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Antifungal Properties of Some Tropical Plant Extract against Pathogenic Strains of *Candida Albican*

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Abstract:
The effect of aqueous and ethanolic extracts of air-dried and pulverized stem bark extracts of Anogeissus leiocarpus, Parkia biglobosa, Bridelia feruginea and leaf of Acalypha wilkesinia on Candida albicans, the inducer of intertrigo candida disease in children was investigated. The zones of inhibition produced by various plant components showed that for ethanolic extract, only Anogeissus leiocarpus bark (ANB) at 30 mg/ml with zone of inhibition 10.33±0.58 surpassed nystatin (500 IU/ml) with zone of inhibition of 9.33±0.58 while Anogeissus leiocarpus bark (ANB) at 15 - 25 mg/ml with zone of inhibition of 7.00±0.00 - 9.00±0.00 was more effective than ketoconazole (USP 200mg) with zone of inhibition of 6.67±0.57 as well as Anogeissus leiocarpus leaf (ANL) at 20-30 mg/ml with zone inhibition of 6.67±0.58 - 8.67±0.58 and Acalypha wilkesinia leaf (ACL) at 30 mg/ml with zone of inhibition of 7.00±0.00. For the aqueous extract Anogeissus leiocarpus bark (ANB) at 20-30 mg/ml with zone inhibition of 9.67±0.58 - 11.67±0.58 and Anogeissus leiocarpus leaf (ANL) at 30 mg/ml with zone of inhibition of 10.00±0.00 surpassed nystatin (500 IU/ml) with zone of inhibition of 9.33±0.58 while Anogeissus leiocarpus bark (ANB) at 10-15 mg/ml with zone of inhibition of 7.00±1.00 - 8.00±1.00 was more effective than ketoconazole (USP 200mg) with zone of inhibition of 6.67±0.57 as well as Acalypha wilkesinia leaf (ACL) at 20-25 mg/ml with zone of inhibition of 7.33±0.58 - 8.33±0.58 and Anogeissus leiocarpus leaf (ANL) at 20-25 mg/ml with zone inhibition of 7.67±0.58 - 9.00 ±0.00. The fungitoxicity tests have shown that extracts from Anogeissus leiocarpus leaf and stem-bark as well as Acalypha wilkesinia leaf can replace synthetic drugs in the management of intertrigo infection.

1. Introduction

Fungal diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide (Meena *et al.,* 2010). Fungal infections have increased worldwide largely because of the increasing size of people at risk, including immune compromised patients receiving parenteral hyper alimentation and/or broad-spectrum antibiotics (Bouza *et al.,*2008). Other reasons are increase in immunosuppressive conditions like AIDS and other factors such as organ transplantation, leukemia, diabetes and intravenous drug misuse among others (Razzaghi-Abyaneh *et al.,* 2014).

An important group of the skin pathogens are the fungi, among which dermatophytes and *candida spp.* are prominent (Fan *et al.,* 2008, De-Toledo *et al.,* 2011). Under certain circumstances usually associated with a compromised host immune system, *Candida albicans* and related species can become pathogenic, causing oral, vaginal and/or systemic candidiasis (Rekha and Vidyasagar, 2013). *Candida albicans* is notorious for causing candidiasis, it can affect esophagus with the potential of becoming systemic, causing a more serious condition called Candidemia (Pappas, 2006). It can also cause a variety of infections that range from non-life threatening mucosal candidiasis like vaginal yeast infections, thrust, skin and diaper rash to lethal disseminated candidiasis in those with compromised immune system who have an implantable medical device such as peace maker or artificial joint, or who use broad spectrum antibiotics (Bhaven *et al.,* 2010).

Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to those agents (Al-Bari *et al.,* 2006). However, since many of the available antifungal drugs have undesirable side effect or are very toxic, produce recurrence, show drug-drug interactions or lead to the development of resistance, some show ineffectiveness (Muschietti *et al.,* 2005) and have become therefore less successful in therapeutic strategies. It is therefore necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages. The present investigation is focused on the screening of *Anogeissus leiocarpus* (Marke in Hausa), *Parkia*

biglobosa (Dorowa in Hausa), *Bridelia feruginea* (Kirni in Hausa), and *Acalypha wilkesinia* (Jiwinini in Hausa) against human fungal pathogen *Candida albicans.*

2. Materials and Methods

2.1. Cultures

One standard strain of *C. albicans* was obtained from Microbiology Laboratory of Nigerian Institute for *Trypanosomiasis* Research (Federal ministry of science and technology) Kaduna. These isolates were maintained on Sabouraud dextrose agar SDA (BIOMARK Laboratories, India) at 4^0C. Colonies from the SDA plates were stained by gram staining techniques following the procedure by (Fox and Bahets, 1995).

