REVIEW PAPER

Design, Optimization and Evaluation of a Liposomal lipid- wax Bioadhesive Nanopatch of Thymol for Sustained Topical Management of Dermatophyte and Candida Infections

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Abstract

Superficial fungal infection caused by dermatophytes and Candida albicans remain a substantial world wide issue. Typical treatments require lengthy treatment regimens and conditions such as insufficient drug retention on the skin, frequent application of treatment, and the development of drug resistance associated with most current topical antifungal therapies. This study is investigating the development, optimisation, and performance evaluation of a thymol-loaded liposome-formed lipid-wax bioadhesive nanopatch for sustained topical antifungal therapy. Due to a number of factors, including low solubility in water, volatility, and low levels of retention within human epidermal tissues, there really is no current formulation for this agent that meets these requirements. This means that there is great potential for developing new systems to increase the stability, depth of penetration and efficacy of this agent.

Thymol-loaded liposomes were produced using thin-film hydration method, and optimised by entrapment efficiency, vesicle size and polydispersity index. The finalised liposomes were incorporated into biocompatible lipid and wax materials to form a nanopatch with lipid-wax properties. Bioadhesive materials were incorporated into the formulation as a means of prolonging their adherence to the treatment area. The formulation was then evaluated for surface morphology using SEM/TEM and mechanical strength and adhesive forces were assessed. The pharmacodynamics of the therapeutic agent were evaluated by in vitro with drug release experiment and ex vivo through skin permeation studies with Franz diffusion cells; antifungal activity was evaluated using Candida albicans.

The created nanopatch has been shown to benefit from longer release of active drugs, greater penetration into the skin, and greater efficacy against fungal infections than typical formulations using these types of delivery systems. This dual delivery format, combining encapsulation of drugs in liposomes with use of a bioadhesive matrix formed with lipids and waxes, will provide longer retention of drug at the infection site, allowing for better control of when the drug will be released. In summary, the current study provides evidence of a plant-derived topical (non-systemic) system, designed to be more effective and safer for treating topical fungal infection, improving patient adherence and outcome.

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1. Introduction

1.1 Disease

1.1.1 Overview and Pathogen Characteristics

Candida albicans, also known as C. albicans, is a dimorphic commensal organism found naturally on the skin, mucous membranes, intestines, and vagina of healthy humans (S. Kashem et al., 2016). Candida albicans is polymorphic, it has two distinct morphologies under normal circumstances of health in healthy individuals; while it has the ability to be an opportunity pathogen that can infect people with infections ranging in severity from superficial skin infections to systemic infections, between both kinds of infections (e.g., yeast vs. hypha) (François L. Meyer et al., 2013). C. albicans has unique characteristics that include phenotypic switching, which allows C. albicans to be resistant to human immune systems, and can also be both yeast form and hyphal form (O. Sadik et al., 2018).

a) Cutaneous Candidiasis: Clinical Manifestations

Candidiasis of the skin is a major type of superficial candida infection of the skin, primarily found in areas with limited air exposure combined with high-humidity conditions. The infection usually occurs in areas where there are skin folds, such as between the legs, in the armpits, or in other areas of the body where the temperature and humidity are conducive to the growth of the fungus (N. A. D. AL-Garawi et al., 2022).

b) Epidemiology and Risk factor

C. albicans is the most common causes of skin and oral infections in both HIV positive individuals and other immunocompromised populations, with HIV and other immunocompromised individuals having substantially higher rate of Candida infection than the general population (O. Sadik, et al. 2018). Moreover, patients whose immune systems are depressed from chemotherapy, immunosuppressive medicines or dysbiosis due to antibiotic therapy can have difficulty treating their C. albicans infection (Hui Lu, et.Al, 2023). It is estimated that about 20%-25% of the population worldwide will have a fungal skin infection with most fungal infections caused by C. albicans (N. A. D. Al-Garawi et.al, 2022).

c) Adhesion and Colonization

Through multiple types of adhesins to tissues on the skin surface, Candida albicans have a high affinity for the skin surface, so they can attach. The ALS (Agglutinin-Like Sequences) family of adhesins, especially Als3, have important roles in adhesion/invasion processes. The fungus can strongly adhere (to keratinocytes and other structural elements) on the skin or keratinocytes and initiate its colonization and then biofilm production (N.A.D. Al-Garawi et al., 2022).

d) Morphological Transition

The ability to switch from a yeast (unicellular) to hyphae (multicellular) is an important virulence factor for the organism. Certain conditions (temperature > 35 °C, presence of serum, environmental pH, availability of nutrients) trigger the conversion from yeast to hyphal form (Al Garavi et al., 2022).

e) Enzymatic Virulence Factors

C. albicans utilises different types of hydrolytic enzymes to penetrate and access food resources from host tissue, with the most prevalent enzymes of this type produced by C. albicans consisting of secreted aspartyl proteases, phospholipases, and lipases. Each of these enzymes serves to disrupt host cell membrane integrity and break down host tissues. C. albicans can also secrete a damaging pore-forming toxin, tyrosinases, that causes direct mechanical destruction of host cell membranes. (N. A. D. Al-Garawi et al., 2022).

f) Host Immune Response to Cutaneous Candidiasis

C. albicans infections may be fought off by the skin immune system due to its highly developed methods of defence. The cutaneous immune response contains both parts of the immune system (innate and adaptive) with a focus on the role of IL-17 in mediating the responses (S. Kashem et al., 2016).

1.2 Spread: National and International

Superficial candidiasis, which is caused by Candida albicans, is a major part of the burden caused by fungal infections globally (G. Garber et al., 2012). Candida has been shown to be one of the most common pathogens found in clinical practice.

1.2.1 Global Context of Superficial Candidiasis

Candidiasis is a type of fungus that can infect many parts of your body. Infections occur most commonly in the vagina, or on the skin or in your mouth. Candidiasis is one type of superficial mycosis, or fungus, which when grouped together, affect millions of people around the world (Noor Ul Islam et al., 2025) (G. Garber et al., 2012). Superficial mycoses are located on the skin, keratinous structures, or mucous membranes. Therefore, candidiasis is one of the most commonly diagnosed dermatological conditions in the world.

1.2.2 Epidemiological Patterns in Tropical Regions

The amount of data that is India-based and specifically implicates Candida albicans is limited, however, there is available epidemiological evidence regarding superficial fungal infections occurring in tropical and sub-tropical areas of the world that provide relevant context. Superficial mycoses (superficial fungal infections) including candidiasis, are very common in many parts of the world; however, they are particularly prevalent in the tropical & sub-tropical regions (B Sharma, et al, 2021).

There are several environmental conditions in both tropical and sub-tropical climate areas that promote the growth and spread of fungi, including: warm humid climates; and, in many tropical and subtropical countries, there is poor sanitation infrastructure (B. Sharma, et al, 2021). Therefore, the significant population density and climatic conditions in countries like India may also lead to higher rates of transmission; thus, resulting in a higher prevalence of superficial fungal infections throughout the country.

Consequently, given that there is an increasing prevalence of superficial mycoses globally, the trends associated with the prevalence of these infections are becoming an increasing public health concern globally and in India (B Sharma, et al, 2021).

1.2.3 Risk Factors and Predisposing Conditions

There are many factors that affect how superficial candidiasis develops and spreads. Environmental conditions play a major part in the development and growth of Candida; for example, in warm, humid climates, Candida can grow and live better (B. Sharma et al, 2021). Poor hygiene, crowded populations, and insufficient sanitation also increase the chance of spreading and establishing an infection.

Factors related to the host play a major role in determining how susceptible one is to infection from Candida. Both primary and acquired immunodeficiency conditions significantly raise the chances that an individual will develop candidiasis and have complications from it (Katarzyna Rychlik et al., 2025). Whatever the type of immunodeficiency, diabetes mellitus is a significant contributor to growth of candidiasis because high glucose levels found in diabetic tissues and abnormal immune function coincide with an increase in the proliferation of Candida.

Socio-economic status (access to health care, hygiene, and type of living conditions), is also a major factor in the development and severity of surface candidiasis (Katarzyna Rychlik et al., 2025). This becomes an even greater factor in developing countries, where implementing preventative measures will be more difficult than in developed nations.

1.2.4 Clinical Manifestations and Impact

Even though superficial candida infections usually do not cause death or serious illness, they can greatly impact the quality of life for an infected person (G. Garber, et al.2012); the ongoing nature of many candida infections, frequent reoccurrence, symptoms of itching, burning, discomfort, affect the ability to perform daily activities and mental well-being.

1.2.5 Public Health Implications

Superficial fungi infections represent an increasing public health challenge throughout the world (Noor Ul Islam et al., 2025). They pose a significant threat to global public health that needs coordinated global prevention, diagnosis and treatment strategies.

Superficial candidiasis has both direct and indirect economic impacts: direct is the cost of diagnosis and treatment of superficial candidiasis; indirect costs relate to lost productivity and diminished quality of life associated with these infections. In resource-poor countries, these infections will likely disproportionately impact people at risk because they do not have adequate access to appropriate healthcare services.

1.3 Fatality

No specific fatality data for skin infections caused by Candida albicans can be found in the literature, although there is a substantial amount of literature documenting the high rates of mortality for invasive candidiasis occurring in immunosuppressed persons.

Invasive fungal infections, particularly those caused by Candida albicans, are associated with high mortality rates, reaching up to 60% in severely immunocompromised patients (Hamied et al., 2021; Parambath et al., 2024). As far as localized skin infections are concerned, reported an 11.4% prevalence C. albicans infection among type 2 diabetic patients who had a diagnosis of diabetes, but did not present fatality data (Shna Rasoulpoor et al., published in 2021). There is little information about mortality rates associated with localized infections caused by cutaneous candidiasis. Most of the research thus far has concentrated on the mechanisms by which cutaneous candidiasis (the disease) develops and the accompanying immune response following the initial infections (Kashem et al., 2016; Lu et al., 2023)"

1.3.1 Mortality Rates for Invasion Candidiasis

When discussing mortality statistics, the greatest concern was found to be associated with cases of invasive candidiasis rather than those that presented as superficial skin infections.The morbidity associated with invasive fungal diseases is significant and the mortality rate in patients with severely immunocompromised hosts can exceed 60% (A. Hamied et al., 2021). There is a large amount of evidence regarding high mortality rates from the invasive infectivity of Candida albicans (Parambath et al., 2024). Both invasive and superficial infections can contribute significantly to the mortality rates among those with compromised immune systems and support the need to differentiate between systemic infections and localized infections (Staniszewska et al., 2014).

