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ANTIOXIDANT AND ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANT EXTRACTS AGAINST PHYTOPATHOGENIC FUNGI.

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Abstract

Background: Medicinal plants are used by many ethnic groups as a source of medicine for the treatment of various ailments in both humans and domestic animals. These plants produce secondary metabolites that have antimicrobial properties, thus screening of medicinal plants provide another alternative for producing chemical fungicides that are relatively non-toxic and cost-effective.
Materials and methods: Leaf extracts of selected South African plant species (*Bucida buceras*, *Breonadia salicina*, *Harpephyllum caffrum*, *Olinia ventosa*, *Vangueria infausta* and *Xylotheca kraussiana*) were investigated for activity against selected phytopathogenic fungi (*Aspergillus niger*, *Aspergillus parasiticus*, *Colletotricum gloeosporioides*, *Penicillium janthinellum*, *P. expansum*, *Trichoderma harzianum* and *Fusarium oxysporum*). These plant fungal pathogens causes major economic losses in fruit industry such as blue rot on nectaries and postharvest disease in citrus. Plant species were selected from 600 evaluated inter alia, against two animal fungal pathogens (*Candida albicans* and *Cryptococcus neoformans).* Antioxidant activity of the selected plant extracts were investigated using a qualitative assay (2, 2-diphenyl-1-picrylhydrazyl (DPPH)). Bioautography assay was used to determine the number of antifungal compounds in plant extracts.
Results: All plant extracts were active against the selected plant phytopathogenic fungi. Moreover, *Bucida buceras* had the best antifungal activity against four of the fungi, with minimum inhibitory concentration (MIC) values as low as 0.02 mg/ml and 0.08 mg/ml against *P. expansum*, *P. janthinellum*, *T. harzianum* and *F. oxysporum.* The plant extracts of five plant species did not possess strong antioxidant activity. However, methanol extract of *X. kraussiana* was the most active radical scavenger in the DPPH assay amongst the six medicinal plants screened. No antifungal compounds were observed in some of the plant extracts with good antifungal activity as shown in the microdilution assay, indicating possible synergism between the separated metabolites.
Conclusion: The results showed that acetone was the best extractant. Furthermore, our findings also confirm the traditional use of *Breonadia salicina* and demonstrate the potential value of developing biopesticides from plants.

Key words: Antifungal activity, Antioxidant activity, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Minimum inhibitory concentration (MIC), bioautography assay.

Introduction

Plants are a primary source of new natural medicinal products (Hostettman, 1999). Of 119 drugs still extracted from plants and used globally, 74% were discovered during an attempt to identify the chemical substances amongst medicinal plants responsible for combating human diseases (Farnsworth, 1990). More importantly, some of the plants natural products have various biological activities such as anti-inflammatory, anticarcinogenic and anti-atherosclerotic, antibacterial, antifungal, antiviral, antimutagenic and antiallergic activities (Ikken et al., 1999; Noguchi et al., 1999; Mishra et al., 2009). These biological activities may be associated with their antioxidant activity (Chung et al., 1998). The antimicrobial activities of plant extracts have formed the basis of many applications, including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Lis-Balchin and Deans 1997, Reynolds, 1996).

Plant pathogens such as fungi, bacteria, nematodes and viruses cause various diseases in plants or may damages the plants (Montesinos, 2003). More importantly, fungi are the main pathogens that harm the plants. Fungal diseases cause a considerable loss of crop yields in agricultural industries worldwide. For example, fungi such as *Fusarium* spp., growing on plants, are able to produce mycotoxins that can seriously harm consumers. *P. digitatum* and *P. expansum* cause spoilage of oranges in citrus industry (Mahlo and Eloff, 2014). Aflatoxin B_1 and B_2 and fumitoxins produced by *A. flavus* and *A. fumigatus* are some examples of mycotoxins (Singh et al., 1991). Antimycotics play an important two major roles in agriculture; firstly, they are used to control fungal growth on plants and fruits. Secondly, they can be used to prevent or to ease the problem of post harvest spoilage of plants and fruits (Hof 2001). The presence and growth of this fungus in food and animal feed threatens human and animal health, respectively.

