uses against respiratory infections including tuberculosis, and stomach disorders and diarrhoea/dysentery (**Table 1**).

The inhibitory properties of 37 plant extracts were evaluated against three Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacterial strains. Sixteen plant extracts (44%) inhibited the growth of at least one Gram-positive microorganism, while twelve plant extracts (32%) showed inhibitory properties against Gram-negative bacteria. Nineteen extracts were not active against any microbial strain (**Table 2**). The greater sensitivity of gram-positive bacteria is not unexpected as the lipopolysaccharide layer and many lipoproteins of the outer surface of Gram-negative bacteria renders these bacteria more resistant than Gram-positive bacteria (Cos *et al*. 2006a). The frequencies of plant extracts inhibiting the growth of the five bacterial strains were as follows: *B. cereus* (12) and *P. aeruginosa* (12) > *S. aureus* (9) > *B. subtilis* (6) > *E. coli* (2). *B. cereus* and *S. aureus* produce toxins which induce diarrhoea, while the latter is also the causative agent for many skin infections, septic wounds and abscesses (Lowy 1998; Kotiranta *et al*. 2000). Twelve plant extracts inhibited the growth of *P. aeruginosa*, which causes muscle weakness and bone-, ear-, eye- and skin infections, particularly in immunocompromised persons, that are amongst the most difficult to treat with conventional antibiotics due to *P. aeruginosa*'s efficient efflux-systems (Livermore 2002).

Six extracts (*A. erioloba* (leaves), *D. capensis* (leaves), *E. burkei* (roots), *E. elephantina* (roots), *L. javanica* (leaves) and *P. limbata* (roots)) showed inhibitory properties against *B. subtilis* (**Table 2**), which is generally not considered a pathogenic organism. Nevertheless, *B. subtilis* infections do occur in immunocompromised individuals and have been implicated in cases of food-poisoning (EPA 1997). Only two plant extracts derived from the roots of *Elephantorrhiza elephantina* and *Persicaria limbata* inhibited the growth of *E. coli*, the bacterium responsible for food-borne diseases resulting in diarrhoea and dysentery (Evans and Evans 2007). Interestingly, the same plant extracts were the only ones which displayed inhibitory activities against all five test organisms, which points not to a specific mode of action against *E. coli*, but rather towards broad spectrum antimicrobial properties of the extracts. To the best of our knowledge the multiple antimicrobial activities of *P. limbata* root extract has not been reported previously, while more information is available about *E. elephantina.* This is one of the four species of the genus *Elephantorrhiza* found in Southern Africa. *Elephantorrhiza* species are known to produce flavonoids, gallic acid derivatives, esterified sugars and simple phenolic derivatives (Majinda *et al.* 2001). Water/ethanolic extracts from *Elephantorrhiza goetzei* showed considerable activities against many standard Gram-positive and -negative laboratory bacterial strains. A number of active compounds have been isolated (Majinda *et al.* 2001).

Anti-*Candida* activities

The susceptibilities of two *Candida* species, *Candida albicans* and *Candida mycoderma*, against 37 plant extracts are summarised in **Table 2**. Both fungi are opportunistic pathogens in HIV/AIDS patients – *C. albicans* to a higher extent. *C. albicans* was inhibited by 26 plant extracts (70%) while *C. mycoderma* was susceptible to 23 plant extracts (62%). Extracts from eight plant species, *Acrotome inflata*, *Bridelia mollis*, *Dichrostachys cinerea*, *Dicerocaryum eriocarpum*, *Dicoma capensis*, *Gomphrena celosioides*, *Tagetes minuta* and *Waltheria indica* specifically inhibited *Candida* growth and were not active against any of the bacterial strains investigated in this study (**Table 2**). *G. celosioides* root and leaf extracts specifically inhibited *C. albicans* growth, but rather weakly. With regards to the clinical importance of *C. albicans* infections we determined Minimal Inhibitory Concentrations (MICs) and Minimal Fungicidal Concentrations (MFCs) of extracts which gave rise to the largest zones of inhibition. As evident from **Table 3** MICs varied between 0.039 and 2.5 mg/ml with *Ocimum canum* leaf extract showing the lowest MIC value of 0.039 mg/ml followed by *D. cinerea* and *E. burkei* root extracts both with a MIC value of 0.078 mg/ml. All extracts with a MIC below 1 mg/ml were fungicidal at the same concentration as their respective MICs.

