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In vitro antifungal activities of some mushroom extracts against yeast isolates among women with vulvovaginal candidiasis in a teaching hospital

Antifungal actions of mushroom extracts on vulvovaginal candidiasis

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Abstract
Objective: Vulvovaginal candidiasis is a common fungal opportunist infection of the female genital parts caused by *Candida* species. Although it is usually mild, severe cases leading to complications do occur. Studies on natural therapy against candidiasis are rare. Hence, this study was aimed at evaluating the antifungal potentials of *Ganoderma applanatum, Ganoderma lucidum, Pleurotus* and *Polyporus* mushrooms against *Candida* species isolated from vaginal specimens.
Method: High vaginal swab (150) samples were collected from patients, with a median age of 34.5 years, streaked on chocolate and sabouraud dextrose agar and incubated between 24 hours and 7 days. Ethanol mushroom extracts (40mg/L) were concentrated using a rotary evaporator Antifungal drugs; fluconazole, nystatin and ketoconazole and the extracts were tested against the isolates.
Results: *Candida albicans, Candida glabrata, Candida tropicalis,* and *Candida krusei* were isolated from the samples; having a prevalence of 27(51.9%), 10(19.2%), 8(15.4%), and 7(13.5%) respectively. Reproductive and teen ages had a higher and lower prevalence of 30(57.7%) and 2(3.8%) respectively. The susceptibility test revealed that 67.3%, 44.2% and 75.0% of the total yeast isolates were susceptible to nystatin, ketoconazole and fluconazole respectively. *Candida albicans, C. tropicalis, C. glabrata* and *C. krusei* were most susceptible to fluconazole (81.5%), fluconazole (100%), ketoconazole (60%) and nystatin (85.7%) respectively. *C. albicans, C. glabrata, C. krusei*; and *C. tropicalis,* were most susceptible to *Ganoderma lucidum, Polyporus;* and *Pleurotus* species respectively.
Conclusion: Vulvovaginal candidiasis can be controlled by natural antifungal therapy. The tested mushrooms can be formulated into drugs against the infection.

Keywords: *Vulvovaginal candidiasis, Mushroom, Candida species, Age groups, Diagnosis, Antifungal drugs*

Plain English Summary

Vulvovaginal candidiasis is a common occurrence among females of reproductive age. There are conventional antifungal agents used effectively against candida infections. However, there are limitations of such antifungal agents used in the treatment of candidiasis these days, because of the development of resistant strains. Thus, the consideration of natural therapy has become necessary.

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Many previous studies have established the antifungal activities of mushrooms to combat fungal diseases. In this study, the potential, potency and effectiveness of selected mushroom species extracts were tested against different species of *Candida*, with great outcomes.
Therefore, the potential of these mushrooms might be more useful as antifungal agents in clinics for the effective treatment of candidiasis.

