

*Full Length Research Paper*

# **Antifungal susceptibility pattern and identification of *Candida* species in women of child-bearing age suffering from malaria, typhoid and diabetes in Nsukka, Nigeria**

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**Candidiasis in women of child-bearing age is a major problem in most hospitals in Nigeria. High vaginal swabs were analyzed for *Candida* presence in women of child bearing age using Chromogenic agar. Isolated *Candida* spp. were analyzed phylogenetically to trace their evolutionary relatedness. Growth rate assay was done using Sabouraud and Dextrose medium supplemented with NaCl and glucose. The resistance pattern of the *Candida* spp. was tested using fluconazole and extracts (methanol and ethyl) from *Byrsocarpus coccineus*. Out of the 79 fungal samples analyzed, 21 (26.6%) were *C. tropicalis*, 25 (31.6%) were *C. krusei*, 10 (12.7%) were *C. parapsilosis* and 8 (10.1%) were *C. albicans*. About 15 (19.0%) had mixed morphology on CHROM agar *Candida* and could not be identified. The healthy women presented very low percentage (8.9%) of *Candida* species. The sequenced *Candida* spp. had 100% sequence identity to *C. tropicalis*. High concentration of glucose (1 g/ml) and NaCl (0.5 M) decreased the growth of the fungus. In conclusion, *C. tropicalis* and *C. krusei* were more prevalent in the malaria patients. *C. albicans* were found mostly in the typhoid patients. The plant extracts showed lower antifungal activity in comparison with fluconazole but they showed a huge antifungal potential.**

**Key words:** *Byrsocarpus coccineus*, *Candida*, diabetes, fluconazole, malaria, phylogeny, typhoid.

## **INTRODUCTION**

Vaginal candidiasis is a very common health problem in both immunocompetent and immunocompromised individuals, while oral candidiasis is much more common

in immunocompromised individuals (Denning et al., 2018; Muzny and Schwebke, 2015; Fidel, 2002). *Candida* colonization of the vaginal and oral mucosa occurs in

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healthy women of all ages, over 75% of women will experience at least one vaginal infection during their lifetime (Vermitsky et al., 2008). Prevalence studies indicate that 10 to 55% of healthy and asymptomatic women have vaginal cultures positive for *C. albicans* (Muzny and Schwebke, 2015). *C. albicans* is the most frequently isolated *Candida* spp. in human candidiasis (more than 50%) and it has been reported to be the most pathogenic species of the genus *Candida* (Ben-Ami, 2018; Deorukhkar and Saini, 2015). However, other *Candida* spp., like *C. glabrata* (10 to 30%), *C. tropicalis* (10 to 20%), *C. parapsilosis* (10 to 20%) have been reported. Vaginal colonization with *Candida* in women has been reported to be due to among other factors, regular use of antibiotics (Canela et al., 2018). The use of antibiotics has been reported to cause destruction of bacterial flora which creates a more favorable environment for *Candida* overgrowth (Canela et al., 2018). It is therefore apparent that *Candida* infections mainly occur as opportunistic infections due to altered conditions of the host. Human infections by *Candida* spp. are usually endogenous because *Candida* is frequently part of the normal flora of the mouth, throat, large intestine, vagina and the skin of humans (Shao et al., 2007). However, infections are more common in patients whose immune systems are compromised (Kim and Sudbery, 2011). Sexual transmission from patients with vaginitis to their sexual partners has been reported (Murray et al., 2002). Most *Candida* spp. have inherent resistance to available antifungal azoles. In *C. albicans*, development of secondary resistance to fluconazole treatment is commonly encountered in HIV-infected patients (Pereira et al., 2010). Among the non-albicans species, *C. tropicalis* and *C. parapsilosis* appear to be susceptible to azoles but *C. tropicalis* is less susceptible to fluconazole than *C. albicans*. However, *C. glabrata* is intrinsically more resistant to most antifungal agents, particularly to fluconazole. *C. krusei* is also resistant to fluconazole. *C. lusitanae* which accounts for 1 to 2 % of all candidemia is susceptible to azoles, but has a higher intrinsic resistance to amphotericin B (Cruciani and Serpelloni, 2008). Studies have reported the emergence of antifungal-resistant *C. albicans* fungemia in bone marrow transplant recipients being administered long-term fluconazole prophylaxis (Cornely et al., 2012; Alexander et al., 2013). However, the number of cases of fluconazole-resistant candidemia caused by *C. albicans* in patients with cancer who are receiving azole prophylaxis still remains small (Cornely et al., 2012; Alexander et al., 2013). *C. krusei* is innately resistant to fluconazole, in addition to intrinsic resistance, nearly about 20% strains of *C. glabrata* can acquire resistance during the course of therapy (Deorukhkar et al., 2014). *C. tropicalis* is generally considered as a fluconazole-susceptible species. However, recent studies have documented emergence of fluconazole resistance (Deorukhkar et al., 2014; Silva et al., 2012). *C. parapsilosis*

