

Original Research Article

Antifungal Potential of Some Nigerian Indigenous Plants: A Remedy for Candidiasis

ABSTRACT

The **Background:** Invasive candidiasis has been recognized as a major cause of mortality and morbidity in hospital settings across the globe. Aside ~~from~~ *C. albicans*, other *Candida* species (*C. glabrata*, *C. parapsilosis*, and *C. tropicalis*) associated with invasive candidiasis have been reported as ~~a~~ public health challenge.

Objective: This study ~~was aimed at evaluating~~ **aimed to evaluate** the antifungal activities of extracts of some indigenous plants using ~~the~~ agar disc diffusion method.

Methods: Fresh samples of the plant parts were collected, identified, air dried, pulverized and extracted using standard methods. The extracts were screened against clinical isolates of *C. albicans*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* using agar disc diffusion method.

Results: All plant extracts exhibited varying ~~zones of inhibition~~ **inhibition zones** ranging from 8.0 - 24 mm against test isolates. Fractions of *Acalypha wilkesiana* Macrophylla (AWR_{F5}) showed the lowest activities against all the test isolates with zones of inhibition ranging from 10.0-13.0 mm while AWR_{F6} fraction of the same plant demonstrated highest antifungal activities against all the test isolates with zones of inhibition ranging from 14.0-24 mm followed by fractions of *Acalypha wilkesiana* Hoffmannii (AWG_{F6}), which demonstrated high (14.0 - 20.0 mm) antifungal activities.

Conclusion: The Plants understudied possessed antifungal potentials and can serve as lead in the development of phytomedicines to combat candidiasis decrease medical as well as financial burden, hence improving the management of cirrhotic patients. These predictors, however, need further work to validate reliability.

Keywords: Invasive candidiasis, Antifungal activities, Plant extracts, Inhibition, *Candida* sp.

1. INTRODUCTION

Within the last three decades, humans have witnessed a significant increase in the occurrence of fungal infections. Some of which are superficial (affecting the nails, hair, skin, and mucosal membranes) while others are systemic (affecting major organs of the body) [1, 2, 3]. Among the fungal infections, ~~the~~ incidence of *Candida* sp. (causative agents of Candidiasis) is on a high increase causing high mortality and morbidity among critically sick patients. This increase in mortality and morbidity rate among patients is often associated

with [an](#) increase in invasive procedures and the number of immunocompromised patients, excessive use of [broad-broad](#)-spectrum antimicrobials, among many other factors [4].

Candida species are dimorphic opportunistic yeast, which are members of [the](#) normal microbiota of the skin, gut, throat, mouth and vagina without causing any problem [5]. However, their status could change from being a normal microbiota to pathogenic microorganisms when [the](#) certain condition of their host changes. Such as: [the](#) emergence of underlining diseases, impaired function of the liver and immune system among many others [6]. Once conditions in their host becomes favorable, *Candida* sp. often proliferate and invade the cells, tissues and even organs of their host often resulting in varying health disorders from discomforting diaper rash, oral and vaginal thrush [7, 8] to [life-life](#)-threatening invasive Candidiasis [9, 10, 11].

Invasive candidiasis simply refers to infection of the blood stream by species of *Candida* (known as Candidaemia) ~~as well as their invasion into deep layers of human cells and tissues causing peritonitis, intra-abdominal abscess andnd their invasion into deep layers of human cells and tissues causing peritonitis, intra-abdominal abscess~~ even osteomyelitis. Invasive candidiasis has been recognized as a major cause of mortality and morbidity in hospital settings across the globe [9]. Aside [from](#) *C. albicans*, which are commonly associated with invasive Candidiasis, other *Candida* species such as *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, and *C. krusei* have all been reported by Cortegiani *et al.*, [4], Dabas *et al.*, [5], Singh *et al.* [7] and Kohli *et al.*, [10] to cause invasive candidiasis across the globe.

Developing countries such as Nigeria are not immune to the occurrence of the aforementioned *Candida* sp. responsible for invasive candidiasis. Studies by Oladele *et al.*, [11], Ezenwa *et al.*, [12], Sule *et al.*, [13], and Uchegbu *et al.*, [14], all reported the incidence of one or more of the causative agents of candidiasis in Nigeria. However, clinicians have found candidiasis difficult to eradicate using conventional drugs owing to the constant abuse of antimicrobial products, which have now resulted in emergence of resistant strains of *Candida* sp. Nevertheless, efforts are constantly being made in search of novel anti-candidiasis substances [s](#) from natural sources, which have seen plants as major reservoirs and sources of anti-candidiasis substances.