1.3.2 Pathogenesis and Virulence Factors

There are a number of virulence factors that influence how serious these infections become. There are a variety of ways that C. albicans will cause "infection ranging from superficial to systemic threatening", based on the pathogenic mechanisms involved: adhesion, invasion, hydrolases secreted into the host by C. albicans, yeast-to-hyphal transition, therefore forming biofilms. (François L. Mayer et al., 2013)

when there are "dysbiosis of bacteria due to antibiotics, immunosuppression caused by medically prescribed drugs, and/or procedures that compromise the integrity of the mucocutaneous barrier", C. albicans can transition from a being a commensal organism to becoming a pathogenic organism, therefore producing debilitating mucocutaneous disease and/or life-threatening systemic infections. (Lopes et al., 2021)

1.3.3 Immune response and Host Defence

C. albicans infections are predominantly opportunistic fungal pathogens leading to disease manifestations including both disseminated candidiasis and chronic mucocutaneous candidiasis (CMC). They also indicate that the immune system responds differently depending on where the infection is occurring because immune responses are compartmentalized by their site of infection; therefore, skin infections tend to result in different outcomes than systemic infections (S. Kashem et al.2016)

1.3.4 Clinical Implications

C. albicans is capable of causing several types of infections on mucosal and skin surfaces, as well as deep inside the body. However, it presents the most risk to patients who suffer from advanced stages of HIV or who have weakened immune systems due to chemotherapy, other medicines that suppress the immune system, or antibiotics which disturb/reduce the amount of normal "healthy" bacteria in the body. (Hui Lu et al. 2023)

1.4 Resistance

1.4.1 Epidemiological Context

Candida albicans is a natural inhabitant of the human body and is typically found on skin and mucous membranes of the mouth (oral cavity), intestines (digestive tract), and vagina (also referred to as the urogenital tract, as well as internally) and maintains a balanced relationship with the immune system of its host (Hui Lu et al., 2023). The delicate balance that exists between C. albicans and the immune system may be lost in individuals who are immune-compromised, resulting in a variety of manifestations, including cutaneous candidiasis; this can be a serious clinical challenge because of the current emergence of antifungal resistance patterns. The clinical spectrum of candidiasis includes various forms of disease such as mucosal candidiasis (oral or vaginal), oropharyngeal (oral) candidiasis (also referred to as thrush), vulvovaginal (vaginal) candidiasis, cutaneous candidiasis, (skin), invasive candidiasis (throughout the body), disseminated candidiasis (throughout the body), and candidemia (presence of C. albicans in the bloodstream) (M. GuruprasadB et al., 2020). Cutaneous candidiasis is a growing concern among the at-risk population; people with HIV/AIDS, patients receiving chemotherapy (cancer treatment), or treatment with immunosuppressive drugs, and patients that experience antibiotic-induced dysbiosis (antibiotics disrupt the normal balance of bacteria in the gut and lead to an overabundance of Candida species, including C. albicans), are all at risk for cutaneous candidiasis (Hui Lu et al., 2023).

Epidemiological data shows that C. albicans is the most prevalent Candida species (37% of clinically diagnosed C. albicans species) and is the most commonly isolated organism in the Candida genus (Somanon Bhattacharya et al., 2020). The pathogen is adaptable, and strains of C. albicans have different morphological characteristics and abilities to form biofilms based on where the organisms were obtained, such as from skin versus from the vagina (O. Sadik et al., 2018). This adaptability contributes significantly to the development of antifungal resistance, particularly in topical skin infections where local drug concentrations and tissue penetration present unique challenges.

1.4.2 Fundamental Resistance Mechanism in Cutaneous Candidiasis

1.4.2.1 Biofilm Formation and Architecture

Biofilm formation is one of the major virulence factors that play a role in antifungal resistance in C. albicans infections (R. Pereira et al., 2020 & J. Chandra et al., 2001) The process of developing a biofilm can be broken down into four separate and distinct temporal phases, all contributing to the overall resistance phenotype. (J. Chandra et al., 2001)

The biofilm development process creates a type of complex architecture; additionally, the biofilm's extracellular matrix composition serves as a formidable barrier to antimicrobial penetration. (J. Chandra et al., 2001; H. Taff et al., 2013; D. Sandai et al., 2016)

The biofilm matrix has multiple roles in the development of resistance. The matrix serves as a physical barrier and thus limits the movement and diffusion of drugs into the deepest layers of the biofilm which contain metabolically active C. albicans cells. (H. Taff et al., 2013 & E. Borghi et al., 2016) In addition to serving as a physical barrier, the matrix is composed of extracellular DNA, proteins, and polysaccharides, which serve to bind antifungal agents, effectively sequestering the antifungal and decreasing its bioavailability. (H. Taff et al., 2013) Sequestration of antifungal agents is particularly troublesome for topical application since drug concentrations can be limited by skin penetration barriers from the beginning.

Within the biofilm structure, C. albicans cells display increased levels of efflux pump activity and alter their metabolic states as a result. (P. Mukherjee et al., 2003 ; H. Taff et al., 2013) The metabolic plasticity of the biofilm associated C. albicans cells allows these cells to adapt to nutrient-limited conditions as well as respond to oxidative stress; both of which are commonly present in skin infection sites. This metabolic plasticity allows cells to survive antifungal treatment while serving as reservoirs for future C. albicans infections. (D. Sandai et al., 2016)

1.4.2.2 Morphological Plasticity and Pathogenesis

C. albicans's ability to switch between yeast and hyphal forms is an important virulence factor associated with the onset of multi-drug resistance (MDR) to treatment. (P. Chong et al., 2018 & R. Calderone & W. Fonzi, 2001) This phenotype is more than just a morphological change; it is also accompanied by significant changes in how the organism expresses genes, metabolizes nutrients, and responds to anti-fungal drugs. (R. Calderone & W. Fonzi, 2001)

The switching between yeast and hyphal forms is controlled by complex biological processes that respond to different environmental conditions, such as temperature, pH level, nutrients, or host factors. For example, the environment surrounding the skin can provide specific information that can predispose to change from yeast to hyphal and result in increased virulence and antifungal resistance. Furthermore, when in the hyphal form, there is an increased capacity for invading surrounding tissues and an altered susceptibility to anti-fungal medications compared to when in the yeast form. (R. Calderone & W. Fonzi, 2001)

Recent research has also identified specific genes that control this transition from yeast to hyphal form and, therefore, provide potential therapeutic targets. By understanding these genetic switches and their decision points in this transition from yeast to hyphae, healthcare providers can use this information to develop educational programs to help prevent the development of resistance in the treatment of dermatophytic infections, especially when the environment encourages the development of fungi in their different forms. (P. Chong et al., 2018).

1.4.2.3 Molecular Mechanisms of Azole Resistance

a) Efflux Pump Systems

The azole antifungal category of drugs is widely used for all kinds of multiple Candida species infections, including use as topical agents in acute cutaneous candidal infections (D. Sanglard et al;, 2017). However, multiple mechanisms of resistance towards these antifungal agents have developed over time, the predominant mechanism being the emergence of sophisticated efflux pump systems to pump the azole out of the cell.

C. albicans has 2 families of efflux pump systems, including ATP-binding cassette (ABC) and major facilitator superfamily (MFS) transporters. The ABC transporters are CDR1 and CDR2, which are regulated by the transcription factor TAC1. If gain-of-function mutations occur in the TAC1 gene, including T225A, V736A, N972D, N977D, G960E or G980W, this leads to the permanent overexpression of the ABC efflux transporters (Somanon Bhattacharya et al,. 2020)

Furthermore, MFS efflux transporter MDR1 is regulated by a transcription factor called MRR1, with gain-of-function mutations such as P6835 and P683H allowing overexpression of the MDR1 efflux transporter and azole drug resistance. All of these gain-of-function mutations have been documented within clinical isolates from a wide variety of infection types, including skin infections, demonstrating clinical significance. (Somanon Bhattacharya et al;, 2020),

In a study of matched clinical isolates of C. albicans from patients who had received azole treatment, there was a consistently increased expression of the CDR1, CDR2 and MDR1 genes in resistant isolates. This is direct evidence of the clinical relevance of efflux pump mediated drug resistance as it relates to real life treatment. (Somanon Bhattacharya et al., 2020)

b) Target Modification and Overexpression

The azole antifungal class of drugs targets the enzyme that converts 14 α-demethylase into ergosterol. The ERG11 gene encodes the enzyme 14α-demethylase, which is the main target of azole antifungal drugs (Sanglard et al., 2017). The mechanism through which resistance to azoles can occur is through two main pathways relative to the 14α-demethylase enzyme: structural modifications in the enzyme causing a resistance phenotype and quantitative changes in the amount of 14α-demethylase produced.

In total there have been numerous studies performed characterizing the point mutations that occur in the ERG11 gene, with 63 patient isolates resistant to fluconazole, 55 of which had at least 1 mutation that resulted in having a different amino acid than the non-mutated parent strain. (Sanglard et al, 2017) Studies utilizing molecular modelling approaches demonstrate the majority of the ERG11 point mutations causing azole resistance cluster in 3 areas of the 14α-demethylase complex: (1) the predicted active site of the enzyme, (2) the exclusively fungal external loop of the enzyme, and (3) the proximal surface of 14α-demethylase.

Another mechanism of resistance to azole antifungal agents through the overexpression of the ERG11 gene, is due to the presence of activating mutations in the transcriptional regulator UPC2 (Sanglard et al, 2017). 47 out of 63 fluconazole resistant patient isolates had more than a twofold overexpression of the ERG11 gene, with 29 being identified as having missense mutations within the UPC2 gene. During the study a total of 8 different amino acid substitutions within UPC2 were identified, where 7 of these substitutions were associated with increased ERG11 expression; increased ergosterol levels; and decreased susceptibility to fluconazole.

c) Ergosterol Biosynthesis Pathway Alterations

The ergosterol biosynthesis pathway can develop resistance through mechanisms other than ERG11 polymorphisms. The C5 sterol desaturase enzyme (encoded by ERG3), plays an important role in the production of toxic sterol intermediates during the synthesis of ergosterol. As such, if azole antifungals inhibit ERG11, then ERG3 and ERG6 typically create toxic sterol products that help form the fungicidal activity of azoles. If ERG3 is disrupted, that prevents the formation of toxic sterols, leading to an increased resistance to azole antifungals. (Bhattacharya et al. 2020)

Similarly, ERG6, the A24 sterol C-methyl transferase, is another mechanism by which azole resistance can occur (Bhattacharya et al., 2020). In C. albicans, a heterozygous deletion of ERG6 creates significant azole resistance, suggesting that a loss of function of ERG6 is responsible for preventing the formation of toxic sterols, rather than by affecting the activity of an efflux pump.

An emerging area of resistance research is the mechanisms of sterol importation (Bhattacharya et al., 2020). While most other Candida species only import sterols when they are in the absence of oxygen C. albicans is capable of importing sterols in both the presence and absence of oxygen, potentially allowing for the compensation of decreased ergosterol synthesis due to azole treatment. This sterol importing mechanism is particularly relevant for cutaneous infections, as availability of serum and cholesterol may provide an avenue for sterol importation.

1.4.2.4 Genomic Plasticity and Chromosomal Alterations

a) Aneuploidy and gene Dosage Effects

Genomic variations (such as loss of heterozygosity (LOH) or aneuploidy) represent advanced resistance mechanisms capable of quickly changing the susceptibility of these organisms to drug treatments. Chromosomal alterations leading to LOH or aneuploidy may both increase the number of copies of resistance genes and/or eliminate the presence of susceptibility alleles. These changes provide the organism with a significant selective advantage when exposed to drugs. (Somanon Bhattacharya et al. in 2020)

Examples of clinically significant aneuploidies associated with increasing the amount of drug resistance include the trisomy of chromosome 5, which contains two important genes (ERG11 and TAC1) involved in azole resistance. Because both of these genes exist in duplicate on the chromosome, they are each expressed at twice their normal rate, thereby resulting in increased resistance to azole drugs (like fluconazole).

Additionally, the trisomy of chromosome 3, which harbors the genes for two efflux pumps (CDR1 and CDR2), would result in increased expression of both efflux pump genes, ultimately leading to increased resistance to azole drugs.

Changes to chromosome 4 have also been shown to confer azole resistance; however, the underlying cause of these changes is not yet completely understood. The presence of either trisomy or monosomy of chromosome 4 has been shown to increase the level of resistance to azole drugs, which suggests that there may be many genes interacting with each other to confer such resistance, and these interactions will need to be evaluated in more detail.

b) Loss of Heterozygosity Events

Heterozygous resistant mutations may quickly change to homozygous mutations as a result of LOH events; this greatly increases resistance levels (Somanon Bhattacharya et al., 2020). LOH has been detected in clinical isolates also present at TAC1, ERG11 and MRR1 loci, which correlate with phenotypic resistance increase. These events may arise as spontaneous events or via selection under drug pressure; therefore, LOH represents a rapid mechanism for adaptation.