Anti-mycobacterial activities

Thirty-three plant extracts were screened for inhibitory properties against *Mycobacterium aurum*. *M. aurum* is a fast-growing, non-pathogenic mycobacterium, which has very similar drug susceptibility characteristics as *Mycobacterium tuberculosis*. Because of the structural similarities of mycolates, which are responsible for the permeability of the cell envelope for antimicrobials, *M. aurum* has been recommended as the most suitable model organism to identify new potential therapeutics against *M. tuberculosis*, particularly cell wall inhibitors (Chung *et al*. 1995; Gupta *et al.* 2009). Seventeen plant extracts (51%) inhibited the growth of *M. aurum*, seven of them (21%) with a MIC of 0.156 mg/ml and below (**Table 4**).

E. burkei root and *O. canum* leaf extracts showed the lowest MIC values of 0.039 mg/ml. While *E. burkei* extract was bactericidal at the same concentration, the MBC of *O. canum* was with 0.312 mg/ml higher than its MIC. The anti-mycobacterial activity of *O. canum* reported here is consistent with a previously published activity of volatile oils in leaves of this species inhibiting an avian mycobacterial strain MT B19-3 (Gupta and Viswanathan 1956) and anti-mycobacterial activities of other *Ocimum* species (Gautam *et al*. 2007), while to the best of our knowledge the anti-mycobacterial activity of *E. burkei* has not been reported before.

Assessment of activities and cytotoxicity

The size of zones of inhibition in antimicrobial cultures induced by plant extracts did not always correspond with their respective MIC values (compare **Table 2** and **Tables 3, 4**). One reason for this might be the fact that particularly more hydrophobic and amphipathic compounds often show significant deviations from predicted behaviour during diffusion in agar (Bauer *et al*. 2008). Additionally concerns have been raised about the presence of compounds in the agar medium that may inactivate active plant compounds and about the general stability of active compounds in agar (Cos *et al.* 2006a; McGaw *et al.* 2008c).

There is no uniform opinion on what constitutes a significant bioactivity. While MIC values of anti-fungal extracts below 500 µg/mg were proposed to reflect strong inhibitory activity (Algiannis *et al.* 2001), more stringent criteria sug-

Table 1 Ethnobotanical information of investigated plants from Botswana.

