COMPARATIVE STUDY OF ANTIFUNGAL EFFECT OF *Curcuma longa* ETHANOLIC EXTRACT WITH *lactobacillus* ON *Candida albicans* ISOLATES

BY

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A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR'S DEGREE IN MEDICAL LABORATORY SCIENCE (NMLS) UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

SUPERVISED BY

DR (MRS) N. A. OLISE

SEPTEMBER, 2025.

CERTIFICATION

This is to certify that this project work was carried out by **SANIYO AVWEROSUO JENNIFER** with Matriculation Number **BMS1906415** under the supervision of **DR. (MRS) N.A. OLISE** in partial fulfillment of the requirement for the award of Bachelor of Medical Laboratory Science (BMLS) degree of the Department of Medical Laboratory science, School of Basic Medical Sciences, University of Benin, Ugbowo, Benin city, Edo State.

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EXTERNAL EXAMINER **DATE**

DEDICATION

I whole heartedly dedicate this project work to God Almighty for making this project a huge success and to my family for their endless love, support and encouragement.

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My deepest appreciation goes to the Almighty GOD for granting me the strength, wisdom, and perseverance to successfully complete this Project work. Without Him all efforts will amount to nothing.

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May the good Lord bless you all.

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ABSTRACTS

C. albicans remains a major opportunistic pathogen implicated in superficial and systemic infections, often exacerbated by antifungal resistance. This study evaluated the antifungal activity of ethanolic turmeric (*Curcuma longa Linn.*) extract and its combination with *Lactobacillus bulgaricus* secondary metabolites against clinical isolates of *C. albicans*. Fresh turmeric rhizomes were authenticated and extracted using ethanol, yielding 3.43% dried extract, while secondary metabolites of *L. bulgaricus* were prepared from probiotic cultures. Standardized clinical isolates of *C. albicans* (n = 5) were obtained from wound swab, high vaginal swab (HVS), ear swab, catheter tip, and urine samples collected at the University of Benin Teaching Hospital. Antifungal susceptibility testing was performed using agar well diffusion, minimum inhibitory concentration (MIC), and minimum fungicidal concentration (MFC) assays. Statistical analysis was carried out using Chi-square, ANOVA, and independent-samples t-test, with significance set at $p < 0.05$. Results revealed that ethanolic turmeric extract inhibited *C. albicans* growth in a concentration-dependent manner, with 40.0% inhibition at 25mg/0.25mL and 60.0% inhibition at both 50mg/mL and 100mg/0.25mL ($p < 0.05$). Combination with *Lactobacillus* metabolites enhanced inhibition to 60.0% at 25mg/0.25mL and 80.0% at both 50mg/0.25mL and 100mg/0.25mL, though overall differences between extract alone and the combination were not statistically significant ($p = 0.749$). Fungicidal activity was more at higher doses, with ethanolic extract achieving 100.0% inhibition at 200mg/0.25mL, while the combination exhibited earlier fungicidal effects, reaching 100.0% inhibition at 100mg/0.25mL. Isolate source influenced susceptibility, with High vaginal swab isolate showing highest sensitivity (MIC: 75.0%), while urine isolates were most resistant (MIC: 0.0%) ($p = 0.044$). These findings demonstrate that ethanolic turmeric extract possesses antifungal activity against *C. albicans*, which is further enhanced when combined with *L. bulgaricus* secondary metabolites. The dose-dependent inhibition observed suggests potential synergistic effects, though statistical comparisons did not reveal significant superiority of the combination over the extract alone. This highlights the therapeutic promise of plant–probiotic synergy as a natural alternative to conventional antifungals in the management of candidiasis. Further in vivo studies and mechanistic investigations are recommended to optimize dosage and evaluate clinical applicability.

CHAPTER ONE

INTRODUCTION

1.1. Background of Study

Candidiasis, is a fungal infection primarily caused by *Candida albicans*, which can affect mucous membranes, skin, systemic organs, the gastrointestinal, urinary tracts, and bloodstream, potentially becoming life-threatening if untreated (Waykar and Kumarapillai, 2024). The infection is particularly prevalent in individuals whose immune system have been impaired or weakened, and a number of virulence factors facilitate its pathogenicity which include adhesins, morphogenesis, and phenotypic switching (Waykar and Kumarapillai, 2024). The incidence of candidiasis is of major concern with a noted increase in more virulent forms of the infection. Contradictorily, while *C. albicans* is typically a normal member of the human microflora, it can become pathogenic under certain conditions, resulting in candidiasis (Waykar and Kumarapillai, 2024). The ability of the organism to switch between yeast and hyphal forms plays a key role in its pathogenicity and the severity of infections (Waykar and Kumarapillai, 2024). Moreover, the emergence of resistant strains has rendered current treatments less effective, necessitating the exploration of new drug targets and the development of novel antifungal agents (Waykar and Kumarapillai, 2024). Currently, the most commonly used antifungals that are effective against *Candida* spp., are azoles, polyenes, 5-flucytosine, and echinocandins. Compared to the emergence of antimicrobial resistance in infections caused by bacteria, the rise of antifungal resistance in *Candida* spp. is also emerging as a serious global public health concern (Seyedjavadi *et al*., 2024). Antifungal resistance is an increasing problem with the fungus *Candida*. Infections caused by *Candida* may show resistance to antifungal agents, especially the

azole class of drugs. Resistance to drugs like fluconazole and echinocandins has significantly limited available treatment options (Dhasarathan *et al*., 2021).

Any plant that contain a chemical that can be utilized for therapeutic purposes or serve as a starting material to the manufacture of valuable pharmaceuticals in one or more of its organs is considered medicinal (Salih *et al*., 2025). Plants are known for decades as the only source of medicines by traditional people. Moreover, plants are still used as primary sources of treatment/remedies by several countries, particularly in Africa and Asia (Soliman *et al*., 2017). Several plant species showed effective anti-candidal activities. Many of these plant bioactive compounds can target and interfere with critical processes in *Candida* biological functions such as cell wall integrity, cell membrane plasticity, cell metabolic processes, respiratory chain, adherence to host cell, germination and biofilm formation, or induction of programmed cell death (apoptosis) (Soliman *et al*., 2017). Amongst the various medicinal plants that have been utilized in traditional and modern therapies, *Curcuma longa linn.* is one whose therapeutic activity has been identified in treating several human ailments (Iweala *et al*., 2023). *Curcuma longa linn.,* commonly known as Turmeric, is a rhizomatous herbaceous perennial plant belonging to the *Zingiberaceae* family (Iweala *et al*., 2023). The rhizome of the plant, recognized by its vibrant yellow colour, is used not only as a cooking spice, but also as a therapeutic agent in several traditional medicine practices (Murtadlo *et al*., 2023). Research has explored its anti-inflammatory, antioxidant, anticancer, and neuroprotective properties. *Curcuma longa* has attracted significant interest due to its antimicrobial properties. In the age of increasing antimicrobial resistance, discovering new antimicrobial agents is of utmost significance, and *Curcuma longa* present as a promising natural candidate (Murtadlo *et al*., 2023). Studies demonstrated that curcumin, as the major constituents of turmeric, displays potent antifungal

activity against the pathogenic fungal strains of *C. albicans, Aspergillus spp., Paracoccidioides brasiliensis, and Cryptococcus neoformans* (Cheraghipour *et al*., 2020).

Probiotic bacteria are defined by the World Health Organization as 'Live microorganisms' that, when administered in adequate amounts, confer a health benefit on the host. The most common probiotic genera *Lactobacillus* are believed to inhibit pathogens by competitive exclusion at various sites, studies has shown that *lactobacillus* produces antimicrobial substances that inhibit the growth of pathogenic and saprophytic microorganisms (Radi and Abdelmonem, 2017). Other compounds like organic acids and hydrogen peroxide are also included in these antimicrobial effects. (Radi and Abdelmonem, 2017) *Lactobacillus* is a type of bacterium that produces lactic acid, it is commonly found in the human gastrointestinal tract, the vagina microbiota and in fermented dairy products such as cheese, yoghurt, and kefir (Chu *et al*., 2024). *Lactobacillus* exhibits inhibitory activity against pathogens including *Candida albicans* through multiple mechanisms including the production of hydrogen peroxide, lactic acid, and antibacterial compound such as bacteriocins or bacteriocin-like molecules, non-bacteriocin molecules, and non-lactic acid molecules (Denkova *et al*., 2013).

The novelty of this study lies in the innovative combination of probiotics and turmeric extract, which demonstrated synergistic antimicrobial effects on pathogens including *Candida albicans* (Bhola and Bhadekar, 2025).

Hence, this study aims to investigate the comparative antifungal efficacy of this novel combination therapy against clinical isolates of *Candida albicans*. It seeks to determine whether the combined use of ethanolic turmeric extract with Lactobacillus can produce synergistic antifungal effects, potentially offering an affordable, natural, and safe alternative or adjunct to conventional antifungal drugs.

1.2. Statement of Problem

Candida albicans have developed drug resistance mechanisms with a repertoire of strategies to overpower the effects of various classes of drugs particularly the azole class of drugs and the echinocandin. The opportunistic *C. albicans* and hospital-acquired infections claim millions of lives every year worldwide. So, there is an imperative need to develop effective treatment modalities. Azole drug-resistant *Candida* infections reduce treatment options, and patients with candidemia are less likely to survive (Dhasarathan *et al*., 2021). In certain groups of vulnerable patients, it causes severe, life-threatening bloodstream infections and subsequent infections in the internal organs (Kim and Sudbery, 2011). The impacts of Candida infections on human health are of increasing concern, and resistance of pathogenic Candida to all licensed systemic antifungals has been documented (Fisher et al., 2022). Studies have shown that the extracts of rhizome turmeric have effective antifungal activity against *Candida albicans* (Kasta. 2020) However, most studies focus on either ethanolic extract alone, with limited research on the combined effect of the extract with other natural antifungal agents. Similarly, *Lactobacillus* which has a direct anti-candidal activity which is primarily caused by bacterial metabolites that kill or inhibit the growth of yeast cells or prevent attachment, dimorphic transition, and biofilm formation (Vazquez-Munoz and Dongari-Bagtzoglou, 2021). Despite the individual antifungal potentials of ethanolic turmeric extract and Lactobacillus, there is limited scientific evidence evaluating the combined antifungal effect of ethanolic and aqueous turmeric extracts with *Lactobacillus* on clinical isolates of *Candida albicans*. The lack of comparative data on their individual versus combined efficacy hinders the development of novel, effective, and affordable treatment alternatives for fungal infections.

1.3 Justification of Study

Fungal infections caused by *Candida albicans* are becoming increasingly difficult to manage due to the growing resistance to commonly used antifungal drugs such as fluconazole and amphotericin B. This challenge is especially significant among immunocompromised patients and in regions with limited access to effective medical treatments. As the effectiveness of synthetic antifungal agents continues to decline, there is a pressing need to explore alternative therapeutic options that are both effective, accessible and safe.

Turmeric (*Curcuma longa linn.*), a well-known medicinal plant, has gained scientific interest for its broad-spectrum antimicrobial and anti-inflammatory properties. Its major active compound, curcumin, has been shown to possess antifungal activity against *Candida albicans*. However, the extraction method significantly influences the phytochemical profile and potency of turmeric. While ethanolic extracts are typically rich in curcuminoids. Combining the ethanolic extract of turmeric with natural antifungal products may result in broader and more effective antifungal action.

Additionally, probiotics such as *Lactobacillus* species are known to play a protective role in the human microbiome by inhibiting the growth of pathogenic fungi. These beneficial bacteria exert their antifungal effects through the production of organic acids, hydrogen peroxide, and antimicrobial peptides. Their natural antagonistic activity against *Candida albicans* makes them a promising candidate for combination therapy with plant-based agents.

Despite the proven individual potentials of turmeric extract and *Lactobacillus*, limited studies have evaluated their combined antifungal effects, particularly against clinical isolates of *Candida albicans*. Exploring this novel combination could lead to the development of a natural, low-cost,

and synergistic antifungal treatment that may reduce dependence on conventional drugs and help combat antifungal resistance.

This study is therefore justified by the urgent need for new antifungal strategies; Explores a novel combination of plant-based and probiotic therapies that may offer an affordable and accessible treatment option to contribute to reducing the overreliance on synthetic antifungal agents.

1.4 Aim of the Study

The aim of this study is to evaluate and compare the antifungal efficacy of ethanolic extract of turmeric (*Curcuma longa linn.*), in combination with *Lactobacillus bulgaricus*, against clinical isolates of *Candida albicans.*

1.5 Specific Objectives

1. To determine the antifungal activity of ethanolic turmeric extract against *Candida albicans* from clinical specimens.

2. To investigate the synergistic antifungal activity of ethanolic turmeric extract in combination with *Lactobacillus* against clinical isolates of *Candida albicans*.

3. To determine the minimum inhibitory concentration (MIC), minimum fungicidal concentration (MFC), and zone of inhibition for each treatment group.

1.6 Research Questions

1. What is the antifungal efficacy of ethanolic turmeric extract against *Candida albicans*?

2. Does the combination of ethanolic turmeric extract with *Lactobacillus* result in a synergistic antifungal effect against *Candida albicans*?

3. What are the MIC, MFC, and zones of inhibition for each treatment tested?

1. 7 Research Hypotheses

H_0: Ethanolic turmeric extract and the combination of ethanolic turmeric extract with *Lactobacillus* do not show significant antifungal activity against clinical isolates of *Candida albicans* and

H_1: Ethanolic turmeric extract and the combination of ethanolic turmeric extract with *Lactobacillus* exhibit significant antifungal activity against clinical isolates of *Candida albicans*.

CHAPTER TWO

LITERATURE REVIEW

2.1 *Candida albicans*

2.1.1. Morphology

Candida albicans is a pleomorphic fungus, being able to grow either as a budding yeast, or as a pseudomycelium of elongated and conjoined yeast cells or as true hyphae formed of generate parallel-sided tip-growing filaments (Da Silva Dantas *et al*., 2016). The fungus is a human opportunist pathogen that exist morphologically either as yeast, pseudohyphae, or true hyphae in vitro and in vivo, depending on environmental factors (Sachivkina *et al*., 2021).

Yeast cells typically have a round-to-oval cell morphology which is as a result of budding and nuclear division. On the other hand, hyphae consist of tubular cells that remain firmly attached following cytokinesis without a constriction at the site of separation from the mother cell. Pseudohyphae has characteristics that resembles both yeasts and hyphae, which are branched chains of elongated yeast cells with constrictions at the septum (Chow *et al*., 2021). *Candida albicans* can also form chlamydospore, which are thick-walled, spherical asexual spores that develop on pseudohyphal support cells, they are formed when environmental conditions are unfavorable (Nadeem *et al*., 2013).

The yeast cells (blastoconidia) range from 2 to 8 μm in diameter. Rarely, under routine cultivation, they produce larger cells called "giant" blastoconidia which can measure up to 30 μm (Bottone et al., 1999). Hyphae have a smaller diameter compared to yeast, which ranges from 2.5–3.5 μm with length extending more than 100 μm (Cottier and Hall, 2020).

2.1.2 Growth Characteristics

Candida albicans derives its name from two Latin words **candidus**, meaning bright or almost glistening white and albicō, meaning becoming white (Hutchison, 1998). The colonies of *Candida albicans* on Sabouraud dextrose agar (SDA) appear white to cream in color, round, convex, and soft, with smooth to wrinkled surface. They have a characteristic yeast-like odor (Ajah *et al*., 2020), on CHROMagar *Candida albicans* produces smooth, convex, green colored colonies which help distinguish it from other *Candida* Species (Hospethal *et al.*, 2002). The fungus grows best in aerobic conditions, but it does grow to a limited extent under anaerobic growth (Anand and Prasad, 1991)

It is well known that neutral pH conditions (pH 7) favor the development of hyphae in vitro, whereas acidic conditions (pH below 6.5) inhibit hyphal formation and promote yeast-form growth. Yeast-form growth is also encouraged by temperatures below 35°C and poor nutrient conditions (Nadeem *et al*., 2013). When Candida albicans is incubated in human or sheep serum at 37°C for 3 hours, it produces germ tubes. Germ tubes are filamentous outgrowths that extends from the yeast cells. It is a transitional phase from budding yeast cell to hyphal cell and regarded as a virulence factor (Munin *et al*., 2007).

