Microbiology Research Journal International

Volume 24, Issue 10, Page 49-58, 2024; Article no.MRJI.123875
ISSN: 2456-7043, NLM ID: 101726596
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

In-vitro Antifungal Activity of Ethanol Plant Extracts of *Moringa oleifera, Vernonia amygdalina* and *Ocimum gratissimum* against Some Clinical *Candida* Species

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Authors' contributions

This work was carried out in collaboration among all authors. Authors JOE and CCU designed the study, while author JOE wrote the protocol, did the literature searches and wrote the manuscript. Authors JOE and CCU supervised the entire study. All authors managed the analysis of the study. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/mrji/2024/v34i101490

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/123875

Original Research Article

Received: 23/07/2024
Accepted: 26/09/2024
Published: 28/09/2024

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Cite as: *Ezeadila, Joachim Ohiakwu, Christian Chibuzo Uba, Onyekachukwu Izuchukwu Udemezue, Peace Chidimma Ilo, Christian Chinedu Orji, and Chukwuebuka Chisom Anene. 2024. "In-Vitro Antifungal Activity of Ethanol Plant Extracts of Moringa Oleifera, Vernonia Amygdalina and Ocimum Gratissimum Against Some Clinical Candida Species". Microbiology Research Journal International 34 (10):49-58. https://doi.org/10.9734/mrji/2024/v34i101490.*

ABSTRACT

Vaginal candidiasis, primarily caused by microorganisms belonging to the *Candida* genus, is a common fungal infection prevalent among millions of women worldwide and can lead to significant morbidity. The treatment of *Candida* infections has often relied on antifungal drugs such as azoles and echinocandins. However, the emergence of resistance among *Candida* species to these drugs poses a significant challenge to effective treatment. This study was, thus, aimed at evaluating the *in vitro* antifungal activity of ethanol extracts of some selected medicinal plants against *Candida* species isolated from high vaginal swabs of some women attending a hospital in Enugu State, Nigeria. Six (6) isolates resistant to three or more commercial antifungal drugs were selected for this study. These isolates include *Candida tropicalis* (2), *Candida albicans* (2), *Candida Parapsilosis* (1) and *Candida krusei* (1). The plants used were *Moringa oleifera* leaves, *Vernonia amygdalina* and *Ocimum gratissimum*. The leaves were dried, pulverized and 300g of each was extracted using ethanol in a Soxhlet extractor at 70°C for 6hrs. The concentrated extract of each plant was reconstituted in Dimethyl sulfoxide and different concentrations of 400 mg/ml, 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.25 mg/ml were obtained using double fold serial dilution. The susceptibility of the *Candida* species to the ethanol plant extracts was carried out using the agar well diffusion method. The results showed that the Extract of *Moringa oleifera* had the highest inhibition zone diameter (19 mm) at 200 mg/ml against resistant *Candida albicans*[1], followed by the extract of *Vernonia amygdalina* and *Ocimum gratissimum* with inhibition zone diameters of 18.67 mm and 18 mm at 100 mg/ml and 200 mg/ml respectively. *Candida tropicalis*[1] was sensitive to all the plant extracts at all concentrations, while *Candida parapsilosis* was resistant to the extract of *Ocimum gratissimum* at all concentrations. The activity of the extracts of *Moringa oleifera* and *Ocimum gratissimum* against *Candida tropicalis*[1] was concentration dependent. This study also revealed that *Candida krusei* was resistant to all the extracts of the plants at the highest concentration of 200 mg/ml. The antifungal activities of these plant extracts implies these plants have great therapeutic potential that can be harnessed. This study, thus, recommends that these plants be investigated further for possible use in the formulation of antifungal drugs especially, against those diseases caused by *Candida* species that have developed resistance to the commonly used antifungal drugs.

Keywords: Vaginal candidiasis; Candida species; resistance; ethanol plant extracts; Enugu State.

1. INTRODUCTION

Vaginal infections caused by fungal pathogens, particularly those belonging to the *Candida* genus, are prevalent among women and can lead to significant morbidity [1,2]. It has also been reported that *Candida* species are the primary causative agents of vaginal candidiasis, which is a common fungal infection affecting millions of women worldwide [3]. *Candida albicans* is the most common cause of vaginal candidiasis, accounting for approximately 80-90% of cases [3]. However, other *Candida* species such as *Candida* glabrata, *Candida* tropicalis, and *Candida* krusei, also emerge as significant pathogens [4]. In Nigeria, the increasing incidence of candidiasis has been linked to various factors, including antibiotic overuse, which disrupts normal flora, and the rising prevalence of conditions such as diabetes and HIV/AIDS, which compromise the immune system [1,2].