Background

Vulvovaginal candidiasis, which is the yeast infection of the vagina and tissues at the opening of the vagina (vulva) caused by *Candida*, was first described in 1849 by Wilkinson (1). In 1875, Haussmann demonstrated the causative organism in both vulvovaginal and oral candidiasis is the same (2, 3). The rates of candidiasis increased with the advent of antibiotics after World War II, however, the rates decreased in the 1950s after the development of nystatin (4, 5, 6). Just like some other microorganisms, over the years, the classification of the genera and species has evolved. The genus *Candida* includes about 150 different species; however, only a few are known to cause human infections. *Candida albicans* is the most significant pathogenic species (7). Other species pathogenic in humans include *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida dubliniensi*, and *Candida lusitaniae*.
Candida albicans is a normal opportunistic microflora in humans and animals could be reservoirs or vectors for its transmission (8, 9). *Candida albicans* is usually transmitted from mother to infant through childbirth and remains as part of a normal human's microflora. The overgrowth of *Candida albicans* leads to symptoms of disease, such as changes in the normal acidity of the vagina. *Candida albicans* infections are very rarely spread through sexual intercourse. People-to-people acquired infections mostly happen in hospital settings where immunocompromised patients acquire the yeast from healthcare workers. About 40% incident rate has been reported (10). The number of cases of vaginal yeast infection is not entirely clear because it is not a reportable disease and it is commonly diagnosed clinically without laboratory confirmation (11). Candidiasis is one of the three most common vaginal infections along with bacterial vaginosis and trichomonas**,** with approximately 20% of women getting infected yearly (11). About 75% of women have at least one infection of vulvovaginal candidiasis in their lifetime (12). According to population-based research conducted in various regions of the world, there are differences in the distribution of *Candida* spp. that cause candidemia (13). One of the main keys to protecting an individual from being burdened by the microorganism is keeping a healthy lifestyle. Good hygiene, proper nutrition, and careful antibiotic use prevent *Candida albicans* from outcompeting other commensal microorganisms (14).
Fungal infections have been increasing in recent years because of the growing number of immunocompromised patients due to HIV infection, cancer chemotherapy and organ or bone marrow transplantations (15). In such persons, *Candida* infections are very common causing oral, vaginal and/or systemic candidiasis. Currently, the world is witnessing heightened antimicrobial drug resistance. According to a study, the emergence of resistance often reflects evolutionary processes that take place during antibacterial drug therapy (16). Antimicrobial treatment may be selected for fungi strains with physiological or genetically enhanced capacity to survive high doses of antifungal (17). Survival of fungi and bacteria often results from inheritable resistance (18).
Antimicrobial resistance is probably the biggest threat to human existence on planet Earth today. The use of conventional synthetic drugs in offering remedies to the existing human disease therapy has proved not to be very fruitful (19). The pathogens are increasingly sharpening their tools to ensure they resist any such drug that finds its way into the market. As a result, the use of naturally produced antimicrobials has gained a lot of popularity, the reason this study was conceived.
Candida albicans can successfully evade much of the immune system's immunological surveillance, so it can commensally exist on mucosal surfaces. Studies have shown that the innate and adaptive immune systems play a role in the clearing of fungal growth (20).
Mushrooms are higher fungi belonging to the class Basidiomycetes. Their medicinal properties are one reason they have been used for centuries, especially in traditional Chinese and Japanese medicines. Medicinal mushrooms contain amino acids, calcium, zinc, selenium, magnesium, and B vitamins including folate. They are also low in sugar, provide prebiotics and so support the formation of healthy probiotic bacteria. Some of their active constituents modulate or optimize immune function. Certain mushrooms even have antifungal benefits including anti-yeast activity against *Candida* (21). For example, the medicinal mushroom *Ganoderma lucidum* (reishi), has adaptogenic properties and can support the immune system. *Grifola frondosa* (maitake), *Trametes versicolor* (turkey tail) and *Cordyceps sinensis* (caterpillar

mushroom) have been used for their immune-supporting properties also. *Lentinula edodes* (shiitake), *Pleurotus ostreatus* (oyster) and *Flammulina velutipes* (enoki) mushrooms have also been used for medicinal purposes as well as taste. Mushrooms have long been used as a valuable food source and as traditional medicines around the world, especially in Japan and China (22, 23). Records of health-promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol-lowering, immunostimulatory effects (24, 25), and immunomodulatory and anti-plasmodial potentials have been reported for some species of mushrooms (26, 27).

Some research work has been carried out on diseases caused by *Candida* species including the antibiotic sensitivity test. However, the use of natural therapy against diseases and causal organisms is yet to be explored. Hence this study is meant to identify the various *Candida* species implicated in vaginal candidiasis and to determine their sensitivity to some commonly-used antifungals in comparison with selected mushroom extracts. The study emphasizes the need to consider natural therapy as a means to overcome the challenge of antifungal resistance which has become a menace. This research therefore focused on the use of mushrooms to combat the pathogenic *Candida* species and recommendations for the development of drugs from mushrooms.

Material and methods

Specimen collection

One hundred and fifty (150) high vaginal swab (HVS) specimens were collected with swab sticks, using a sterile speculum, from the patients attending Gynaecology, Antenatal and General out-patient clinics of the hospital by the qualified medical personnel/staff at the clinics. The ages of study subjects ranged from 10 to 60 years with a median age of 34.5 years. The samples were immediately transferred into Amie's transport medium and taken to the laboratory for processing.

The patients' consent was sought before the study and sample collection. They were established to have vaginal itching through the complaint and clinical explanation. The patients used were not on antibiotic or anti-fungal therapy in the last 2 weeks.

Isolation and identification of isolates

The specimens were routinely cultured for *Candida* species using Sabouraud Dextrose Agar and incubated in paired sets, one set at 25^{o}C for 3 to 7 days and the other at 37°C for 24 hours. On chocolate agar plates, it was incubated at 37°C for 48 hours. The other microbial isolates other than yeast cells were processed adequately. CHROMagar Candida (CAC, Becton Dickinson, Heidelberg, Germany) was also used in culturing *Candida* species to identify colony colour and morphology (28).

