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Anti-mycotic and Sporocidal properties of three South African Medicinal Plants against Moulds associated with Fungal Ear Infections

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ABSTRACT

Background: Gasteria bicolor *Haw.*,Lachnostylishirta *(L.f.) Müll. Arg. and* Sansevieriaaethiopica*(Thunb.) are commonly used for the treatment of ear infections (otomycoses) in Eastern Cape of South Africa but, with no scientific proof to justify the empirical evidence.* **Objective of the study:** *This study aimed at screening both acetone and methanolic extracts of the medicinal plants against both mycelia and spores of six fungal isolates frequently implicated in fungal ear infections.* **Methodology:** *Standard microbiological methods were used in this study to assess different indices of antifungal.* **Results:** *Methanolic extract of* G. bicolor *was the most effective extract, while methanolic extract of* S. aethiopica *had the least activity. Methanolic extracts of the plants were more effective that the corresponding acetone extracts except for the* S. aethiopica. *Acetone extract had the least minimum inhibitory concentration (MIC) against the pathogens and the ratio of minimum Fungicidal Concentration (MFC) to MIC for the extracts showed that they exhibiting fungicidal effects on the test fungi. Methanolic extract of* G. bicolor *has total inhibition on the germination of spores of* A. fumigates *DMS 790 and* A. niger *ATCC 16404 at the maximum concentration tested (10 mg/mL). Extracts of* S. aethiopica *had higher inhibitory effects on the spores of the pathogens compare to extracts of other plants.* **Conclusion:** *These extracts demonstrated the potency to inhibit the germination of spores that could lead to further propagation of the pathogenic moulds. This study justified the folkloric usage of these plants in treatment of ear infections.*

Introduction

Otomycosis is an infection of the pinna, the external auditory meatus, however, it may occur in the middle ear if the tympanic membrane is perforated (Aneja *et al*., 2010). It is usually suspected when the discharging ear does not respond to local antibiotic treatment (Sengupta and Kacker, 1978; Talwar*et al*., 1988). Causative agents of fungi ear infections have been identified to be belong mainly to the genera of *Aspergillus, Penicillium, Mucor, Rhizopus, Scopulariopsis*and *Absidia,* though species of *Aspergillus* being most frequent (Aneja*et al.,* 2010). Otomycosis has a global prevalent especially among children more often among those below the age of seven years (Ifante-Rivard and Fernandez, 1993; Li *et al*., 2001). Their high susceptibility has been reported to be as a result of their short Eustachian tube and low immunity (Weiner and Collison, 2003; Aich*et al*., 2009; Osazuwa *et al*., 2011).

Predisposing factors to otomycosis include; a humid climate, presence of cerumen, instrumentation of the ear, compromised immunity, use of topical antibiotic/steroid and swimming (Stern and Lucente, 1988; Kindo *et al*., 2007). Apart from the fact that ear infections are usually painful, they also caused aural fullness, pruritus, thick fibrinous accumulation of debris and hearing loss (Talwar*et al*., 1988; Paulose*et al*., 1989; Kaur *et al*., 2000; Fasunla*et al*., 2007).

Medicinal plants are relative cheap, safe and effective in the treatment of various diseases. They have the widest usage among the resource-poor populations of the world (Areekul*et al.,* 2009; Murdiyanto*et al.,* 2014; Nyamukuru *et al.,* 2017; Ududua *et al.,* 2019). The resistance of pathogens to conventional antimicrobials, poverty and circulation of fake and substandard antimicrobials have been identified as the major factors that have led to the increase in the folkloric usage of medicinal plants (Janakiraman*et al.,* 2012). Medicinal plants contains biologically active phytochemicals used extensively for the treatment of different infectious diseases (Gundidza, 1986; Ududua*et al.,* 2019). New classes of antimicrobials are now being discovered from medicinal plants (Runyoro*et al.,* 2006; Douglas and Jeruto, 2016). Considering the huge cost of the antifungal agents currently in use, the use of these extracts among other plant extracts may minimize the huge cost of treatment, thus would make treatment of such fungal infections affordable to the masses especially in developing countries.

The entnobotanical survey conducted in the Nkokonbe Municipality of Eastern Cape of South Africa showed that *Gasteria bicolor*, *Sansevieria aethiopica* and *L. hirta*(L.f.) Müll. Arg. to be most mentioned plants which are used for the treatment of ear infections. Despite the acclaimed folkloric usage of these plants in the treatment of fungal infections of the ear they have not received scientific evidence to justify this claim which this study try to address.