The treatment of *Candida* infections has historically relied on antifungal medications such as azoles and echinocandins. However, the emergence of antifungal resistance among *Candida* species poses a significant challenge to effective treatment. Resistance mechanisms, including biofilm formation and mutations in drug targets, have been documented, leading to treatment failures and recurrent infections [5]. The increasing resistance of *Candida* species to conventional antifungal agents has led to a growing need for novel antifungal agents [6]. This situation necessitates the exploration of alternative therapeutic options, particularly those derived from natural sources, as there is a growing interest in herbal medicine in Nigeria, where traditional practices often complement modern healthcare [7].

Medicinal plants have been a rich source of novel antifungal compounds, with various studies demonstrating their efficacy against *Candida* species [6,8,9]. These plants have been used for

centuries to treat various fungal infections, including vaginal candidiasis [8].

Among the plants traditionally used for medicinal purposes, *Moringa oleifera, Vernonia amygdalina*, and *Ocimum gratissimum* are well-studied in the Nigerian context for their pharmacological properties. *Moringa oleifera*, commonly known as the drumstick tree, is noted for its high nutritional value and medicinal applications. Recent studies in Nigeria have highlighted its antifungal properties, particularly against various *Candida* species, demonstrating its potential as a natural therapeutic agent [10]. A study by [8] investigated the *in vitro* antifungal activity of ethanol extracts from Garcinia kola, Psidium guajava, and Ocimum gratissimum against *Candida albicans* isolated from vaginal swabs. The results showed that the ethanol extracts exhibited significant antifungal activity against *Candida albicans*, with Garcinia kola showing the highest activity.

Similarly, *Vernonia amygdalina*, often referred to as bitter leaf, has been recognized for its antimicrobial activities in Nigerian ethnomedicine. Research indicates that its extracts exhibit significant antifungal effects, attributed to its rich content of secondary metabolites such as flavonoids and terpenoids [11]. These compounds have been shown to disrupt the integrity of fungal cell membranes, enhancing their efficacy against resistant strains. Similarly, [12], showed the *in vitro* antimicrobial activities of *Vernonia amygdalina* on selected clinical isolates, including *Candida albicans.*

Ocimum gratissimum, commonly known as holy basil, is another plant with notable antifungal properties. Its essential oils have been reported to possess strong inhibitory effects against various fungal pathogens, including *Candida* species. The bioactive compounds found in *Ocimum gratissimum*, such as eugenol and rosmarinic acid, contribute to its antimicrobial activities [13]. Also, [14] reported the sensitivity of *Candida albicans* to six extracts of locally used antifungal plants, including *Moringa oleifera* and *Ocimum gratissimum*.

The traditional use of these plants in Nigeria highlights the importance of integrating indigenous knowledge with scientific research to identify effective treatments for fungal infections. This study was, thus, aimed at evaluating the *in vitro* antifungal activity of ethanol extracts of *Moringa oleifera, Vernonia amygdalina* and *Ocimum gratissimum* against *Candida* species isolated from high vaginal swabs of some women attending a hospital in Enugu State, Nigeria.

2. MATERIALS AND METHODS

2.1 Source of Test Microorganisms

The test microorganisms were isolated from high vaginal swab (HVS) specimens of women attending the Obstetrics and Gynecology Unit of the University of Nigeria Teaching Hospital (UNTH) Ituku/Ozalla, Enugu State, Nigeria as described [15]. The antifungal sensitivity of these isolates to some commercial antifungal drugs was tested using disc diffusion method as described by [16]. Six (6) isolates resistant to three or more commercial antifungal drugs were selected for this study. These isolates include *Candida tropicalis* (2), *Candida albicans* (2) *Candida Parapsilosis* (1) and *Candida krusei* (1). *Candida tropicalis* and *Candida albicans* are differentiated as *Candida tropicalis*^{1} and *Candida tropicalis*^{2} and *Candida albicans*^{1} and *Candida albicans*^{2} respectively.

2.2 Collection of Plant Materials

Fresh leaves of *Moringa oleifera* were collected from around the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria while *Vernonia amygdalina* leaves were collected from Umukwa village in Awka, Anambra State, Nigeria. The fresh leaves of *Ocimum gratissimum* were bought from “Ogige market” (a local market) in Nsukka, Enugu state, Nigeria. The plants were identified by Late Mrs Aziagba Bibian (a plant taxonomist) in the Department of Botany, Nnamdi Azikiwe University, Awka. The herbarium numbers of the plants were as follows: *Moringa oleifera* (NAU H No 01A), *Vernonia amygdalina* (NAU H No 47A) and *Ocimum gratissimum* (NAU H No 35A).

The leaves were dried under shade for seven days and milled into powder with the aid of an electric Qlink blender (Model QBL-20L40P) as described by [17] and reported by [18]. The pulverized leaves of each plants were transferred into a pre-weighed clean container, weighed, appropriately labelled, covered tightly and kept at room temperature for further use.