Family	Botanical name	Local name	Voucher	Plant part tested	Major traditional use (Reference)
Fabaceae	*Acacia erioloba* E. May	Mokala, mogotlho, omumbonde,//ah, /ana (!kung Bushmen), go, mosu	E6 81	Bark	Pulverized burnt bark is a remedy for headaches. Gum treats gonorrhea (Roodt 1998b)
			E7 81	Leaves	-
			E10 81	Seeds	Crushed mature pod powder treats ear infections (Roodt 1998b)
			E10 81	Fruits	-
Mimosaceae	*Albizia anthelmintica* Brongn.	Mositanokana	E1 Aar	Roots	Painful body sores, including sores caused by cancer are washed with the solution of cold water in which bark and roots have soaked (Hedberg and Staugard 1989)
Lamiaceae	*Acrotome inflata* Benth.	Leatla, seromo, Makhudungwane	E12 71	Fruit	Unspecified plant parts treat blackleg in cattle (Roodt 1998a)
Asphodelaceae	*Aloe littoralis* Bak.	Mokgopha, mopane aloe	E7 87	Leaves	Leaf and root decoctions taken orally for roundworm infestation. Leaf sap is applied to breasts to hasten weaning
Asphodelaceae	*Aloe marlothii* Berger	Mokgopha	E4 84	Leaves	Leaf shoot are used as a stomach ailment across Africa
Asclepiadaceae	*Asclepias fruticosa* L.	Mositanokana	E1 66	Roots	Roots and leaves infusion treat diarrhea (Roodt 1998a), tuberculosis (Watt and Breyer-Brandwijk 1962) and stomach/general pains (Van Wyk 2009), roots are boiled to treat gonorrhea (Roodt 1998a) or powder is applied topically to treat gonorrhoea (Hedberg and Staugard 1998)
			E7 66	Leaves	
Caesalpinaceae	*Bauhinia petersiana* Bolle	Motshentshe, dikgose, mugutswe, mopondopondo, motlhwa-o jewa, motsope, mohuthi, mogotswe, nsekesa, mochancha	E6 67	Bark	Liquid from water-soaked bark treats poor eyesight, in the form of a lotion it treats wounds (Roodt 1989b)
			E7 67	Leaves	-
Euphorbiaceae	*Bridelia mollis* Hutch.	Mokokonane, mogwnanengebe, mokomanawe, mokokwele, mopororo, mokororo, nkunbakumba	E7 69	Leaves	-
Euphorbiaceae	*Croton gratissimus* Burch.	Moologa mmakwana, mhakwana	DCr150608	Roots	Roots and bark infusions treat respiratory disorders (Roodt 1998b)
Fabaceae	*Dichrostachys cinerea* (L.) Wight & Arm.	Moselesele, mpangale	Dr300508	Roots	Roots are boiled against pneumonia, gonorrhea and internal abscesses and dysentery (Roodt 1998b)
			DL300508	Leaves	Chewed leaves are applied onto sites of snake bites and serve as natural painkiller (Roodt 1998b)
Pedaliaceae	*Dicerocaryum eriocarpum* Decne. Abels	Makanje	Der08	Roots	Boiled roots treat sexually transmitted diseases (STDs, Hedberg and Staugard 1989); are used to heal umbilicus and are taken orally to bring out sores during children's infectious diseases, e.g. measles (Mankudu Glickman, pers. comm.)
Compositae	*Dicoma capensis* Less.	Seromo	DL300508F	Fibre	Boiled fruits/flowers are applied topically to treat persistent wounds and open sores (Mankudu Glickman, pers. comm.)
			Df300508	Fruits	
Fabaceae	*Elephantorrhiza burkei* Benth.	Mosidigodimo, mosidi, mositsane, Mosetlha -o-monnye	Ebr300508	Roots	Roots are boiled and taken by couples together. It strengthen bones, muscles and nerves, especially after pregnancy (Mankudu Glickman, pers.comm.)
Fabaceae	*Elephantorrhiza elephantina* (Burch.) Skeels	Mosidi, mositsane tjizezana, chizezana, motshijane, mosibe	Ee300508	Roots	Grated roots are steeped in water and are used externally to treat skin problems or boiled roots are taken internally to treat diarrhea, dysentery, stomach disorders and ulcers (Van Wyk 2009) or against erectile dysfunction (Mankudu Glickman, pers. comm.)
Euphorbiaceae	*Euphorbia tirucalli* L.	Motsetsi, moremotala, ngocha	Eu E4	Stems/twigs	-
Amaranthaceae	*Gomphrena celosioides* Marf.	Mositanoka	E176	Roots	-
			E676	Bark	-
			E776	Leaves	-
Verbenaceae	*Lippia javanica* (Burm.f.) Spreng	Mosukudu, mosukutshane, mosukujane, mabele-a-dinonyane	LJ1	Leaves	Leave infusion used to treat coughs, colds and fever, influenza, measles, malaria and stomach ache (Van Wyk 2009)
Fabaceae	*Mundulea sericea* (Willd.) A. Chev.	Mositatlou, mosikatse, mosikase, moswaatlou, maibana, mohato	E6 82	Bark	-
			E7 82	Leaves	Emetic to treat poisoning (Bester and Grobler 2008)

Table 1(Cont.)