2.1.3 Pathogenicity

Candida Species causes majority of fungal infections that affect humans, especially *Candida albicans* which causes infections in people whose immune system has been compromised. *C. albicans* also an opportunistic fungus when there is an imbalance between the host's immunity and the commensal microenvironment, thus causing superficial or disseminated infection (Macias-Paz *et al*., 2022).

The ability of *Candida albicans* to cause infection is due to a wide range of virulence factors ranging from morphological transition from yeast to hyphal forms, formation of biofilms, secretion of hydrolytic enzymes, thigmotropism, and the expression of adhesins and invasins on the cell surface (Mayer *et al*., 2013). It can also be influenced by rapid adaptation to environmental changes such as change in pH, robust stress response machineries, metabolite flexibility and nutrient acquisition (Mayer *et al*., 2013).

The adhesive molecules (adhesins) on the surface of *C. albicans* facilitates its attachment to host cells and extracellular matrix components. Attachment initiated the process of colonization and subsequent infection. Various factors, including host surface proteins, extracellular matrix proteins (such as fibronectin and laminin), and carbohydrates (like mannose), contribute to the adherence of *C. albicans* to the host tissues (Mayer *et al*, 2013). *C. albicans* invade epithelial cells by active penetration or induced endocytosis and secretes enzymes known as secreted aspartyl proteinase (Saps) and phospholipase that damage the epithelial cells (Zhu and Filler, 2009). C. albicans causes two broad classes of infections: mucosal and systemic infections. Skin candidiasis is uncommon and occur only in a small proportion of patients with certain inborn errors of immunity. Mucosal candidiasis includes vulvovaginal candidiasis, oropharyngeal candidiasis and esophageal candidiasis. Systemic candidiasis affects sterile body sites such as the bloodstream, spleen, central nervous system, liver and/or kidney (Lopes and Lionakis, 2021).

Candida albicans possess the ability to switch into elongated, filamentous structures known as hyphae. This hyphae form is predominantly found in an infectious tissue resulting in the fungus ability to invade and damage tissues which in turn results to disease manifestation (Fróis-Martins *et al*., 2024).

Candida albicans induces anti-inflammatory cytokine release by favoring toll-like receptor (TLR2) instead of TLR4 recognition thereby suppressing inflammatory responses and enhancing its immune evasion strategies (Dühring *et al*., 2015). *C. albicans* has the ability y mask its pathogen-associated molecular patterns (PAMPs) and produce extracellular matrix to shield the fungus from recognition and phagocytosis by immune cells such as macrophages and neutrophils (Lopes and Lionakis, 2021).

In addition to shielding itself from the host immune defenses and external antimicrobial agents, *C. albicans* exhibits the ability to form biofilms, which are intricate assemblies of microbial cells encases within a matrix of extracellular polysaccharide (Tsui *et al*., 2016).

Candida albicans has the ability to produce diverse toxins that contribute to its pathogenicity. Among these is candidalysin, a peptide toxin that impairs host cell membrane, leading to pore formation and eventually the death of the cell. This results to tissue damage and inflammatory responses (Fróis-Martins *et al*., 2024).

2.1.4 Mechanism of Pathogenicity

In most healthy individuals, *Candida albicans* lives as a harmless commensal organism, making it a member of the microbiota in these individuals. However, in certain conditions, *Candida albicans* can cause infections that range from mild superficial infections of the skin to life-threatening systemic infections. Several factors and activities have been discovered to contribute to the pathogenicity of this fungus, they include the expression of molecules which help the organism adhere to and invade host cells, the secretion of hydrolases (hydrolytic enzymes), the ability to switch from yeast to hypha form, contact sensing and thigmotropism, biofilm formation and phenotypic switching (Mayer *et al*., 2013).

- **Adhesion to Host Cells**

Candida albicans possess some specialized proteins called 'adhesins' which it mediates its adherence when it comes in contact with host cells and inanimate objects (Mayer *et al*., 2013). One of the most studied adhesins are agglutinin-like sequence proteins (ALS) family which consist of eight members (ALS1 -9). *Candida albicans* possess ALS5--7 and ALS9 on its cell surface (Macias-Paz *et al*., 2022). ALS3 is only expressed in the hyphal phase of the fungus. It binds E-cadherins on epithelial cells and N-cadherins on endothelial cells Macias-Paz *et al*., 2022). ALS3 induces endocytosis of the fungus into the host cell (Wilson *et al*., 2016).

Another important adhesin protein possess by *Candida albicans* is the hyphal wall protein 1 (Hwp1) whose N-terminal region serves as a substrate for epithelial cell transglutaminase leading to the covalent binding of *Candida albicans* hyphae to the epithelial cells proteins of the host (Mayer *et* al., 2013; Macias-Paz *et al*., 2022) this is essential in the development of oropharyngeal candidiasis but not for disseminated candidiasis (Macias-Paz *et al*., 2022). Other adhesin proteins include Ssa1 (Hsp70-like heat shock protein) (Macias-Paz *et al*., 2022), GPI-linked proteins (Eap1, Iff4 and Ecm33), non-covalent wall-associated proteins (Mp65, a putative β-glucanase, and Phr1, a β-1,3 glucanosyl transferase), cell-surface associated proteases (Sap9 and Sap10) and the integrin-like surface protein Int1. (Mayer *et al*., 2013).

- **Morphological Switching**

The ability of *Candida albicans* to switch over from yeast to hyphal stage is crucial for its virulence and pathogenicity (Pattnaik *et al*., 2021). The yeast form of *Candida albicans* is typically considered as the commensal form which allows the colonization of mucosal surfaces on the host. However, the hyphal form is regarded as the invasive capacity of the fungus which allows it to penetrate host barriers and invade deeper tissues. Environmental factors that facilitate

morphological transition of the fungus include host body temperature, pH, nutrient availability and quorum sensing (Lopes and Lionakis, 2021). The Hyphae aids the fungal to evade phagocytes and facilitate the death of macrophage via a two-step process: first by initiating pyroptosis and second by penetrating the macrophage membrane (Wilson *et al*., 2016)

The formation of hyphae is associated with the expression of other virulence-enclosed genes including hypha-associated secreted aspartyl protease genes (SAP4-6) the superoxide dismutase gene SOD5, hyphal wall protein Hwp1, the agglutinin-like sequence protein Als3, and the hypha-associated proteins Ece1 and Hyr1 (Mayer *et al*., 2013; Wilson *et al*., 2016).

- **Biofilm Formation**

Biofilms are three dimensional structures that consist of communities of microbes that adhere to surfaces and are enclosed in a matrix of extracellular polysaccharide which act as structural support and protection for the cells enclosed in the biofilm (Tsui *et al*., 2016).

The ability of *Candida albicans* to form biofilm is considered an important virulent factor (Gulati *et al*., 2018). The formation of biofilm occurs in a series of steps which occurs within 24 to 48 hours (Tsui *et al*., 2016). These steps include the adherence of single fungal yeast cells to the substrate to form a basal foundation layer, this is followed by the proliferation of these yeast cells across the surface, filamentation to form hyphae which then initiates the biofilm production (which several hundred micron thick) by accumulating extracellular polysaccharide matrix, the last step is the dispersion of non-adherent yeast Cells from the outer layer of the biofilm into the surrounding to colonize other surfaces and form new biofilms (Mayer *et al.*, 2013; Tsui *et al*., 2016; Gulati *et al*., 2018). *Candida albicans* biofilms can form on both organic and inorganic surfaces including catheters, dentures, tissues and mucosal cell surfaces (Mayer *et al*., 2013; Gulati *et al*., 2018). Studies have shown that the biofilms are associated with candidemia and

disseminated invasive disease (Tsui *et al*., 2016). The biofilms consist of multiple cell types (spherical yeast-form cells, oval pseudohyphal cells, and cylindrical hyphal cells) (Gulati *et al*., 2018). This matrix controls cell dispersion, protects against the immune system, and participates in developing resistance to azoles, polyenes, and pyrimidine analogs by not allowing the passage of these antifungal drugs (Macias-Paz *et al*., 2022).

Several transcription factors control biofilm formation which include the transcription factors Bcr1, Tec1 and Efg1.50. Recently new previously unknown regulators of biofilm formation have been identified and they include Ndt80, Rob1 and Brg1. Deletion of any of these regulators (BCR1, TEC1, EFG1, NDT80, ROB1 or BRG1) resulted in defective biofilm formation in in vivo rat infection models (Mayer *et al*., 2013).

- **Secretion of Hydrolytic Enzymes**

Candida albicans secret various extracellular hydrolytic enzymes which act as important virulence factor contributing to its pathogenicity (Pandey *et al*., 2018). These enzymes include proteases, phospholipases, lipases, and hemolysins (Macias-Paz *et al*., 2022). The enzymes act by degrading the cellular component of the host tissues thus facilitating the survival, adherence, invasion and dissemination of the fungus (Pandey *et al*., 2018).

The proteases also called secreted aspartic proteases (Saps) consist of a family of ten members, Sap1 - 10 (Mayer *et al*., 2013), of these, Sap9 and Sap10 remain bound to the cell surface and also associate with biofilm formation (Macias-Paz *et al*., 2022) while Sap1 to Sap8 are released into the surrounding medium (Mayer *et al*., 2013), owing to the fact that they do not contribute to the virulence of *Candida albicans*. The proteases degrade human proteins such as hemoglobin, albumin, keratin, collagen, laminin, fibronectin, mucin, and almost all immunoglobulins (Macias-Paz *et al*., 2022).

The phospholipases (PLs) consist of a family of four different classes (A, B, C and D), but only the five member of class B (PLB1 - PLB5) are extracellular and play a role in the pathogenicity of *Candida albicans* (Mayer *et al*., 2013). The PLB are enzymes that contribute to tissue invasion by hydrolyzing one or more water bonds in glycerophospholipids. Studies have shown that they disrupt the host membranes, facilitate the penetration of the hyphae into the cytoplasm indicating that the null mutant of the same (PLB−/−) exhibited attenuated virulence in animal models (Macias-Paz *et al*., 2022).

The lipases consist of a family of ten members, LIP1 - LIP10 (Mayer *et al*., 2013). The catalyze the hydrolysis of the ester bonds of triglycerides resulting in the release of fatty acids, the lipases induces cytotoxicity and favors the buildup of lipid droplets in the cytoplasm of liver cells and macrophages (Macias-Paz *et al*., 2022).

In systemic infections *Candida albicans* produces hemolysin which lysis the blood cellular component allowing the fungus to acquire iron by degrading haemoglobin for its survival (Pandey *et al*., 2018).

- **Contact Sensing and Thigmotropism**

Thigmotropism is the ability of an organism to sense and react to changes in surface contours or ridges to help the organism locate epidermal entry points and initiate invasive growth (Davies, 1999). Studies have shown that *Candida albicans* is thigmotropic. When the yeast cells come in contact with a surface, it forms hyphae via contact sensing, thee hyphae then grows along certain topologies (such as the presence of ridges). This mechanism enables the fungus to invade certain substrates such as agar or mucosal surfaces thus contributing to its pathogenicity (Mayer *et al*., 2013). Contact to solid surfaces also initiate biofilm formation. Studies have shown that thigmotropism by *Candida albicans* is regulated by the uptake of extracellular calcium through

the calcium channels Cch1 and Mid1. Additional mechanisms include the polarisome Rsr1/Bud1-GTPase module (Mayer *et* al., 2013).

- **Immune Evasion**

Contributing to its pathogenicity, *Candida albicans* is capable of evading the immune system the host especially the alternative pathway of the complement activation. This is done by masking the β-glucans in the inner layer using the mannan-enriched outer layer of the yeast cell (Gaffar *et* al., 2025). This immune evasion strategy is particularly significant in individuals with MBL deficiency, as their lectin pathway of complement activation is also compromised (Gaffar et *al*., 2025). Masking of their cell wall components prevent recognition of fungal pathogen-associated molecular patterns (PAMPs) by host epithelial and immune cell pattern recognition receptors (PRRs) which is essential for stimulating protective anti-fungal immune responses (Lopes and Lionakis, 2021). The alteration of cell wall components by *Candida albicans* is induced by certain metabolic cues. This immune evasion strategy involves harnessing host signals such as changes in oxygen availability, carbon source, hormone levels, stress, pH or iron level changes (Lopes and Lionakis, 2021).

Another strategy of immune evasion used by *Candida albicans* is the use of Saps produced by the fungus notably Sap1, Sap2 and Sap3 to degrade host complement proteins C3b, C4b, and C5, inhibiting he formation of MAC, thus blocking the classical and alternative pathways of complement. Additionally, Sap2 has been shown to cleave immunoglobulins GIgG, which is important for complement activation and also exhibits proteolytic activity against IgA, an essential immunoglobin in mucosal immunity, thereby potentially compromising host defence at mucosal surfaces (Lopes and Lionakis, 2021).

2.2 Resistance of *Candida albicans* to Conventional Antifungal Therapies

Conventional Antifungal Drugs

Antifungal drugs are medications that are used to treat fungal infections they act by inhibiting fungal growth or killing fungi (Seladi-Schulman, 2019). They selectively eliminate fungal pathogens from a host without causing much harm to the host (Dixon and Walsh, 1996). Antifungal drugs act by targeting specific fungal cell components or processes to prevent their growth or totally eliminate the fungus. They target the cell membrane, cell wall and Inhibit the synthesis of certain enzymes, nucleic acids and other cellular components of fungi. It's important to note that specific antifungal drugs and their mechanism of action vary based on their targets and the fungus being treated (Nahar *et al*., 2023). There are various antifungal drugs, they include the azoles, polyenes, pyrimidine analogues and the echinocandins which are the most widely used antifungal medications, along with a few newly developed antifungal drugs against *Candida* infections (Hossain *et al*., 2022).

The Azoles represent the largest class of antifungal drugs and also the first to be formulated (Hossain *et al*., 2022). They are five-membered organic rings broadly classified into two main groups, the imidazoles which containsl two nitrogen moleculs and the triazoles which contain three nitrogen molecules (Dixon and Walsh, 1996). The azoles disrupt the fungal cell membrane by binding to the iron protoporphyrin site of the fungal cytochrome P450 enzyme (14α-demethylase), this inhibiting the conversion of lanosterol to ergosterol leading to the change in stability permeability and function of the cell membrane and the enzyme attached to it (Hossain *et al*, 2022). Examples of imidazoles include miconazole, clotrimazole, and ketoconazole (Dixon and Walsh, 1996) which are used to treat superficial fungal infections (Hossain *et al*., 2022).

Examples of triazoles include itraconazole and fluconazole which exhibit a broader spectrum activity and are used to treat both systemic and mucosa fungal infections (Hossain *et al*., 2022).

The polyenes are macrolides, amphipathic organic compounds (Hossain *et al*., 2022) that target the ergosterol, a unique component of fungal membrane by binding to it thereby creating a pore leading to change in the permeability and integrity of the membrane. (Osset-Trénor *et al*., 2023). The most common polyene is amphotericin B initially derived from Strptomyces nodosus. It has a strong antifungal effect against *Candida* Species a d used to treat systemic infections (Hossain *et al*., 2022; Osset-Trénor et al., 2023). Other polyenes include natamycin and nystatin used to treat superficial fungal infections (Hossain *et al*., 2022).