Materials and Methods

Study Area and Collection of Plant materials

Fresh leaves of samples of *S. aethiopica* and *G. bicolor* with the bark of *L. hirta* were collected at the Alice Township in the Municipality of Nkokobe, Eastern Cape Province of South Africa. The plant samples were identified and voucher specimen deposited in the Giffen Herbarium of the University Of Fort Hare, Alice, South Africa. The plant materials were died separately in the oven at 40^{o}C.

Dried samples were pulverized into a fine powder and 40 g was submerged in 500 mL of each of the extractants (acetone and methanol) separately and allowed for extraction for 72 h on an orbital shaker (Stuart Scientific Orbital Shaker, Greater Manchester UK). The extracts were filtered through Whatman No. 1 filter paper. The extracts were evaporated to dryness under reduced pressure at 40 °C using a rotary evaporator (Laborota 4000-efficient, Heldolph, Germany). The extract of the plant was kept in the refrigerator at 4 ^{o}C until used.

Source of the Test Fungi

The isolates used for this work were collected from the Department of Biochemistry and Microbiology, University of Fort Hare, Alice. The fungi which include *Absidiacorymbifera*DSM 1144, *Aspergillus flavus* ATCC 9643, *Aspergillus fumigatus*DMS 790, *Aspergillus niger* ATCC 16404, *Penicilliumchalybeum*PMC 006 and *Penicilliumexpansum*PRC 004 were maintained on Potato Dextrose Agar (Oxoid, UK) .

Determination of antifungal property of the extracts

Food poison assay

The reconstituted extract solution was filtered by 0.45 µm membrane filter for sterility. Sterility of the extracts was confirmed according to Ronald (1995) by introducing 2 mL of the extract into 10 mL of sterile nutrient broth and incubated at 37 °C for 24 h as observed for sterility as sterile extract produces no sign of growth. The method of Nene and Thapilyal (2002) was used to screen for antifungal activity of the different extracts of the medicinal plants. Sterile extract was mixed with sterilized Potato Dextrose Agar (PDA) (Oxoid Ltd, Basingstoke, Hampshire, England) to achieve a concentration range of 0.1 to 10 mg/mL. Amphotericin B at different concentrations was also added into another set of plates to serve as control. The Inoculation was done at the center of each plate with a 10 mm mycelium block for each fungus. Mycelium block was prepared with 10 mm cork borer from the advancing edges of a five day old culture of the test fungi on PDA. The blocks were placed at the center of each Petri plate in an inverted position and incubated at 25°C for 5 days. The PDA plate without extract was also maintained at the same condition to serve as control and the experiment was performed in triplicate. After incubation the diameter of fungal mycelia was measured in millimetre and the percentage of inhibition of mycelial growth calculated. The observed data were subjected to Probit analysis according to Finney (1974) to get the concentrations that will inhibit 50% of the fungal growth (IC_{50}).

Determination of the Minimum Inhibitory Concentration

Modified method of Granade and Artis (1980) was used to determine the minimal concentration of extract that will inhibit the growth of the fungi. Hyphae were collected from the edges of actively growing fungal colony and inoculated into 20 ml Potato Dextrose broth (PDB) in 50 ml conical flask. The flasks were incubated at 35 °C for 4 days gently shaken twice a day to allow constant aeration and to prevent spore formation. The culture was centrifuge for 20 min at 7500 rpm after incubation. The pellet was washed twice and later homogenized in sterile distilled water. The optical density was adjusted to 0.60 at 450 nm wavelength to standardize the suspension.

To achieve concentrations ranging between 0.78812 to 25.00 mg/mL, sterile extract was diluted in sterile PDB in test tubes. Each tube containing 5 mL appropriate extract in PDA was inoculated with 100 µl of the standardized culture of a test fungus. Tubes containing PDB without extract and those containing amphotericin B served as negative and positive controls respectively. All the tubes were incubated at 25 °C for 72 h and the first tube in the series with no sign of visible growth was taken as the MIC.

Determination of Minimum Fungicidal Concentration (MFC)

Minimum fungicidal concentration (MFC) was determined by taken one standard loopful of culture from each of the first three broth tubes that showed no growth in the MIC tubes and inoculated on fresh potato dextrose agar plates. After incubation at 26 ^{o}C for 48 h, the least concentration of the extracts that showed no growth on the agar was taken as the MFC. The ratio of MFC to MIC was determined to predict effect of the extract on the test bacteria. The results of MFC/MIC ratios were classified according to Shanmughapriya *et al*. (2008).