2.2. Collection, Identification and Treatment of Plant Materials

Fresh plant parts were collected at Trial Afforestation Research Station, Forestry Research Institute of Nigeria, Afaka Kaduna. The plants were authenticated by a taxonomist in the Department of biological sciences NDA Afaka Kaduna to confirm their identities with voucher numbers NDA BIO 1514, 1515, NDA BIO 1516 and NDA BIO 1517 for *A. leiocarpus*, *P. biglobosa*, *B. feruginea*, and *A. wilkesinia* respectively. The plant parts were chopped and shade-dried at room temperature for 2 weeks then grounded using mortar and pestle to a fine powder in accordance to method described by (Al-Hussaini and Al-Mohana 2010). The grounded samples were then transported for extraction process at the Chemistry Laboratory of Faculty of Science NDA Postgraduate School Kaduna.

2.3. Solvent Extraction of Plant Materials

The grounded powder was weighed on Satorius balance type (BA 610), 100 g each of the dried samples were dissolved in 500ml of 95% ethanol and also 100g of each of the dried samples were dissolved in 1000ml of distilled water separately. After the plant materials were successively extracted with ethanol and distilled water separately, the extract was filtered through (Whatman® No.1, England) in Buchner funnel. This was followed by concentration of the ethanol filtrate on Rotary evaporator type Buchi-R-Switzerland at 50 °C to recover the solvent used and the aqueous filtrate was concentrated using water bath. The filtrate stock solution was kept air dried for further analysis (Al-Hussaini and Al-Mohana, 2010).

2.4. Fungitoxicity Test

Different concentration of each plant extract was prepared for studying their antifungal activity following the method described by Titilawo *et al*., (2011

2.5. Determination of Minimum Inhibitory Concentration (MIC)

The least concentration of the plant extracts that does permit any visible growth of the inoculated test organism in the broth medium was regarded as the MIC in each case. Control experiments were performed without the plant extracts Mann *et al*., (2008).

2.6. Determination of Minimum Fungicidal Concentration (MFC) of the Extracts

The contents of the tubes that showed no visible fungal growth or turbidity in the minimum inhibitory concentration experiment were cultured into prepared Sabouraud dextrose agar plate to assay for the fungicidal effect of the extracts. The plates containing the test organisms were incubated at 37^0C for 48h. The minimum fungicidal concentration was regarded as the lowest concentration that did not yield any fungal growth on the solid medium used Mann *et al*., (2008).

2.7. Statistical Analysis

The experiment was replicated thrice and the mean was obtained for statistically analysis. Duncan's multiple range test ($P<0.05$) was used to determine areas where there was significant difference.

3. Results

3.1. Susceptibility testing of aqueous and ethanolic plant extracts and antifungal drugs in culture media on C. albicans

Table 1 and 2 shows inhibition zones (mm) of *C. albicans* growth produced by aqueous and ethanolic plant extracts in culture media. Almost all the plant extracts exhibit antifungal effects against *C. albicans* (table 1 and 2). In particular, aqueous extracts offer effective bioactive compounds for growth inhibition of *C. albicans* especially *A. wilkesinia leave, A. leiocarpus* leave and bark (Table 1). Even at low concentrations, these plant extracts showed antifungal activity nearly equal to that of the commercial fungicide used as a positive control (Nystatin and Ketoconazole). The most active extract was that obtained from both the aqueous and ethanolic extracts of *A. leiocarpus* bark with the highest zone of inhibition (11.67±0.58), and (10.33±0.53) at 30 mg/ml concentration, followed by *A. leiocarpus* leave extract with zone of inhibition about (10.00±0.00) and (8.67±0.58) while *A. wilkesiana* leave extract also showed strong activity and gives inhibition zones about (9.33±0.58) and (7.00± 0.00). *B. feruginea* leave and bark showed moderate activity and gives inhibition zones about (5.00±0.00), (3.67±0.57)

and (3.00±0.00) at 30 mg/ml concentration, but at 2.5 and 5 mg/ml concentration of ethanolic extract of *B. feruginea* leave did not give any significant inhibition. Both ethanolic and aqueous extracts of *P. biglobosa* leave and bark extract have no effect at 2.5, 5, 10 mg/ml but little inhibition of (0.33±0.58) (0.67±0.57), (1.00±0.00), (1.67±0.00) and (2.00±0.00) was observed at 15, 20, and 30 mg/ml respectively.