The pyrimidine analogues are the third group of antifugal drugs to be formulated. They can disrupt pyrimidine metabolism and inhibit DNA, RNA and proteins synthesis in fungi. It has been how to have a strong antifungal effect against Candida Species (Wu *et al*., 2022; Osset-Trénor *et al*., 2023). They most commonly used pyrimidine analogue is flucytosine (5FC) which act by entering cell via permease enzyme, it is then converted to 5-fluorouracil (5FU) by the enzyme cytosine deaminase. 5FU is then converted to 5-fluorouridylic acid (FUMP) by UMP pyrophosphorylase which is further phosphorylated and incorporated into DNA and RNA leading to inhibition of protein synthesis (Hossain *et al*., 2022; Osset-Trénor *et al*., 2023).

The echinocandins are lipopeptde compounds that are relatively new class of antifugal drugs (Hossain *et al*., 2022). They act by targeting β (1,3)-glucan, a key component of the fungal cell wall which is essential for its stability and integrity (Osset-Trénor *et al*., 2023). Echinocandins inhibits the synthesis of β (1,3)-glucan leading to instability of the cell wall which in turn results in cell lysis and death (Mourad and Perfect, 2017). Echinocandins only exerts its effect on a particular cell wall manufacturing pathway found in fungal cells this making it non-toxin to

mammalian cells (Hossain *et al*., 2022). They have a significant fungicidal activity against *Candida* Species and are currently used as first line antifungal therapy against Candidemia (Mourad and Perfect, 2017) and can alsom be used in combination with other antifungal agents to treat invasive fungal infections (Osset-Trénor *et al*., 2023). Examples of drugs in this class are: caspofungin, micafungin and anidulafungin (Grover, 2010).

2.3 Antifungal Resistance of Candida albicans

Candida albicans, a prevalent fungal specie, has the ability to develop resistance to antifungal drugs. Various mechanisms have been employed by the fungus to resist the effect of various antifungal drugs including the azoles, polyenes, echinocandins and pyrimidines (Costa-De-Oliveira and Rodrigues, 2020).

Resistance of *Candida albicans* to the azole drugs is the highest and most common since they are frequently used. The mechanisms involved include the mutation of drug target genes ERG11 and ERG3 and over-expression of ERG11, and up-regulation drug efflux genes CDR1 and CDR2 (Dhasarathan *et al*., 2021). In *C. albicans* overexpression of ERG11 can result from mutations in the transcription factor Upc2 which binds to the azole-responsive enhancer element (ARE) located in the ERG11 promoter. When exposed to azole, Upc2 also binds to two separate regions on its own promoter allowing it to autoregulate expression, this leads to increased production of lanosterol 14α-demethylase, and resulting in azole resistance (Costa-De-Oliveira and Rodrigues, 2020). Several factors lead to the overexpression of efflux pumps, a well-known mechanism in *C. albicans* is Gain-of-function (GOF) in Tac1p (a zinc-cluster transcription factor that regulates CDR1 and CDR2). Missense mutations in TAC1 (switch from heterozygosity to homozygosity) lead to a high expression of CDR1 and CDR2 and result in the expulsion of antifungal drugs out of the fungal cell (Li *et al*., 2025). Additionally, the overexpression of the MDR1 gene which

encodes a multidrug efflux pump result in fluconazole resistance in *C. albicans* (Dhasarathan *et al*., 2021). Furthermore, the ability of *C. albicans* to form biofilm is major contributor to drug resistance. This is because the biofilm acts as a physical barrier to drug penetration protecting the fungal cells this rendering the cells less susceptible to antifungal drugs. Also, increased expression of FKS1 contribute biofilm matrix sequestration, this preventing the antifungal drugs from reaching the fungal cells (Kaur and Nobile, 2022).

Candida albicans has also shown resistance to flucytosine, this results from the mutation in the enzyme uracil phosphoribosyltransferase (Fur1p) Which prevents the conversion of 5-fluorouracil to 5-fluorouridine monophosphate. (Costa-De-Oliveira and Rodrigues, 2020).

Mutations in the EGR3 Gene (which encodes a C-5 sterol desaturase, an enzyme involved in ergosterol biosynthesis), which lower the concentration of ergosterol in the fungal membrane often results in resistance to amphotericin B in *Candida albicans* but this is usually rare (Costa-De-Oliveira and Rodrigues, 2020). Furthermore, C. albicans develop resistance to the echinocandins. This is achieved by mutations in the FKS1 gene, the catalytic subunit of β-(1,3)-glucan synthase, and in a lesser extent in FKS2, resulting in amino acid substitutions in conserved regions hot spot 1 (HS1) and hot spot 2 (HS2). These strategies collectively contribute to the development of antifungal resistance in C. albicans, thus preventing the successful treatment a management if infections caused by the fungus (Costa-De-Oliveira and Rodrigues, 2020).

2.4 *Curcuma longa*

2.4.1 Description of *Curcuma longa plant*

Curcuma longa, commonly known as turmeric, is a rhizomatous herbaceous perennial plant that belongs to the *Zingiberaceae* ginger family. The plant grows up to two meters, it does not

possess a stem or rhizome stock but has erect leafy shoots having about twelve (12) leaves in each leafy shoot (Iweala *et al*., 2023). Each leafy shoot also known as pseudostem is divided into the leaf sheath, petiole, and leaf blade. The leaves are alternate and arranged in two rows. A false stem develops from the leaf sheaths. The petiole measures about 50–115 cm (20–45 in.) long (Tung *et*. *al* 2019). The leaves grow up to one meter, they are oblong or lanceolate in shape, they possess a dark green colour from the upper part and pale green colour from the lower part. The leaf sheath and petiole have a length almost the same length with the leaf blades (Iweala *et al*., 2023). The simple leaf blades measure about 76–115 cm (30–45 in.) long and rarely up to 230 cm (91 in.). They have a width of 38–45 cm (15–18 in.) (Dada Khalandar *et al*., 2018). The plant has a sterile, pale yellow and reddish flower, whereas its flowering bract is green tinged with a purplish colour (Iweala *et al*., 2023). The flowers grow on a spike like stalk measuring about 10 -15cm long. The flowers produce small, ovoid, brown seeds which are not viable thus making the flowers sterile (Tung *et al*., 2019). The plant is mainly grown for its rhizome, which is located below the soil (Iweala *et al*., 2023. Turmeric is derived from the rhizome. The primary rhizome is ovate and mostly pear shaped, known as "bulb" and the secondary one are cylindrical (Nisar *et al*., 2015) Tee rhizome is tuberous characterized with a rough and segmented skin. They possess a yellowish brown colour on the outer part whereas the interior is dull orange in colour. Smaller tubers branch off the main rhizome. The rhizome is 2.5–7.0 cm (1–3 inches) long and 2.5 cm (1 inch) in diameter, with the distal end being tapered or pointed. The dried rhizome of turmeric can be processed into a yellow powder that has a sweet, somewhat acrid, and bitter flavor (Prasad and Aggarwal, 2011). The plant is native to Tamil Nadu, a tropical state in southeast India, and requires temperatures between 20 and 30°C as well as a lot of rainfall each year. A fraction of the trees is propagated the next season after being harvested each year for

their roots. Although it doesn't yield seeds, turmeric is sterile and grows quickly from its rhizomes. It is believed to have developed through vegetative propagation and selection of a hybrid between wild turmeric (*Curcuma aromatica*), which is indigenous to the eastern Himalayas, India, and Sri Lanka (Tung *et al*., 2019).

Curcuma longa, is grown in tropical and subtropical regions across the world and thrives in warm climates. There are other cultural names for it, including kunyit in Indonesian, haldi in Hindi, manjal in Tamil, kyoo in Japanese, and turmeric in English (Tian *et al*., 2025).

2.4.2 Taxonomic Classification

Curcuma longa linn. is classified in the following taxonomical hierarchy:

Kingdom: Plantae

Subkingdom: *Tracheobionts*

Phylum: *Tracheophyta*

Class: *Liliopsida*

Order: *Zingiberales*

Family: *Zingiberaceae*

Genus: *Curcuma*

Species: *Curcuma longa* (Nisar et al., 2015)

2.4.3 Geographical Distribution

It is thought that *Curcuma longa* originated in South Asia, specifically India. The plant is widely cultivated in China, Sri Lanka, West and East Africa and other tropical countries (Iweala *et al*., 2023). It is currently grown in Bangladesh, Burma, Pakistan, Indonesia, and Sri Lanka. Over 90% of the world's total output comes from India (Hegde *et al*., 2012). It is primarily grown in China, India, and Pakistan in tropical and subtropical regions of the world. In India and Pakistan, it is

typically known as haldi (Nisar *et al*., 2015). In China, turmeric is widely cultivated in Sichuan, Yunnna, Fujian, Guangdong, Taiwan, and other proviences (Tian *et al*., 2025). It is thought that turmeric originated in South East Asia and then traveled to neighboring regions of China, Japan, Indochina, and other South Pacific Islands before reaching tropical West Africa and East Africa. It was only recently introduced to Central America and the Caribbean Islands. In South East Asia and Indo-China (Velayudhan *et al*., 2012).

2.4.4 Ethnobotanical Uses

In Pakistan, wounds and pimples are treated with powdered *C. longa* extract. According to an ethnomedicinal survey, wounds and injuries could be treated with a paste made from the rhizome of *C. longa*. Its leaves' juice purifies the blood and has anthelmintic properties. In the Philippines, the juice made from powdered *C. longa* is also used to treat arthritis (Iweala *et al*., 2023). Turmeric's medicinal applications were initially recorded in "Atharveda." The common cold, stomachache, flatulence, indigestion, hepatic disorders, jaundice, bilious attack, gallstones, rheumatism, irregular menstruation, dermatological disorders (poor ulcers, pimples, and skin infections), external injuries (sprains, wounds, swellings, and cuts), and inflammatory diseases (rhinitis, arthritis, and inflammatory bowel disease) have all been treated with turmeric in the Ayurvedic system (Tian *et al*., 2025). Turmeric is frequently used to color food. Tetrahydrokurcumin, an odorless and heat-resistant antioxidant compound, is also present in turmeric. It tastes sharp like ginger and has a subtle scent (Güneri, 2020). In India, turmeric has been used since ancient times in relation to Sakthi worship, or the worship of the pre-Aryan divine mother or goddess, and later as a commercial commodity used as a condiment and coloring agent. Turmeric is widely used as a spice and condiment by people who eat rice. It gives a variety of dishes color, flavor, and taste. In the past, it was frequently used as a dye in the

weaving industry, but other synthetic dyes have since taken its place (Velayudhan *et al*., 2012). Turmeric has also been used to treat burns, cuts, and bruises in South Asia. Turmeric has been used extensively in Japan to treat digestive issues and is also popular as a tea, especially in Okinawa. Turmeric was used as an anxiety and hematuria remedy in Korea. Turmeric was used by the ancient Hawaiians to treat gastrointestinal ulcers, ear infections, and sinus infections. Turmeric was used as a spice and herb in Nigeria to treat inflammation and joint pain. Turmeric has historically been used in numerous significant rituals, including marriages, in India. Turmeric was also used in religious ceremonies, such as those in Buddhism and Hinduism. Turmeric powder represented fertility, wealth, and purity in Buddhism and Hinduism. Similarly, turmeric has long been used as a face mask to improve skin tone and minimize imperfections (Tian *et al*., 2025).

(a) **(b)**

Figure 1: (a) Leaves of *Curcuma longa linn* (*Curcuma Longa*.)

(b) Rhizome of *Curcuma longa linn* (Mans *et al*., 2019).

2.4.5 Phytochemical Constituents of *Curcuma longa*

A vast class of chemical compounds that are found naturally in plants and give them color, flavor, aroma, and texture are known as phytochemicals (Barbieri *et al*., 2016). They are a large group of naturally occurring non nutrient, biologically active compounds found in plants which act as natural defense system for the host plants (Adebisi *et al*., 2021). These substances have evolved over thousands of years to protect plants against the effects of bacteria, fungi, viruses, and free radicals. Fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs, spices, and plant-based drinks like tea and wine all contain them in large quantities (Barbieri *et al*., 2016) with the spread of pharmacological action against any disease state. They provide protection from competitors, microbes, and herbivores; control fertilization, treatment, and the rhizosphere climate; and direct development (e.g., by delaying seed germination until a suitable time) (Reddy *et al*., 2023). Numerous studies have demonstrated for years that turmeric is incredibly rich in valuable phytochemicals with pharmacological properties, such as terpenes (such as ar-, α- and β-turmerone, α-zingiber, and β-sesquiphellandrene), flavonoids, coumarins, saponins, tannins, and steroids, as well as polyphenols (such as curcuminoids) (Delgado *et al*., 2021) and alkaloids (Kasta, 2020).

- **Curcuminoids**

Curcuminoids are bioactive phenolic compounds. There are 15 curcuminoids isolated and identified in turmeric (C. longa), but the three major curcuminoids: curcumin (CUR), demethoxycurcumin (DMC), and bisdemethoxycurcumin (BMC) (Zhang & Kitts, 2021). Curcumin or diferuloylmethane with chemical formula of (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) and other curcuminoids constitute the main phytochemicals of Curcuma longa L. (Moghadamtousi *et al*., 2014). These curcuminoids have anti-inflammatory,

anti-protozoal, antifungal, antibacterial, and anti-venom qualities, among other qualities. In aqueous solutions, curcuminoids are less stable and poorly soluble. On the other hand, they are highly soluble in organic solvents such as acetone, methanol, and ethanol. Ethanol is known to produce the most curcuminoids during the extraction process among these organic solvents (Garimella and Pradhan, 2024). Some of which will be discussed below:

- **Flavonoids**

Flavonoids are essential aromatic compounds which function in the development and growth of plants. They are a class of secondary metabolites found in plants possessing anti-inflammatory and antioxidant qualities (Sulaymanov *et al*., 2024). They are a class of polyphenolic compounds which are widely found in fruits, vegetables, and other food crops. Flavonoids have beneficial biochemical effects on a variety of diseases, including cardiovascular disease and atherosclerosis (Shen *et al*., 2022). More than 10,000 flavonoid compounds have been identified and isolated thus far (S. Chen *et al*., 2023). Flavonoids also possess anticancer, anti-aging, neuroprotective, immunomodulatory, antidiabetic, antibacterial, anti-parasitic, antifungal, and antiviral properties. Flavonoids have a number of antifungal mechanisms, including damaging the plasma membrane, causing various mitochondrial dysfunctions, and inhibiting the formation of cell walls, cell division, and the synthesis of RNA and proteins (Dias *et al*., 2021).

- **Saponins**

The term 'saponin' is derived from the Latin word 'sapo' meaning soap, and is associated with the detergent-like characteristics. Saponins are a diverse group of compounds found throughout the plant kingdom. They are distinguished by their structure, which includes a lipophilic triterpene or steroid aglycone linked to one or more hydrophilic sugar moieties. Based on the aglycone's structure, saponins can be broadly divided into two groups. They are called steroid saponins and

triterpenoid saponins. A third group, known as the steroidal amines or alkaloid saponins, is acknowledged by some authors. A broad spectrum of pathogens, including bacteria, viruses, fungi, and protozoa, are susceptible to the antimicrobial effects of saponins. They may be used to create novel antimicrobial agents and enhance current therapies because they damage microbial cell membranes and prevent their replication (Timilsena *et al*., 2023). Additionally, saponins have anticancer qualities that include anti-angiogenesis, anti-metastasis, anti-proliferation, and anti-reversal of multidrug resistance (MDR). These outcomes are caused by immune-modulatory effects, bile acid binding, apoptosis induction, cell differentiation promotion, and reduction of carcinogen-induced cell proliferation (Elekofehinti *et al*., 2021). As an antibacterial and antifungal, saponin works by causing the bacterial cell wall to lyse and AKP (alkaline phosphate) to leak out. When the concentration of saponin rises, the protein dissolves and intercellular compounds diffuse through the cell wall and outer membrane. Cell death results from the cytoplasm leaking out of the cell as a result (Kasta, 2020).