Family	Botanical name	Local name	Voucher	Plant part tested	Major traditional use (Reference)
Lamiaceae	*Ocimum canum* Sims	Mogatololo, sebeitona, badingwana	E7 74	Leaves	Dried or pulverized leaves are burnt and smoke inhaled or leave infusion is taken to treat chest complaints and blockage of air passages (Roodt 1998a)
Sapindaceae	*Pappea capensis* Eckl. & Zeyh.	Mopenoene, mopenwaeng, mothata, ntorido, molalakgaka, mopanyobojalwa	E678	Bark	-
			E778	Leaves	Powdered leaves are mixed with water and used against bleeding/hemorrhage (Hedberg and Staugard 1989)
Polygolaceae	*Persicaria limbata* (Meisn.) H. Hara	Kubutona, letetemetso	E1 73	Roots	Roots and leaves are pounded, mixed with fat and applied to swollen neck and throat (Roodt 1998a)
			E7 73	Leaves	
Polygolaceae	*Securidaca longipedunculata* Fresen.	Mmaba, maabo, magolela, bogokgwe, mofufu	Slrb	Root bark	Root infusion treats cough, chest complaints, tooth aches, rheumatism and headache (Van Wyk 2009) and tuberculosis (Hedberg and Staugard 1989)
Asteraceae	*Tagetes minuta* L.	-	E12 72	Fruit	Whole plant is nematocidal (Oduor-Owino 1993)
Combretaceae	*Terminalia sericea* Burch. ex DC.	Mogonono	E1 Tsr	Roots	Roots treat diarrhea and stomach disorders (Van Wyk 2009), a hot infusion of the root bark treats pneumonia (Roodt 1998b)
Sterculiaceae	*Waltheria indica* L.	Seretwane	E7 80	Leaves	Powdered leaves are applied topically on septic wounds, whole plant decoction treats diarrhea (Maregesi *et al*. 2007)
Rhamnaceae	*Ziziphus mucronata* Willd.	Mokgalo	E7 77	Leaves	Paste of leaves treats boils, carbuncles and swollen glands, leaf infusion is taken against chest complaints (Roodt 1998b)

- no specific use in Botswana published

Table 2 Zones of inhibition (mm[a, b]) induced by crude ethanol extracts[c] from selected Botswana medicinal plants against fungi and bacteria.

Plant species	Plant part	Microorganisms						
		Candida albicans	***Candida mycoderma***	***Bacillus subtilis***	***Bacillus cereus***	***Escherichia coli***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Acacia erioloba	Bark	2.5 ± 0.5	-	-	2.0 ± 0.0	-	-	1.5 ± 0.0
	Leaves	1.5 ± 0.0	1.5 ± 0.0	1.0 ± 0.0	1.5 ± 0.0	-	1.5 ± 0.0	2.0 ± 0.0
	Seeds	-	2.5 ± 0.0	-	2.0 ± 0.0	-	-	-
	Fruit	3.0 ± 0.0	2.5 ± 0.0	-	-	-	2.5 ± 0.0	1.5 ± 0.0
Acrotome inflata	Fruit	2.0 ± 0.3	3.5 ± 0.0	-	-	-	-	-
Aloe littoralis	Leaves	-	2.5 ± 0.0	-	-	-	-	-
Aloe marlothii	Leaves	-	-	-	-	-	-	-
Asclepias fruticosa	Roots	-	-	-	-	-	-	-
	Leaves	-	-	-	-	-	-	-
Bauhinia petersiana	Bark	1.0 ± 0.3	-	-	1.0 ± 0.0	-	-	1.0 ± 0.0
	Leaves	1.0 ± 0.3	-	-	1.0 ± 0.0	-	2.0 ± 0.0	1.0 ± 0.0
Bridelia mollis	Bark	-	3.0 ± 0.0	-	-	-	-	-
	Leaves	-	-	-	-	-	-	-
Croton gratissimus	Leaves	3.5 ± 0.2	3.5 ± 0.0	-	-	-	-	-
Dicerocaryum eriocarpum	Roots	4.5 ± 0.0	2.0 ± 0.0	-	-	-	-	-
Dichrostachys cinerea	Roots	2.0 ± 0.0	5.0 ± 0.0	-	-	-	-	-
Dicoma capensis	Roots	-	-	-	-	-	-	-
	Leaves	2.0 ± 0.7	2.0 ± 0.3	1.0 ± 0.0	-	-	-	-
	Fibre	1.0 ± 0.0	2.5 ± 0.0	-	-	-	-	-
	fruits	3.0 ± 0.2	3.0 ± 0.0	-	-	-	-	-
Elephantorrhiza burkei	Roots	3.5 ± 0.3	3.0 ± 0.0	2.5 ± 0.0	3.0 ± 0.3	-	3.5 ± 0.0	3.5 ± 0.0
Elephantorrhiza elephantina	Roots	3.0 ± 0.2	3.0 ± 0.0	1.0 ± 0.0	2.0 ± 0.0	1.0 ± 0.0	3.0 ± 0.0	2.5 ± 0.0
Euphorbia tirucalli	Stems/Twigs	-	-	-	-	-	-	-
Gomphrena celosioides	Roots	1.5 ± 0.0	-	-	-	-	-	-
	Leaves	1.5 ± 0.0	-	-	-	-	-	-
Lippia javanica	Leaves	-	-	1.0 ± 0.0	-	-	-	-
Mundulea sericea	Bark	2.0 ± 0.0	2.0 ± 0.0	-	1.5 ± 0.0	-	-	-
	Leaves	1.0 ± 0.0	-	-	1.0 ± 0.0	-	-	-
Ocimum canum	Leaves	2.0 ± 0.5	3.0 ± 0.0	-	4.5 ± 0.0	-	3.5 ± 0.0	1.0 ± 0.0
Pappea capensis	Bark	1.0 ± 0.3	3.5 ± 0.0	-	-	-	2.0 ± 0.0	1.5 ± 0.0
	Leaves	1.5 ± 0.3	3.0 ± 0.3	-	-	-	2.0 ± 0.0	1.0 ± 0.0
Persicaria limbata	Roots	2.0 ± 0.7	3.0 ± 0.0	2.0 ± 0.0	3.0 ± 0.7	2.0 ± 0.3	3.5 ± 0.0	4.0 ± 0.0
	Leaves	1.0 ± 0.0	-	-	-	-	-	-
Securidaca longipedunculata	Root bark	2.0 ± 0.3	3.0 ± 0.3	-	-	-	-	1.0 ± 0.0
Tagetes minuta	Fruit	1.0 ± 0.5	7.0 ± 0.0	-	-	-	-	-
Waltheria indica	Leaves	2.0 ± 0.3	2.5 ± 0.3	-	-	-	-	-
Ziziphus mucronata	Leaves	-	2.0 ± 0.0	-	2.0 ± 0.3	-	-	-

