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Full Length Research Paper

Antifungal activities of selected Venda medicinal plants against *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans* isolated from South African AIDS patients

A. Samie[1]*, T. Tambani[1], E. Harshfield[2], E. Green[3], J. N. Ramalivhana[4] and P. O. Bessong[1]

[1]AIDS Virus Research Laboratory, Department of Microbiology, University of Venda, Thohoyandou , South Africa.
[2]Department of Chemical engineering, University of Virginia, Charlottesville, VA, USA.
[3]Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa.
[4]College of Agriculture and Life Sciences, University of South Africa, Private bag X6, Florida, Johannesburg, South Africa.

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Infection with HIV leads to immunosuppression and up to 90% of HIV infected individuals contract fungal infections of which 10 - 20% die as a direct consequence of these infections. In the present study, 76 extracts from 30 plants used by Venda traditional healers for the treatment of fungal related ailments, were tested for their antifungal activities against clinical isolates of *Candida albicans*, *Candida krusei* and *Cryptococcus neoformans* using the agar diffusion and the microdilution methods. The minimum fungicidal concentrations as well as the time kill curves of the thee most active plants were also determined. Extracts from 25 plants (83.3%) were active against *C. albicans*, *C. krusei* or *C. neoformans*. Thirty two extracts were active against *C. neoformans*, while 15 were active against *C. albicans* and 12 were active against *C. krusei*. *Warburgia salutaris*, *Cassine transvaalensis*, *Piper capense*, *Maerua edulis*, *Pseudolachnostylis maprouneifolia*, *Berchemia discolor* and *Lippia javanica* were not only inhibitory to fungal growth but also had fungicidal effects against one or all the 3 fungi tested (MIC/MFC between 0.11 and 7.5 mg/ml). Hexane extracts were also active indicating that many of the antifungal components of these plants are non-polar compounds. Time-to- kill experiments indicated an intense time-dependent fungicidal effect against *C. albicans*, achieving over a 5 h-period a 6 $\log_{10}$-unit decrease in CFU/ml at a concentration of 0.4 mg/ml for *W. salutaris*. The present study justifies the traditional use of these plants for the treatment of opportunistic infections in the region.

Key words: Candidiasis, Cryptococcosis, HIV/AIDS, medicinal plants, MIC, MFC, Venda, South Africa.

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) caused by the human immunodeficiency virus (HIV) is a devastating epidemic in Africa and South Africa has been reported to have had the fastest growing rate of HIV/AIDS epidemic in the world (Abdool-Karim, 2000; UNAIDS, 2006). Up to 90% of all HIV patients contract fungal infections during the course of the disease, of which 10 - 20% die as a direct consequence of fungal infections (Hamza et al., 2006; Back-Brito et al., 2008). *Candida albicans* and *Cryptococcus neoformans* are the most common opportunistic infections in HIV/AIDS patients (Fan-Harvard et al., 1991) and management of such infections particularly in HIV/AIDS patients is faced with some difficulties, such as resistance to antifungal agents, drug toxicity and high cost of antifungal agents (Debruyne, 1997; Traeder et al., 2008). Therefore, there is a need to search for alternative

*Corresponding author. E-mail: samieamidou@yahoo.com or samie.amidou@univen.ac.za. Tel: +27159628186 or +27760952916. Fax: 27 15 962 8648.

control methods and the utilization of medicinal plants by the local populations constitutes an important source of new active and renewable antifungal drugs.

Many potent drugs including anti-malarial, anti-bacterial and anti-diabetic compounds have been purified from medicinal plants (Schmidt et al., 2008). For centuries, plants have been used by indigenous people to produce medicines that were used to treat different kinds of ailments. Up to 80% of populations living in Africa rely on medicinal plants (Elujoba et al., 2005). In Venda, geographically located in the Northeast of South Africa, the use of medicinal plants is very common based on local traditions as well as the availability of highly diverse genetic resources (van Vuuren, 2008). Traditional doctors and elders use several plants to treat different types of infections including those caused by fungal organisms. The preferred parts of specific medicinal plants are collected each at its favorable harvest season, dried by directly exposing them to sunlight, pounded using pestle and mortar and then stored in containers in powder form for future usage. The part of the plant used varies among species and traditional healers and also depends on the nature and state of the disease (Mabogo, 1990).

Previous studies on Venda medicinal plants have concentrated on the antibacterial activity and very few studies have targeted the antifungal activities of Venda medicinal plants (Obi et al., 2003; Tshikalange et al., 2005; McGaw et al., 2007). Plants are not only important to the millions of people to whom traditional medicine serves as the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals (de Boer et al., 2005). Commercially available antifungal drugs are expensive and some fungal species are developing resistance against them and therefore screening for more medicinal plants with antifungal activities can help in the identification and development of more active and affordable drugs. In the present study, medicinal plants used by traditional healers in the Venda region were investigated for their antifungal activities against *C. albicans*, *Candida krusei* and *Cryptococcus neoformans* isolated from South African AIDS patients.

MATERIALS AND METHODS

Ethnobotanical data and plant material collection

Ethnobotanical information on medicinal plants that are used by the Vha-Vendas to treat fungal infections was attained though visits and interviews with two, male traditional doctors aged between 45 and 60 years from two different areas in the Vhembe district (Thohoyandou and Nzhelele) situated in the North-eastern part of South Africa 60 km from the Kruger National Park. Identification and collection of medicinal plants was done under the supervision of the traditional healers to avoid confusion and nature destruction. The plants were further identified by botanists from the Department of Botany (University of Venda) and the Thohoyandou Botanical Garden. The voucher specimens of the plants investigated were kept in the Department of Botany, University of Venda and the Thohoyandou Botanical Garden.

Fungal organisms

The fungal organisms used in the present study were isolated from AIDS patients with oro-pharyngeal thrush and cryptococcal meningitis. *C. albicans* was isolated from a 28 year old female (CD4 count of 54/mm^3) while *C. krusei* was isolated from a 45 years old male patient (CD4 count of 115/mm^3). *Candida* species were identified using CHOM agar following the manufacturer's instructions (CHOMagar, Paris, France). *C. neoformans* was isolated from the cerebrospinal fluid obtained from a 29 year old female patient (CD4 cells count 94/mm^3) with cryptococcal meningitis. The *Candida* species were susceptible to nystatin, while *Cryptococcus* was susceptible to 5 -fluorocytosine. Nystatin and 5 -fluorocytosine were used as positive controls in the present study.

