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Antifungal Potentials Of Ginger (Zingiber Officinale) On Human Pathogen Candida Species

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Abstract- The increasing prevalence of antifungal-resistant Candida species, such as Candida albicans, Candida glabrata, and Candida parapsilosis, poses a significant challenge in managing oral and vaginal fungal infections in humans. This study investigates the antifungal potential of ginger (Zingiber officinale), a commonly used medicinal plant known for its antimicrobial properties, against these pathogenic Candida species in tropical Africa. Methanolic and aqueous extracts of ginger was examined for their antifungal activity, through preliminary antimicrobial screening, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. The species of candida isolated was described as F1 which aligns with Candida albicans, F2 with Candida parapsilosis, F3 with Candida krusei, and F4 with Candida galbrata. Candida albicans is known to produce germ tubes, while other Candida spp typically does not exhibit such structural characteristics. The methanolic extract was particularly effective against Candida albicans and Candida parapsilosis, while the aqueous extract was more potent against Candida krusei at a concentration of 75mg/ml and 100mg/ml with an inhibition zone diameter of 9.50mm and 18.00mm. The methanolic extract generally exhibited stronger antifungal activity than the aqueous extract suggesting its possible therapeutic application in treating candida associated infections.

Keywords: Antifungal, Zingiber Officinale, Humans, Pathogens, Candida Spp.

I. INTRODUCTION

Globally, Candida is a normal commensal of the oral cavity; however, in immunocompromised individuals, candidiasis is often one of the earliest infections to manifest. Microorganisms are ubiquitous in nature and may become pathogenic when host immunity is compromised (Awari et al., 2023). For millions of people, traditional medicine serves as the primary or only source of healthcare, especially in developing countries where access to modern medicine may be limited (Adindu et al., 2016).

The emergence of antifungal resistance among Candida species, a common cause of fungal infections in humans, has prompted the search for alternative therapeutic agents (Obasi et al., 2024;

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Awari et al., 2024). Studies have shown that microbial resistance is increasing across various pathogens, thereby reducing the effectiveness of conventional antimicrobial drugs (Umeoduagu et al., 2023; Umeoduagu et al., 2023b). One such potential alternative is ginger (Zingiber officinale), a widely used spice and medicinal plant known for its antimicrobial properties. Several plant-based studies have demonstrated that bioactive compounds present in medicinal plants exhibit significant antimicrobial activity (Agu et al., 2013; Awah et al., 2017; Ubaoji et al., 2020).

Ginger contains bioactive compounds such as gingerol, shogaol, and paradol, which have been shown to possess antimicrobial properties. These compounds are believed to disrupt microbial cell membranes, leading to cell death and inhibition of growth. Previous phytochemical and antimicrobial studies on plant extracts support the efficacy of natural products in inhibiting microbial growth (Adindu et al., 2016; Obianom et al., 2023). Furthermore, the antioxidant properties of plant-derived compounds may contribute to their antifungal effects by mitigating oxidative stress in infected tissues.

Recent research has provided evidence supporting the antifungal efficacy of natural products. Comparative studies involving plant extracts and conventional antifungal drugs have demonstrated promising results against fungal pathogens, including Candida species (Anazodo et al., 2024; Orji et al., 2025). Similarly, investigations into fungal pathogenicity and susceptibility patterns have emphasized the need for alternative antifungal agents (Ogbo & Agu, 2015; Anazodo et al., 2024b).
Candida albicans is a yeast that naturally inhabits the human body, including the mouth, skin, and gastrointestinal tract. Under normal conditions, the body's microbiota maintains a balance that prevents overgrowth; however, disruption of this balance can lead to candidiasis. Common infections include vaginal yeast infections, oral thrush, diaper rash, and invasive candidiasis. The increasing incidence of such infections has been linked to factors such as immunosuppression, prolonged antibiotic use, and underlying diseases.