[a] Between the edge of the paper disc and the edge of the inhibition area; [b] Data are presented as means ± standard deviation; [c] 500 µg/disc; -: no inhibition

Table 3 Anti-*Candida albicans* activities of selected medicinal plant extracts.

Plant species	Plant part	MIC[a] (mg/ml)	MFC[b] (mg/ml)
Acacia erioloba	Bark	0.312	0.312
	Fruit	1.25	n.d.
Acrotome inflata	Fruit	1.25	n.d.
Croton gratissimus	Leaves	2.5	n.d.
Dicerocaryum eriocarpum	Roots	0.156	0.156
Dichrostachys cinerea	Roots	0.078	0.078
Dicoma capensis	Fruits	1.25	n.d.
Elephantorrhiza burkei	Roots	0.078	0.078
Elephantorrhiza elephantina	Roots	1.25	n.d.
Ocimum canum	Leaves	0.039	0.039
Persicaria limbata	Roots	0.156	0.156
Waltheria indica	Leaves	0.156	0.156

[a] Minimal inhibitory concentration
[b] Minimal fungicidal concentration
n.d.: not determined as MIC >1 mg/ml

Table 4 Anti-*Mycobacterium aurum* activities of selected medicinal plant extracts.

Plant species	Plant part	MIC[a] (mg/ml)	MBC[b] (mg/ml)
Acacia erioloba	Bark, leaves, fruits, seeds	-	n.d.
	Roots	0.625	n.d.
Acrotome inflata	Fruit	-	n.d.
Albizia anthelmintica	Roots	0.156	0.156
	Leaves	0.625	n.d.
Aloe littoralis	Leaves	-	n.d.
Aloe marlothii	Leaves	2.5	n.d.
Asclepias fruticosa	Roots	0.156	0.156
Bauhinia petersiana	Bark, leaves	-	n.d.
Bridelia mollis	Bark, leaves	-	n.d.
Croton gratissimus	Leaves	2.5	n.d.
Dicerocaryum eriocarpum	Roots	0.156	0.312
Dichrostachys cinerea	Roots	0.156	0.156
Dicoma capensis	Fruits, flowers	5	n.d.
	Fibre	5	n.d.
Elephantorrhiza burkei	Roots	0.039	0.039
Elephantorrhiza elephantina	Roots	2.5	n.d.
Euphorbia tirucalli	Stem, twigs	-	n.d.
Gomphrena celosioides	Roots, bark, leaves	-	n.d.
Lippia javanica	Leaves	-	n.d.
Ocimum canum	Leaves	0.039	0.312
Pappea capensis	Leaves	0.312	0.312
Persicaria limbata	Roots	0.156	0.156
Securidaca longipedunculata	Roots	-	n.d.
Terminalia sericea	Roots	2.5	n.d.
Ziziphus mucronata	Leaves	0.625	n.d.