Some farmers use chemical fungicides in plant agriculture to control fungal diseases, for example, fire blight or blister spots in fruit trees (such as apple, pear and peach). However, many fungicides are toxic to humans and they can cause environmental contamination or may result in fungicide residues on food products (Moenne-Loccoz et al., 1998). On the other hand, biological control using microorganisms to repress plant disease, offers an alternative, environmentally friendly strategy for controlling agricultural phytopathogens (Chang et al., 2006). The screening of medicinal plants is also another alternative that may produce chemical fungicides that are relatively non-toxic and cost-effective.

There are few different classes of effective antifungal drugs available for the treatment of fungal diseases of plants, animals and humans. Thus, it is important to develop new sources of antifungal agents. Further development of antifungal compounds with diverse chemical structures and novel mechanisms of action is necessary because there has been an alarming

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increase in the incidence of new and re-emerging infectious diseases as well as resistance to currently used drugs. The investigations on new antifungal substances should be continued and all possible strategies and techniques need to be explored further.

In this contribution, we investigate the antifungal and antioxidant activity of leaf extracts of the six South African tree species: *Bucida buceras* L. (Combretaceae), *Breonadia salicina* (Vahl) Hepper and J.R.I Wood (Rubiaceae), *Harpephyllum caffrum* Bernh. ex Krauss (Anacardiaceae), *Olinia ventosa* (L.) Cufod (Oliniaceae), *Vangueria infausta* Burch. (Rubiaceae) and *Xylotheca kraussiana* Hochst (Flacourtiaceae) using qualitative antioxidant method (DPPH) and serial dilution assay. These plant species were selected based on excellent activity observed against two animal fungal pathogens (*Candida albicans* and *Cryptococcus neoformans)*. Moreover, if these plant species possess strong activity against plant fungal pathogens; further phytochemical analysis and isolation of antifungal compound will be investigated.

Materials and methods

Plant collection

Leaf materials of the selected plant species were collected from the Lowveld National Botanical Garden in Nelspruit, Mpumalanga during the summer on a sunny day after all traces of moisture has evaporated. In order to ensure effective drying, leaves were collected in open mesh orange bags and kept in the shade to minimize photo-oxidative changes. Collected plant material was examined and the old, insect and fungus-infected leaves were removed. Plant materials were dried at room temperature (c. 25°C) for about a week in a forced air draught in a purpose-built drying machine until the leaves were brittle enough to break easily. The dried plant material was ground to a fine powder (diameter c. 0.1 mm) using a laboratory grinding mill (Telemecanique/MACSALAB model 200 LAB) and stored in airtight bottles in the dark.

Extraction procedure

Each finely ground plant material (4 g) were extracted with 40 ml of solvents of increasing polarities: hexane, dichloromethane, acetone and methanol (technical grade-Merck) in polyester plastic tubes, while shaking vigorously for 3-5 min on a Labotec model 20.2 shaking machine at high speed. After centrifuging at 3500 rpm for 5 min, the supernatants were decanted into labelled, weighed glass vials. The process was repeated three times on the marc and the extracts were combined. The solvent was removed under a stream of cold air at room temperature. Plant extracts were re-dissolved in acetone for further microbiological assays and phytochemical analysis.

Phytochemical analysis

Chemical components of each plant extracts were analyzed Thin Layer Chromatography (TLC) plates (ALIGRAM _SIL g/UV 254-MACHEREY-NAGEL, Merck), that were developed with three solvent systems of different polarities: Ethyl acetate:methanol:water: 40:5:4:4 [EMW] (polar), Chloroform:ethyl acetate:formic acid: 5:4:1 [CEF] (intermediate polarity:acidic), Benzene:ethanol:ammonia hydroxide: 90:10:1 [BEA] (nonpolar/basic) (Kotze and Eloff, 2002).

