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Research Paper

In vitro antifungal activity of *Croton macrostachys* and *Allium sativum* extracts against *Candida albicans* and *Trichophyton mentagrophytes* isolates

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Abstract. *In-vitro* studies on the efficacy of crude extracts of *Croton macrostachys* and *Allium sativum* against *Candida albicans* and *Trichophyton mentagrophytes* was conducted in JimmaTown, Ethiopia. The leaves and the bulb cloves of *Croton macrostachys* and *Allium sativum,* respectively, were collected and the crude plant extracts were prepared. The *in-vitro* antifungal activity was evaluated at six different concentrations by agar disc diffusion method for several replicates, and the activity obtained was not concentration dependent except methanolic extract of *Croton macrostachys* on the strain of mould. The results were compared with standard antifungal drug (Ketoconazole) and distilled water. *T. mentagrophytes* was more susceptible fungal strain, while *Candida albicans* was more resistant one. The results revealed that the methanolic extract of *A. sativum* demonstrated more growth inhibitory activity against both fungi strains which studied than methanolic and aqueous extract of *C. macrostachys*. There was no significant variation in the zone of inhibition between the different concentrations ($p > 0.05$). However, there was high significant variation between the plant species, method of extraction and the tested fungus ($p < 0.001$). Findings from this study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides.

Keywords: *Candidia albicans*, crude plant extracts, fungi, *Trichophyton mentagrophytes,* Jimma.

INTRODUCTION

Economically fungi are important in that some of them have direct public and animal health importance, some others are responsible for toxin production, and others are contaminants of foods (Magwaza *et al*., 2017). For instance, yeasts of a genus *Malassezia* inhibit the skin growth for variety of mammals and birds, and are considered as opportunistic pathogens in animals and human beings (Alcazar-Fuoli and Mellado, 2014). *Cryptococcus neoformans* is also an opportunistic fungal pathogen, which causes fatal meningoencephalitis in patients with compromised immune responses (Heung, 2017). *Candida albicans* has a worldwide distribution and is common commensal of GIT and urogenital tracts of human (Whittington *et al*., 2014). In addition, fungal infections have been damaging the different plant species. For instance, the imperfect fungi called *Phaeoisariopsis griseola*, is one of most widely distributed and damaging disease of common bean (*Phaseolus vulgaris)*, which causing a yield losses as high as 80% all over the world (Schwartz *et al*., 2006).

Fungal infections remain a significant cause of morbidity and mortality of animals despite of advances in medicine and the emergence of new antifungal agents (Alcazar-Fuoli and Mellado, 2014). The most responsible fungal groups causing morbidity are *Aspergillus* and *Candidia* species (Pal, 2017). Of these, the zygomyces

Absidira corymbifera, *Rhizopus oryze, Rhizommucor puzzles* and *Mortirella wolfii* are the most common infectious, which cause systemic meiotic disease. Additionally, the infections most commonly recorded in bovine mycotic abortion are *Mucor*, *Aspergilus* species, *Petrilellidium bode*, *Candida parapoilosis* and *M. wolfii* (Foley and Schlafe, 1987). For instance, many different species of fungi have been isolated from conjunctiva sac of horses, including *Aspergilus* species and other molds, such as *Cladosporium*, *Mucor, Fusarium, Alternaria* and *Candida* species (Khosravi *et al*., 2014). Another common type of infection is a yeast infection, which caused by the fungus *C. albicans* (Ogundana *et al*., 2004).

Candida albicans, the agent of candidiasis, is an increasingly important disease, because it is a frequent opportunistic pathogen in its nature (Kabir *et al*., 2012). It is a common commensal of the gastrointestinal and urogenital tracts of human (Black, 2003), and is the cause of Candiasis in women (Neerja *et al*., 2006). In addition, *Candida tropicalis* is one of the non-albicance Candida strains currently emerging in fungal infections (Deorukhkar *et al*., 2014). The treatment of systemic mycosis has been largely by amphotericin-B and nystatin, but the newer azoles compounds (Enilconazole, Fluconazole, Itraconazole and Ketoconazole) being administered orally appears to be highly effective and easy (Radostits *et al*., 2007). However, the cost of conventional drugs and its resistance to pathogens is the major problem, especially in rural areas where high proportion of livestock is reared and far from the modern drugs (Landers *et al*., 2012). Since a strain of *C. albicans* with multiple antibiotic resistances is increasing, it is of great importance to find effective treatments for these pathogens. To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is matter urgency. Therefore, researchers are increasingly turning their attention to folk medicines, looking for new leads to develop better drugs against microbial infections.