1.4.3 Resistance to Alternative Antifungal Classes Polyene Resistance mechanisms

Although azoles are the primary concern with regard to resistance to topical antifungals, there is also the possibility of resistance to polyenes, which have some mechanistic similarities (Somanon Bhattacharya et al., 2020). Polyenes, such as amphotericin B and nystatin, act on ergosterol in the plasma membrane by forming pores causing cellular death.

1.5 Chronic and Severe Infection State

1.5.1 Nature of Severe cutaneous Candidiasis

Superficial cutaneous infections, severe infective cutaneous infections with a mortality rate of 30-60%, and extremely remote cutaneous infection with a mortality rate of 30-40% have been associated with C. albicans in extreme circumstances.

1.5.2 Pathogenesis and Virulence Mechanisms

Several virulence factors associated with severe cutaneous candidiasis allow for invasion of tissue and evasion of the immune system (François L. Mayer et al., 2013)

1.5.3 Pathogenic Mechanisms

a) Adhesion and Invasion: C. albicans has been observed to invade host tissues via two different mechanisms. Endocytosis (induced by C. albicans) & active penetration (A. Hamied et al., 2021)

b) Morphological Switching: Transition from yeast form to hyphal form, where filamentous form is more invasive & more capable of penetrating host tissue (François L. Mayer et al., 2013)

c) Hydrolytic Enzyme Secretion: The secretion of extracellular enzymes by C. albicans causes degradation of proteins contained in the host, including hemoglobin and keratin, facilitating invasion of host tissues (A. Hamied et al., 2021).

d) Biofilm Formation: Biofilm structures formed by C. albicans provide an organized structure which is resistant to antimicrobial treatment (O. Sadik et al. 2018)

Serve Complications

- CMC can be seen often among patients whose immune systems do not function properly (Kashem, 2016).
- When a patient has disseminated candidiasis, fungus cells enter through their skin (Lu, 2023).
- For patients suffering from candidiasis, the presence of pain and/or sensitivity to touch (allodynia) is most severe in patients with vulvovaginal infections (Kashem, 2016).

1.5.4 High Risk Population and Prevalence

a) Prevalence Data:

- Meta-analysis of 13 studies with - a total of 1,348 Type 2 Diabetic Patients (Shna Rasoulpoor and al., 2021) showed the prevalence rate to be 11-4%.
- The incidence in HIV patients was found to be significantly higher than in other groups (O. Sadik et al. 2018).

b) Severe Infection Risk Factors:

- Immunosuppression & Immunocompromised States
- Diabetes Mellitus (Major Metabolic Risk Factor)
- HIV/AIDS
- Chemotherapy & Immunosuppression Treatments
- Dysbiosis due to Antibiotics
- Long Term Catheterization & Invasive Medical Procedures. (M. Staniszewski et al. 2014 and others)

1.6 Extreme Conditions and Manifestations of Tropical Candida albicans Infections

1.6.1 Theoretical Framework of Extreme Predisposing Conditions

Diabetic Mellitus is another extreme condition that creates an environment conducive for severe cutaneous candidiasis to develop and thrive. This model indicates that diabetic patients have multiple predisposing factors to this infection: hyperglycemia is a nutrient-rich source for fungal growth, the acidic environment in which C. albicans thrives is created due to diabetic ketoacidosis, impaired neutrophil function provides diminished cellular defenses against fungal infections, and diabetic microangiopathy results in compromised tissue perfusion and delayed healing (Sakina Shahabudin et al, 2024), as shown by the epidemiological data that shows an 11.4% prevalence rate of C. albicans skin infections in patients with type II Diabetes, representing a significantly increased risk of infection than the general population (Shna Rasoulpoor et al, 2021).

1.6.2 Pathophysiological Mechanisms in Extreme Conditions

High temperatures, altered pH levels, increased levels of carbon dioxide and amino acids are all examples of environmental factors which can quickly alter growth patterns and result in increased rates of filamentation (R. Calderone & W. Fonzi, 2001). Furthermore, all of these conditions cause significant morphological changes from yeast to hyphal form, along with changes to the cellular model, (i.e. cellular metabolism and gene expression), which provide the organism greater ability to adhere to host tissues, produce hydrolase enzymes for further adherence to host tissues, and penetrate epithelial barriers. (R. Calderone & W. Fonzi, 2001 & P. Chong et al., 2018)

The theoretical model predicts that when C. albicans is forced into extreme conditions, it will utilize both induced endocytosis and penetration mechanisms at the same time, therefore providing it with different strategies for invading host tissues. Induced endocytosis is a tactic used by C. albicans; it uses the signalling pathways of the host organism to cause the internalization of C. albicans cells into the cells of the host organism, and the penetration mechanism depends on C. albicans producing hydrolytic enzymes (e.g. proteases, phospholipases and hyaluronidases) which will degrade specific components of host tissue and the ECM. (R. Calderone & W. Fonzi, 2001).

1.6.3 Chronic Mucocutaneous Candidiasis: The Paradigm of Extreme Disease

Chronic mucocutaneous candidiasis (CMC) is believed to be the most severe level of infection caused by topical C. albicans; it typically occurs in individuals whose IL-17 signaling pathways have genetic defects or who have severe immunodeficiency due to other means (S. Kashem et al., 2016). CMC therefore allows us to understand how C. albicans can cause prolonged, non-treatable infections at multiple anatomical sites when normal immune control mechanisms are entirely absent.

1.6.4 Systemic Effects and Complications

The theoretical framework for extreme candidiasis includes the possibility of progression from localized cutaneous infection to systemic disease with potentially life-threatening complications. The theoretical model predicts that C. albicans may breach epithelial barriers and enter the bloodstream under extreme conditions, leading to disseminated candidiasis with mortality rates of approximately 40-60% (Hur Lu et al., 2023; and an additional source).

There is also evidence that the systemic effects of extreme topical candidiasis are the result of direct fungal invasion as well as an intense systemic inflammatory concomitant of cutaneous infection. Previous research supports the theory that the severe inflammatory response caused by severe cutaneous infection triggers the release of pro-inflammatory cytokines and the activation of the complement and coagulation systems, ultimately causing sepsis syndrome and multiorgan failure.

1.7 Neuroimmune Interactions in Extreme Disease

Recent progress in the understanding of severe topical candidiasis indicates that the interactions of the immune system with the nervous system are important in relation to the development and pathogenesis of this disease S. Kashem et al., 2016. The theoretical model indicates that C. albicans may directly activate sensory neurons via pattern recognition receptors and by-products of metabolism producing pain and neuropeptides, which will either affect local immune responses or lead to systemic inflammatory cascades. Furthermore, the theory of neuroimmune interaction provides an explanation for the fact that individuals suffering from severe cutaneous candidiasis may experience disproportionate levels of pain and inflammation when compared with the actual extent of the tissue involved. Furthermore, it is proposed that normal links between immunological and neurological systems may become disrupted in patients with extreme forms of topical candidiasis when there is a release of neuropeptides leading to amplification of local immune responses and conditions that support the continued growth of fungi.

1.8 Therapeutic Implications and Resistance Mechanisms

A detailed theoretical understanding of extreme candidiasis has far-reaching implications for treatment options and reveals why traditional antifungal treatments may be insufficient in very advanced cases (Hui Lu et al., 2023). Theoretical modeling indicates that treatment failure is not due to straightforward antifungal resistance; rather, it results from complex interactions among biofilm development, immune suppression/dysfunction, changes in tissue pharmacokinetics, and the organism's ability to rapidly adapt to therapeutic pressures.

1.9 Need for Treatment

The main types of antifungal medications for candidiasis (candidiasis) have a cure rate of 73-100%. (E.Taudorf et al., 2019) Medications that are primarily used for treating fungal infections are clotrimazole (also called clotrimazole), nystatin (oral solution), and miconazole (oral gel). All three medications have few side effects; however, use them alone as well as in combination with antibacterial medications or corticosteroids will not affect their efficacy. (H.Lu et al., 2023)

Within the treatment regimen, treating patients who are susceptible to developing candidiasis is essential because candidiasis can lead to serious complications in these individuals. In immunocompromised patient populations, such as persons with HIV/AIDS or who are receiving chemotherapy, candidiasis can progress into severe and, in some cases, life-threatening conditions, resulting in high mortality rates (M.Staniszewska et al., 2014) C. albicans skin infections need to be treated due to the complex pathogenicity mechanisms that this organism employs. For example, C. albicans has a wide array of virulence features, such as “adhesion to and invasion of host cells, secretion of hydrolases, transitioning from yeast to hyphae, tactile response and growth along surfaces, forming biofilms, phenotypic switching and many other fitness characteristics” (François L. Mayer et al., 2013). Therefore, this organism is capable of causing persistent disease or infections that may live on and continue to reproduce despite being under attack by the host’s immune system.

1.9.1 Clinical consequences and disease progression

It is important to treat cutaneous candidiasis in order to prevent potentially serious outcomes from untreated infections. Cutaneous candidiasis is due to the fungal organism Candida albicans and can result in a broad and diverse array of infections, ranging from mild cutaneous irritations to severe, invasive systemic disease, especially in individuals with some degree of immune system compromise (i.e., those infected with HIV/AIDS, those undergoing active chemotherapy, etc.). (H.Lu et al., 2023 & M. Staniszewska et al. 2014)

Untreated cutaneous candidiasis can progress from superficial cutaneous involvement to invasive life-threatening infections, resulting in a high possible rate of mortality (>50% in some studies) with delay in treatment. In fact, whereas the vast majority of patients can be treated with topical antifungal drugs (i.e., clotrimazole, miconazole, etc.), if a patient does not receive early management, he/she may develop more severe forms of cutaneous candidiasis which will ultimately be more challenging to manage. (C. Spampinato et al. 2013)

Thus, there is great importance in obtaining early and appropriate management of cutaneous candidiasis in order to prevent advancement of the infection into more serious forms.

1.9.2 Vulnerable Groups and Their Risk Factors

Vulnerable patient populations require special attention to their treatment. A recent study explores how diabetes mellitus, a metabolic disorder, has been shown to cause immunosuppression, which allows for Candida colonization and subsequent skin infections. They further explain, "diabetes is a significant risk factor for the disease process of oral and esophageal candidiasis and other forms of cutaneous candidiasis, including vulvovaginal candidiasis, Candida balanitis, and nail, hand, and foot infections." (Sakina Shahabudin et al. 2024)

Individuals who are at high risk for infection may exhibit an increased susceptibility to many types of infections. There are many possible risk factors associated with an increased chance of contracting an infection. Some of these include immunosuppressive or corticosteroid drugs, long-term catheter use, invasive medical procedures, broad-spectrum antibiotics, deep skin burns, local GI tract disorders, diabetes mellitus, premature infants with very low birth weights, immunologically compromised persons, and individuals with HIV (M. Staniszewska et al.,2014), All of these risk factors make it critical for an individual to receive treatment to prevent complications within 24-48 hours of developing symptoms.

1.9.3 Immune System Considerations

The immune response developed against C.albicans adds further logic for the required treatment of this organism's infections. C. albicans is a dimorphic commensal fungus that colonizes the skin, mucosa, and reproductive tract of healthy humans; however, this organism may pose a threat when the host’s immune defenses are compromised. Their research found that "the immune response to infection varies based on the location of the infection" suggesting that different approaches to the therapy of cutaneous infections are required. (S.Kashem et al., 2016)

Candida yeast normally resides within the human body and the human immune system keeps Candida yeast from growing. If the body's immune system fails, Candida will colonize and eventually lead to an infection. Thus, when the human immune system fails to provide an adequate immunological balance, treatment is required for restoration of the balance. (Selva Prasanthi Parameshwaran et al., 2022)

1.9.4 Resistance and Treatment Challenges

Additionally, the occurrence of antifungal resistance creates urgency for proper treatment choices. There have been increasing observed rates of resistance to antifungal drugs currently in use for the management of fungal infections. (M.Staniszewska et al., 2014) "This has created a widespread problem necessitating search for new antifungal compounds". Emerging trends in invasive candidiasis have led to an increase in the number of infections caused by non-albicans Candida species globally. (C.Bustamante 2005) Therefore, "with regard to the inherent reduced susceptibility of non-albicans Candida species to antifungal agents".