- **Alkaloids**

Alkaloids are naturally occurring specialized metabolites having nitrogen as a defining element in their chemical structures (Bhambhani *et al*., 2021). Alkaloids can be classified as isoquinolines, pyrroles, pyridines, quinolines, indoles, and ten other types based on their distinct chemical structures (Yan *et al*., 2021). 2-(2'-methyl-1'-propenyl)-4,6-dimethyl-7-hydroxyquinoline is produced from the dried root of C. longa (Sun *et al*., 2016). About 20% of plant species contain trace amounts of alkaloids (Heinrich *et al*., 2021). Alkaloids have a wide range of biological activities, such as immunomodulatory, hepatoprotective, antidiabetic, cardioprotective, anti-inflammatory, anticancer, antimicrobial, antimalarial, and analgesic qualities (Letchuman *et al*., 2024).

- **Tannins**

Tannins are water-soluble polymeric phenolic compounds with astringent qualities that are found in many plants as secondary metabolites (Ojo, 2022). Tannins have been shown in numerous studies to have broad-spectrum antibacterial properties. By blocking enzymatic activity and nucleic acid synthesis, tannins have been described as antiviral, antifungal, and antibacterial substances. Moreover, tannins have the ability to break down the membranes of microorganisms, which results in cellular release and, eventually, cell death. Moreover, tannins can bind to enzymes, cell membranes, microbial cells, and cell wall proteins, preventing physiological functions and limiting growth and development (Huang *et al*., 2024). Astringent herbs with a tannin component are used to treat intestinal conditions like dysmenorrhea and diarrhea (Kasta, 2020).

- **Terpenes**

The largest natural products are terpenes, which can have linear hydrocarbon skeletons or carbocyclic ones, among other structural variations. There are roughly 55,000 members (Ninkuu *et al*., 2021). Terpenes are used in pharmaceutical drugs for their anti-plasmodial, antiviral, antidiabetic, neuroprotective, and analgesic properties (Hilal *et al*., 2024).

2.4.6 Antifungal Effects of *Curcuma longa*

Studies has shown that Curcumin, a major constituent of turmeric has potential antifungal effects and has shown a potent antifungal effect against various *Candida* species including *Candida albicans* (Moghadamtousi *et al*., 2014). The agar dilution method was used to test the ethanolic turmeric extract's antifungal activity against *Candida albicans*. To exclude ethanol's antifungal properties, an alcoholic control was made. The fungal colonies' size and number decreased as the alcoholic extract of turmeric's concentration rose. The concentration of turmeric was inversely

correlated with the size and quantity of colonies. Only the alcohol-containing control table displayed the highest growth. Therefore, alcohol was not the cause of the inhibition of *Candida* growth. The ethanolic extract of turmeric was used because it contains a variety of chemical components, such as phenolic compounds and their derivatives, weak acid esters, fatty acid, terpenes, and others. These crude extracts can impact multiple target sites against the fungus because they contain a wide variety of chemical compounds. Turmeric seems to have specific antifungal qualities. At lower concentrations, it exhibits static effects; at higher concentrations, it exhibits fungicidal effects Murugesh *et al*., 2019).

Mechanism of Action

An important component of turmeric, curcumin, inhibits ERG3 by lowering its expression levels, which depletes ergosterol and causes other biosynthetic intermediates to build up (Sharma 2011). Curcumin directly targets and damages the plasma membrane of *candida albicans* leading to leakage of potassium ions from the fungal cytosol and dissipation of the membrane potential (Lee and Lee, 2014). Curcumin also inhibit the development of hyphae through targeting the global suppressor thymidine uptake 1 (TUP1) (Moghadamtousi *et al*., 2014). Curcumin inhibits the proteolytic enzyme activities of phospholipases and SAPs without decreasing gene expression (Chen *et al*., 2018).

According to transcriptional analyses, curcumin upregulates genes linked to aggregation (ALS5 and AAF1) while downregulating important adhesins (ALS1 and ALS3). All of the information presented above demonstrated that curcumin has antiadhesive properties and stimulates the transcription of genes essential to the processes involved in the formation of biofilms. Therefore, curcumin and related polyphenols have the potential to be developed for use in oral healthcare to enhance current preventive measures for candidal biofilms on the surface of dentures (Murugesh

et al., 2019). Curcumin has been demonstrated to mechanistically cause Candida cells to produce reactive oxygen species (ROS), which results in oxidative stress and early apoptosis. Increased expression of oxidative stress-response genes, including CAP1, SOD2, and CAT1, provided evidence for this. The role of ROS was confirmed by the fact that the antifungal effects were partially reversible with antioxidants. Curcumin also prevented the morphological transition of Candida albicans from yeast to hyphae, which is a crucial virulence factor. Curcumin's impact on the transcriptional repressor TUP1, a global regulator of filamentation, was implicated in this inhibition, which happened independently of ROS generation. Even in hypha-inducing conditions, curcumin successfully inhibited hyphal development by downregulating TUP1 activity (Sharma *et al*., 2010)

2.4.7 Synergistic Effect of *Curcuma longa* with Other Antifungal Agents

The primary motivation for examining the potential synergistic effect of *C. longa* rhizome with current fungicides was its potent antifungal activity and minimal adverse effects. The MIC values of the fungicides against clinical isolates of *Candida albicans* were reduced by the synergistic activity of curcumin with five azole and two polyene medications, including voriconazole, itraconazole, ketoconazole, miconazole, fluconazole, amphotericin B, and nystatin. The accumulation of ROS, which will be inhibited by the addition of an antioxidant, may be linked to the synergistic action of curcumin with amphotericin B and fluconazole. While fluconazole and curcumin occasionally showed additive effects rather than synergistic activity, the combination of curcumin and amphotericin B also demonstrated synergistic activity against tested *Candida* species. These findings demonstrated that curcumin, when combined with currently available fungicidal agents, can have a more substantial impact on systemic fungal infections such as candidemia and candidiasis (Moghadamtousi *et al*., 2014).

2.5 Probiotics (*Lactobacillus*) in Antifungal Therapy

2.5.1 Lactobacillus

Probiotics are described as "live microorganisms that confer a health benefit on the host when administered in adequate amounts." Probiotics' antimicrobial activity is a crucial characteristic that includes strengthening the host's immune system to effectively fight off infections, producing antimicrobial compounds like bacteriocins, competitively excluding pathogens, and improving the intestinal barrier's ability to fend off infections (Fijan, 2023).

Members of the *lactobacilli* group, which includes, but is not limited to, specific strains of the following species: *Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus gasseri, Lactobacillus crispatus,* and others, and *Bifidobacterium* genera are the most widely used probiotics (Fijan, 2023).

Lactobacillus is a genus of rod-shaped, gram-positive, non-spore-forming, facultative anaerobic bacteria of the phylum ‘Firmicutes (Dempsey and Corr, 2022) now phylum Bacillota (Ibrahim *et al*., 2024). The *Lactobacillus* genus is a member of the Bacilli class, phylum Firmicutes, order *Lactobacillales* and family *Lactobacillaceae* (De Mesquita *et al*., 2017). There are over 300 species of *Lactobacilli* in 25 genera (Mejía-Caballero and Marco, 2025). *Lactobacilli* are the largest genus in the lactic acid bacteria (LAB) group because they break down carbohydrates to produce lactic acid. According to their metabolism, *Lactobacillus* species can be traditionally categorized into three groups. The facultatively heterofermentative group, which ferments carbohydrates to produce lactic acid, ethanol/acetic acid, and carbon dioxide as by-products under specific conditions or with specific substrates (e.g., *L. casei and L. plantarum*), the obligate homofermentative group, which ferments carbohydrates to produce lactic acid as the primary by-product (e.g., *L. acidophilus and L. salivariu*s), and the obligately heterofermentative

group, which always ferments carbohydrates to produce lactic acid, ethanol/acetic acid, and carbon dioxide as by-products (e.g. *L. reuteri and L. fermentum*) (Dempsey and Corr, 2022). They are a component of the natural bacterial flora found in humans, and they play a regulatory role in preventing pathogens from colonizing hosts. They also have positive effects, like promoting host tissues or enhancing nutrient assimilation during digestion (Colautti *et al*., 2022). *Lactobacillus* inhabits the human oral cavity, digestive tract, female genital tract and skin. It can also be found in fermented foods like yogurt and sauerkraut, raw milk, and plant material. These bacteria thrive in low-oxygen, high-sugar, high protein environments (Segovia *et al*., 2025). *Lactobacillus* species have a mutualistic relationship with humans (Dempsey and Corr, 2022). The ability to coaggregate and self-aggregate, which enables *lactobacilli* to stick to other microorganisms or to one another, is another crucial mechanism. *Lactobacilli*'s ability to stick to the mucosa thanks to these adhesive qualities reduces pathogen adhesion and creates a microenvironment where the close proximity of the secreted substances increases their inhibitory effects (Colautti *et al.*, 2022). *Lactobacillus bulgaricus* is a well-known member of the lactic acid bacterium family, widely used in the food industry, particularly in the manufacture of yogurt and various other fermented dairy products (Liu *et al.*, 2024). *L. bulgaricus* has been proposed to control a variety of human pathogens, including fungi (Divyashree *et al.*, 2023). We divide lactobacilli-mediated anti-Candida activity into two categories: direct and indirect. Bacterial metabolites primarily cause direct anticandidal activity by killing or inhibiting yeast cell growth, as well as preventing attachment, dimorphic transition, and biofilm formation. Indirect activity mechanisms rely on Lactobacillus-host interactions because they modulate immune processes or epithelial responses, preventing *Candida* growth on mucosal surfaces and protecting the mucosal barrier (Vazquez-Munoz and Dongari-Bagtzoglou, 2021).

2.5.2 Mechanism of Antifungal Action of *Lactobacillus*

Lactobacilli produce a variety of active compounds (primary and secondary metabolites) that exhibit broad antimicrobial activity. Several of these metabolites work well against Candida albicans as well. The most researched metabolites are bacteriocins and bacteriocin-like peptides (Vazquez-Munoz and Dongari-Bagtzoglou, 2021). In addition, *lactobacilli* generate inorganic substances like hydrogen peroxide, which in excess causes oxidative stress and genotoxicity. These substances exhibit widespread antimicrobial activity against fungi and bacteria (Vazquez-Munoz and Dongari-Bagtzoglou, 2021). By fermenting sugars into lactic acid, *Lactobacillus bulgaricus* reduces the pH of the surrounding environment to less than 4.5. The growth, enzyme activity, and transition to the invasive hyphal form of *Candida albicans* are all hindered by this acidic environment. In addition to weakening *Candida*'s cell membrane and increasing its susceptibility, the lower pH may also cause *L. bulgaricus* to produce additional antimicrobial compounds and The probiotic effect of *Lactobacillus* strains is largely due to lactic acid accumulation, with high local concentrations preventing *Candida* overgrowth nearby. (Zangl *et al*., 2019). *Lactobacillus* species, including *L. bulgaricus*, can produce hydrogen peroxide (H_2O_2) under aerobic or microaerophilic conditions. In vitro, H_2O_2 damages pathogens like *Candida albicans* through oxidative stress. Epidemiological studies link H_2O_2-producing strains with healthy vaginal flora (Miko and Barakonyi, 2023). *Lactobacillus delbrueckii* strains, including *L. bulgaricus*, inhibited *Candida albicans* most strongly when producing high levels of hydrogen peroxide. This reactive oxygen species damages fungal cells and contributes significantly to anticandidal activity in vitro (Strus *et al*., 2005) This metabolite alter the physiology of the fungus by inducing oxidative stress or by ATP depletion leading to cytotoxicity or growth inhibition (Vazquez-Munoz and Dongari-Bagtzoglou, 2021). Several studies have reported that

Lactobacillus species secrete antimicrobial peptides, including bacteriocins and bacteriocin-like substances (BLS), into their culture supernatants, and these molecules can have anticandidal activity (Vazquez-Munoz and Dongari-Bagtzoglou, 2021). Bacteriocins, a highly diverse class of bactericidal peptides or proteins, are also produced by *Lactobacillus*. They function by blocking cell wall biosynthesis, disrupting metabolic processes, and causing cytoplasmic membrane permeability (by pore formation) (Darbandi *et al*., 2021). Additionally, bacteriocins and bacteriocin-like substances (BLS) produced by probiotic *lactobacilli* can inhibit *Candida albicans* biofilms, suppress adhesion receptors, and alter the immune system (Hefzy *et al*., 2021). Biosurfactants, which are substances with both hydrophilic and hydrophobic moieties that lower surface tension, are produced by *Lactobacillus*. In addition to having direct anticandidal action, biosurfactants prevent *Candida albicans* from adhering to abiotic surfaces and tissues. Changes in the charge of the cell wall may result in adherence reduction, which makes the cell unable to overcome its electrostatic repulsion barrier with the substrate (Vazquez-Munoz and Dongari-Bagtzoglou, 2021).

2.6 Combination Therapy Approaches

2.6.1 Rationale for combining natural products with probiotics

Candida albicans is an opportunistic fungal pathogen that causes a variety of infections, especially in people with weakened immune systems. The growing resistance of *Candida* to conventional antifungal agents and the frequent relapse of infections highlight the need for alternative therapeutic strategies. By producing lactic acid, hydrogen peroxide, and antimicrobial peptides, probiotics particularly *Lactobacillus* species have shown inhibitory activity against *Candida*. However, turmeric's (*Curcuma longa*) antifungal, antibacterial, and anti-inflammatory qualities are well known. Recent evidence showed that turmeric extract enhanced the

antibacterial activity of *Lactobacillus acidophilus* against *Cutibacterium acnes*, producing a synergistic effect greater than either treatment alone. Importantly, turmeric did not impair probiotic growth, suggesting its potential role as a prebiotic that supports probiotic action (Kim *et al*., 2020). These promising findings, there is limited research on the combined effect of *Lactobacillus* and turmeric against fungal pathogens such as *C. albicans*. Exploring this synergy may reveal a novel synbiotic-based approach with improved antifungal efficacy and fewer side effects compared to conventional antifungal drugs. Emerging evidence suggests that combining probiotics with phytochemicals such as turmeric may exert synergistic effects. For example, in a murine asthma model, the co-administration of *Lactobacillus rhamnosus* GG and crude turmeric extract led to greater suppression of inflammatory markers and immune cell infiltration than either treatment alone, confirming that probiotic–turmeric combinations can achieve enhanced outcomes (Yazdi *et al*., 2020). Similarly, in functional food research, a bioavailable curcumin formulation enhanced the viability and activity of lactic acid bacteria while simultaneously inhibiting spoilage yeasts and fungi, demonstrating that turmeric can support probiotic growth while also providing antifungal protection (Buniowska-Olejnik *et al*., 2023). Most recently, an in vitro dermatological study showed that a *Lactobacillus*–turmeric synbiotic lysate displayed superior antimicrobial activity, including antifungal effects against *Candida* species, outperforming either component alone and even surpassing commercial treatment (Bhola & Bhadekar, 2025).

Together, these findings provide strong evidence that a turmeric–Lactobacillus combination offers a dual mechanism of action: direct antifungal activity through curcumin and probiotic metabolites, and indirect potentiation, whereby turmeric enhances probiotic viability and efficacy.