Plant sample preparations

Plant materials collected from Nzhelele and Thohoyandou between May and August 2007 were dried at room temperature for about 2 weeks or using an incubator at 40°C for 2 to 3 days. The dried plant materials were ground into powder form using a grinder followed by a warring blender.

Extraction

50 g of the ground material of each plant was extracted in 500ml of each of two solvents (acetone and hexane) under continuous shaking for 24 h. The extract was filtered through a 22 µm paper filter. The filtrate was evaporated to dryness using a rotatory evaporator at 40°C. The residues in the form of powder or oily material were preserved in sterile glass bottles in a cool dark place until use.

Antifungal activity test

Agar diffusion and micro-dilution methods were used to determine the antifungal activities of the medicinal plant extracts against *C. albicans*, *C. krusei* and *C. neoformans*. Sabouraud dextrose broth (SDB) was used for the preparation of fungal cultures and for the determination of the MIC and was prepared following the manufacturer's instructions. Sabouraud dextrose agar (SDA) was used to determine the activity of the plant extracts against the fungal organisms and was prepared following the instruction of the manufacturer.

Agar diffusion assay

Using the micropipette, 100 µl of 1Mcfarland solution of *C. albicans* culture (in SDB) was spread over the surface of an agar plate using a sterile hockey stick. The same procedure was followed for *C. krusei* and *C. neoformans*. Using a sterile 5 cm plastic pipette, four holes were punched (2 mm in diameter) in each of the culture plates. In the first hole, 10 µl of the drug was added as positive control; 10 µl of DMSO was added as a negative control in the second hole; 5 and 10 µl of the plant extract were added in the third and last holes. The culture plates were then incubated at 37°C and the results were observed after 24 h. The clear zone around the plant extract was measured in mm and indicated the activity of the plant extract against the fungal organisms. The experiments were done in triplicate.

Microdilution assay

The microdilution method was employed to determine the minimum

inhibitory concentration (MIC) of the plant extracts using 96 well microtitration plates as previously described (Samie et al., 2005). Briefly, 185 µl of the broth was added into each well in the first row of microtitration plate and 100 µl to the rest of the wells from the second row downwards. 15 µl of the plant extracts was then added into each well on the first row (row A), starting with the positive control (Nystatin, Roche), followed by the negative control (the 20% DMSO used to dissolve the plant extracts) and the plant extracts in the rest of the wells on that row. A twofold serial dilution was done by mixing the contents in each well of the first row and transferring 100 µl to the second well of the same column and the same was done up to the last well of the same column and the last 100 µl from the last well was discarded. Then 100 µl of yeast suspensions was added. The results were observed after 24 h incubation at 37°C followed by the addition of 40 µl of a 0.2% Iodo Nitro Tetrazolium (INT) solution after a further incubation of 4 h at 37°C. The wells that did not show any colour change after INT was added indicating the concentration of the plant extract that was able to inhibit fungal growth whereas the pink colour change indicated fungal growth.

Determination of the MFC (minimum fungicidal concentration)

The MFC was determined by inoculating the contents from the MIC plates onto SDA plates and the results were observed after 24 h incubation at 37°C. The presence of the fungal colonies on agar plates was an indication that the plant extract only inhibited the growth of the fungi without killing them and the absence indicated that the plant extract was able to kill the fungal organisms. The smallest concentration of the plant extract that was able to kill the microorganisms was considered as the minimum fungicidal concentration.

Time - kill experiment

Determination of the rate of kill of the crude extract was done following the procedure described by Aiyegoro et al. (2008) with slight modifications. Briefly, inocula were prepared as described above. The resultant suspension was diluted 1:100 with fresh sterile broth and used to inoculate 50 ml volumes of SDB incorporated with extract at MFC to a final cell density of approximately 2×10^6 cfu/ml. The flasks were incubated at 37°C on an orbital shaker at 120 rpm. A 500 µl sample was removed from cultures at 0, 2, 5, 10 and 24 h, diluted serially and 100 µl of the diluted samples were plated on SDA plates and incubated at 37°C for 24 h. Controls included extract free Mueller Hinton broth seeded with the test inocula.

RESULTS

Ethnobotanical information on plants used to treat fungal and similar infections in the Venda region

The visits and interviews with traditional healers revealed about 30 extracts used for the treatment of fungal related ailments. Some of the plants have also been described by Mabogo (1990). Table 1 describes the plants as well as their traditional use and the part used. Most of the plants were used for several ailments and in most cases many plants were used at the same time for different ailments and the combination of the plants varied with the ailment and the traditional healer. Of the 30 plants tested in the present study, 11 have been tested by other authors (Gundidza and Sibanda, 1991; Motsei et al., 2003; Hamza et al., 2006; Steenkamp et al., 2007). Information on plants previously tested with proven activity is presented in Table 2. However, only 10 according to previous studies had proven anti-*C. albicans* activity while one of them (*Strychnos decussata*) did not show activity even though in the present study we found that this plant was active against *C. neoformans* (disc diffusion method: Table 3) as well as the two *Candida* species tested (microdilution method: Table 4).

