Susceptibility Pattern of *Candida albicans* from vaginal candidiasis to azole antifungal agents and Extracts of *Lannea welwitschii* (Hiern) Engl. (Anacardiaceae)

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Abstract

Increase in resistance of *Candida* species to antifungal drugs including those of azole group commonly employed to treat vaginal candidiasis is a global health challenge, necessitating the need to seek alternative therapeutic approaches from medicinal plants. This study investigated the susceptibility pattern of *Candida albicans* to selected azole antifungal agents and extracts from a Nigerian medicinal plant, *Lannea welwitschii*. Thirty-five clinical isolates of *C. albicans* collected from University College Hospital, Ibadan, were further identified using Sabouraud dextrose agar, CHROM-agar (*Candida*), catalase, germ tube and lactose fermentation tests. Susceptibility of the isolates to azole antifungal drugs (fluconazole and voriconazole) and methanol extract of *L. welwitschii* stem bark at 100 and 50mg/ml were determined using agar-well diffusion method. Minimum inhibitory concentrations (MICs) of the extract and Miconazole (control) were determined using agar dilution method. Using CLSI breakpoint standard; for Voriconazole (25µg), 82.85% of the isolate were resistant, 5.71% intermediate and 11.43% susceptible while for Fluconazole(1µg), 88.57% resistant, 5.71% intermediate and 2.85% susceptible. The isolates showed 83-89% susceptibility to the plant's extract. The MIC of *L. welwitschii* and Miconazole on seven (7) selected isolates were 3.1-12.5mg/ml and 1.0-1.6µg/ml respectively. The study showed an increase in resistance of *Candida* species to azole antifungal agents when compared to previous studies. Extract of *Lannea welwitschii* showed good anti-candida activity comparable to Miconazole, even on isolates that were resistant to Fluconazole and Voriconazole, and as such may be investigated for treating vagina candidiasis.

Keywords: *Candida albicans*, Fluconazole, Voriconazole, *Lannea welwitschii*, antifungal

INTRODUCTION

Recent global estimates of fungal infection worldwide have found about 700,000 cases of invasive candidiasis occurring annually [1]. Vaginal candidiasis is an excessive growth of the yeast in the vagina resulting in irritation. Vulvovaginal candidiasis affects 70–75% of women at least once during their life time especially during childbearing age with a global prevalence estimate of 134 million cases [2]. The pathogenesis of the disease is still under study but genetics play a bit of role. Predisposing factors for increased rates of vulvovaginal candidiasis are an ageing but sexually active population, antibiotic misuse, and increased numbers of patients who are diabetics [2]. Immunity, especially cell-mediated plays a predominant role in host defense mechanism against mucosal *Candida* infections and it was

revealed that protection against vaginitis coincides with a non-inflammatory innate presence, whereas symptomatic infections correlates with a neutrophil infiltrate in the vaginal lumen and elevated vaginal burden [3].

Therapy for serious *Candida* infections has been difficult because of the limited number and uses of available antifungal agents. Amphotericin B (a polyene antibiotics), which is the main drug of treatment, is associated with many toxicities and requires intravenous administration. Flucytosine, which would have been a main drug in treating fungal infection is limited by its bone marrow toxicities and the high rate of spontaneous mutation to resistance such that it cannot be used alone. With the introduction of azole antifungal agents in 1980s, with oral bioavailability, broad spectrum activity and less toxicity, fungal chemotherapy was enhanced [4,5]. However, with increasing reports of resistance of *Candida* and other fungi to the azole antifungals, research to develop new, safe and effective antifungal agents has increased tremendously. To date, more antifungal drugs have been and are being introduced; for example, terbinafine (1991), caspofungin (2001), voriconazole (2002), posaconazole (2006) and isavuconazole (2015) are some of the new agents. Yet, resistance development remained a concern. *Candida albicans* use different resistant mechanisms like multidrug resistant protein and membrane potential to efflux azoles, changing the affinity of the target CYP51 [6]. Azole resistance in *C. albicans* has now been well reported in women with vulvovaginal candidiasis and the emergence of fluconazole-resistant *Candida glabrata* is frequently documented [6].