The positive control Nystatin and ketoconazole showed moderate activity similar to that found in *A. wilkesiana* and *A. leiocarpus* and gives inhibition zones about (9.33±0.58) and (6.67±0.57). Therefore, those readings in *A. wilkesiana* at 30 mg/ml, *A. leiocarpus* bark at 20 to 30 mg/ml and *A. leiocarpus* leave at 25 to 30 mg/ml worked as good as or even better than the control drugs. All others even at high concentrations did not make any significant difference (Table 1). In Table 2 only *A. leiocarpus bark* at 25 to 30 mg/ml can be compared with Nystatin, Others like *A. wilkesiana* at 30 mg/ml, *A. leiocarpus* bark at 15 to 30 mg/ml and *A. leiocarpus* leave at 20 to 30 mg/ml could only be compared with ketoconazole. Differences in Table 1 and Table 2 established potency of aqueous extract over the ethanolic extracts.

Plant parts	**Extract concentrations (mg / ml)**						
	2.5	5	10	15	20	25	30
ACL	2.33^{c}±0.58	3.67^{b}±1.15	4.67^{b}±1.15	6.00^{c}±1.00	7.33^{d}±0.58	8.33^{d}±0.58	9.33^{e}±0.58
ANB	3.67^{d}±0.58	5.33^{c}±1.15	7.00^{c}±1.00	8.00^{d}±1.00	9.67^{e}±0.58	10.67^{e}±0.58	11.67^{g}±0.58
ANL	2.67^{c}±0.58	3.67^{b}±0.58	5.00^{b}±1.00	6.33^{c}±0.58	7.67^{d}±0.58	9.00^{d} ±0.00	10.00^{f}±0.00
PBL	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.33^{a}±0.58	1.00^{a}±0.00	1.00^{a}±0.00	2.00^{a}±0.00
PBB	0.00^{a}±0.00	0.33^{a}±0.58	0.33^{a}±0.58	1.00^{ab}±0.00	1.00^{a}±0.00	2.00^{b}±0.00	3.00^{b}±0.00
BFL	1.00^{b}±0.00	1.00^{a}±0.00	1.00^{a}±0.00	1.33^{ab}±0.58	2.00^{b}±0.00	2.67^{b}±0.58	3.67^{c}±0.57
BFB	1.00^{b}±0.00	1.00^{a}±0.00	1.00^{a}±0.00	2.00^{b}±0.00	3.00^{c}±0.00	3.67^{b}±0.58	5.00^{d}±0.00

Positive and negative control	Inhibition zone (mm)
Nystatin (500 IU / ml)	9.33±0.58
Ketoconazole USP 200 mg	6.67±0.57
Distilled water	0.00±0.00

Table 1: Zones of inhibition (mm) of C. albicans growth in aqueous plant extracts and antifungal drug culture media

a, b, c, d means within a column with different superscripts are significantly different ($p<0.05$). Values are means ± standard deviation of three replicates.
ACL=*Acalypha wilkesinia leave, ANL=Anogeissusleiocarpus leaves, ANB=Anogeissus leiocarpus bark, BFL Bridelia feruginea leave,* BFB =*Bridelia feruginea bark,* PBL=*Parkia biglobosa leave,* PBB=*Parkia biglobosa bark.*