Before orthodox medications, the use of plant extracts in folk medicine has been and is still in use by many Nigerians today. This is due to their economic cost and versatility in [the](#) treatments of various ailments. The therapeutic properties of plants have been reported to be attributes of the phytochemicals (i.e., tannin, saponins, phenols, alkaloids, terpenes among many others) and essential oils they possess. These phytochemicals are said to be produced to ward off herbivores and microbial attacks. As such, studies of Spampinato and Leonardi [2], Sharanappa and Vidyasagar [15], and Aboh *et al.*, [16] have all demonstrated antimicrobial activities of different plants at varying concentration.

Thus, this study was carried out to evaluate the antifungal activities of various plants extracts and fractions in a bid to proffer therapeutic alternatives and solution in the treatment of candidiasis.

2. MATERIAL AND METHODS

2.1. Media and chemicals

Sabouraud dextrose agar (SDA) was obtained from Oxoid, Germany. Chloramphenicol, sodium chloride (NaCl) and organic solvents (chloroform, n-hexane, ethanol, ethyl acetate

and methanol) were all of analytical grade obtained from Sigma Aldrich Laboratories, Germany.

2.2. Plant collection and identification

Plants selected for this research were based on knowledge gathered from herbal practitioners and published evidence on their topical application against skin infections. Fresh parts (leaves) of the plants used in this research were collected from different places in Abuja. The plants were taken to the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, for identification and authentication. Voucher specimens of *Leptadenia hastata*, *Lippia multiflora*, *Azelaia africana*, *Acalypha wilkesiana Hoffmannii*, *Alchornea laxiflora*, *Acalypha wilkesiana Macrophylla*, *Crinum jagus*, *Newbouldia laevis*, *Ricinus communis*, *Cassia alata*, *Acacia nolitica* and *Luffa cylindrica* were all deposited in the herbarium for reference purposes.

2.3. Preparation of plant extract

The fresh plants collected were rinsed using running water to remove dirt and other debris before drying them separately for 1 week under open air away from direct sun contact. After drying, the leaves were grinded using porcelain mortar and pestle to reduce the sizes of the leaves, thereby increasing the surface area in contact with solvents to be used. Cold maceration method as explained by Aboh *et al.*, [17] was used. About 200 g of the dried leaves were weighed and dispensed into 500 mL conical flask. A 400 mL of designated solvent (i.e. chloroform, n-hexane, ethanol, ethyl acetate and methanol) was added and stirred gently to ensure it reaches every part of the conical flask. The conical flask was corked and kept in the dark for 48 hours after which, its contents were sieved using a Muslim cloth before using Whatman No. 1 filter paper through a funnel. The filtrate was concentrated using rotary evaporator and dried with water bath at 70°C before storing in the refrigerator at 4°C until use.

2.4. Preparation and standardization of *Candida* species

The fungi species used in this research were obtained from Department of Microbiology and Biotechnology, NIPRD, Abuja. They include: *Candida albicans*, *Candida pseudoparapsilosis*, *Candida parapsilosis*, and *Candida glabrata*. The methods of Aboh *et al.*, [16] were used in the standardization of the test isolates to 0.5 McFarland standards. A colony from a 48 h culture plate was aseptically inoculated into 5 mL of sterile normal saline, followed by adjustment of the turbidity to 0.5 McFarland.

2.5. Antifungal assays

The antifungal properties of the various plant extracts were ascertained using disc diffusion method as demonstrated by Sashikaladevi *et al.*, [18]. Sterile swab sticks were used to inoculate the standardized test isolates onto surfaces of the gelled SDA plates. Various discs impregnated with varying plant extracts at designated concentration were placed at equidistant onto SDA plates using a sterile forceps. Standard antibiotic discs were used as the control. The plates were then incubated in the incubator in an inverted position at 37°C for 24 hours before measuring the zone(s) of inhibition around the impregnated discs.

3. RESULTS AND DISCUSSION

The antifungal activities of methanol, n-hexane, and ethyl acetate crude extracts and fractions of various plants are represented in Table 1. The plant extracts and fractions exhibited varying degree of antifungal activities against the *Candida* sp. tested at different concentration (5, 10, 16, 20 and 32 mg/mL) with diameter zones of inhibition ranging from 8.0 - 24.0 mm were recorded for all solvents. However, methanol crude extracts of *Cassia*

alata and *Azelia africana*, ethyl acetate crude extract of *Newbouldia laevis* and fraction of AWG_{F5} were inactive against the entire *Candida* sp. tested.

