

## Research Article

# Antimicrobial Susceptibility of Clinical Isolates of *Candida* species to Ethanolic extracts of *Ziziphus abyssinica* leaves

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### ABSTRACT

This study determined the sensitivity profile of clinical isolates of *Candida* species to the leaf extracts of *Z. abyssinica*. Three hundred (300) clinical samples consisting of the vaginal swab (163), oral swab (49), urine (87) and blood samples (1) were collected and screened for the presence of *Candida* species using standard procedures. The yeast cells were identified by Gram staining, microscopy, colony morphology on SDA, germ tube test and API20C KIT. The plant material was extracted using ethanol and was followed by phytochemical screening. The susceptibility test was carried out using the disc diffusion method according to standard guidelines. A total of 93(31%) *Candida* species were isolated as follows; *C. albicans* (60.2%) has the highest percentage occurrence, followed by *C. tropicalis* (12.9%), *C. krusei* (9.7%), *C. glabrata*, *C. pelliculosa*, *C. lusitanniae* and *C. guilliermondi* (4.3%) each. Similarly, 19% were isolated from vaginal swabs, urine sample 8.3%, oral swabs 3.3% and blood sample 0.3%. The phytochemical screening of the extracts revealed that all the extracts contained reducing sugar, resins and alkaloids, flavonoids, glycosides tannin, saponins, steroids and terpenoids. The antifungal susceptibility test showed that ethanol extract recorded higher activity (20mm-9mm zone of inhibition diameter) with MIC of 3.125mg/ml. The GC-MS result of the extract showed that the most active compounds are esters of carboxylic acids. Fluconazole showed the highest overall activity in 68.8% of all the isolates and 38.7% of the isolates were resistant to ketoconazole. It can be concluded that clinical *Candida* species isolated from various samples in this study showed appreciable susceptibility to leaf ethanol extract of *Z. abyssinica* with a positive correlation to the most active orthodox antifungal namely fluconazole. The extract could thus be recommended as a candidate for the search for potent antifungal drugs.

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## INTRODUCTION

The plant kingdom has provided different compounds with known therapeutic properties like analgesics; anti-inflammatories, and their products are chemically safer than orthodox/synthetic drugs (Srinivan *et al.*, 2001). Plants are making the backbone of the traditional medicinal system (Nayak *et al.*, 2011). Due to the increased prevalence of drug-resistant microorganisms, there is a great need to search for new effective drugs having natural or synthetic origins (Rai *et al.*, 2004). Accordingly, the World Health Organization has recommended that plants should be evaluated to better discover their pharmacological features including safety and efficacy to enhance the success in chemotherapy of even drug-resistant microorganisms (Nascimento *et al.*, 2000).

The spread of multidrug-resistant strains of *Candida* and the reduced number of drugs available makes it necessary to search for new sources of antifungals and compounds that inhibit these resistance mechanisms. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization instruction resulting in several preclinical studies that have provided the scientific basis for the efficacy of many plants used in folk to treat infections (Akko *et al.*, 2003).

*Candida* species are members of the normal flora of the skin, mucous membranes and gastrointestinal tract, they colonize the mucosal surface of humans during or soon after birth, and the risk of endogenous infection is always there. Candidiasis is the most common systemic mycosis, and the most common agents are *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. guilliermondii*, and *C. dubliniensis* (Brooks *et al.*, 2010).

Most societies use traditional medicines to cure various kinds of illnesses including Nigeria. They depend heavily on traditional medicines due to their availability, cheapness as well as fewer side effects compared with synthetic medicines.

*Z. abyssinica* is among the medicinal plants used traditionally in the treatment of various microbial infections among the Hausa people of Northern Nigeria. For instance, water from boiled leaves of *Z. abyssinica* has been used for sit baths and rinse of mouth for the treatments of vaginal and oral thrush respectively. Hence, there is a need to scientifically investigate and ascertain the effectiveness of the plant against different species of *Candida* (a major cause of vaginal and oral thrush) isolated from various sites of the human body.

## MATERIALS AND METHODS

### Ethical Clearance

Ethical approval was sourced from Aminu Kano Teaching Hospital Research Ethical Committee before the start of the study. In addition, informed consent was sought from the patients before their enrolment.