is reported to have high minimum inhibitory concentration to echinocandins (Deorukhkar and Sani, 2016). For treatments in both human and animals, therapeutic options become limited when there is cross resistance to all antifungal azoles.

Therefore, medicinal plants may provide an alternative to antifungal drugs due to the increasing resistance towards the available azoles. *Byrsocarpus coccineus* is an indigenous tropical plant found in West Africa (Kossivi et al., 2014). Previous studies have reported considerable antimicrobial activities of the extracts of *B. coccineus* on *C. albicans*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* (Ahmadu et al., 2006). The antimicrobial property of *B. coccineus* has been reported to be due to a coumaryl derivative and flavonoid glycoside isolated from its leaves (Akindele and Adeyemi, 2006). Other phytochemical constituents like tannins, sugar and saponins have been reported to contribute to its antimicrobial activity (Ahmadu et al., 2006). *B. coccineus* has also been used in the treatment of diarrhea (Akindele and Adeyemi, 2006). Although *B. coccineus* has been shown to be a promising candidate for antifungal therapy, no study has compared its efficacy with the commercially available antifungal azoles.

In the present study, a comparative analysis of *Candida* spp from high vaginal swab (HVS) of healthy women (20 to 30 years) with normal clinical conditions was made with the *Candida* spp. isolated from HVS of women within the same age group with clinical conditions such as malaria, typhoid and diabetes. Also, the resistance pattern of the *Candida* spp was investigated using the common antifungal agent fluconazole and extracts from the medicinal plant, *B. coccineus*. The result from this work will give an insight into the relationship between *Candida* infections in women and some common clinical conditions in the tropics as well as give insight on the potentials of using this plant as a replacement for common azoles that are no more efficacious in treatment due to resistance.

## MATERIALS AND METHODS

### Sample collection

The study was conducted at the Daughters' of Devine Love (DDL) Hospital, Ehalumona, Nsukka. Female outpatients 20 to 30 years of age with asymptomatic candidiasis without any history of antifungal, antibiotic and antimalarial therapy for the past one month were enrolled in the study. Patients were diagnosed with diabetes on clinical presentation by a certified endocrinologist and clinically confirmed by a satisfied physician to be suffering from either malaria or typhoid. Potential participants were approached in the clinic waiting room and consent granted. Clinical samples of high vaginal swabs (HVS) were self-collected by the female patients. Swab sticks of about 3 cm in length were self-inserted into the upper part of the vaginal lumen and gently rotated. The swab stick was then withdrawn carefully to avoid contamination with the external genitalia and immediately inserted into its plastic pack, labeled with the patient's identification tag, age and medical history

and was quickly transported to the laboratory for further processing. In parallel, samples of HVS of healthy females (females without any history of illness or any history of medication of any kind) within the same age bracket were collected from female students living in Awolowo Hall of the University of Nigeria Nsukka as control. The study protocol was approved at each of the study sites by the local institutional review board. There were 100 female participants in this trial.

#### Fungal isolation and morphological characterization on CHROM agar *Candida*

Each of the samples was inoculated into petri plates of Sabouraud dextrose agar (SDA) (Oxoid, Basingstoke, United Kingdom) containing 5 mg/ml of chloramphenicol and incubated at 28°C under aerobic condition for 48 h. Single colonies of the obtained cultures were transferred into freshly prepared SDA, incubated for another 48 h to produce pure cultures. Individual colonies from the pure cultures were inoculated into plates of CHROM agar *Candida*/differential media (CM10002 BRILLIANCE™ *CANDIDA* AGAR; Oxoid UK) reconstituted with 70% ethanol. The fungi were grouped together based on their colours on the CHROM agar *Candida* and were later classified to different *Candida* species based on colour following already existing information in the literature (Daef et al., 2014).