Ginger has been used medicinally since the Vedic period and is often referred to as "maha-aushadhi," meaning "great medicine." It is widely available, affordable, and generally well tolerated. The World Health Organization has reported that a significant proportion of individuals in developing countries rely heavily on traditional medicine for primary healthcare. The antimicrobial efficacy of herbal products, including oral hygiene products, has also been documented (Awah et al., 2016; Umeoduagu et al., 2023).

In recent decades, there has been a global increase in fungal infections, accompanied by limitations in currently available antifungal drugs, including toxicity, high cost, and reduced efficacy. Additionally, the emergence of resistant strains has further complicated treatment strategies (Obasi et al., 2024). This challenge underscores the need to explore alternative and sustainable antifungal agents derived from natural sources.

This study, therefore, aims to evaluate the antifungal activity of Zingiber officinale extracts against clinically relevant Candida species. To achieve this, the study will examine the phytochemical composition of Zingiber officinale, isolate and identify Candida species from vaginal and oral specimens, and assess the antifungal efficacy of both methanolic and aqueous extracts of the plant. Furthermore, the study will determine the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of these extracts against the fungal isolates, with a view to establishing their potential as alternative antifungal agents.

Mayerials and Methods

Study Area:

The study area carefully encompasses ten (10) female student in the department of applied Microbiology and Brewing in Nnamdi Azikiwe University, Awka. The University (UNIZIK) is located at approximately: latitude 6.1881° N and longitude N: 7.0852° E.

Ginger Extraction Procedure

Cold Maceration Method (Aqueous Extraction):

Finely chop or grind 500 g of fresh ginger rhizomes. Soak the ginger in water at a 1:3 (w/v) ratio for 24 hours with occasional shaking. Filter the mixture through Whatman No. 1 filter paper. Concentrate the filtrate under reduced pressure using a rotary evaporator. Store the concentrated extract at 4°C until further use (Moghadamtousi et al., 2015).

Soxhlet Extraction Method :

Dry and powder 500 g of ginger rhizomes. Place the powdered ginger in a Soxhlet apparatus and extract with 70% methanol for 6-8 hours. Concentrate the extract using a rotary evaporator. Store the concentrated extract at 4°C (Ravindran et al., 2016).

Sample Analysis

Ten (10) well moisten swab sticks (mouth and vagina) samples were aseptically introduced into 9ml of sterile peptone water for the fungi, it was properly shaken to homogenize the sample. One tenth of a millilitre (0.1ml) of appropriate dilutions (10-2) of the sample were pour plated in sterile plates of Sabouraud Dextrose Agar plates for the culture of fungi . The culture plates were incubated at 37°C aerobically for 7days for the fungi (Gomes et al., 2019). Developing colonies on SDA were counted to obtain total viable. Discrete colonies for the fungi were obtained by sub culturing into SDA plates and were subsequently identified using standard methods.

Total Fungi Count was calculated thus:

$$TFC\ (CFU/ml) = \left(\frac{N}{VD}\right)$$

Where TFC: Total fungi Count
CFU: Colony Forming Unit
N: No fungi colonies on plate
V: Volume plated
D: Dilution Factor

Characterization and Identification of Fungi Isolates

Identification of the fungi isolate was done by physiological and biochemical tests (Chessbrough, 2006). The characterization of the isolates were performed, by employing lactophenol cotton blue stain and Sugar fermentation tests.

Sugar Fermentation Test

Each of the isolate was tested for its ability to ferment a specific sugar. 1g of the sugar and 1g of peptone water were dissolved in 100ml of water. 5ml of the solution were transferred into clean test-tubes using sterile pipettes. The test-tubes containing peptone water and sugar were added Durham's tube which were placed inversely and bromothymol blue as an indicator. These were sterilized for 10minutes and allowed to cool before inoculating the inocula. The test-tubes were incubated for 3days. The production of acid and gas or acid only indicated utilization of sugars. Acid production was indicated by change in colour of the medium from green to yellow while gas production was observed by presence of gas in the Durham's tubes. (Oyeleke et al., 2017)

