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Antifungal activities of fifteen Southern African medicinal plants against five *Fusarium* species

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In the present study, 35 extracts from 15 different medicinal plants traditionally used in the Venda region for the treatment of fungal related ailments were tested for antifungal activities against five *Fusarium* spp. including *Fusarium oxysporum, Fusarium verticillioides, Fusarium graminearum, Fusarium nygamai* and *Fusarium proliferatum* using the hole plate diffusion method. The microdilution method was used to determine the minimum inhibitory concentration and the minimum fungicidal concentration. Growth inhibition assay was conducted using an agar diffusion method. Out of the 35 extracts tested, 31 (88%) were active against at least one of the five *Fusarium* spp. However extracts of *Warburgia salutaris*, *Rhoicissus tridentata, Syzygium cordatum, Ximenia caffra* and *Terminalia sericea* gave zone of inhibition ranging from 10 to 20 mm with minimum inhibitory concentration (MIC) ranging from 3.75 to 0.06 mg/ml. Extracts from *W. salutaris* bark in hexane and *Terminalia sericea* bark in acetone were fungicidal to at least one of the *Fusarium* spp. tested at concentrations between 0.06 and 7.5 mg/ml. The present study has revealed the importance of these medicinal plants in the possible control of fungal diseases caused by the tested *Fusarium* spp. and further justifies the use of these medicinal plants by the local populations. Further studies are needed to identify the active components of these plants that could be used as lead compounds in the development of new antifungal agents.

Key words: *Fusarium*, medicinal plants, South Africa, Venda, antifungal.

INTRODUCTION

Organisms of the genus *Fusarium* are among the most common emerging fungal pathogens in immunocompromised patients (Langner et al., 2009). They are also an economically important genus, with many species responsible for plant diseases amounting to millions of dollars each year around the world (Chehri, 2011). They are also responsible for infections in animals and humans, where they cause a broad spectrum of infections. In human, two modes of acquisition of the infection have been described: (i) respiratory, following inhalation of conidia as evidenced by the sinopulmonary involvement in several patients; and (ii) cutaneous, as in cases following cellulitis of the toe or finger, in catheter-related infections, and in patients with burns (Girmenia et al., 1992). The prevalence of *Fusarium* spp. in grain foods and the involvement of the gastrointestinal tract in some cases raise the possibility, though unlikely, of a gastrointestinal route of infection. Thus far, *Fusarium* spp. is the only opportunistic mold that can be easily recovered from the bloodstream (Sahin and Akova, 2006). Usually, patients with positive blood cultures tend to have concomitant skin lesions. It has been speculated that the

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toxins produced by *Fusarium* spp. may enhance the breakdown of tissues and thus facilitate entry of fusaria into the systemic circulation (Kocić-Tanackov et al., 2011).

The treatment of *Fusarium* infections in humans as well as plants generally necessitates the use of drugs and other chemicals which might have secondary undesirable effects in human or in the environment. Furthermore, they are generally resistant to many drugs used for treatment in both immune-competent and immune-compromised patients. Studies have shown that the continuous application of chemicals could lead to the destruction of the ecosystem, and might result in the emergence of new strains of fungi that are difficult to control (Rebib et al., 2012). The use of medicinal plants is part of the African tradition and the Venda region of South Africa has a great variety of vegetation used by local population to treat and prevent several types of diseases (Obi et al., 2003). Due to the high diversity of medicinal plants in South Africa, most of the medicinal plants have been used by the populations in the treatment of fungal related diseases. Therefore, there is a high need to test for those medicinal plants against *Fusarium* spp. which is pathogenic to human, animal and plants.

Southern Africa has one of the richest plant diversity in the world and a high percentage of these species have been implicated in traditional medicine of the region for several centuries (Samie et al., 2005). The use of herbal medicine is an integral part of the culture of the people and an estimated 80% of South Africans use herbal remedies for their physical and physiological health care at different stages of their life. The high human and flora diversity of the country is probably responsible for the immense knowledge in native medicine (Lewu and Afolayan, 2009). Some extracts of Medicinal plants such as *Mitracarpus villosus* leaves have shown antifungal activities against *Fusarium solani* with the minimum inhibitory concentrations (MICs) ranged from 0.50 to 4.0 mg/ml with fungicidal at higher concentrations (Irobi and Daramola, 1994). The present study determined the antifungal activity of fifteen medicinal plants traditionally used in the Venda region for the treatment of different types of infections against five *Fusarium* species responsible for human, animal and plant diseases.

MATERIALS AND METHODS

Plant collection

The medicinal plants used in the present study have been previously tested against bacterial organisms in the Department of Microbiology. Briefly, plants used by local population to cure different ailments were collected with the help of botanists and traditional healers in the Venda region between March, 2007 and October, 2007. The plants were identified by taxonomist of the Department of Biological Sciences, University of Venda. Voucher specimens were deposited at the herbarium of the Thohoyandou Botanical garden and the University of Venda. Names and parts of the plants are indicated in Table 1.