Antifungal activity of medicinal plants

Out of the 76 plant extracts from 30 plants tested against the 3 fungal organisms, 38 (50%) extracts from 26 (83.3%) plants were active against at least one of the organisms. 33 extracts were active against *C. neoformans* with zones of inhibition varying from 5 to 20 mm. 13 extracts were active against *C. albicans* and 10 were active against *C. krusei*. The highest activity was obtained with *Piper capense* acetone root extract with a 22 mm zone of inhibition against *C. albicans* and *C. krusei*. *Warburgia salutaris* hexane bark extracts was the most active against *C. neoformans*. *Cassine transvaalensis* (was active against all the three fungi although only the hexane extract from the bark was active and the acetone extract was not active against any of the tested fungi. *Berchemia discolor* and *P. capense* also inhibited all the tested fungi. In many instances, the acetone extracts were active against *C. neoformans*. This was observed with *Banksia micrantha*, *Diospyros mespiliformis*, *Passiflora mixta*, *Schotia brachypetala*, *Ziziphus mucronata*, *Sclerocarya birrea*, *R. tridentate*, *R. rogesii*, *Pygeum africanum* and *Bauhinia galpinii.* However, hexane extracts were more active such as observed with *C. transvaalensis*, *Ximenia caffra*, *Euclea divinorum*, *S. decussata*, *Terminalia sericea*, *Amorpha fruticosa*, *Pseudolachnostylis maprouneifolia*, *B. discolor*, *B. molis* of which the hexane extracts was more active against *C. neoformans*, while hexane extracts of *Lippia javanica*, *D. mespiliformis*, *P. maprouneifolia* were active against *C. albicans* or *C. krusei*. Table 3 presents the results obtained from the agar diffusion method with the growth inhibition zones expressed in mm. For *C. albicans* and *C. krusei* nystatin was used as positive control while fluorocytosine was used as positive control for *C. neoformans*.

Minimum inhibitory concentrations of the medicinal plants

To determine the minimum inhibitory concentration, concentrations of the plant extracts varying between 7.5 and 0.05 mg/ml were tested. Table 4 shows the different MICs of the extracts against the three fungal organisms. The lowest MICs were obtained with *W. salutaris* hexane

Table 1. Ethnobotanical information on the plants used.

Scientific name (Family)	Common name (Venda/English)	Voucher No	Plant part used	Traditional use
	Venda			
Cassia petersiana (Caesalpinioideae)	Munembenembe Monkey pod	SA03	Bark	Root for aphrodisiac, gonorrhoea, syphilis, stomach ache and epilepsy.
Warburgia salutaris (Canellaceae)	Mulanga Pepper bark	SA08	Bark and Leaves	Bark for aphrodisiac, venereal diseases, colds, sore throat, malaria.
Senna didymobotrya (Fabaceae)	Tshiduwana Peanut butter cassia	SA09	Root	Roots are used to treat sexually transmitted infections.
Maerua edulis (Capparaceae)	Mutshalimela Sozwe Tree	SA01	Tube	Fungal infections, wounds, venereal diseases.
Cassine transvaalensis (Celastraceae)	Mulumanamana/ Mukuvhazwivhi Transvaal saffron	BP05	Bark	Piles, venereal diseases, anthhelmintic, laxative, stomach ache, cough, diarrhoea, kidney and bladder infections.
Ximenia caffra (Olacaceae)	Mutshili Sour plum	AS15	Root and Leaves	Blood in feces, menorrhage, cough, infertility, venereal diseases, scurvy.
Rhoicissus tridentata (Vitaceae)	Murumbulambudzana Bushman's grape	AS18	Root and Tubes	To treat diarrhoea, prevent miscarriages (Mabogo, 1990).
Euclea divinorum (Ebenaceae)	Mutangule Magic guari	SA02	Leaves	Noisy stomach, headache, general cleansing, tooth ache.
Ficus sycomorus (Moraceae)	Muhuyu Broom cluster fig	SA04	Bark	Stomach pains, weight in children.
Strychnos decussata (Loganiaceae)	Muvhavhanyane Cape teale	ST05	Bark	Sore throat, fever, headache, wounds, vaginal infections.
Sucuridaca longepedunculata (Polygalaceae)	Mpesu Violet tree	ST06	Bark and Root	Aphrodisiac, tuberculosis, gonorrhoea.
Terminalia sericea (Chenopodiaceae)	Mususu Silver Terminalia tree	ST07	Bark and Root	Infected wounds, menorrhage, to dress on magical wounds (Mabogo, 1990).
Rhus rogersii (Anacardiaceae)	Muthasiri	TT01	Bark	General pain, watery diarrhoea.
Peltophorum africanum (Caesalpinoidiae)	Musese Weeping wattle	BP01	Bark	Colds, fever, sore throat, sores, ulcers, blisters in the oral cavity, gonorrhoea (Mabogo, 1990).
Grewia vilosa (Tiliaceae)	Mupunzu Mallow raisin	SA01	Root	Vaginal discharge, oral sores.
Piper capense (Piperaceae)	Mulilwe Bospeper	TT02	Root	Wounds, vaginal discharge, infertility, sore throat and tongue sores, infertility (Mabogo, 1990).
Asclepias fruticosa (Asclepiadaceae)	Mutshule Tennis Ball Bush	ST04	Root	Infertility, stomach troubles (Mabogo, 19900, vaginal infection.
Bauhinia galpinii (Caesalpinodiae)	Mutswiriri Pride of De Kaap	AS04	Bark and Leaves	Diarrhoea, infertility (Mabogo, 1990).
Bridelia micrantha (Euphorbiaceae)	Munzere coast gold leaf	BP03	Bark	Burns, infected wounds, tooth ache, eye pains, headaches, fevers (Mabogo, 1990), stomach pains.
Lipia javanica (Verbenaceae)	Musudzungwane Fever tea/ Lemon Bush	AS19	Leaves	Fever, malaria, influenza, measles, lung infections, asthma, chronic coughs and pleurisy, skin disorders stings and bites (Van Wyk et al., 1997) .

Table 1. Contd.