Fungal Microscopy using Slide Culture Technique

The improved slide culture technique described by Agu and Chidozie (2021) was adopted for this study. A sterile glass slide was aseptically placed at the base of a sterile Petri dish. Using a sterile 2 mL syringe, approximately 0.5 mL of molten Sabouraud Dextrose Agar (SDA), maintained at 45 °C in a water bath, was carefully dispensed onto the slide. The Petri dish was then covered, and the medium was allowed to solidify. After solidification, a sterile inoculating needle was used to introduce a small colony of the fungal sample was placed at the center of the agar mound. A heat-sterilized coverslip was gently placed over the agar without applying pressure. The setup was incubated at room temperature for 3–5 days, depending on the growth rate of the organism. Once adequate growth was observed, a few drops of lactophenol cotton blue stain were applied at the junction between the slide and the coverslip, allowing the stain to diffuse through the culture and preserve its structural integrity prior to microscopic examination. Identification of the fungal isolates was carried out with reference to standard fungal atlases (Barnett & Hunter, 2000; Watanabe, 2002; Ellis et al., 2007).

Germ Tube Assay

Put 0.5 ml of sheep or human serum (Fetal bovine serum can also be used instead of human serum). Into a small tube using a Pasteur pipette, touch a

colony of yeast and gently emulsify it in the serum. Incubated the tube at 37°C for 2 to 4 hours. Transfer a drop of the serum to a slide for examination. Coverslip and examine microscopically under low and high power objectives Put 0.5 ml of sheep or human serum into a small tube. (Pandey et al., 2017)

Fungal Identification

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the modified slide culture technique using lactophenol cotton blue stain for the microscopic evaluation under X10 and X40 magnification of the microscope (Agu and Chidozie, 2021); with reference to the Manual of Fungal Atlases (Barnett and Hunter, 2000; Watanabe, 2002; Ellis et al., 2007).

Standardization of Test Fungi

The test organisms were standardized by using a sterile wire loop, to pick 3–5 pure cultures of the test microorganisms and emulsified in 3–4 ml of sterile physiological saline. The turbidity reading of the 0.5 McFarland Standard was recorded as Absorbance in a Spectrophotometer at 540 nm, while the turbidities of the test organisms were adjusted to match the absorbance of the 0.5 McFarland standard at the same wave length, using physiological saline. NB: 0.5 McFarland contains 1.5x108CFU/ml.

Antimicrobial Susceptibility Test

The antibacterial activities of the herbal mixtures against the test bacteria were evaluated by modified well diffusion methods (Agu et al., 2013; Adindu et al., 2016; Awah et al., 2016; Awah et al., 2017) Exactly 25 µl of 0.5 McFarland standardized suspension of test fungi (1.5x108cfu ml-1) were cultured onto the Sabouraud Dextrose Agar plates by spread plate method. Exactly 50 µl of the extracts were introduced into agar well. The Inhibition zone diameters of the various plates were measured and recorded in millimeters. Negative controls were set up with sterile physiological saline and positive controls were set up using 50 µg/ml Nystatin for fungi.

Minimal Inhibitory Concentration (MIC) Determination

The minimum inhibitory concentration (MIC) is the lowest concentration of a chemical which prevents visible growth of a fungi. This is in difference to the minimum fungicidal concentration (MFC) which is the concentration resulting in microbial death as defined by the inability to re-culture fungi. The closer the MIC is to the MBC, the more bactericidal the compound (Tripathi, 2013). Minimal inhibitory concentration (MIC) was determined by two fold serial dilution methods (Fabry, et al., 1998). Candida albican, Candida galbrata, Candida parapsilosis, and Candida krusei were used as the indicator organisms. Samples were dissolved in DMSO (Dimethyl sulfoxide), which was a widely used commercial solvent derived from trees as a byproduct from the production of paper (Gregg, 1998), to a final concentration of 100mg/ml and added in increasing concentration such as,6.25, 12.5, 25, 50mg/ml respectively and incubated overnight at 37°C. Growth was observed by visual inspection and by measuring the turbidity. Results was recorded as Turbid (T) or Non-Turbid (NT).