Preparation of extracts

Plant material was washed with distilled water and air dried in the laboratory for two week and ground in a Wiley grinder with a 2 mm wire mesh. Fifty gram of each ground material was soaked in 500 ml of hexane, acetone or methanol for at least 72 h with frequent shakings. The samples were then suction filtered through Whattman no.1 filter paper. The filtrate was evaporated to dryness under reduced pressure at 40°C using a rotary evaporator. A stock solution of 0.2 g/ml in dimethylsulfoxide (DMSO) was made for each extract (Samie et al., 2005). A total of 35 extracts were prepared from 15 medicinal plants.

Microorganisms preparation

The fungal organisms were provided by the Department of Botany of the University of Pretoria. The fungal organisms used in the study were field isolates and included *F. verticillioides, F. nygamai, F. oxysporum, F. proliferatum* and *F. graminearum.* Fresh *Fusarium* cultures were prepared from the stock cultures in brain heart infusion broth (BHIB; Oxoid, England)). Briefly, small piece of pure culture was taken and placed in 50 ml of BHIB. The culture was incubated at 30°C for 24 h. One ml of culture was added in approximately 9 ml of fresh BHIB to prepare a 0.5 Mac Farland standard of the organism. All tests were conducted in duplicate.

Antimicrobial activity determination

Hole plate diffusion method

The hole plate diffusion method was used to determine the activity of the medicinal plants against the fungal organisms. Briefly, PDA (potato dextrose agar; Oxoid, England) plates were inoculated with 1000 µl of a 0.5 Mac Farland standard of the organisms grown in brain heart infusion broth. Afterward, 6 wells of approximately 5 mm in diameters and 2.5 mm deep were made on the surface of the solid medium using the tip of a sterile plastic pipette. Each well was then filled with 20 µl of the test extract or controls. Sterile dimethylsulfoxide (DMSO) was used as negative control and Nystatin was used as positive control. The plates were then incubated at 30°C for 2 to 3 days. After 3 days, the radial zone of inhibition was measured by using a ruler and the diameters of inhibition zone was determined in millimeters. Extracts with zones of inhibition greater or equal to 6 mm diameters were regarded as active (Apak and Olila, 2006).

Determination of minimum inhibition concentration (MIC) using the microdilution method

The microdilution method was used to determine the MIC as previously described (Eloff, 1998). Briefly, 185 µl of brain heart infusion broth was placed in the first well of each column of the microtitre plates and 100 µl in the rest of the wells. Fifteen (15) µl of extract was placed in the first well and mixed. Thereafter, 100 µl of the mixture was transferD to the next well until to the last well to achieve a serial two fold dilution in the wells. One hundred (100) µl

Table 1. Ethnobotanical data of the plants used in the present study.

Scientific names (family)	Common names (Venda/English)	Plant parts used	Traditionally used in
Peltophorum africanum (Caesalpinoidiae)	Musese, weeping wattle	Bark	Colds, fever, Sore throat, Sores, ulcers, blistersin the oral cavity, gonorrhoea (Mabogo, 1990)
Pouzolzia mixta (Utriciae)	Muthanzwa, soap nettle	Leave, stem, root	Diarrhoea, dysentery (Mabogo, 1990)
Rhoicissus tridentata (Vitaceae)	Murumbulambudzana, bushman's grape	Leaves, tubers, root	To treat diarrhoea, prevent miscarriages (Mabogo, 1990)
Rhus rogersii (Anacardiaceae)	Muthasiri	Bark	General pain, watery diarrhoea (Mabogo, 1990).
Schlerocarya birrea (Anacardiaceae)	Mufula, Marula tree	Bark	Fever, stomach ulcers, wounds, infertility (Mabogo, 1990)
Schotia brakipetala (Caesalpinoidiae)	Mulubi, weeping boer bean	Bark	Heart disorders, dysentery, diarrhoea (Mabogo, 1990)
Securidaca longepedunculata (Polygalaceae)	Mpesu, violet tree	Root, bark	Aphrodisiac, tuberculosis, gonorrhoea (Mabogo, 1990).
Sida alba	Lukandalula	Leaves	Dysentery, diarrhoea (Mabogo, 1990)
Strychnos decussata (Loganiaceae)	Muvhavhanyane, Cape teale	Bark	Sore throat, fever, headache, wounds, vaginal infections (Mabogo, 1990).
Syzygium cordatum	Mutu	Bark, leaves	Stomach troubles, cold and fever, babies' food, diarrhoea, wounds (Mabogo, 1990).
Terminalia sericea (Chenopodiaceae)	Mususu, silver terminalia tree	Bark	Infected wounds, menorrhage, to dress on magical wounds (Mabogo, 1990)
Warburgia salutaris (Canellaceae)	Mulanga, pepper bark	Barks, leaves	Bark for aphrodisiac, venereal diseases, colds, sore throat, malaria (Mabogo, 1990).
Ximenia caffra	Mutswili	Roots, leaves	Diarrhoea, dysentery, fever, cough, venereal disease (Mabogo, 1990).
Ziziphus mucronata (Rhamnaceae)	Mukhalu Buffalo/Cape thorn	Bark	Boils, skin infections, tubercular gland swellings, measles, dysentery, lumbago and chest complaints (Mabogo, 1990).
Zornia milneana	Lukandulula	Whole plant	Diarrhea (Mabogo, 1990).