Pouzolzia mixta (Utriciae)	Muthanzwa Soap nettle	AS17	Leaves	Diarrhoea, dysentery (Mabogo, 1990).
Bridelia mollis (Euphorbiaceae)	Mukumbakumba Velvet sweet berry	SA11	Leaves	Anti-emetic, piles, dysentery, burning and itching, wounds (Mabogo, 1990).
Diospyros mespiliformis (Ebenaceae)	Musuma Jackalberry Tree/ African ebony	SA12	Bark and Leaves	Bark, leaves and roots helps stop bleeding, heal wounds and treat ring worm, dysentery and fever, remedy for leprosy, fruit treat fungal infections on skull (Mabogo, 1990).
Pseudolachnostylis maprouneifolia (Euphorbiaceae)	Mutondowa Kudu berry	ST09	Leaves	Bark for noisy stomach, venereal diseases, root for pneumonia (Mabogo, 1990).
Berchemia discolor (Rhamnaceae)	Munie Bird plum	AS21	Bark and Leaves	Infertility (Mabogo, 1990), vaginal infections.
Capparis tomentosa (Capparaceae)	Muobadali Woolly caper-bush	SA13	Root	Worms, swollen ankles, infertility (Mabogo, 1990).
Schotia brakipetala (Caesalpinoidiae)	Mulubi Weeping boer bean	SA14	Bark	Heart disorders, dysentery, diarrhoea (Mabogo, 1990).
Ziziphus mucronata (Rhamnaceae)	Mukhalu Buffalo/ Cape thorn	SA15	Bark	Boils, skin infections, tubercular gland swellings, measles, dysentery, lumbago and chest complaints.
Schlerocarya birrea (Anacardiaceae)	Mufula Marula tree	SA16	Bark	Fever, stomach ulcers, wounds, infertility (Mabogo, 1990).

Table 2. Reports from the literature showing similar ethnomedical claims and proven laboratory results showing antifungal activity for some of the plants reported by Venda traditional healers.

Plant name	Literature reports of related ethnomedical uses and proven antifungal activity
Berchemia discolor	Water extracts were found active against *C. albicans* (Gundidza and Sibanda, 1991).
Bridelia micrantha	Water extract of the bark showed activity against one of the five clinical strains of *C. albicans* tested (Steenkamp et al., 2007).
Cassine transvaalensis	Methanol extract of the bark showed activity against the ATCC *C. albicans* strain but not the clinical strains (Steenkamp et al., 2007).
Ficus sycomorus	Water extracts of the leaves had an MIC of 3.72mg/ml against *C. albicans* clinical strains (Steenkamp et al., 2007).
Peltophorum africanum	The water extract of the root was found active against all the four clinical *Candida* strains while the methanol extract was active against the standard ATCC strain and one clinical strain (Steenkamp et al., 2007).
Piper capense	Both the methanol and water extracts were found active against the entire *C. albicans* strains used (Steenkamp et al., 2007).
Rhoicisus tridentata	Methanol extract exhibited antifungal activity against *C. albicans* and *Saccharomyces cerevisiae* (Lin et al., 1999) and *C. krusei* (Hamza et al., 2006).
Sclerocaria birrea	Stem bark ethanol extracts exhibited strong activity against *C. albicans* (Adoum et al., 1997) and *C. krusei* (Hamza et al., 2006).
Securidata longipedunculata	Root bark extracts exhibited antifungal activity against *Saccharomyces cerevisiae* (Taniguchi et al., 1978).
Warburgia salutaris	Bark and leaf extracts were found active with leaves from wild plants more active than those from the garden plant (Motsei et al., 2003).

bark extract (0.12 mg/ml against *C. krusei*) and *P. capense* acetone root extract (0.12 mg/ml against *C. neoformans*). *W. salutaris* hexane bark extract showed an MIC of 0.23 mg/ml against both *C. albicans* and *C. neoformans*, while *P. capense* acetone root extract showed an MIC of 1.88 mg/ml against both *Candida* species tested in the present study. The hexane extract of *P. capense* root was not as active as the acetone extract against *C. neoformans* (MIC 7.5mg/ml) but was more active against *Candida* sp. with MIC of 3.75 mg/ml.

Table 3. Antifungal activity of medicinal plants as determined by the agar diffusion method (diameter in mm). These were the results obtained following 24 h incubation at 37°C on Sabouraud dextrose agar. The results presented are for the active plant extracts.

Plant name	Solvent and plant part used	Zone of inhibition (mm)					
		C. albicans		*C. krusei*		*C. neoformans*	
		5 µl	10 µl	5 µl	10 µl	5 µl	10 µl
A. fruticosa	Hexane (Root)	0	0	0	0	7	12
B. discolor	Hexane (Bark)	0	0	0	0	7	12
B. discolor	Acetone (Leaves	0	0	0	0	0	7
B. discolor	Hexane (Leaves)	10	12	7	10	7	10
B. galpinii	Acetone (Bark)	0	8	0	0	0	12
B. micrantha	Acetone (Bark)	0	0	0	0	10	12
B. mollis	Hexane (Leaves)	0	0	0	0	7	0
C. tomentosa	Hexane (Root)	0	0	0	0	0	7
C. transvaalensis	Acetone (Bark)	0	0	0	0	0	0
C. transvaalensis	Hexane (Bark)	12	16	8	14	14	16
D. mespiliformis	Hexane (Leaves)	7	7	0	0	0	0
D. mespiliformis	Acetone(Bark)	0	0	0	0	10	12
E. divinorum	Hexane (Leaves)	0	0	0	0	0	8
G. Villosa	Hexane (Root)	0	0	0	0	0	8
L. Javanica	Hexane (Leaves)	0	10	0	7	0	0
P. africanum	Acetone (Bark)	0	0	0	0	14	18
P. capense	Acetone (Root)	22	24	24	22	22	16
P. capense	Hexane (Root)	8	12	7	12	0	8
P. maprouneifolia	Hexane (Leaves)	0	10	0	7	0	7
P. mixtra	Acetone (Leaves)	0	0	0	0	18	14
R. rogersii	Acetone (Bark)	0	0	0	0	10	11
R. tridentata	Acetone (Root)	0	0	0	0	8	8
R. tridentata	Acetone (Tubes)	0	0	0	0	8	10
S. birrea	Acetone (Bark)	0	0	0	0	12	14
S. birrea	Hexane (Bark)	0	0	0	0	0	7
S. brachypetala	Acetone (Bark)	0	0	0	0	14	7
S. brachypetala	Hexane (Bark)	0	0	0	0	0	7
S. decussata	Hexane (Bark)	0	0	0	0	0	12
T. sericea	Hexane (Root)	0	0	0	0	0	8
W. salutaris	Acetone (Bark)	7	22	12	10	8	12
W. salutaris	Hexane (Bark)	16	10	14	12	22	22
W. salutaris	Acetone (Leaves)	15	15	10	14	10	12
W. salutaris	Hexane (Leaves)	16	22	12	17	0	7
X. caffra	Acetone (Root)	0	8	0	0	0	0
X. caffra	Hexane (Root)	0	0	0	0	0	8
X. caffra	Hexane (Leave)	0	0	0	0	7	12
Z. mucronata	Acetone (Bark)	0	0	0	0	12	0
Nystatin	-	-	22	-	22	-	-
Flucytosine	-	-	-	-	-	-	22