#### Sequencing the fungal internal transcribed spacer region

To confirm the result from the screening on CHROM agar *Candida*, molecular identification was carried out on one isolate from the *C. tropicalis* group. From a 3 day old SDA (Oxoid, Cambridge, UK) culture grown at 25°C, genomic DNA was extracted using hexadecyltrimethylammonium bromide (CTAB)-based method (Nygren et al., 2008). The concentration of the extracted DNA was determined spectrophotometrically using NanoDrop (Thermo Scientific, Wilmington, DE). PCR amplification of the highly conserved fungal internal transcribed spacer (ITS) region was done using primer pairs ITS1F and ITS4 (Gardes and Bruns, 1993; White et al., 1990) as previously described (Kårén et al., 1997). Amplified PCR products were purified using ethanol-sodium acetate precipitation protocol and sequencing was performed by MacroGen Europe (Amsterdam, The Netherlands).

#### Phylogenetic analysis

The generated sequence was submitted to GenBank at the National Center for Biotechnology Information (NCBI) with the accession number MK101318, and used for BLASTN analysis at NCBI. Additional ITS sequences from *Candida* spp. were retrieved from GenBank at NCBI. Sequence alignment was performed using MUSCLE algorithm (Edgar et al., 2004). Phylogenetic analysis was performed using neighbour-joining method implemented in MEGA ver. 5.0 (Tamura et al., 2011). The analysis was run using maximum composite likelihood substitution model with uniform rates among sites and homogeneous patterns among lineages. Gaps were deleted in pairwise comparisons and statistical support for the phylogenetic grouping was assessed by bootstrap analysis using 1000 replication.

#### Growth rates in NaCl and glucose solutions

For growth rate measurement, 6 ml of freshly prepared Sabouraud dextrose (SD) broth was added in 3 sterile test tubes. Each test tube was made up to 10 ml with final concentrations of 0.1M, 0.2M and 0.5M NaCl respectively. Exactly 0.1 ml of 10<sup>4</sup> CFU of the

identified *C. tropicalis* isolate was inoculated into each test tube and incubated overnight (O/N) at 37°C. A similar experimental set up containing 10 ml of SD broth without NaCl solution was prepared as the control and inoculated with 10<sup>4</sup> CFU of cells, the culture was incubated O/N at 37°C. At time intervals of 0, 3, 6 and 9 h, 2 ml of the content of each test tube was placed into a cuvette and the quantity of *C. tropicalis* cells in the solution was determined using spectrophotometer (UNICO® series spectrophotometer) at 600 nm. The growth rate assay was repeated at the same time intervals using 0.2, 0.5 and 1 g/ml glucose concentrations with a control group containing SD broth without glucose. There were 3 biological replicates for each experimental set up.

#### Preparation of plant extracts

Leaves of *B. coccineus* Schum. and Thonn. was harvested from Orba in Udenu Local Government Area of Enugu state, Nigeria. The plant was identified by Mr. A.O. Ozioko, a plant taxonomist at the International Center for Ethno-medicine and Drug Development, Nsukka (ICEMDD). The leaves were rinsed with running tap water and air-dried on a clean surface at room temperature for 7 days. The dried leaves were then pulverized with an electric blender to obtain a fine powder. Exactly 500 g of the pulverized leaves were subjected to extraction using soxhlet extractor (Electrothermal heating mantle, model MS-9506). The samples were packed into the soxhlet column to 2/3 of its volume and filled with 95% methanol, the extraction solvent. The soxhlet was then placed on the heating mantle adjusted to 40°C. The methanol was allowed to reflux repeatedly, until it was clear and free from the extracts. The extracted content was then subjected to rotary evaporator (Bibby sterlin Ltd, England, RE.200) until the methanol was completely evaporated to get the solidified crude extracts. The crude extract obtained was stored in sterilized amber coloured bottles and maintained at 4°C in a refrigerator. The extraction process was repeated with ethyl acetate as the extraction solvent.