of the microorganism's culture in the broth was added. The plates were then incubated until the next day. The next day, 50 µl of iodo-nitro tetrazolium (INT) was added to each well. The minimum inhibitory concentration was determined as the smallest concentration of the extracts that inhibited the growth of the organisms.

Determination of minimum fungicidal concentration (MFC) by microdilution

The MFC was determined from the microdilution plates used for the MIC. The cultures from the wells in the microtitration plate that showed no visible growth were inoculated in PDA plates. The Petri dishes were then incubated at 30°C for 7 days and observed for growth. The smallest dilutions that did not show any growth were considered to be the MFC (Rukayadi et al., 2008).

Growth inhibition assay

Twenty eight medicinal plant extracts that showed activity using the above described hole plate diffusion method and microdilution method were tested against the five *Fusarium* species to determine the rate of growth inhibition using the agar dilution methods. Briefly, potato dextrose agar (PDA) was prepared and left to cool down to 50°C and was mixed with the plant extracts to give a predetermined concentration. Then 10 ml of the mixture was poured in the Petri dishes and let to solidify. A piece of the *Fusarium* culture on the agar was cut and placed in the middle of the plate containing the extract. Then the plates were incubated at 25°C for 7 days. Radial growth of the fungal organisms was recorded everyday for 7 days or until the plates were overgrown. Negative and positive controls were run along each fungal isolate and crude extract following the procedure described by Amjad et al. (2005).

Statistical analysis

All the tests were conducted in duplicates. The data were analysed using the Statistical package for Social Sciences (SPSS) program. The Chi square was used and the p values were determined. The difference between two variables was considered significant when the p value was less than 0.05.

RESULTS

Antifungal activity of the plant extracts as determined by the hole plate diffusion method

Out of the 36 medicinal plant extracts tested, 14 (39%) extracts were active against *Fusarium* spp. based on the hole plate diffusion method. Table 2 shows the diameter zone of inhibition of the fungal organisms by the plants extracts as determined by the hole plate method. The hexane extract of the *W. salutaris* bark was the most active with a diameter of zone of inhibition ranged from 12 to 20 mm against all five *Fusarium* spp. tested while

Table 2. Antifungal activity of medicinal plants extracts active against *Fusarium* species determined by the hole plate diffusion method (mm).

Plant (Part used and solvent)	*F. ver*	*F. nug*	*F. oxy*	*F. prol*	*F. gram*
P. africanum acet.	0	0	0	0	12
P. mixta (L) acet	0	0	0	10	0
P. mapnounefolia hex.	0	0	0	0	10
R. tridentate (R) meth.	0	0	0	0	15
S. birrea (B).acet.	12	0	0	0	0
S. longipeduculanta (R) acet.	0	0	0	8	0
S. longipeduculanta (R) hex.	15	0	20	15	0
S. cordatum (L) meth.	0	0	0	15	20
W. salutaris (B) hex.	15	20	12	12	13
W. salutaris (B) acet.	0	10	10	0	0
W. salutaris (L) hex.	0	12	10	0	0
X. caffra (R) acet.	0	0	0	0	20
X. caffra (R) hex.	7	7	14	0	0
X. caffra.sond (L) acet.	0	0	0	15	15
Positive control (Nystatin)	20	16	20	20	20

The results are presented as inhibition zone diameters in mm. Keys: F. ver = *Fusarium verticillioides,* F. nug= *Fusarium nygamai,* F. oxy= *Fusarium oxysporum,* F. prol = *Fusarium proliferatum,* F. gram = *Fusarium graminearum.* Hex = hexane, acet = acetone, meth = methanol, B = bark, L = leaves, S = stem and T= tubers

the leaves extract of the same plant was active with the diameter of zone of inhibition of 12 mm against *F. oxysporum*. Most plants were active against *F. graminearum* with the 14 extracts showing a growth inhibition zone varying between 7 and 20 mm. *F. nygamai* was the most resistant with only 4 extracts showing activity against this fungus and *W. salutaris* bark extract in hexane showed the strongest activity (20 mm zone of inhibition). The hexane extract of the root of *S. longipeduculanta* showed the highest activity against *F. oxysporum*.