2.3 Extraction of Plant Materials and Percentage Yield

After weighing the pulverized plant leaves, 300g was extracted using analytical grade of ethanol

(Anala® BDH Chemicals Ltd, Pool, England) in a Soxhlet extractor at 70°C for 6hours. The plant weight to solvent volume was in the ratio of 1:5. That is, for every 100g of pulverized plant leaves, 500ml of ethanol was used for the extraction. The extract recovered was then concentrated by surface evaporation to dryness under room temperature. This helped remove the solvent, leaving a solvent-free extract. The concentrated extract was transferred to a sterile container and kept in the refrigerator at 20°C until required for analysis.

The yield of crude plant extract was obtained by measuring its dry weight before and after extraction and the % yield of extract was given as

$$\% \text{ yield of extract} = \frac{crude\ extract\ weight}{initial\ dry\ weight} \times 100$$

2.4 Preparation of Stock Solution of the Extracts

Stock solutions of the ethanolic extracts of the leaves of *Moringa oleifera*, *Vernonia amygdalina*, and *Ocimum gratissimum* were prepared by weighing out 1.6 g of each extract using an electronic weighing machine. This was then dissolved completely in 4 ml of Dimethyl sulfoxide (DMSO) (JHD Guangbong Guanghue Sci-TechCo, Ltd, Shamtou Guangdong, China) in sterile bottles to give a stock concentration of 400 mg/ml of the individual extracts. A double fold serial dilution was performed on the stock solution by transferring 2 ml of the solution into an equal volume (2 ml) of DMSO in another bottle. This resulted in a concentration of 200 mg/ml. Progressively, different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml and 12.25 mg/ml were obtained.

2.5 Standardization of Inoculum and *In vitro* Antifungal Susceptibility Testing Using the Plant Extracts

Using a sterile wire loop, discrete colonies each of 24 hours pure culture of the *Candida* isolates were picked and inoculated into 5ml of sterile 0.85% saline. The turbidity of the suspension was adjusted and then matched visually with 0.5 McFarland standard, which is equivalent to 1 x 10^6 colony-forming units per ml (CFU/ml).

The antifungal potency of the plant extracts was evaluated against the selected six (6) *Candida* species that were resistant to four or more of the commercial antifungal drugs as already mentioned. This was carried out using agar well diffusion method as described by [19]. A pure culture of the selected resistant strains of the *Candida* species was exposed to different dilutions of the individual crude plant extracts for antimicrobial evaluation. Mueller Hinton agar (20 ml) in Bijou bottles- was previously prepared and allowed to cool to warm touch, and it was introduced into sterile Petri dishes. Subsequently, 0.1ml of standardized inoculum (containing approximately 1 x 10^6 cfu/ml) was introduced into each of the Mueller Hinton agar medium and shaken for even distribution in the Petri dish. This was allowed to cool and gel. A sterile cork borer dug wells of 9 mm in diameter each into the Mueller Hinton agar. The individual plant extracts were introduced into the corresponding labeled wells using a sterile micropipette. In one of the wells in each Mueller Hinton agar plate, 0.2ml of DMSO was introduced to serve as negative control. This was carried out in duplicates and the plates were then incubated at 25°C ± 2°C for 24hrs. After the incubation period, each plate's inhibition zone diameter (in mm) was measured and the mean recorded.

2.6 Statistical Analysis

All values of inhibition zone diameters for the plant extracts were expressed as mean ± Standard deviation and difference between the inhibition zone diameters across the plant extracts were also considered significant at $p < 0.05$ using one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of the Plant Extracts

Table 1 shows the percentage yield of the individual plant extract. *Moringa oleifera* had the highest percentage yield of 15.0% followed by *Vernonia amygdalina* (12.0%) and *Ocimum gratissimum* (11.67%).

3.2 Inhibition Zone Diameter (IZD) of the Ethanol Plant Extracts against Resistant Strains of *Candida albicans*

The inhibition zone diameter (IZD) of the plant extracts against some resistant strains of the isolated *Candida albicans* is presented in Tables 2 and 3. Extract of *Moringa oleifera* had the highest inhibition zone diameter (19 mm) at