#### Antifungal susceptibility test

One isolate from each of the five *Candida* spp identified on the CHROM agar *Candida*, including the isolate identified by molecular sequencing, were selected for the susceptibility study. Exactly 0.5 ml of O/N cultures of each *Candida* isolate containing 8.0 x 10<sup>5</sup> CFU was spread on freshly prepared SDA using a sterile spreader. In each petri plate containing the *Candida* culture, 4 holes (A, B, C and D), 3 mm in size were made with sterile borer at each side of the plate, 4 cm from the center. The holes were inoculated with 0.1 ml each of H<sub>2</sub>O, 500 mg/ml *B. coccineus* extracts (methanol and ethyl acetate) and 500 mg/ml of fluconazole, respectively. The experiment was repeated with 300, 200, 100 and 50 mg/ml each of *B. coccineus* extracts and fluconazole, respectively and the zones of inhibition were measured. There were 3 biological replications for each experimental set up.

#### Statistical analysis

Data was analyzed using descriptive statistics and represented as means and standard deviations. Statistical differences between means obtained from the zones of inhibition were determined using Mann-Whitney test.

## RESULTS

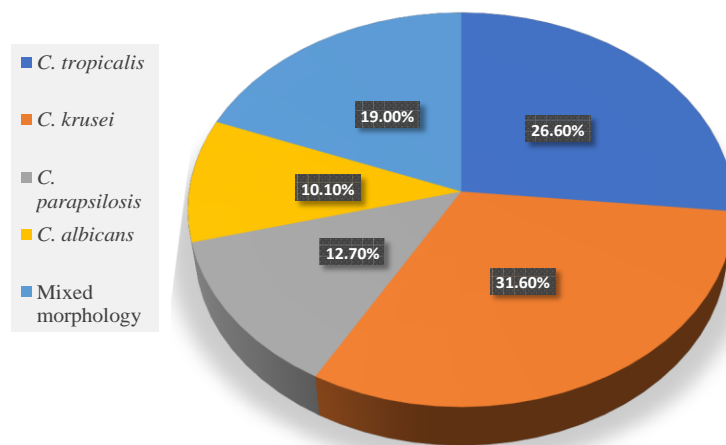
### Fungal isolation and identification

Based on differences in colour on CHROM agar *Candida*,

**Table 1.** *Candida* species isolated from patients (child bearing mothers) with different clinical conditions.

Condition	<i>C. tropicalis</i>	<i>C. krusei</i>	<i>C. albican</i>	<i>C. parapsilosis</i>	Variable <sup>a</sup>	Mixed <sup>b</sup>
Malaria	12	10	-	5	-	3
Typhoid	-	4	8	-	4	4
Diabetes	5	8	-	3	-	3
Healthy	4	3	-	2	-	-
Total	21	25	8	10	4	10

Total number of *Candida* spp isolated from each treatment condition. The *Candida* spp were identified based on morphological appearance on CHROMagar *Candida*. a= observed colour on CHROMagar *Candida* representing more than one *Candida* species in the literature. b= mixed, *Candida* culture contaminated with more than one *Candida* species.



**Figure 1.** The percentage distribution of *Candida* spp isolated from this study. Percentages were calculated from the total number of each *Candida* species isolated from each of the treatment conditions.

the fungal isolates were separated into different *Candida* species. Out of the 100 patients sampled, 21 showed no growth of *Candida* and were dropped from further analysis. About 79 samples showed growth of *Candida*, 21 (26.6%) were *C. tropicalis*, 25 (31.6%) were *C. krusei*, 10 (12.7%) were *C. parapsilosis* and 8 (10.1%) were *C. albicans* (Table 1, Figure 1). Four samples appeared yellow on CHROM agar *Candida* and could not be classified because they exhibited variable status in the literature. Also, 15 (19.0%) had mixed morphology on CHROM agar *Candida* and were not discernable. *C. tropicalis* and *C. krusei* were more prevalent in the malaria patients (Table 1), compared with the other categories. *C. albicans* was only found in the typhoid patients. About 8.9% of the women sampled in the study were in the control group.