Plant parts	**Extract concentrations (mg / ml)**						
	2.5	5	10	15	20	25	30
ACL	1.33^{bc}±0.58	2.00^{c}±0.00	2.67^{b}±0.58	3.67^{c}±0.58	5.00^{c}±0.00	6.00^{d}±0.00	7.00^{c}±0.00
ANB	2.33^{d}±1.15	3.33^{d}±1.15	4.33^{c}±1.15	7.00^{e}±0.00	8.00^{e}±0.00	9.00^{f}±0.00	10.33^{e}±0.58
ANL	2.00^{cd}±0.00	2.67^{cd}±0.57	4.00^{c}±1.00	5.67^{d}±0.58	6.67^{d}±0.58	7.67^{e}±0.00	8.67^{d}±0.58
PBL	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.67^{a}±0.58	1.00^{a}±0.00	1.00^{a}±0.00
PBB	0.00^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00	0.67^{b}±0.58	1.00^{a}±0.00	1.00^{a}±0.00	1.67^{a}±0.57
BFL	0.00^{a}±0.00	0.00^{a}±0.00	0.67^{a}±0.57	1.00^{b}±0.00	1.67^{b}±0.58	2.00^{b}±0.00	3.00^{b}±0.00
BFB	1.00^{b}±0.00	1.00^{b}±0.00	1.00^{a}±0.00	1.00^{b}±0.00	2.00^{b}±0.00	3.00^{c}±0.00	3.67^{b}±0.58

Positive and negative control	Inhibition zone (mm)
Nystatin (500 IU / ml)	9.33±0.58
Ketoconazole USP 200mg	6.67±0.57
Distilled water	0.00±0.00

Table 2: Zones of inhibition (mm) of C. albicans growth in ethanolic plant extracts and antifungal drug culture media

a, b, c, d means within a column with different superscripts are significantly different ($p<0.05$). Values are means ± standard deviation of three replicates.
ACL=*Acalypha wilkesinia leave, ANL=Anogeissus leiocarpus leaves, ANB=Anogeissus leiocarpus bark, BFL Bridelia feruginea leave,* BFB =*Bridelia feruginea bark,* PBL=*Parkia biglobosa leave,* PBB=*Parkia biglobosa bark.*

3.2. Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of plant extracts against C. albicans

The minimum inhibitory concentrations of the plant extracts varied. Some plants were more efficacious than others as their MIC varied. Plants like *B. feruginea* and *P. biglobosa* did not inhibit candidal growth at concentrations less than (15 mg/ml), but A. *leiocarpus* and *A. wilkesinia* had minimum inhibitory concentrations that were between (0.15 – 2.5 mg/ml) and these were considered very efficacious (Figure 1 and 2).

The minimum fungicidal concentration of the extracts proved to possess more fungicidal action against *C. albicans* when they are assayed. The extract of *A. leiocarpus* was the most active fungicidal with minimum fungicidal concentration of (0.15 – 0.65 mg/ml) followed by *A. wilkesinia* with a minimum fungicidal concentration of (1.25 – 2.5 mg/ml). Relatively higher value of minimum fungicidal concentration was observed in the extract of *B. feruginea* and *P. biglobosa* which possess the lowest fungicidal action against *C. albicans* among the extracts assayed against the organism with minimum fungicidal concentration of (5.00-15.0 mg/ml) and (10.00-20.00 mg/ml) (Figures 1 and 2).