Minimum inhibitory concentration of the plant extracts against *Fusarium* spp.

Many extracts that were active using the hole plate diffusion method also gave high MICs values with the microdilution method. However, more extracts showed activity when tested with the microdilution method compared to the hole plate agar diffusion method. Out of the 36 medicinal plants extracts tested, 27 (75%) extracts showed activity against at least one of the five fungal organisms tested by the microtitration plate method. Among those that showed activities, acetone extract of the *P. africanum* bark, *P. mixta, T. sericea* and *Z. milneana* showed strong activities with MICs values less than 1 mg/ml against at least one species of *Fusarium* tested. The methanol extract of *P. mixta* roots was active with MIC value of 0.95 mg/ml against *F. nygamai* and *F. proliferatum* while against other three *Fusarium* spp. tested; the MIC values were higher (3.75 mg/ml). Acetone extract of *R. tridentate* tubers also showed high activities with MICs values varying from 0.95 to 3.75 mg/ml against five *Fusarium* spp. tested. The acetone extract of *T. sericea* bark gave MIC values varying from 0.95 to 1.9 mg/ml against all five *Fusarium* spp. tested. The acetone extract of the *P. africanum* bark extract was active against all five *Fusarium* spp. Tested, with the MIC of 1.9 mg/ml. The acetone and hexane extracts of the *S. longipedunculata* roots were active against all species of *Fusarium* tested except *F. graminearum* with MICs ranging from 1.9 to 3.75 mg/ml. Table 3 shows the MIC for the different extracts.

Fungicidal activity of the medicinal plants

Out of the 27 extracts that were active with MIC equal or less than 7.5 mg/ml, 13 extracts showed fungicidal activity against the *Fusarium* species tested (Table 4). The acetone extract of the *P. africanum* bark and hexane extract of the *S. longipedunculata* roots were fungicidal against four *Fusarium* spp. tested except against *F. oxysporum*. The methanol extract of the *P. mixta* roots also gave high fungicidal activities against *F. nygamai*

Table 3. Minimum inhibitory concentrations (MIC) of medicinal plant extracts active against *Fusarium* species using micro dilution method. The MICs are in mg/ml.

Plants extract (part used and solvent)	*F. ver*	*F. nug*	*F. oxy*	*F. prol*	*F. gram*
P. africanum (B) acet	1.9	1.9	1.9	1.9	1.9
P. africanum (B) meth	7.5	0.95	>7.5	7.5	3.75
P. africanum (R) meth	>7.5	>7.5	>7.5	>7.5	3.75
*P. mixta(*R) meth	>7.5	>7.5	>7.5	>7.5	>7.5
P. mixta (R) meth	3.75	0.95	3.75	0.95	3.75
P. mixta (S) meth	7.5	>7.5	3.75	3.75	7.5
P. mixtra (L) acet	>7.5	>7.5	3,75	>7.5	7.5
P. angolensis (B) meth	>7.5	>7.5	7.5	>7.5	7.5
R. tridentate (T) acet	3.75	1.9	0.95	1.9	0.95
R. tridentate (R) meth	7.5	>7.5	>7.5	7.5	7.5
R. rogersii(B).acet	>7.5	>7.5	7.5	>7.5	>7.5
S. birrea (B) acet	>7.5	>7.5	>7.5	>7.5	3.75
S. brakepetale (B) acet	>7.5	>7.5	>7.5	>7.5	7.5
S. longipeduculanta (R) acet	3.75	7.5	3.75	1.9	>7.5
S. didymobotrya (R). hex.	>7.5	>7.5	>7.5	>7.5	7.5
S. alba .acet	3.75	7.5	1.9	1.9	0.95
S. decussate (B)hex	7.5	>7.5	>7.5	3.75	>7.5
S. cordatum(L) meth	7.5	>7.5	7.5	>7.5	>7.5
T. sericea (B) acet	1.9	0.95	1.9	1.9	1.9
W. salutaris (B).acet	>7.5	>7.5	3.75	7.5	3.75
W. salutaris (L).acet	>7.5	>7.5	0.48	0.95	>7.5
X. caffra (R) .acet	7.5	7.5	1.9	3.75	>7.5
X. caffra (R).hex.	>7.5	>7.5	0.95	7.5	>7.5
X. caffra (L) .acet	>7.5	>7.5	>7.5	>7.5	3.75
Z. munoronata (B).acet	7.5	7.5	>7.5	7.5	>7.5
Z. milneana. acet	3.75	1.9	0.48	1.9	7.5
Z. milneana. meth.	>7.5	>7.5	>7.5	1.9	>7.5
Nystatin (Positive control) (µg/ml)	20.4	19.1	18.8	19.4	16.2