The acetone extract of *W. salutaris* bark was not as active as the hexane extract and had an MIC of 3.75 against all the 3 fungal organisms tested. The leaves of *W. salutaris* were not as active as the bark and showed an MIC of 1.88 mg/ml against *C. krusei* for the hexane extract and 1.88 mg/ml against *C. neoformans* for the acetone extract. The hexane extract from the leaves was not active against *C. neoformans* at the concentration tested. *Securidaca longepedunculata* acetone bark extract showed an MIC of 0.23 mg/ml against both *C. albicans* and *C. krusei* while the MIC against *C. neoformans* was 1.88 mg/ml and the hexane extract of

Table 4. Minimum inhibitory concentration (mg/ml) of the active plant extracts against the thee fungal organisms.

Plant name	Solvent and plant part used	*C. albicans*	*C. krusei*	*C. neoformans*
B. discolor	Acetone (Bark)	3.75	3.75	> 7.5
B. discolor	Hexane (Bark)	7.5	3.75	7.5
B. discolor	Acetone (Leaves)	0.23	0.47	7.5
B. discolor	Hexane (Leaves)	3.75	3.75	3.75
B. galpinii	Hexane (Bark)	1.88	1.88	> 7.5
B. galpinii	Acetone (Leaves)	0.23	0.23	1.88
B. galpinii	Hexane (Leaves)	1.88	> 7.5	1.88
B. micrantha	Acetone (Bark)	> 7.5	0.94	> 7.5
B. micrantha	Hexane (Bark)	3.75	> 7.5	> 7.5
B. mollis	Acetone (Leaves)	3.75	3.75	3.75
B. mollis	Hexane (Leaves)	> 7.5	> 7.5	3.75
C. petersiana	Acetone (Bark)	> 7.5	> 7.5	1.88
C. tomentosa	Acetone (Root)	3.75	> 7.5	> 7.5
C. transvaalensis	Hexane (Bark)	0.46	1.88	1.88
L. javanica	Acetone (Leaves)	> 7.5	1.88	> 7.5
L. javanica	Hexane (Leaves)	3.75	3.75	> 7.5
M. edulis	Acetone (Root)	1.88	3.75	7.5
M. edulis	Hexane (Root)	3.75	0.94	7.5
P. capense	Acetone (Root)	1.88	1.88	0.12
P. capense	Hexane (Root)	3.75	3.75	7.5
P. maprouneifolia	Acetone (Leaves)	1.88	1.88	7.5
P. maprouneifolia	Hexane (Leaves)	> 7.5	3.75	> 7.5
P. mixtra	Acetone (Leaves)	> 7.5	> 7.5	> 7.5
P. mixtra	Hexane (Leaves)	3.75	> 7.5	> 7.5
R. rogersii	Acetone(Bark)	> 7.5	> 7.5	7.5
R. tridentata	Acetone (Tubes)	> 7.5	3.75	> 7.5
R. tridentata	Hexane (Tubes)	3.75	3.75	> 7.5
S. brakipetala	Hexane (Bark)	3.75	> 7.5	> 7.5
S. decussata	Acetone (Bark)	3.75	3.75	> 7.5
S. decussata	Hexane (Bark)	3.75	1.88	> 7.5
S. didymobotyra	Acetone (Root)	7.5	7.5	7.5
S. didymobotyra	Hexane (Root)	7.5	7.5	> 7.5
S. longepedunculata	Acetone (Bark)	O.23	0.23	1.88
S. longepedunculata	Acetone (Root)	> 7.5	> 7.5	1.88
T. sericea	Acetone (Root)	1.88	0.94	> 7.5
T. sericea	Acetone (Bark)	0.94	3.97	1.88
W. salutaris	Acetone (Bark)	3.75	3.75	3.75
W. salutaris	Hexane (Bark)	0.23	0.12	0.23
W. salutaris	Acetone(Leaves)	7.5	7.5	1.88
W. salutaris	Hexane (Leaves)	7.5	1.88	> 7.5
X. caffra	Acetone(Root)	0.94	0.94	3.75
X. caffra	Hexane (Root)	> 7.5	> 7.5	7.5
Nystatin		0.23 µg/ml	0.23 µg/ml	-
Flucytosine		-	-	1.88 µg/ml

the same bark was not active. The leaves of *S. longepedunculata* (both hexane and acetone extracts) were not active against both *Candida* species however, the acetone extracts of bark and root showed activity against *C. neoformans* with an MIC of 1.88 mg/ml. *B. galpinii* was active against all the 3 fungi with highest activity for this plant observed against both *Candida* species with MIC of 0.23 mg/ml for the acetone leaf extract. The same extract showed an MIC of 1.88 mg/ml against *C. neoformans*. The hexane extract of the leaves

Table 5. Minimum fungicidal concentration (mg/ml) of the active plants against the thee fungal pathogens investigated.