Determination of Sporocidal Activity of the Extracts

Modified method of Moyo*et al*. (2012) was used to harvest and standardize the spores of the fungi. The test fungi were cultivated

on PDA and the plates incubated at for 7 days at 26 °C. The spores produced were harvested by flooding the surface of the plates gently with 10 ml of sterile mixture of 1.0 % DMSO. The harvested fungal spores were standardized to absorbance of 0.1 at OD_{600} nm wavelength. A 100 µl of the standardized spore suspension was mixed with 10 ml of PDA which contain different concentrations of the extract. This was pour to make a thin agar in the Petri plate and incubated at 25 ^{o}C for 24 h. A 6 mm agar plug was cut and remove from the plate after incubation, placed on a clean glass slide and covered with cover slip. The specimen was view under a microscope (Stereo Microscope SZMT2) at X 40 magnification. Number of germinated spores was counted per field and percentage inhibition of spore germination was calculated as:

% inhibition = [1 - (Number of germinated spores/total spore per view] × 100

Statistical Analyses

The values were analyzed using Statistical Package for the Social Science (SPSS) version 14. The results were subjected to Analyses of variance test and the post hoc (multiple comparisons) test was done by Dunnett's test. The significance level was fixed at p=0.05.

Results

Methanolic extract of *G. bicolor* proved to be most effective among the plant extract tested with IC_{50} values ranging from 0.15 and >10.00 mg/mL. The minimum and maximum values were observed against *P. expansum* PRC 004 and *P. chalybeum* PMC 006 respectively. The activity of methanolic extract of *L. hirta* expressed in term of IC_{50} ranged between 0.98 and 6.15 mg/mL as shown in Table 1. The extract had the highest inhibitory concentration against *A. niger* ATCC 16404. Methanolic extracts of the plants were more effective than the corresponding acetone extracts except for the *S. aethiopica.* Methanolic extract of *S. aethiopica* had IC_{50} of value greater than 10.00 mg/mL against *A. fumigates* DMS 790, *A. niger* ATCC 16404 and *Ab. corymbifera* DSM 1144. The order of susceptibility of the test fungi to the extracts was *Ab. corymbifera* DSM 1144 < *P. chalybeum* PMC 006 < *A. niger* ATCC 16404 < *A. fumigates* DMS 790< *A. flavus* ATCC 9643 < *P. expansum* PRC 004. Methanolic extract of *G. bicolor* was the most effective in inhibiting the mycelial growth of the test fungi, while the least activity was noticed in methanolic extract of *S. aethiopica*. Among the test fungi, *P. expansum* PRC 004 was overall most susceptible to the extracts followed by *A. flavus* ATCC 9643. *Aspergillus* species tested were susceptible to the extracts though at varying degrees.

Table 1: Inhibitory concentration (IC_{50}) of extracts of *G. bicolor, L. hirta*and *S.aethiopica*against mycelial growth of the test fungi (mg/mL)

		Fungi isolates					
Extracts	**Extracts**	***A. niger*** **ATCC 16404**	***A. flavus*** **ATCC 9643**	***P. chalybeum*** **PMC 006**	***A. fumigates*** **DMS 790**	***P. expansum*** **PRC 004**	***Ab. corymbifera*** **DSM 1144**
	Acetone	6.35	0.98	4.49	9.17	5.21	>10.00
S. aethiopica	Methanol	>10.00	6.67	6.15	>10.00	5.93	>10.00
	Acetone	7.62	6.49	7.65	6.09	5.69	5.86
G. bicolor	Methanol	0.16	0.36	>10.00	0.59	0.15	4.62
	Acetone	>10.00	10.00	>10.00	6.91	0.76	9.91
L. hirta	Methanol	6.15	0.98	4.86	0.44	1.43	5.76
Amph. B (AmB) µg/mL		1.31	1.65	1.76	4.75	5.74	1.64

In Table 2, the MIC and MFC of each of the extracts of the medicinal plants were reported. Except *P. chalybeum* PMC 006 and *Ab. Corymbifera* DSM 1144, acetone extracts had the least MIC against the pathogens. The MFC/MIC ratio for the extracts showed that most of the extract exhibiting fungicidal effects on the pathogen because they have values higher than 1. The MFC/MIC observed in this study ranged from 1.00 and 8.00.