200 mg/ml against resistant *Candida albicans*[1], followed by extract of *Vernonia amygdalina* and *Ocimum gratissimum* with inhibition zone diameters of 18.67 mm and 18 mm at 100 mg/ml and 200 mg/ml respectively. *Candida albicans*[1] was totally resistant to *Ocimum gratissimum* at 25 mg/ml and 12.25 mg/ml (Table 2). There was also total resistance by *Candida albicans*[2] to extracts of *Vernonia amygdalina* (at 200 mg/ml and 100 mg/ml) and *Ocimum gratissimum* (at 25 mg/ml and 12.25 mg/ml). The highest IZD (18.33mm) against *Candida albicans*[2] was exhibited by extracts of *Vernonia amygdalina* at 50 mg/ml followed by *Ocimum gratissimum* (18 mm) at 200 mg/ml. The extract of *Moringa oleifera* showed the least IZD (11.67 mm) at 12.25 mg/ml (Table 3). Similar researches support the antifungal activity of the extract of *Moringa oleifera* against *Candida albicans*. In a study carried out by [20] in Dutse, Jigawa State, the ethanol extract of *M. oleifera* was found to possess antifungal activity against *Candida albicans* with the inhibition zone diameter being up to 22 mm at a concentration of 5000 µg/ml. Similarly, [21] showed the ethanolic leaf extracts of *Moringa oleifera* exhibited antifungal activity against *Candida albicans*, giving an inhibition zone diameter of 11 mm at 100 mg/ml concentration. However, present study's findings do not agree with that of [22] who reported that *Candida albicans* (MTCC No. 183) was resistant to both aqueous and ethanolic extracts of *Moringa oleifera*. Also, [21] reported that *Candida albicans* was resistant to ethanolic extracts of *Moringa oleifera* at concentrations of 50, 25 and 12.5 mg/ml, unlike in the present study in which ethanolic extracts of *Moringa oleifera* at concentrations of 50, 25 and 12,5 mg/ml showed activity against both *Candida albicans*[1] and *Candida albicans*[2].

The present study agrees with some other studies that showed that ethanolic extracts of *V. amygdalina* have antifungal properties against *Candida albicans*, though with lower IZDs. For example, [23] showed that ethanolic extracts of *V. amygdalina* had antifungal activity against *Candida albicans* with inhibition zone diameters (IZD) of 10.67±1.15mm at 100 mg/ml. In another study by [12] in Maiduguri, Borno State, ethanolic leaf extracts of *V. amygdalina* displayed zones of inhibition of 12.4mm against *Candida albicans* isolated from urine specimens. Contrary to the result of this study, the findings of [24] showed that clinical wound isolates of *Candida albicans* from patients in the surgical wards at Nnamdi Azikiwe Teaching Hospital (NAUTH), Nnewi were resistant to both the ethanol and methanol extracts of *V. amygdalina* at all concentrations (6.25 mg/ml to 100 mg/ml). However, there was also total resistance by *Candida albicans*[2] to extracts of *Vernonia amygdalina* at concentrations of 200 mg/ml and 100 mg/ml.

Table 1. Percentage yield of the plant extracts

Plants	Initial Dry Weight (g)	Crude Extract Weight (g)	Percentage yield (%)
Moringa oleifera	300	45	15.0
Vernonia amygdalina	300	36	12.0
Ocimum gratissimum	300	25	11.67

Table 2. Inhibition zone diameter of the ethanol plant extracts against resistant *Candida albicans*[1]

	Inhibition Zone Diameter (mm)				
Plant Extracts	**200 mg/ml**	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.25 mg/ml**
Moringa oleifera	19 ± 0.0	17.33±0.577	15.67± 0.0	15 ± 0.0	14.33±0.577
Vernonia amygdalina	15.33±0.577	18.67±0.577	17 ± 1.0	15 ± 0.0	13 ± 0.0
Ocimum gratissimum	18 ± 0.0	16 ± 0.0	14 ± 0.0	0 ± 0.0	0 ± 0.0

Table 3. Inhibition zone diameter of the ethanol plant extracts against resistant *Candida albicans*[2]

Plant Extract	Inhibition Zone Diameter (mm)				
	200 mg/ml	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.25 mg/ml**
Moringa oleifera	15.00 ± 0.000	16.33 ± 0.577	14.67± 0.577	14.00 ± 0.000	11.67± 0.577
Vernonia amygdalina	0.00 ± 0.000	0.00 ± 0.000	18.33 ±0.577	17.00 ± 0.000	15.33 ±0.577
Ocimum gratissimum	18.00 ± 0.000	15.67 ± 0.577	14.00 ±0.000	0.00 ± 0.000	0.00 ± 0.000

The result of the study of [18] conforms with that of the present study. Their results showed that ethanolic extract of the leaves of *Ocimum gratissimum* was active against both the clinical isolate of *Candida albicans* and the control strain (*Candida albicans* ATCC 90028) giving inhibition zone diameters of 16mm and 13mm at 100 mg/ml and 13mm and 10mm at 50 mg/ml respectively. Both the clinical isolate and control strains were resistant to concentrations of 25, 12.5 and 6.25 mg/ml of the extracts as also recorded in this study.