Bioactive plant constituents play an important role in the treatment of various microbial infections, including those caused by *Candida* species [7]. Many Nigerian medicinal plants are used to treat infections (oral and vulvo-vaginal thrush) caused by *Candida albicans* [8]. *Lannea welwitschii* (Hiern) Engl. (Anacardiaceae) is a tropical African plant used in traditional medicine in South West Nigeria for treating wounds and other infections and has been confirmed to have antimicrobial activity [9]. It is important to search for, especially among the traditionally used medicinal plants, therapeutic alternatives to treat vaginal candidiasis and to overcome resistance to azole antifungal drugs. The aim of this study was to determine the susceptibility pattern of *Candida albicans* from vaginal candidiasis to some commonly used azole antifungals and methanol extract of *Lannea welwitschii* as a preliminary step towards discovering new agents to treat azole resistant *Candida* infections.

MATERIALS AND METHODS

Plant material collection, authentication and extraction

Lannea welwishii was collected from Botanical Garden, University of Ibadan and was identified in Forestry Research Institute of Nigeria (FRIN) with a deposited voucher specimen number FHI-107973. The bark of *L. welwitschii* (1kg) was air-dried at room temperature, pulverized to coarse powder and then extracted with methanol using a Soxhlet apparatus. The extract was concentrated over a rotary evaporator, air-dried and stored at 4^{o}C for use.

Isolate collection

Clinical isolates of *Candida albicans* were collected from the stock in Medical Microbiology Department, University College Hospital (UCH), Ibadan. The study was conducted in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan. The collected isolates were suspended in tryptone soy broth and then incubated for 24hrs at 37°C, after which it was subcultured on Sabouraud dextrose agar (SDA) (Oxoid,

Basingstoke, UK) and incubated at 37°C for 24hrs. The isolates were purified and subcultured on SDA slant for subsequent testing. *Candida albicans* ATCC 18804 was used as a reference (standard) strain.

Identification of isolates

Identification of the yeast cells was done using methods such as Biochemical (Lactose fermentation and Catalase) tests, Cultural method on CHROM-agar (*Candida)* (Oxoid, Basingstoke, UK) and Germ tube test.

Cultural method

The isolates were streaked on a plate of SDA and incubated at 37°C for 24 hs. Pure yeast colonies were then collected and stored for further testing.

Lactose fermentation test

The isolates were cultured in peptone water to which normal saline (0.9%w/v), phenol red, and lactose have been added, and were incubated with Durham tubes included for gas collection for 24hrs at 37°C. The results were interpreted using changes in colour and production of gas as seen in the Durham tubes.

Catalase test

The test was done by placing a drop of hydrogen peroxide on a microscope slide. Using a sterile wire loop, the colony of each *Candida* isolate was smeared into the drop. The specimens were observed for bubbles production. The microorganism was said to be catalase-positive if it produced bubbles and catalase-negative if no bubbles were produced.

Germ tube test

The clinical isolates were incubated in tryptone soy broth for 2-3hrs at 30-37°C and then smeared on a microscope slide and stained with lactophenol cotton blue stain and mounted for examination under microscope. The presence of *C. albicans* was observed under a microscope as short, slender, tube-like structures which are germ tubes. These germ tubes are the elongated daughter cells from the mother cell without constriction at their origin, unlike the pseudo-hyphae which are constricted at the origin of the mother cells.

CHROM-agar *Candida*

CHROM-agar *Candida* was prepared according to the manufacturer's directions, poured in sterile petri-dishes and allowed to set. Each of the isolates was spread (using a sterile glass spreader) on the CHROM-agar *Candida* plates and were then incubated at 37°C for 24-48 hs. The *C. albican* isolates were then identified as they produced the characteristic green colonies.

Susceptibility testing of the isolates to conventional antifungal agents

Modified Kirby-Bauer disc diffusion method was used to determine the susceptibility of the Candida isolates to Fluconazole (25µg) and Voriconazole (1µg) discs (Oxoid) following CLSI guidelines. Sabouraud dextrose agar (SDA), prepared according to the manufacturer's instruction was poured into each sterile Petri-dish and allowed to set. From an overnight culture of each *Candida* isolate corresponding to the turbidity of 0.5 McFarland (1.0×10^8 cfu/ml), 0.1 ml of 10^{-2} dilution of each isolate was spread on each corresponding plate. The plate was divided into two and labeled as FLU (fluconazole) and VOR (voriconazole), and the corresponding antifungal discs were carefully fixed on the inoculated agar surface as labeled and the plates were incubated at 37°C for 24 hs. The activities of the antifungal discs were read as zone of inhibition in millimeters and were interpreted with CLSI [11] reference to

the following categories: Susceptible (S), Intermediate (I) and Resistant (R).