Table 2: Minimum inhibitory and minimum fungicidal concentrations of *G. bicolor, L. hirta* and *S. aethiopica* against the test fungi

		Fungal isolates					
Extracts	**Effects**	***A. niger*** ATCC 16404	***A. flavus*** ATCC 9643	***P. chalybeum*** PMC 006	***A. fumigates*** DMS 790	***P. expansum*** PRC 004	***Ab. corymbifera*** DSM 1144
	MIC	12.5	3.125	6.25	12.50	12.5	12.50
	MFC	12.5	6.25	6.25	25.00	25.00	>25.00
SEA	MFC/MIC	1.00	2.00	1.00	2.00	2.00	>2.00
	MIC	12.50	12.50	25.00	12.50	6.25	12.50
	MFC	25.00	25.00	25.00	25.00	12.50	12.50
SEM	MFC/MIC	2.00	2.00	1.00	2.00	2.00	1.00
	MIC	12.50	12.50	25.00	12.50	12.50	12.50
	MFC	12.50	25.00	>25.00	25.00	12.50	12.50
GBA	MFC/MIC	1.00	2.00	>1.00	2.00	1.00	1.00
	MIC	0.78812	1.5625	12.50	1.5625	1.5625	12.50
	MFC	1.5625	1.5625	25.00	6.25	6.25	12.50
GBM	MFC/MIC	2.00	1.00	2.00	4.00	4.00	1.00
LHA	MIC	12.50	12.50	12.50	12.50	1.5625	12.50

	MFC	12.50	>25.00	>25.00	>25.00	6.25	25.00
	MFC/MIC	1.00	>2.00	>2.00	>2.00	4.00	2.00
	MIC	12.50	1.5625	12.50	1.5625	3.125	12.50
	MFC	12.50	12.50	12.50	1.5625	6.25	12.50
LHM	MFC/MIC	1.00	8.00	1.00	1.00	2.00	1.00
	MIC	0.0025	0.0025	0.0025	0.005	0.0100	0.0025
	MFC	0.005	0.0025	0.0100	0.0010	0.0010	0.0050
AmB	MFC/MIC	2.00	1.00	4.00	2.00	1.00	2.00

MIC = minimum inhibitory concentration, MFC = minimum fungicidal concentration, SEA= acetone extract of S. aethiopica, SEM = methanolic extract of S. aethiopica, GBA = acetone extract of G. bicolor, GBM = methanolic extract of G. bicolor, LHA = acetone extract of L. hirta, LHM = methanolic extract of L. hirta, AmB = amphotericin.

The extracts at different concentrations inhibited spores of the fungi (Fig. 1-6). At low concentrations the spore germination was higher and subsequently the sporocidal effects showed a dose dependent trends. At minimum concentrations of the extracts of the plants there was no total inhibition. Methanolic extract of *G. bicolor* inhibited the spores of the fungi better than acetone extracts. At the maximum concentration tested (10 mg/mL) of methanolic extract of *G. bicolor* there was no germination of spores of *A. fumigatus*DMS 790 and *A. niger*ATCC 16404

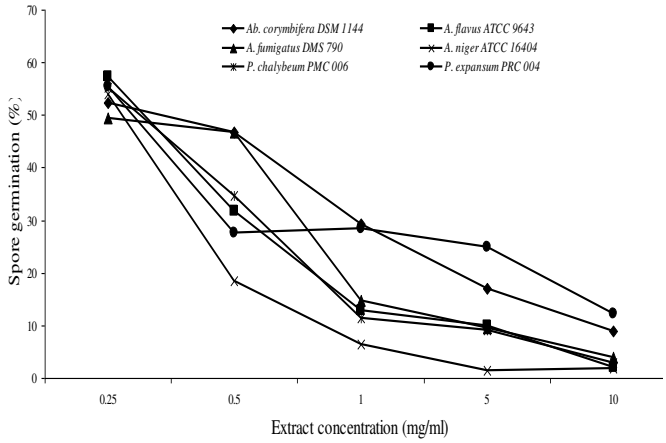

Fig 1: Spore germination inhibitory activity of acetone extract of *G. bicolor* leaf against fungal isolates

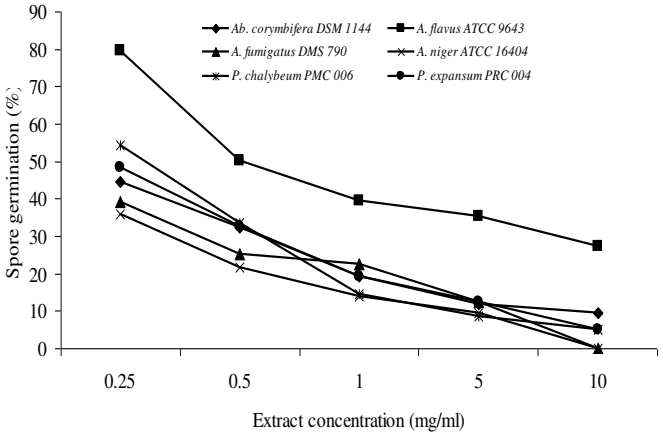

Fig 2: Spore germination inhibitory activity of methanolic extract of *G. bicolor* leaf against fungal isolates