The inclusion criteria were adult consenting females who present with symptoms of candidiasis and their samples were brought to the Microbiology Laboratory of Aminu Kano Teaching Hospital, the patients were residents but not necessarily indigenes of Kano State.

### Sample Collection

Patients recommended for diagnosis of candidiasis symptoms were recruited for the study. Vaginal, oral swabs, urine and blood samples were collected by a laboratory technologist. The samples were collected from the Microbiology Laboratory of Aminu Kano Teaching Hospital, Kano.

### Sample Size Determination

A total of 300 samples were collected from various sites (vaginal, oral, urine and blood) for possible isolation of *Candida* species. The sample size was calculated using the equation below

$$Ss = z^2 p (1-p) / d^2$$

where Ss= sample

size, z=standard normal deviation at 95 =1.96,

p= current prevalence rate = 24% and

d= error margin =0.005

### **Isolation and Identification of yeast cells**

The samples were streaked on already prepared Saboraud Dextrous Agar (SDA) supplemented with chloramphenicol (1 mg/mL) and incubated at room temperature for 24 hrs. The incubation period was extended for up to 3 days for a culture that showed no growth earlier than 24 hours (Manolokaki, 2010).

Gram staining and Germ Tube Tests were carried out. Yeast isolates were identified to species level using API 20C Aux kit Biometriux (France) according to the manufacturer's specifications. Yeast isolates were first subcultured on SDA to obtain fresh colonies of the organism. The pure isolates were stored in skimmed milk media at -80°C.

### **Antifungal Susceptibility Testing**

#### **Preparation of stock solutions of the antifungal agents and sensitivity disc**

The antifungal drugs were obtained (purchased) from a reputable pharmaceutical store in Kano. The stock solution of the antifungals was prepared at the Drug and Manufacturing Unit of Aminu Kano Teaching Hospital using ketoconazole U.S.P 200mg tablets(fungizole®), fluconazole 50 mg capsule(Diflucan), Itraconazole 100mg capsule (Itacare), and Amphotericin B in respective solvents to arrive at the desired concentration in 10 microliter required to impregnate 6mm filter paper disc.

#### **Inoculum Preparation and Standardization**

This was carried out according to the methods of Clinical and Laboratory Standards Institutes (CLSI, 2006). A loopful of a fresh overnight culture of *Candida* colonies was suspended in a test tube containing 5 ml of 0.85% normal saline. Then it was compared and adjusted to 0.5 Macfarland's standard visually.

#### **Antifungal Bioassay**

A sterile cotton swab stick was dipped into the suspension of the standardized organism to be tested. The cotton swab stick was then raised above the suspension and pressed along the inner wall of the test tube to remove excess

moisture. This was inoculated on a dried plate of Muller Hinton agar supplemented with 2% Glucose and 0.5g methylene blue by swabbing at 60° in three dimensions for uniformity as recommended by the Clinical Laboratory Standard Institute. The glucose supports the growth of *Candida* species while methylene blue enhanced zone and edge clarity. Sterile single filter paper discs impregnated with required antifungal concentrations were aseptically placed on each inoculated plate with the aid of sterile forceps and incubated at 37°C for 24hrs. The zone of inhibition developed by each antifungal disc was measured with a calibrated ruler and then interpreted based on the interpretive breakpoints recommended by CLSI (2006).

#### **Collection, identification and processing of the plant materials**

The plant was collected from the premises of Bayero University, Kano (BUK). It was then taken to the herbarium section of the Department of Plant Biology of Bayero University, Kano for identification and confirmation. Confirmation of the taxonomic identity was achieved by comparison with the voucher number of *Ziziphus abyssinica* (BUKHAN 0233). The fresh leaves of the plant were collected, washed with distilled water and then dried under shade at an ambient temperature for two weeks. This was later pounded using a clean mortar and pestle into powder form and stored in a refrigerator until use (Mukhtar and Tukur, 1999).

#### **Extraction of the Plant Materials**

Fifty grams (50g) of the powdered plant materials were dissolved in 500 ml of ethanol and left to stand for 14 days with regular shaking. This was then filtered using a Whatman filter paper No. 1 and the filtrate was allowed to evaporate to dryness in a water bath at 30°C until the solvent escapes completely leaving behind the ethanol extract. The extract was placed in an air-tight container and stored in the

refrigerator at 4°C for future use (Fatope and Hamisu, 1993).