Direct Gram staining was carried out as described by (29). All the wet preparations were made and examined microscopically with X10 and X40 objectives by making a smear of the vaginal swab on a clean grease-free slide, allowed to dry and fixed directly by passing through bunsen flame. Crystal violet, Lugol's iodine solution, acetone and safranin were used on the fixed smear. The slide was later rinsed under running tap water and blotted carefully. This slide was examined under immersion oil objectives (X100). Positive slides showed purple large oval yeast cells. Wet smears were made immediately after the specimens were collected and cultured. About ten drops of saline were taken from the Bijou - bottle containing normal saline using a sterile Pasteur pipette for each specimen collected. The emulsified drops were placed on clean grease-free slides covered with cover-slip and examined microscopically with X10 and X40 objectives (29). Wet preparation showed budding yeasts and usually hyphae with buds at points of constriction.

Germ Tube Test was done as previously described (29). For germ tube production, a colony of yeast cells is inoculated into a small quantity of serum in a bijou bottle containing peptone water. This was incubated at 37°C for 2 hours, after which the culture was examined microscopically in a wet preparation at X40 objective. Nearly all strains of *Candida albicans* produce germ tubes which is the primary aim of this experiment. This method helps in differentiating *Candida albicans* and *Candida stellatoides* from other *Candida* species. Both positive and negative controls were set up along with the test sample. A positive control was set up by using known *Candida albicans* while a negative control was set up also. Standard control organisms used were from the store culture from the Department of Medical Microbiology of the hospital. Rapid urease and rapid selective urease tests were used for the diagnosis (30).

The sugar fermentation test was carried out by inoculating the test organism into the peptone water sugar (2% carbohydrates) in cotton-wool pigged tubes each containing a Durham tube. These were incubated at 37^{0}C overnight and the production of acid and gas. A positive fermentation test was indicated by pink-red colouration. *Candida albicans* ferments glucose and maltose with the production of acid and gas and does not ferment lactose. The fermentation of maltose differentiates it from *Candida*

stellatoidea.

Preparation of mushroom ethanol extracts and Antifungal assay

Several batches of *Pleurotus spp; Ganoderma lucidum, Ganoderma applanatum* and *Polyporus* species were purchased fresh from supermarkets; and from the University of Ibadan Botanical Garden. They were correctly identified by Botanists at the Department of Biological Sciences, Bowen University. They underwent ethanol extraction of potential antimicrobial components. The fruit bodies of the mushrooms were cleaned and cut into small pieces using scalpels before drying in the oven at 40^0C for 2 days. The dried fruiting bodies were crushed using a pistil and a mortar. Separately, 10.0 g of mushroom powder was placed in Erlenmeyer flasks, onto which 100ml of ethanol had been placed. The flasks were covered with aluminium foil and allowed to stand for 3 days for extraction. The mixture was filtered through Whatman filter paper no. 1 and the filtrate was concentrated in a rotary evaporator before weighing 4g of the resulting concentrates into 100ml of the solvent.

The *in-vitro* antifungal susceptibility pattern of *Candida* isolates to the mushroom extracts was determined by placing the extract-impregnated paper discs on Sabouraud dextrose agar seeded with yeast isolates after the plates were dried at ambient temperature for 15 minutes. For the conventional antifungal agents; fluconazole, ketoconazole, and nystatin (USP, Rockville, USA), the single agar discs were aseptically placed on the solidified agar medium. The diameter zone of inhibition was measured by a metal calliper after 24 and 48 h of incubation at 35°C. Filter paper disks soaked in sterile distilled water without antifungal agents were placed on a culture plate to serve as the control. Standard strains of *C. albicans* ATCC 10231 were used for quality control. Criteria of susceptibility and resistance of antifungal agents were determined according to zone diameter interpretation for fungi of Rosco Diagnostica Company (Neosensitabs, Denmark) as shown in Table 1.

Table 1: Zone diameter interpretation for fungi

Drug	Potency	Zone diameter in mm		
		Sensitive	Intermediate	Resistant
Fluconazole	**25 µg/disk**	**≥19**	**15–18**	**≤19**
Ketoconazole	**10 µg/disk**	**≥28**	**21–27**	**≤20**
Nystatin	**50 µg/disk**	**≥15**	**10–14**	**No**

Inoculum Preparation

Yeast inocula were prepared by suspending five colonies of each isolate from 24-hour-old pure culture in sterile saline and suspensions were then adjusted to the turbidity of a 0.5 McFarland standard to obtain 1×10^6 to 5×10^6 cells per mL. Yeast suspensions were then streaked onto Sabouraud Dextrose agar (Oxoid, Basingstoke, UK).

Data management and statistical analysis

Data obtained were entered into SPSS statistical package version 20.0 and analyzed by the same software. Descriptive analysis of frequency and bi-variate analysis were performed on all the data. Results were presented in graphs, charts and descriptive tables.