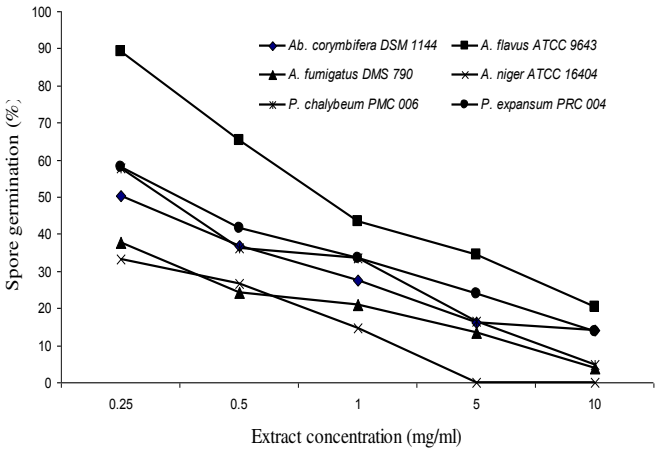

Fig 3: Spore germination inhibitory activity of acetone extract of *L. hirta*bark against fungal isolates

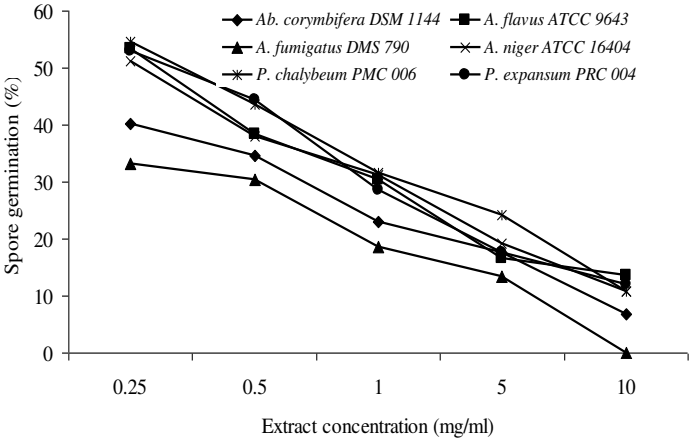

Fig 4: Spore germination inhibitory activity of methanolic extract of *L. hirta*bark against fungal

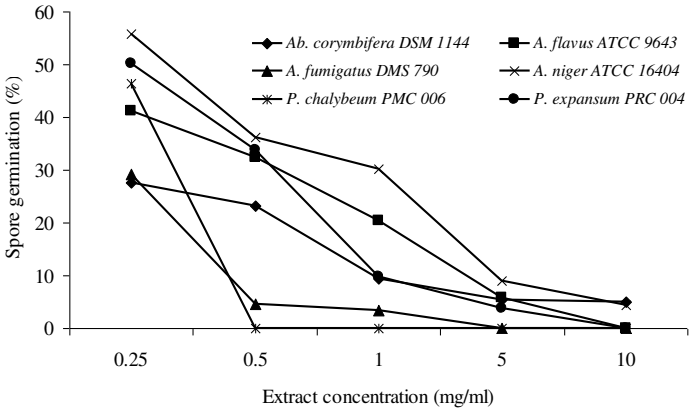

Fig 5: Spore germination inhibitory activity of acetone extract of *S. aethiopica* leaf against fungal isolates

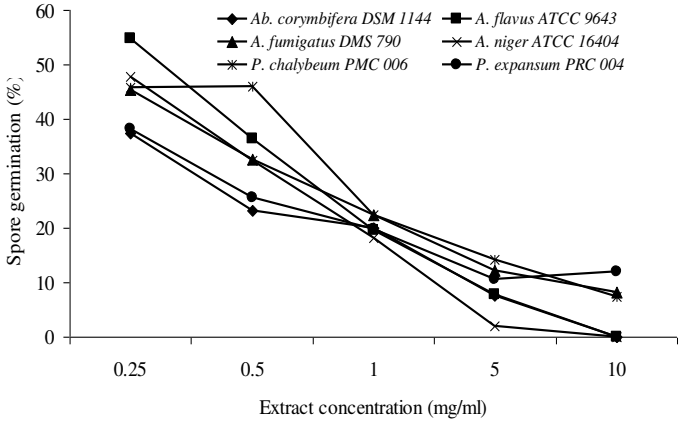

Fig 6: Spore germination inhibitory activity of methanolic extract of *S. aethiopica* leaf against fungal isolates

Sansevieria aethiopica extracts had more inhibitory activity on the spores of the pathogens compare to other fungi. Only spores of *Ab. Corymbifera* DSM 1144 and *A. niger*ATCC 16404 did germinate at 10 mg/mL of the extract of *S. aethiopica*. Spores of the three out of the six oomycotic agents were inhibited by the methanolic extract of *S. aethiopica*. The difference in the inhibition of the germination of spore by the six extracts was not significant at $p<0.05$. The spores harvested were from young fungal culture.