Plant name	Solvent and Plant part used	*C. albicans*	*C. krusei*	*C. neofomans*
B. discolor	Hexane (Leaves)	7.5	7.5	7.5
B. discolor	Hexane (Bark)	7.5	-	-
C.transvaalensis	Hexane (Bark)	3.75	7.5	1.88
L. javanica	Acetone (Leaves)	-	7.5	-
M. edulis	Hexane (Root)	-	-	7.5
P. capense	Acetone (Root)	3.75	3.75	0.11
P. capense	Hexane (Root)	7.5	7.5	7.5
P.maprouneifolia	Hexane (Leaves)	7.5	7.5	-
W. salutaris	Acetone (Bark)	7.5	7.5	3.75
W. salutaris	Hexane (Bark)	0.93	0.46	0.46
W. salutaris	Acetone (Leaves)	-	-	7.5

was not as active as the acetone extracts but showed an MIC of 1.88 mg/ml for both *C. albicans* and *C. neoformans* and was not active against *C. krusei* at the highest concentration tested (MIC > 7.5 mg/ml). The hexane extract of the bark was more active with MIC of 1.88 mg/ml against both *C. albicans* and *C. krusei* but was not active against *C. neoformans*. The other most active plants included *B. discolor* that appeared to be active against all the 3 fungal organisms with the highest activity against *C. albicans* with MIC of 0.23 mg/ml for the acetone extract of the leaves. *T. sericea* acetone extracts of the bark as well as *X. caffra* acetone extract of the root showed good activity with MICs of 0.94 mg/ml against *C. albicans*. *M. edulis* hexane extract from the root also showed good activity against *C. krusei* with MIC of 0.94 mg/ml.

Fungicidal activity of the medicinal plants

The capacity of the plant extracts to kill the fungal organisms instead of inhibiting their growth was further measured and indicated that 7 of the 30 plants tested had such activity at concentrations varying between 0.11 and 7.7 mg/ml (Table 5). These plants included *W. salutaris*, *M. edulis*, *C. transvaalensis*, *P. capense*, *P. maprouneifolia*, *B. discolor* and *L. javanica*.

Time - kill experiments

The killing curves were determined for all the plants that showed fungicidal activity against *C. albicans* but significant changes appeared only for three extracts including *C. transvaalensis*, *M. edulis* and *W. salutaris* at the concentration of 0.4 mg/ml. Figures 1, 2 and 3 represent the killing curves for the three extracts that showed significant killing activity on *C. albicans* at the concentrations of 1.88, 3.75 and 0.46 mg/ml respectively. *W. salutaris* was active up to the concentration of 0.2 mg/ml but it was able to kill all the cells (99.99%) at a concentration of 0.4 mg/ml after 5 h of incubation.

DISCUSSION

Over the last two decades, fungal infections with special reference to *Candida* sp. and *C. neoformans* have become important public health concerns (Pfaller and Diekema, 2004). In South Africa, these infections are among the most common opportunistic infections in the immuno-compromised individuals causing oropharyngeal candidiasis and cryptococcal meningitis due to *Candida* species and *C. neoformans*, respectively (McCarthy et al., 2006). Although *C. albicans* is the most common cause of candidiasis, other *Candida* species particularly *Candida glabrata* and *C. krusei* have emerged as important cause of oropharyngeal candidiasis and candidemia and *C. krusei* has been shown to be the most resistant to antifungal drugs (Fan et al., 2008; Kliemann et al., 2008). The objectives of the present study were to identify different plants used by local traditional healers in the Venda region for the treatment of fungal related ailments and to determine the *in vitro* activities of these plants against three fungal organisms including *C. albicans*, *C. krusei* and *C. neoformans* isolated from South African AIDS patients. It was observed that of most of the plants, at least one extract was active against one of the fungal organisms.

Previous studies on Venda medicinal plants have targeted bacterial organisms while very few studies have been conducted on parasitic and fungal organisms. For example, Obi et al. (2003) indicated that *Warburgia salutaris* and *Peltophorum africanum* were active against most of the bacterial organisms tested. The present study is thus one of the few to study the antifungal activity of medicinal plants from this region. Out of the 30 plants

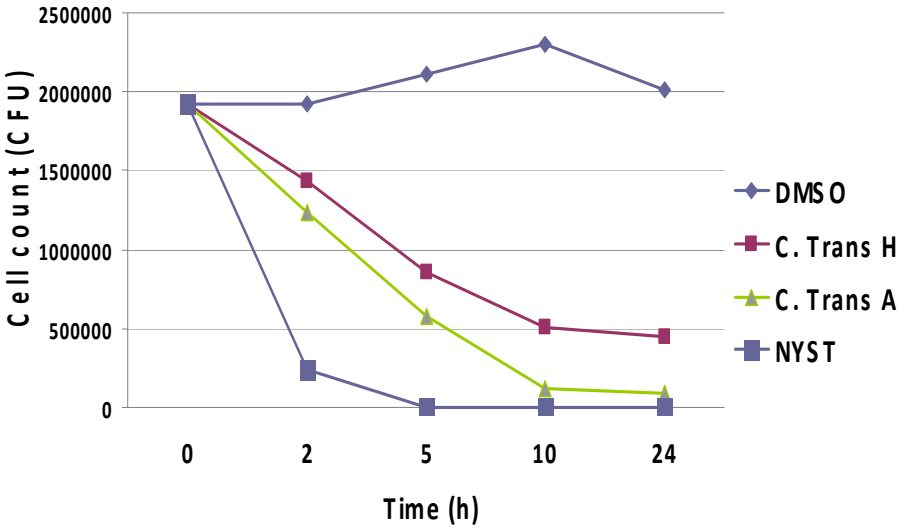

Figure 1. Time to kill curve for *C. transvaalensis* hexane extract (C. Trans H), *C. transvaalensis* acetone extract (C. Trans A) against *C. albicans* as compared to that of the negative control (DMSO) and the positive control nystatin (NYST). The concentration of the extracts used in these experiments was 2XMIC (1.88 mg/ml). Nystatin was used at a concentration of 0.2 µg/ml while DMSO was used at 10%.