Development of the chromatograms was under eluent saturated conditions. Each sample (100 µg) were applied on the TLC plates in a c.1 cm band and developed using the above eluent solvent systems. The separated components were visualized under visible and ultraviolet light (254 and 360 nm, Camac Universal UV lamp TL-600). Vanillin-sulphuric acid spray reagent (Stahl, 1969) was used to detect compounds that were not visible under UV light,

Fungal strains and inoculum quantification

The selected plant fungal pathogens, *A. niger, A. parasiticus, C. gloeosporioides, T. harzianum, P. expansum, P. janthinellum* and *F. oxysporum*, were obtained from the Department of Microbiology and Plant Pathology at the University of Pretoria. These fungi are among the most important pathogenic fungi of economical significance to plants. Fungal strains were maintained on Potato Dextrose (PD) agar. Haemocytometer cell-counting, with some modifications was used for counting the number of cells for each fungal culture (Aberkane et al., 2002). The inoculum of each isolate was prepared by first growing the fungus on potato dextrose (PD) agar slants for seven days at 35°C. The slant was rubbed carefully with a sterile cotton swab and transferred to a sterile tube with fresh PD broth (50 ml). The sterile tubes were then shaken for 5 min and appropriate dilutions were made in order to determine the number of cells by microscopic enumeration using a haemocytometer. The final inoculum concentration was adjusted to approximately 1.0×10^6 cells/ml.

Determining antifungal activity

Microdilution assay

The plant extracts (100 ml) were serially diluted 50% with water in 96 well microtitre plates (Eloff,1998), and 100 ml of fungal culture was added to each well. Amphotericin B was used as the reference antibiotic and 100% acetone as the negative control. It was previously shown (Eloff et al., 2007) that the final concentration of acetone in the microplate well that the fungi are subjected to has no influence on the growth of fungi. As an indicator of growth, 40 ml of 0.2 mg/ml p-iodonitrotetrazolium violet (INT) dissolved in water was added to the microplate wells. The covered microplates were incubated for three to five days at 35°C at 100% relative humidity after sealing in a plastic bag to minimize fungal contamination in the laboratory. The MIC was recorded as the lowest concentration of the extract that inhibited antifungal growth. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms (Eloff, 1998). Where fungal growth is inhibited, the solution in the well remains clear or shows a marked reduction in intensity of colour after incubation with INT.

In order to determine which plants can be used for further testing, not only the MIC value is important, but also the total activity. Since the MIC value is inversely related to the quantity of antifungal compounds present, the quantity of antifungal

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compounds present was calculated by dividing the quantity extracted in milligrams from 1g leaves by the MIC value in mg/ml. The total activity is used to determine to what volume an extract from 1 g of plant material can be diluted and still inhibit the growth of the test organism (Eloff, 1999). Furthermore, it can also be used to evaluate losses during isolation of active compounds and the presence of synergism (Eloff, 2004).

The total activity can be calculated as:

$$\text{Total activity} = \frac{\text{Quantity of material in mg extracted from 1 g of plant material}}{\text{Minimum inhibitory concentration (mg/ml)}}.$$

In the previous study, the total activity of the six plant species (*Bucida buceras*, *Breonadia salicina*, *Harpephyllum caffrum*, *Olinia ventosa*, *Vangueria infausta* and *Xylotheca kraussiana*), were calculated (Mahlo et al., 2010). Total activity for crude extract in this case provided an indication of the volume to which the crude extract or fraction can be diluted and still kill the microorganism.