3.3 Inhibition Zone Diameter (IZD) of the Ethanol Plant Extracts against Resistant Strains of *Candida tropicalis*

Tables 4 and 5 show the IZD of the plant extracts against resistant strains of *Candida tropicalis*[1] and *Candida tropicalis*[2], respectively. *Candida tropicalis*[1] was sensitive to all the plant extracts at all concentrations. The IZDs produced by extracts of *Moringa oleifera* and *Ocimum gratissimum* against *Candida tropicalis*[1] were concentration-dependent, ranging from the least IZD of 11.33 mm (at 12.25 mg/ml) for *Ocimum gratissimum* to the highest IZD of 17.00mm (at 200 mg/ml) for extracts of *Moringa oleifera* and *Vernonia amygdalina* (Table 4). For *Candida tropicalis*[2], the IZD ranged from 11.67mm for *Moringa oleifera* at 12,25 mg/ml to 17.67mm for *Vernonia amygdalina* at 200 mg/ml. *Candida tropicalis*[2] was resistant to extract of *Moringa oleifera* at 200 mg/ml (Table 5). This agrees with the work of [25] who demonstrated the antifungal activity of chloroform and ethanolic extracts (especially from leaves and flowers) of *Moringa oleifera* against some strains of *Candida* species including *Candida tropicalis*. In India, [22] reported that both aqueous and ethanolic extracts of *Moringa oleifera* had little activity against *Candida tropicalis* (MTCC No.1000). In their findings, [26] showed that the *Ocimum gratissimum* essential oil had fungicidal activity against some *Candida* species including *Candida tropicalis*. However, *Candida tropicalis* was the least susceptible.

3.4 Inhibition Zone Diameter (IZD) of the Ethanol Plant Extracts against *Candida parapsilosis*

The IZD of the plant extracts against resistant *Candida parapsilosis* is shown in Table 6. The microorganism was resistant to the extract of *Ocimum gratissimum* at all concentrations (Fig. 1), while the extracts of both *Moringa oleifera* and *Vernonia amygdalina* had activity at all concentrations with various IZDs ranging from 14.33 mm (at 12.25 mg/ml) to 19.00 mm (at 200 mg/ml) and from 12.33mm (at 12.25 mg/ml) to 19.00 mm (at 200 mg/ml) respectively (Fig. 2). This agrees with the study of [25] who demonstrated the antifungal activity of chloroform and ethanolic extracts of *Moringa oleifera* against some strains of *Candida* species (*Candida ciferrii*, *Candida famata*, *Candida guilliermondii*, *Candida parapsilosis* and *Candida tropicalis*). Similarly, [26] showed that *Candida parapsilosis* was most susceptible to *Ocimum gratissimum* essential oil.

3.5 Inhibition Zone Diameter (IZD) of the Ethanol Plant Extracts against *Candida krusei*

Candida krusei was totally resistant to all the extracts of the plants at 200 mg/ml concentration and also resistant to *Ocimum gratissimum* at 12.25 mg/ml. It is expected that the higher the concentration, the higher the IZD. However, at the highest concentration of 200 mg/ml, *Candida krusei* was totally resistant to all the plant extracts. A possible explanation may be that at that high concentration, the diffusion of the extracts through the medium was very slow and the growth of the microorganism was faster than the extract could diffuse [27]. The highest IZD (18 mm) was shown by extract of *Vernonia amygdalina* at 100 mg/ml followed by *Ocimum gratissimum* (16mm) at the same concentration. Extract of *Moringa oleifera* at 12.25 mg/ml showed the least IZD (11.67 mm) against *Candida krusei* (Table 7). A study by [26] showed that the *Ocimum gratissimum* essential oil had

Table 4. Inhibition zone diameter of the ethanol plant extracts against Resistant *Candida tropicalis*[1]

	Inhibition Zone Diameter (mm)				
Plant Extracts	**200 mg/ml**	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.25 mg/ml**
Moringa oleifera	17.00 ±0.00	15.67±0.577	15.00±0.00	14.67±1.53	14.00 ±0.00
Vernonia amygdalina	17.00 ±0.00	15.67 ±0.577	15.00 ±0.00	14.67±1.53	14.00±0.00
Ocimum gratissimum	15.00±0.00	13.67±0.577	13.00±0.00	11.67±0.577	11.33±0.00

Table 5. Inhibition zone diameter of the ethanol plant extracts against resistant *Candida tropicalis*[2]

Plant Extract	Inhibition Zone Diameter (mm)				
	200 mg/ml	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.25 mg/ml**
Moringa oleifera	0.00 ± 000	17.00 ± 0.000	15.33 ±0.577	14.00 ±1.732	11.67 ±1.155
Vernonia amygdalina	17.67 ± 0.577	16.33 ±0.577	16.00 ±0.000	15.00 ±0.000	13.00 ±0.000
Ocimum gratissimum	17.00 ±.000	16.00 ±.000	14.00 ±1.000	13.67 ±0.577	12.33 ±.577