F. ver = Fusarium verticillioides, F. oxy = Fusarium oxysporum, *F. nug = Fusarium nygamai*, *F. gram = Fusarium graminearum*. *F. prol = Fusarium proliferatum,* Hex =hexane, acet =acetone, meth = methanol, B = bark, L = leaves, S = stem and T = tubers.

and F. proliferatum with MFCs values of 0.48 and 3.75 mg/ml, respectively. Acetone extract of the *W. salutaris* leaves was also fungicidal against *F. verticilloides*, *F. oxysporum* and *F. proliferatum* with MFCs value of 3.75, 0.95 and 0.95 mg/ml, respectively.

Growth inhibition assays of the active extracts against *Fusarium* spp.

The capacity of the extracts to inhibit the growth of the fungal organisms was evaluated in an agar medium. On the growth inhibition curves it can be seen that the curve for the negative control was generally above the curves for the active extracts (Figure 1a and b). Extracts like acetone extract of the *S. cordatum* bark, hexane extract of the *S. longipeduculanta* roots, acetone extract of *B. discolour* roots *and* acetone of *E. divinorum* (leaves and bark) were less inhibitory to the fungal organisms tested compared to the other extracts.

DISCUSSION

Fusarium species are common fungal pathogens in both humans and animal as well as in plants of agricultural importance. Recent studies have indicated that *Fusarium* spp*.* are emerging opportunistic pathogens among immunocompromised patients such as those with human immune deficiency virus (HIV) and acquired immune

Table 4. Minimum fungicidal concentrations (MFC) of medicinal plants extract that were able to completely kill the fungal organisms. The MFCs are in mg/ml.

Plant extracts (part used and solvent)	*F. ver*	*F. nug*	*F. oxy*	*F. prol*	*F. gram*
P. africanum (B) acet.	3.75	3.75	>7.5	3.75	3.75
P. mixta (S) meth.	>7.5	>7.5	>7.5	>7.5	7.5
P. mixta (R) meth.	>7.5	0.48	>7.5	3.75	7.5
R. tridentate (R) meth.	>7.5	>7.5	7.5	>7.5	>7.5
S. birrea (B) acet.	>7.5	>7.5	>7.5	>7.5	3.75
S. longipeduculanta (R) hex.	3.75	3.75	>7.5	3.75	3.75
S. cordatum (L) Meth.	3.75	>7.5	>7.5	3.75	>7.5
W. salutaris (L) acet.	3.75	>7.5	0.95	0.95	7.5
W. salutaris (B) acet.	>7.5	7.5	>7.5	7.5	7.5
X. caffra (R) acet.	7.5	7.5	7.5	7.5	>7.5
X. caffrasond (L) acet.	>7.5	>7.5	>7.5	>7.5	7.5
Z. mucronata (B) acet.	>7.5	>7.5	>7.5	>7.5	0.95
Z. milneana acet.	7.5	>7.5	7.5	7.5	>7.5
Nystatin (µg/ml)	32.6	28.8	32.6	31.8	30.8

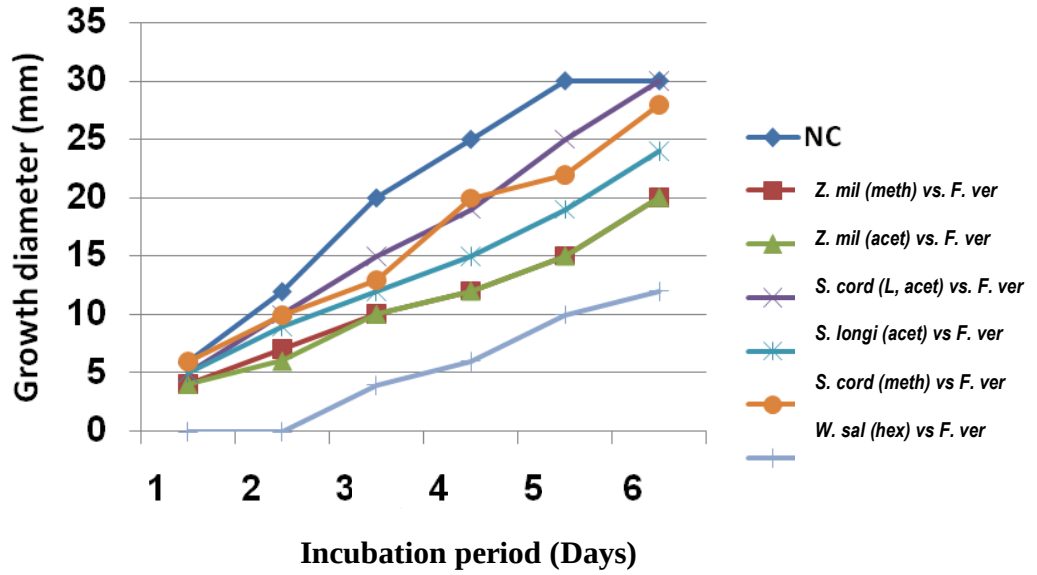
The results represented here show the concentrations in the wells that did not show any visible growth of the microorgnisms after subculturing from the MIC plates. Key: F. ver = *Fusarium verticillioides,* F. oxy = *F. oxysporum*, F. nug = *F. nygamai,* F. gram = *F. graminearum*. F. prol = *F. proliferatum.* Hex = hexane, acet = acetone, meth = methanol, B = bark, L = leaves, S = stem and T= tubers.

