Available online at www.sciencedirect.com
ScienceDirect
South African Journal of Botany xx (2009) xxx – xxx
SOUTH AFRICAN JOURNAL OF BOTANY
www.elsevier.com/locate/sajb

Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*

M.M. Suleiman [a,b], L.J. McGaw [a], V. Naidoo [a], J.N. Eloff [a,*]

[a] *Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa*
[b] *Permanent Address: Department of Physiology and Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria*

Received 4 May 2009; received in revised form 22 June 2009; accepted 6 July 2009

Abstract

Aspergillus fumigatus causes severe problems in poultry production systems. Seven South African tree species were selected from the database of the Phytomedicine Programme based on its antifungal activity against the fungus *Cryptococcus neoformans*. The acetone leaf extracts of the selected species had minimum inhibitory concentrations (MICs) of 0.16 mg/ml and lower in the preliminary screening. The antibacterial and antifungal activities of hexane, dichloromethane, acetone and methanol extracts of the leaves were determined using a two-fold serial microdilution method against a range of commonly encountered animal pathogenic fungi (*A. fumigatus*, *Candida albicans*, *C. neoformans*, *Microsporum canis* and *Sporothrix schenckii*) and four nosocomial bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*). The plant species investigated were *Combretum vendae* (A.E. van Wyk) (Combretaceae), *Commiphora harveyi* (Engl.) Engl. (Burseraceae), *Khaya anthotheca* (Welm.) C.DC (Meliaceae), *Kirkia wilmsii* Engl. (Kirkiaceae), *Loxostylis alata* A. Spreng. ex Rchb. (Anacardiaceae), *Ochna natalitia* (Meisn.) Walp. (Ochnaceae) and *Protorhus longifolia* (Bernh.) Engl. (Anacardiaceae). All the extracts had activity against at least one of the test organisms over an incubation period of 24 or 48 h. The MIC values of the non-polar and intermediate polarity extracts of *O. natalitia*, *K. anthotheca*, *C. vendae*, *C. harveyi*, and *P. longifolia* had MICs as low as 0.08 mg/ml against at least one of the tested bacteria. Furthermore, the acetone extracts of *L. alata*, *K. wilmsii*, *O. natalitia* and *C. vendae* had antifungal activities with MIC values ranging from 0.04 to 0.08 mg/ml against at least one of the tested fungi. The average MIC values of the plant extracts against the different bacteria ranged from 0.17 to 2.11 mg/ml, while the range was 0.23–1.98 mg/ml for fungi. The Gram-positive bacteria (*S. aureus* and *E. faecalis*) were more susceptible to the plant extracts than the Gram-negative bacteria (*E. coli and P. aeruginosa*). *E. faecalis* was the most susceptible microbe and *C. vendae* extracts were the most active against nearly all the bacteria tested. The acetone extract of *L. alata* was the most active against fungal pathogens, with activity against at least 3 fungal organisms. *L. alata* was selected for further work to isolate compounds active against *A. fumigatus* and other fungal pathogens.

Keywords: Antibacterial; Antifungal; Medicinal plants; Minimum inhibitory concentration; Microdilution assay

1. Introduction

With the development of relatively effective and safe antibiotics in the 1940's, medical treatment had been revolutionalised leading to a drastic drop in morbidity and mortality previously induced by microbial diseases (Rang et al., 2003). Unfortunately, this development was rapidly hampered by the emergence of drug-resistance microbes (Walsh, 2000). This resistance has resulted in an increased incidence of infectious diseases with some pathogens (Kunin, 1993; Archibald et al., 1997; Sahm et al., 1999). With the evolutionary process that enables microbes to adapt genetically to changes in their environment, the unwise use of antibiotics inevitably selects for resistant microbes (Clardy et al., 2006). As a result new drugs have to be consistently developed to counteract the development of resistance and to possibly reduce the cost of controlling the disease. (Cowan, 1999).

In addition to the pathogenic bacteria, opportunistic fungal infections are becoming more important especially due to the immune deficiency induced by HIV-AIDS (Groll et al., 1996). Invasive pulmonary aspergillosis (IPA) is a serious fungal infection of immunocompromised patients usually caused by *Aspergillus fumigatus* with ever increasing incidence (Stevens, 1990; Denning, 1998). In contrast, however, there are only a limited number of

* Corresponding author. Tel.: +27 12 5298244; fax: +27 12 5298525.
E-mail address: kobus.eloff@up.ac.za (J.N. Eloff).

0254-6299/$ - see front matter © 2009 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2009.07.001

antimicrobial drugs which are active against fungal pathogens (Denning, 1998). Although conventional antifungals remain the standard therapy for many invasive or life-threatening mycoses, these drugs are associated with significant toxicity (Dismukes, 2000). Against this backdrop, there is the need to develop cheaper, safer and effective antifungal drugs that could be used to control opportunistic fungal infections.

Plants have an almost limitless ability to synthesize secondary chemical substances, which play a pivotal role in their ecophysiology (Briskin, 2000). Accordingly, secondary products may have both a defensive role against herbivores, pathogen attack, and interplay competition and an attractant role toward beneficial organisms such as pollinators or symbionts (Wink and Schimmer, 1999). Some of the secondary-derived compounds may therefore have beneficial effects in the treatment of microbial infections in animals and humans (Cowan, 1999; Kuete et al., 2007). Recently, the interest in these metabolites has increased following searches for new antimicrobial agents from plant sources (Hostettmann et al., 2000).

Southern Africa is exceptionally rich in plant and animal diversity. It has the richest temperate flora in the world, with a floristic diversity of about 24,000 species and intraspecific taxa in 368 families. The region has only 2.5% of the world's land surface area and contains more than 10% of the world's vascular plant flora (Germishuizen and Meyer, 2003). Southern Africa also contains a major proportion of the 50,500 taxa present in sub-Saharan Africa (Klopper et al., 2006).