Table 6. Inhibition Zone Diameter of the Ethanol Plant Extracts against Resistant *Candida parapsilosis*

Plant Extract	Inhibition Zone Diameter (mm)				
	200 mg/m	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.25 mg/ml**
Moringa oleifera	19.00± 0.000	17.33± 0.577	15.67± 0.577	15.00± 0.000	14.33± 0.577
Vernonia amygdalina	19.00±1.000	17.67± 0.577	15.33±1.528	13.33±1.528	12.33± 0.577
Ocimum gratissimum	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

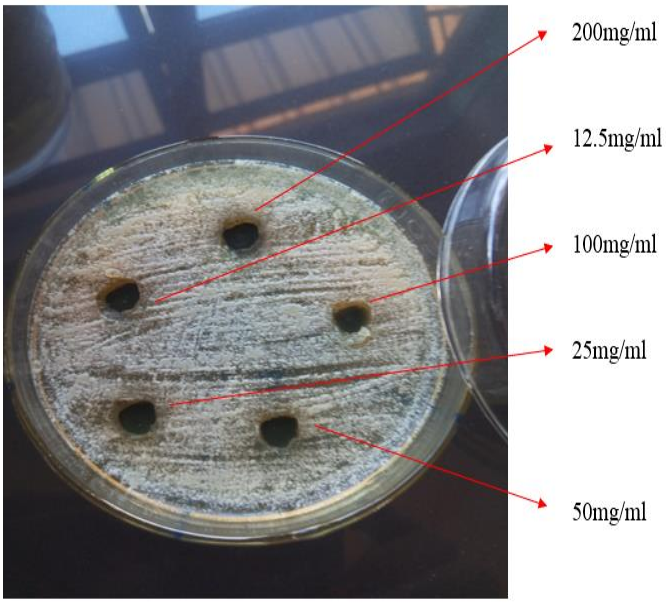

Fig. 1. Total resistance (No inhibition zone diameters) to the Ethanolic extracts of *Ocimum gratissimum* (at all Concentrations) by *Candida parapsilosis*

fungicidal activity against *Candida krusei, Candida parapsilosis*, *Candida tropicalis* and *Candida albicans*. Also, the ethanolic and methanolic extracts of the leaves of *Ocimum gratissimum* showed antifungal effect against *Candida krusei* producing inhibition zone diameters of 13.00±2.00mm and 14.67±1.53mm respectively ([23]). However, the result of the present study doesn't agree with that of [23], who reported that both the ethanolic and methanolic extracts of *V. amygdalina* had no activity against *Candida krusei*.

Table 7. Inhibition zone diameter of the ethanolic plant extracts against resistant *Candida krusei*

Plant Extract	Inhibition Zone Diameter (mm)				
	200 mg/ml	**100 mg/ml**	**50 mg/ml**	**25 mg/ml**	**12.25 mg/ml**
Moringa oleifera	0.00 ± 0.000	14.33 ± 0.577	13.00 ±0.000	12.00± 0.000	11.67 ±0.577
Vernonia amygdalina	0.00 ± 0.000	18.00 ± 0.000	15.00 ±0.000	14.00± 0.000	13.00± 0.000
Ocimum gratissimum	0.00 ± 0.000	16.00 ± 0.000	14.00± 0.000	13.00± 0.000	0.00± 0.000

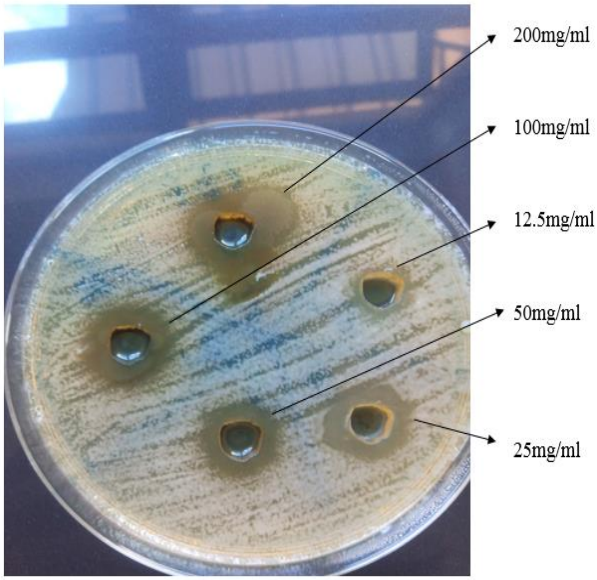

Fig. 2. Inhibition Zone Diameter of Ethanolic extracts of *Moringa oleifera* (at different Concentrations) against *Candida parapsilosis*

