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

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Authors' contributions

This work was carried out in collaboration among all authors. Author MIA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MIA and JUE performed the laboratory study. Author JUE and JGY managed the literature review and analyses of the study. Author POO supervised and reviewed the work. All authors read and approved the final manuscript.

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ABSTRACT

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. *glabarata, C. parapsilosis, and C. tropicalis*) associated with invasive candidiasis have been reported as a public health challenge.
Objective: This study 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. glabarata, C. parapsilosis* and *C. tropicalis* using agar disc diffusion method.
Results: All plant extracts exhibited varying inhibition zones ranging from 8.0 - 24.0 mm against the tested 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

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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* Hoffmanii (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) [1-3]. 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 [4]. 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-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 become favorable, *Candida* sp. often proliferate and invade the cells, tissues and even organs of their host, resulting in varying health disorders ranging from discomforting diaper rash, oral and vaginal thrush [7,8] to life-threatening invasive Candidiasis [9-11].

Invasive candidiasis simply refers to infection of the blood stream by species of *Candida* (known as Candidaemia) and 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*. *glabarata, 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 like Nigeria is 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 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 attributed 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 reported by 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 put forward alternative therapeutics, that can be potent to treat candidiasis in the treatment of candidiasis.

2. MATERIALS 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 - Nigeria. 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, Afzelia africana, Acalypha wilkesiana* Hoffmanii*, 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 sunlight. After drying, the leaves were grounded 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 reported 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 homogeneity. The conical flask was corked and kept in the dark for 48 hours after which, it 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] was used for 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 where 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 concentrations 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 *Afzelia africana,* ethyl acetate crude extract of *Newbouldia laevis* and fraction of AWG_{F5} were inactive against the entire *Candida* sp. tested.

C. albicans was resistant to all the crude extracts of *Ricinus communis* and ethyl acetate crude extracts of *Cassia alata, Crinum jagus, Afzelia 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

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

Plant	Extract	Concentration (mg/mL)	Test Microorganisms			
			C. parapsilosis	*C. pseudoparapsilosis*	*C. glabrata*	*C. albicans*
			Zones of inhibition (mm)			
Cassia alata	Met	16	-	-	-	-
	Hex	16	18.0	14.0	12.0	16.0
	Etyl Ace.	16	16.0	10.0	13.0	-
Crinum jagus	Met	16	16.0	8.0	12.0	8.0
	Hex	16	16.0	13.0	12.0	24.0
	Etyl Ace.	16	12.0	8.0	-	-
Afzelia Africana	Met	16	-	-	13.0	-
	Hex	16	15.0	8.0	10.0	8.0
	Etyl Ace.	16	-	7.0	20.0	-
Newbouldia laevis	Met	16	10.0	12.0	10.0	18.0
	Hex	16	-	16.0	8.0	8.5
	Etyl Ace.	16	-	-	-	-
Ricinus communis	Met	16	20.0	10.0	18.0	-
	Hex	16	8.0	8.0	17.0	-
	Etyl Ace.	16	16.0	-	-	-
Lippia multiflora	Met	16	10.0	18.0	10.0	-
	Hex	16	12.0	13.0	16.0	12.0
Luffa cylindrica	Met	5	10.0	18.0	13.0	14.0
	Ethyl Ace.	20	9.0	10.0	18.0	-
Alchornea laxiflora	Met	16	10.0	9.0	-	-
	Hex	16	16.0	9.0	18.0	18.0
Leptadenia hastata	Hex	16	14.0	10.0	12.0	15.0
	Etyl Ace.	16	18.0	10.0	12.0	-
Acacia nilotica	Met	16	-	14.0	9.0	-
Acalypha wilkesiana M	AWR_{F5}	32	10.0	10.0	13.0	-
	AWR_{F6}	16	14.0	22.0	22.0	24.0
	AWR_{F8}	32	12.0	16.0	18.0	9.0
Acalypha wilkesiana H	AWG_{F2}	16	13.0	18.0	12.0	18.0
	AWG_{F5}	20	-	-	-	-
	AWG_{F6}	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)

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* Hoffmanii (AWG_{F6}) also demonstrated high antifungal activities with zones of inhibition ranging from 14.0-20.0 mm.

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, inhibited the growth of *Candida* sp. tested (i.e. *Candida albicans, Candida pseudoparapsilosis, Candida parapsilosis, and Candida glabrata*) 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 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 *Afzelia africana*, 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 to established that antifungal activities of *L. hastata* are concentration and time dependent. Thus, 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. Moreover, 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. The understudied plants can thus 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

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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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