Original Research Article

IN VITRO ANTIFUNGAL ACTIVITY OF SOME ETHANOL PLANT EXTRACTS AGAINST CANDIDA SPECIES ISOLATED FROM HIGH VAGINAL SWABS OF WOMEN ATTENDING A HOSPITAL IN ENUGU STATE, NIGERIA.

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ABSTRACT

Vaginal candidiasis, primarily caused by microorganisms belonging to the *Candida* genus, is a common fungal infectionprevalent among millions of women worldwide and can lead to significant morbidity. The treatment of *Candida* infections has most 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 wasthus, 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 that were resistant to three or more of the 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 the leaves of *Moringaoleifera*, *Vernoniaamygdalina* and *Ocimumgratissimum*. The leaves were dried, pulverized and 300g of each was extracted using ethanol in a Soxhlet extractor at 70°C for 6hrsThe concentrated extract of each plant was reconstituted in Dimethyl sulfoxide and different concentrations of 400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.25mg/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 (19mm) at 200mg/ml against resistant *Candida albicans*[1], followed byextract of *Vernoniaamygdalina* and *Ocimumgratissimum* with inhibition zone diameters of 18.67mm and 18mm at 100mg/ml and 200mg/ml respectively. *Candida tropicalis*[1] was sensitive to all the plant extracts at all concentrationswhile *Candida parapsilosis* was resistant to the extract of *Ocimumgratissimum* at all concentrations. The activityof the extracts of *Moringaoleifera*and *Ocimumgratissimum* 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 200mg/ml. The antifungal activities of these plant extracts implies these plants have great therapeutic potentials that can be harnessed.This study, thus, recommends that these plants be investigated further for possible use in the formulation of antifungal drugsespecially against those diseases caused by *Candida* species that have developed resistance to the commonly used antifungal drugs.

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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 candidiasiswhich 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*are also emerging 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, Vernoniaamygdalina*, and *Ocimumgratissimum* 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, Psidiumguajava, and Ocimumgratissimum 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, which can be 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. [12], showed the *in vitro* antimicrobial activities of *Vernonia amygdalina* on selected clinical isolates including *Candida albicans.*

Ocimumgratissimum, 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 *Ocimumgratissimum*, 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 which included *Moringaoleifera* and *Ocimumgratissimum*.

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 wasthus, aimed at evaluating the *in vitro* antifungal activity of ethanol

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extracts of some selected medicinal plants against *Candida* species isolated from high vaginal swabs of some women attending a hospital in Enugu State, Nigeria.

2. MATERIAL 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 that were resistant to three or more of the 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 two*Candida tropicalis*and the two *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, NnamdiAzikiwe University, Awka, Anambra State, Nigeriawhile *Vernoniaamygdalina* leaves were collected from Umukwa village in Awka, Anambra State, Nigeria. The fresh leaves of *Ocimumgratissimum* were bought from "Ogige market" (a local market) in Nsukka, Enugu state, Nigeria. The plants were identified by Late Mrs.AziagbaBibian (a plant taxonomist), in the Department of Botany, NnamdiAzikiwe University, Awka. The herbarium numbers of the plants were as follows: *Moringa oleifera* (NAU H No 01A), *Vernonia amygdalina* (NAU H No 47A) and *Ocimumgratissimum* (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 of the plants were transferred into a pre-weighed clean container and weighed, appropriately labelled, covered tightlyand kept at room temperature for further use.

2.3 Extraction of Plant Materials and Percentage Yield

After weighing the pulverized plant leaves, 300g of each was extracted using analytical grade of ethanol (Anala® BDH Chemicals Ltd, Pool, England) in a Soxhlet extractor at 70°C for 6hrs. 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 to remove the solventleaving 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

$$\% \text{ yield of extract} = \frac{crudeextractweight}{initialdryweight} \times 100$$

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2.4 Preparation of Stock Solution of the Extracts

Stock solutions of the ethanolic extracts of the leaves of *Moringa oleifera*, *Vernoniaamygdalina*, and *Ocimumgratissimum* were prepared by weighing out 1.6g of each of the extract using electronic weighing machine. This was then dissolved completely in 4ml of Dimethyl sulfoxide (DMSO)(JHD GuangbongGuanghueSci-TechCo, Ltd, Shamtou Guangdong, China) in sterile bottles to give a stock concentration of 400mg/ml of the individual extracts. A doublefold serial dilution was performed on the stock solution by transferring 2ml of the stocksolution into an equal volume (2ml) of DMSO in another bottle. This resulted in a concentration of 200mg/ml. Progressively, different concentrations of 100mg/ml, 50mg/ml, 25mg/ml and 12.25mg/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 24hours pure culture of the *Candida* isolates was 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 standardwhich is equivalent to 1 x 10^6 colonyforming 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 bottlespreviously prepared and allowed to cool to warm touch were introduced into sterile Petri dishes. Subsequently, 0.1ml of standardized inoculum (containing approximately 1 x 10^6cfu/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. Wells of 9 mm in diameter each were dug into the Mueller Hinton agar using a sterile cork borer.Using a sterile micropipette, 0.2ml of the different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12.25mg/ml) of the individual plant extracts were introduced into the corresponding labeled wells. 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, the inhibition zone diameter (in mm) produced in each plate was measured and the mean recorded.