Results

The results showed isolation of Candida species infection and susceptibility pattern of conventional antifungals and mushroom extracts from the hospital.

Figure 1 shows the prevalence of the normal and pathogenic Candida species isolated from high vaginal swab (HVS) samples of the patients. The percentage of the isolated Candida species capable of causing diseases is 34.7% (52) while the normal (non-pathogenic) species is 65.3% (98).

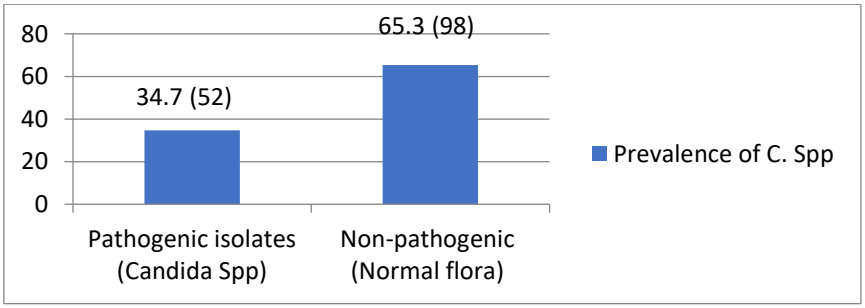


Figure 1: Prevalence of *Candida* species from high vaginal swab (HVS) sample

The fermentation reaction of the isolates is shown in Table 2. All the isolates fermented glucose and maltose, while none of them fermented lactose sugar.

Table 2: Fermentation reaction for *Candida* species

	Glucose	Sucrose	Lactose	Maltose	Urease	Germ Tube Test
Candida albicans	$+^{AG}$	$-^{A}$	-	$+^{AG}$	-	+
Candida stellatoidea	$+^{A}$	-	-	$+^{AG}$	-	+
Candida tropicalis	$+^{AG}$	$+^{AG}$	-	$+^{AG}$	-	-
Candida krusei	+		-	$+^{AG}$	+	-
Candida glabrata	+		-	$+^{AG}$	+	-

AG = Acid and gas, A = Acid, - = Negative, + = Positive

The prevalence of *Candida* species infection was observed based on different age groups of which 30-39 had the highest prevalence of 30 (57.7%), followed by age groups 20-29 having 11(21.2%), then age groups 40-49 having 5(9.6%), age groups 50-59 having 4 (7.7%) and age groups 10-19 had the lowest 2 (3.8%) of *Candida* species (Figure 2).

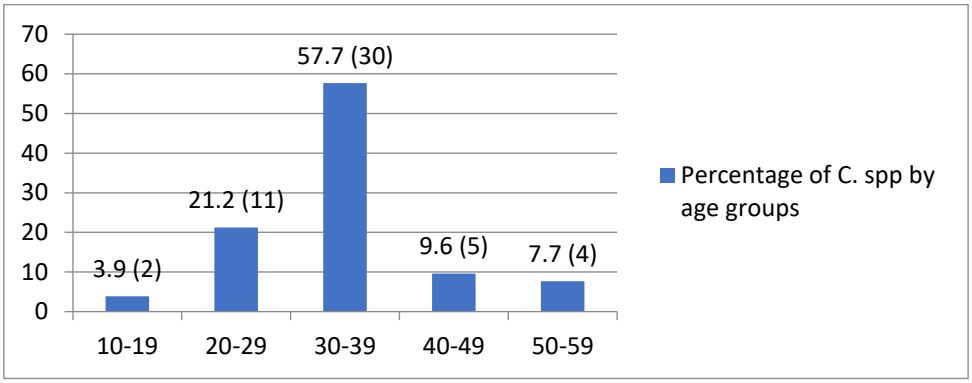


Figure 2: The percentage prevalence of *Candida* species based on age groups

Figure 3 represents the prevalence of *Candida* species. *Candida albicans* showed the highest prevalence of 27(51.9%), then *Candida glabrata,*10 (19.2%). The prevalence of *Candida tropicalis* (8; 15.4%) and *Candida krusei* had the lowest prevalence of 7 (13.5%) of the four species isolated.