#### **Phytochemical Screening of the Plant Extract**

This was carried out to determine the presence of steroids (Sofowora, 1993), Flavonoids, Saponins, Glycosides, Resins, Reducing Sugars, Terpenoids and Tannins (Garba *et al.*, 2011).

#### **Preparation of Plant Extract Concentrations**

The stock solution was prepared by dissolving 0.1g of the dried extract in 2ml of each of the solvents in sterile bijou bottles (distilled water for aqueous and dimethylsulfoxide, DMSO for ethanol extract). This yielded a concentration of 50mg/ml. From the stock solution, 1ml was then transferred into another bottle containing 1ml of the solvent which gave a concentration of 25mg/ml, also, 1ml from 25mg/ml of each of the extracts was transferred into another bottle containing 1ml of solvent which gave a concentration of 12.5mg/ml, from 12.5mg/ml, 1ml was transferred into another bottle containing 1 ml of the solvent which gave a concentration of 6.25mg/ml (Sulaiman *et al.*, 2014).

#### **Antifungal Bioassay for the plant extracts**

The antifungal bioassay for the plant extract was carried out using the same procedures as that of commercial antifungal agents described in item 3.6.4 above, except that for the plant extract, each concentration was carried out in triplicate and the disc was impregnated with 0.01ml of each of the extract using a micropipette. After incubation, the mean diameter of the zone of inhibition (expressed in millimetres) for each of the extracts was measured (using mm-rule) against the isolates. Fluconazole 25ug/ml and distilled water were used as positive and negative controls.

The determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) was carried out according to the method of Hena *et al.* (2010) and CLSI (2006).

#### **Gas Chromatography Separation Analysis (GC-MS)**

The ethanol extract of *Z. abyssinica* was subjected to gas chromatography/mass spectrometry to separate and identify the components responsible for the antifungal activity of the extract.

#### **Statistical analysis**

Analysis of variance (ANOVA) was used to analyze the result statistically as described by Mukhtar, (2008). The potency of the antifungal agents and that of the plant extract was compared, and the relationship between the species isolated and the sites of isolation as well as their susceptibilities to the two solvent extracts was also compared at 5% probability using Ms Excel software.

## **RESULTS**

### **Frequency of *Candida* species within the clinical samples**

The frequency of *Candida* species isolated from the clinical samples is presented in Table 1. There was a 19% occurrence of *Candida* species in the 163 vaginal swabs collected. Out of eighty-seven (87) urine samples collected, 25(8.3%) had *Candida* species. Similarly out of 49 oral samples, 10(3.3%) harboured the yeast.

### **Distribution of *Candida* species among clinical specimens**

*Candida* species isolated from different sources are presented in Table 2. A total of 93 different species are isolated which include 56(18.6%) *C. albicans*, 38(12.6%) of which are from the vaginal swab, 12(4.0%) from urine and 6(2.0%) from oral samples. Twelve (4.0%) *C. tropicalis* were isolated, 6(2.0%) from vaginal sample, 5(1.66%) urine and 1(0.33%) from oral swabs. Nine (3.0%) species of *C. krusei* were also isolated 5(1.66%) from vaginal

samples, 3(1.0%) from urine and 1(0.33%) from an oral swab. Four (1.33%) *C. glabrata* were also isolated which include 2(0.66%) species from vaginal and urine samples each. Four (1.33%) species also of *C. guilliermondi* were isolated. One (0.33%) specie each from vaginal, oral, urine and blood sample was isolated. Four (1.33%) *C. pelliculosa* were isolated which

include 2(0.66%) from the vaginal sample and 1(0.33%) specie each from the urine and oral sample. Four (1.33%) *C. lusitanniae* were isolated which include 3(1.0%) from vaginal and 1(0.33%) from urine samples. From the result, *Candida* species were isolated more in vaginal specimens followed by urine and oral samples.