Thus, investigating the synergistic antifungal potential of *Lactobacillus bulgaricus* and turmeric against *Candida albicans* is both scientifically justified and clinically relevant.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was conducted in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin, Benin City, Edo state, Nigeria.

3.2 Ethical Approval

Ethical Approval was gotten from School of Basic Medical Sciences, University of Benin, Benin City.

3.3 Sample Collection

Clinical isolates of *Candida* albicans for this study was obtained from the Medical Microbiology Laboratory of the University of Benin Teaching Hospital (UBTH), Ugbowo, Benin City, Nigeria. UBTH is a foremost tertiary healthcare and academic institution located in Ugbowo, a prominent suburb of Benin City in Edo State. The hospital is affiliated with the University of Benin and serves as a major referral center for diagnostic, clinical, and academic activities in the South-South region of Nigeria.

The following clinical isolate types were included:

Wound swabs – collected from infected skin or soft tissue lesions

High vaginal swabs – obtained from patients presenting with symptoms of vulvovaginal candidiasis

Ear swabs – collected from patients with signs of otomycosis or fungal ear infections

Catheter tips – from patients with indwelling urinary catheters or central lines, suspected of fungal colonization or infection.

Urine samples – from patients of suspected urinary candidiasis All collections followed standard aseptic techniques to minimize contamination.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

Clinical isolates: Clinical isolates of Gram-positive ovoid yeast cells that were confirmed as *Candida albicans* by standard laboratory identification methods including germ tube test, CHROMagar and sugar fermentation were obtained.

3.4.2 Exclusion Criteria

Clinical isolates: Isolates that were not identified as *Candida albicans* (e.g., *C. glabrata, C. tropicalis*) and cultures showing mixed microbial growth that interferes with the isolation or purity of *Candida albicans* were excluded from this study.

3.5 Collection and Identification of Rhizome turmeric (Curcuma longa linn)

Fresh turmeric rhizomes (Curcuma longa Linn.) were collected from Oba market in Benin city, Edo State, Nigeria during the month of July, 2025. Care was taken to select healthy, mature rhizomes free from disease, insect damage, or physical blemishes. Upon collection, the turmeric samples were transported to the laboratory in clean, dry, and breathable paper bags to prevent moisture retention and fungal contamination.

3.5.1 Authentication

The collected rhizomes were taxonomically identified and authenticated by Prof. Akinnibosun Henry Adewale (FLS, MRSB; London) of the Department of Plant Biology and Biotechnology, Faculty of Life sciences in the University of Benin, Benin city, Edo State. A Voucher Specimen was prepared, assigned the number, UBH-C397 and deposited at the departmental herbarium for future reference and verification.

3.6 Preparation of Ethanolic Turmeric Extract

The rhizomes of *Curcuma longa Linn.* were washed thoroughly with clean water, then peeled and sliced into thin pieces. The sliced turmeric was air-dried at room temperature in a clean, dust-free environment until completely dry. The dried slices were then ground into a fine powder using a mechanical grinder. 700g of turmeric powder was macerated using 2L of Ethanol solvent in a maceration jar for 72hours with occasional stirring to enhance extraction. The mixture was filtered first through muslin cloth and then through Whatman No. 1 filter paper. The filtrate (ethanol extract) was concentrated using a water bath set at 40–50°C to remove the ethanol and obtained a thick crude extract. The dried extract was weighed (Ethanol extract was 24g) and stored in sterile, airtight containers at room temperature until it required for use. For testing, the extract was reconstituted in sterile distilled water to prepare different concentrations of 25 mg/250uL, 50 mg/250uL, 100 mg/250uL, 200 mg/250uL, and 400 mg/250uL respectively

3.7 Determination of Extraction Yield

The percentage yield of the extract was calculated to determine the efficiency of the extraction for the solvent. The yield was calculated ethanol extract using the following formula:

Percentage Yield (%) = (Weight of the dried extract / Weight of the initial powdered plant material) x 100

Weight of dried extract = 24g

Weight of initial powdered plant = 700g

Percentage Yield (%) = (24/700) x 100 = 3.43%

3.8 Sterilization of Materials

All materials used were adequately and appropriately sterilized before and after use. Petri dishes, glass wares, and metal equipment were thoroughly washed, rinsed, and autoclaved at 121°C for

20 minutes. New gloves were used for each sample analysis and media was prepared according to the manufacturer's instructions and autoclaved at 121°C for 15 minutes.

3.9 Preparation of *Lactobacillus* Secondary Metabolite

A probiotic strain of *Lactobacillus* precisely *Lactobacillus bulgaricus* was isolated from a probiotic supplement (yoghurt). The selected *Lactobacillus* strain was confirmed by Gram staining and catalase testing (Gram-positive rods, catalase-negative) was activated and cultured by inoculating a loopful of the yoghurt into de Man, Rogosa and Sharpe (MRS) Agar selective for Lactobacillus and incubated anaerobically at 37°C for 24-48hours, then a loopful of the bacterial culture was inoculated into de Man, Rogosa and Sharpe (MRS) broth to promote secondary metabolite formation. The broth culture was centrifuged at 3000–4000 rpm for 15–20 minutes to separate the bacterial cells from the culture supernatant. The supernatant, which contains the secondary metabolites (e.g., lactic acid, hydrogen peroxide, bacteriocins), was carefully decanted and collected. To ensure sterility and remove any remaining bacterial cells, the supernatant was filtered using a 0.22 µm membrane filter. The filtered metabolite solution was stored at 4°C for short-term use. The *Lactobacillus* supernatant was used at various concentrations in combination with turmeric extracts.

3.10 Preparation of Test Organisms

Confirmed clinical isolates of Candida albicans obtained from wound swab, high vaginal swabs, ear swab, catheter tip and urine sample were used for antifungal testing. Each isolate was maintained in Sabouraud Dextrose Broth (SDB) and frozen. Prior to use, test microorganisms were sub-cultured from stock onto sterile Sabouraud Dextrose Agar (SDA) plates, incubated at 37°C for 24–48 hours. After incubation, a few well-isolated colonies were picked and suspended

in sterile normal saline. The turbidity of the suspension was adjusted to match 0.5 McFarland standard, which corresponds to approximately 1×10^6 CFU/mL.

3.11 Test for Antifungal Activity

3.11.1 Determination of Inhibition Zone Diameters (IZD)

Antimicrobial Susceptibility test was carried out using agar well diffusion method (Murray, 2009) with some modifications. Sterile Sabouraud Dextrose Agar was prepared and poured into different petri dishes aseptically, each containing 30ml and allowed to set. The petri dishes were dried in hot air oven for about 10minutes at 40°C. Each of the dried plates were then streaked evenly with each test organism respectively using a swab stick aseptically. A sterile cork borer (10mm) was used to bore 6 wells (evenly spaced) in each agar plate, the disk was removed and each of the well was sealed with 2 drop of molten agar. Five of the wells were filled with 25mg/250ul, 50mg/250ul, 100mg/250ul, 200mg/250ul, and 400mg/250ul of the ethanolic extracts respectively and the sixth well was filled with the standard, Ketoconazole. The plates were incubated at 37°C for 24 hours.

The procedure was repeated using a combination of the ethanolic extract and *lactobacillus* supernatant (secondary metabolite) at ratio 1:1 in concentrations of 25mg/250ul, 50mg/250ul, 100mg/250ul, 200mg/250ul, and 400mg/250ul respectively. The plates were incubated at room temperature for 24 hours.

The inhibition zone diameters (IZD) was be measured and recorded.

3.11.2 Determination of Minimum Inhibitory Concentration (MIC)

Agar dilution method of Afyon and Meyer (1997) was used in this study for the determination of Minimum Inhibitory Concentration (MIC) of the extract and combination treatment. A 2-fold serial dilution of the extract was prepared to give concentrations of 12.5mg, 25mg, 50mg, 100mg,

and 200mg. Double strength agar was prepared according to manufacturer's instruction. Calculated volumes of each extract concentration and double strength agar (1 gram of extract + 20ml molten agar) was poured into five petri dishes respectively and allowed to set. This was repeated using a combination of the ethanolic extract and lactobacillus supernatant (secondary metabolite) at ratio 1:1 in concentrations of 12.5mg, 25mg, 50mg, 100mg, and 200mg respectively. Each of the test organisms prepared to a standard concentration was streaked with the aid of a sterile wire loop on well labeled different sections of each plate. The dilution plates were incubated at 37°C for 24 hours. After incubation, the plates were visually examined for growths in the inoculated spots. The lowest concentration of the extracts that inhibits growth was considered as the MIC.

3.11.3 Determination of Minimum Fungicidal Concentration (MFC)

The MFC was determined using agar well dilution method. It was determined from the agar dilution of the MIC tests by sub-culturing into agar plates that did not contain any test extract or combination treatment. The dilution plates were then incubated at 37°C for 24 hours. After incubation, the plates were visually examined for growths in the inoculated spots. The lowest concentration of the extract and combination treatment that showed no growth were considered as the MFC respectively

3.12 Statistical Analysis

The data obtained from the experiments was recorded and analyzed using appropriate statistical methods, including analysis of various (ANOVA) followed by post-hoc tests for multiple comparisons. Results will be considered statistically significant at $p<0.05$.

CHAPTER FOUR

RESULTS

Table 4.1 shows the percentage yield of crude extract and physical properties of *Curcuma longa linn*. Rhizome.

The Ethanolic extract of *Curcuma longa linn* yielded 3.43% of extract, was orange-brown in colour, had a spicy aromatic smell with a thick consistency and smooth texture.

Table 4.1: Physical properties and percentage yield of *Curcuma longa linn*. Rhizome.

Property	Ethanolic Extract
Odour	A Spicy aromatic smell
Solvent	Ethanol
Texture	Smooth
Colour of crude extract	Orange-brown
Consistency	Thick
Percentage yield	3.43%

Table 4.2 shows the Minimum Inhibitory Concentration (MIC) of the ethanolic turmeric extract. Ethanolic turmeric extract inhibited *Candida albicans* growth beginning at 25 mg, where 40.0% of isolates showed no growth. Inhibition increased with concentration, reaching 60.0% at both 50 mg and 100 mg. For the combination of ethanolic extract with *Lactobacillus*, inhibition was slightly higher at each concentration, with 60.0% inhibition at 25 mg and 80.0% inhibition at both 50 mg and 100 mg The association between concentration and inhibition was statistically significant (Chi-square, $p < 0.05$), indicating a clear dose response effect. However, comparison between ethanolic extract alone and the combination treatment showed no significant overall difference (Chi-square, $p = 0.749$).

Table 4.3 illustrates the fungicidal activity of ethanolic turmeric extract and its combination with *Lactobacillus* against *Candida albicans*. The ethanolic extract demonstrated fungicidal effects only at higher concentrations, with 20.0% inhibition observed at 50 mg and 40.0% inhibition at 100 mg. Complete fungicidal inhibition (100.0%) was achieved at 200 mg. In contrast, the combination of ethanolic extract with *Lactobacillus* exhibited enhanced fungicidal activity across all tested concentrations. At 25 mg, the combination inhibited 40.0% of isolates, increasing to 60.0% at both 50 mg and 100 mg, and reaching total inhibition (100.0%) at 200 mg. Statistical analysis confirmed a significant dose-dependent relationship between concentration and fungicidal activity (Chi-square test, $p < 0.05$). However, comparison between the ethanolic extract alone and its combination with *Lactobacillus* revealed no statistically significant overall difference in fungicidal effect (Chi-square test, $p = 0.256$).

As shown in **Table 4.4**, ethanolic turmeric extract produced no inhibition zones at concentrations up to 100 mg. At 200 mg, a mean zone of 2.20 ± 2.20 mm was recorded, which increased to

7.00 ± 2.88 mm at 400 mg. The combination treatment produced larger zones at corresponding concentrations, with 4.80 ± 2.15 mm at 200 mg and 10.20 ± 2.36 mm at 400 mg. The ketoconazole control at 25mg produced the largest inhibition zone (17.00 ± 1.14 mm). ANOVA revealed a statistically significant difference in mean inhibition zones across concentrations (**$p < 0.001$**). Post hoc Tukey HSD analysis showed that the zone diameter at 400 mg was significantly higher compared to 25, 50, and 100 mg (**$p < 0.05$**), but not significantly different from 200 mg (**$p > 0.05$**). Furthermore, ketoconazole (500 mg) was significantly different from all plant-based concentrations ($p < 0.05$).

Independent-samples t-test also demonstrated that the combination treatment achieved significantly larger inhibition zones than ethanolic extract alone (**$p = 0.021$**).

As shown in **Table 4.5** susceptibility varied by source of *Candida albicans*. MIC data indicated that isolates from HVS were most susceptible (75.0% inhibition), whereas urine isolates were most resistant (0.0% inhibition). This difference was statistically significant (Chi-square, $p = 0.044$).

For MFC, inhibition ranged from 0.0% (wound swab) to 37.5% (HVS and ear swab), but differences between sources were not significant ($p = 0.382$). Mean inhibition zones ranged from 2.30 ± 0.82 mm (ear swab) to 6.30 ± 1.03 mm (urine). However, differences were not statistically significant (ANOVA, $p = 0.428$). Across all sources, the ketoconazole control consistently produced the highest inhibition zones.

Table 4.2: Minimum Inhibitory Concentration (MIC) of Ethanolic Turmeric Extract and Combination with *Lactobacillus* Against *Candida albicans*

Concentration (mg/mL)	Ethanolic Extract n (%)	Extract + Lactobacillus n (%)	p-value
12.5	0 (0.0)	0 (0.0)	0.749
25	2 (40.0)	3 (60.0)	
50	3 (60.0)	4 (80.0)	
100	3 (60.0)	4 (80.0)	

P < 0.05 is significant

Table 4.3: Minimum Fungicidal Concentration (MFC) of Ethanolic Turmeric Extract and Combination with *Lactobacillus*

Concentration (mg/0.25mL)	Ethanolic Extract n (%)	Extract + Lactobacillus n (%)	p-value
12.5	0 (0.0)	0 (0.0)	0.392
25	0 (0.0)	0 (40.0)	
50	1 (20.0)	1 (20.0)	
100	2 (40.0)	5 (100.0)	
200	5 (100)	5 (100)	

P < 0.05 is significant

Table 4.4: Mean Zone of Inhibition (Mean ± SEM) at Different Concentrations

Concentration (mg/0.25mL)	Ethanolic Extract (mm)	Extract + Lactobacillus (mm)	P value
25	0.00 ± 0.00	0.00 ± 0.00	0.001
50	0.00 ± 0.00	0.00 ± 0.00	
100	0.00 ± 0.00	0.00 ± 0.00	
200	2.20 ± 2.20	4.80 ± 2.15	
400*	7.00 ± 2.88	10.20 ± 2.36	
Ketoconazole*	17.00 ± 1.14	17.00 ± 1.14	

p < 0.001 (significant). * Tukey post hoc, 400 mg (extract) was significantly different from 25, 50, and 100 mg (p < 0.05). Ketoconazole (25mg) was significantly higher than all plant-based concentrations (p < 0.05).