From data of approximately 350 plant species tested for biological activity obtained in an ongoing tree screening project of the Phytomedicine Programme, seven plant species (Table 1) with minimum inhibitory concentrations of 0.16 mg/ml and lower against *Cryptococcus neoformans* were selected for evaluation of their potential action against other important pathogenic bacteria and fungi. Although the selection was based on previously reported activity against the fungus *C. neoformans* (Pauw and Eloff, unpublished data), some of these plant species are also used by indigenous healers for different disease conditions (Van Wyk et al., 2000) and a summary of their traditional use is presented in Table 1. Leaves of the selected tree species were screened for activity against five important fungal pathogens (*A. fumigatus*, *Candida albicans*, *C. neoformans*, *Microsporum canis* and *Sporothrix schenckii*). Additionally we also investigated the antibacterial activity against four important nosocomial bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*). The focus was however on *A. fumigatus*, one of the most common pathogenic fungal species in humans and animals (Rippon, 1982). It also plays a role in the economically important disease aspergillosis in poultry. The aim of this publication was therefore to select the tree species with the best potential for developing a commercially useful antifungal product. To promote the sustainable use of plants only tree leaves were considered in this study.

2. Materials and methods

2.1. Plant collection

Commiphora harveyi (Engl.) Engl. (Burseraceae), *Combretum vendae* (A.E. van Wyk) (Combretaceae), *Khaya anthotheca* (Welm.) C.DC (Meliaceae), *Loxostylis alata* A. Spreng. ex Rchb. (Anacardiaceae) and *Protorhus longifolia* (Bernh.) Engl. (Anacardiaceae) leaves were collected at the University of Pretoria's Botanical Garden (Department of Botany), South Africa. *Kirkia wilmsii* Engl. (Kirkiaceae) and *Ochna natalitia* (Meisn.) Walp. (Ochnaceae) were collected at the Lowveld National Botanical Garden in Nelspruit, South Africa. All plant leaves were collected in summer (November 2006) between 9:30 am and 12:30 pm. Samples of the plants were identified and authenticated by Ms. Lorraine Middleton, the herbarium curator, and Magda Nel at the Botanical Garden of the University of Pretoria. Voucher specimens of the plants were deposited at the Schweikert Herbarium of the Department of Plant Sciences, University of Pretoria, South Africa. The botanical names of the plants, tree reference numbers, and the plant parts used are presented in Table 1.

2.2. Plant storage

Immediately after collection and transportation to the laboratory, leaves were separated from stems and dried at room temperature under natural ventilation. The dried plant leaves were milled to a

Table 1
Botanical names and traditional use of the plants studied.

Botanical name	Family	Voucher specimen number	Traditional medicinal uses	Reference
Combretum vendae A.E. van Wyk	Combretaceae	PRU96507	Leprosy, ophthalmic remedy, and blood purification	Watt and Breyer-Brandwijk (1962)
Commiphora harveyi (Engl.) Engl.	Burseraceae	PRU96506	Used as disinfectant for wounds, anthelmintic and treatment of snake bite	Watt and Breyer-Brandwijk (1962)
Khaya anthotheca (Welm.) C.DC	Meliaceae	PRU96509	Skin diseases, black quarter, helminthosis	(Watt and Breyer-Brandwijk, 1962, Nfi et al., 2001)
Kirkia wilmsii Engl.	Kirkiaceae	PRU96503	Treatment of malaria and feverish conditions.	Clarkson et al. (2004)
Loxostylis alata A. Spreng. ex Rchb.	Anacardiaceae	PRU96508	Stimulation of immune system, relief of pain during child birth	(Pooley, 1993; Pell, 2004)
Ochna natalitia (Meisn.) Walp.	Ochnaceae	PRU96504	Infusions and decoctions for headache, and respiratory diseases	Watt and Breyer-Brandwijk (1962)
Protorhus longifolia (Bernh. ex C. krauss) Engl.	Anacardiaceae	PRU96505	Treatment of diarrhoea and heartwater	Dold and Cocks (2001)

Please cite this article as: Suleiman, M.M., et al., Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*, South African Journal of Botany (2009), doi:10.1016/j.sajb.2009.07.001

fine powder in a Macsalab mill (Model 200 LAB), Eriez®, Bramley, and stored at room temperature in closed containers in the dark until used.

2.3. Plant extraction

Plant samples from each species were individually extracted by weighing four aliquots of 1 g of finely ground plant material and extracting each aliquot with 10 ml of acetone, hexane, dichloromethane (DCM) or methanol (technical grade, Merck) respectively in centrifuge tubes. Tubes were vigorously shaken for 1 h using a Labotec model 20.2 shaking machine at a moderate speed. Extracting at lower speed for a longer period allows the solvent to penetrate more into the plant tissues, allowing the extraction of more of the compounds contained in the plant species (Silva et al., 1998). After centrifuging at 3500 ×*g* for 10 min, the supernatant was decanted into pre-weighed labelled glass vials. The whole process was repeated three times on the marc to exhaustively extract the plant material. The solvent was removed under a stream of air in a fume cupboard at room temperature to quantify the extraction.