Bioautography

TLC plates (10 × 10 cm) were loaded with 100 µg of each of the extracts with a micropipette in a line c. 1 cm wide. The prepared plates were developed using different mobile systems of varying polarity: CEF, BEA and EMW. The chromatograms were dried at room temperature under a stream of air overnight to remove the remaining solvent. Fungal cultures were grown on potato dextrose (PD) agar for 3-5 days. Cultures were transferred into PD broth from agar with sterile swabs. The developed TLC plates were sprayed with a concentrated suspension containing c. 1.0×10^6 cells/ml of actively growing fungi. The plates were sprayed until wet, incubated overnight, sprayed with 2 mg/ml solution p-iodonitrotetrazolium violet and further incubated overnight or longer at 35°C in a clean chamber at 100% relative humidity in the dark. White areas indicated where reduction of INT to the coloured formazan did not take place due to the presence of compounds that inhibited the growth of the test fungi. The plates were sealed in plastic to prevent the spreading of the fungi in the environment and to retain the humidity and then scanned to produce a record of the results.

Assay for free radical scavenging (DPPH)

The antioxidant activities of each plant extracts were determined using the qualitative assay 2, 2-diphenyl-1-picrylhydrazyl (DPPH). This assay is preferred because it is used to provide stable free radicals (Fatimi et al., 1993).

A solution of 0.2% DPPH in methanol was prepared and then sprayed on the plates (until they became wet) and allowed to dry in a fume cupboard. The presence of antioxidant compounds was indicated by yellow bands against a purple background on TLC plates..

Results and Discussion

Microdilution assay was used for screening antifungal activity of plant extracts against selected phytopathogenic fungi. Hexane and methanol extracts of *B. salicina* had shown good activities against the three most sensitive organisms, *P. janthinellum*, *T. harzianum* and *F. oxysporum* (Table 1). Moreover, acetone and methanol extracts of *H. caffrum* had good antifungal activity against the three most sensitive test organisms (with MIC values ranging between 0.02 and 0.08 mg/ml). The DCM extract of *H. caffrum* had lower activity against *T. harzianum* (MIC = 0.63 mg/ml). Four extracts of *O. ventosa* had highest activity against *T. harzianum* (MIC values of 0.04 and 0.08 mg/ml). The acetone and DCM extracts of *V. infausta* also had moderate activity against *A. parasiticus* and *T. harzianum* (MIC values of 0.16 mg/ml). All extracts of *O. ventosa* had the highest activity compared to the other plant extracts against the tested fungi.

The highest average MIC values (1.19 mg/ml) were observed in hexane extracts and lowest in acetone extracts with MIC value of 0.71 mg/ml against the tested microorganisms. Plant extracts with low MIC values could be a good source of bioactive components with antimicrobial potency. In particular, acetone extracts have shown potentially interesting activity compared to extracts prepared using other solvents. Based on the MIC results, acetone was the best extractant, and additional positive features include its volatility, miscibility with polar and non-polar solvents and its relative low toxicity to test organisms (Eloff, 1999). Previously, it was indicated that acetone was not toxic to the test microorganisms at the concentration used in the assay (Mahlo et al., 2013).

In the current study, negative controls showed that acetone alone was not harmful to the plant pathogens at the highest percentage tested, confirming previous results (Eloff et al., 2007). However, plant extracts are traditionally prepared with water as infusions, decoctions and macerations. Therefore, it would be difficult for the traditional healer to be able to extract those compounds which are responsible for activity in the acetone and methanol extracts (Aliero and Afolayan, 2005). Many traditional healers use water to extract plant material, since water is not toxic, not expensive and is the only extractant available. In some cases animal fat is mixed with plant material and under these conditions the non-polar compounds could become available.