Natural products have been served as a major source of drugs for centuries, and about half of the pharmaceuticals in use today are derived from natural products (Veeresham, 2012). The study has indicated that about 25 to 50% of current pharmaceuticals are derived from plants (Gakuubi *et al*., 2017). Microbiologists and natural product chemists trying to discover more about phytochemicals, which could be developed for treatment of infectious diseases (Dhinahar and Lakshmi, 2011). Recent trends favor the use of plant extracts and their essential oils show antifungal activity against a wide range of fungal effects (Gakuubi *et al*., 2017). Many of these plant materials used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicines (Mann *et al*., 2008). Several authors also studied the effect of different plant extracts on the growth of fungi (Aye, and Matsumoto, 2011; Parveen *et al*., 2013; Saha *et al*., 2008). Findings from this study confirmed that plant extracts can be used as natural fungicides to control pathogenic fungi, thus reducing the dependence on the synthetic fungicides (Gakuubi *et al*., 2017). There are also some folkloric herbs still in use without any scientific evidences. Therefore, pharmacological and clinical data should be conducted for herbal medicinal products to remove the impedimentation of integration of herbal medicines in to conventional medical practices (Parveen *et al*., 2015). However, there is no information on antifungal effects of *Croton macrostachys* and *A. sativum* on fungal isolates of *C. albicans* and *Trichophyton menthagrophytes*. Therefore, this study was aimed at assessing the *in-vitro* antifungal activity of *C. macrostachys* and *A. sativum* on the growth of *C. albicans and T. mentagrophytes* in Jimma Town, Ethiopia.

MATERIALS AND METHODS

Description of the study area

The present study was carried out at Jimma town Southwestern part of Ethiopia, which is located on 346 km away far from the capital Addis Ababa between latitude of 70°13′ to 80°56′ N and longitude of 35°52′ to 37°37′ E, with an elevation ranging from 880 to 3360 m above sea level. The study area receives a mean annual rainfall of about 1530 mm. The mean annual minimum and maximum temperature of the study area were 14.4 and 26.7°C, respectively. There area is characterized by mixed farming systems of crop production and livestock rearing. The most important crops grown are; maize, teff, sorghum, horse bean and chick pea. Majority of the animals kept in the region are cattle, sheep and there are some goats, horses and donkeys.

Study design

An experimental study was used to determine the antifungal activity of selected plants that was conducted between November 2010 and May 2011 in Jimma University College of Agriculture and Veterinary Medicine. *A.sativum (Qullubbii adiiin Oromic, Netch shunkurt in Amharic)* and *Croton macrostachys (Bakkanniisa in Oromic, Bisanna in Amharic),* were collected from different places of Jimma town, and identified and authenticated by a botanist in the Department of Botany, Jimma University, Ethiopia. The seeds of *C. macrostachys* are used as purgatives, anthehelmentics and mulluscides (Amin *et al*., 2002). The two plants were extracted by methanol and distilled water at different concentrations in order to compare and contrast them with their activity against both fungi growth. The test organisms used for screening the antimicrobial

activity of the extracts were fungal isolates identified as *C. albicans* and *T. mentagrophytes* that were acquired from Ethiopian health and nutrition research institute, Addis Ababa.

Pre-extraction preparation of the two herbal medicine

After collection of the plants, leaves and shoots of the fresh *C. macrostachys* were washed using distilled water, drained and cut in to very small pieces using a knife, then dried at room temperature (25°C methanol and distilled water) for seven days in the laboratory. In addition, *A. sativum* purchased from the shops of Jimma town were separated into its cloves, peeled to remove its cover and cut into small pieces using a knife. After appropriate drying, the dry products of both plants were grinded in to a thin powder by using a pestle and mortar, sealed properly, and kept in plastic vials until used for preparation of the crude extracts (Charimbu *et al*., 2009).