2.4. Micro-organisms and medium

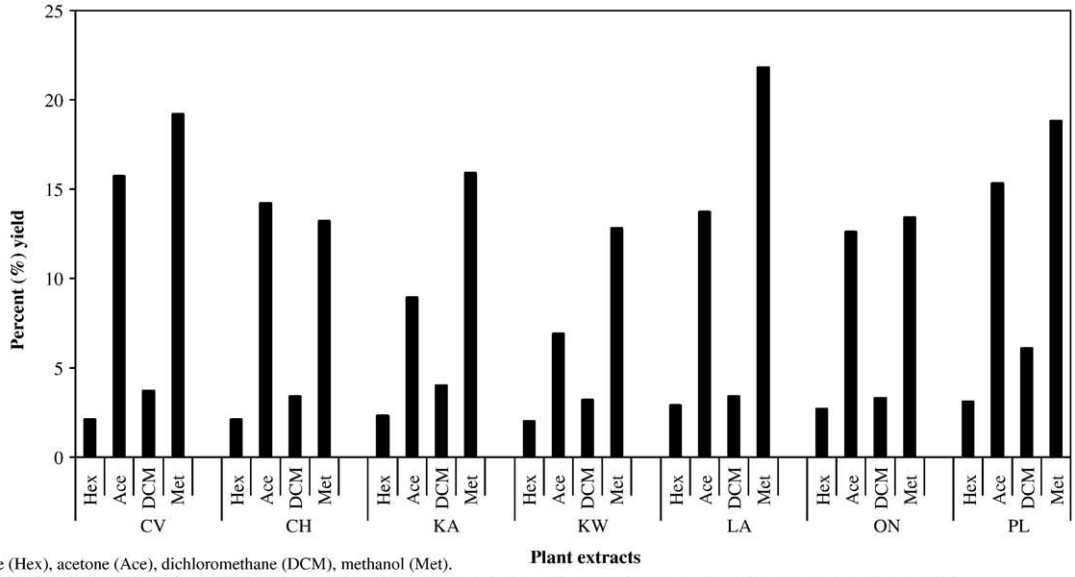
The bacterial organisms used in this study were obtained from the Department of Microbiology at the Medical Campus, University of Pretoria. They included the Gram-positive bacteria, *S. aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212), and the Gram-negative bacteria, *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 27853). All bacterial cultures were maintained on Mueller Hinton (MH) agar and subcultured before use in MH broth (Oxoid, Basingstoke, UK). The five fungal organisms that were used included *A. fumigatus*, *M. canis*, *C. albicans*, *C. neoformans* and *S. schenckii*. All fungal organisms were isolated from animal clinical cases prior to treatment, by the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria. These fungi are important disease-causing pathogens of animals and man. Sabouraud dextrose (SD) agar (Oxoid, Basingstoke, UK) was used as the maintenance growth medium for the fungi.

2.5. Antimicrobial sensitivity test

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of extract that inhibits visible growth of the micro-organism. For the different microbial species, the MIC was determined using the serial microdilution assay (Eloff, 1998a).

2.5.1. Bacterial organisms

Different plant extracts (hexane, acetone, dichloromethane and methanol) were dissolved in acetone to a concentration of 10 mg/ml. Acetone is non-toxic to the micro-organisms at the concentrations used in this assay (Eloff, 1998b; Masoko et al., 2005). One hundred µl of each plant extract was serially diluted 2-fold with sterile distilled water in 96-well microtitre plates. One millilitre of concentrated bacterial culture grown at 37 °C for 3 days was transferred to 100 ml of fresh MH broth and 100 µl of the resultant culture was added to each well. Densities of bacterial cultures used for the screening were approximately: *S. aureus*, 3×10^{12} cfu/ml; *E. faecalis*, 2×10^{10} cfu/ml; *P. aeruginosa*, 5×10^{13} cfu/ml; *E. coli*, 3×10^{11} cfu/ml (Shai et al., 2008). Gentamicin at 0.1 mg/ml (Virbac®) and acetone were used as positive and negative control agents, respectively. After incubation overnight at 37 °C, *p*-iodonitrotetrazolium violet (INT, Sigma) at a concentration of 0.2 mg/ml was used as an indicator of bacterial growth. Forty µl of INT was added to each of the microtitre wells. Thereafter, the plates were incubated at 37 °C and the MIC was assessed 1 and 2 h after the addition of INT. Bacterial



Hexane (Hex), acetone (Ace), dichloromethane (DCM), methanol (Met).
Loxostylis alata (LA), *Kirkia wilmsii* (KW), *Ochna natalitia* (ON), *Khaya anthotheca* (KA), *Combretum vendae* (CV), *Commiphora harveyi* (CH) and *Protorhus longifolia* (PL)

Fig. 1. Percentage yield extracted by different solvents from leaves of different South African plant species.

Please cite this article as: Suleiman, M.M., et al., Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*, South African Journal of Botany (2009), doi:10.1016/j.sajb.2009.07.001

cultures react with INT and give red or purple colouration within 10–60 min (Eloff, 1998a,b).

2.5.2. Fungal organisms

Fungal cultures were transferred from agar culture plates to fresh SD broth and 100 µl of the broth was added to each well. Densities of fungal cultures used for the screening were approximately: *A. fumigatus*, 8×10^4 cfu/ml; *C. albicans*, 3×10^4 cfu/ml; *C. neoformans*, 3×10^4 cfu/ml; *M. canis*, 2×10^5 cfu/ml; *S. schenckii*, 1×10^5 cfu/ml. Amphotericin B (0.16 mg/ml) and acetone were used as positive and negative control substances, respectively. Forty µl of *p*-iodonitrotetrazolium violet INT (0.2 mg/ml) was added to each of the microtitre wells to serve as an indicator of fungal growth. The minimum inhibitory concentration (MIC) is defined as the lowest concentration of extract to inhibit bacterial growth in the microtitre plate as indicated by a reduction in the red colour of the INT formazan (Masoko et al., 2005) and was assessed after 24 and 48 h incubation period.

3. Results and discussion

3.1. Mass extracted

The amounts of dried plant material extracted by each of the solvents (hexane, acetone, dichloromethane and methanol) used in this study are shown in Fig. 1. Methanol extracted the largest quantity of plant material. The largest quantity was extracted from *L. alata* (218 mg) representing 21.8%. The masses extracted by acetone and dichloromethane extracts were generally second and third, respectively, in all the plants except *C. harveyi*. The result was comparable to what was reported by Kotze and Eloff (2002) where methanol was shown to be the best extractant for *Combretum erythrophyllum* found in South Africa. In contrast, acetone extracted more plant material from *C. harveyi* (142 mg) representing 14.2%. In a study with 27 members of the Combretaceae family, acetone extracted more plant material than the other solvents used (Eloff, 1999). The lowest amount of extract was obtained from the hexane extraction of *K. wilmsii* (20 mg) representing 2% of extractable material. In traditional medical practice, water is used as the major extractant. The implication of this is that potential active compounds that are not hydrophilic may not be extracted and a plant disregarded as not being active biologically, especially if the polar extracts are not active in the bioassay. Such a problem was circumvented in our study by extracting the plant leaves in parallel with solvents of low to high polarity. Acetone was used to re-dissolve the extracts of hexane, acetone, dichloromethane and methanol prior to bioassay.