Out of the seven plant pathogens used, only three fungi (*P. janthinellum*, *T. harzianum* and *F. oxysporum*) had significant sensitivity to the plant extracts (average MIC values of 0.28 mg/ml, Table 3). *F. oxysporum* was reasonably sensitive. This fungus has been reported to cause wilting diseases in several crops. *F. oxysporum* also causes the wilting disease of lentil, tomato and banana. (Abd-Ellatif et al., 2011). The results showed that the other four fungi (*A. niger*, *A. parasiticus* and *C. gloeosporioides*) were more resistant to all of the extracts (Table 2). *A. niger* was also reported to be resistant to DCM, aqueous and methanolic extracts of 14 plants used traditionally in Paraguay (Portillo et al., 2001). Previous study indicated that *Bucida buceras*, *Breonadia salicina*, and *Xylotheca kraussiana* have been reported to have strong antifungal activity against *A. fumigatus* (Mahlo et al., 2013)

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Table 1: Minimum inhibitory concentration (MIC) of six plant species against plant pathogenic fungi (values duplicate SD = 0)

Micr[1]	MIC (mg/ml)																							
	Bucida buceras					*Breonadia salicina*				*Harpephyllum caffrum*				*Olinia ventosa*				*Vangueria infausta*				*Xylotheca kraussiana*					Amp
	Time[2] (h)	Extractant[3] A	H	D	M	A	H	D	M	A	H	D	M	A	H	D	M	A	H	D	M	A	H	D	M	Ave	
A. p	24	0.63	1.25	1.25	1.25	0.63	2.50	0.63	1.25	1.25	2.50	1.25	0.63	1.25	1.25	0.63	1.25	1.25	2.50	0.63	0.63	0.16	0.63	0.32	0.32	1.06	0.02
A. n	48	0.63	2.50	2.50	1.25	2.50	1.25	1.25	2.50	1.25	2.50	1.25	0.63	1.25	2.50	1.25	2.50	1.25	2.50	2.50	2.50	0.63	2.50	1.25	1.25	1.63	0.02
C. g	48	0.63	2.50	2.50	0.63	1.25	2.50	1.25	1.25	1.25	0.63	1.25	2.50	1.25	2.50	1.25	2.50	1.25	2.50	2.50	2.50	1.25	2.50	0.63	0.63	1.54	<0.02
P. e	24	0.08	0.63	0.32	0.32	1.25	2.50	2.50	2.50	2.50	1.25	2.50	0.63	0.32	1.25	1.25	0.63	2.50	1.25	1.25	1.25	0.63	2.50	1.25	1.25	1.27	<0.02
P. j	48	0.02	0.08	0.32	0.02	0.08	0.08	0.08	0.08	0.04	0.32	0.16	0.08	0.08	0.32	0.16	0.32	0.63	0.63	0.63	0.32	0.32	0.63	0.63	0.63	0.28	<0.02
T. h	48	0.02	0.08	0.63	0.02	0.63	0.32	0.63	0.63	0.08	0.63	0.63	0.08	0.04	0.04	0.04	0.08	0.32	0.32	0.32	0.32	0.16	0.32	0.16	0.32	0.28	<0.02
F. o	24	0.02	0.63	0.32	0.04	0.32	0.08	0.16	0.16	0.02	0.32	0.16	0.04	0.08	0.63	0.32	0.16	0.32	0.63	0.32	0.32	0.63	0.32	0.32	0.32	0.28	<0.02
Ave		0.29	1.02	1.05	0.5	0.92	1.25	0.89	1.12	0.88	1.09	0.99	0.62	0.61	1.14	0.70	0.99	1.04	1.37	1.09	1.05	0.54	1.23	0.65	0.67	0.91	

[1]Microorganisms: *A. p. = Aspergillus parasiticus, A. n. = Aspergillus niger, C. g. = Colletotrichum gloeosporioides, P. e. = Penicillium expansum, P. j. = Penicillium janthinellum, T. h. = Trichoderma harzianum* and *F. s. = Fusarium oxysporum*

[2]Time: MIC values after 24 h were sometimes not distinct, so the plates were left to incubate for a further 24 h before MIC was read

[3]Extractant: A = acetone, H = hexane, D = dichloromethane, M = methanol

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Table 2: Average MIC values of acetone, hexane, dichloromethane and methanol extracts against seven plant pathogens