C. albicans was ~~found to be~~ resistant to all the crude extracts of *Ricinus communis* and ethyl acetate crude extracts of *Cassia alata*, *Crinum jagus*, *Azelia africana*, *Newbouldia laevis*, *Luffa cylindrica*, and *Leptadenia hastata*. Among the fractions tested for antifungal activity, fractions of *Acalypha wilkesiana* Macrophylla (AWR_{F5}) showed the lowest activities against all the test isolates with zones of inhibition ranging from 10.0 - 13.0 mm while AWR_{F6} fraction of the same plant demonstrated high antifungal activities against all the test isolates with zones of inhibition ranging from 14.0 – 24.0 mm. Fractions of *Acalypha wilkesiana* Hoffmannii (AWG_{F6}) also demonstrated high antifungal activities with zones of inhibition ranging from 14.0-20.0 mm.

Table 1.- Antifungal activities of various plant extracts against test isolates

Plant	Extract	Concentration (mg/mL)	Test Microorganisms			
			<i>C. parapsilosis</i>	<i>C. pseudoparapsilosis</i>	<i>C. glabrata</i>	<i>C. albicans</i>
Zones of inhibition (mm)						
<i>Cassia</i>	Met	16	-	-	-	-

<i>alata</i>	Hex	16	18.0	14.0	12.0	16.0
	Etyl	16	16.0	10.0	13.0	-
	Ace.					
<i>Crinum jagus</i>	Met	16	16.0	8.0	12.0	8.0
	Hex	16	16.0	13.0	12.0	24.0
	Etyl	16	12.0	8.0	-	-
	Ace.					
<i>Afzelia Africana</i>	Met	16	-	-	13.0	-
	Hex	16	15.0	8.0	10.0	8.0
	Etyl	16	-	7.0	20.0	-
	Ace.					
<i>Newbouldia laevis</i>	Met	16	10.0	12.0	10.0	18.0
	Hex	16	-	16.0	8.0	8.5
	Etyl	16	-	-	-	-
	Ace.					
<i>Ricinus communis</i>	Met	16	20.0	10.0	18.0	-
	Hex	16	8.0	8.0	17.0	-
	Etyl	16	16.0	-	-	-
	Ace.					
<i>Lippia multiflora</i>	Met	16	10.0	18.0	10.0	-
	Hex	16	12.0	13.0	16.0	12.0
<i>Luffa cylindrica</i>	Met	5	10.0	18.0	13.0	14.0
	Ethyl	20	9.0	10.0	18.0	-
	Ace.					
<i>Alchornea laxiflora</i>	Met	16	10.0	9.0	-	-
	Hex	16	16.0	9.0	18.0	18.0
<i>Leptadenia hastata</i>	Hex	16	14.0	10.0	12.0	15.0
	Etyl	16	18.0	10.0	12.0	-
	Ace.					
<i>Acacia nilotica</i>	Met	16	-	14.0	9.0	-
<i>Acalypha wilkesiana</i> M	AWR _F	32	10.0	10.0	13.0	-
		5				
	AWR _F	16	14.0	22.0	22.0	24.0
		6				
	AWR _F	32	12.0	16.0	18.0	9.0
		8				
<i>Acalypha wilkesiana</i> H	AWG _F	16	13.0	18.0	12.0	18.0
		2				
	AWG _F	20	-	-	-	-
		5				
	AWG _F	16	20.0	14.0	18.0	19.0

Key: Met (methanol), Hex (n-hexane), Ethyl Ace. (Ethyl acetate), AWR (*Acalypha wilkesiana* Macrophylla (Red variety) Fraction 5, 6 and 8), and AWG (*Acalypha wilkesiana* Hoffmannii (Green variety), Fraction 2, 5 and 6).

Plants over time have shown how essential they are to the pharmaceutical industry. They serve as cheap but yet efficient reservoirs for discovery of essential bioactive compounds that can be used in the formulation of drugs, which has been demonstrated in this study. The plant extracts used were able to inhibit the growth of *Candida* sp. tested (i.e. *Candida albicans*, *Candida pseudoparapsilosis*, *Candida parapsilosis*, and *Candida glabrata*) although at varying degrees using [a](#) different solvent.

The variation of antifungal activities of plant extracts have been associated to the type of plant, geographical location, season of the year, moisture content, type of soil, method of processing among other factors can affect the quality and quantity of bioactive compounds present plants [19]. As such, it is of no surprise that the plant extracts we evaluated differ in terms of antifungal activities. Another factor to be considered is the type of solvent used for the extraction since polarity affects the solubility of compounds present in plant. As such, a plant extracted with different solvent can exhibit varying degrees of activities as this was evidence in this study. For example, antifungal activities of methanol extract of *Azelia africana*, which showed no activity, however differ with 15.0 mm zone of inhibition measured for extracts of n-hexane of the same plant.