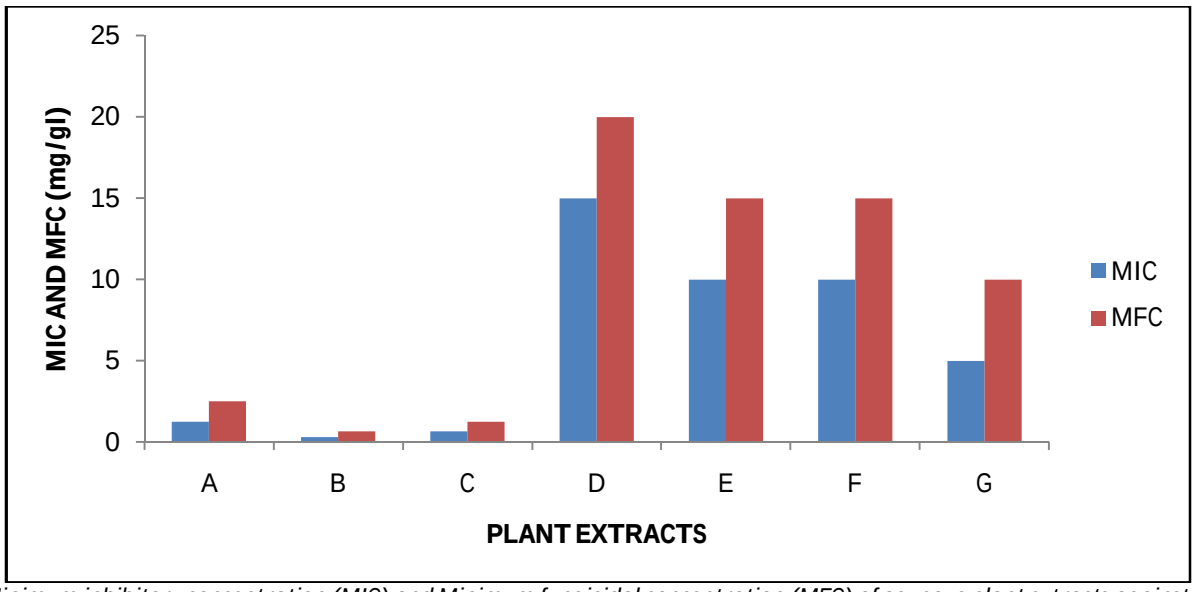

Table 3: Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of aqueous plant extracts against C. albicans

Key

A: *Acalypha wilkesinia leave* B: *Anogeissus leiocarpus leave* C: *Anogeissus leiocarpus bark*
D: *Bridelia feruginea leave* E: *Bridelia feruginea bark* F: *Parkia biglobosa leave*
G: *Parkia biglobosa bark*

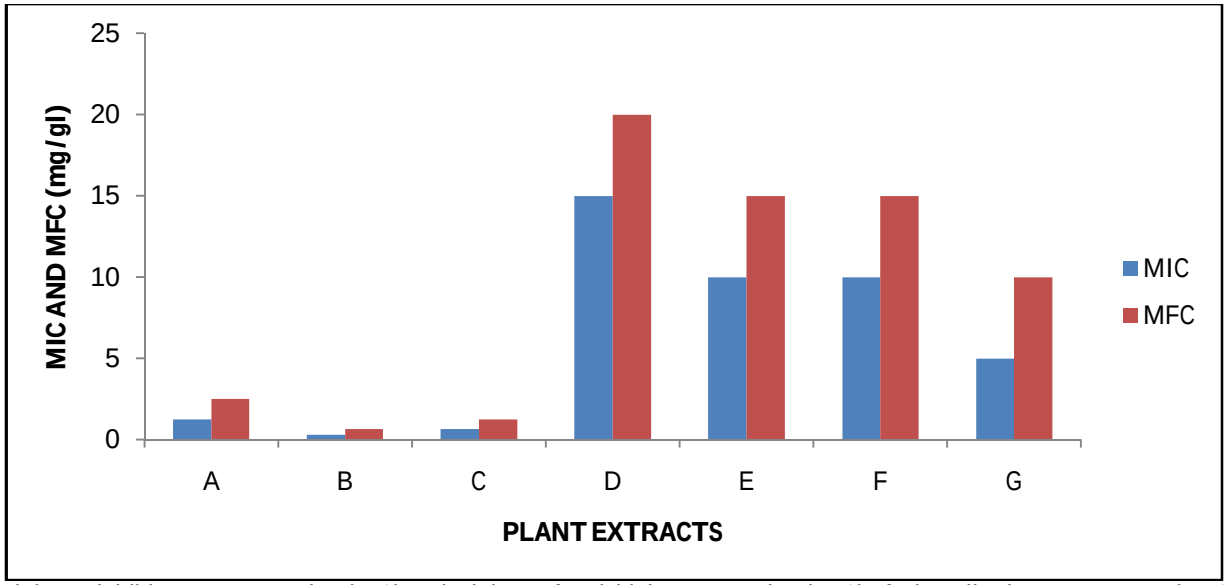

Table 4: Minimum inhibitory concentration (MIC) and Minimum fungicidal concentration (MFC) of ethanolic plant extracts against C. albicans

Key

A: *Acalypha wilkesinia leave* B: *Anogeissus leiocarpus leave* C: *Anogeissus leiocarpus bark*
D: *Bridelia feruginea leave* E: *Bridelia feruginea bark* F: *Parkia biglobosa leave*
G: *Parkia biglobosa bark*