3.2. Minimum inhibitory concentration

3.2.1. Bacterial species

The plant extracts differed greatly in their activity against the test bacteria and the best bacterial inhibition was observed with

Table 2
Minimum inhibitory concentrations (average of triplicate determinations) of four different extracts from seven South African plants tested against bacteria.

Microorganism	Time (h)	MIC (mg/ml)															
		Combretum vendae				*Commiphora harveyi*				*Khaya anthotheca*				*Kirkia wilmsii*			
		H	A	D	M	H	A	D	M	H	A	D	M	H	A	D	M
P. aeruginosa	1	0.31	0.16	0.16	1.25	0.31	0.63	0.63	2.50	0.31	0.16	0.16	1.25	0.16	0.24	0.31	0.63
	2	0.63	0.31	0.16	1.25	0.63	1.25	0.63	2.50	0.63	0.31	0.16	1.25	0.31	0.47	0.63	2.50
S. aureus	1	0.31	0.63	0.31	1.25	0.31	0.63	0.08	2.50	0.04	0.16	0.08	1.25	0.63	0.31	0.63	1.25
	2	0.31	1.25	0.63	2.50	0.31	0.63	0.16	2.50	0.08	0.16	0.08	1.25	1.25	0.31	0.63	1.25
E. coli	1	0.78	0.63	0.31	1.25	2.50	1.25	1.25	2.50	2.50	1.25	1.25	2.50	0.16	0.31	0.63	1.25
	2	1.25	0.63	0.63	2.50	2.50	2.50	1.25	2.50	2.50	2.50	0.63	2.50	0.16	0.31	1.25	2.50
E. faecalis	1	0.08	0.12	0.08	0.16	0.06	0.08	0.08	0.63	0.16	0.16	0.16	1.25	0.31	0.31	0.31	1.25
	2	0.16	0.12	0.08	0.31	0.31	0.24	0.16	1.25	0.16	0.31	0.16	2.50	0.31	0.31	0.31	1.25
Average		0.48	0.48	0.30	1.31	0.87	0.90	0.53	2.11	0.80	0.63	0.34	1.72	0.41	0.32	0.59	1.49

Microorganism	Time (h)	MIC (mg/ml)												Gentamicin
		Loxostylis alata				*Ochna natalitia*				*Protorhus longifolia*				
		H	A	D	M	H	A	D	M	H	A	D	M	
P. aeruginosa	1	0.31	0.31	0.47	0.31	0.16	0.31	0.31	1.25	0.16	0.08	0.16	2.50	0.015
	2	0.63	0.31	0.47	0.31	0.31	1.25	0.63	1.25	0.63	0.16	0.31	2.50	0.06
S. aureus	1	0.08	0.06	0.63	2.50	0.16	0.31	0.16	1.25	0.63	0.63	0.63	1.25	0.007
	2	0.1	0.06	1.25	2.50	0.16	1.25	0.63	2.50	0.63	0.63	0.31	1.25	0.025
E. coli	1	0.08	0.06	0.63	1.25	0.31	0.63	2.50	2.50	0.63	0.31	0.31	1.25	0.025
	2	0.16	0.08	0.08	1.25	0.63	0.63	1.25	2.50	0.63	0.31	0.63	2.50	0.05
E. faecalis	1	1.25	0.16	0.16	0.16	0.08	0.16	0.24	0.63	0.31	0.63	0.63	1.25	0.003
	2	2.5	0.31	0.63	0.63	0.08	0.16	0.31	1.25	1.25	1.25	0.63	2.50	0.006
Average		0.64	0.17	0.54	1.11	0.24	0.59	0.75	1.64	0.61	0.50	0.45	1.88	

MIC assessment was done 1 and 2 h after INT (indicator of bacterial growth) was added to the bacterial cultures.
H = hexane extract; A = acetone extract; D = dichloromethane extract; M = methanol extract.

Please cite this article as: Suleiman, M.M., et al., Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*, South African Journal of Botany (2009), doi:10.1016/j.sajb.2009.07.001

MIC = 0.04 mg/ml by the hexane extract of *K. anthotheca* against *S. aureus*. There are no validated criteria for the MIC end points for *in vitro* testing of plant extracts. However, an attempt was made to grade MIC of plant extracts/compounds by Holetz et al (2002). He proposed: good antimicrobial activity = MIC less than 0.1 mg/ml; moderate antimicrobial activity = MIC of 0.1 to 0.5 mg/ml; weak antimicrobial activity = MIC of 0.5 to 1 mg/ml; MIC of greater than 1 mg/ml was considered inactive.

Among the tested extracts, the hexane extracts of *C. vendae*, *C. harveyi*, *K. anthotheca*, *O. natalitia* and *L. alata*, the acetone extracts of *C. harveyi*, *L. alata* and *P. longifolia*, and the dichloromethane extracts of *C. vendae*, *C. harveyi* and *L. alata* showed the best antibacterial activity against at least one of the tested pathogens. The MIC values of these extracts were the lowest, ranging from 0.04 to 0.01 mg/ml (Table 2). The extracts of *L. alata* had very promising results with good antibacterial activity in 3 out of 4 of the extracts tested. The hexane, acetone and dichloromethane extracts of *L. alata* had MIC values as low as 0.08 mg/ml against *S. aureus*, *E. faecalis* and *E. coli*. The reference antibiotic (gentamicin) had an MIC of 0.025 mg/ml against the mentioned pathogen. Perhaps when the active compound(s) are isolated in pure forms from the crude extracts they might show increased antimicrobial action. The action of most of the extracts appeared to be bacteriostatic, as growth of the bacteria and resulting red colour formation appeared to resume after the 24-h incubation period with INT (Table 2).