deficiency syndrome (AIDS) patients (Tezcan et al., 2009). However the control and treatment of these species is problematic due their resistance against most fungal drugs (Nucci and Anaissie, 2007). The use of medicinal plant in the treatment of different infection is still most common in Venda. In the present study (77.1%) of plants extracts showed antifungal activities against at least one of the five *Fusarium* species. This indicates the importance of these plants in their possible usage in the control of infections caused by *Fusarium* spp. In fact most of the plants under investigation have been studied against other pathogens mainly bacteria and yeast organisms (Samie et al., 2010). However, very few or no studies have determined the activities of these plants against the *Fusarium* spp tested.

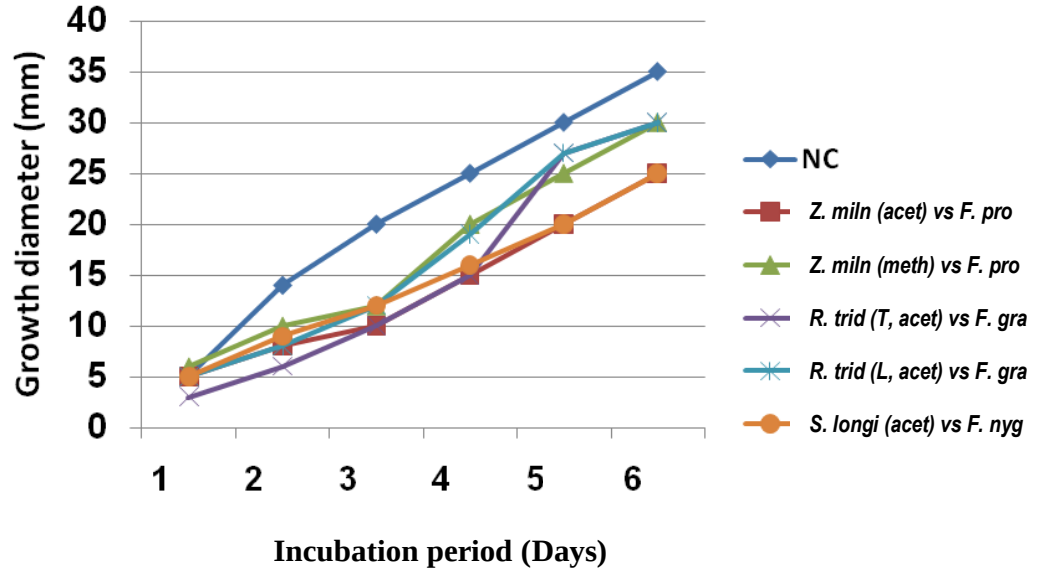
W. salutaris was the most active against the organisms in all tests performed. Other studies have indicated that *W. salutaris* had strong antibacterial activities. However, no study has tested the activity of this plant against the *Fusarium* spp. investigated in the present study. Previous studies by Viresh and Bharti (2009) indicated that hexane extract of *W. salutaris* bark contain aromatic compound and isoprene compounds which had antifungal activities against *F. moniliforme* and *Aspergillus flavus* determined by microdilution. The activities observed could be due to compounds such as sesquiterpene lactone which were isolated from the plant and shown antiplasmodial activity (Sekhoacha et al., 2007). A compound called muzigadial has been isolated from *W. salutaris* as well as other related species (Rabe and van Staden, 2000). The stem bark of *W. salutaris* collected in Venda yielded a new drimane sesquiterpenoid lactone, salutarisolide, along with four known drimane sesquiterpenoids warburganal, mukaadial, polygodial and isopolygodial (Mashimbye et al., 1999). Studies by Samie et al. (2010) have also demonstrated strong activities of *W. salutaris* against *Candida albicans* and *Cryptococcus neoformans*. However, there is need for further studies to determine the mechanisms of action of the extracts and compounds against *Fusarium* spp. as well as other fungal organisms.