2.0 Thymol

2.1 Chemical Structure and Nomenclature

Thymol is the IUPAC name of this compound (chemical name) and has the chemical formula $C_{10}H_{14}O$, whereas Thymol or 2 isopropyl 5 methyl phenol) is a monoterpene intermediate product of cymene that belongs to the group of phenols and has therefore phenolic properties in aromatics and is chemically related to hydroxylated terpenoids, carvacrol for example (M.F. Nagoor Meeran et al in 2017; B. Salehi et al. 2018; Marwa Waheed et al. 2024). Thymol molecular weight of 150.22 g/mol and the compound has a hydroxyl (-OH) at position 1, isopropyl ($-CH(CH_3)_2$) at position 2 and methyl ($-CH_3$) groups at position 5 on the phenol molecule. B. Salehi et. al, 2018. This structural configuration makes thymol structurally identical to carvacrol, with the only difference being in the positions of the -OH and $-CH_3$ functionalities. Chemically, it is found as a natural halogenated, volatile compound, which means that it possesses natural antinociceptive properties (that is: the compounds can relieve pain without producing a feeling of depressant activity). The molecular structure of thymol can be expressed as a single, three-dimensional structure, and therefore can be classified (according to the IUPAC nomenclature) as a monoterpenoid phenol occurring naturally due to the natural degradation process of certain organic matter (and an aromatic compound based upon their composition of carbon C and hydrogen H atoms) and having a melting temperature range between 49ºC and 51ºC (322K and 324K) (at a room temperature of 120ºF and 124ºF) with a density of 0.96 g/cm³ at 25ºC, and also possessing various physio-chemical properties, were reported recently (M. F. Nagoor Meeran et al in 2017).

2.2 Physical and Chemical Properties

Thymol (unlike other phenolic compounds) is a white crystalline solid that contains an aromatic character and the distinct flavor of thyme. The density of thymol is approximately 0.96g/cm³ at 25ºC, its melting point is between 49ºC and 51ºC (approximately 322 to 324 Kelvins, or 120 to 124 Degrees Fahrenheit), and its chemical components are readily soluble in both alcohols and alkaline solutions, as well as in a number of other organic solutes (due to phenolic deprotonation). On the contrary, due to phenol deprotonation, thymol has very limited solubility in water (at neutral pH). Additionally, thymol is able to absorb maximum UV radiation at a wavelength of 274nm (M. F. Nagoor Meeran et al in 2017).

2.3 Origin

a) Natural Sources and Plant Origins

A significant amount of thymol found in essential oil is obtained from the Lamiaceae (mint) class of essential oils, which includes plants from the Thymus, Ocimum, Origanum, and Monarda genera (A. Marchese et al, 2016). Thymol has also had many plants from other flowering plants in the Verbenaceae, Scrophulariaceae, Ranunculaceae, and Apiaceae families A. Marchese et al, 2016. The primary source of thymol found within the greater essential oil; Thymus cognatus L. (Thymus) which contain on average: 10-64% of the total essential oil B. Salehi et al, 2018. Other credible sources of thymol: Trachyspermum ammi, Baccharis grisebachii, Centipeda minima (Jyoti et al, 2019), Ocimum gratissimum L., Carum copticum L., several species within the Satureja L. and Oliveria decumbens Vent A. (Escobar et al., 2020). Other plant sources of thymol, Euphrasia rostkoviana, Monarda didyma, M. fistulosa, Nigella sativa, Origanum compactum, O. dictamnus, O. onites, O. vulgare, Thymus glandulosus, T. hyemalis, and T. zygis (M. T. Islam et al, 2018).

b) Biosynthesis and Chemical Formation

The The Recognized Scheme of The Biosynthesis of Thymol Through Hydroxylation of P-Cymene After Aromatizing Gamma-Terpenene Into P-Cymene and Shows the Natural Conversion to P-Cymene and in the Metabolic Pathways Of Thymol Producing Fountain Become Establish. (M. F. Nagoor Meeran et al., 2017). **c) Synthetic Production**

There are numerous ways in which thymol can be synthesized for industrial use, all of which involve chemical reactions utilizing one of the following chemicals: p-cymene, m-cresol, isopropyl alcohol, propylene, or through the documented use of supercritical carbon dioxide (CO_2) extraction methods (M. F. Nagoor Meeran et al., 2017). These methods represent viable alternatives to producing commercially available forms of thymol derived from natural sources.

2.4 Pharmacokinetics and Metabolism

2.4.1 Absorption and Distribution

Thymol is quickly absorbed after ingestion and reaches a maximum plasma concentration (Tmax) within 30 minutes to 1.97 hours. The bioavailability of thymol in plasma measured as thymol sulfate is approximately 16% (M. F. Nagoor Meeran et al., 2017).

2.4.2 Metabolic Pathway

Thymol undergoes significant first-pass metabolism; it is primarily metabolized via sulfation and glucuronidation. The main metabolites of thymol are thymol sulfate, thymol glucuronide, and thymol thymohydroquinone sulfate. Thymol is rarely found as free in plasma, while the bulk of the circulating form of thymol is present as thymol sulfate (M. F. Nagoor Meeran et al., 2017).

2.4.3 Elimination

The average half-life of thymol is approximately 10.2 hours with renal clearance of 0.271 ± 0.7 L/h. The majority of the administered dose (approximately 16.2 ± 4.5%) is excreted in urine as conjugated metabolites within 24 hours after administration; most of this excretion occurs within the first 6 hours of dose administration (M. F. Nagoor Meeran et al., 2017).

2.5 Chemical Classification

Thymol is one of the most important natural dietary components present in the species of thyme and has been used many times throughout history within Traditional Medicine (M. F. Nagoor Meeran et al., 2017). Thymol is classified as a phenolic monoterpene, which are some of the most common groups of organic compounds known to have multiple biological activities; the pharmacological activities of thymol arise from the presence of a phenolic hydroxyl group within its chemical structure, as compounds with phenolic hydroxyl groups are known to protect the body against the potentially harmful effects of free radicals (M. F. Nagoor Meeran et al., 2017).

2.6 Regulatory Status and Safety

The European Union (EU) and the Food and Drug Administration (FDA) consider thymol to be a safe food additive classified as 'Generally Recognized As Safe' (GRAS) (M. T. Islam et al., 2018). The FDA's determination of GRAS status indicates that thymol is widely accepted to have an established safety history with little to no toxicity during normal use (M. F. Nagoor Meeran et al., 2017).

2.7 Biosynthetic Pathways

Biosynthetic pathways of thymol are based on the Mevalonic acid pathway. The construction of these compounds through Mevalonic acid biosynthesis results from Isopentenyl diphosphate (IPP) and Dimethylallyl diphosphate (DMAPP). The last hydroxylated product of thymol in the final product is combined through a series of steps that include cyclizing geranyl diphosphate and aromatizing α-Terpinene using P450 enzymes (CYP71D180 & CYP71D181). In addition, cytochrome P450 monooxygenase-mediated hydroxylation is considered the rate-limiting step (Limiting Factor) in the overall biosynthesis of thymol and thus determines total thymo concentration in tissues from thyme plants (W.Hikal et al. 2021).

Traditional methods used to extract and isolate thymol from plant material are based on Steam distillation. This process involves passing the vapour Phase of Steam through the plant material, thus volatilizing essential oil constituents. Once vapour extraction has been achieved, the resulting vapour from the plant material is collected via condensation, producing an emulsion that includes both essential oil and water from the plant (Jyoti & et al. 2019).

Supercritical CO_2 extraction of thymol and its isolated constituents is an advanced extraction method that provides improved purity of extracted products, eliminates thermal degradation and provides short extraction times with efficient extraction and maintenance of compound integrity. Microwave and Ultrasonic-Assisted extractions represent a suite of growing advanced extraction techniques for extracting compounds from plant materials (Jyoti et al. 2019).

2.7.1 Advanced Formulation Technologies

Encapsulating thymol in tiny spheres using the natural polymers methylcellulose and hydroxypropyl methylcellulose phthalate greatly improves the bioavailability of thymol; with spray drier encapsulation being used for controlled release and increased bioavailability over regular thymol, helping fight intestinal infection (M. F. Nagoor Meeran et al. 2017).

Encapsulating matrix polymers with hydroxypropyl methylcellulose (HPMC) and ethyl cellulose (EC) by using an emulsion solvent evaporation technique is effective for producing controlled release compounds. Drug release rate behavior, entrapment efficiency, and particle size optimization are all significantly impacted by the types of polymers used for encapsulation of the matrix.

The Dihydrochakra (DTH), which is a hybrid of diacerein and thymol, is an example of a new method of co-drug development, where thymol has been esterified with the carboxylic acid of diacerein to create a prodrug that has lower levels of irritation and faster absorption than diacerein alone. The lipid affinity of thymol increases diacerein's lipophilicity, thereby increasing its bioavailability and absorption levels through the body's tissues (M. F. Nagoor Meeran et al. 2017).

2.8 Molecular Interactions and Binding Studies

a) Affinity of Protein Binding

The results obtained from molecular docking studies suggest that the greatest binding affinity of thymol towards PARP-1 (6BHV) is equal to −6.9 kcal/mol; and that significant hydrogen bond interactions with TYR896 (2.949 Å) and HIS862 (2.663 Å) (i.e. amino acid residues) were observed. Additional hydrophobic interactions with TYR896, TYR907, and ALA898 were also identified. (M. T. Islam et al., 2018)

b) Membrane Interactions

Thymol incorporation into artificial membranes increased the binding affinity of neurotransmitter receptors. At an effective dose of 1 mM, it reduced both the temperature of phase transition and the surface tension of membranes by approximately 25%. The change in membrane fluidity was determined by both the size and shape of molecules in relation to that of the lipids, thus decreasing the surface tension from 72 to 53 dye/cm (M. F. Nagoor Meeran et al., 2017).

2.8 Industrial Applications and Quality Control

Thymol is used as a natural preservative and flavoring agent in the food industry. The effectiveness of thymol as an antimicrobial agent is broad-spectrum, providing activity against both gram-positive and gram-negative bacteria. The Minimum Inhibitory Concentrations (MIC) against several microorganisms have been reported to be as low as 0.31 mg/ml (S. aureus) and 5.00 mg/ml (E. coli) respectively (M. F. Nagoor Meeran et al., 2017). Thymol plays a major role in food preservation, especially in protecting foods from pathogenic microorganisms, during both the cultivation and storage (A. Escobar et al., 2020).

Thymol is used in many different types of pharmaceutical products; e.g., topical ointments, oral hygienic products (toothpaste and mouthwash), and numerous medical products due to its strong anti-microbial activity. In dentistry, it is highly valued for its effectiveness in treating infections of the oral cavity (Nagoor Meeran, 2017). Thymol is also used in commercial mosquito repellent products, because of its natural ability to repel these insects (Marchese et al., 2016).

Thymol is utilized in the cosmetic industry in many different types of products, including soaps, shampoos, and deodorants due to its antiseptic and aromatic properties. Its pleasant scent and ability to kill microorganisms makes it a suitable ingredient for a variety of personal care products (Nagoor Meeran, 2017).