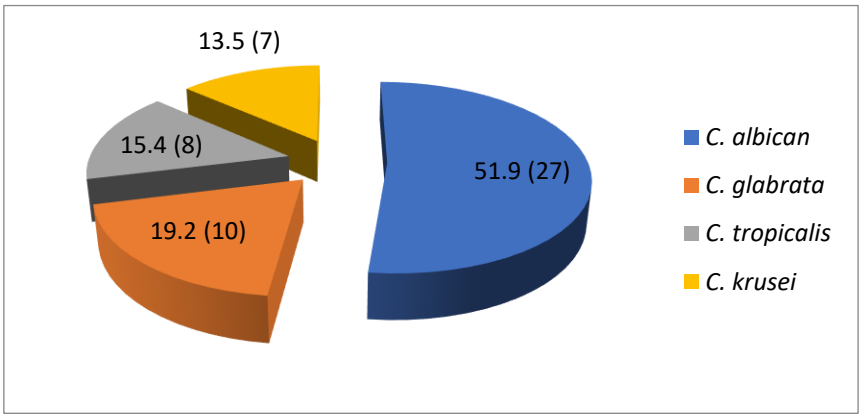


Figure 3: The distribution of the isolated C*andida* species

In vitro susceptibility of isolates to conventional antifungals

The susceptibility of yeast isolates to the three tested conventional antifungal drugs is summarized in Table 3. Of the total yeast isolates, 67.3% were susceptible and 15.4% were resistant to fluconazole. Against nystatin, there was 75.0% and 19.2% susceptibility and resistance respectively. The yeast isolates were most resistant to ketoconazole, having 28.8% resistance and 44.2% susceptibility. More yeast isolates were susceptible to nystatin 39 (75.0%) were being susceptible, while 3 (5.0%) and 10 (19.2%) were intermediate and resistant to the

drug, respectively. Nystatin was the most effective drug against *C. tropicalis* with no resistant strains to the drug being observed. Nystatin also exhibited the greatest activity against *C. albicans* with 22(81.5), 1(3.7) and 4(14.8) susceptible, intermediate and resistant. Nystatin was also a relatively active drug against *C. krusei* to which 5(71.4%) out of 7 isolates were susceptible to the drug. Among the four major isolates of the present study, *C. glabrata* was less sensitive to Fluconazole (40.0%), while the sensitivity of *C. albicans, C. krusei and C. tropicalis* to the drug was 66.7%, 85.7% and 87.5%, respectively. Ketoconazole was relatively the least effective drug against *C. albican* and *C. krusei* isolates in this study. The sensitivity of *C. albicans, C. glabrata*, *C. tropicalis* and *C. krusei* to the drug was 29.6%, 60.0%, 90.0% and 42.9%, respectively. Among the antifungal agents, more intermediate strains were produced against ketoconazole in which 37.0% of *C. albicans* were intermediate to the drug.

Table 3: *In vitro* susceptibility of conventional antifungal drugs against vaginal *Candida* species

***Candida* species**	**Susceptibility pattern**	**Conventional antifungals**		
		N	**K**	**F**
	S	18 (66.7)	8 (29.6)	22 (81.5)
C. *albicans* (N=27)	I	4 (14.8)	10 (37.0)	1 (3.7)
	R	5 (18.5)	9 (33.3)	4 (14.8)
C. *glabrata* (N=10)	S	4 (40.0)	6 (60.0)	4 (40.0)
	I	3 (30.0)	1 (10.0)	2 (20.0)
	R	3 (30.0)	3 (30.0)	4 (40.0)
C. *tropicalis* (N=8)	S	7 (87.5)	8 (100.0)	8(100)
	I	1 (12.5)	0 (0.0)	0(0)
	R	0 (0.0)	0 (0.0)	0(0)
C. *krusei* (N=7)	S	6 (85.7)	3 (42.9)	5 (71.4)
	I	1 (14.3)	1 (14.3)	0 (0.0)
	R	0 (0.0)	3 (42.9)	2 (28.6)
Total (N=52)	S	35 (67.3)	23(44.2)	39 (75.0)
	I	9 (17.3)	12 (23.0)	3 (5.0)
	R	8 (15.4)	15 (28.8)	10 (19.2)

N: Nystatin; F: Fluconazole; K: Ketoconazole; S: Sensitivity; I: Intermediate; R: Resistance

In vitro Susceptibility of isolates to mushroom extracts

The susceptibility of yeast isolates to the four mushroom extracts is summarized in Table 4. 50% (26/52) of the total yeast isolates were susceptible, 32.7% (17/52) were intermediate, and 17.3% (9/52) were resistant to *Polyporus species*. 22 (43.0%), 25 (48.0%), and 5 (9.0%) yeast isolates were susceptible, intermediate, and resistant to *Ganoderma* species respectively. Yeast isolates were more resistant to *Pleurotus* species in which 23(44.2%) were resistant, while 13(25%) and 16(30.8%) were susceptible and intermediate respectively. 18 (34.6%), 17(32.7%), and 17(32.7%) yeast isolates were susceptible, intermediate, and resistant to *Ganoderma lucidum.* More yeast isolates were susceptible to *Polyporus species* of which 26 (50.0%) were susceptible, while 17(32.7%) and 9(17.3%) were intermediate and resistant to the mushroom extract respectively. *Polyporus species* was the most effective drug against *C. glabrata* and *C. krusei*. *Polyporus species were* also relatively active against *C. tropicalis* with 50% susceptibility.