Preparation of crude plant extracts

To obtain different concentrations of crude plant extracts, different concentrations like 5, 10, 15, 20, 25 and 30% by methanol (99.5%) and distilled water were used as solvents. Then, 5 g of each of the powdered plant material was macerated in 100 ml of methanol in 500 ml flasks, kept on a rotator shaker, and shacked for 24 h to prepare 5% concentration of methanolic extracts. Thereafter, it was filtered through gauze placed in a tea strainer and centrifuged at 3000 rpm for 5 min, so that the plant material, which passed through the gauze and tea strainer was sedimented at the bottom of test tubes. The supernatant was collected and evaporated until to give the crude plant extract. The same procedure was continued to prepare all the rest concentrations for both methanolic and aqueous extract of both plant species by changing their weight together with change of concentrations (Charimbu *et al*., 2009).

Microbial culture and growth conditions

The work surface was sterilized by 70% alcohol, and 9.75 g of Sabouraud's dextrose agar media was mixed with 150 ml of distilled water and boiled in bath water and stirrer. Then, it was autoclaved at 121°C for 15 min. After autoclaving, chloramphenicol was added to the medium under laminar flow hood to make the medium selective for fungi. About 15 ml of the medium was poured into sterilize Petridish up to a level of approximately 4 mm and kept until it was solidified. Dishes with solidified agar were incubated at 25°C for 24 h for sterility checkup. *C. albicans* and *T. mentagropytes* were inoculated on the media using sterile wire loop, and the inoculated dishes were incubated at 25°C for 24 h. Finally, the obtained colonies were identified culturally and stored in the refrigerator at 4°C.

Antifungal assay of the tested plant extracts

To evaluate the antifungal activity, sterile agar plates were used according to the disc diffusion assay (Bauer *et al*., 2001). A loopful *C. albicans* and *T. mentagrophytes* from Petridis were inoculated in to test tubes containing Sabouraud's broth media, and incubated at 25°C for 24 h. After 24 h, the diluted broth cultures were aseptically poured and spread on sets of Sabouroud's dextrose agar plates. Surplus suspension was decanted from the surface of the agar plates which were allowed to dry at room temperature for 5 minutes. Later sterile, Whitmann No.1 filter paper discs (5.0 mm diameter) were impregnated with different concentrations of the plant extracts and allowed to air dry in the laminar flow hood for 3 min (Souza *et al*., 2005). Other discs impregnated in aqueous suspension of standard ketoconazole served as a positive control and discs impregnated in distilled water served as a negative control. The impregnated discs in the ketoconazole were placed firmly at the center of the culture plates, whereas discs impregnated in different concentrations of crude plant extracts and discs impregnated in distilled water were placed firmly near the periphery of the culture plates. The plates were incubated at 25°C for 72 h. Following incubator period of 72 h, plates were removed from the incubator, and antifungal activity was evaluated by measuring zones of inhibition of fungal growth. Clear zones within which fungal growth absent were measured and recorded as the diameter (mm) of complete growth inhibition. The whole experiment was replicated five times to minimize error.

Statistical analysis

Descriptive statistical methods was used by Microsoft office excel (2003) for data analysis, and the results was present as percentages and tables for illustration. The laboratory results were analyzed using SPSS version 16.0. The significance of association between and among the considered variables was determined using Chi-square ($\chi 2$).

RESULTS

On the bases of this study, all the tested concentrations (5, 10, 15, 20, 25 and 30%) of the two plants extracts (*C. macrostachys* and *A. sativum*) inhibited the two fungal strains growth with varying degree of sensitivity except aqueous extraction of *C. macrostachys* on the *C. albicans*. Out of the all 240 replicates tested with different

Table 1. Effect of the plant species extracts (*C. macrostachys* and *A. sativum)* on *C. albicans* and *T. mentagrophyts* fungal species.

Extraction method	Total replicates	Mean ± SD	Negative	Positive (%)	P-value
Methanolic	120	74 ± 65.05	28 (23.33)	92 (76.67)	
Aqueous	120	87 ± 46.67	54 (45.0)	66 (55.0)	0.000
Total	240		82 (34.17)	158 (65.8)	

Table 2. Effect extraction methods of *C. macrostachys* and *A. sativum* on *C. albicans* and *T. mentagrophyts* fungal species.