Table 4.5: Susceptibility of *Candida albicans* Isolates from Different Sources

Source	MIC n (%)	MFC n (%)	Mean Zone ± SEM (mm)
Wound swab	4 (50.0)	0 (0.0)	2.60 ± 0.82
HVS	6 (75.0)	3 (37.5)	4.90 ± 1.03
Ear swab	4 (50.0)	3 (37.5)	2.30 ± 0.82
Catheter tip	3 (37.5)	1 (12.5)	2.40 ± 0.82
Urine	0 (0.0)	1 (12.5)	6.30 ± 1.03
P value	0.044	0.382	0.428

P < 0.05 is significant

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

The results obtained in this study demonstrate that ethanolic turmeric extract exhibits dose-dependent antifungal activity against *Candida albicans*, consistent with prior studies that have established the inhibitory potential of *Curcuma longa* extracts. For instance, Salih *et al*. (2025) reported significant growth inhibition of *Candida* species with ethanolic turmeric extract, highlighting its potential as a phytotherapeutic alternative to conventional antifungals (Salih *et al*., 2025). Similarly, Kasta (2020) confirmed that ethanolic turmeric extracts exert inhibitory effects against *C. albicans*, in addition to bacterial pathogens, suggesting a broad antimicrobial spectrum (Kasta, 2020). The dose-dependent response observed in the current study is further corroborated by Murugesh *et al*. (2019), who demonstrated that higher concentrations of ethanolic turmeric extract produced stronger antifungal effects against *C. albicans* (Murugesh, Annigeri, and Mangala, 2019). However, these effects are attributed to curcumin and other bioactive compounds in turmeric, which disrupt fungal cell wall integrity and inhibit biofilm formation (Sadanandan *et al*., 2023). Notably, *C. albicans* biofilms are highly resistant to conventional antifungal drugs, underscoring the importance of phytochemicals as complementary therapeutic options (Sadanandan *et al*., 2023). The enhanced inhibition observed with the combination of ethanolic turmeric extract and *Lactobacillus* suggests a synergistic effect between phytochemicals and probiotic microorganisms. Bhola and Bhadekar (2025) demonstrated that a *Lactobacillus*-turmeric synbiotic exhibited superior antimicrobial activity compared to turmeric alone, particularly in dermatological applications, highlighting the therapeutic potential of probiotic–phytochemical synergies (Bhola and Bhadekar, 2025). This is

consistent with Ogidi *et al*. (2021), who showed that combining turmeric derivatives with other antifungal agents enhances efficacy against pathogenic fungi, suggesting that turmeric may serve as an effective adjuvant (Ogidi *et al*., 2021).

Despite the observed trend toward greater inhibition in the combination group, the lack of a statistically significant difference compared to turmeric extract alone suggests that while probiotics may potentiate antifungal effects, their influence may depend on strain specificity, concentration ratios, and host micro-environmental factors. This aligns with evidence that *Lactobacillus* spp. inhibit *C. albicans* primarily through acidification, bacteriocin production, and competitive exclusion, which may vary in magnitude depending on co-administered agents (Ilham *et al*., 2018).

The findings further demonstrated that ethanolic turmeric extract exhibited limited fungicidal activity at moderate concentrations, achieving only 20.0% inhibition at 50 mg/0.25mL and 40.0% at 100 mg/0.25mL, but complete inhibition at 200 mg/0.25mL. These results align with prior studies that established a concentration-dependent antifungal activity of turmeric extracts against Candida albicans. Salih *et al*. (2025) observed that ethanolic turmeric extract effectively suppressed clinical Candida isolates in a dose-dependent manner, underscoring its potential as a natural antifungal agent (Salih *et al*., 2025). Similarly, Kasta (2020) confirmed that ethanolic rhizome extracts significantly inhibited *C. albicans* growth, validating the broad antifungal potential of turmeric (Kasta, 2020).

The complete fungicidal inhibition observed at 200 mg/0.25mL is consistent with the concept of a threshold concentration required for curcumin and associated bioactive compounds to exert maximal disruption of fungal cell membranes and biofilms. Al-Najjar *et al*. (2024) found that both aqueous and alcoholic turmeric extracts demonstrated potent antifungal effects against C.

albicans, even in nystatin-resistant strains, reinforcing the therapeutic relevance of turmeric in overcoming antifungal resistance (Al-Najjar *et al.,* Alalwany, 2024).The combination of ethanolic extract with Lactobacillus revealed enhanced fungicidal activity at lower concentrations, achieving 40.0% inhibition at 25 mg/0.25mL and 60.0% at both 50 mg/0.25mL and 100 mg/0.25mL, with complete inhibition at 200 mg/0.25mL. This synergistic effect is in line with previous reports that probiotic strains such as *Lactobacillus* spp. can potentiate antifungal efficacy by lowering pH, producing organic acids, and inhibiting fungal adhesion. Ogidi *et al*. (2021) demonstrated similar synergism, showing that turmeric essential oil in combination with antifungal agents produced enhanced inhibition of pathogenic fungi (Ogidi *et al*., 2021). Furthermore, Jyotirmayee and Mahalik (2022) noted that curcumin biotransformation by *Lactobacillus fermentum* enhances bioactivity, suggesting that probiotics may augment turmeric's antifungal potency by modulating its metabolic profile (Jyotirmayee and Mahalik, 2022). While the current study did not find a statistically significant overall difference between treatments, the trend toward stronger inhibition in the combination group supports the growing evidence for plant–probiotic synergism. Such findings highlight the potential of turmeric–*Lactobacillus* formulations as novel antifungal therapies, particularly against drug-resistant Candida infections.

From the results obtained the ethanolic turmeric extract exhibited limited inhibitory activity against *Candida albicans* at lower concentrations, a modest mean zone of 2.20 ± 2.20 mm at 200 mg/mL, and a larger mean zone of 7.00 ± 2.88 mm at 400 mg/0.25mL. In contrast, the combination of turmeric extract with *Lactobacillus* demonstrated superior inhibition, producing zones of 4.80 ± 2.15 mm at 200 mg/0.25mL and 10.20 ± 2.36 mm at 400 mg/0.25mL. These results suggest that while turmeric alone has fungistatic potential, its efficacy improves

considerably when administered in combination with probiotics. Previous research supports these findings. Salih *et al*. (2025) similarly reported dose-dependent inhibition zones produced by ethanolic turmeric extract against clinical Candida isolates, though consistently smaller than those observed with standard antifungal drugs such as ketoconazole (Salih *et al*., 2025). Consistently, Siddique *et al*. (2021) found that turmeric extracts inhibited C. albicans, but with smaller inhibition zones compared to ketoconazole, which was confirmed as the most potent antifungal control (Siddique *et al*., 2021). The significantly larger inhibition zones recorded for the combination of turmeric extract with *Lactobacillus* align with evidence of synergistic interactions between phytochemicals and probiotics. Bhola and Bhadekar (2025) demonstrated that a *Lactobacillus*-turmeric synbiotic achieved higher antimicrobial activity compared to turmeric extract alone, reinforcing the hypothesis that probiotics potentiate turmeric's efficacy (Bhola and Bhadekar, 2025). This synergism may be attributed to the ability of *Lactobacillus* spp. to acidify the local environment, inhibit fungal adhesion, and enhance curcumin bioavailability. Similarly, Ogidi *et al*. (2021) found that turmeric essential oil combined with antifungal formulations produced stronger inhibition zones against pathogenic fungi, supporting the concept of combinatory approaches to enhance antifungal potency (Ogidi, Ojo, and Ajayi-Moses, 2021).

Despite the promising results, ketoconazole remained significantly more effective, producing the largest inhibition zone (17.00 ± 1.14 mm). This finding is consistent with previous research that consistently identifies ketoconazole as a potent antifungal with superior inhibitory potential compared to plant-derived extracts (Trigo-Gutierrez *et al*., 2021). Nonetheless, the observed synergy between turmeric and Lactobacillus highlights the potential of plant–probiotic formulations as adjunct or alternative therapies, particularly in contexts of antifungal resistance where synthetic agents may fail (Trigo-Gutierrez *et al*., 2021).

The observed variation in susceptibility of Candida albicans isolates from different clinical sources to ethanolic turmeric extract highlights the heterogeneity of fungal responses depending on the site of isolation. MIC data showed that isolates from high vaginal swabs (HVS) were the most susceptible (75.0% inhibition), whereas urine isolates were completely resistant (0.0% inhibition). This variation was statistically significant ($p = 0.044$), suggesting that host microenvironment and site-specific adaptation may influence fungal sensitivity to plant-derived antifungals. Salih *et al*. (2025) similarly reported differential inhibitory responses among Candida species isolated from diverse clinical specimens when tested against turmeric ethanolic extract, noting stronger inhibition in mucosal isolates compared to urinary ones (Salih *et al*., 2025). For minimum fungicidal concentration (MFC), inhibition ranged from 0.0% (wound swab isolates) to 37.5% (HVS and ear swab isolates), though the difference was not statistically significant. Murugesh *et al*. (2019) also emphasized that fungicidal activity of Curcuma longa extracts was strain- and concentration-dependent, with mucosal isolates exhibiting higher susceptibility than isolates from non-mucosal sources (Murugesh *et al*., 2019). These findings highlight the potential influence of biofilm-forming ability and resistance traits, which vary across clinical sites.

The consistently larger inhibition zones produced by ketoconazole control reaffirm its superior antifungal efficacy across all sources. Siddique *et al*. (2021) confirmed similar findings in their comparative evaluation of plant extracts, showing that while turmeric displayed antifungal activity, ketoconazole consistently produced larger inhibition zones across clinical isolates of Candida (Siddique *et al*., 2021). Recent advances suggest that curcumin's antifungal efficacy may also be enhanced when used in combination with synthetic antifungals. Tsopmene and Tokam Kuaté (2024) demonstrated that curcumin and piperine exerted strong antibiofilm activity

and enhanced the efficacy of azole antifungals against resistant C. albicans clinical isolates (Tsopmene and Tokam Kuaté, 2024). This suggests that while turmeric extract alone may show variable efficacy across clinical isolates, its adjuvant use alongside established antifungals such as ketoconazole could overcome site-related resistance differences.

Overall, the findings revealed that susceptibility of Candida albicans to turmeric extract is not uniform across clinical sources, as vaginal isolates appear more responsive, while urinary and wound isolates are more resistant. These results are consistent with the broader literature that highlights isolate-specific responses and supports the potential of turmeric extract as a complementary, though not stand-alone, antifungal strategy.

5.2 Limitations

This study was limited by:

1. Limited sample size: Only a small number of isolates of Candida albicans were examined, which might not fully reflect the variety of clinical strains.

2. Extraction method: Curcuma longa was only extracted using ethanol; other solvents might produce different or more potent bioactive compounds.

3. Microbial variability: The antifungal activity of Lactobacillus may be influenced by variations in its concentration and viability.

4. Generalization limitation: Without additional in vivo and clinical research, laboratory experiment results might not be directly applicable to clinical practice.

5.3 Conclusion

In conclusion this study confirmed that ethanolic turmeric extract exhibits dose-dependent antifungal activity against *Candida albicans*, with enhanced efficacy when combined with *Lactobacillus*. While the extract alone required high concentrations to achieve fungicidal activity,

the combination consistently produced larger inhibition zones at lower doses, suggesting probiotic-mediated synergism. Despite these benefits, ketoconazole remained significantly more effective across all assays, underscoring the limitations of turmeric-based treatments as standalone agents. Variation in susceptibility among clinical isolates, with vaginal isolates showing higher sensitivity than urinary or wound isolates, further highlights the complexity of host–pathogen interactions.

Overall, turmeric extract, particularly in combination with *Lactobacillus*, holds promise as an adjunctive therapy, potentially enhancing efficacy and mitigating resistance when used alongside conventional antifungals.

5.4 Recommendation

The findings of this study provide evidence that ethanolic turmeric extract and *Lactobacillus bulgaricus* secondary metabolites possess promising antifungal activity against *Candida albicans*, suggesting their potential as natural therapeutic alternatives. Based on these results, several recommendations are proposed. Firstly, further in vivo investigations and clinical trials should be undertaken to validate the antifungal efficacy, safety, and pharmacokinetic properties of turmeric–probiotic combinations, as in vitro results alone are not sufficient for clinical application. Secondly, research should focus on isolation, characterization, and mechanistic evaluation of the bioactive compounds within turmeric responsible for antifungal activity, as well as the metabolites produced by *Lactobacillus*. This will help to clarify whether observed effects are synergistic or additive in nature. Finally, considering the increasing problem of antifungal resistance, health authorities, pharmaceutical researchers, and policymakers should support further research into herbal–probiotic alternatives. Integrating these into antifungal drug

development pipelines could provide safer, more accessible, and cost-effective solutions for managing candidiasis and related fungal infections.

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APPENDIX I

ANTIFUNGAL SUSCEPTIBILITY

Table 1: Inhibition Zone Diameter (IZD) of Ethanolic Extract on the Clinical Isolates at Different Concentrations

Isolates/Concentration	**25mg**	**50mg**	**100mg**	**200mg**	**400mg**	**Keto(25mg)**
CA 1/ Wound swab	___	___	____	____	____	17mm
CA 2/ High vaginal swab	____	____	____	____	11mm	19mm
CA 3/ Ear swab	____	____	____	____	11mm	20mm
CA 4/ Catheter tip	____	____	____	____	____	15mm
CA 5/ Urine	____	____	____	11mm	13mm	14mm

KEY:

CA - *Candida albicans*

Table 2: Inhibition Zone Diameter (IZD) of Ethanolic Extract + *Lactobacillus* Supernatant on the Clinical Isolates at Different Concentrations

Isolates/Concentration	**25mg**	**50mg**	**100mg**	**200mg**	**400mg**	**Keto(25mg)**
CA 1/ Wound swab	___	___	____	11mm	15mm	17mm
CA 2/ High vaginal swab	____	____	11mm	13mm	14mm	19mm
CA 3/ Ear swab	____	____	____	____	12mm	20mm
CA 4/ Catheter tip	____	____	____	11mm	13mm	15mm
CA 5/ Urine	____	____	11mm	13mm	15mm	14mm

KEY:

CA - *Candida albicans*

Table 3: Minimum Inhibitory Concentration (MIC) of Ethanolic Extract on the Clinical Isolates

Isolates/Concentration	**12.5mg**	**25mg**	**50mg**	**100mg**
CA 1/ Wound swab	G	G	G	G
CA 2/ High vaginal swab	G	NG	NG	NG
CA 3/ Ear swab	G	G	NG	NG
CA 4/ Catheter tip	G	NG	NG	NG
CA 5/ Urine	G	G	G	NG

KEY:

CA - *Candida albicans*

G- Growth

NG- No Growth

Table 4: Minimum Inhibitory Concentration (MIC) of Ethanolic Extract + *Lactobacillus* Supernatant on the Clinical Isolates

Isolates/Concentration	**12.5mg**	**25mg**	**50mg**	**100mg**
CA 1/ Wound swab	NG	NG	NG	NG
CA 2/ High vaginal swab	G	NG	NG	NG
CA 3/ Ear swab	G	G	NG	NG
CA 4/ Catheter tip	G	G	NG	NG
CA 5/ Urine	G	G	NG	NG

KEY:

CA - *Candida albicans*

G- Growth

NG- No Growth

Table 5: Minimum Fungicidal Concentration (MFC) of Ethanolic Extract on the Clinical Isolates

Isolates/Concentration	**12.5mg**	**25mg**	**50mg**	**100mg**	**200mg**
CA 1/ Wound swab	G	G	G	G	NG
CA 2/ High vaginal swab	G	G	NG	NG	NG
CA 3/ Ear swab	G	G	G	NG	NG
CA 4/ Catheter tip	G	G	G	G	NG
CA 5/ Urine	G	G	G	G	NG

KEY:

CA - *Candida albicans*

G- Growth

NG- No Growth

Table 6: Minimum Fungicidal Concentration of Ethanolic Extract + *Lactobacillus* Supernatant on the Clinical Isolates

Isolates/Concentration	**12.5mg**	**25mg**	**50mg**	**100mg**	**200mg**
CA 1/ Wound swab	G	G	G	NG	NG
CA 2/ High vaginal swab	G	G	G	NG	NG
CA 3/ Ear swab	G	G	NG	NG	NG
CA 4/ Catheter tip	G	G	G	NG	NG
CA 5/ Urine	G	G	G	NG	NG

KEY:

CA - *Candida albicans*

G- Growth

NG- No Growth

APPENDIX II

PREPARATION OF MEDIA

Sabouraud Dextrose Agar Preparation
Sixty-five (65g) grams of the nutrient agar powder was weighed and suspended in 1 liter of distilled water. The solution was mixed properly and allowed to dissolve completely. It was then sterilized by autoclaving at 121°C for 15 minutes. The molten liquid was poured into the petri dish and allowed to solidify. The plates were stored in a refrigerator.