3. RESULTS AND DISCUSSION

3.1 Percentage Yield of the Plant Extracts

The percentage yield of the individual plant extract is shown in Table 1. *Moringa oleifera* had the highest percentage yield of 15.0% followed by *Vernonia amygdalina* (12.0%) and *Ocimumgratissimum* (11.67%).

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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
Ocimumgratissimum	300	25	11.67

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

The inhibition zone diameter (IZD) of the plant extracts against some resistant strains of the isolated *Candidaalbicans* is presented in Tables 2 and 3 and Plate 1. Extract of *Moringa oleifera* had the highest inhibition zone diameter (19mm) at 200mg/ml against resistant *Candida albicans*[1], followed by extract of *Vernoniaamygdalina* and *Ocimumgratissimum* with inhibition zone diameters of 18.67mm and 18mm at 100mg/ml and 200mg/ml respectively. *Candida albicans*[1] was totally resistant to *Ocimumgratissimum* at 25mg/ml and 12.25mg/ml (Table 2). There was also total resistance by *Candida albicans*[2]to extracts of *Vernonia amygdalina* (at 200mg/ml and 100mg/ml) and *Ocimumgratissimum* (at 25mg/ml and 12.25mg/ml). The highest IZD (18.33mm) against *Candida albicans*[2] was exhibited by extracts of *Vernonia amygdalina*at50mg/ml followed by *Ocimumgratissimum* (18mm) at 200mg/ml. The extract of *Moringa oleifera* showed the least IZD (11.67mm) at 12.25mg/ml (Table 3). Similar researches support the antifungal activity ofextract of *Moringaoleifera* 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 22mm at a concentration of 5000µg/ml. Similarly, [21] showed the ethanolic leaf extracts of *Moringa oleifera* exhibited antifungal activity against *Candida albicans*giving inhibition zone diameter of 11mm at 100mg/ml concentration. However, the findings of the present study does 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 12.5mg/mlunlike in the present study in which ethanolic extracts of *Moringa oleifera* at concentrations of 50, 25 and 12,5mg/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*has 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 100mg/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 (6.25mg/ml to 100mg/ml). However, there was also total resistance by *Candida albicans*[2]to extracts of *Vernonia amygdalina* at concentrations of 200mg/ml and 100mg/ml.

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The result of the study of [18]is in conformity with that of the present study. Their results showed that ethanolic extract of the leaves of *Ocimumgratissimum* 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 100mg/ml and 13mm and 10mm at 50mg/ml respectively. Both the clinical isolate and control strains were resistant to concentrations of 25, 12.5 and 6.25mg/ml of the extracts as also recorded in this study.

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Table 2. Inhibition Zone Diameter of the Ethanol Plant Extracts against Resistant *Candida albicans*[1]

	Inhibition Zone Diameter (mm)				
Plant Extracts	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.25mg/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
Ocimumgratissimum	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]

	Inhibition Zone Diameter (mm)				
Plant Extract	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.25mg/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
Ocimumgratissimum	18.00 ± 0.000	15.67 ± 0.577	14.00 ±0.000	0.00 ± 0.000	0.00 ± 0.000

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 *Moringaoleifera* and *Ocimumgratissimum* against *Candida tropicalis*[1] were concentrationdependent, ranging from the least IZD of 11.33mm (at 12.25 mg/ml) for *Ocimumgratissimum* 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 12,25mg/ml to 17.67mm for *Vernonia amygdalina* at 200mg/ml. *Candida tropicalis*[2] was totally resistant to extract of *Moringa oleifera* at 200mg/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,

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[26]showed that the *Ocimumgratissimum* essential oil had fungicidal activity against some *Candida* species including *Candida tropicalis*though*Candida tropicalis* was the least susceptible.