Method of extraction	Total replicates	Mean ± SD	Negative	Positive (%)	P-value
Methanolic	120	74 ± 65.05	28	92 (76.67)	
Aqueous	120	87 ± 46.67	54	66 (55.0)	0.001
Total	240		82	158 (65.8)	

Table 3. Screening of *C. albicans* medicinal plants for antifungal activity against strain of yeast.

Fungal species	Plant species	Diameter of zone of inhibition (mm) at different concentrations												Control result
		Methanolic extract						Aqueous extracts						
	Concentration	5	10	15	20	25	30	5	10	15	20	25	30	
C. albicans	*C. macrostachys*	-	8	6	6	6	6	-	-	-	-	-	-	
	A. sativum	10	10	9	13	9	9	7	8	-	11	-	8	
Control	2% Ketoconazole	18												+ve
	Distilled water	-												-ve

+ve = Positive, -ve = Negative, - = Zero zone of inhibition. Values are mean of the five replicates.

Table 4. Screening of *T.mentagrophyts* medicinal plants for antifungal activity against strain of mould.

Fungal species	Plant species	Diameter of zone of inhibition (mm) at different concentrations												Control result
		Methanolic extract						Aqueous extract						
T. mentagrophyts	Concentration (%)	5	10	15	20	25	30	5	10	15	20	25	30	
	C. macrostachys	-	10	12	13	15	11	12	8	8	6	9	-	
	A. sativum	15	15	15	18	14	14	10	13	13	16	10	14	
Control	2% Ketoconazole	22												+ve
	Distilled water	-												(-ve)

+ve = Positive, -ve = Negative, - = Zero zone of inhibition. Value are mean of the five replicates.

concentrations, 158 (65.8%) of them showed inhibition zone and 82 (34.2%) of them did not show inhibition zone against the test fungus (Tables 1 and 2).

The result showed that the antifungal activity of the two crude plant extracts determined by disc diffusion method. Both the aqueous and methanolic extracts of *A. sativum* exhibited antifungal activities against almost the tested *C. albicans* except 15 and 25% of the plant extracts. However, the 5% methanolic extracts of *C. macrostachys* and all of its concentrations of aqueous extracts were not demonstrated inhibitory activity against *C. albicans* (Table 3).

In other way, both aqueous and methanolic extracts of *A. sativum* showed good inhibitory activity at all concentrations against the tested *T. mentagrophyts*. In addition, *C. macrostachys* showed antifungal activity with both extraction methods except at 25% of its aqueous extraction on *T. mentagrophyts*. This indicates that the yeast strain (*C. albicans*) is more resistant than the mould strain (*T. mentagrophytes*). According to this study, *A. sativum* showed more antifungal activity on both *C. albicans* and *T. mentagrophyts* than that of *C. macrostachys* by both methanolic and aqueous extraction methods (Table 4).

Generally, there was no a significant variation in the zone of inhibition between the different concentrations which studied ($p > 0.05$). However, there was highly significant variation observed between the plant species,

extraction methods and the tested fungus ($p < 0.001$).

DISCUSSION

The fungicidal activity of some plant extracts in controlling different plant pathogens have been reported by several researches (Okigbo and Emoghene, 2004; Parveen *et al*., 2013; Cannon *et al*., 2007). The active compounds present in plants are influenced by many factors, which include the age of plant, extraction solvent, method of extraction and time of harvesting plant materials (Okigbo and Ajalie, 2005). Many researchers have reported the presence of antifungal in different plant extracts, which cause inhibition of radial growth and spore germination, and the reduction of in rot development by the pathogen *in-vitro* (Aye and Matsumoto, 2011; Parveen *et al*., 2013; Saha *et al*., 2008). The differences observed in fungitoxic activity to the extracts were likely to be due to the solubility of the active compounds in aqueous and organic solutions or the presence of inhibitors to the fungitoxic principle (Amadioha, 2002).

The tested organisms in the present study (*C. albicans* and *T. mentagrophytes*) have been indicated in causing of dermatomysitis (Abd Elmegeed *et al*., 2015). Our finding indicated that the presence of active antimicrobial agents in *C. macrostachys* and *A. sativum* plants thereby justifying their use for the treatment of *C. albicance* and *T. mentagrophytes* infections. A similar study of screening natural plant extracts against different fungal pathogens was well recorded in literature (Rani and Murti, 2006).