Microorganism	Average
Aspergillus parasiticus	1.06
Aspergillus niger	1.63
Colletotrichum gloeosporioides	1.54
Penicillium expansum	1.27
Penicillium janthinellum	0.28
Trichoderma harzianum	0.28
Fusarium oxysporum	0.28
Average	0.91

The average MIC values of each plant species using different extractants are shown in Table 3. Based on the results obtained, *B. buceras* was the most active plant extract (average MIC value 0.72 mg/ml), followed by *X. kraussiana* (0.78 mg/ml), *O. ventosa* (0.86 mg/ml), *H. caffrum* (0.89 mg/ml) and *V. infausta* (1.14 mg/ml). The highest total activity was found in the acetone extract of *H. caffrum* (22 000 ml/g) against *F. oxysporum*. The lowest total activity was observed in the methanol extract of *O. ventosa* (133 ml/g) against both *A. niger* and *C. gloeosporioides*. In the previous study, the total activity of *Bucida buceras*, *Breonadia salicina*, *Harpephyllum caffrum*, and *Olinia ventosa*, were calculated (Mahlo et al., 2010). These observations are consistent with the variation of the MIC values in Table 1, that is, where the MIC value is low, the total activity is high.

Table 3: The average MIC values (mg/ml) of plant extracts prepared using different extractants against seven plant pathogens

Extractants	Plant species					
	Bucida buceras	*Breonadia salicina*	*Harpephyllum caffrum*	*Olinia ventosa*	*Vangueria infausta*	*Xylotheca kraussiana*
A	0.29	0.92	0.88	0.61	1.04	0.54
H	1.02	1.25	1.09	1.14	1.37	1.23
D	1.05	0.89	0.99	0.7	1.09	0.65
M	0.5	1.12	0.62	0.99	1.05	0.67
Average	0.72	1.05	0.89	0.86	1.14	0.78

Extractant: A = acetone, H = hexane, D = dichloromethane, M = methanol

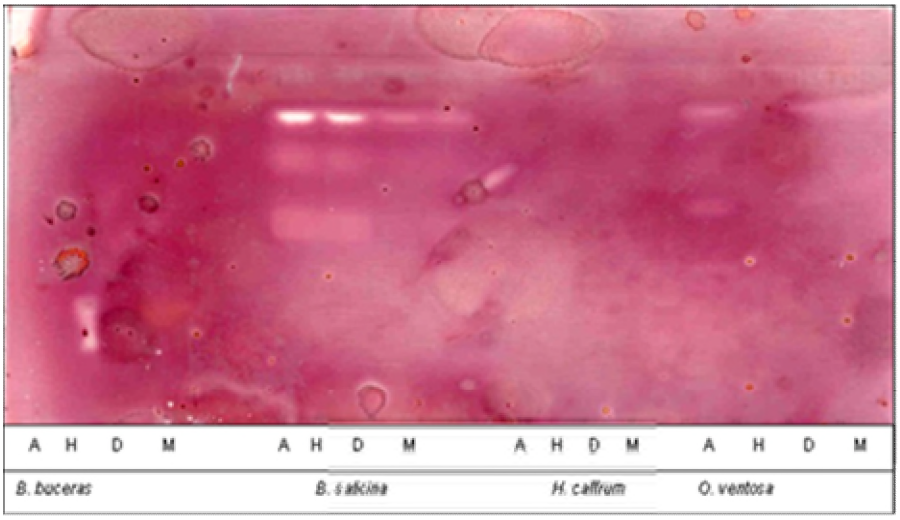

Figure 1: Bioautograms of extracts of *Bucida buceras*, *Breonadia salicina*, *Harpephyllum caffrum* and *Olinia ventosa*. Chromatograms were developed in CEF and sprayed with *Penicillium janthinellum*. White areas indicate inhibition of fungal growth. Lanes from left to right: acetone (A), hexane (H), DCM (D) and methanol (M)