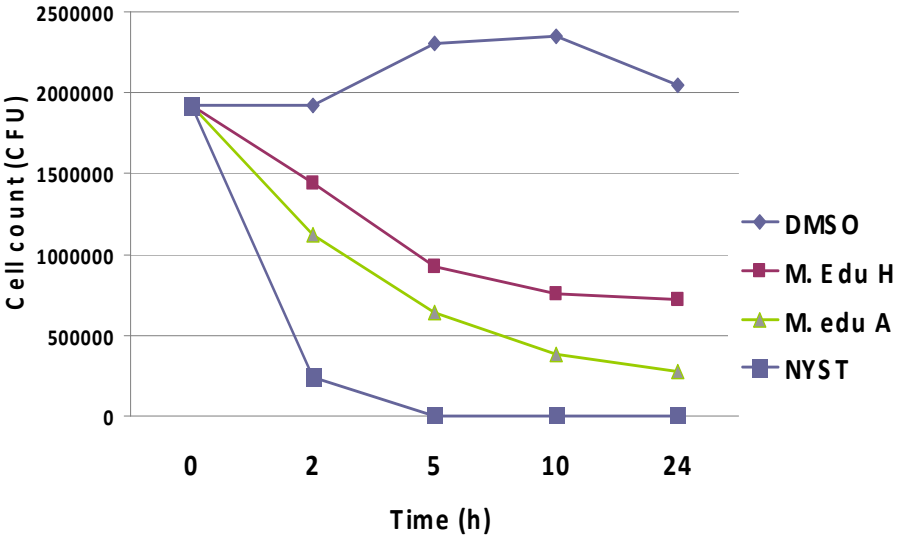

Figure 2. Time to kill curve for *M. edulis* hexane extract (M. Edu H), *M. edulis* acetone extract (M. Edu A) against *C. albicans* as compared to that of the negative control (DMSO) and the positive control nystatin (NYST). The concentration of the extracts used in these experiments was 2XMIC (3.75 mg/ml). Nystatin was used at a concentration of 0.2 µg/ml while DMSO was used at 10%.

identified and tested in the present investigation, 6 plants including *B. micrantha*; *C. transvaalensis*; *Ficus sycomorus*; *P. africanum*; *P. capense* and *S. decussata* were previously tested by Steenkamp et al. (2007) against *C. albicans*. In their study, water and methanol extracts were used while in the present study, acetone and hexane which are two solvent systems of opposite polarity were used. This is important in determining the activity of compounds of different polarities. Furthermore; the plants extracts were tested against 3 different fungal organisms, identifying plants with activity against *C. krusei* and *C. neoformans*. This study has further confirmed the antifungal activity of *P. capense* against *C. albicans* and determined its activity against *C. krusei* and

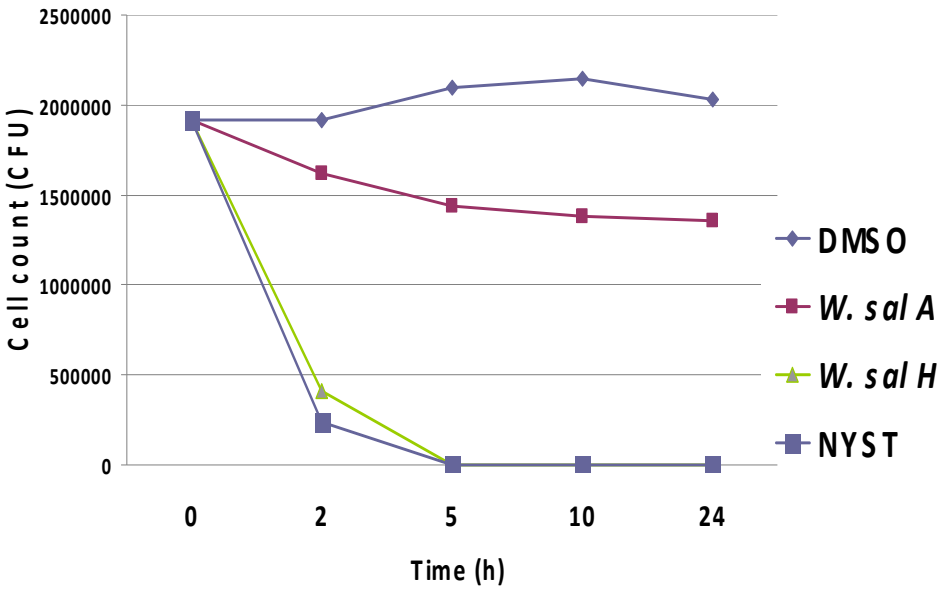

Figure 3. Time to kill curve for *W. salutaris* hexane extract (W. sal H), *W. salutaris* acetone extract (W. sal A) against *C. albicans* as compared to that of the negative control (DMSO) and the positive control nystatin (NYST). The concentration of the extracts used in these experiments was 2XMIC (0.46 mg/ml). Nystatin was used at a concentration of 0.2 µg/ml while DMSO was used at 10%.

C. neoformans. In their study, Steenkamp et al. (2007) did not find any activity with *C. transvaalensis* water and methanol extracts against the clinical *C. albicans.* However it was found that the hexane extracts of this plant was very active against all the fungal organisms with strongest activity against *C. albicans* isolate (MIC 0.46 mg/ml) while acetone extracts did not show any activity indicating that the active compounds in this plant should be non-polar. Steenkamp et al. (2007) also did not find any activity against *C. albicans* clinical isolates when *S. decussata* was used. In the present study, hexane extract of *S. decussata* was active against both *C. albicans* and *C. krusei* but not against *C. neoformans*. Also *B. micrantha* showed strong activity against C*. krusei* with MIC value of 0.94 mg/ml for the acetone extract while the hexane extract showed moderate activity against *C. albicans* clinical isolate (MIC 3.75 mg/ml).

In the present study it was found that 10 out of the 30 plants tested had MIC less than 1 mg/ml against one or more fungal organisms tested (Rios and Recio, 2005). Similar study by Steenkamp et al. (2007) found that the methanol extracts of *Combretum molle* (root), *P. capense* (bark), *Solanum aculeastrum* (fruits), *Syzygium cordatum* (bark) and *Zanthoxylum davyi* (bark) as well as the aqueous bark extract of *Afzelia quanzensis* and root extract of *Tabernaemontana elegans* inhibited clinical *C. albicans* strains at concentrations less than 1 mg/ml. This study thus has investigated new plants that have not been tested before against fungal organisms and confirm the antifungal activity of some plants such as *P. capense* and *W. salutaris* previously described.