This study recorded a resistant trend exhibited by *C. albicans* against all the plant extracts of ethyl acetate. Since other solvents (i.e. methanol and n-hexane) showed activities, this simply means the bioactive compounds responsible for anti-candida activities were not soluble in ethyl acetate. This result is however, contrary to that of Haruna *et al.*, [20], which recorded 7.0 ± 0.4 mm zone of inhibition for ethyl acetate crude extract of *A. wilkesiana* against *C. albicans*. Similarly, Aboh *et al.*, [21] recorded (20.00 ± 0.00 mm) zone of inhibition of ethyl acetate crude extract of *L. cylindrical* against *C. albicans* ATCC 2876. This could be as a result of difference in the strains of *C. albicans* or the period of collection of the plant. - It is however interesting to note that the methanol and n-hexane crude extracts of *A. wilkesiana* in the study of Haruna *et al.*, [20] were not active against *C. albicans* but were active in this study, although, the fractions was utilized. This brings us to the importance of identifying bioactive compounds present in different part of plants.

Parts of plants (i.e. root, leaves, and barks) also display varying antifungal activities. Study of Umaru *et al.*, [22] demonstrated antimicrobial activities of n-hexane extract of *Leptadenia hastata* stem-bark. In their study, they were able to establish that antifungal activities of *L. hastata* are concentration and time dependent. The longer the exposure of bioactive compounds with a simultaneous increase in the extract concentration the higher the zones of inhibition recorded. Although time intervals and range of concentrations was not used in this study, nonetheless, parts of plant used for antimicrobial studies can also influence the activities of plant extracts. Umaru *et al.*, [22] demonstrated n-hexane stem bark of *L. hastata* at 25 ppm to exhibit 0.40 ± 0.00 mm zone of inhibition against *C. albicans* after 24 hours. However, in this study using the same plant but at concentration of 16 mg/mL, 15 mm zone of inhibition was recorded after 24 hours.

4. CONCLUSION

Plant extracts used in this study have antifungal activities and can be used as antifungal agents against *Candida* sp. responsible for invasive candidiasis.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE:

The study highlights the efficacy of "herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

REFERENCES

1. Yousufi MK. To study antibacterial activity of *Allium sativum*, *Zingiber officinale* and *Allium cepa* by Kirby-Bauer method. IOSR J Pharm Biol Sci. 2012; 4(5); 6-8.
1. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, Azeredo J. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. Federation of European Microbiological Societies (FEMS) Microbiology Reviews, 2012. **36**; 288-305.
2. Spampinato C, Leonardi D. *Candida* Infections, Causes, Targets, and Resistance Mechanisms: Traditional and Alternative Antifungal Agents. BioMed Research International. 2013, Article ID 204237. <http://dx.doi.org/10.1155/2013/204237>
3. Sharma, A. Oral candidiasis: An opportunistic infection: A review. International Journal of Applied Dental Sciences, 2019; **5(1)**: 23-27.
4. Cortegiani A., Misseri G, Fasciana T, Giammanco A, Giarratano A, Chowdhary A. Epidemiology, clinical characteristics, resistance, and treatment of infections by *Candida auris*. Journal of Intensive Care, 2018; 6:69-75. <https://doi.org/10.1186/s40560-018-0342-4>
5. Dabas PS. An approach to etiology, diagnosis and management of different types of candidiasis. Journal of Yeast and Fungal Research; 2013; **4(6)**: 63-74.