**Table 1:** Frequency of occurrence of *Candida* species among clinical samples from patients at AKTH

Source	Frequency	Percentage
Blood (n=1)	1	0.3
Oral (n=49)	10	3.3
Urine (n=87)	25	8.3
Vaginal (n=163)	57	19.0
Total (n=300)	<b>93</b>	<b>31.0</b>

**Table 2:** Frequency and Distribution of identified *Candida* species among clinical specimens (n = 300)

Isolates	Blood	Oral	Urine	Vagina	Total frequency (%)
<i>C. albicans</i>	0(0.0)	6(2.0)	12(4.0)	38(12.6)	56(18.6)
<i>C. glabrata</i>	0(0.0)	0(0.0)	2(0.66)	2(0.66)	4(1.33)
<i>C. guilleirmondi</i>	1(0.33)	1(0.33)	1(0.33)	1(0.33)	4(1.33)
<i>C. krusei</i>	0(0.0)	1(0.33)	3(1.0)	5(1.66)	9(3.0)
<i>C. lusitanniae</i>	0(0.0)	0(0.0)	1(0.33)	3(1.0)	4(1.33)
<i>C. pelliculosa</i>	0(0.0)	1(0.33)	1(0.33)	2(0.66)	4(1.33)
<i>C. tropicalis</i>	0(0.0)	1(0.33)	5(1.66)	6(2.0)	12(4.0)
<b>Total</b>	<b>1(0.33)</b>	<b>10(3.3)</b>	<b>25(8.3)</b>	<b>57(19.0)</b>	<b>93(31.0)</b>

Values in ( ) are percentages

### Physical Characteristics of the Extracts

The physical characteristics of *Z. abyssinica* ethanol leaf extracts are indicated in

Table 3. The ethanol extract was a dark green colour and gummy and hard texture. The extract is soluble in DMSO and has a slightly repulsive odour.

**Table 3:** Physical characteristics of the plant extracts

Extract	Color	Texture	Solubility	Smell/odor
<i>Z. abyssinica</i> ethanolic extract	Dark green	Hard and gummy	Dimethyl sulphoxide	Slightly repulsive

#### Phytochemical components of Ethanol Extracts of *Z. abyssinica* leaf

Phytochemical components of *Z. abyssinica* ethanol leaf extracts are presented in Table 4.

Reducing sugars, alkaloids and Resins, Saponin, tannin, glycosides and flavonoids were detected. Steroids and terpenoid were not detected in the extract.

**Table 4:** Phytochemical components of the ethanol extract of *Z. abyssinica* leaf

Phytochemical	Inference
Saponins	+
Tannins	+
Reducing Sugar	+
Glycosides	+
Alkaloids	+
Flavonoids	+
Terpenoids	-
Resins	+
Steroids	-

Key: + present, -absent

#### Bioassay of *Z. abyssinica* extracts against the isolates

Table 5 presents the anti-*Candida* activity of *Z. abyssinica* leaf ethanol extract. From the Table, *C. krusei* showed the highest zone of inhibition diameter (20mm) at a concentration of

50mg/ml, for ethanolic extract. These showed that *C. krusei* and *C. albicans* are more susceptible to ethanolic leaf extract of *Z. abyssinica*. On the other hand, *C. glabrata* and *C. guilliermondi* showed the least zone of inhibition diameter of 14mm for ethanolic extract.

**Table 5:** Susceptibility Profile of Clinical isolates of *Candida* species to various concentrations of leaf ethanol extract of *Z. abyssinica*

Yeast	Concentrations (mg/ml)				Fluconazole
	50	25	12.5	6.25	6.25µg/ml
<i>C. albicans</i>	18.00±1.00	15.00±2.64	12.00±1.00	09.00±.00	25
<i>C. tropical</i>	16.00±1.00	12.00±3.46	12.33±6.11	09.00±.00	32
<i>C. krusei</i>	20.00±2.00	13.00±1.44	15.00±2.00	13.00±1.00	21
<i>C. glabrata</i>	14.00±0.00	13.00±1.00	10.00±1.00	10.00±0.00	21
<i>C. pelliculosa</i>	15.00±2.64	12.00±3.46	11.33±1.52	10.00±0.00	26
<i>C. lusitanninec</i>	16.00±1.00	14.00±2.00	11.00±1.00	09.00±.00	23
<i>C. guilleirmondi</i>	14.00±2.00	13.00±1.00	10.00 ±000	09.00±1.00	26

**Minimum Inhibitory Concentrations (MIC) and Minimum Fungicidal Concentration (MFC) of ethanol extract of *Z. abyssinica***

The minimum inhibitory concentrations of *Ziziphus abyssinica* ethanolic extract is presented in Table 6. The MIC for the plant

extracts ranged from 3.125mg/ml to 12.5mg/ml with *C. glabrata* and *C. krusei* having the lowest value of 1.56mg/ml and *C. guilliermondi* having the highest value of 12.75mg/ml. The MFC ranged from 12 to 50mg/ml.