The differences observed in the activities of the plant extracts in this study, when compared with the findings of other studies may be due to different strains of the *Candida* species used in the different studies. Also, the antimicrobial activity of the plant extracts can be influenced by the method of their preparation as well as the choice of solvents used [28,29]. The age of the plant, as well as the time of harvest, can determine the amount of active constituents (phytochemical substances) and hence, the potency of the plants [30]. In cases where there was no activity by any of the plant extracts, it may be due to the absence of some secondary metabolites or the presence of some in low concentration; or it may be due to the type of strains used or a slight change in any of the factors that are likely to affect rate of microbial growth or rate of diffusion of the test agent [18]. The antifungal activities shown by these plant extracts is not unrelated to the presence of secondary metabolites (such as saponins, flavonoids, tannins, carbohydrates, glycosides, reducing sugar and other active ingredients of plants) which are responsible for the antimicrobial activities shown by these extracts [17].

4. CONCLUSION

In the present study, the Extract of *Moringa oleifera* had the highest inhibition zone diameter (19mm) at 200 mg/ml against resistant *Candida albicans*[1], followed by the extract of *Vernonia amygdalina* and *Ocimum gratissimum* with inhibition zone diameters of 18.67mm and 18mm at 100 mg/ml and 200 mg/ml respectively. *Candida tropicalis*[1] was sensitive to all the plant extracts at all concentrations while *Candida parapsilosis* was resistant to the extract of *Ocimum gratissimum* at all concentrations. The IZDs produced by extracts of *Moringa oleifera* and *Ocimum gratissimum* against *Candida tropicalis*[1] were concentration dependent. This study also revealed that *Candida krusei* was totally resistant to all the extracts of the plants at the highest concentration of 200 mg/ml.

The ethanol extracts of the three plants in this study showed varying degrees of antifungal activity. Thus, the activities of these plant extracts against these *Candida* species implies that plants have great therapeutic potentials that can be harnessed for the formulation of drugs especially against diseases caused by yeasts resistant to the commonly used antifungal drugs like Fluconazole. This study recommends that these plants be investigated further for possible use in the formulation of antifungal drugs.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

ACKNOWLEDGEMENTS

This work was supported by a grant award by the Education Trust Fund (established by the Federal Government of Nigeria).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dhanasekaran S, Selvadoss PP, Manoharan SS, Jeyabalan S, Devi Rajeswari V. Revealing anti-fungal potential of plant-derived bioactive therapeutics in targeting secreted aspartyl proteinase (SAP) of *Candida albicans*: A molecular dynamics approach. Journal of Molecular Graphics and Modelling. 2023; 118:108282.
2. Eze EE, Okwu DE, Okwu IM. The role of antifungal resistance in recurrent *Candida* infections in Nigeria: A review. African Journal of Microbiology Research. 2022; 16(5):151-160.
3. Sobel JD. Vulvovaginal candidiasis. Lancet. 2016;387(10034):1362-1371.
4. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Nagy E, Dobiasova S, et al. *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: Geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. Journal of Clinical Microbiology. 2010; 48(10):3575-3582.
5. Selvaraj G, Wilson J, Kanagaraj N, Subashini E, Thangavel S. Enhanced antifungal activity of *Piper betle* against candidiasis infection causing *Candida albicans* and in silico analysis with its virulent protein. Journal of Fungi. 2022; 8(12):1348.
6. Kumar A, Singh, R, Kumar P. Antifungal activity of medicinal plants against *Candida* species: A review. Journal of Mycology. 2020;2020: 1-12.
7. Akinmoladun OF, Olarewaju FO, Abiola O. Ethnopharmacological survey of herbal antifungal treatments in Nigeria: A systematic review. Journal of Ethnopharmacology. 2023;300:115858.
8. Esimone CO, Nworu CS, Okoye FBC. In vitro antifungal activity of some medicinal plants against *Candida albicans*. Journal of Ethnopharmacology. 2017;206:241-248.
9. Okeke MI, Nwachukwu SC, Eze EA. Antifungal activity of crude extracts of some medicinal plants against *Candida* species. Journal of Mycology. 2018;1-8.
10. Sarkar S, Chaudhuri B, Guchhait P, Das S. Antibacterial and antifungal activities of Avicennia marina extract against various multiple drug resistant microorganisms. Natural Product Research. 2023;37(2): 203-208.
11. Ameer MR, Moghul NB, Javed A, Azhar Butt M, Abbas HB. The therapeutic potential of the medicinal plant *Justicia adhatoda* and its antibacterial and antifungal activities: A comparative study. Journal of Ethnopharmacology. 2022;295: 115378.
12. Ghamba PE, Balla H, Goje LJ, Halidu A, Dauda MD. *In vitro* Antimicrobial Activities of *Vernonia amygdalina* on Selected Clinical Isolates. International Journal of Current Microbiology and Applied Sciences. 2014;3(4):1103-1113.
13. Sabreen S, Niaz S, Hussain A, Muhammad I, Nayab GE. Antifungal potential of selected medicinal plants against *Candida* albicans and HPLC analysis. Journal of Medicinal Plants Research. 2022;16(1):1-9.
14. Vroumsia T, Moussa D, Bouba G, Daniel EM, Ebot AC, Eneke T, et al. Prevalence of Vulvovaginal Candidiasis amongst Pregnant Women in Maroua (Cameroon) and the Sensitivity of *Candida albicans* to Extracts of Six Locally Used Antifungal Plants. International Research Journal of Microbiology. 2013;4(3):89-97.
15. JO Ezeadila, CA Oyeka, I Okoli, LC Chidi-Onuorah. Prevalence of vaginal *Candida* colonization among women Attending University of Nigeria Teaching Hospital, Enugu State, Nigeria. Global Journal of Advanced Research.2020a;7(4):88-95.
16. JO Ezeadila, I Okoli, CA Oyeka. Antifungal resistance pattern of *Candida* Species Isolated From High Vaginal Swabs Of Women Attending A Hospital In Enugu State, Nigeria. Journal of Advances in Microbiology. 2020b;20(I9):62-72.
17. Nweze EI, Okafor JI, Njoku O. Antimicrobial Activities of Methanolic Extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida* Benth Used in Nigeria Herbal Medicinal Practice. Journal of Biological Research and Biotechnology. 2004;2(1):36-39.