The Gram-negative organisms (*E. coli* and *P. aeruginosa*) were more resistant to the extracts than the Gram-positive organisms (*S. aureus* and *E. faecalis*) as indicated by their high MIC values. Gram-negative bacteria are relatively resistant to plant extracts owing to the presence of an outer membrane which is known to present a barrier to penetration of numerous antimicrobial molecules, and the periplasmic space contains enzymes which are capable of breaking down foreign molecules introduced from outside (Nikaido, 1996). *S. aureus* exhibited the highest susceptibility to the plant extracts used in other studies conducted (Stickler and King, 1992; Martínez et al., 1996; Chariandy et al., 1999). Similar results were obtained in this study.

3.2.2. Fungal species

In the present investigation, the 28 extracts screened had activity against at least one of the test organisms (Table 3). The acetone extracts of *L. alata*, *K. wilmsii*, *O. natalitia*, and *C. vendae* had high antifungal activity with MIC values ranging from 0.04 to 0.8 mg/ml against one or more of the tested micro-organisms. In a similar study, a member of the Anacardiaceae family, *Sclerocarya birrea* exhibited very good antifungal activity against some

Table 3
Minimum inhibitory concentrations (average of triplicate determinations) of four different extracts from seven South African plants tested against some animal pathogenic fungi.

Microorganism	Time (h)	MIC (mg/ml)															
		Combretum vendae				*Commiphora harveyi*				*Khaya anthotheca*				*Kirkia wilmsii*			
		H	A	D	M	H	A	D	M	H	A	D	M	H	A	D	M
A. fumigatus	24	0.31	0.26	0.31	0.63	1.88	2.5	1.25	2.50	0.31	0.31	0.31	0.13	0.16	0.13	0.21	1.04
	48	0.42	0.37	0.31	0.63	2.5	2.5	1.67	2.50	1.25	1.67	1.25	2.08	1.25	1.25	2.50	2.50
C. albicans	24	0.21	0.26	0.26	0.63	0.52	0.16	0.32	1.25	0.63	0.83	0.63	1.67	0.31	0.83	1.25	1.25
	48	0.31	0.31	0.63	1.25	0.52	0.13	0.37	2.08	2.08	1.46	1.67	2.50	0.83	1.25	1.67	2.50
C. neoformans	24	0.08	0.31	0.31	1.25	2.08	0.21	1.25	0.84	0.05	0.16	0.31	1.04	1.25	0.07	0.63	0.31
	48	0.16	0.63	1.67	2.50	2.08	0.63	2.50	0.84	0.11	0.31	0.63	2.50	2.50	0.13	2.08	1.25
M. canis	24	0.16	0.08	0.11	0.31	0.11	0.16	0.63	1.25	0.21	0.26	0.63	1.04	1.04	0.08	0.84	1.25
	48	0.31	0.21	0.11	1.25	0.16	0.16	1.67	2.50	0.31	0.37	1.88	2.50	2.50	0.37	1.04	2.50
S. schenckii	24	0.13	0.31	0.63	1.11	0.63	0.31	1.04	2.50	0.16	0.11	0.63	0.94	1.25	0.26	1.25	0.31
	48	0.16	0.63	0.63	2.5	1.67	0.84	2.08	2.50	0.26	0.16	1.25	1.04	2.50	0.63	2.50	2.50
Average		0.23	0.34	0.50	1.21	1.22	0.76	1.28	1.88	0.54	0.56	0.92	1.54	1.50	0.50	1.40	1.54

Microorganism	Time (h)	MIC (mg/ml)												Amphotericin B (mg/ml)
		Loxostylis alata				*Ochna natalitia*				*Protorhus longifolia*				
		H	A	D	M	H	A	D	M	H	A	D	M	
A. fumigatus	24	1.25	0.05	0.52	0.42	2.08	1.25	1.25	2.50	1.67	0.31	0.63	1.25	0.005
	48	2.08	0.16	1.25	2.50	2.50	2.50	2.08	2.50	2.50	2.50	1.67	2.50	0.01
C. albicans	24	2.50	0.31	1.25	1.67	0.31	1.25	1.25	1.25	2.50	1.67	0.52	1.25	0.025
	48	2.50	1.04	2.50	1.67	0.31	1.25	2.08	2.50	2.08	1.67	0.52	1.25	0.005
C. neoformans	24	1.67	0.21	0.63	1.67	0.84	0.31	0.42	1.67	2.08	0.21	0.52	0.31	0.00063
	48	2.50	0.52	1.25	2.50	1.25	1.25	0.62	2.50	2.50	0.31	0.63	0.63	0.025
M. canis	24	1.04	0.07	1.04	0.63	0.08	0.07	0.11	0.13	0.31	0.63	0.94	0.31	0.00031
	48	2.50	0.07	1.25	1.25	0.52	0.09	0.52	0.52	1.04	1.67	1.67	2.50	0.00063
S. schenckii	24	1.25	0.04	0.13	1.25	0.08	0.26	0.42	1.25	1.04	1.25	0.84	1.25	0.00063
	48	2.50	0.08	0.31	2.5	0.21	0.31	1.25	2.50	2.50	1.25	0.84	2.50	0.005
Average		1.98	0.26	1.01	1.61	0.82	0.85	1.00	1.73	1.82	1.15	0.88	1.38	

H = hexane extract; A = acetone extract; D = dichloromethane extract; M = methanol extract.