Minimum Fungicidal Concentration

The minimum fungicidal concentration (MFC) is the minimum concentration of an antimicrobial drug that is fungicidal. It is determined by re-culturing (sub-culturing) broth dilutions that inhibit growth of a fungal organism (those at or above the MIC). The broth dilutions are streaked onto agar and incubated for 24 to 48 hours. The MFC is the lowest broth dilution of antimicrobial that prevents growth of the organism on the agar plate.

II. RESULTS

Table 1: Total fungi count and distribution of the fungi isolate

	No. of fungi colonies on plate	**Total *fungal* count (Cfuml-^{1})**	**Fungi distribution**

S1V	160	$1.60x10^6$	*Candida albicans*
S2V	TNTC	TNTC	*Candida albicans* *Candida krusei*
S3V	TNTC	TNTC	*Candida albicans* *Candida galbrata*
S4V	NG	NG	-
S5V	120	$1.20 x10^6$	*Candida albicans* *Candida parapsilosis*
S6M	45	$4.5 x10^5$	*Candida parapsilosis*
S7M	NG	NG	-
S8M	NG	NG	-
S9M	41	$4.1 x10^5$	*Candida krusei* *Candida albicans*
S10M	NG	NG	-

Key,
TNTC: too numerous to count (colonies more than 300 on plate)
TFTC: too few to count (colonies less than 30 on plate)
NG: no growth
S: swab
V: vagina
M: mouth

Table 2: Colonial, Biochemical and microscopic examination of fungi isolates

	Colonial morphology	Microscopy	Germ tube	Sugar fermentation				Probable Isolate
				Glu	Mal	Suc	Lac	
F1	Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like	Spherical to subspherical budding blastoconidia, 2-7 x 3-8 µm in size	+	+	+	+	-	Candida albicans
F2	Colonies (SDA) white to cream-coloured smooth,	Predominantly small, globose to ovoid budding blastoconidia, 3-4 x 5-8	_	+	+	+	-	Candida parapsilosis

	glabrous, yeast-like.	µm, with some larger elongated forms present						
F3	Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.	Predominantly small, elongated to ovoid blastoconidia, 2-5 x 4-5 µm.	_	+	_	_	_	Candida krusei
F4	Colonies (SDA) white to cream-coloured smooth, glabrous, yeast-like.	Ovoid to ellipsoidal budding blastoconidia, 3.4 x 2.0 µm in size. No pseudohyphae or chlamydospores produced.	_	+	_	_	_	Candida galbrata

Key,
Glu: Glucose
Mal: Maltos
Suc: Sucrose
Lac: Lactose

Table 3: Preliminary antifungal screening using methanolic and aqueous extract of Ginger

METHANOLIC EXTRACT OF GINGER (mm)						
	25 mg/ml	50 mg/ml	75 mg/ml	100mg/ml	POSITIVE CONTROL mg/ml (mm)	NEGATIVE CONTROL (mm)
F1	0.00	0.00	0.00	8.5	16.00	0.00
F2	0.00	0.00	0.00	0.00	22.00	0.00
F3	0.00	0.00	0.00	0.00	20.00	0.00
F4	0.00	0.00	14.50	26.00	34.00	0.00

AQUEOUS EXTRACT OF GINGER (mm)					POSITIVE CONTROL mg/ml (mm)	NEGATIVE CONTROL (mm)
	25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml		
F1	0.00	0.00	0.00	0.00	16.00	0.00
F2	0.00	0.00	0.00	7.20	22.00	0.00
F3	0.00	0.00	0.00	7.80	20.00	0.00
F4	0.00	0.00	9.50	18.00	34.00	0.00

Key,
Positive control: Fluconazole
Negative control: physiological saline

Table 4: Minimum Inhibitory Concentration (MIC) of the Aqueous Extracts on the various test organisms