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Table 4. Inhibition Zone Diameter of the Ethanol Plant Extracts against Resistant *Candida tropicalis*[1]

	Inhibition Zone Diameter (mm)				
Plant Extracts	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.25mg/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
Ocimumgratissimum	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]

	Inhibition Zone Diameter (mm)				
Plant Extract	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.25mg/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
Ocimumgratissimum	17.00 ±.000	16.00 ±.000	14.00 ±1.000	13.67 ±0.577	12.33 ±.577

3.4Inhibition 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 *Ocimumgratissimum* at all concentrations(Fig. 1) while the extracts of both *Moringa oleifera* and *Vernonia amygdalina* had activity at all concentrations with various IZDsranging from 14.33mm (at 12.25 mg/ml) to 19.00mm (at 200 mg/ml) and from 12.33mm (at 12.25mg/ml) to 19.00mm (at 200mg/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 *Ocimumgratissimum* essential oil.

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

	Inhibition Zone Diameter (mm)				
Plant Extract	200mg/m	100mg/ml	50mg/ml	25mg/ml	12.25mg/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
Ocimumgratissimum	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0

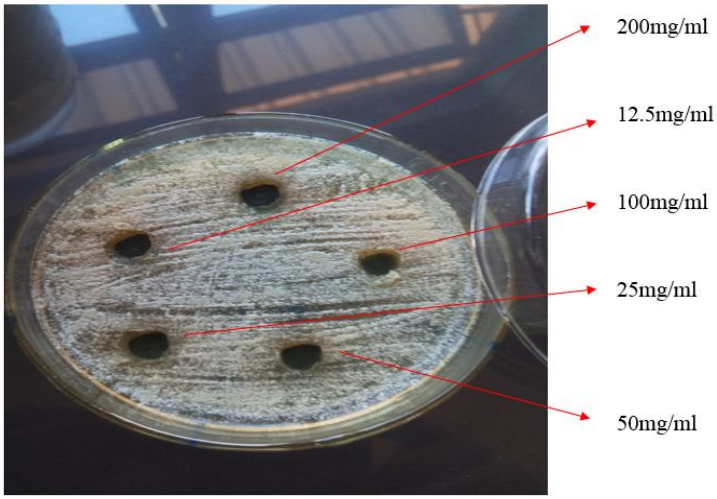

Fig 1.Total Resistance (No Inhibition Zone Diameters) to the Ethanolic Extracts of *Ocimum gratissimum* (at all Concentrations) by *Candida parapsilosis*

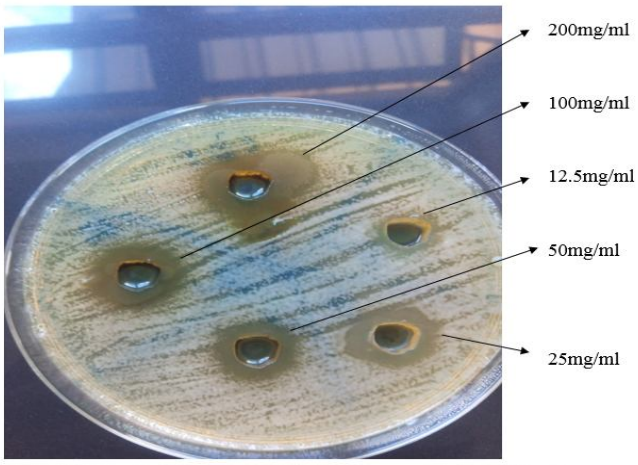

Fig. 2. Inhibition Zone Diameters of Ethanolic Extracts of *Moringa oleifera* (at Different Concentrations) against *Candida parapsilosis*.

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

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Table 7. Inhibition Zone Diameter of the Ethanolic Plant Extracts against Resistant *Candida krusei*

	Inhibition Zone Diameter (mm)				
Plant Extract	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.25mg/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
Ocimumgratissimum	0.00 ± 0.000	16.00 ± 0.000	14.00± 0.000	13.00± 0.000	0.00± 0.000

The differences observed in the activities of the plant extracts in this study, when compared with the findings of other studies may be as a result of 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 plantas well as the time of harvestcan 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 have been shown to be responsible for the antimicrobial activities shown by these extracts [17].

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4. CONCLUSION

In the present study, the Extract of *Moringa oleifera* had the highest inhibition zone diameter (19mm) at 200mg/ml against resistant *Candida albicans*[1], followed byextract of *Vernoniaamygdalina* and *Ocimumgratissimum* with inhibition zone diameters of 18.67mm and 18mm at 100mg/ml and 200mg/ml respectively. *Candida tropicalis*[1] was sensitive to all the plant extracts at all concentrationswhile *Candida parapsilosis* was resistant to the extract of *Ocimumgratissimum* at all concentrations.The IZDs produced by extracts of *Moringaoleifera*and *Ocimumgratissimum* 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 200mg/ml.

Comment [S80]: Add(the)

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

Comment [S81]: Delete

Comment [S82]: activity

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Comment [S84]: delete (those)

Comment [S85]: add(that)

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Comment [S86]: I suggest to add modern references and increase their numbers

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