2.9 Toxicological Profile

2.9.1 Acute Toxicity Data

Large toxicology studies on animals indicate various species-specific lethal doses (LD50) of (thymol) ranging from 980 mg kg-1 for rats; 88 mg kg-1 for guinea pigs; approximately 1200 mg kg-1 for male ddY mice; 1050 mg kg-1 for female ddY mice; 250 mg kg-1 for cats; 750 mg kg-1 for rabbits; and approximately 640 mg kg-1 for mice (M.F. Nagoor Meeran et al., 2017). All results indicate the degree of acute toxicity produced by thymol is moderate, although significant variation exists by species.

Long-term safety studies have demonstrated that thymol has been safely used for hundreds of years across many cultures and civilizations. According to the Environmental Protection Agency (EPA), there are no known adverse effects when thymol is used properly in animals and humans; furthermore, its historical use by Ancient Egyptians for mummification confirms its long-established safety record (M.F. Nagoor Meeran et al., 2017).

2.9.2 Genotoxicity Assessment

Genotoxicity studies report conflicting results, depending on dose and assay type. In one assay type (the DNA repair assay), thymol at therapeutic concentrations (0.1 mM) was shown to reduce the occurrence of double-stranded break (DSB) events in human lymphocytes, conversely at concentrations greater than 0.2 mM; thymol has been shown that it may have a genotoxic effect. The SOS chromotest (an in vitro assay) indicates that thymol has a very weak potential for producing genotoxicity (M.F. Nagoor Meeran et al., 2017).

2.10 Cellular and Molecular Mechanisms

2.10.1 Membrane Effects

Thymol has many different ways of interacting with cellular membranes that lead to a variety of biological effects. It can increase the permeability of cell membrane lipids, which affect both erythrocyte (red blood cell) hemolysis (cell death) in a manner dependent on the concentration of thymol present (i.e., 0.06-1 mM vs 2-4 mM for hemolysis protection versus hemolysis respectively). Additionally, it has been shown to decrease the surface tension of cell membranes and, as a result, alter membrane fluidity; specifically, erythrocytes are best protected from hemolysis at a concentration of 1 mM (M. F. Nagoor Meeran et al., 2017).

2.10.2 Interaction with Ion Channels

Thymol has been shown to interact with numerous ion channels in a complex fashion. For instance, thymol has been demonstrated to block sodium voltage-gated channels with IC_{50} values of 104 and 149 μM for skeletal muscle and neuronal sodium channels, respectively. In addition, thymol also inhibits calcium and potassium currents through rat skeletal muscle fibers at EC_{50} values of 193±26 μM and 93±11 μM, respectively (M. F. Nagoor Meeran et al., 2017).

2.10.3 Calcium Homeostasis

Thymol significantly alters intracellular calcium homeostasis (the balance of calcium in and out of a cell) by stimulating the release of calcium from the sarcoplasmic reticulum through ryanodine receptor activation at an EC_{50} value of 158±16 μM. Consequently, upon activation of ryanodine receptors, calcium is released from the sarcoplasmic reticulum in skeletal muscle via depolarization-induced calcium release and diminished calcium-dependent ATPase activity (M. F. Nagoor Meeran et al., 2017).

2.11 Multi-Target Mechanisms Against Candida albicans

2.11.1 Cell Membrane Disruption

The primary antifungal mechanism of Thymol is the disruption of the fungal cell membrane by multiple pathways. Thymol interacts with the polar headgroups of the negatively charged phospholipids through hydrogen bonds or electrostatic interactions. This causes (1) monolayer expansion of the plasma membrane, (2) decreased membrane elasticity, and (3) local aggregation within the membrane. As a result, both fluidity is increased and stability is decreased in the membrane (Chun Chen et al., 2026). Thymol inhibits the biosynthesis of ergosterol, a major component of the fungal cell membrane, by downregulating genes involved in the synthesis of sterols. The resulting dysfunction of the plasma membrane and the increased permeability of the plasma membrane are also due to thymol inhibiting the production of ergosterol (Chun Chen et al., 2026). The minimum inhibitory concentrations of thymol against Candida species are between 32 and 256 μg/mL. The mechanisms of action of thymol against C. albicans include (1) induction of a reactive oxygen species (ROS) burst and (2) inhibition of the efflux pumps (Chun Chen et al., 2026). Studies indicate that thymol has a dose-dependent mechanism of action where higher concentrations will have a greater damaging effect on cell membranes. (Nagoor Meeran et al., 2017). The ability of thymol to disrupt the membrane potential and ionic homeostasis is a critical component of the antifungal activity of thymol against C. albicans.

2.11.2 Impairing Cell Wall Synthesis

Thymol doesn't only attack the cellular membrane, it also causes disruption to the fungal cell wall by impairing the ability of C. albicans to synthesize cell wall components, which is essential to their life and structural integrity (Chun Chen et al., 2026). The fungal cell wall is comprised of chitin, β-glucans, and mannoproteins, which provide structure and protection against environmental sources of stress. Thymol is capable of causing multiple types of impairments to the products of cell wall synthesis. First and foremost, it has an impact on the enzymes that are responsible for synthesizing chitin, specifically, on the activity of chitin synthase, the enzyme that links N-acetylglucosamine units together to form chitin polymers. Secondly, thymol targets β-glucans, principally through their complex assembly pathway by targeting glucan synthase complexes and, in doing so, contributes to weakened cell walls. (Chun Chen et al., 2026).

Overall, the impairment of cell wall synthesis has a negative effect on fungal cell viability. C. albicans cells that have weakening cell walls will be more prone to osmotic stress or mechanical damage. This is particularly true during cell division when the cell wall needs to be remodeled and reconfigured, hence, cells that are undergoing cell division will be especially vulnerable to thymol treatment.

2.11.3 Energy Metabolism Disruption and Mitochondrial Dysfunction

Another mechanism of antifungal activity for thymol is its effects on mitochondrial function. In addition, mitochondria are the primary energy-producing organelles in fungi and other eukaryotic cells and, therefore, represent a prime target for antifungal agents (Chun Chen et al., 2026).

Disruption of the mitochondrial membrane potential via thymol leads to a malfunctioning electron transport chain and decreased ATP synthesis. ATP is required for many of the cellular processes, such as active transport, biosynthesis, and maintenance of cellular structure, that are disrupted by a depletion of energy due to disruption of energy metabolism, resulting in cellular dysfunction and eventual cell death.

Research indicates that the effects of thymol can induce mitochondria to swell, alter their membrane permeability, and release pro-apoptotic factors (M.F. Nagoor Meeran et al., 2017). Thus, the mitochondrial damage leads to depletion of energy and activation of cell death pathways, resulting in both a mechanism of action against fungi and a mechanism for inducing death in fungi.

2.11.4 Biofilm Inhibition and Disruption

Biofilms created by Candida albicans contain complex three-dimensional communities of microorganisms and provide significant clinical challenges.Candida albicans biofilms are formed through the same stepwise process described previously. The process for Candida biofilm formation involves a series of four steps: 1) Initial adhesion of yeast cells to a surface, 2) Proliferation and formation of microcolonies, 3) Maturation of the biofilm through hyphal development and production of an extracellular matrix (ECM), and 4) Dispersal of cells from the biofilm to create new sites of infection (M. Gulati & C. Nobile, 2016; C. Nobile & A. Johnson, 2015; N. Ponde et al., 2021).

The composition of the extracellular matrix, which forms the overall architecture of the biofilm, includes multiple morphological forms of the cells present in the biofilm (e.g. round yeast cells, oval pseudohyphal cells, and elongated hyphal cells) (C. Nobile & A. Johnson, 2015). The composition of the extracellular matrix contains approximately 55% proteins and glycoproteins, 25% carbohydrates, 15% lipids and 5% nucleic acids. There are over 500 proteins contained within the extracellular matrix structure (N. Ponde et al., 2021).

Thymol is a potential anti-biofilm agent with two modes of action: First, thymol inhibits the adhesion of fungal cells to surfaces, which is a critical step in developing a biofilm. By preventing the attachment of fungal cells, thymol has the potential to stop the formation of biofilm communities entirely (C. Nobile & A. Johnson, 2015). Second, thymol has shown significant efficacy against established extracellular matrices (ECMs) in mature biofilms. Disruption of the ECMs will lead to the dispersal of biofilms and enhance the susceptibility of the cells in the biofilm to currently available antifungal agents. This would be particularly useful because it would enhance the usefulness of currently available antifungal agents for treating biofilm infections. (M. Gulati & C. Nobile, 2016)

2.11.5 Virulence Factor Suppression

Candida albicans employs numerous virulence factors that help to establish infection, such as adhesion, morphogenic changes, secreted hydrolytic enzymes, and biofilm creation (Calderone and Fonzi 2001; Preeti Patra & Rajen Dey, 2024) these facilitate tissue invasion, immune evasion, and continual colonization. (dos Santos Silva et al. 2024)

Thymol is known to act against multiple targets of the key virulence factors of Candida albicans. In addition to its ability to inhibit the growth of fungi, thymol can inhibit biofilms formed by the fungi in a concentration dependent manner and can activate the p38 MAPK signaling pathway leading to enhanced immune responses by the host and the restoration of gene expression changes and phosphorylation levels that occur during an infection (Shu et al.).Thymol inhibits the formation of germ tubes an important morphogenic switch that yeast undergoes to transition from its yeast form to its hyphal form necessary for invading tissue (Abdulrahman M Abu Shaban and H. Darmani, 2024). Thymol also inhibits the dimorphic transition between yeast and hyphae and exhibits a strong antibiofilm activity at higher concentrations. Additionally, thymol downregulates the expression of virulence associated genes (i.e., ACT1) needed for filamentation and pathogenicity (Abdulrahman M Abu Shaban and H. Darmani, 2024).

2.11.6 Key Enzyme Inhibition

Through unique mechanisms of action, thymol possesses a very diversified antifungal action against Candida albicans. It acts to disrupt multiple essential cellular pathways simultaneously and decrease the likelihood of developing resistance. In particular, it disrupts ergosterol biosynthesis, mitochondrial respiratory function, and DNA damage repair, which are critical to maintaining the integrity of the plasma membrane and normal metabolic function (Chun Chen et al., 2026). Its ability to inhibit ergosterol biosynthesis is now well-documented and it has demonstrated very potent antifungal activity with low minimum inhibitory concentrations. The addition of ergosterol to cells significantly reverses the antifungal activity of thymol and confirms that it specifically targets the ergosterol pathway (R. D. de Castro et al., 2015). Thymol also downregulates key genes that encode for proteins involved in the synthesis of sterols (such as ERG1), thus compromising the formation and function of the membrane (Fatemeh Asmiteh et al., 2024). In addition to disrupting membranes, thymol disrupts genetic stability mechanisms through an inhibitory effect on telomerase activity as a consequence of decreasing the expression of the EST2 gene, leading to telomere shortening and increased aging of the cells (Emad Darvishi et al., 2013). Visible damage to cells from fungi has been documented, including membrane permeabilization and surface irregularities, indicating that there are direct effects on the integrity of the cell envelope (P. Braga & D. Ricci, 2011). Furthermore, thymol has been found to have potent antibiofilm activity, demonstrated through an ability to decrease metabolic activity in established biofilms, providing additional evidence that this compound has direct antifungal action (P. Braga et al., 2008). Evidence from in vivo studies suggests host survival from infection can be increased by thymol, and that thymol can support immune responses supporting its clinical therapeutic plausibility (C. Shu et al ., 2016). Overall, these conclusions indicate that thymol has more than one mechanism for its antifungal actions, including disruption of fungal membrane integrity; disruption of fungi's capacity to produce energy; interference with stability of fungi's genome; disruption of formation of fungal biofilms; and that these various mechanisms may reduce the probability of development of resistance to thymol.