Susceptibility testing of the isolates to plant's extract

Susceptibility testing of the isolates and the reference *Candida* to *L. welwitschii* extract was done by agar-well diffusion method. Generally, CLSI guidelines and references were followed [10,11]. Using agar-well method, 50mg/ml and 100 mg/ml of *L. welwischii* extract was used and 0.05mg/ml of miconazole was used as the control. Sabouraud dextrose agar was prepared and poured into the sterile Petri dishes and allowed to set. From an overnight culture of each *Candida* isolate corresponding to the turbidity of 0.5 McFarland (1.0 x 10^8 cfu/ml), 0.1 ml of 10^{-2} dilution of the isolate was spread using a surface spreader on the surface of each corresponding SDA plates. Using agar-well method, a sterilized cork-borer of 8mm diameter was used to cut 3 equidistant wells in each compartment of the seeded agar plate. The discs of agar were removed into disinfectant solution. Each concentration (100 µl) of plant extract was dispensed into the wells with corresponding labels. The control (miconazole powder at 0.05 mg/ml) was also dispensed to fill ¾ of its labeled well. The plates were left at room temperature for about an hour to allow the extract and miconazole solution to diffuse through the medium. The plates were then incubated at 37°C for 24 hs and the zones of inhibition in millimetres were read.

Determination of minimum inhibitory concentration (MIC)

Determination of minimum inhibitory concentration of miconazole

Using agar dilution method, seven (7) concentrations of miconazole was prepared by dilution of the stock solution (1 µg/ml) with methanol. Each of the Petri-dish was divided into seven (7) equal compartments using a marker and labeled according to the seven (7) various candida isolates, already sterilized SDA agar prepared according to manufacturer's specification was mixed with each concentration of miconazole and poured into sterile Petri-dishes and then allowed to set firmly. Each inoculum was then spread on the agar surface, corresponding to the label of the isolates on the plates. Positive and negative plates were used as control and the plates were incubated for 48 hs at 37°C then the result was read as diameter of zone of inhibition in millimeters and recorded.

Determination of minimum inhibitory concentration of *Lannea welwitschii*

Using agar dilution method, seven (7) concentrations of *L. welwitschii* extract was prepared by dilution of the stock solution (50,000 µg/ml) with methanol. From *L. welwitschii* extract stock solution prepared with methanol, 5mls was transferred to another 5mls methanol which was mixed thoroughly and another 5mls was taken from the concentration of miconazole solution produced to another 5mls of methanol and the procedure was repeated for subsequent tubes to produce two-fold dilution of miconazole solution.

Each Petri-dish was divided into seven (7) equal compartments using a marker and labeled according to the seven (7) various candida isolates, already sterilized Sabouraud's dextrose agar prepared according to manufacturer's specification was mixed with each concentration of the *L. welwitshii* extract and poured into sterile Petri-dishes and then allowed to set firmly. Each inoculum was then spread on the agar surface, corresponding to the label of the isolates on the plates. Positive and negative plates were used as control and the plates were incubated for 48 hs at 37°C then the result was read as diameter of zone of inhibition in millimeters and recorded.

RESULTS

Identification of the isolates

All the isolates cultured on SDA had the characteristic colony typical of *Candida* and as green colonies on CHROM-agar *Candida*. Twenty-nine isolates produced germ tube on examination after incubation in tryptone soy broth for 2-3 hours and staining its smear on microscope slide with Lactophenol cotton blue stain while the remaining six isolates (isolate no 4,7,10,13,16 and 27) did not (Table 1). Only one isolate (18) showed changes in colour of the medium to yellow and production of gas as indicated by the durham tubes in the medium in Lactose fermentation test and all the 35 isolates were catalase positive.

Susceptibility of *Candida* isolates to standard Fluconazole and Voriconazole discs

In this study, the susceptibility of the isolates to azole antifungals (Fluconazole 25 µg and Voriconazole 1 µg discs) showed that only 25.71 % of the *Candida* isolates were susceptible. Using Clinical and Laboratory standards institute (CLSI) M27-A3 zone diameter interpretive breakpoint standard; 82.85 % of the isolates were resistant, 5.71 % were intermediate and 11.43 % was susceptible to Voriconazole, while 88.57 % of the isolates were resistant, 5.71 % were intermediate and 2.85 % were susceptible to Fluconazole. The reference organism, *C. albicans* ATCC 18804 was found sensitive to the drugs (Table 2).