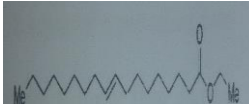
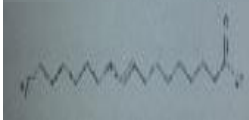
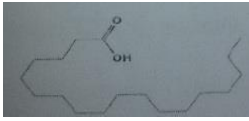
**Table 6:** MIC and MFC of *Z. abyssinica* ethanol leaf extract in relation to specific *Candida* species

Isolates	MIC	MFC
<i>C. albicans</i>	3.125	12.5
<i>C. tropicalis</i>	6.25	12.5
<i>C. krusei</i>	1.56	3.125
<i>C. glabrata</i>	1.56	12.5
<i>C. pelliculosa</i>	6.25	50
<i>C. lusitanniae</i>	3.125	6.25
<i>C. guilliermondi</i>	12.75	50

**Gas Chromatography Separation (GC-MS)**

The GC-MS results revealed compounds such as octadecanoic acid methyl ester, stearic acid methyl ester, and octadec-9-enoic acid methyl ester and octadecanoic acid stearic acid in the ethanolic extract of *Z. abyssinica* leaf extract. The aqueous extract contained 9-octadecanoic acid ethyl ester, 2- otadecadecem-1-ol and propylheptol ester.

**Table 7:** Some of the compounds identified from *Z. abyssinica* Ethanol crude extract

S/No.	Retention time	Areas %	Molecular weight	IUPAC Name	Molecular formular	Chemical structure
1	15.815	7.80	296	9 –octadecanoic acid methyl ester	C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>	
2	16.073	10.19	298	Stearic acid methyl ester	C <sub>19</sub> H <sub>38</sub> O <sub>2</sub>	
3	16.217	20.96	282	Octadec – 9ecoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	
4	16.433	27.16	284	Octadecanoic acid stearic	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	



## DISCUSSION

The current study revealed a prevalence rate of *Candida* species as 31%. *Candida* species are members of the normal flora of the skin, mucous membranes and gastrointestinal tract, they colonize the mucosal surface of humans during or soon after birth, and the risk of endogenous infection is always there (Brooks *et al.*, 2010). In comparison, Khara (2017) in India reported a 14.26% rate which is lower than the current study and Kelvin (2015) from Zambia reported a 93.7% prevalence rate which is much higher than the current study. This might be due to differences in geographical location.

The distribution of species in this study were; *C. albicans* 60.2%, *C. tropicalis* 12.9%, *C. krusei* 9.7%, *C. glabrata*, *C. guilliermondi*, *C. lusitanniae* and *C. pelliculosa* 4.3% each. Tak *et al.* (2014) reported *Candida* species distribution as *C. albicans* (13%), *C. tropicalis* (38%), *C. glabrata* (11.4%), *C. guilliermondi* (1.8%) and *C. lusitanniae* (0.4%). Chakarboti, (2014) in India reported the distribution of *Candida* species as *C. albicans* (20%), *C. tropicalis* (41.6%), *C. glabrata* (7.8%), *C. krusei* and *C. guilliermondi* (1.74%) each. Kelvin (2015) reported the distribution of *Candida* species isolated from clinical samples as follows *C. albicans* (66.7%), *C. lusitanniae* (12.2%); *C. glabrata* (6.7%), *C. tropicalis* (5.6%), *C. guilliermondii* (3.3%) and *C. pelliculosa* (1.1%). The differences in the prevalence rate may be due to geographical location, sources of the isolates as well as the methods used in the isolation of *Candida* species.