Please cite this article as: Suleiman, M.M., et al., Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*, South African Journal of Botany (2009), doi:10.1016/j.sajb.2009.07.001

selected fungal pathogens (Hamza et al., 2006). The hexane extracts of *C. vendae*, *K. anthotheca* and *O. natalitia* had MIC values ranging from 0.05 to 0.09 mg/ml. The hexane extract of *C. vendae* had the lowest average MIC of 0.23 mg/ml against all the tested pathogens. As in the bacterial assays, most of the methanol extracts were relatively inactive against all the tested pathogens. However, the methanol extract of the stem bark of *K. anthotheca* was reported by Hamza et al (2006) to be very active against *Candida krusei* but inactive against other pathogenic yeast namely *C. albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *C. neoformans*. The highest average MIC value of 1.73 mg/ml was obtained with the methanol extract of *O. natalitia*. Plant extracts with MIC of 0.78 mg/ml against *C. albicans* are regarded to have good activity (Buwa and Van Staden, 2006). Similarly, Hamza et al. (2006) reported that extracts having MIC of 0.5 mg/ml or less as being strong inhibitors of fungal growth. Their report was based on classification of MIC earlier reported by Aligiannis et al. (2001) who proposed that plant extracts having MIC of 0.5 mg/ml as strong inhibitors; moderate inhibitors have MIC between 0.6 and 1.5 mg/ml. Extracts having MIC above 1.6 mg/ml are considered weak inhibitors.

M. canis had the highest susceptibility to the extracts, being sensitive to 5 of the tested extracts at concentrations as low as 0.05–0.08 mg/ml, while *C. albicans* had the lowest sensitivity to the plant extracts. *C. neoformans*, *S. schenckii* and *A. fumigatus* were sensitive to 3, 2 and 1 of the tested extracts respectively, with MIC values lower than 0.1 mg/ml. No growth inhibition was detected in the negative control wells. The antifungal activities of the plant extracts screened were not as effective as that of amphotericin B which is the reference compound (Table 3). The positive control (amphotericin B) had MIC values of 0.01–0.0003 mg/ml against the tested fungi. Similarly, as was mentioned for antibacterial screening, the action of most of the extracts on fungi appears to be fungistatic, as growth of the organisms and resulting red colour formation appeared to resume after the 48-h incubation period with INT (Table 3).

3.3. Total activity

For ethnopharmacological research to be locally relevant, not only the MIC is important, but also the quantity extracted from each plant species. Total activity is calculated by dividing the quantity extracted from 1 g of plant material with the MIC value in mg/ml (Eloff, 2001). This value indicates the volume to which the active constituent present in 1 g of the plant material can be diluted and still inhibit the growth of the test organism.

For bacterial organisms, the total activity of the plants ranged from 5 to 2283 ml/g (Table 4). The highest total activity of 2283 mg/ml was produced by the acetone extract of *L. alata* against *S. aureus* and *E. faecalis*. It therefore means that 1 g of *L. alata* acetone extract can be diluted in 2283 ml of the solvent used and still inhibit the growth of the organisms. Similarly, the total activities of the plant extracts against fungi ranged from 5 to 3425 ml/g (Table 5). *L. alata* was the most active, with the acetone extract having a total activity of 3425 ml/g against *M. canis* over an incubation period of 24 h. This is a step

Table 4
Total activity in ml/g of seven South African plants screened for antibacterial activity.

Microorganism	Time (h)	Total activity (ml/g)															
		Combretum vendae				*Commiphora harveyi*				*Khaya anthotheca*				*Kirkia wilmsii*			
		H	A	D	M	H	A	D	M	H	A	D	M	H	A	D	M
P. aeruginosa	1	68	981	231	154	68	225	54	5	74	556	250	127	125	288	103	203
	2	33	506	231	154	33	114	54	5	37	287	250	127	65	147	51	51
S. aureus	1	68	249	119	154	68	225	425	5	575	556	500	127	32	223	51	102
	2	68	126	30	77	68	225	213	5	288	556	500	127	16	223	51	102
E. coli	1	27	249	119	154	8	114	27	5	9	71	32	64	125	223	51	102
	2	17	249	59	77	8	57	27	5	9	36	63	64	125	223	26	51
E. faecalis	1	263	1308	463	1200	350	1775	425	21	144	556	250	127	65	223	103	102
	2	131	1308	463	619	68	592	213	11	144	287	250	64	65	223	103	102
Average		84	622	214	323	84	416	180	8	160	363	262	103	77	221	67	102

Microorganism	Time (h)	Total activity (ml/g)											
		Loxostylis alata				*Ochna natalitia*				*Protorhus longifolia*			
		H	A	D	M	H	A	D	M	H	A	D	M
P. aeruginosa	1	94	442	72	703	169	406	106	107	194	1913	381	75
	2	46	442	72	703	87	101	52	107	49	956	197	75
S. aureus	1	363	2283	54	87	169	406	206	107	49	243	97	150
	2	290	2283	27	87	169	101	52	54	49	243	197	150
E. coli	1	363	2283	54	174	87	200	13	54	49	494	197	150
	2	181	1713	425	174	43	200	26	54	49	494	97	75
E. faecalis	1	23	856	213	1363	338	788	138	213	100	243	97	150
	2	12	442	54	346	338	788	106	107	25	122	97	75
Average		171	1343	121	455	175	374	88	100	71	588	170	113

H = hexane extract; A = acetone extract; D = dichloromethane extract; M = methanol extract.

Please cite this article as: Suleiman, M.M., et al., Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*, South African Journal of Botany (2009), doi:10.1016/j.sajb.2009.07.001

Table 5
Total activity in ml/g of seven South African plants screened for antifungal activity.