The yield of the plant on extraction with methanol is 8.45% as shown in Table 3.

Anti-Candida activity of *Lannea welwitschii* and Miconazole

Miconazole was used as a positive control in this study as it is one of the azole antifungals commonly used in the management of vagina candidiasis. Miconazole had anti-candida activity on 77.1 % of the isolates. The extract of *L. welwitschii* showed anti-candida activity on 83-89 % of the isolates (Tables 2 and 4).

Table 1: Germ tube test

Isolate	Result	Isolate	Result
1	+	19	+
2	+	20	+
3	+	21	+
4	-	22	+
5	+	23	+
6	+	24	+
7	-	24	+
8	+	26	+
9	+	27	-
10	-	28	+
11	+	29	+
12	+	30	-
13	-	31	+
14	+	32	+
15	+	33	+
16	-	34	+
17	+	35	+
18	+		

Key: + = A short hyphal (filamentous) extension arising laterally from a yeast cell: - = No hyphal (filamentous) extension

Table 2: Susceptibility of *Candida* isolates to fluconazole and voriconazole discs

ISOLATES	VOR	FLU
1	R	R
2	R	R
3	R	R
4	R	R
5	S	S
6	R	R
7	R	R
8	R	R
9	R	R
10	S	R
11	R	R
12	R	R
13	R	R
14	R	R
15	R	R
16	R	R
17	I	I
18	R	R
19	R	R
20	R	R
21	R	R
22	R	R
23	R	R
24	R	R
25	R	R
26	S	R
27	I	I
28	R	R
29	R	R
30	R	R
31	S	I
32	R	R
33	R	R
34	R	R
35	R	R
ATCC	S	S

Key: FLU = Fluconazole (25µg); VOR = Voriconazole (1µg); ATCC 18804 = Reference *C. albicans;* S = Susceptible; I = Intermediate; R = Resistant.

Table 3: Yield of plant on extraction

Plant	Weight of plant sample (g)	Weight of extract (g)	Yield (%)
***Lannea welwitschii* stem bark**	1000	84.535	8.45

Table 4: Anti-candida activity of the plant extracts and miconazole

Isolate	LW(50mg/ml)	LW(100mg/ml)	Mico (0.05mg/ml)
	Diammeter of zone of inhibition(mm)		
1	10	15	-
2	10	10	13
3	17	19	-
4	10	15	-
5	15	19	10
6	12	13	15
7	19	25	14
8	-	18	-
9	15	13	25
10	12	20	25
11	10	21	13
12	12	15	15
13	12	17	13
14	-	10	14
15	-	-	12
16	15	15	15
17	10	12	-
18	11	19	18
19	16	16	30
20	10	17	19
21	10	15	20
22	17	17	15
23	15	15	10
24	17	13	10
25	12	17	15
26	12	19	19
27	-	-	11
28	15	19	12
29	10	16	20
30	-	-	-
31	13	15	-
32	13	15	11
33	-	-	-
34	10	15	15
35	-	13	16
ATCC	16	19	19

Key: LW = *Lannea welwitschii* stem bark; ATCC= *C. albicans* ATCC 18804; Mico = Miconazole; - = No zone of inhibition

Minimum inhibitory concentration of *Lannea welwitschii* extract and Miconazole.

The minimum inhibitory concentration of the *L. welwitschii* extract was 3.1-12.5mg/ml and that of miconazole was 1.0-1.6µg/ml using seven (7) selected isolates (Table 5).

Table 5: MIC of Miconazole and *Lannea welwitschii* extract on *Candida* isolates

Isolates	Mico (mg/ml)	LW (mg/ml)
1	1.563	3.125
2	1.563	3.125
3	1.563	12.500
4	1.563	12.500
5	1.563	3.125
6	1.000	3.125
7	1.563	3.125

Key: LW = *Lannea welwitschii* stem bark; Mico = miconazole

DISCUSSION

Identification

Cultural method

The first step in the identification of the isolates was culturing them on Sabouraud Dextrose Agar (SDA) for growth and macroscopic examination. Specifically, SDA is a selective medium for fungi, containing glucose and peptone (to grow the fungal cells) and with its low pH (5.6), SDA inhibits the growth of most other organisms, especially bacteria. Most fungi produce characteristic colonies on SDA. In this study, the isolates when grown on SDA showed buttery, cream-coloured, smooth and soft colonies which are characteristic of *Candida* species. All the isolates cultured on SDA had the characteristic colony typical of *Candida* and it is a simple, but not conclusive method for identifying *Candida*. These characteristics colonies of *Candida* on SDA were also described by Ilona, 2006 [13].