2.11.7 Apoptosis Induction

Thymol causes the fungus C. albicans to undergo programmed cell death, or apoptosis, through various routes. Though fungi do not utilize the same method for execution of apoptosis as do mammalian cells, they have a number of ways to initiate programmed cell death through different types of stressors. (Chun Chen et al 2026).

Thymol will initiate apoptosis through different cellular stressors, such as oxidative stress, loss of energy and damage to membranes. All of these disrupt the natural cell cycle, which will ultimately culminate in a defined manner, resulting in fragmentation of DNA; blebbing, or attachment to the cell membrane; and/or shrinking of the cell. (H. Mahmud et al., 2009)

Ros will be produced as a result of thymol's ability to induce the production of ROS, resulting in oxidative damage that will result in activation of pathways that lead to the execution of cell death. This additional mechanism of action will enhance this compound's ability to kill other fungal cells in addition its own cytotoxic effects (M. F. Nagoor Meeran et al., in 2017).

2.12 Comprehensive Evidence Base

2.12.1 Systematic Review Evidence

It has long been recognized that thymol possesses potent activity against fungi, especially Candida albicans, and can inhibit other microbes as well. An extensive body of literature has established thymol as one of the most effective naturally occurring phenolic agents for antifungal use. A systematic review of numerous studies involving plant extracts has determined that thymol is one of the major phenolic constituents demonstrating high antifungal efficacy in both in vitro and in vivo studies, providing evidence of its ability to be used as a potential therapeutic agent. Collectively, these data clearly demonstrate that there is a robust body of evidence from experiments supporting the use of thymol as a highly effective antifungal agent (Hsuan-Min Hsu et al., 2020)

Various extracts containing thymol were proven to show consistent antifungal activity from multiple studies conducted by various research teams. Furthermore, thymol is one of the top three or four identified antifungal compounds (e.g., gallic acid and flavonoids) and therefore has high therapeutic potential. Also, thymol's effectiveness against various extraction methods and different types of plants utilized by the different researchers indicates that it has consistently demonstrated strong and reproducible antifungal activity. (L. Mezzomo et al., 2025; Chun Chen et al., 2026)

2.12.2 Meta-Analysis Quantitative Evidence

Quantitative assessments of thymol's antifungal activity against Candida albicans have been conducted using meta-analytical methods confirming its inhibitory capacity in all studies included in the meta-analysis. A total of seven studies evaluated thymol's antifungal performance through standardized reports of minimum inhibitory concentration (MIC) with established MIC values of 2 mg/mL (both MIC50 and MIC90). Therefore, it appears that thymol has inhibited fungal growth at both 50% and 90% levels with statistical significance ($P < 0.001$) which, in turn, provides evidence that the antifungal activity of thymol observed in these studies is a result of true biological effect and not of random chance. Although MIC values were higher for thymol than those seen with other natural plant-derived antifungal products such as garlic, thymol produced measurable antifungal effects across each of the studies included in this meta-analysis. Therefore, thymol represents a viable candidate for additional study and development for treatment as an antifungal medication. (S. Mohammadi et al., 2018)

2.13 Essential Oil Research Context

When discussing the antifungal properties of Thymol, it is important to understand the larger context of essential oil research. One recent study, (Gao-wei Hou et al.,) evaluated a number of essential oils for their efficacy as antifungals against C. albicans (Candida albicans). Thymol was determined to be a major active ingredient in the majority of essential oils evaluated, thereby establishing its significance. The authors of the review supported their conclusion by documenting the multiple mechanisms through which thymol-containing essential oils exert their antifungal activities, including both direct antifungal activity and modulation of the host's immune response.

Additional studies on the effects of essential oils have shown that those containing thymol have broader impacts than simply killing the fungus and extend to include the modulation of the host's microbiome and immune system. In this respect, the extensive range of thymol's effects demonstrates its potential effectiveness in successfully treating difficult fungal infections, especially those in which host-related factors are critical to progression of disease and success of treatment.

2.14 Synergistic Potential

The combination of thymol with conventional antifungal agents is known to have potent synergistic effects, leading to increased overall therapeutic efficacy against Candida albicans. Studies have shown that thymol in combination with each of the conventional antifungal drugs has greater antifungal activity than monotherapy (Hsuan-Min Hsu et al., 2020). The mechanism of this synergism is due in large part to the different modes of action of each agent, as thymol and the standard antifungal agents have different ways through which they exert their antifungal activity. For example, azoles (most common class of antifungal agents) inhibit ergosterol biosynthesis and echinocandins disrupt the synthesis of the fungal cell wall; however, thymol will disrupt fungal membrane integrity, metabolic processes, and multiple cellular functions simultaneously (A. Blanc et al., 2023).

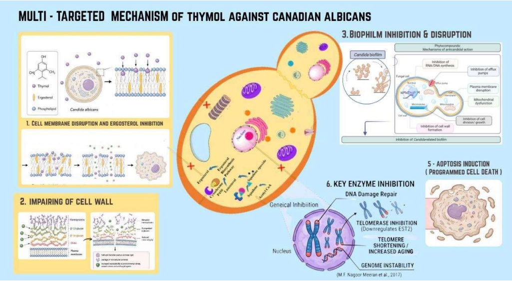
This additive mechanism will allow for the enhancement of antifungal activity and the increase in efficacy of the agents, as well as the ability to overcome resistance mechanisms associated with agents acting on a singular target. Additionally, thymol enhances the activity of many antifungal agents, including fluconazole and amphotericin B (Hsuan-Min Hsu et al., 2020). Ultimately, this synergistic effect will allow for a reduction in the dose of both thymol and traditional antifungal agents without losing or improving the antifungal activity, which may subsequently lower toxicity and improve clinical outcomes.

This tactic is very useful in the treatment of infections caused by biofilm-associated organisms because traditional antifungal medications often have less effect because of restricted penetration and developing resistance. Therefore, including thymol as a synergistic agent offers an exciting new opportunity for increasing the effectiveness of antifungal treatment in difficult cases and long-term infections.

2.15 Delivery Systems and Formulation Advances

The capacity for therapy from thymol has been greatly improved by the use of delivery systems and formulation technologies. (Gao-Wei Hou et al. 2024) The effectiveness of nanotechnology in increasing thymol's bioavailability, stability, and targeted delivery has been reported in the literature. Research indicates that nanoformulations of thyme extracts show greater antifungal effects than conventional formulations. A systematic review of seven studies looking at nanotechnology on herbal extracts indicated that nanoparticles significantly improved the qualities of plants and prolonged the duration of antifungal effect. One of the reasons for the increased activity is that the very small size of the nanoparticles (<200 nm) allows them to passively travel through the cell membranes and increases the area that the nanoparticles can come into contact with a target organism. (Hsuan-Min Hsu et al., 2020).

To help overcome some of the difficulties associated with thymol (e.g., volatility, poor solubility in water, and susceptibility to degradation), many encapsulation technologies have been developed including natural polymer microencapsulation to improve bioavailability and allow for controlled release. (M.F. Nagoor Meeran et al. 2017) The advances made by these formulations are improving the use of thymol in clinical practice by reducing the inherent physicochemical limitations.



2.16 Economic and Global Health Perspectives

Thymol-derived antifungals could provide positive impacts to the economy and global health.

a) Cost-Effectiveness

Natural plant materials (e.g., thymol) can be more cost effective than synthetic antifungal agents especially in low-resource settings. (Hsuan-Min Hsu, 2020) Widespread access to thymol-containing plants would allow for local production of antifungal agents, thereby decreasing reliance on expensive imported medications.

b) Global Access

Widespread distribution of thymol-containing plants could improve access to antifungal medications in areas where traditional antifungal treatments are inaccessible and/or unaffordable. (Gao-wei Hou, 2024) This has particular importance in light of the high burden of fungal diseases on a global scale and the limited availability of antifungal treatments.

2.17 Efficacy

Today, fungal infections are at their highest ever and worldwide, the greatest of all the opportunistic fungi is Candida albicans (C. Tsui et al., 2016). The ability of this yeast to grow in two forms is one reason why it continues to be a serious clinical challenge for providers. Candida albicans can cause two types of infections in patients with suppressed immunity; (1) mucosal infections can be found on the skin and in the mouth, (Joonhee Kim & P. Sudbery, 2011) (2) Systemic infections can lead to severe systemic (blood) infections.

2.17.1 Quantitative Assessment of Antifungal Potency

Researchers have conducted many studies on thymol's antifungal activity against Candida albicans and determined its minimum inhibitory concentration (MIC) values. In a comprehensive meta-analysis (Mohammadi et al.,2018) the MIC_{90} and MIC_{50} values of thymol against C. albicans were determined to be 2 mg/ml based on their analysis of seven studies. This indicates moderate inhibition of C. albicans compared to other compounds, such as garlic, which has much lower MIC values (0.062 and 0.0312 mg/ml for MIC_{90} and MIC_{50}, respectively) and greater inhibition than thymol. When evaluating anticandidal compounds like thyme based on MIC, however, it is critical to consider the multiple mechanisms of action and safety profile of each compound.

Thymol penetrates the outer membrane of C. albicans by several mechanisms, including disrupting the cell wall and cell membrane, acting as an anti-inflammatory agent, preventing biofilm formation, and inhibiting enzymes involved in C. albicans biology (Sana Kauser et al., 2024). As a result, variations in thymol's MIC against C. albicans due to experimental variables (methodologies, characteristics of the strains tested), may impact the overall efficacy of thymol depending on the specific strains being tested. For example, Chen et al. found that the MIC range of thymol against different fluconazole-resistant isolates of C. albicans ranged from 32-256 µg/ml, thus highlighting that different strains of C. albicans may have differing sensitivities to compounds that possess anticandidal activity. Hence, the importance of standardizing testing protocols before evaluating the efficacy of thymol and other candida-killing agents will help guide future investigations into establishment of efficacy of these agents against a wide range of candida strains in patients with candida-related infections.

2.17.2 Clinical Evidence and Therapeutic Applications

Multiple clinical studies have indicated the effectiveness of thymol-containing essential oils in the treatment of different forms of candidiasis;

therefore there exists indirect support for thymol's therapeutic potential (E. Ferreira et al. 2021). There have been several clinical trials that have tested Zataria multiflora essential oil, which contains high levels of thymol, to treat prosthetic stomatitis caused by Candida spp. In these studies, participants receiving Z. multiflora gel (0.1% concentration) applied 4 times daily for 2 weeks experienced statistically significant decreases in clinical signs and fungal colony counts relative to control subjects (E Ferreira et al 2021). While these studies do not evaluate the specific impact of thymol, they provide valuable clinical support for thymol-containing formulations to be used in treating oral candidiasis. Similarly, other thymol-containing formulations, such as Pelargonium graveolens essential oil, have demonstrated efficacy in treating denture-related oral candidiasis based on clinical investigations. Based on clinical and microbiological parameters, patients who received therapy with Pelargonium graveolens essential oil experienced statistically significant reductions in erythema, pain, and Candida colony counts after treatment compared to baseline, (E Ferreira et al 2021). The clinical efficacy of the various formulations containing thymol could support improved therapeutic outcomes if thymol alone were to be further developed and characterised.

There are a number of clinical scenarios where thymol has potential for therapeutic purposes against the pathogenic yeast, Candida albicans. Each of these clinical scenarios reflects a unique set of challenges and opportunities to optimize treatment use with thymol. In superficial mucocutaneous infections, thymol's well-established safety profile and potent antifungal properties lend themselves to topical applications where adequate local concentration can be achieved without any risk of systemic toxicity (M. Martínez-Pabón et al., 2020).