De Man, Rogosa and Sharpe Agar Preparation
Fifty-two grams (52g) of the powdered medium was weighed and suspended in one liter of distilled water. It was heated with frequent agitation and boiled for one minute to completely dissolve the medium. The mixture was autoclaved at 121ºC for 15minutes. It was allowed to cool to 45ºC and poured into petri dishes. The poured plates were allowed to solidify and were used to culture the samples after drying

Preparation of Stock Solution of Ketoconazole

Stock solution of 5ml Ketoconazole was prepared containing 25 mg/mL by dissolving 125 mg of Ketoconazole in 0.5ml of 10% DMSO (Dimethyl Sulfoxide) and 4.5ml of sterile water.

Preparation of McFarland Solution

A 0.5 McFarland standard solution was prepared by adding 0.05ml of 1.175% (weight/volume) Barium Chloride dihydrate salt ($BaCl_2 \cdot 2H_2O$) to 9.95ml of 1% Sulfuric acid (H_2SO_4). The solution was mixed completely to form a turbid suspension in a test tube which was then placed in a test tube rack and kept at room temperature before use.

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APPENDIX III

MATERIALS

Microbiological Media

Sabouraud Dextrose Agar (SDA), Sabouraud Dextrose Broth (SDB), De Man, Rogosa and Sharpe Agar (MRSA), De Man, Rogosa and Sharpe Broth (MRSB)

Equipment and Apparatus

Portable autoclave, rotary evaporator, weighing balance, hot air oven, mortar and pestle, mechanical grinding machine, refrigerator, micropipette

incubator, whatman filter paper, cotton wool, pipette tip, cork borer (10mm in diameter), transparent millimeter rule, grease pencil, sterile swab sticks, tripod stand, bunsen burner, foil paper, heating mantle, water bath, spatulas, porcelain dishes, flask brushes

Glassware

Conical flask, bottles (MacCartney, universal and Bijou) as well as test tubes, pipettes, glass stirrers, porcelain dish, pestle, maceration jars, glass funnels, beakers, measuring cylinders, and Petri dishes.

Chemicals and Reagents

All solvents used were of analytical grade.

Ethanol, Tween 80, distilled water, disinfectant: Purit, standard antifungal agent (ketoconazole), soap and detergent.

APPENDIX IV

PLANT AUTHENTICATION

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London)
Faculty of Life Sciences,
Department of Plant Biology and Biotechnology,
P. M. B. 1154 Ugbowo, 300283 Benin City,
Edo State, Nigeria.

University of Benin

Department of Plant Biology and Biotechnology

Herbarium Unit

Faculty of Life Sciences

University of Benin, Benin City, Edo State

Plant Name: *Curcuma longa* Linn.

Family: Zingiberaceae

Common Name: Turmeric

Voucher Number: UBH-C397

Student Name: Saniyo Avwerrosuo Jennifer

Plant Identification and Voucher Number Issued by:

20/05 /2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MECOSON, LMBOSON, MAEIAN; MFBAN Nigeria).

APPENDIX V

ETHICAL APPROVAL

RESEARCH ETHICS COMMITTEE
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF BENIN, BENIN CITY, NIGERIA.

Chairman: Prof. F. A Imarhiagbe
MBChb, FMCP
Cert Clin Res and ethics (NIH), MD.
0803449092

P.M.B 1154, BENIN CITY
Email: researchethics.cms@gmail.com

Our Ref: CMS/REC/01/VOL.2/836 **Date:** 5th September, 2025

Re: COMPARATIVE STUDY OF ANTIFUNGAL EFFECT OF *Curcuma longa* ETHANOLIC EXTRACT WITH *lactobacillus* ON *Candida albicans ISOLATES*

Name of Principal Investigator: **SANIYO AVWEROSUO JENNIFER**
Department of Med Lab Sci,
School of Basic Medical Science
College of Medical Sciences,
University of Benin

REC Approval No: CMS/REC/2025/836

This is to inform you that the research described in the submitted proposal, the Informed Consent Forms and other participant information materials have been reviewed and approved by the College Research Ethics Committee, University of Benin.

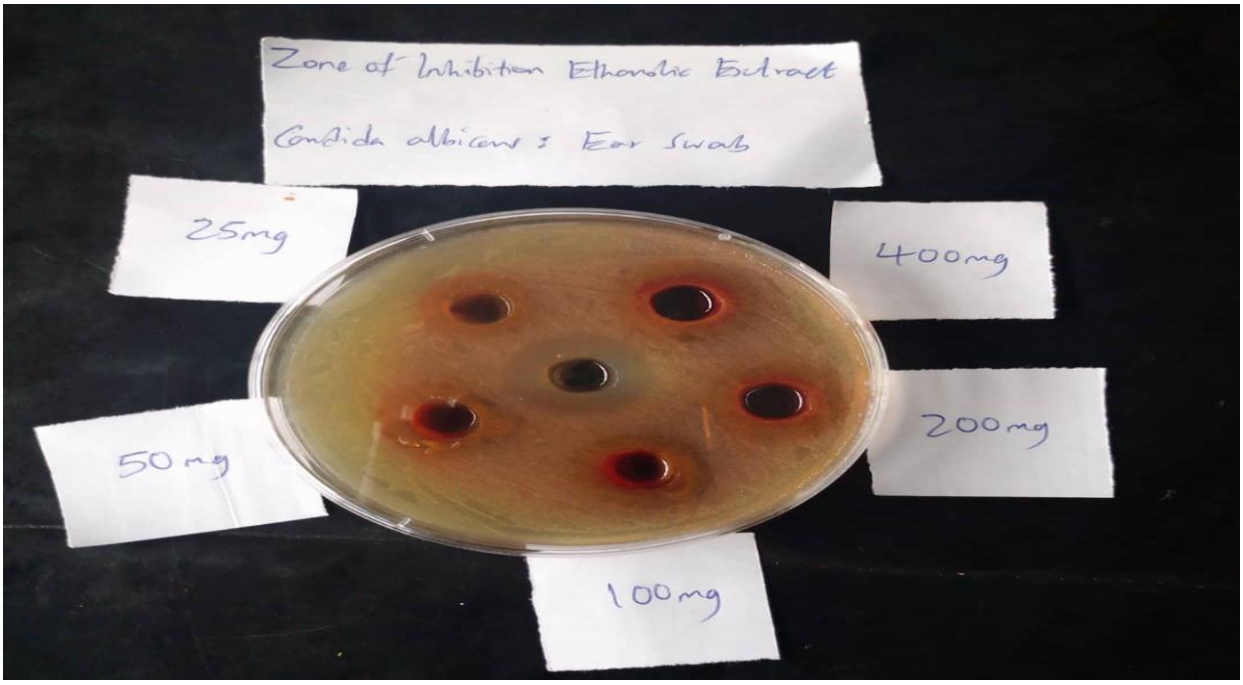
This approval dates from **5th September, 2025 to** 4th September, 2026. In multi-year research, Endeavour to submit your annual report to the REC early in order to obtain renewal of your approval and avoid disruption of your research.

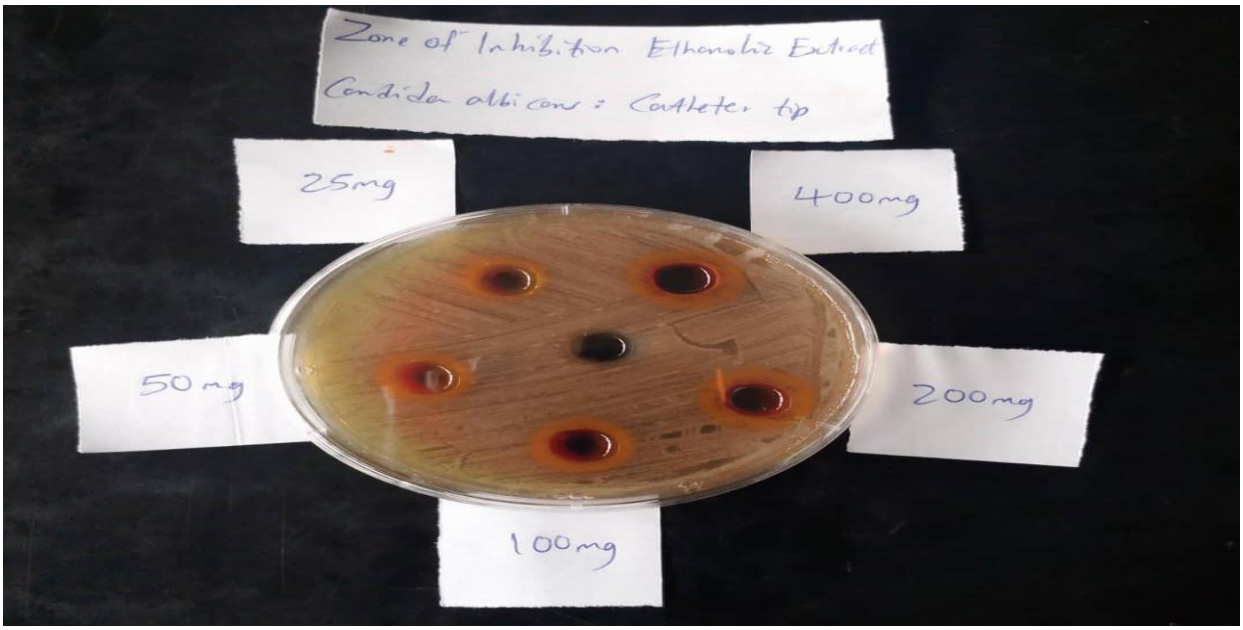
The National Code of Health Research Ethics requires you to comply with all institutional guidelines, rules and regulations and with the tenets of the code including ensuring that all adverse events are reported promptly to the REC. No, changes are permitted in the research without prior approval by REC except in circumstances outlined in the code. REC reserves the right to conduct compliance visit to your research site without prior notice. Thank you.