Ethanolic extracts of the leaves of *Bridellia micantha* had shown activities against several bacterial spp (Mathabe et al., 2006). However, very few studies have been conducted against *Fusarium* spp. In the present study, we found that *B. micantha* hexane leaves extracts had an antifungal activity against *F. graminearum*. There have been several reports on the natural occurring plant chemicals found in this plant. These include phenolic acids which include gallic acid, ellagic acid, protocaechoc acid and flavonoids which include quercetin, hyperin and guaijaverin (Lamikanra et al., 1990; Banzouzi et al., 2002). In a previous study, extract of *B. micantha* leaves showed activity against *F. moniliforme* (Viresh and Bharti, 2009).

In the present study, hexane extracts of *Syzygium cordatum* barks and leaves were active against all five



(a)



(b)

Figure 1. Growth inhibition curves obtained for the plant extract against different Fusarium spp. The plants are: W. sal = *Warburgia salutaris* (bark) in hexane, Z. Mil or Z. miln =*Zornia milneana* (whole plant) L.J.A =*Lippia javanica* (leaves) in acetone, S. cord = *Syzygium cordatum* , S. longi =*Securidaca longipedunculata* .R. trid= *Rhoicicus tridentate*, F. ver = *F. verticillioides,* F. oxy = *F. oxysporum*, F. nug = *F. nygamai,* F. gram = *F. graminearum*. F. prol = *F. proliferatum.* Hex = hexane, acet = acetone, meth = methanol, B = bark, L = leaves, S = stem and T= tubers, NC = Negative control.

species of *Fusarium* with high inhibition zone while the methanol extracts were fungicidal against *F. verticillioides* and *F. proliferatum*. Their activity could be due to compounds such as friedelin, epi-fridelinol, β-sitosterol, arjunolic acid, gallic acid, ellagic acid (hexahydroxydiphenic acid), glucose and a gallic acid –allagic acid complex previously identified in this plant (Candy et al., 1968). In previous study, leucodelphinidin and methanol extracts of the stem bark and leaves of this plant were the most active against bacterial species (Samie et al.,

2005). Acetone and hexane extracts of *Carisa edulis* were active against *F. graminearum*. Previous studies have indicated that this plant contains benzenoids, penylpropanoid, lignans, sesquiterpenes and cumarins steroids, terpenes, tannins, flavonoids and cardiac glycosides (Bentley, 1984). These compounds could be responsible for the activity observed. However, further studies are needed to confirm the mode of action as well as the compounds specifically responsible for the anti-fusarial activities.

In South Africa, *Cissampelos torulosa* is used to treat stomach and skin cancer (De Wet et al., 2009). Some studies have indicated that *Cissampelos* families have good antimicrobial activities. For example, hydrophilic extract of *C. torulosa* have shown good antiplasmodial activities (Tshibangu et al., 2002). In the present study, the methanol and acetone extracts of *C. torulosa* were active against *F. proliferatum* when tested by both the hole plate method as well as the microdilution method. Previous studies on the South African Menispermaceae (including the *Cissampelos*) have been said to be attributed to its rich variation in alkaloids (de Wet et al., 2009). For example, bisbenzyltetrahydroisoquinoline alkaloids have been described in *Cissampelos mucronata* (Ferreira et al., 1965). However, this is the first report of the anti-*Fusarium* activity of *C. torulosa*.

The bark and roots of *Euclea divinorum* are used in traditional medicine for the treatment of diarrhea, convulsions, cancer, skin diseases and gonorrhoea (Mabogo, 1990). In this study, an acetone extracts of *E. divinorum*e leaves were active against *F. proliferatum* which has been found to be responsible for disseminated and skin infections in human particularly those with HIV (Summerbell et al., 1988; Guarro et al., 2000). Previous studies of *E*. *divinorum* and other *Euclea* species have indicated that this plant yield some naphthoquinones, triterpenes and flavonoids as secondary metabolites (Mebe et al., 1997) which support the pharmacological activities for these plants. *Lippia javanica* leaves are commonly used as a mosquito repellent by the population in Southern African. Previous studies have indicated that acetone and methanol extracts of this plant were active against most of the bacteria tested (Samie et al., 2005).

In another study, ethyl acetate and dichloromethane extracts of this plant also showed antibacterial activities against*, Staphylococcus aureus, Escherichia coli*, *Bacillus subtilus, Micrococcus luteus and S. marcercens* (Viresh and Bharti, 2009). In the present study acetone extracts has great activity against most of the species of *Fusarium* with the MICs less than 1 mg/ml. Studies in the *L. javanica* collected from the Venda region indicated that the volatile oil of this plant was active against bacterial organisms such as *E. coli*, *S. aureus* as well as *Plasmodium falciparum* responsible for malaria. The major component isolated from the oils was 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one (Manenzhe et al., 2004).