CHROM-agar *Candida*

CHROM-agar *Candida* is a chromogenic medium that uses colour to distinguish the various *Candida spp*. based on the principle that yeasts produce enzymes that react with chromogenic agents in CHROM-agar *Candida* to produce colonies of different colours. In this study, the 35 isolates showed green colonies on CHROM-agar *Candida*. The colour of the *C. albicans* colony produced is similar to that described by Nadeem *et al*., 2010 in a study of use of CHROM-agar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resources-limited setting [14].

Germ Tube Test

Germ tube test is confirmatory for *Candida albicans*, which produce short, slender tube-like structured with no constriction from the origin point, called germ tubes. In this study, 29 out of the 35 isolates produced germ tubes after incubation in tryptone soy broth for 2-3 hours, stained with Lactophenol cotton blue and examined under a microscope (40x objective). The 6 isolates that were negative to germ tube test are isolates number 4, 7, 10, 13, 16 and 27. The formation of germ tube by *Candida albicans* was also reported by Kyoung-Ho *et al*., 1999 [15], where it was submitted that germ tube induction of *C. albicans* may involve complicated pathways of dimorphism, triggered by distinct environmental factors.

Lactose Fermentation Test

This is an easy method of differentiating *Candida albicans* from other species of

Candida but it is not definitive, as *Candida dubliensis* also do not ferment lactose. In this study, only isolate 18 showed changes in colour of the medium to yellow and production of gas as indicated by the Durham tubes in the medium. This is similar to a work by Saravana, [16] where the carbohydrate assimilation test for *C. albicans* was done, the test was positive for dextrose, maltose, galactose, sucrose, xylose and trehalose while tests were negative for lactose, melibiose, cellobiose, inositol, reffinose and dulcitol.

Catalase test

In this study, all the 35 isolates of *C. albicans* were catalase positive. This method is a rapid method and is typical of the organism.

Susceptibility of *Candida* isolates to standard Fluconazole and Voriconazole discs

Certain factors tend to reduce the availability of the antifungal agents below that of the effective therapeutic concentrations and yeasts thereby undergo only a limited exposure to the antifungals during therapy [18]. In this study, the susceptibility of the isolates to azole antifungals was done using Fluconazole 25 µg and Voriconazole 1µg disc from Oxoid pharmaceuticals. Fluconazole and voriconazole showed anti-candida activity against 25.71 % of the isolates. Using Clinical and Laboratory standards institute (CLSI M27-A2) zone diameter interpretive breakpoint standard, 82.85 % of the isolates were resistant, 5.71 % were intermediate and 11.43 % were susceptible to voriconazole and 88.57 % of the isolate were resistant, 5.71 % were intermediate and 2.85 % were susceptible to fluconazole. This result is similar to a study done by Ekanola *et al*. [19] where a total of 107 identified *Candida* strains (*C. albicans* 87 (81.3 %), *C. glabrata* 2 (1.97 %), *C. pseudotropicalis* 11 (10.3 %) and *C. tropicalis* 7 (6.54 %) were used in the study. The *Candida* strains were assayed for their in vitro susceptibility and resistance rates to antifungal drugs. Out of a total of 18 antifungal drugs tested for their in vitro inhibitory activities against the 107 *Candida* strains, significantly high resistance rates were recorded, while only few of the *Candida* strains were susceptible to the antifungal drugs overall. Resistance rate of 36.4-100 % were recorded against the fluconazoles. However, considering the report of Mandras *et al*. [20] that, "despite the widespread use of fluconazole for more than a decade, it was found that there was no evidence that *C. albicans* (98 % susceptible) has developed increased resistance to fluconazole", there has been a paradigm shift in resistance of *Candida* species to fluconazole and other azole antifungals. This was also in line with the recommendation of Pfaller *et al*. 2011 [12]. The high level of and the mechanisms involved in the resistance of *Candida* species to azole antifungal drugs indicated multiple steps plus cross resistance [21].