4.Discussion

Candida albicans remains the most common infection-causing fungus, about 45% of clinical infections are caused by this pathogen (Gupta *et al*., 2004). Despite serious environmental implications associated with the excessive use of chemical fungicides still remains the first line of defense against fungal pathogens. Moreover, these fungicides when ingested by human beings and animals through food and water cause various ailments in the body. Search of natural fungicidal principle, from the plant sources would definitely be a better alternative to these hazardous chemicals (Mishra *et al*., 2009). Mahmoudabadi *et al*. (2007) and Al-Bayati and Al-Mola (2008) demonstrated that ethanolic extracts of medicinal herbs inhibit growth of *C. albicans*. The present study showed that similar extracts from *A. wilkesinia leaves, A. leiocarpus leaves and bark* at the concentrations of 30, 25, 20, 15, 10, 5, and 2.5 mg/ml for each extract have promising antifungal activity against *C. albicans*. This study also revealed that increase in the antifungal activity of the extracts was enhanced by increase in the concentration of the extracts. Banso *et al*. (1999) also have reported that higher concentrations of antimicrobial substances show appreciable growth inhibition. The fact that extracts of *A. wilkesinia* and *A. leiocarpus* exhibited antifungal properties justify their traditional use as medicinal plants. This may be due to the presence of active principles in the plant materials. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Ibrahim *et al.,* 1997; Oguntipe *et al.,* 1998). Plant products presently remain the principal source of pharmaceutical agents used in orthodox medicine (Ibrahim *et al*., 1997; Oguntipe *et al.,* 1998). Several species of the genus *Acalypha* have been studied and it has been demonstrated that they have antioxidant, wound healing, post-coital antifertility, neutralization of venom, antibacterial, antifungal and antitrypanosomal activities (Shirwaikar *et al.,* 2004; Perez and Vargas, 2006; Marwah *et al*., 2007).

The minimum inhibitory concentration values of the plant extracts against the test organisms showed that fungi vary widely in the degree of their susceptibility to antifungal agents. This agrees with the report that antimicrobial agents with low activity against an organism have high minimum inhibitory concentration while a highly antimicrobial agent has a low minimum inhibitory concentration Banso *et al*., (1999) and Prescott *et al.,* (2002). When the broth culture of the extract and the test organism used in the minimum inhibitory concentration tests were sub cultured on a solid medium for the assessment of the minimum fungicidal concentration of the extracts, the result indicated that the minimum fungicidal concentration of the extracts were obtained at higher concentrations than in the minimum inhibitory concentration studies. This observation therefore suggests that the antifungal substances contained in the extracts were fungistatic at lower concentrations while becoming fungicidal at higher concentrations of the extracts. The standard antifungals drugs used in this study were found that nystatin was more effective when compared with the used ketoconazole. Ketoconazole belongs to azole antifungal agent, Azoles have direct effect on the fatty acids of cell membranes (Bodey, 1993), and they inhibit ergosterol biosynthesis through their interactions with the enzyme lanosteroldemethylase, which is responsible for the conversion of lanosterol to ergosterol in the fungal cell membrane, leading to the depletion of ergosterol in the membrane (Andriole, 1999; Wynn *et al*., 2003). Our results agree with Shadomy, (1971) in producing inhibitory effect against *C. albicans in vitro*, but Jabra-Rizk *et al*. (2004) demonstrated that Candida cells in biofilm developed resistance against azoles especially fluconazole and clotrimazole *in vivo*, but the formation of biofilms can be inhibited by azoles (Bruzual *et al*., 2007). Similar observations have been reported by Banso and Adeyemo, (2000). Nystatin was chosen as the control against C. *albicans* as it combines with the fungal cell membranes exhibiting both fungicidal and fungistatic activities *in vitro* (Jagedesh, 1991; Shu *et al*., 2001). Many studies have demonstrated the *anti-Candida* activity of Nystatin through the effect on the biosynthesis of fatty acids and phospholipids causing abnormal growth of *C. albicans* (Koul *et al*., 1995; Mahmoudabadi and Drucker, 2006) by increasing membrane fluidity (Van den *et al.,* 1983).

5.Conclusion

Our present study has demonstrated the antifungal potentialities of *A. leiocarpus*, *P. biglobosa*, *B. feruginea*, and *A. wilkesinia*, which has broadened our understanding on the role of the plants in the development of new antifungal therapies.

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