3. Formulation

3.1 Advantages of thymol liposomal formulations

a) Enhanced Bioavailability and Solubility

Improved solubility and bioavailability of drugs are two advantages of nano-sized formulations of thymol for drug delivery system (NDDS) applications. By utilizing NDDS, it is possible to circumvent the limitations of poor solubility and bioavailability of thymol by developing well-designed nanocarrier systems (M. B* et al., 2023). When encapsulating thymol in nanocarrier systems designed specifically to negate the negative effects associated with its hydrophobic nature and low water solubility, these challenges can be addressed. In addition to overcoming the inherent limitations associated with the solubility of thymol, nano-encapsulation provides an increased ability to distribute drugs throughout the body more readily since the nanoencapsulation will enhance the ability for drug concentrations needed for therapeutic benefits to be achieved at sites of infection. Improved solubility is essential for achieving effective drug delivery to infected tissues and biofilms in patients with Candida albicans infections, because achieving an adequate concentration of drug that penetrates through the biofilm or infected tissue is critical to the success of the treatment.

b) Superior Antifungal Efficacy

Thymol nanoformulations exhibit significantly enhanced antimicrobial activities when compared with free thymol by virtue of the greater surface area–to–volume ratio of nanoparticles allowing for more efficient interactions with fungal cell membranes resulting in improved therapeutic outcomes. There is evidence that encapsulated thymol shows more potent antifungal effects against different species of Candida, with lower minimum inhibitory concentrations required for achieving fungicidal activity. (Aidan Hajibinabi et al. 2022) Contributing to this enhanced efficacy are several factors including improvement in cellular uptake; prolonged release profiles; and the ability to overcome the cellular defense mechanisms that usually limit the efficacy of standard antifungal treatments. The ability of nanoformulated thymols to offer superior antifungal activity is especially crucial in situations where there is a presence of resistant Candida albicans strains for which standard treatment options may be ineffective.

c) Targeted Drug Delivery and Controlled Release

Through nanotechnology, improved methods for designing drug delivery systems capable of providing precise therapy and regulatingly releasing drugs or other active ingredients can be developed. Such systems can be designed to respond to environmental factors such as changes in pH levels, temperature fluctuations, or the presence of specific enzymes at the site of infection (Yang Gao et al., 2024). When designed properly, these delivery systems can ensure that thymol is delivered exactly where it is needed to provide effective therapy while limiting systemic exposure and side effects.

The controlled release characteristic of nanoparticle formulations also allows for sustained therapeutic concentration over longer periods, thereby reducing the amount of doses that need to be taken and increasing patient compliance. This characteristic will help to improve the treatment of chronic or recurrent Candida infections as there is a tremendous need to maintain consistent antifungal pressure in order to avoid relapse.

d) Biofilm Penetration and Disruption

Candida albicans biofilms are among the toughest factors to combat when treating an infection because of the significant protection that these biofilms offer to antifungal agents. The use of nanoparticles, specifically those with a size range of 10-50 nm, provides enhanced ability compared to larger particle sizes or free drug molecules to penetrate the biofilm structure (Yang Gao et al., 2024).

The ability of thymol nanoparticles to penetrate biofilms allows for improved disruption of the extracellular polymeric matrix, which protects the fungal cells in biofilms. The ability of thymol nanoparticles to effectively penetrate the biofilm matrix is beneficial for treating device-associated infections, chronic wounds, and clinical instances where biofilms are a large therapeutic barrier.

e) Multi-Target Mechanisms of Action

The antifungal activity of metal nanoparticles loaded with thymol occurs through multiple pathways, including the following: Ion release, Induction of oxidative stress, Damage to the membrane, Inhibition of enzyme activity, Interference with cellular processes (P. H. F. Carmo et al., 2023). This multicomponent method decreases the probability for resistance to be developed as it becomes increasingly more complex for fungal pathogens to develop multiple forms of resistance simultaneously due to their ability to develop multiple types of resistance against more than one mechanism of action.

Thymol has an antipathogenic activity in and of itself, this functionality combined with the added functionality provided by the carrier nanoparticles creates a synergistic effect that increases the total therapeutic benefit from both of these active ingredients. This synergy is especially advantageous when treating resistant Candida infections, as a single-target therapeutic will likely not provide adequate treatment.

f) Reduced Systemic Toxicity

Nanoparticle formulations have been shown to decrease the systemic toxicity when administering thymol by allowing for targeted drug delivery and controlled release of the drug. Concentrating the active ingredient in the infected area while minimising the amount of drug distributed to the rest of the body will allow these formulations to provide therapeutic effect using a smaller amount of drug overall (Yang Gao et al., 2024).

The reduction of toxicity is especially relevant when treating vulnerable patient groups, such as immunocompromised, elderly patients and/or those with several comorbidities, as these groups may experience an increased risk of adverse drug reactions when using typical antifungal therapies.

3.2 Disadvantages

a) Manufacturing Complexity and Cost

Thymol formulation production involves advanced manufacturing techniques and advanced technology to provide a controlled environment and significant quality assurance, resulting in much higher manufacturing costs than standard pharmaceutical formulations (Yang Gao et al., 2024). The nanoparticle production process has created multiple opportunities for variation in the overall quality and consistency of the product as a result of the complex nature of the nanoparticle synthesis process, creating the need for very detailed manufacturing protocols and quality assurance processes.

As you move away from the lab into commercial production, there are more difficulties with the management of the precision of particle sizes, distribution of particles, and the drug loading throughout the manufacturing process, which converts large-scale production of the product more difficult to achieve than small scale. These operational challenges can create barriers to the commercial viability of thymol formulations in the marketplace.

b) Stability and Storage Challenges

The multiple inherent instabilities of thymol, which has a high rate of volatility, oxidizes easily, and is reactive to light and temperature can be problematic, even when freshly incorporated into a nanoparticle formulation (Aida Hajibonabi et al., 2023). While the use of nanoencapsulation can decrease the vulnerability of thymol to these degradation pathways, it will not eliminate all stability issues associated with both thymol and the nanoparticle formulation, so that the stability of a nanoparticle formulation can be affected by other factors as well. Such as, aggregation of particles, leakage of drug from the particle, and alteration in the release characteristics of the particle.

Because of the stability issues encountered with nanoparticle formulations, the formulation of nanoparticle formulations will require a combination of appropriate formulation design, appropriate packaging, and possibly refrigerated storage. This can have an impact on both the distribution and cost of the nanomaterials being produced.

c) Regulatory and Safety Concerns

There are currently no comprehensive regulatory guidelines or processes for the approval of new nanoparticle-based pharmaceutical products; therefore, development of nanoparticle formulations is a rapidly developing area and regulatory agencies are still working on establishing guidelines for assessing the safety and efficacy of these products (Carmo et al., 2023). The characteristics of nanoparticles (for example, their ability to be taken up by cells and their ability to be distributed throughout the body and retained for extended periods of time) create questions surrounding the safety of their use and require careful study.

Toxicological studies have to be conducted to evaluate the safety of nanoparticle products and are essential to assess whether there is the potential for nanoparticles to accumulate in tissues, interact with biological systems at the cellular and molecular levels, or have long-term effects after repeated exposure to nanoparticles. The requirement for toxicological studies increases the length of time spent developing thymol nanoparticle products and adds cost to the overall process of developing and commercialising thymol nanoparticle products.

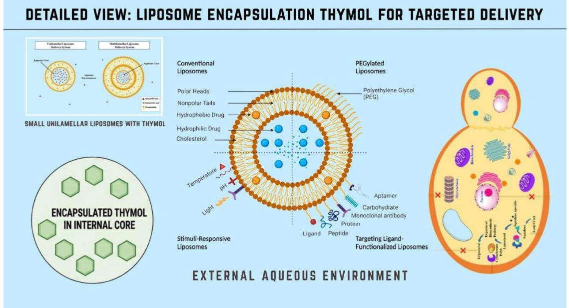
d) Limited Clinical Data

Currently, there is limited clinical evidence available to demonstrate the safety and efficacy of thymol NP dosage forms in treating C. albicans infections, despite promising preclinical findings. Of the few studies available, the majority were performed using in vitro or animal models, with very few human clinical trials reported to date (V. Araújo et al., 2020). Therefore, due to the lack of clinical evidence, it is difficult to predict how thymol NP formulations will perform in clinical practice under conditions of variation among patients and interactions with other medications, which can impact the efficacy of new therapies. Additionally, the limited amount of clinical data available makes it difficult for regulatory agencies to approve new products; thus, physicians do not feel confident prescribing these new types of therapeutic options.

e) Potential for Unexpected Interactions

Nanoparticle formulations are complicated enough that they could result in unexpected interactions with biological systems, with other drugs, or with medical appliances, and these interactions may not show themselves through studies on the individual components, and therefore, may result in adverse experiences or decreased effectiveness (P. H. F. Carmo, et al. 2023).

There has yet to be sufficient investigation conducted into the ability of nanoparticles to alter the pharmacokinetics of traditional drugs, their interaction with proteins in the plasma, or their impact on the functional properties of cellular membranes. Additionally, a thorough review must be conducted regarding whether or not the constituents of nanoparticles may stimulate the immune response or cause an allergic reaction.



3.3 Methodology

3.3.1 Creation of Liposomes of Thymol Acid

The thin-film hydration method was used to make thymol-loaded liposomes. Thymol crystals were added to a solution containing phosphatidylcholine and cholesterol in a 7:3 (wt/wt) ratio which was dissolved in a 2:1 (v/v) chloroform/methanol organic solvent mixture (Greige-Gerges et al., 2013). Lipids/thymol were placed into a round-bottom flask and evaporated under vacuum conditions (40-50 °C, <0.2 Torr) to form a thin film of lipids on the walls of the flask, then dried in a high vacuum for 1 to 2 hours to remove all residual solvents (Engel et al., 2017). The dried films were then rehydrated with pre-warmed phosphate buffered saline (pH 7.4) at temperatures greater than the phase transition temperature of the lipids, and gently rotated for 30-60 minutes to create multilamellar vesicles from swollen lipid layers (Coimbra et al., 2011). To reduce the size of the vesicles and obtain nanosized liposomes, short bursts of probe sonication (in an ice-water bath) were performed until the liposomes became small, uniform unilamellar liposomes (Sennouni et al., 2023). Finally, excess thymol was removed by dialyzing overnight against fresh PBS and was available for subsequent physicochemical analysis (Greige-Gerges et al. 2013).

3.3.2 The inclusion of Liposomes of Thymol into a lipid-wax base in preparation for patches.

After the lipid phase had been developed, the lipid was cooled slowly to approximately 45°C, which is the temperature at which there was enough energy removed from the preformed liposome to not disrupt the liposomal structures before they were added to the undisturbed lipid-polymer matrix (while being slowly stirred), so as to preserve the integrity and structure of the liposomal vesicles, in accordance with established good handling practices for essential oil liposomes (Greige-Gerges et al. 2013). The undisturbed lipid-polymer blend was immediately cast onto the silicone-coated release liner to prepare a uniform film of patch material. The lipid wax nanopatch was prepared with an approximately 35–40°C temperature during the coating of the release liner to allow an even distribution throughout the patch (Sennouni et al., 2023). After the uniformity of the cast layer was completed, the cast layer cooled so that the lipid wax network hardened and dried under controlled conditions to eliminate any remaining moisture without exceeding a temperature that would vaporize thymol and/or cause destabilization of the liposomes (Coimbra et al., 2011). Once the patch was hardened, a backing membrane was laminated to the patch and the patch material was cut into uniform patches. These patches possess all attributes specified in lipid-wax nanopatch systems and can be used as stable forms of antifungal agent applications delivered through the skin by creating a thymol-liposome lipid-wax polymer-nanopatch system for delivery of dermally administered antifungal agents.