The present study showed *Candida albicans* (60.2%) as the most common isolated species followed by *C. tropicalis* (12.9%). This is not surprising as Sundave *et al.* (2017) and Sajjan *et al.* (2014) reported *C. albicans* as the most common isolated species. On the contrary, Badiie *et al.* (2017) from Iran, Won *et al.* (2015) from Korea and Khara *et al.* (2017) from India reported *C. krusei*, *C. parapsilosis* and *C. glabrata* as the most

common isolated species. This showed that *Candida* species distribution may differ due to the differences in geographical location. The findings showed the percentage of non-*albicans Candida* (NAC) as 44% which is contrary to the research findings of Golia, (2013), who reported the percentage of NAC as 75%.

About the source of the specimen, a total of 57 out of 163 species were isolated from vaginal samples, which include *C. albicans* 38(67.8%), *C. tropicalis* 6(10.62%), *C. krusei* (8.77%), *C. lusitanniae* 3(5.26%), *C. pelliculosa* and *C. glabrata* 2(3.5%) species each and *C. guilliermondi* 1 (1.75%) species. Chong *et al.* (2003) isolated *C. albicans*, *C. glabrata*, *C. lusitanniae*, *C. krusei* and *C. parapsilosis* from vaginal samples, with *C. albicans* having the highest occurrence as in the present study. On the other hand, Madhavan *et al.* (2010) reported *C. glabrata* as the most common species isolated from vaginal samples. Vaginal candidiasis is the most common opportunistic fungal infection that affects millions of women worldwide every year. Approximately 75% of adult women have at least one episode of vulvovaginal candidiasis in their lifetimes and about half of these women (40-50%) experience more than one recurrence, 5% - 8% have multiple episodes each year. Vaginal candidiasis occurs when there is an overgrowth of *Candida* species present in the vagina as normal commensal. Pregnancy, use of contraceptive drugs with high estrogen, antibiotics, uncontrolled diabetes, immunosuppressive drugs, unsafe or excessive sexual intercourse, chronic anaemia and seasonal allergy are the predisposing factors of vaginal candidiasis (Ane-amyangwe *et al.*, 2015).

A total number of 25 *Candida* species were isolated from 79 urine samples. *Candida albicans* is the most predominant isolated species which account for 8.3%. Other species isolated in the urine were *C. tropicalis* (20%),

*C. krusei* (12%), *C. glabrata* (8%), then *C. guilliermondi*, *C. pellicidosa* and *C. lusitanniae* (4%) each. Ozhak-baysan, (2012) reported almost similar distribution of *Candida* species isolated from urine samples as follows; *C. albicans* (44%), *C. tropicalis* (20%), *C. glabrata* (18%) and *C. guilliermondi* (1%). Nemati *et al.*, (2008) also isolated *Candida* species in the same distribution, only that they do not isolate *C. krusei* in urine. *Candida* species accounted for 40% of all urinary tract infections and can reach the urinary tract via the ascending route, from the urethra to the bladder, as *Candida* species are filtered by kidneys and excreted in the urine. The risk factors include advanced age, female sex, urinary tract abnormalities, drainage catheters, malignancy and use of broad-spectrum antibiotics (Nemati *et al.*, 2008).

*Candida guilliermondi* was the only species isolated from a blood sample. To support this finding, Jaswinder (2012) also isolated *C. guilliermondi* but together with *C. parapsilosis*. Rajeevan *et al.* (2017) reported the prevalence of *C. guilliermondi* from a blood sample as 3.1%. On the other hand, Sri Janani, *et al.* (2015) did not isolate *C. guilliermondi* from a blood sample, but *C. albicans* 39%, *C. tropicalis* 33%, *C. parapsilosis* 11%, *C. krusei* 4% and *C. glabrata* 3%. *C. guilliermondi* is a relatively rare species which is isolated from a blood specimen. It is isolated more frequently in patients with prior cardiovascular or gastrointestinal surgery (Kothavade *et al.*, 2007). The risk factors for bloodstream candidiasis include intravascular and central venous catheters, malignancy, total parental nutrition, diabetics, ventricular assist devices etc.

A total of 10 out of 49 *Candida* species were isolated from the oral sample. *Candida albicans* were found to be the predominant species isolated (3.3%). The majority of yeasts isolated from oral cavity swabs were *C. albicans*, but it was often recovered in

association with other yeasts. The next predominant species after *C. albicans*, were *C. tropicalis*, *C. krusei*, *C. guilliermondi*, and *C. pelliculosa* with 10% prevalence each. Manikandam and Amsath (2013) reported the distribution of *Candida* species as follows *C. albicans* 54%, *C. glabrata* 15%, *C. Parapsilosis* 12%, *C. krusei* 6.7% and *C. tropicalis* 6.7%. Raju and Rajappa (2011) reported a similar pattern of distribution of the species. This could be due to variations in the geographical distribution of various *Candida* species. The relationship between the isolates and the source of isolation is significant ( $p=0.047$ ). Most species were isolated from the vaginal sample with *C. albicans* as the most isolated species.