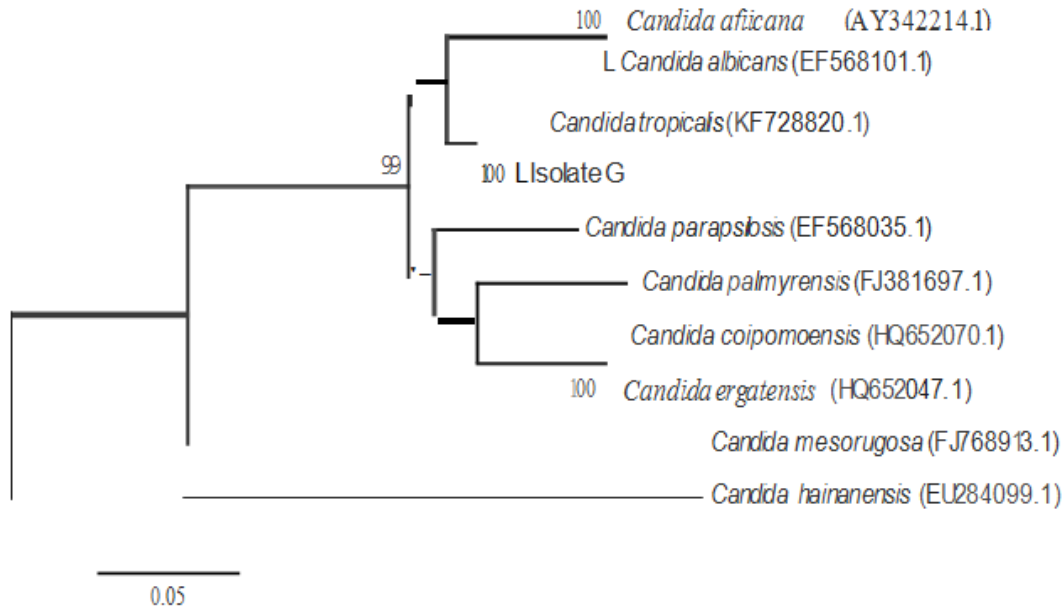
### Molecular identification

A BLASTN search of the NCBI database with the ITS sequence from the sequenced isolate revealed high

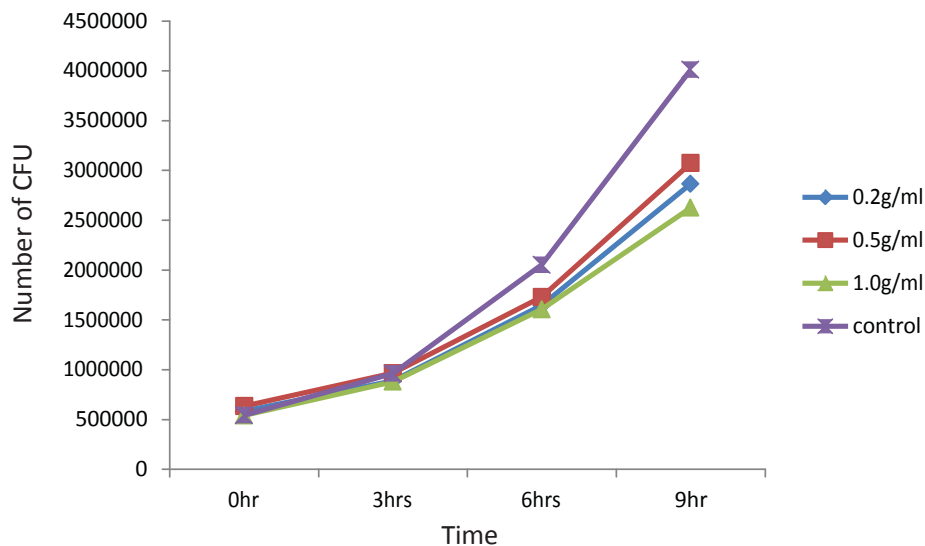
sequence similarity (100% identity, E-value = 0) to the sequence of *C. tropicalis* with accession number KF728820.1 (Figure 2). The phylogenetic analysis confirmed the identification of the isolate as *C. tropicalis* (Figure 2).