Table 4: I*n vitro* susceptibility of mushroom extracts against vaginal *Candida* species

Candida species		**Mushroom extracts**			
	Susceptibility pattern	A	E	G	P
	S	11(40.7)	10(37.0)	12(44.4)	2(7.4)
C. *albicans*(N=27)	I	13(48.2)	17(63.0)	8(29.6)	7(30)
	R	3(11.1)	0(0)	7(30)	18(66.6)
C. *glabrata*(N=10)	S	7(70)	6(60)	1(10)	3(30)
	I	2(20)	4(40)	2(20)	4(40)
	R	1(10)	0(0)	7(70)	3(30)
C. *tropicalis*(N=8)	S	4(50)	3(37.5)	2(25)	6(75)
	I	1(12.5)	2(25)	4(50)	2(25)
	R	3(37.5)	3(37.5)	2(25)	0(0)
C. krusei (N=7)	S	4(57.1)	3(42.8)	3(42.8)	2(28.6)

	I	1(14.3)	2(28.6)	3(42.8)	3(42.8)
	R	2(28.6)	2(28.6)	1(14.4)	2(28.6)
Total (N=52)	S	26(50)	22(43.0)	18(34.6)	13(25)
	I	17(32.7)	25(48.0)	17(32.7)	16(30.8)
	R	9(17.3)	5(9.0)	17(32.7)	23(44.2)

A: *Polyporus* species; E: *Ganoderma applanatum;* G: *Ganoderma lucidum;* P: *Pleurotus* species; S: Sensitivity; I: Intermediate; R: Resistance

Among the four major isolates of the present study, *C. albicans* was less sensitive to *Pleurotus* species (7.4%), while the sensitivity of *C. tropicalis, C glabrata and C. krusei* to the *Pleurotus* species was 75.0%, 30.0% and 28.6% respectively. *Pleurotus* species also exhibited the greatest activity against *C. tropicalis* with no resistant strain found. The sensitivity of *C. albicans, C. glabrata*, *C. tropicalis and C krusei* to the *Ganoderma* species was 37.0%, 60.0%, 37.5% and 42.8%, respectively. *Ganoderma* species also exhibited the greatest activity against *C. glabrata* with no resistant strain found. The sensitivity of *C. albicans, C. glabrata*, *C. tropicalis* and *C. krusei* to *Ganoderma lucidum* was 44.4%, 10.0%, 25.0% and 42.8% respectively.

Discussion

Vulvovaginal candidiasis (VVC) is a common fungal infection among women worldwide. The infection usually occurs at the lower genital and is reported in 35% - 80% of cases without any symptoms (31). Symptomatic vulvovaginal candidiasis has been found in up to 70% of the sexually active women (32). Chronic vulvovaginal candidiasis severely affects the quality of life for millions of women in the world and is frequently refractory to antifungal treatments (33). *Candida albicans* can colonize on the mucous membrane of genitourinary tracts of healthy humans. *Candida* virulence factors are adherence, enzyme production, proteinase secretion, dimorphisms with antigenic variations, and cell surface composition (34). Changing from yeast to hypha is under the control of a complex set of environmental signals and has long been believed to be the main virulence factor of this pathogen (35).

CHROMagar Candida is a differential ready-to-use chromogenic medium designed to identify *Candida albicans*, *Candida tropicalis,* and *Candida krusei* by colony colour and morphology. Typically, on chromogenic agar, colonies of *Candida albicans* are green; *Candida tropicalis* are dark blue; and pink, irregular and dry colonies indicate *Candida krusei.* Other *Candida* species produce bluish-green, beige, yellow, maroon, or violet colonies (36). This was per this study as *Candida albicans, Candida tropicalis, Candida glabrata and Candida krusei* were isolated.