Microorganism	Time (h)	Total activity (ml/g)															
		Combretum vendae				*Commiphora harveyi*				*Khaya anthotheca*				*Kirkia wilmsii*			
		H	A	D	M	H	A	D	M	H	A	D	M	H	A	D	M
A. fumigatus	24	68	604	119	305	11	57	27	5	74	287	129	1223	125	531	152	123
	48	50	424	119	305	8	57	20	5	18	53	32	76	16	55	13	51
C. albicans	24	100	604	142	305	40	888	106	11	37	107	63	95	65	83	26	102
	48	68	506	59	154	40	1092	92	6	11	61	24	64	24	55	19	51
C. neoformans	24	263	506	119	154	10	676	27	16	460	556	129	153	16	986	51	413
	48	131	249	15	77	10	225	14	16	209	287	63	64	8	531	15	102
M. canis	24	131	1963	119	619	191	888	54	11	110	342	63	153	19	863	38	102
	48	68	748	30	154	131	888	20	5	74	241	21	64	8	186	31	51
S. schenckii	24	162	506	33	173	33	458	33	5	144	809	63	169	16	265	26	413
	48	131	249	15	77	13	169	16	5	88	556	32	153	8	110	13	51
Average		117	636	77	232	49	540	41	9	123	330	62	221	30	366	38	146

Microorganism	Time (h)	Total activity (ml/g)											
		Loxostylis alata				*Ochna natalitia*				*Protorhus longifolia*			
		H	A	D	M	H	A	D	M	H	A	D	M
A. fumigatus	24	23	2740	65	519	13	101	26	54	19	494	97	150
	48	14	856	27	87	11	50	16	54	12	61	37	75
C. albicans	24	12	442	27	131	87	101	26	107	12	92	117	150
	48	12	132	14	131	87	101	16	54	15	92	117	150
C. neoformans	24	17	652	54	131	32	406	79	80	15	729	117	606
	48	12	263	27	87	22	101	53	54	12	494	97	298
M. canis	24	28	1957	33	346	338	1800	300	1031	100	243	65	606
	48	12	1957	27	174	52	1400	63	258	30	92	37	75
S. schenckii	24	23	3425	27	174	338	485	79	107	30	122	73	150
	48	12	1713	14	87	129	406	26	54	12	122	73	75
Average		16	1414	32	187	111	495	68	185	26	254	83	234

H = hexane extract; A = acetone extract; D = dichloromethane extract; M = methanol extract.

towards the rational use of plants in traditional primary health care and could be of benefit in enabling rural use of the plants as information regarding the usefulness of the plant could be handed to rural people. Higher values of total activity indicate increased usefulness and potential economic value.

This study investigated the *in vitro* antimicrobial activity of selected plant species, and has supplied preliminary evidence of the efficacy of these plant species for the traditional treatment of various bacterially-related diseases. However, *in vivo* data is necessary to determine the potential usefulness of these plants for treatment of infectious diseases. One of the inherent problems associated with *in vitro* testing is the absence of body metabolic processes. More importantly, factors such as absorption and metabolism may be responsible for discrepancies between *in vitro* and *in vivo* activity (Houghton et al., 2007). However, *in vitro* activity may serve as a lead towards the discovery of plant chemical agents that are potentially active *in vivo*.

In terms of conservation, the results show that leaf material of these plants is useful for antimicrobial uses because this material can be used without any detrimental effect on the plant (Holetz et al., 2002).

4. Conclusion

The plant extracts tested had varying levels of activity against bacteria. The hexane extract of *K. anthotheca* had the highest antibacterial activity with lowest MIC against *S. aureus*. The acetone extract of *L. alata* had the highest antifungal activity with lowest MIC against *S. schenckii*. In line with the aim of this publication *Loxostylis alata* was selected for further work. In subsequent work the antifungal compounds have been isolated and excellent results were obtained in protecting poultry against aspergillosis (Suleiman, 2009).

Acknowledgements

The National Research Foundation (NRF) and University of Pretoria provided funding. The curators of the University of Pretoria Manie van der Schijff Botanical Garden and the Lowveld National Botanical Garden, Nelspruit allowed us to collect tree leaves.

References

Aligiannis, N., Kalpotzakis, E., Mitaku, S., Chinou, I.B., 2001. Composition and antimicrobial activity of the essential oils of two *Origanum* species. Journal of Agricultural and Food Chemistry 40, 4168–4170.

Archibald, L., Phillips, L., Monnet, D., McGowan, J.E., Tenover, F.C., Gaynes, R.P., 1997. Antimicrobial resistance in isolates from inpatients and outpatients in the United States: increasing importance of the intensive care unit. Clinical Infectious Diseases 24, 211–215.

Briskin, D.P., 2000. Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant Physiology 24, 507–514.

Please cite this article as: Suleiman, M.M., et al., Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*, South African Journal of Botany (2009), doi:10.1016/j.sajb.2009.07.001

Buwa, L.V., Van Staden, J., 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. Journal of Ethnopharmacology 103, 139–142.

Chariandy, C.M., Seaforth, C.E., Phelps, R.H., Pollard, G.V., Khambay, B.P.S., 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. Journal of Ethnopharmacology 64, 265–270.

Clardy, J., Fischbach, M.A., Walsh, C.T., 2006. New antibiotics from bacterial natural products. Nature Biotechnology 24, 1541–1550.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12, 564–582.

Clarkson, C., Maharaj, V.J., Crouch, N.R., Grace, M.O., Pillay, P., Matsabisa, M.G., Niresh, B., Smith, P.J., Folb, P.I., 2004. *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. Journal of Ethnopharmacology 92, 177–191.

Denning, D.W., 1998. Invasive aspergillosis. Clinical Infectious Diseases 26, 781–803.

Dismukes, W.E., 2000. Introduction to antifungal drugs. Clinical Infectious Diseases 30, 653–657.

Dold, A.P., Cocks, M.L., 2001. Traditional veterinary medicine in the Alice district of Eastern Cape Province, South Africa. South African Journal of Science 97, 375–379.

Eloff, J.N., 1998a. A sensitive and quick method to determine minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64, 711–714.

Eloff, J.N., 1998b. Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.

Eloff, J.N., 1999. The antibacterial activity of 27 southern African members of the Combretaceae. South African Journal of Science 95, 148–152.