18. Nweze EI, Eze EE. Justification for the use of *Ocimum gratissimum* L in Herbal Medicine and its Interaction with Disc Antibiotics. BMC Complementary and Alternative Medicine. 2009;9**:**37.
19. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis.2016;6:71–79.
20. Aisha SB, Kutama AS, Kabir S, Paul AT. Phytochemical screening and antifungal activity of *Moringa oleifera* on Some Selected Fungi in Dutse, Jigawa State. Global Advanced Research Journal of Agricultural Science. 2016;5(6):243-248.
21. Bassey EE, Mohammed GA, Mohammed HB, Bashir MA, Buhari BY, Okeke I, Umeh SO, Abubakar M, Mbanusi B.C omparative phytochemical screening and *in vitro* antimicrobial activity of aqueous, ethanolic and ethyl acetate extracts of Stem Bark and Leaves of Horse Radish (*Moringa oleifera*) Plant. Academic Journal of Life Sciences. 2016;2(9):61-76.
22. Pinal P, Nivedita P, Dhara P, Sharav D, Dhananjay M. Phytochemical analysis and antifungal activity of *Moringa oleifera.* International Journal of Pharmacy and Pharmaceutical Sciences. 2014;6(5):144-147.
23. Erute MA, Egboduku OW. Screening of some Nigerian Medicinal Plants for Anti-*Candida* Activity. American Journal of Drug Discovery and Development. 2013;3(2):50-71.
24. Oshim IO, Desmond CO, Nwobu RAU, Ezugwu UM, Urama EU. Kinetics of Minimum Inhibitory Concentration, Minimum Bactericidal Concentration and Minimum Fungicidal Concentration of *Vernonia amygdalina* (Bitter leaf) on Microorganisms Isolated from Wound Infections. International Journal of Surgical Research. 2016;5(1):8-14.
25. Rocha MFG, de Alencar LP, Brilhante RSN, Sales J, de Ponte YB, Rodrigues PH, et al. *Moringa oleifera* Inhibits Growth of *Candida* spp. and *Hortaea werneckii* Isolated from *Macrobrachium amazonicum* Prawn Farming with a Wide Margin of Safety. Ciência Rural, Santa Maria. 2014; 44(12):2197-2203.
26. Nakamura CV, Ishida K, Faccin LC, Cortez DACG. Rozental S, de Souza W, Ueda-Nakamura T. *In vitro* activity of essential oil from *Ocimum gratissimum L* against four *Candida* species. Research in Microbiology. 2004;155:579–586.
27. Trease EG, Evans CW. Laboratory methods in antimicrobial chemotherapy. Churchill Livingstone, Edinburgh. 1978:35-38.
28. Anibijuwon II, Duyilemi OP, Onifade, AK. Antimicrobial Activity of Leaf of *Aspilia Africana* on Some Pathogenic Organisms of Clinical Origin. Ethnobotanical Leaflets. 2010;14: 390-401.
29. Foo RQ, Manogaran E, Gabriel AA. Antimicrobial and antioxidant studies of *Vernonia amygdalina*. Journal of Applied Pharmaceutical Science. 2014;6(4):360-371.
30. Geyid A, Abebe D, Debella A, Nakonnen A, Abberra F, Teka F, et al. Screening of some medicinal plants of Ethiopia for their antimicrobial properties and chemical profiles. Journal of Ethnopharmacology. 2005;97: 421-425.

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