[a] Minimal inhibitory concentration
[b] Minimal bactericidal concentration
n.d.: not determined as MIC >0.5 mg/ml or no activity has been detected
- : no inhibition

gested to consider MICs of plant extracts below 50 µg/ml against Gram-negative, mycobacteria and fungi (Cos *et al.* 2006b) or below 100 µg/ml against *mycobacteria* (McGaw *et al.* 2008b) as suitable for detailed phytochemical analysis and evaluation for potential therapeutic applications. Applying the most stringent criteria, *O. canum* leaf and *E. burkei* root extracts would constitute the most interesting activities inhibiting the growth of *C. albicans* and *M. aurum*.

A further characterisation of the two extracts would be worthwhile because they seem to contain compound(s) which are active against both HIV/AIDS opportunistic pathogens, provided the extracts inhibit the pathogenic *M. tuberculosis* as well.

O. canum leaf and *E. burkei* root extracts did not show *in-vitro* cytotoxicity up to a concentration of 1000 µg/ml using confluent Human Embryonic Kidney (HEK293) cells as an experimental system. This signifies a favourable therapeutic index [ratio of CC_{50} and effective concentration at which inhibition of 50% of microorganisms is observed (EC_{50})] of > 25 for *O. canum* leaf extract inhibiting *C. albicans* and *E. burkei* root extract exercising antimycobacterial activity. The lack of cytotoxicity in the concentration range measured also indicates that the rather broad antimicrobial activity of *E. burkei* root extract (inhibitory activity against seven out of eight test microorganisms) is not the consequence of a non-specific acute toxicity of the extract. This makes *E. burkei* an attractive plant species to consider for the development of herbal broad spectrum antibiotics. However, more pharmacological and toxicity studies are necessary to confirm these results.

CONCLUSION

The anti-infective screening of selected plants from Botswana reported in this paper revealed a number of plant species as sources which could yield standardized herbal preparations (Taylor 2003) or drugs potentially useful for the management of bacterial and fungal infections. Particularly, inhibitory activities against *C. albicans* and *M. aurum*, a model system for the causative agent of tuberculosis, *M. tuberculosis*, are of interest for Botswana as a country with a high rate of HIV infections. Remedies which could help in combating opportunistic infections, such as candidiasis and tuberculosis are urgently needed. Our findings are consistent with the general ethnomedical uses of plants in the management of skin infections, wound, sores, stomach disorders, diarrhoea and respiratory conditions. However, closer inspection shows that not all plant uses match their respective anti-infective profiles. One example is *Securidaca longipedunculata*, which is popularly used in Botswana for the treatment of cough, chest complaints and tuberculosis. The root extract from this plant did not show antimycobacterial activity. It has been suggested that in such cases, most likely the plants are used to treat the symptoms of the disease rather than actually cure the disease itself (Newton *et al.* 2002). It is also thinkable that some plant species may not contain compounds which inhibit the growth of or kill *mycobacteria*, but may have modulatory effects on the immune system. On the other hand, the use of *O. canum* leaves in the treatment of chest complaints and respiratory conditions might be consistent with the low MIC value against *M. aurum*. The ethnomedical uses compiled in this report represent rather a snapshot of some traditional therapeutic applications. Without doubt, more comprehensive surveys on ethnomedical plant uses in Botswana would substantiate correlations between traditional medical plant uses and bioactivities. We therefore recommend conducting such surveys. In this study only 26 plant species were investigated. Two promising bioactivities from *E. burkei* and *O. canum* were identified which could be further investigated as antimycobacterials and antifungals. We, therefore, hope that this study will encourage more research on bioactivities of medicinal plants, which can serve as a scientific basis for innovations to the benefit of communities in Botswana.

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