The phytochemical screening of *Z. abyssinica* ethanol leaf extract revealed that the extracts contained reducing sugars, alkaloids and resins. In addition, the ethanol extract contained saponins, flavonoids tannin and glycosides, which is in agreement with the findings of Rizk (1986) and Tiwari *et al.* (2011) who also reported the presence of these components in the ethanolic extract of *Z. abyssinica*. Ethanol can pull out these phytochemicals when used as a solvent for extraction. Kwa *et al.* (2012) reported ethanol as the best solvent that dissolves multi-variable type of compounds. The effectiveness of ethanol might be attributed to two reasons; firstly, the nature of biologically-active components and secondly, the strength of the extraction capacity of the ethanol could have allowed more of the bioactive constituents responsible for the antimicrobial activities.

Secondary plant metabolites contain the chemical compounds responsible for the healing properties of the medicinal plant as they produce specific pharmacological effects in humans and animals (Ahmad *et al.*, 2014). Importantly the presence of these biologically active phytochemicals has been reported by several researchers to account for the therapeutic effects of many medicinal plants (Maganha, 2010; Gavamakulya, 2012).

On the other hand, this research work showed that ethanol extract of *Ziziphus abyssinica* has some degree of anti-*Candida* properties with high activity (from the zone of inhibition diameter). This might be due to the presence of saponins, tannins glycosides and flavonoids, which have been proven to have antimicrobial activities. The zones of growth inhibition produced by the extracts against the *Candida* species indicated the potency of the active components (phytochemicals) in *Ziziphus abyssinica*. It has been seen from previous literature (Gundida *et al.*, 1991; Beentje (1994) that *Z. abyssinica* has significant activity against *Staphylococcus aureus* and *Candida albicans*.

The presence of flavonoids and tannins may be a good source of phenylpropane and flavones antioxidants. This might support their uses in the treatment of oral candidiasis, sore or inflamed throat and tonsillitis (Amin, 1991). Moreover, the GCMS analysis of the crude ethanolic and aqueous leaf extracts of *Z. abyssinica* showed that the most active components present are mostly carboxylic acids of octadecanoic acids and n-propyl heptol. These compounds were reported to have possessed antimicrobial activity (Awa *et al.*, 2012), and antifungal, anti-inflammatory and antioxidant properties (Ya *et al.*, 2005). Hence, the anticandidal activity of *Z. abyssinica* in this study might be due to the presence of these bioactive compounds in the extract.

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The MIC value of ethanolic *Z. abyssinica* leaf extract ranged from 3.125 to 12.5 mg/ml.

The sensitivity pattern of the *Candida* species isolated to fluconazole showed some are sensitive, resistant or intermediate. However, fluconazole showed the highest overall sensitivity in 68.8% of all the isolates. Sundav (2017) and Tak *et al.* (2014) showed overall fluconazole sensitivity at 96.4% and 93.9% respectively. The present study showed *C. tropicalis*, *C. pelliculosa* and *C. guilliermondi*. *C. krusei* to be 100 % sensitive to fluconazole, while *C. lusitanniae*, *Candida albicans* and *C. glabrata* were 88.9%, 75%, 53% sensitive.

This indicated that *Candida glabrata* have the highest resistance rate to fluconazole.

Statistically, there is no significant difference between the ketoconazole and the ethanol leaf extract.

## CONCLUSION

From the results of this study, it can be concluded that the most common *Candida* species isolated from the clinical sample in this study include *Candida albicans*, *C. tropicalis* and *C. krusei*. The isolates were sensitive to ethanol leaf extract of *Ziziphus abyssinica* and fluconazole. Several compounds were detected using GS-MS analyses, which are mostly carboxylic acids of octadecanoic acid. Further research should be carried out with other parts of the plant, namely, the root, stem bark and fruit.

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