Of the two plants, the aqueous and methanolic extracts of *A. sativum* showed an induced zone of inhibition on both fungal strains. However, the effect of plant extracts was varied with the different fungal strains and extraction solvents. The results of this study corresponds with work done by Slusarenko *et al*. (2008) who tested the effectiveness of garlic juice against range of plant pathogenic bacteria, fungi and oomycetes *in-vitro*.

The minimum inhibitory concentration (MIC) of *A. sativum* was also observed with both test organisms and extraction solvents. The effects of the antifungal compounds may be on spore germination leading to its inhibition or may be due to the effect of these compounds on the cell wall altering its permeability (William, 2008.). The inhibition zones induced by both plant extracts were observed to reduce in size, and the time implying the active ingredient was losing its potency with time. Although the methanolic and aqueous extracts of *C. macrostachys* showed zones of inhibition on the strain of mould, but the aqueous extracts of the plant did not show any inhibition zone on the *C. albicans*. This may probably due to the fact that the extracts were in suboptimal doses to effect the inhibition of the organism. Moreover, the test organism used may have been a highly resistant strain (Cannon *et al*., 2007). The result is in agreement with that of Parveen *et al*. (2013).

Thus, from the overall results obtained, it is evident that the two plants screened possess antimicrobial agents against some pathogenic organisms associated with skin infections. They therefore justify their popular use by local herbalists in the treatment of skin diseases.

CONCLUSIONS

In this study, the leaf of *C. macrostachys* and bulb cloves of *A. sativum* showed good antimicrobial activity against *C. albicans* and *T. mentagrophtes*, whereas the aqueous extraction of *C. macrostachys* leaf has no any activity against *C. albicans*. The demonstration of satisfactory growth inhibition against *C. albicans* and *T. mentagrophtes* at different concentrations would see justify their potential in the synthesis of new phytoremedies and their use in treatment of microbial infections. These findings suggest that there is a potential in the discovery of novel antimicrobial agents from the two herbal medicines that are found in Ethiopia.

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REFERENCES

Abd-Elmegeed AM, Ouf SA, Moussa TAA, Eltahlawi SMR (2015). Dermatophytes and other associated fungi in patients attending to some hospitals in Egypt. Braz. J. Microbiol. 46(3):799-805.

Alcazar-Fuoli L, Mellado E (2014). Current status of antifungal resistance and its impact on clinical practice. Brit. J. Haematol. 166:471-484.

Amadioha AC (2002). Fungitoxic effects of some leaf extracts against Rhizaopusoryzae causing tuber rot of potato Arch. Phytopathol. 0:1-9.

Amin MA, Dafalla AA, Abdel MO (2002). Preliminary report on the molluscidal properties of habatelmeluk *Jaropha spp.* Trop. Med. Hyg. 66:805-812.

Aye SS, Matsumoto M (2011). Effect of some plant extracts on *Rhizoctonia spp*. and *Sclerotium hydrophilum*. J. Med. Plants Res. 5(16):751-3757.

Bauer AW, Kirby WMM, Sheris JC, Turck M (2001). Antimicrobial susceptibility testing by standardized single method. Clin. Pathol. 45:493-496.

Cannon RD, Lamping E, Holmes AR, Niimi K, Tanabe K, Niimi M, Monk BC (2007). *Candida albicans* drug resistance – another way to cope with stress. Microbiol. 153:3211-3217.

Charimbu MK, Wagrara IN, Otaye DO (2009). Antifungal activity of various crude plant extracts against phaeoisariopssisgriseola pathogenic on common bean. Department of Biological sciences, Egerton University, Egerton, Kenya.

Dhinahar S, Lakshmi T (2011). Role of botanicals as antimicrobial agents in management of dental infections – a review. Int. J. Pharm. Bio Sci. 2(4):690-704.

Foley GL, Schlafe DH (1987). Candida Abortion in Cattle. Vet. Pathol. 24:532-536.