Discussion

In this study we investigated the *in vitro* assessment of the extracts of the three medicinal plants used, generally for the treatment of ear infections in the Eastern Cape of South Africa, against fungi associated with ear infections. The extracts demonstrated potential to eliminate germination of spores that could lead to further propagation. The extracts of the plants showed a dose dependent antifungal properties. Relatively the extracts of *G. bicolor* has a better than the extracts of other two plants screened. This may be due to the presence of high amount of aromatic phenolic compounds in it (Dagne*et al.,* 1996). *Gasteria bicolor* was reported by Otang*et al*. (2012) to possess antifungal properties and used in the treatment of dermatophytic fungi. In this study we observed *Aspergullus*species screened were susceptible to the extracts however, at different rates. The differences in the activities of the extracts may be due to presence of different bioactive compounds. The genus *Aspergillus* is a common cause of human fungal infections; it produces a severe outcome especially in the susceptible and immunocompromised patients (Aneja*et al*., 2010; Dal *et al*., 2012; Nyamukuru *et al.,* 2017). The genus has been reported to be most frequently isolated in the cases of otitis (Ling and Sader, 2008).

This study gives a picture that the activities of the extracts of the medicinal plants screened are comparable with the standard antifungal drug (Amphotericin B). However, we observed a significantly low activity of acetone extract of *L. hirta* and methanolic extract of *S. aethiopica* this supports the previous work of David and Afolayan (2015). The extracts of these plants were able to inhibit fungi causing otomycoses. Fungi ear infections are wrongly treated for bacterial infections and can lead to increase in treatment time and irreversible damages in the auditory canal (Li *et al*., 2001; Dal *et al*., 2012).

Extracts of *G. bicolor* has higher antifungal activities that extracts of other plants. *Absidiacorymbifera* followed by *P. chalybeum* showed the highest overall resistance to the extracts. Prior exposure of pathogen to antimicrobial agents may lead to increase resistance to other antimicrobials and hence may increase the MICs of the antimicrobials against them. Most of the MFC/MIC values observed in this study were higher 1 indicating that the extracts had fungistatic effect on the test pathogens. The MFC/MIC value higher than 1 is an indication of fungistcidal activity as reported by Shanmughapriya*et al*. (2008).

The results of the sporocidal activity of the extracts showed a concentration dependent trend. Increasing concentration of the plant extracts produced increased in growth inhibition and sporocidal effects. Un-Nisa*et al.* (2010) reported the inhibitory effects on spore germination by extracts of neem, mint, mehendi, safeda and garlic. In like manner extract of onion and ginger also showed inhibitory effect on the spore germination and mycelial growth of pathogenic fungi (Karade and Sawant, 1999; Hasan *et al*., 2005; Tagoe *et al*., 2011). The susceptibility of the spores is age dependent, the age of spores has been reported to affect the germinability of fungal spores according to Siqueira *et al*. (1985). Fungal spores tested in this study were harvested from relatively young cultures hence the variability in the susceptibility of the spores cannot be as a result of differences in the age of the fungi (spores).

The anti-otomycotic effects of the extracts of the plants screened showed effects on both hyphae and spores of the test fungi. The mechanisms of action of the extracts may be due to cell wall disruption by causing transmembrane pores, protein leakage and hyphae rupture (Roberts and Selitrennikoff, 1990; Abada *et al*., 1996). Some biological antifungal agents have also been reported to cause cell membrane disruption and negatively altered the pH gradient across the cell wall/membrane of fungi (Roberts and Selitrennikoff, 1990; Hejgaard *et al*., 1991).

Conclusion

The results of this study showed that both acetone and methanolic extracts of the three medicinal plants were very

active against both the growth of the hyphae and germination of the spores. The extracts of these plants could serve as alternative treatment of fungal ear infections and/or other mycotic infections and also justify their uses. These plants could be further investigated to get the active compounds that responsible for the antifungal activity and also determine the actual targets on the fungal cell.