Some species of the *Ficus* family showed extremely high antibacterial activity as reported by previous studies. These included *Ficus abotifolia, Ficus platyphylla, Ficus polita, Ficus sycomorus* and *Ficus thoningii* (Kubmarawa et al., 2007). In the present study, the acetone extracts of *F. sycomorus* bark showed less activity against all the five *Fusarium*. The stem-bark of *Peltophorum africanum* contains gallotannin, bergenin and catechin (Theo et al., 2009). *P. africanum* bark was active against four *Fusarium* spp. except *F. oxysporum* and the MFC value was 3.75 mg/ml. The present study is the first to report on the anti-fusarium activity of this plant.

The populations of Limpopo province, South Africa use *Ximenia caffra* in the treatment of dysentery and cholera. In previous studies, the extracts of *X. caffra* were active against *S. aureus*, *Vibro cholera* and *Shigella dysentery* (Mathabe et al., 2006). In the present study, acetone extracts of *X. caffra* was active against *F. graminearum* which appeared to be much resistant to other plant extracts. In previous study by Fabry et al. (1996), *X. caffra* was active against several species of *Candida.* Okemo et al. (1996) indicated that the methanolic extracts of this plant contained tannins and water extracts contain coumarins, which might be responsible for the pharmacological activities of the plant. Previous studies on *Pouzolzia mixta* have indicated that this plant was not active against bacterial organisms (Samie et al., 2009). However, in the present study methanol extract of *P. mixta* roots was active against *F. nygamai* and *F. proliferatum* with lower MIC value of 0.95 mg/ml and an MIC value of 3.75 mg/ml against the other three *Fusarium* species studied.

Rhoicicus tridentate is used by populations in South Africa for gynecological purposes and diarrhea. In the present study, the acetone extract of the *R. tridentate* tubers was active against all *Fusarium* spp. tested with the MICs values varying from 0.95 to 3.75 mg/ml, although it did not show a zone of inhibition in the hole plate diffusion assay. This indicates the possibility that the active compounds might not be readily diffused in the hydrophilic agar matrice and might therefore be hydrophobic compounds. Several compounds have been isolated from extracts of this plant and included proanthocyanldin monomers: (-)-eplgallocatechin, (+)-gallocatechln, (+)-catechin hydrate, (+)-mollisacacidin, (+)-eplcatechin, (-)-fisetinidol and epicatechin-3-O-gallate; and dimers: procyanidin B3, procyanidin B4, flsetlnidol-(4a-8)catechin and flsetinidol-(4β-8)catechin, as well as gallic acid and 74% polymeric proanthocyanidins (Brookes and Katsoulis, 2006). Although the activities of these compounds have not been demonstrated against the fungal species tested in the present study, it is possible that they might contribute to antifungal activities

of the extracts.

Terminalia sericea Burch. Ex.DC (Combretaceae) extracts are used to treat bacterial infections/woods, diarrhea, and diabetes by centuries (Likoswe et al., 2008). Previous studies have indicated that methanol extract of the *T. sericea* had the highest antifungal activity against *C. albicans, Cryptococcus neoformans and Aspergillus fumigatus* (Eloff et al., 2005). In the present study, acetone extracts of the *T. sericea* bark exhibited antifungal activity with low MIC values of 0.95 mg/ml against *F. nygamai* and 1.9 mg/ml against the other species of *Fusarium* tested using the MIC determination also during identification of percentage of inhibition. Compounds so far isolated from *T. sericea* include a triterpene sericoside and resveratrol-3-O-_-d-rutinoside, a hydroxystilbene glycoside (Bombardelli et al., 1975). These compounds identified might be responsible for the antifungal activity, but further studies on further isolation, identification and separation of active pure compounds against *Fusarium* spp. is needed.

In the present study, we have demonstrated that several plants traditionally used in the Venda region for the treatment of fungal and related diseases in humans are active against *Fusarium* spp. Therefore, these plants could be used in the control of infections by *Fusarium* spp. in humans, animals and plants. This study also justifies the use of these plants by traditional healers in the region in the management of fungal and other infectious diseases. The hexane extract of the bark of *W. salutatis* was the most active against all the *Fusarium* tested. In addition, *F. graminearum* is one of the most intensively studied fungal pathogens as it is responsible for causing head blight on wheat, barley, rice, and ear rot on maize. The active plants found in the present study could be used in further studies to identify potential drugs that are more effective and affordable to the local population for the management of infections by this organism.

Acetone extract of the *Piper capense* roots, *Pelto-phorum africanum* bark and *Securidaca longipedunculata* roots were fungicidal to one or more of the fungal organisms tested. The acetone extracts of the *T. sericea* bark and *P. capense* roots showed high activity during killing curve determination. Further studies are needed to isolate and identify the active compounds from these extracts that could lead in the development of new and effective antifungal drugs.

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