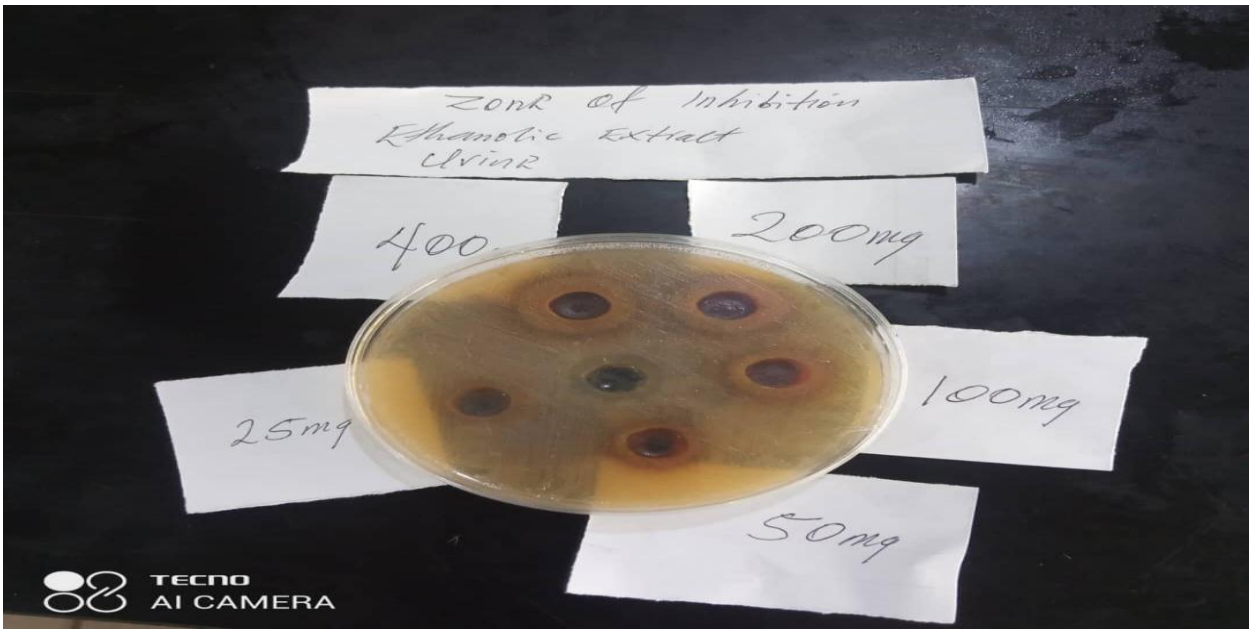
PROF. F.A IMARHIAGBE
Chairman, REC

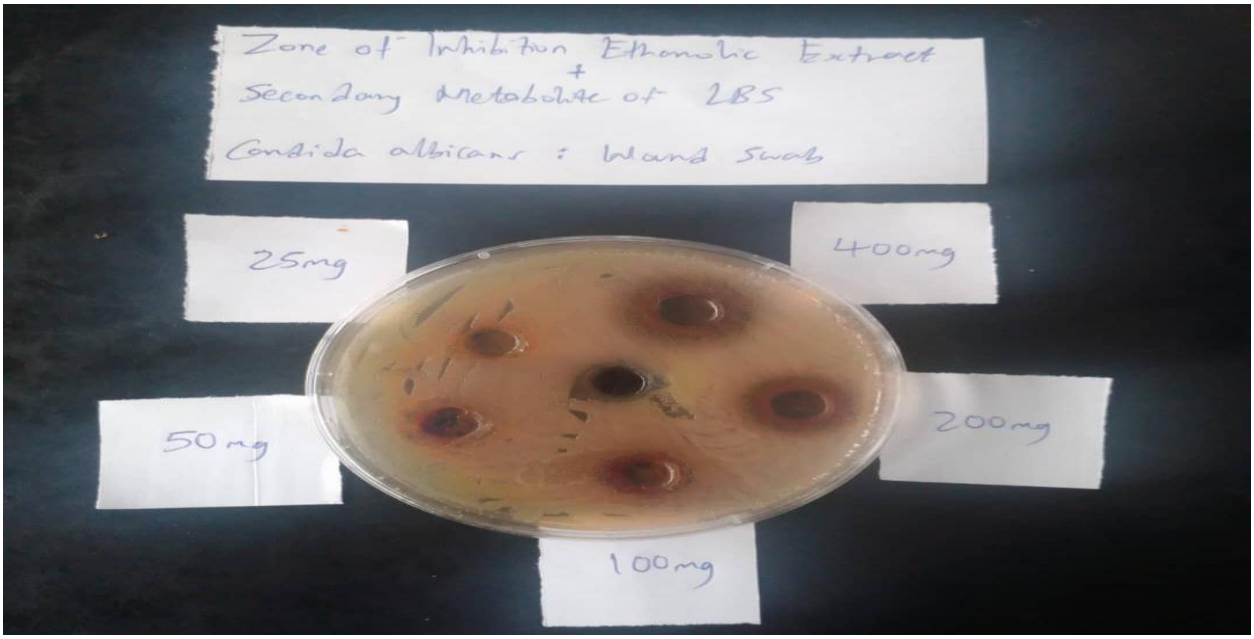
APPENDIX VI

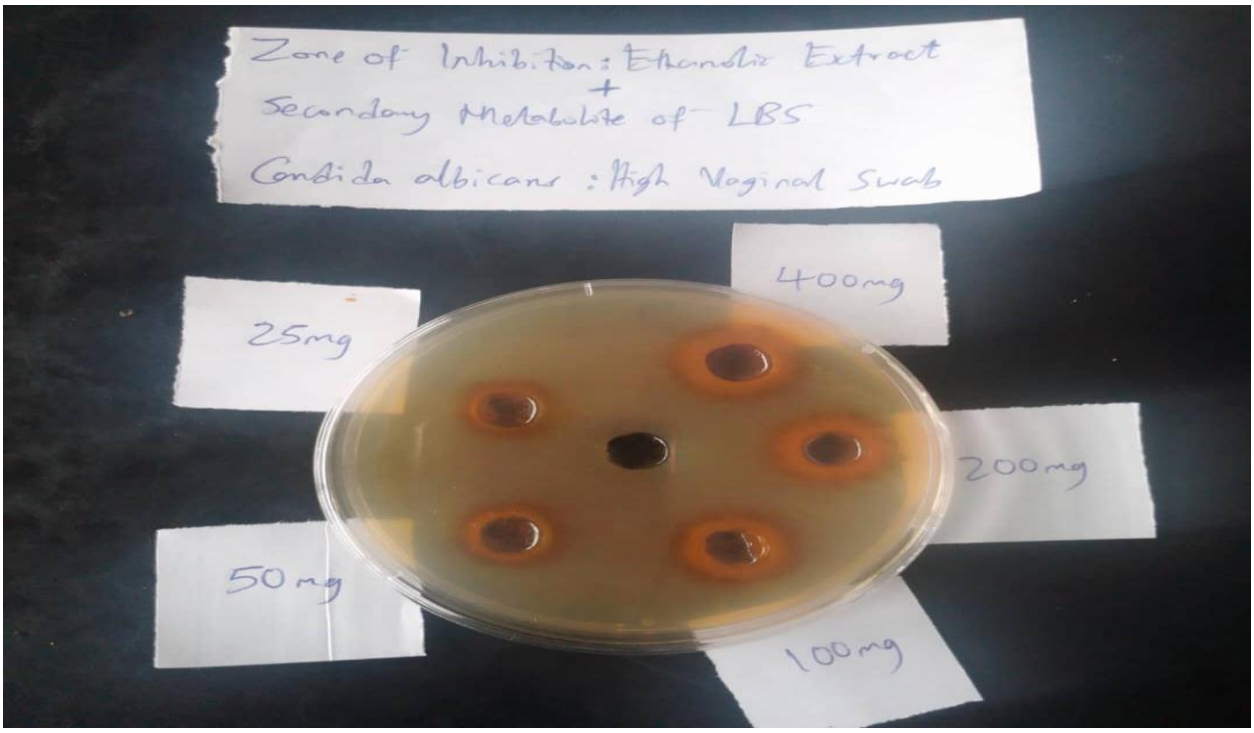
ZONE OF INHIBITION PLATES

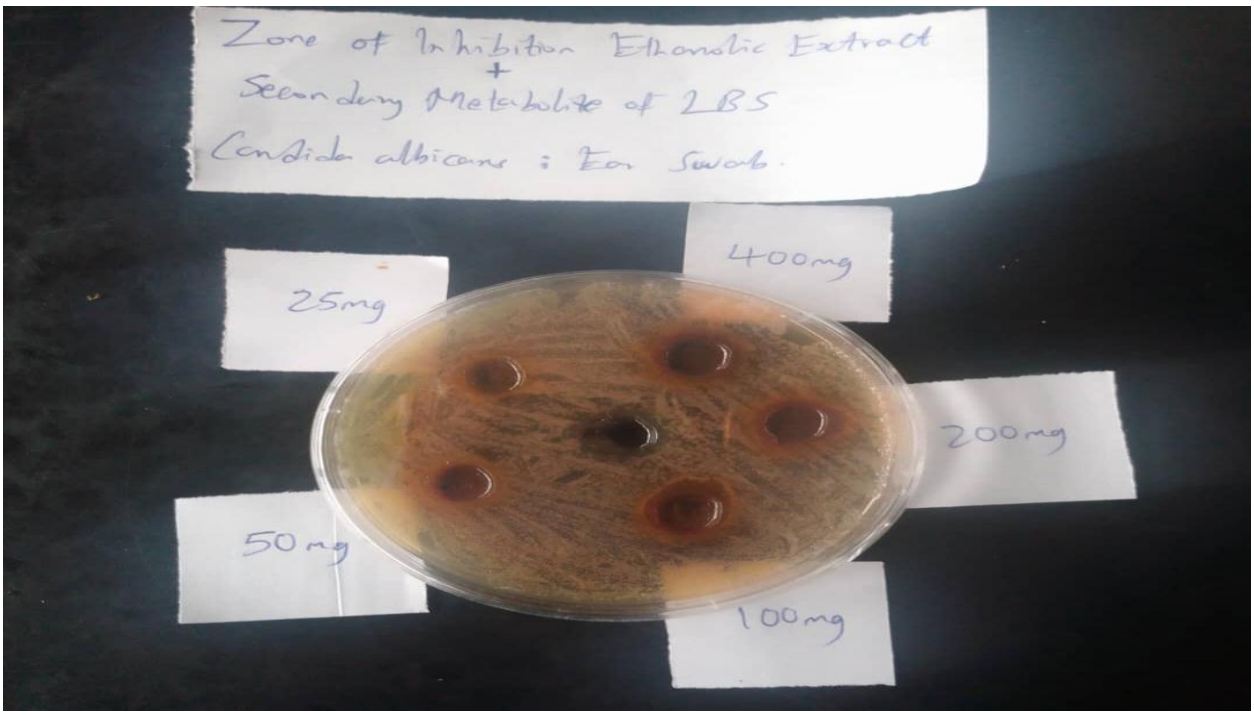


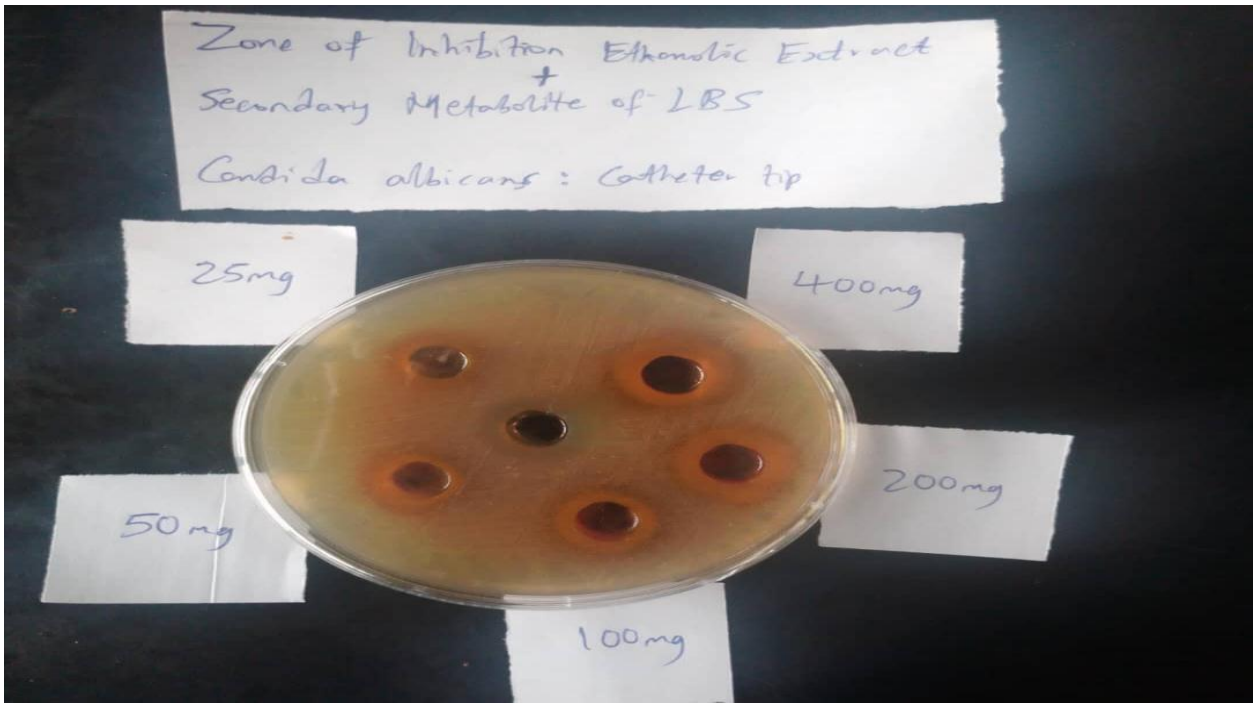



Zone of Inhibition
Ethanolic Extract
Urine
400
200mg
25mg
100mg
50mg
TECNO
AI CAMERA

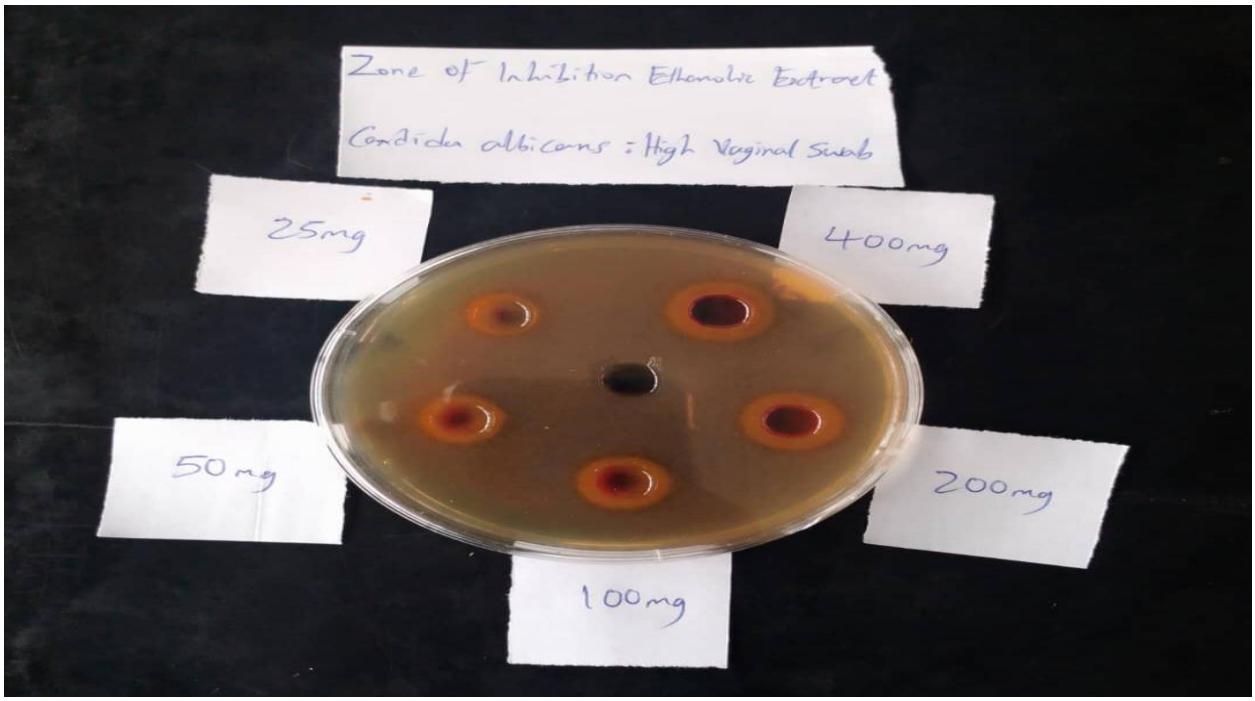

Zone of Inhibition Ethanolic Extract
+
Secondary Metabolite of LBS
Candida albicans : Wound Swab
25mg
400mg
50mg
200mg
100mg

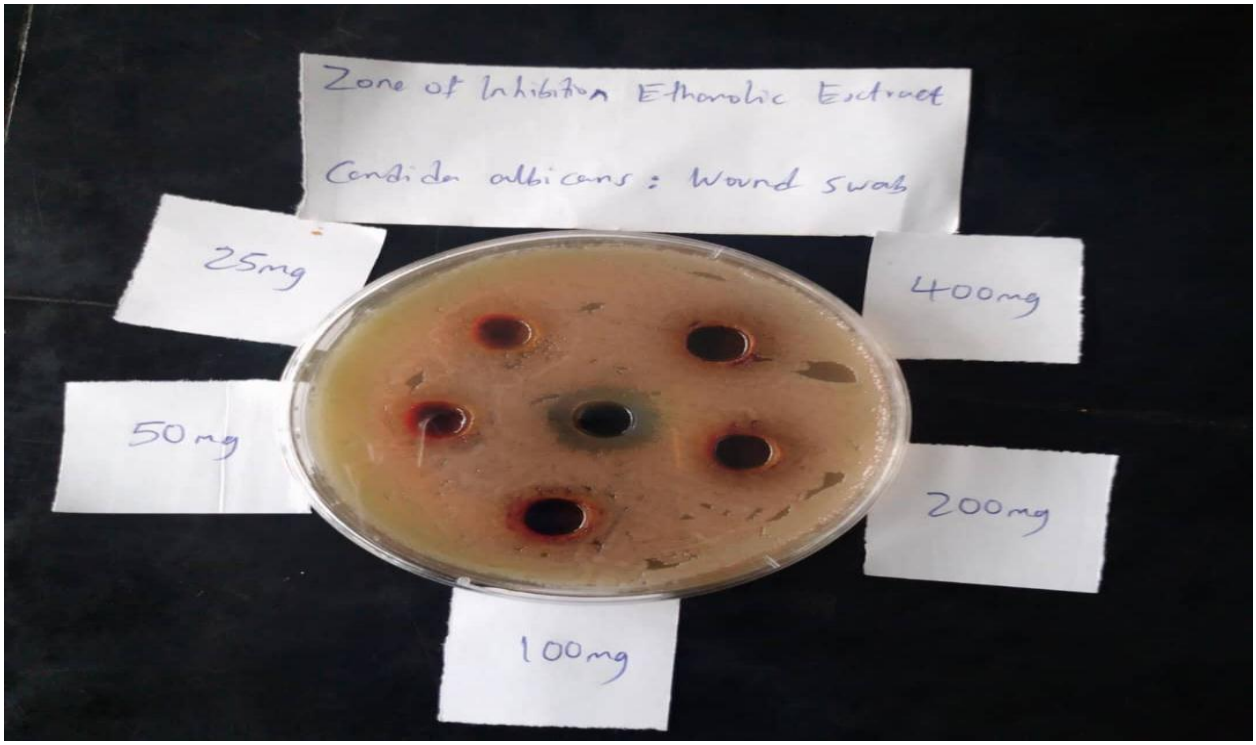

Zone of Inhibition: Ethanolic Extract
+
Secondary Metabolite of LBS
Candida albicans : High Vaginal Swab
400mg
25mg
200mg
50mg
100mg


Zone of Inhibition Ethanolic Extract
+
Secondary Metabolite of LBS
Candida albicans : Ear Swab.
400mg
25mg
200mg
50mg
100mg


Zone of Inhibition Ethanolic Extract
+
Secondary Metabolite of LBS
Candida albicans : Catheter tip
25mg
400mg
200mg
50mg
100mg


Zone of Inhibition
Ethanolic Extract + Lactobacillus
Urine
200mg
100mg
25mg
50mg
TECNO
AI CAMERA


Zone of Inhibition Ethanolic Extract
Candida albicans : High Vaginal Swab
25mg
400mg
50mg
200mg
100mg


Zone of Inhibition Ethanolic Extract
Candida albicans : Wound swab
25mg
400mg
50mg
200mg
100mg