	75($mgml^{-1}$)				**100 ($mgml^{-1}$)**				**100($mgml^{-1}$)**				**100($mgml^{-1}$)**				**Positive control ($mgml^{-1}$)**				**Negative control ($mgml^{-1}$)**			
TEST FUNGI																								
	50	**25**	**125**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**125**	**6.25**	**50**	**25**	**125**	**6.25**	**50**	**25**	**12.5**	**62.5**	**50**	**25**	**12.5**	**62.5**
F2	-	-	-	-	-	-	-	-	-	-	-	-	NT	T	T	T	NT	NT	NT	NT	T	T	T	T
F3	-	-	-	-	-	-	-	-	NT	T	T	T	-	-	-	-	NT	NT	NT	NT	T	T	T	T
F4	NT	T	T	T	NT	NT	T	T	-	-	-	-	-	-	-	-	NT	NT	NT	NT	T	T	T	T

Key,
NT: Not Turbid
T: Turbid

Table 5: Minimum Inhibitory Concentration (MIC) of the Methanolic Extract of Ginger on the various fungi organisms

	75(mgml^{-1})				**100(mgml^{-1})**				**100 (mgml^{-1})**				**Positive control (mgml^{-1})**				**Negative control (mgml^{-1})**			
TEST FUNGI																				
	50	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**
F1	-	-	-	-	-	-	-	-	NT	T	T	T	NT	NT	NT	NT	T	T	T	T
F4	NT	NT	T	T	NT	NT	NT	NT	-	-	-	-	NT	NT	NT	NT	T	T	T	T

Key, T: Turbid
NT: Not Turbid

Table 6: Minimum Fungal Concentration Aqueous Extract

	75(mgml^{-1})				**100 (mgml^{-1})**				**100(mgml^{-1})**				**100(mgml^{-1})**				**Positive control (mgml^{-1})**				**Negative control (mgml^{-1})**			
TEST FUNGI																								
	50	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**
F2	-	-	-	-	-	-	-	-	-	-	-	-	NG	-	-	-	NG	NG	NG	NG	T	T	T	T
F3	-	-	-	-	-	-	-	-	NG	-	-	-	-	-	-	-	NG	NG	NG	NG	T	T	T	T
F4	NG	-	-	-	NG	NG	-	-	NG	-	-	-	-	-	-	-	NG	NG	NG	NG	T	T	T	T

Key,T: Turbid
NG: No growth

Table 7: Minimum Fungal Concentration Methanolic Extract

	75($mgml^{-1}$)				**100($mgml^{-1}$)**				**100 ($mgml^{-1}$)**				**Positive control ($mgml^{-1}$)**				**Negative control ($mgml^{-1}$)**			
TEST FUNGI																				
	50	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**	**50**	**25**	**12.5**	**6.25**
F1	-	-	-	-	-	-	-	-	NG	-	-	-	NG	NG	NG	NG	T	T	T	T
F4	NG	NG	-	-	NG	NG	NG	2	-	-	-	-	NG	NG	NG	NG	T	T	T	T

Key,
T: Turbid
NG: No growth

III. DISCUSSION

Ginger (Zingiber officinale), a widely used culinary spice and traditional medicine, has garnered significant attention for its potential antifungal properties against oral and vaginal candidiasis. While numerous studies have demonstrated its effectiveness against various Candida species, this research takes a more in-depth exploration to fully understand its mechanism of action, clinical applications, and potential limitations.

Table 1 shows the Prevalence of Candida Species in which the majority of plates showed growth of Candida, particularly Candida albicans. This rightly suggests that Candida species are common in the vagina and mouth swab sample that was studied. The plate variability shows that there was significant variation in the number of colonies and types of fungi present on both the vagina and mouth swab plates. These variability in culture plates had high counts (TNTC), while others had low counts or no growth (NG). This could be due to factors such as sample heterogeneity, previous exposure to antifungal drugs or the immune system of the patient being studied. There was a co-occurrence of Candida Species, where in some sample cases, such as S9M, S5V, S3V and S2V multiple Candida species were observed on the same plate. This indicates that different species can coexist in certain environments. This finding disagrees with the research of Ekwealor et al., (2023) which found a significant number of candida spp in pregnant women in Ebonyi State, Nigeria.