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References

Abada, L. R., D'Urzo, M. P., Liua, D., Narasimhan, M. L., Reuveni, M., Zhua, J. K., Niua, X., Singhb, N. K., Hasegawaa, P. M. and Bressan, R. A. (1996). Antifungal activity of tobacco osmotin has specificity and involves plasma membrane permeabilization. *Plant Science*, 118: 11-23.

Aich, M. L., Biswas, A. C., Ahmed, M., Joarder, M. A. H., Datta, P. G. and Alauddin, M. (2009). Prevalence of otitis media with effusion among school going children in Bangladesh. *Bangladesh Journal of Otorhinolaryngology*, 15: 31 – 34.

Aneja, K. R., Sharma, C. and Joshi, R. (2010). Fungal infection of the ear: a common problem in the north eastern part of Haryana. *International Journal of Pediatric and Otorhinolaryngology*,74: 604-607.

Areekul, V., Jiapiyasakul, P. and Chandrapatya, A. (2009). *In vitro* antimicrobial screening of selected traditional Thai Plants. *Thai Journal of Agricultural Science*, 42(2): 81-89.

Dagne, E., VanWyk, B., Mueller, M. and Steglich, W. (1996).Three dihydroanthracenones from *Gasteria bicolor. Phytochemistry*,41(3): 795 799.

Dal, T., Tekin, A., Deveci, O., Bulut, M., Fırat, U. and Mete, M. (2012). Septic arthritis caused by *Aspergillus fumigatus*in an immunosuppressive patient: A case report and review of the literature. *Journal of Microbiology and Infectious Diseases*, 2(1): 29-32.

David, O. M. and Afolayan, A. J. (2015). Evaluation of antibacterial potentials and antioxidant property of extracts of *Lachnostylishirta* (L.F.) Muell. Arg. stem bark. *International Journal of Advanced Biological Research,* 5(4): 350-357.

Douglas, K. and Jeruto, J. (2016). Phytochemistry and antimicrobial activity of extracts from medicinal plants *Tithoniadiversifolia* and *Oleaafricana*. *Brazillian Journal of Pharmaceutical Research*. 12: 1–7.

Fasunla, J., Ibekwe, T. and Onakoya, P. (2007). Otomycosis in Western Nigeria. *Mycoses*, 51: 67–70.

Finney, D. J. (1974). *Probit analysis: a Statistical Treatment of the Sigmoid Response Curve.* Cambridge University Press, London, pp. 333.

Granade, T. C. and Artis, W. M. (1980). Antimycotic susceptibility testing of dermotophytes in microcultures with a standardized fragmented mycelial. *Antimicrobial Agents and Chemotherapy,* 17(4): 725 – 729.

Gundidza, M. (1986). Screening of extracts from Zimbabwean higher plants II: Antifungal properties. *Fitoterapia,*57: 111-113.

Hasan, M. M., Chowdhury, S. P., Alam, S., Hossain, B. and Alam, M. S. (2005). Antifungal effects of plant extracts on seed-borne fungi of wheat seed regarding seed germination, Seedling health and vigour index. *Pakistan Journal of Biological Science*,8: 1284-1289.

Hejgaard, J., Jacobsen, S. and Svendsen, I. (1991). Two antifungal thaumatin-like proteins from barley grain. *FEBS Letter*, 291: 127-131.

Ifante-Rivard, C. and Fernandez, A. (1993). Otitis media in children: frequency, risk factors and research avenues. *Epidemiological Review*, 15: 446 – 65.

Janakiraman, N., Sahaya, S. S. and Johnson, M. (2012). Anti-bacterial studies on *Peristrophebicalyculata*(Retz.) Nees. *Asian Pacific Journal of Tropical Biomedicine,* 2012: S147-S150

Karade, V. M. and Sawant, D. M. (1999). Effect of some plants on the spore germination of *Alternariaalternata*. *Plant Diseases Research*, 14: 75-77.

Kaur, R., Mittal, N., Kakkar, M., Aggarwal, A. K. and Mathur, M. D. (2000). Otomycosis: a clinicomycologic study. *Ear Nose Throat Journal*, 79: 606–609.

Kindo, A. J., Shams, N., Anandi, L. and Kalyani, J. (2007). A stitch in time saves nine- patient not responding to antibiotic treatment think of fungus. *Sri Ramachandra Journal of Medicine*. Nov: 20-31

Li, W. C., Chiu, N. C., Hsu, C. H., Lee, K. S. and Hwang, H. K. (2001). Pathogens in the middle ear effusion of children with persistent otitis media: Implications of drug resistance and complications. *Journal of Microbiology, Immunology and Infection*, 34: 190 – 194.