Gakuubi MM, Maina AW, Wagacha JM (2017). Antifungal Activity of Essential Oil of *Eucalyptus camaldulensis* Dehnh against Selected *Fusarium spp*. Int. J. Microbiol. 2017:1-7.
Heung LJ (2017). Innate Immune Responses to Cryptococcus. J. Fungi 3:24-35.
Kabir MA, Hussain MA, Ahmad Z (2012). *Candida albicans*: A Model Organism for Studying Fungal Pathogens. ISRN Microbiology 2012:1-15.
Khosravi D, Sharifzadeh NA, Gharagozlou F (2014). Ocular fungal flora from healthy horses in Iran. Sci. direct 30:1-5.
Landers F, Cohen B, Wittum TE, Larson EL, Faan CIC (2012). A Review of Antibiotic Use in Food Animals: Perspective, Policy and Potential. Public Health Reputation 127(1):4-22.
Magwaza NM, Nxumalo EN, Mamba BB, Msagati TAM (2017). The Occurrence and Diversity of Waterborne Fungi in African Aquatic Systems: Their Impact on Water Quality and Human Health. Int. J. Environ. Res. Public Health 14:546-553.
Mann A, Banso A, Clifford LC (2008). An antifungal property of crude plant extracts from *Anogeissus leiocarpus* and *Terminalia avicennioides.* Tazania J. Health Res. 10(1):34-38.
Neerja J, Aruna A, Paramjeet G (2006). Significance of candida culture in women with vulvo vaginal symptoms. J. Obstet. Gynecol. India 56 (2):139-141.
Ogundana SK, Naqvi SHZ, Ekundayo JA (2004). Fungi associated with soft rot of yams in storage Nigeria. Transform. Br. Mycol. Soc. 54:445-451.
Okigbo RN, Ajalie AN (2005). Inhibition of some human pathogens with tropical plant extracts *Chromolinaena odorata* and *Citrus aurantifolia* and some antibiotics. Int. J. Mol. Med. Adv. Sci. 1(1):34-40.
Okigbo RN, Emoghene AO (2004). Antifungal activity of leaf extracts of some plant species on *Mycosphaerella fijiensis* Morelet, the causal organism of black sigatoka disease of Banana (*Musa acuminate*). KMITL Sci. J. 4:20-31.
Pal M (2017). Morbidity and Mortality due to Fungal Infections. J. Appl. Microbiol. Biochem. 1(1:2):1-3.
Parveen A, Parveen B, Parveen R, Ahmad S (2015). Challenges and guidelines for clinical trial of herbal drugs. J. Pharmacol. Bioallied Sci. 7(4):329333.
Parveen S, Wani AH, Ganie AA, Pala SA, Mir RA (2013). Antifungal activity of some plant extracts on some pathogenic fungi. Archiv. Phytopathol. Plant Protect. 47(3):279-284.
Radostits OM, Gay CC, Hinchcliff KW, Costable PD (2007). Mycosis, In: veterinary medicine, text book of cattle, horse, sheep, goat and pig.10th ed. pp. 1471-1472.
Rani SA, Murti SU (2006). Antifungal potential of head flower extract of plants. *Acmella Linn*. Afr. J. Biomed. Res. 9:67-69.
Sachin C, Saini DS, Mathew S (2014). Non-albicans Candida Infection: An Emerging Threat. Interdisciplinary Perspect. Infect. Dis. 8:1-7.
Saha D, Dasgupta S, Saha A (2008). Antifungal Activity of Some Plant Extracts against Fungal Pathogens of Tea (*Camellia sinensis*). J. Pharm. Biol. 43:87-91.
Schwartz HF, Correa VF, Pineda DDA, Otoya MM, Katherman MJ (2006). Ascochyta, angular and white flies leaf spots in Colombia. Plant Dis. 65:494-496.
Slusarenko AJ, Patel A, Portz D (2008). Control of plant diseases by natural products: Allicin from garlic as a case study. Eur. J. plant pathol. 12(3):1-8.
Souza EL, Lima EO, Freire KR, Sousa CP (2005). Inhibitory action of some essential oils and phytochemicals on the growth of yeast and moulds. Braz. Archive. Biol. Technol. 48:245-250.
Veeresham C (2012). Natural products derived from plants as a source of drugs. J. Adv. Pharm. Technol. Res. 3(4):200-201.
Whittington A, Gow NAR, Hube B (2014). From Commensal to Pathogen: *Candida albicans*. Human Fungal Pathogens, 2nd Edition, Springer-Verlag Berlin Heidelberg.
William Q (2008). Least toxic controls of plant diseases. Brooklyn Botanic garden. Natural disease control 11:225-232.