The study also revealed the isolation of *Candida albicans* and non-*candida albicans* organisms to be the causative fungal agent associated with vulvovaginal candidiasis among women who attend clinics in the hospital. *Candida albicans* having the highest prevalence corroborates different studies that have been done. For example, a study performed traditional tests such as gram-staining, microscopic examination, and culture for species identification and reported *Candida albicans* as the most prevalent species (83.5%), followed by *Candida glabrata* (16%) (37). In another reported study on 288 pregnant women, 175 were positive for vulvovaginal candidiasis giving a prevalence rate of 60.8% (38). *Candida albicans* can colonize on the mucous membrane of genitourinary tracts of healthy humans. *Candida* virulence factors are: adherence, enzyme production, proteinase secretion, dimorphisms with antigenic variations, and cell surface composition (34). A previous study showed that the occurrence of vulvovaginal candidiasis was 25% among women and Candida albicans with a prevalence of 80.7% was the predominant *Candida* species (39). Changing from yeast to hypha is under the control of a complex set of environmental signals and has long been believed to be the main virulence factor of this pathogen (39). In contrast to this study, a report revealed a lower prevalence of *Candida albicans* 13 (6.5%), than that of non-*albicans* candidiasis 15(7.5%) (40). Their study reported that among the non-*albicans* species, *Candida glabrata* was the most common type (10.52%) and *Candida krusei* was the least common type (3.51%).

From this study, the prevalence of *Candida* species infection based on age group distribution showed women of age groups 30-39 having the highest prevalence of 30 (57.7%), followed by age groups 20-29 having 11 (21.2%), then age groups 40-49 having 5(9.6%). In contrast, another study (35) shows the highest age distribution of between 26-30 having 13 (22.0%), followed by 31-35 having 12 (20.3%), then 36-40 having 10 (17.0%). This might be because vulvovaginal candidiasis shows different frequencies from region to region. It can be related to geographical conditions, social and cultural factors, hygiene customs, and diagnostic techniques (41). However, the outcomes of prevalence based on the age distribution from this study agree with the findings of a study (42) that reported a higher prevalence of *Candida* species (33.33%) within the age bracket of 36–40 years. This outcome agreed with the report (42) which reported peak vaginal infections

between ages 20 and 40 years. This may be due to high sexual activity, poor personal hygiene, the use of contraceptives, and drug abuse among this age group.

The susceptibility of yeast isolates to the three conventional antifungal drugs is 67.3% (35/52) of the total yeast isolates were susceptible, 17.3% (9/52) were intermediate, and 15.4% (8/52) were resistant to fluconazole. 39(75.0%), 3 (5.0%), and 10(19.2%) yeast isolates were susceptible, intermediate, and resistant to nystatin respectively. Yeast isolates were more resistant to ketoconazole in which 15 (28.8%) were resistant, while 25 (44.2%) and 12 (23.0%) were susceptible and intermediate, respectively. More yeast isolates were susceptible to Nystatin of which 39 (75.0%) were susceptible, while 3 (5.0%) and 10 (19.2%) were intermediate and resistant to the drug, respectively. Nystatin was the most effective drug against *C. tropicalis* with no resistant strains to the drug being observed. Nystatin also exhibited the greatest activity against *C. albicans* with 22(81.5), 1(3.7) and 4(14.8) were susceptible, intermediate and resistant strains found; nystatin was also relatively active drugs against *C. krusei* to which 5(71.4%) out of 7 isolates were susceptible to the drug. Among the four major isolates of the present study, *C. glabrata* was less sensitive to fluconazole (40.0%), while the sensitivity of *C. albicans, C. krusei and C. tropicalis* to the drug was 66.7%, 85.7% and 87.5%, respectively. Ketoconazole was relatively the least effective drug against *C. albicans* and *C. krusei* isolates in this study. The sensitivity of *C. albicans, C. glabrata*, *C. tropicalis* and *C. krusei* to the drug was 29.6%, 60.0%, 90.0% and 42.9%, respectively. Among the antifungal agents, more intermediate strains were produced against Ketoconazole in which 37.0% of *C. albicans* were intermediate to the drug. Our result on *in vitro* susceptibility of *Candida* species to nystatin antifungal agents was consistent with what was reported previously (43) and showed that *Candida* isolates recovered in the study were highly sensitive to nystatin antifungal agents.

These results would support the effectiveness of topical nystatin therapy for vulvovaginal candidiasis. Recurrent VVC and repeated and/or prolonged exposure of patients to antifungal therapy induce reduced susceptibility of yeasts to azole antifungal agents (44). Selective pressure caused by antifungal agents and azole cumulative doses due to exposure to several courses of short- or long-term suppressive therapies in patients with VVC has been incriminated as a cause of induction of reduced susceptibility of yeasts to azole antifungal agents (45). Ketoconazole was also the least active drug against *C. albicans* which is the most frequently isolated yeast. Though ketoconazole has been widely used for the treatment of vulvovaginal candidiasis because of its low toxicity and ease of administration locally (44). This data suggests that the continued use of ketoconazole may lead to clinical treatment failure which is significantly correlated with reduced susceptibility to ketoconazole and other azoles.