### Growth rates in glucose and NaCl

Including glucose in the growth medium reduced the growth of *C. tropicalis* (Figure 3). The biggest reduction in growth rate was found in the 1.0 g/ml glucose solution (Figure 3), compared with the 0.2 and 0.5 g/ml solutions. There were also observed differences in growth caused by addition of different concentrations of NaCl to the growth media. At 0 to 2 h, the same growth pattern was observed in the 3 salt concentrations, but the fungus showed a slightly higher growth rate in the control media during this time points (Figure 4). At 3h, the fungus showed exponential growth in the medium with 0.2 M and 0.3 M NaCl but the growth rate was drastically reduced by the 0.5 M salt concentration. The highest growth rate



**Figure 2.** Evolutionary relatedness of the isolated *Candida* spp. Phylogenetic analysis was performed using neighbour-joining method implemented in MEGA5.0. Composite likelihood substitution model with uniform rates among sites and homogeneous patterns among lineages were employed in the analysis. Statistical support for the branching was determined by bootstrap analysis using 1000 replication. Branches without support are less than 70%.

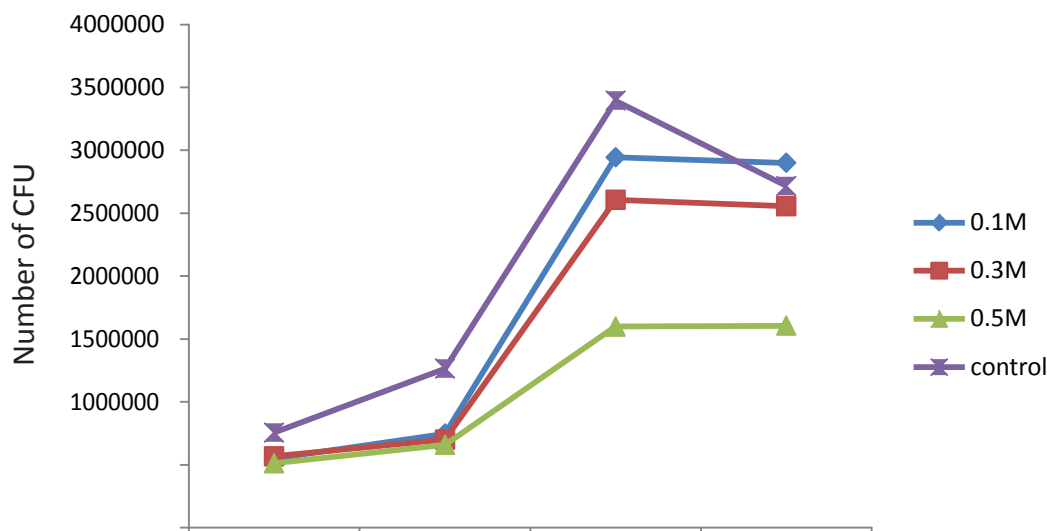


**Figure 3.** Growth curve of *C. tropicalis* in a glucose supplemented media. Growth rate was measured at time points (0, 3, 6 and 9 h) in different glucose concentrations (0.2, 0.5 and 1 mg/ml) by passing 2 ml of the O/N *Candida* culture through a spectrophotometer at 600 nm.

was observed in the control medium (without NaCl) at this time point. At 6 h, a sharp decline was observed in the growth rate of the fungus in the control media while a plateau was evident in the 3 media with different salt concentrations.

### Susceptibility studies

Fluconazole showed statistically significant higher activity than the two extracts from *B. coccineus* at any given concentration ( $P = 0.005$ ). The zones of inhibition ranged



**Figure 4.** Growth curve of *C. tropicalis* in a salt supplemented media. Growth rate was measured at time points (0, 3, 6 and 9 h) in different salt concentrations (0.1, 0.3 and 0.5 M) by passing 2 ml of the O/N *Candida* culture through a spectrophotometer at 600 nm.

**Table 2.** Susceptibility profile of the isolated *Candida* species to fluconazole, methanol and ethyl acetate extracts of *B. coccineus*.