Eloff, J.N., 2001. Antibacterial activity of marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro) (Anacardiaceae) bark and leaves. Journal of Ethnopharmacology 76, 305–308.

Germishuizen, G., Meyer, N.L. (Eds.), 2003. Plants of Southern Africa: an annotated checklist. In: Strelitzia, vol. 14. National Botanical Institute, Pretoria.

Groll, A.H., Shah, P.M., Mentzel, C., Schneider, M., Just Nuebling, G., Huebner, K., 1996. Trends in the postmortem epidemiology of invasive fungal infections at a University hospital. Journal of Infection 33, 23–32.

Hamza, O.J.M., Van den Bout-van den Beukel, C.J.P., Matee, M.I.N., Moshi, M.J., Mikx, F.H.M., Selemani, H.O., Mbwambo, Z.H., Van der Ven, A.J.A.M., Verweij, P.E., 2006. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. Journal of Ethnopharmacology 108, 124–132.

Holetz, F.B., Pessini, G.L., Sanches, N.R., Cortez, D.A.G., Nakamura, C.V., Filho, B.P.D., 2002. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Memórias do Instituto Oswaldo Cruz 97, 1027–1031.

Houghton, P.J., Howes, M.J., Lee, C.C., Steventon, G., 2007. Uses and abuses of *in vitro* tests in ethnopharmacology: Visualizing an elephant. Journal of Ethnopharmacology 110, 391–400.

Hostettmann, K., Marston, A., Ndjoko, K., Wolfender, J.L., 2000. The potential of African plants as a source of drugs. Current Organic Chemistry 4, 973–1010.

Klopper, R.R., Smith, G.F., Gautier, L., Chatelain, C., Spichiger, R., 2006. The African plant checklist and database project. Conference South African Association for Botanists, Port Elizabeth, Programme and Abstracts, pp. 93–94.

Kotze, M., Eloff, J.N., 2002. Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). South African Journal of Botany 68, 62–67.

Kuete, V., Nguemeving, R.J., Beng, V.P., Azebaze, A.G.B., Etoa, F., Meyer, M., Bodo, B., Nkengfack, E., 2007. Antimicrobial activity of the methanolic extract of *Vismia laurentii* De Wild (Guttiferae). Journal of Ethnopharmacology 109, 372–379.

Kunin, C.M., 1993. Resistance to antimicrobial drugs—a worldwide calamity. Annals of Internal Medicine 118, 557–561.

Martínez, M.J., Betancourt, J., Alonso-González, N., Jauregui, A., 1996. Screening of some Cuban medicinal plants for antimicrobial activity. Journal of Ethnopharmacology 52, 171–174.

Masoko, P., Picard, J., Eloff, J.N., 2005. Antifungal activities of six South African T*erminalia* species (Combretaceae). Journal of Ethnopharmacology 99, 301–308.

Nikaido, H., 1996. Outer membrane. In: Neidhardt, F.C. (Ed.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, vol. 1. ASM Press, Washington, DC, pp. 29–47.

Nfi, A.N., Mbanya, J.N., Ndi, C., Kameni, A., Vabi, M., Pingpoh, D., Yonkeu, S., Moussa, C., 2001. Ethnoveterinary medicine in the Northern Provinces of Cameroon. Veterinary Research Communications 25, 71–76.

Pell, S.K., 2004. Molecular systematics of the cashew family. (Anacardiaceae). PhD Thesis, Graduate Faculty of Louisiana State University of Agricultural and Mechanical College, United States of America.

Pooley, E., 1993. Trees of Natal, Zululand and Transkei. Natal Flora Publications Trust, Durban, pp. 244–246.

Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K., 2003. Pharmacology, 5th ed. Churchill, Livingstones. Edinburgh, p. 797.

Rippon, J.W., 1982. Medical mycology: the pathogenic fungi and the pathogenic actinomycetes. W.B. Saunders Co., Philadelphia, pp. 565–568.

Sahm, D.F., Marsillio, M.K., Piazza, G., 1999. Antimicrobial resistance in key bloodstream bacterial isolates: electronic surveillance with the surveillance network database—USA. Clinical Infectious Diseases 29, 259–263.

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*. South Africa Journal of Botany 74, 677–684.

Silva, G.L., Lee, I., Kinghorn, D., 1998. Special problems with the extraction of plants. In: Cannell, R.J.P. (Ed.), Natural Products Isolation. Humana Press, New Jersey, pp. 343–363.

Stickler, D.J., King, J.B., 1992. Bacterial sensitivity and resistance. Intrinsic resistance. In: Russell, A.D., Hugo, W.B., Ayliffe, G.A.J. (Eds.), Principles and Practice of Disinfection, Preservation and Sterilization. Blackwell Scientific Publications, Oxford.

Stevens, D.A., 1990. Fungal infections in AIDS patient. British Journal of Clinical practice (Suppl.) 71, 11–22.

Suleiman, M.M., 2009. The *in vitro* and *in vivo* biological activities of antifungal compounds isolated from *Loxostylis alata* A. Spreng. ex Rchb. PhD Thesis, Phytomedicine Programme, Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria.

Van Wyk, B., Van Wyk, P., Van Wyk, B.-E., 2000. Photographic guide to trees of southern Africa, 1st ed. Briza Publications, Pretoria, p. 196.

Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd ed. Livingstone, London.

Walsh, C., 2000. Molecular mechanisms that confer antibacterial drug resistance. Nature 406, 775–781.

Wink, M., Schimmer, O., 1999. Modes of action of defensive secondary metabolites. In: Wink, M. (Ed.), Functions of Plant Secondary Metabolites and Their Exploitation in Biotechnology. CRC Press, Boca Raton, Florida, pp. 17–112.

Edited by J Van Staden

Please cite this article as: Suleiman, M.M., et al., Evaluation of several tree species for activity against the animal fungal pathogen *Aspergillus fumigatus*, South African Journal of Botany (2009), doi:10.1016/j.sajb.2009.07.001