Ling, S. S. and Sader, C. (2008). Fungi malingnant otitis externa treated with herperbaric oxygen. *International Journal of Infectious Diseases,* 12: 550-552.

Moyo, B., Masika, P. J. and Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera* Lam leaf extracts. *Africa Journal of Biotechnology,* 11(11): 2797-2802.

Murdiyanto, W. K., Arung, E. T. and Kim, Y. (2014). Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. *Food Science and Human Wellness*, 3: 191–196.

Nene, Y. and Thapilyal, L. (2002). *Poisoned Food Technique of Fungicides in Plant Disease Control*. 3rd Ed, Oxford and IBH Publishing Company, New Dehli.

Nyamukuru, A., Tabuti, J., Lamorde, M., Kato, B., Sekagya, Y. and Aduma, P. (2017). Medicinal plants and traditional treatment practices used in the management of HIV/AIDS clients in Mpigi District, Uganda. *Journal of Herbal Medicine.* 7: 51-58.

Osazuwa, F., Osazuwa, E., Osime, C., Igharo, E. S., Imade, P. E., Lofor, P., Momoh, M., Omoregie, R. and Dirisu, J. (2011). Etiologic agents of otitis media in Benin City, Nigeria. *North America Journal of Medical Science,* 3(2): 95-98.

Otang, W. B., Greirson, D. S. and Ndip, R. N. (2012). Enthnobotanical survey of medicinal plants used in the management of opportunistic fungal infections in HIV/AIDS patients in the Amathole District of the Eastern Cape Province, South Africa. *Journal of Medicinal Plant Research,* 6(11): 2071-2080.

Paulose, K. O., Al-Khalifa, S., Shenoy, P. and Sharma, R. K. (1989). Mycotic infection of the ear (otomycosis): a

prospective study. *Journal of Laryngology and Otolaryngology*, 103: 30–53.

Roberts, W. K. and Selitrennikoff, C. P. (1990). Zeamatin, an antifungal protein from maize with membrane permeabilizing activity. *Journal of General Microbiology*, 136: 1771-1778.

Runyoro, D. K. B., Matee, M. I. N., Ngassapa, O. D. Joseph, C. C. and Mbwambo, Z. H. (2006). Screening of Tanzanian medicinal plants for anti-Candida activity. *BMC Complementary and Alternative Medicine*, 6(11): 1-10.

Sengupta, R. P. and Kacker, S. K. (1978). Otomycosis. *Indian Journal of Medical Science*. 32: 5-7

Shanmughapriya, S. A., Manilal, A., Sujith, S., Selvin, J., Kiran, G. S. and Natarajaseenivasan, K. (2008). Antimicrobial activity of seaweeds extracts against multiresistant pathogens. *Annals of Microbiology,* 58: 535-541.

Siqueira, J., Sylvia, D., Gibson, J. and Hubbel, D. (1985) Spores, germination, and germ tubes of vesicular-arbuscularmycorrhizal fungi. *Canadian Journal of Microbiology.* 31: 965–97

Stern, J. C. and Lucente, F. E. (1988). Otomycosis. *Ear Nose Throat Journal*. 67: 804 –10.

Tagoe, D. N. A., Nayar, H. D. and Akpaka, R. (2011). A comparison of antifungal properties of onion (*Allium cepa*), Ginger (*Zingiberofficinale*) and Garlic (*Allium sativum*) against *Aspergillus flavus*, *Aspergillus niger*and *Cladosporiumherbarum*. *Research Journal of Medicinal Plant*,5: 281-287.

Talwar, R., Chakrabarti, A., Kaur, P., Pahwa, R. K., Mittal, A. andMehra, Y. N. (1988). Fungal infections of ear with special reference to chronic suppurative otitis media. *Mycopathologia*, 104: 47-50.

Ududua, U. O., Monanu, M. O. and Chuku, L. C. (2019). Evaluation of acute toxicity of the ethanolic leaf extract of *Brachystegiaeurycoma*in albino Wistar rats. *Journal of Complementary and Alternative Medical Research.* 7(1): 1-7.

Un-Nisa, T., Wani, A. H. and Mir, R. A. (2010).Antimycotic activity of plant extracts on the spore germination of some pathogenic fungi. *Mycopathology*, 8(2): 65-69.

Weiner, R. and Collison, P. J. (2003). Middle ear pathogens in otitis prone children. *South Dakota Journal of Medicine,* 56: 103-107.