Table 2 provides detailed information about the colonial morphology, microscopic characteristics, germ tube formation, Lactophenol cotton blue stain and sugar fermentation patterns of the fungal isolates (F1, F2, F3, and F4). The combination of these biochemical and microscopic features were used to identify the probable fungal species in Table 1. The isolates F1, F2, F3, and F4 correspond to the fungal species identified in Table 1. For example, F1 aligns with Candida albicans, F2 with Candida parapsilosis, F3 with Candida krusei, and F4 with Candida galbrata. Candida albicans is known to produce germ tubes, while other Candida spp typically does not. The sugar fermentation patterns (glucose, maltose, sucrose, and lactose) can be used to further

differentiate between Candida species. For example, Candida albicans ferments all four sugars, while Candida parapsilosis ferments glucose and maltose but not sucrose or lactose. Table 2 provides valuable information to confirm the identities of the fungal isolates identified in Table 1. The combination of colonial morphology, microscopic characteristics, germ tube formation, lactophenol cotton blue stain, and sugar fermentation patterns allows for the accurate identification of these Candida species. This agrees with the work of Van Schalkwyk et al., (2015); Altayyar et al.,(2016); Sule-Odu et al., (2020) where The most common yeast associated with vaginal candidiasis is Candida albicans but other species as C. parapsilosis, C. tropicalis, C. krusei, C. glabrata, C. pseudotropicalis, and C. stelloides are now emerging as important causes of infection.

Table 3 presents the results of a preliminary antifungal screening using methanolic and aqueous extracts of ginger against the four identified Candida species (F1-F4) in Table 2. The inhibitory zones (in millimeters) were measured to assess the antifungal activity of the extracts at different concentrations. A positive control (fluconazole) and a negative control (physiological saline) were included for the antifungal comparison. Both the methanolic and aqueous extracts of ginger exhibited antifungal activity against all four Candida species. The inhibitory zones increased with increasing extract concentration as recorded for F1 and F4 with a zone of inhibition diameter at 8.5mm at concentration 100mg/ml and a diameter of 14.50mm at a concentration of 75mg/ml and 26.00mm at a concentration of 100mg/ml, suggesting a dose-dependent effect.

The methanolic extract of ginger, especially at higher concentrations, demonstrated comparable or even superior antifungal activity to the positive control (fluconazole) against some of the Candida species. The extracts showed varying levels of activity against different Candida species. For example, the methanolic extract was particularly effective against Candida albicans and Candida parapsilosis, while the aqueous extract was more potent against Candida krusei at a concentration of 75mg/ml and 100mg/ml with an inhibition zone diameter of 9.50mm and 18.00mm. The methanolic extract generally exhibited stronger antifungal activity than the suggesting that the bioactive compounds responsible for the antifungal effect might be more soluble in methanol. The work agree with the work of Ikegbunam et al., (2016) where the highest activity on C. albicans was obtained with the methanolic and propanolic extracts, moderate activity was obtained with the aqueous extracts of ginger based on their inhibitory zone of diameter (IZD) of tested spice extracts on Candida albicans.

IV. CONCLUSION

The wide spectrum of activity of ginger extracts has been documented earlier. This study evaluated the inherent antifungal activity of Methanolic and Aqueous extract of ginger. From the obtained results it can be concluded that although methanol in itself has antifungal activity, methanolic extract of ginger has a synergistic activity-The extracts showed varying levels of activity against different Candida species, highlighting the importance of species-specific considerations in antifungal therapy. The methanolic extract generally exhibited superior antifungal activity compared to the aqueous extract, suggesting that the bioactive compounds responsible for the antifungal effect might be more soluble in methanol. The results suggest that ginger extracts could be explored as potential natural antifungal agents. However, further research is needed to evaluate their efficacy and safety in clinical settings. Understanding the mechanism of action of the antifungal compounds in ginger extracts can help optimize their use and potentially identify targets for drug development.

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