Bioautography assay was used to determine the presence of antifungal compounds in different plant extracts (Figure 1). The antifungal compounds separated well in both CEF and EMW solvent system. The antifungal compounds were clearly visible and more active compounds were observed against *A. parasiticus* than *T. harzianum* and *P. janthinellum*. Furthermore, three active compounds separated with CEF had the same R_f values (0.70, 0.85 and 0.95) in the acetone, hexane, DCM and methanol extracts of *B. salicina*. Three other active compounds with common R_f values of 0.54, 0.72 and 0.95 were visible in the acetone, DCM

and methanol extracts of *O. ventosa* against *A. parasiticus* and *P. janthinellum*. Some of the antifungal compounds were observed in chromatograms separated with EMW (more polar compounds). Acetone and hexane extracts had similar active compounds against *P. janthinellum* and *A. niger* with R_f value 0.17. Furthermore, active compounds with the same R_f value of 0.13 were observed in the acetone and hexane extracts against *C. gloeosporioides* and *A. parasiticus*. Bioautograms produced using *P. expansum* and *T. harzianum* showed active compounds in the acetone and hexane extracts, while in the case of DCM and methanol extracts, no active compounds were clearly visible. In CEF bioautograms, acetone extracts of *O. ventosa* inhibited the growth of fungi i.e *P. janthinellum* with R_f value of 0.17 while no compound were observed in hexane, DCM and methanol extracts. Surprisingly, the acetone extract of *B. salicina* and *O. ventosa* showed similar antifungal compound (R_f 0.13 and 0.17) which were active against *A. parasiticus* and *P. janthinellum*. All extracts of *O. ventosa* did not inhibit the growth of the other three fungi, *A. niger*, *C. gloeosporioides* and *P. expansum*.

Breonadia salicina and *O. ventosa* had the most promising number of antifungal compounds in all four extracts (acetone, hexane, DCM and methanol) because they showed compounds that inhibit the fungi. The remaining four plant species (*B. buceras*, *H. caffrum*, *V. infausta* and *X. kraussiana*) showed no activity in the bioautography screening against the seven test organisms, thus the results are considered not significant. However, these plant extracts had good activity in the microplate assay. Possible reasons may be that some of the active compounds were volatile and evaporated during the drying period of the TLC chromatograms prior to bioautography. Secondly, residues of formic acid or ammonia on the TLC plates following evaporation could have inhibited the growth of the plant pathogenic fungi. Biological activity synergism between different compounds in the extracts is also a possible reason. The chromatograms developed in BEA had no zones with strong antioxidant activity in some of the plant extracts. In general, most of the plant extracts did not show the presence of antioxidant compounds on chromatograms. However, methanol extract had a higher antioxidant activity than the acetone, hexane and DCM extracts. Previously, it was reported that extracts of some plant species contain very few antioxidant compounds and inhibition of microbial infections by stimulating the immune system of the host does not appear to be a realistic mechanism for its activity and traditional use (Masoko and Eloff, 2007).

Conclusion

Acetone was the best extractant and it is also low in toxicity to the test organisms. In bioautography, several active compounds were visible in acetone, hexane, dichloromethane and methanol extracts of *B. salicina* while only one antifungal compound was observed in the acetone and hexane extracts of *O. ventosa*. No active compounds were visible in the TLC chromatograms developed in BEA for plant extracts of *V. infausta* and *X. kraussiana*. In both MIC and bioautography assay results, O. *ventosa* and *B. salicina* were the most promising to isolate antifungal compounds therefrom. In isolating antimicrobial compounds from plant extracts, bioautography plays an enormously important role to facilitate the isolation of antimicrobial compounds. Without good bioautography data it is very difficult to ensure success in isolating antimicrobial activity especially if more than one compound is required to express activity. The plant extracts of five of the six medicinal plants did not possess strong antioxidant activity. As expected, the polar methanol extracts of *X. kraussiana* showed the antioxidant activity. Since plant extracts did not show strong antioxidant activity, it was unnecessary to include quantitative antioxidant assays for further screening procedure.

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