Concentrations (mg/ml)	<i>B. coccineus</i> extracts		
	Fluconazole	Methanol extract	Ethyl extract
500	45.0 ± 1.2 <sup>a</sup>	17.44 ± 0.3 <sup>b</sup>	15.0 ± 1.3 <sup>b</sup>
200	40.44 ± 3.4 <sup>a</sup>	14.22 ± 1.1 <sup>b</sup>	14.78 ± 0.6 <sup>b</sup>
100	26.33 ± 1.9 <sup>a</sup>	13.9 ± 0.4 <sup>b</sup>	12.46 ± 0.2 <sup>b</sup>
50	31.83 ± 2.1 <sup>a</sup>	14.22 ± 0.7 <sup>b</sup>	12.33 ± 1.7 <sup>b</sup>

The susceptibility pattern was determined by measuring the zones of inhibition of fluconazole and the *B. coccineus* extracts against the growth of  $8.0 \times 10^5$  inoculum of the *Candida* spp. Numbers with superscripts in each row indicate statistically significant difference at 0.05% level.

from 26.3 to 45.0 mm for fluconazole to 13.9 to 17.4 mm and 12.3 to 15.0 mm for the methanol and ethyl acetate extracts respectively (Table 2). Optimal activity of both fluconazole and the two extracts was observed at 500 mg/ml. At this concentration, the activity of the methanol extract was slightly higher than the activity of the ethyl acetate extract while the activity of the fluconazole control was almost twice the activities of the two extracts.

## DISCUSSION

Candidiasis in women of child-bearing age is a major health problem in most hospitals especially among the immunocompromised patients (Lee et al., 2007). The distribution of *Candida* spp in vaginal candidiasis among women of child-bearing age under certain health conditions has not been fully investigated. In the present

study, the prevalence of *Candida* in women 20 to 30 years of age who were suffering from malaria, diabetes and typhoid was compared. The antifungal susceptibility profile of the identified *Candida* species was also tested using both conventional azole and extracts from *B. coccineus*. Out of the 79 vaginal swabs (VGS) samples that showed presence of *Candida*, *C. krusei* had the highest occurrence 32.6%, followed by *C. tropicalis* 26.6%.

The occurrence of *C. albicans* and *C. parapsilosis* was relatively low 10.1 and 12.7%, respectively. The normal/healthy patients presented low level of *Candida* colonization probably because of intact immune system. Most of the identified *C. tropicalis* were found in the malaria patients, but could not be statistically linked to malaria. Also, most of the *C. albicans* were isolated from the typhoid patients while *C. krusei* was the most abundant species in the patients with diabetes. However,

*C. albicans* have been reported in other studies as the most abundant *Candida* species isolated from the vaginal swab of women with Type 1 diabetes, while *C. glabrata* was shown to be the most common isolate in Type 2 diabetic patients (Bohannon, 1998). Yeast infection in diabetic patients has been reported and *Candida* spp. described as the major infectious agent causing oral and esophageal candidiasis, pyelonephritis as well as cystitis (Casqueiro et al., 2012). In the present study, we did not classify the diabetic participants into Types 1 and 2 diabetics, it is possible that the variations in results could be as a result of differences in race or due to geographical variations. Studies have shown that the distribution of *Candida* species in women with vaginal candidiasis vary with geographic locations as well as the populations studied (Achkar and Fries, 2010). In the United States, Europe and Australia, *C. albicans* is the most abundant *Candida* species identified in 76 to 89% of studied women, followed by *C. glabrata* (7 to 16%) (Corsello et al., 2003; Holland et al., 2003). Furthermore, in HIV infected patients, *C. albicans* and *C. glabrata* were found as the most prevalent *Candida* spp. colonizing the vaginal mucosa of infected patients (Merenstein et al., 2013). To further confirm the results from the morphologic appearance on CHROM agar *Candida*, we sequenced the ITS region of a randomly selected isolate that was presumptively identified as *C. tropicalis* based on its colour on CHROM agar *Candida*. BLASTN search of the sequenced isolate matched with the sequence of *C. tropicalis* in GenBank. CHROM agar *Candida* is a suitable media for the isolation and presumptive identification of *Candida* spp. based on strongly contrasted colony colours produced by reactions of species-specific enzymes with a chromogenic substrate (Khadka et al., 2016). CHROM agar *Candida* medium has been successfully used in the identification and differentiation of *C. albicans*, *C. krusei*, *C. tropicalis*, and *Trichosporon* spp. from other yeast species (Horvath et al., 2003).