In general, our study has shown that nystatin was more active than azole antifungal agents. Therefore, it is advisable to use these drugs as a second-line therapy for vulvovaginal candidiasis which is resistant to fluconazole, and ketoconazole as reported in other studies (46). Further studies are recommended.

The susceptibility of yeast isolates to the four mushroom extracts is 50% (26/52) of the total yeast isolates were susceptible, 32.7% (17/52) were intermediate, and 17.3% (9/52) were resistant to *Polyporus species*. 22 (43.0%), 25 (48.0%), and 5 (9.0%) yeast isolates were susceptible, intermediate, and resistant to *Ganoderma* species respectively. Yeast isolates were more resistant to *Pleurotus* species in which 23 (44.2%) were resistant, while 13 (25%) and 16 (30.8%) were susceptible and intermediate, respectively. 18 (34.6%), 17 (32.7%), and 17 (32.7%) yeast isolates were susceptible, intermediate, and resistant to *Ganoderma lucidum.*

More yeast isolates were susceptible to *Polyporus species* of which 26 (50.0%) were susceptible, while 17(32.7%) and 9(17.3%) were intermediate and resistant to the mushroom extract respectively. *Polyporus species* was the most effective against *C. glabrata* and *C. krusei*. *Polyporus species were* also relatively active against *C. tropicalis* with 50% susceptibility. Among the four major isolates of the present study, *C. albicans* was less sensitive to *Pleurotus* species (7.4%), while the sensitivity of *C. tropicalis, C glabrata and C. krusei* to the *Pleurotus* species was 75.0%, 30.0% and 28.6% respectively. *The Pleurotus* species also exhibited the greatest activity against *C. tropicalis* with no resistant strain found.

The sensitivity of *C. albicans, C. glabrata*, *C. tropicalis and C. krusei* to the *Ganoderma* was 37.0%, 60.0%, 37.5% and 42.8%, respectively. *Ganoderma* species also exhibited the greatest activity against *C. glabrata* with no resistant strain found. The sensitivity of *C. albicans, C. glabrata*, *C. tropicalis* and *C. krusei* to *Ganoderma lucidum* was 44.4%, 10.0%, 25.0% and 42.8% respectively. Mushrooms can be exploited as alternative therapies, and have long been used in ethnomedicine as effective and safe antifungal agents against *Candida* infections. Several workers have reported promising anti-candida activity against mucosal candidiasis through the

use of various mushrooms (47). Since natural products are less expensive and considered safer, they could be explored for their synergistic interactions with drugs of choice for the treatment of *Candida* infections which might result in more cost-effective and safer formulations.

Conclusion

The prevalence of vulvovaginal candidiasis among women has been a serious issue in our society and is fast growing which can result in a lot of future challenges. Emerging of *Candida albicans* and non-*albicans* species among clinical specimens are crucial concern in medical mycology. For better management of fungal infections like vulvovaginal candidiasis, precise identification of clinical isolates and a good choice of antifungal agent after antifungal susceptibility testing are inevitable. Infections with *Candida* are almost endogenous; therefore, identification of the species and corresponding susceptibility patterns to antifungal agents can be helpful for the management of these infections. As demonstrated in this study, resistance to azole drugs such as fluconazole, the most frequent antifungal used in the country, could be suggestive of the need for routine fungal culture and *in vitro* drug susceptibility patterns of yeast in medical centres to manage more efficiently the invasive candidiasis in the above-mentioned patients. Also, it is a suggestion to the use of alternative therapy, especially the use of naturals such as medicinal mushrooms which have proved effective against numerous infections both as prophylaxis and therapeutics. Follow-up studies are needed of women infected with *Candida* yeast species to determine the risk factors for the yeast's overgrowth, as it appears that the examination of behavioural risk factors does not appear to be a fruitful avenue for further inquiry. Since vulvovaginal candidiasis is a prevalent, recurrent, and bothersome fungal infection. The controlling of predisposing factors and personal hygiene are extremely recommended among vulnerable populations. Due to the role of women in childbearing and society, there is a need for prompt attention to proper health care provision among women.

List of Abbreviations

C. albicans: *Candida albicans*
C. kruseiC: *andida krusei*
C. tropicalis: *Candida tropicalis*
C. glabrata: *Candida glabrata*

Declarations

Ethical approval and consent to participate

Ethical approvals were obtained from the Ethical Committee of Bowen University (BUTH/REC-2439). Informed consents of the participants were also sought and obtained.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution- Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

All the authors contributed equally to the work. OOO, IOT, and OAA contributed to the conceptualization of the work, the practical work and the writing of the manuscript. All authors reviewed the manuscript and approved the submitted final version.

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