In this study, *W. salutaris* was observed to have potent fungicidal activities. In traditional medicine, the bark of *W. salutaris* is used as an expectorant and smoked for coughs and colds, including a topical application for sores and inflammation (Rabe and van Staden, 2000). The leaves and bark of *W. salutaris* have been described to contain numerous drimane sesquiterpenoids, including warburganal and polygodial. These compounds might be responsible for the antimicrobial activities observed with this plant. In a study conducted by Kubo and Taniguchi, (1988), polygodial was combined with an antibiotic having poor cell membrane permeability (actinomycin D) in an effort to increase its antibiotic activity by increasing its ability to gain entrance into the cell and a remarkably enhanced efficacy was obtained and therefore this led to a conclusion that polygodial may be acting as an "advance scout," punching holes in the plasma membrane and gaining an entrance into the cell for an antibiotic previously less effective because of problems with cell membrane permeability.

P. capense has been tested by other authors and found to have inhibitory properties against *C. albicans* (Steenkamp et al., 2007). Using different solvent systems it was also found that this plant was active against three different fungal organisms. Further studies on extracts of *P. capense* roots have resulted in the isolation and characterization of the novel sesquiterpene capentin (Chen et al., 1992). Three new lignans have been isolated from the roots of the African shrub *P. capense* and characterized by means of spectroscopic studies and in one case, a single crystal X-ray analysis. These

compounds include a new 8.O.3'-neolignan, further distinguished by an uncommon 1.3.5-trisubstituted ring system and two dihydrobenzofuranoid neolignans (Green and Wiemer, 1991) and > 58% of hydrocarbons were found in the oil of *P. capense* (Amvam Zollo et al., 1998). Although Steenkamp et al. (2007) did not find any anti-*C. albicans* with *B. discolor* also used by Venda traditional healers for the treatment of fungal related ailments, the present study found that this plants showed activity against all the three fungal organisms tested (by agar diffusion and microdilution). This difference might be due to the type of solvent used in the two studies, the location where the plant was collected or the methodology used. Based on present knowledge, no study had identified the compounds responsible for the antifungal activity of this plant. However, several flavonoid compounds were isolated from this plant in Tanzania by Chin et al. (2006). These included five new prenylated flavonoids ((6aS, 11aS)-2-hydroxyleiocarpin Discoloranone A (3*S*)-isodiscoloranone A (3*S*)-discoloranone B and (3*S*)-isodiscoloranone) along with 10 known compounds including nitidulin, amorphigenin and dabinol. Although not verified for their antifungal activity, these or other compounds might be responsible of the observed antifungal activity in this plant.

In this study, the hexane extract of *C. transvaalensis* bark was shown to be active against all the three fungal species, *C. albicans*, *C. krusei* and *C. neoformans* with the MICs ranging from 0.46 to 1.88 mg/ml and inhibition zones from 6 to 14mm. In a study done by Motlhanka et al. (2008) the roots of *C. transvaalensis* exhibited strong antioxidant activity and thus supported the ethnomedical use of this plant to promote good health but they did not test this plant for antifungal activity. The acetone root extract of *T. sericea* inhibited the growth of *C. albicans* and *C. krusei* but not *C. neoformans*. Masoko et al. (2005) found that *T. sericea* extracts were also the most active amongst the six *Termialia* species that were screened against *C. albicans*, *C. neoformans*, *Aspergillus fumigatus*, *Microsporum canis* and *Sporothrix schenkii*, with MIC values ranging between 0.02 to 0.08 mg/ml. In the present study *W. salutari*s was able to kill all the *C. albicans* cells after 5 h while *C. transvaalensis* were able to kill only 75% of the cells after 10 h. The activity of extracts from these plants need to be tested against more fungal organisms such as *Histoplasma capsulatum* which is also very common among AIDS patients in order to identify further beneficial potentials of these plants.

The activity of the plants tested in the present study was not only inhibitory to the fungal organisms but was also fungicidal. Such activities have been previously reported for other plants and the extent of the fungicidal activity has been evaluated by the time-kill experiments (Rukayadi et al., 2006). Studies by Okemo et al. (2001) indicated that the crude extracts of the neem plant *Azadirachta indica* killed a whole population of *C. albicans* at a concentration of 8 mg/ml in 24 h while Patel and Coogan, (2008) found that *Dodonaea viscosa* extracts killed all the *C. albicans* strains within 30 s. In the present study, *W. salutaris* was able to completely kill *C. albicans* cell at a concentration of 0.4 mg/ml. This indicates the possibility of compounds from this plant to kill fungal organisms with special reference to *Candida* sp. at lower concentrations than the crude extract. This activity could be due to the compounds such as muzigadial and warburganal previously isolated from this plant in other studies (Rabe and van Staden, 2000).

Of all the plants tested in the present study, most plants were shown to be active. However; some plants commonly used by the traditional healers such as *F. sycomorus*, *Z. mucronata* and *S. birrea* were not active. Although in previous studies by Steenkamp et al. (2007) water extracts from *F. sycomorus* fruits were active, no activity in the extracts of the back at the concentration tested was found in the present study. This difference in activity could be due to the phytochemical differences between the two parts of the plant and probably geographical differences. Some of the plant may not have any effect on the fungal organisms but may play important roles such as: facilitating diffusion of active compounds into the blood, stabilizing the body temperature, reducing the toxicity and taste improving. This might explain why most medicinal plants are given in a cocktail by the traditional healers. The use of medicinal plants can therefore be highly recommendable for fungal infections and any other disease and the plants that were active can be of great importance for antifungal drug synthesis. Population growth, urbanization and the unrestricted collection of medicinal plants from the wild is resulting in an over-exploitation of natural resources in southern Africa. In southern Africa, the most frequently used medicinal plants are slow-growing forest trees, bulbous and tuberous plants, with bark and underground parts being the parts mainly utilized (Zschocke et al., 2000) and therefore micropropagation of such plants, including the plants that were tested active in this research can therefore be a great step towards conservation sustainability of the plants for future medicinal researches.

This study has identified new plants with antifungal activity that could be used as sources for the isolation of active compounds that may serve as lead compounds in antifungal drug development. This study further justifies the use of these plants by traditional healers in the region. Further studies on their cytotoxicity or toxicity will be beneficial in providing data on the possible harmful effects of these plants commonly used by the numerous local communities.

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