6. Martins N, Ferreira ICFR, Barros L, Silva S, Henriques M. Candidiasis: Predisposing Factors, Prevention, Diagnosis and Alternative Treatment. *Mycopathologia*, 2014; [https:// doi: 10.1007/s11046-014-9749-1](https://doi.org/10.1007/s11046-014-9749-1)
7. Singh DK., Tóth R, Gácsér A. Mechanisms of Pathogenic *Candida* Species to Evade the Host Complement Attack. *Frontiers in Cellular and Infection Microbiology*. 2020; 10:94-101. [https://doi: 10.3389/fcimb.2020.00094](https://doi.org/10.3389/fcimb.2020.00094)
8. Vila, T., Sultan, A. S., Montelongo-Jauregui, D. and Jabra-Rizk, M. A. Oral Candidiasis: A Disease of Opportunity. *Journal of Fungi*. 2020; 6, 15. [https://doi:10.3390/jof6010015](https://doi.org/10.3390/jof6010015)
9. Pappas, P. G., Lionakis, M. S., Arendrup, M. C., Ostrosky-Zeichner, L. and Kullberg, B. J. Invasive candidiasis. *Nature Reviews Disease Primers*, 2018. **4**:18026. [https://doi:10.1038/nrdp.2018.26](https://doi.org/10.1038/nrdp.2018.26)
10. Kohli S, Shinde CV. Candidiasis and Oral Cavity: A Review. *International Journal of Research and Review*. 2019; **6(5)**: 335-340.
11. Oladele R, Otu AA, Olubamwo O, Makanjuola OB, Ochang EA, Ejembi J, Iruhe N, Ajanaku I, Ekundayo HA, Atoyebi AOO, Denning D. Evaluation of knowledge and awareness of invasive fungal infections amongst resident doctors in Nigeria. *Pan African Medical Journal*; 2020. **36(297)**; 1-9. <https://10.11604/pamj.2020.36.297.23279>
12. Ezenwa BN, Oladele RO, Akintan EP, Fajolu IB, Oshun PO, Oduyebo OO, Ezeaka VC. Invasive candidiasis in a neonatal intensive care unit in Lagos, Nigeria. *Nigeria Postgraduate Medical Journal*; 2017. **24**:150-154.
13. Sule H, Kumurya AS, Shema FB. Candidiasis Opportunistic Mycosis within Nigeria: A Review. *UMYU Journal of Microbiology Research*; 2019. **4(1)**: 77-82.
14. Uchegbu UN, Onyemelukwe NF, Amah HC, Uche-Uchegbu N, Uduji HI, Ndukwe CK., Oparaocha SC. Epidemiological Survey of *Candida* Species Isolated from Immunocompromised Hosts at the University of Nigeria Teaching Hospital, Enugu State. *Journal of Quality in Health care and Economics*. 2020; **3(5)**: 182-193.
15. Sharanappa R, Vidyasagar GM. Anti-*Candida* Activity of Medicinal Plants. A Review. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2013. **5(4)**: 9-16.
16. Aboh MI, Oladosu, P, Adeshina GO, Olayinka BO, Olonitola S, Atasie NH. Screening of selected Medicinal plants for their Antifungal Properties.– *Journal of Clinical and Experimental Microbiology*. 2018; 20(1): 54-59 <https://dx.doi.org/10.4314/ajcem.v20i1.8>
17. Aboh MI, Fidelis S, Oladosu OP, Adeshina GO, Olayinka OB, Olonitola SO. Antifungal potentials of *Luffa cylindrica* (Roem) ethyl acetate leaf extract. *The Journal of Phytopharmacology*. 2020; **9(3)**: 178-187.
18. Sashikaladevi G, Muthu AK, Satheeshkumar D, Rekha S, Nandhini IR. Studies on the Antibacterial and Antifungal Activities of the Ethanolic extracts of *Luffa cylindrica* (Linn) Fruit. *International Journal of Drug Development and Research*. 2009; **1(1)**:105-109.

19. Kanyanga CR, Munduku KC, Ehata TM, Lumpu NS, Maya MB, Manienga K, Bumoyi M, Kabangu KO. Antibacterial and antifungal screening of extracts from six medicinal plants collected in Kinshasa-Democratic Republic of Congo against clinical isolate pathogens. *Journal of Pharmacognosy and Phytotherapy*. 2014; **6(3)**: 24-32. [https://doi: 10.5897/JPP2013.0263](https://doi.org/10.5897/JPP2013.0263)
20. Haruna MT, Anokwuru CP, Akeredolu AA, Akinsemolu AA, Alabi OA. Antibacterial and Antifungal Activity of *Acalypha wilkesiana*. *European Journal of Medicinal Plants*. 2013; **3(1)**: 52-64.
21. Aboh MI, Oladosu P, Adeshina G, Olayinka B, Olonitola SO. Phytochemical screening and antifungal activity of leaves extracts of *Luffa cylindrica* (Roem). *African Journal of Microbiology Research*. 2017; **11(47)**: 1681-1687. [https://doi: 10.5897/AJMR2017.8715](https://doi.org/10.5897/AJMR2017.8715)
22. Umaru IJ, Badruddin FA, Umaru HA. Phytochemical, antifungal and antibacterial potential of *Leptadenia hastata* stem-bark extract. *MOJ Toxicology*. 2018; **4(4)**:263–268. [https://doi: 10.15406/mojt.2018.04.00110](https://doi.org/10.15406/mojt.2018.04.00110)