Plant extraction and yield

The yield of *Lannea welwitschii* stem bark after extraction using soxhlet with methanol as solvent is 8.45 % (Table 3) which is in line with, though slightly lower than the yields previously reported Idowu and Idowu [9]. Agyare et al. [17], who extracted by another method involving Ultra-Turrax T-50 under ice cooling, also recorded a lower yield. Soxhlet extraction is an efficient method but thermolabile constituents, if present in the plant materials, may be affected by the extraction temperature. However, it is an economical method as it requires less solvent when compared to maceration.

Anti-candida activity of the plant extracts and miconazole

Miconazole was used as a positive control in this study as it is one of the azole antifungals commonly used in the management of vagina candidiasis. Miconazole had anti-candida activity on 77.1 % of the isolates and the reference organism. The extract of *Lannea welwitschii* showed anti-candida activity on 83-89 % of the isolates. The activity at 100mg/ml was higher than at 50mg/ml (Table 4), which indicated that the anti-candida activity may be concentration dependent and was confirmed by the MIC values. This anti-candida activity of *L. welwitschii* is comparable to that of miconazole, and is similar to a study in which *L. welwitschii* showed broad spectrum activity against both Gram negative and Gram positive bacteria and also on *C. albicans* [9]. Also, in a study to determine the antimicrobial, antioxidant, and in vivo wound healing properties of methanol leaf extracts of *Justicia flava* and *L. welwitschii*, methanol leaf extracts of *L. welwitschii* possessed antimicrobial and wound healing properties which may justify the traditional uses of the plants to treat wounds and infections [17].

Minimum inhibitory concentrations of Miconazole and *Lannea welwitschii* stem bark extract

In this study, the minimum inhibitory concentration (MIC) of miconazole (from miconazole powder dissolved in methanol) on the 7 selected *C. albicans* isolates ranged 1.0 - 1.6 µg/ml using seven (7) selected isolates. This result is similar to that got in a study reported by Paniagua *et al.* [4] where miconazole had a MIC of 1.56 µg/ml.

The minimum inhibitory concentration of the *L. welwitschii* extract was 3.1 - 12.5 mg/ml on the same seven (7) selected isolates. The MIC was moderately low compared with standard drugs, but is similar to that reported by Agyare *et al.* [17] in which the MIC of *L. welwitschii* extract on *Candida albicans* was found to be 2.5 mg/ml. However, considering the bioactivity study of crude extracts in comparison to pure compounds, MIC may be invariably high. It therefore recommended that the active compound(s) be isolated and tested for appropriate evaluation of the antimicrobial activity in terms of MIC.

Comparison of susceptibility of *Candida* isolates to the drugs and plant's extract

Fluconazole (25 µg) and voriconazole (1 µg) showed similar anti-candida activity; with a total of 25.71 % of the isolates being susceptible to both drugs. This is not surprising, considering their closely related structural formula, with the major difference being the substitution of a fluoropyrimidine grouping in place of a triazole moiety in voriconazoe. However, using CLSI [10] zone diameter interpretive breakpoint standard, the difference is as follows; for voriconazole, 82.85 % of the isolates were (R) resistant, 5.71 % (I) intermediate and 11.43 % (S) susceptible; while for fluconazole, 88.57 % of the isolates were (R) resistant, 5.71 % (I) intermediate and 2.85 % (S) susceptible. Therefore, in this study, *Candida albicans* isolates were more susceptible to voriconazole than to fluconazole. The result is in agreement with the report of Greer (2003), who also cautioned that there may be a cross resistance of *C. albicans* to both drugs [22]. Earlier report from Pfaller *et al.* (2002) had proved the efficacy of voriconazole (and ravuconazole) over many antifungal agents in a study involving 6,970 species of *Candida* [23].

However, in this study, 83-89 % of the *Candida* isolates tested were susceptible to *L. welwitschii* extract compared with 77.1 % that were susceptible to Miconazole. Some Candida albicans strains that were resistant

to miconazole, Fluconazole and voriconazole were susceptible to the plant's extracts. This implies that, *Lannea welwitschii* stem bark extract was more active on the Candida isolates than miconazole at the tested concentration.

CONCLUSION

The study showed an increase in resistance level of *Candida* spp. to azole antifungal agents when compared to previous studies. We therefore cautioned against the misuse and abuse of azole antifungal drugs to prevent further development and spread of strains of *C. albicans* that are resistant to them. The extract of *Lannea welwitschii* stem bark showed good anti-candida activity comparable to miconazole, even on isolates that were resistant to fluconazole and voriconazole and as such may be investigated for developing antifungal agents for the treatment vaginal candidiasis.

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