Further analysis of the identified *Candida* spp. was done by tracing its evolutionary relatedness with other *Candida* spp. in the GenBank. Phylogenetic reconstruction showed one cluster separated into two small sub groups. The identified fungal isolate was more closely related to other pathogenic *Candida* spp. like *C. tropicalis*, *C. albicans* and *C. africana* in that order. The other small group comprised of mainly *Candida* spp. that have not been previously reported to be pathogenic in either humans or animals although *C. parapsilosis* which is a pathogenic species nested with this group. Other studies have demonstrated the close relationship between *C. albicans* and *C. tropicalis* using the internal transcribed spacer ITS1 gene (Butler et al., 2009). This intimate evolutionary relationship is also evident in phenotypic and biochemical characteristics of both species.

To determine how carbon and electrolyte sources affect

the growth of the *Candida* spp, growth assays were carried out using graded levels of NaCl (table salt) and glucose. While lower concentrations of glucose in the medium (0.2 and 0.3 g/ml) increased the growth rate of the fungus, concentrations as high as 1 g/ml adversely affected the growth rate. This result was not surprising as more energy and time may be required to metabolize the 1g/ml glucose concentration. Previous studies have shown that moderate carbon source modulates the physiological status of fungus and also plays a crucial role in fungus-host interaction (Ene et al., 2012a, b; Ueno et al., 2011; Vylkova et al., 2011). A similar trend was also observed in the behavior of the fungus in the presence of salt. While growth rate was improved in the presence of 0.2 M and 0.3 M NaCl concentrations, the growth rate was reduced by 0.5 M NaCl. Halotolerant yeasts have been shown to grow in the presence of salt concentrations as high as 4 M NaCl (Lages et al., 1999). In a survey performed on 42 different yeast species, *Candida* halophiles were the only yeasts capable of growing on glucose in the presence of 5 M NaCl (Lages et al., 1999). However, in our studies, growth rate was hampered by 0.5 M concentration of the salt, an indication that the *Candida* spp. used in the growth assay is not a salt tolerant strain. We further analyzed the resistant pattern of the isolated *Candida* spp. using a conventional azole (fluconazole) and two extracts (methanolic and ethyl acetate) from *B. coccineus*. Fluconazole showed a higher activity than the two extracts with zone of inhibition as high as 26.3 to 45.0 mm compared to 13.9 to 17.4mm and 12.3 to 15.0 mm ZI for the methanol and ethyl acetate extracts respectively. Some *Candida* spp., such as *C. glabrata* and *C. krusei* have been shown to acquire decreased susceptibility to fluconazole (Cleveland et al., 2012; Pfaller et al., 2015). *C. glabrata* can survive in the hospital environment due to its high resistance to the commonly used azoles and echinocandins thereby increasing the chance of nosocomial transmission (Pfaller et al., 2011). For effective antifungal therapy therefore, the use of extracts from medicinal plants should be explored. However, our plant of choice *B. coccineus* showed decreased activity compared to the fluconazole. This could probably be as a result of the extraction methods or poor content of bioactive components in the leaves. The ethyl acetate extracts of *B. coccineus* has been found to have very high antimicrobial activity on *C. albicans* (Ahmadu et al., 2006). Also the butanolic extract of the plant has been shown to have high activity on *S. aureus* but the activity on *C. albicans* was quite low (Ahmadu et al., 2006). The antimicrobial activities of these extracts could be as a result of different bioactive components inherent in the leaves. Although we did not carry out phytochemical screening to investigate the bioactive components of the plant, other studies have reported the presence of tannins, saponins, sugars, coumeryl derivatives and flavonoid glycosides to be responsible for the antimicrobial



activities (Ahmadu et al., 2006). In conclusion, although the extracts from *B. coccineus* showed a relatively lower activity compared to the fluconazole, it is a promising candidate for antifungal therapy. Additionally, *C. tropicalis* and *C. krusei* were the most common *Candida* spp. isolated from this study and they were more abundant in the malaria and diabetic patients respectively than in the other health conditions. Other *Candida* spp. like *C. albicans* and *C. krusei* were also isolated but at a relatively lower level.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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