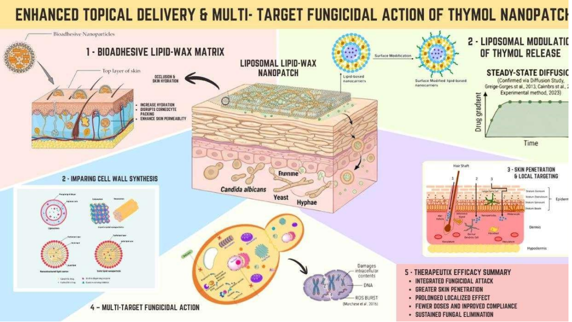
3.3.3 Examine the effectiveness of antifungal patches both in vitro and in vivo.

To test the effectiveness or potential therapeutic activity of the antifungal patches for both in vivo and in vitro studies will consist of microbiologic, permeability, and therapeutic evaluations. The in vitro activity will first be assessed by using the agar well diffusion method to measure the zones of inhibition against C. albicans and other clinically important fungi. This will be performed according to previously validated antifungal screening procedures for thymol-based patches (Sennouni et al., 2023). As these tests are being performed, the permeation characteristics of the topical patches through the skin and drug release from the topical patches will also be evaluated utilizing Franz diffusion cells, which are well-established systems for testing the permeation and/or drug delivery of essential oils some nanocarriers or liposomal formulations, etc. for topical use (Coimbra et al., 2011). For the in vivo experiments on antifungal patches, Wistar rats will be shaved on their dorsal surface (back) and they will then be inoculated with dermatophyte strains through standard pathogen inoculation techniques in accordance with established methodologies using rodent models for antifungal investigators (Thymol Formulation and Effect Document). When visible lesions form, the animals will be split up into groups that will receive a different treatment. There will be seven different treatment groups: Positive Control (placebo), API Control (experimental drug), Nanoparticle Control (experimental nanoparticle), Patch Control (PBTNP), Standard Formulation Control (essential oils), Vehicle Control (placebo), and Excipient Control (control exipients). There will be data collected from the seven treatment groups for every animal and this data will used by the researcher to determine the effectiveness of the patch compared to the other treatment options. All animals will receive treatment over a period of 14 to 21 days and after this time period, an assessment will be make by each researcher for the antifungal efficacy of all treatment groups using KOH mount and fungal culture according to the criteria established for evaluating antifungal therapy based on thymol oil (Sennouni etal.; 2023). Lesion size, erythema, scale swabs, and southern-western blot results will be included with the previous two variables in the assessment of clinical improvement in each of the groups. The safety of all treatment options will be evaluated using standard skin irritation scores for each treated area and comparison with standard histopathological examination of the response of the treated tissue according to the International Society for the Study of Animal Fertility (ISSAF) guidance for evaluating skin irritation and other aspects of topical liposomal and essential oil products (Greige-Gerges etal.; 2013). In conclusion, these in vitro and in vivo assessments provide a clear, scientifically validated method for evaluation via antifungal efficacy and safety of the developed patches.

3.4 Mechanism of Action Of Patch

The composition of the bioadhesive lipid-wax system (in the form of a liposomal lipid-wax nanopatch) facilitates the antifungal activity of thymol via a combination of synergistic effects resulting from controlled release of the drug and multiple pharmacodynamic actions. Following topical administration, the bioadhesive lipid-wax matrix will form a close association (intimate contact) with the stratum corneum creating an environment that permits prolonged retention and localized availability of the drug in the skin. The occlusive nature of the matrix increases the hydration of the skin surface leading to disruption of the close packing of corneocytes within the stratum corneum and thus increasing the permeability of the active pharmaceutical ingredient. The lipid-wax matrix also acts as a reservoir for continuous and sustained release of thymol for long periods of time. The addition of liposomes provides an additional means of modulating the rate of release of thymol, as the release of thymol occurs in a steady-state fashion from the phospholipid bilayer of the liposomes, thus helping to maintain a relatively constant concentration gradient of drug across the skin. The steady-state release of thymol from the liposomes was confirmed using permeation and diffusion studies (Greige-Gerges et al., 2013; Coimbra et al., 2011; Experimental method, 2023). When nanosized liposomes are delivered through the dermis into the skin via dermal drug delivery they also help to improve both penetration and targeting of drugs. The interaction between the phospholipids comprising a liposome and the lipid domains of the stratum corneum will both fluidize the lipids in the stratum corneum and create transient disruptions to the barrier of the skin, thereby facilitating deeper penetration of the liposomes into the epidermis. Liposomes may be able to fuse with biological membranes at these deeper penetrating levels and deliver thymol locally to the site of fungal infection (Greige-Gerges et al., 2013; Coimbra et al., 2011). Thymol demonstrates strong antifungal efficacy against Candida albicans by disrupting the integrity of the fungal cell membrane. Thymol's lipophilic, phenolic structure allows it to be incorporated into the lipid bilayer of the fungi to increase the permeability of the membrane and disrupt its structural integrity by allowing for the exit of its intracellular contents (ions, proteins, etc.). Furthermore, thymol inhibits the biosynthesis of ergosterol, which is necessary for the stability of the fungal membrane, and will lead to the disintegration and lysis of the fungal cell (Ahmad et al., 2011).

This study found that thymol can disrupt membranes and hinder intracellular metabolic activities, such as by inhibiting the enzyme systems of mitochondria and reducing the production of ATP, ultimately leading to a loss of energy. The research also found that thymol creates oxidative pressure resulting in the formation of reactive oxygen species which can damage the lipids, proteins and nucleic acids within the cell (Marchese et al., 2016). It was shown, therefore, that thymol can inhibit adhesion to tissues and decrease the ability of C.albicans to form biofilms, thus resulting in a decreased level of virulence and establishing persistent infections. Integrating sustained-release of thymol from liposomal lipid wax bioadhesive patches facilitates greater penetration into the skin, as well as provides multiple targets for an antifungal effect on C. albicans, to promote greater therapeutic efficacy with fewer doses and improved compliance of patients. The liposomal/lipid wax bioadhesive nanopatch will therefore provide an advanced method for the topical treatment for C. albicans infections.



3.5 Current Research gap

While there has been significant development on vesicular systems for drug delivery, a considerable gap remains regarding the ability to effectively treat dermatophytes and Candida albicans topically. Traditional liposomal formulations have very limited capability to penetrate the skin because of their rigid, multi-layered structure; hence these systems are unable to provide deeper penetration for delivery of active ingredients (Jardan et al 2023; Garg et al 2020). Advanced carriers such as transethosomes provide improved deformability and permeation of vesicles but there has not yet been a sufficient level of investigation into their incorporation into stable, sustained release systems. In addition, there has virtually been no specific research on the creation of nanocarriers that are loaded with thymol and include lipid-wax bioadhesive surfaces to facilitate retention of the drug (and therefore improved therapeutic effect). Most commercially available topical and vesicular systems have poor residence times and rapid clearance of the drug due to a lack of bioadhesive properties. There is also an incomplete understanding of the mechanism of vesicle-skin interactions and drug pathways resulting in an inability to rationally design formulations (Abdalla et al 2021). The vast majority of published data on this topic comprises only in vitro and ex vivo analyses, with little or no evidence from clinical trials. Therefore, development of a thymol-loaded liposomal lipid-wax bioadhesive nanopatch would provide a unique and necessary way to gain improved outcomes with sustained systemic antifungal therapy.

3.6 Novelty

This research shows a high degree of innovation through rational integration of a wide variety of sophisticated pharmaceutical drug delivery technologies into a single therapeutic platform. The study proposes a hybrid system consisting of vaginally-administered; thymol-loaded liposomes and a lipid-wax based bioadhesive nanopatch which has not been explored to any degree in the treatment of superficial infections caused by fungi. Liposomes have been shown to improve drug loading, stability and penetration into the skin; however, when used in conjunction with lipid-wax matrix, they have additional controlled release and greater structural support for the liposomes, thereby creating a dual controlled release delivery system at both nano and macro levels.

The use of thymol as the active ingredient is key since it is a naturally occurring phytochemical with established antifungal properties. All of thymol's inherent disadvantages, such as low solubility in water, rapid evaporation and potential for skin irritation, are mitigated through incorporation into liposomes, therefore making it a safer and more therapeutically efficient alternative.

Additionally, the use of a bioadhesive nanopatch will provide prolonged residence time on the skin, resulting in less drug loss due to external factors (i.e., sweat/water) and increased patient compliance compared to traditional topical forms of therapy (i.e., cream/gel). Moreover, the antifungal formulation developed will provide broad spectrum antifungal activity since the formulation is intended to be active against both dermatophytes and Candida albicans, which are two of the main fungal pathogens associated with superficial fungal infections but typically require different therapies for treatment.

The overall goal of this research is to create a new way to apply topical antifungal medications using an innovative plant-derived and nanocarrier-supported adhesive system that allows for prolonged action and localization of drug delivery to the site of infection, while being safer than existing therapies. This new system will address major shortcomings associated with current treatments, including low retention of medication, frequent administration, and adverse effects or potential side effects.

3.7 Future Prospective

Thymol-loaded liposomal lipid-wax bioadhesive nanopatches offer an innovative approach to applying topical therapies for treating dermatophytosis and Candida albicans for extended periods of time. The use of a liposome as a carrier system allows for improved solubility and stability of thymol, a hydrophobic phytochemical that has been shown to have antifungal activity, and increases its ability to penetrate the skin in the liposome formulation. The addition of lipid-wax matrices provides a controlled release of thymol while remaining in contact with the skin longer, thereby improving the potential for therapeutic success. The optimal formulations have nanoscale vesicle size, high entrapment efficiency, and provide sustained-release characteristics; all of which are critical for effective transdermal delivery (Verma et al., 2021; Sharma et al., 2022).

The bioadhesive nanopatch system can also provide benefits such as extended contact with the skin, fewer doses per day, and improved patient compliance and is particularly useful in treating chronic and recurrent fungal infections where conventional topical treatments often fail due to inadequate penetration and rapid clearance (Patel et al., 2020). In addition, thymol is thought to have antifungal properties through disrupting the cell membrane of the fungus, inhibiting ergosterol synthesis, and increasing membrane permeability, which ultimately leads to fungal cell death (Marchese et al., 2016).

The research efforts to develop novel liposomal systems for delivering drugs directly to a specific location must continue utilizing stimuli-responsive mechanisms targeting the relevant anatomical sites. The combination of microneedle-assisted nanopatching and smart polymer technology could assist with improving the permeability of drugs across the outermost layer of skin (stratum corneum). Combination therapies using thymol together with currently used antifungal agents may provide a synergistic effect and could also provide benefits against antifungal resistance (Khan et al., 2021).

The next phase of this research will involve transferring the above technologies into clinical use and conducting studies on humans for establishing the safety, efficacy, and duration of effects of these treatments. Addressing issues related to large-scale production, stability, and regulatory approvals will be critical to the commercialization of the technologies. With continued advances in computer modeling and computational intelligence, the design and optimization process of these nanocarrier systems could be accomplished in a rational manner, which will facilitate personalization and precision-based topical therapy (Gupta et al., 2023).

4. Conclusion

The creation of a liposomal lipid-wax bioadhesive nanopatch containing thymol is a groundbreaking innovation in topical antifungal delivery systems, specifically, for dermatophyte and Candida albicans treatments. Liposomes are very useful as a drug delivery system for thymol, which is a phytochemical that has excellent antifungal abilities but is hydrophobic; liposomes increase thymol's solubility, stability, and ability to penetrate the skin. Liposomes allow deeper penetration into the skin layers, thus increasing availability of the drug to the site of infection and improving therapeutic benefits.

The use of a lipid-wax matrix also adds to the efficacy of the system by allowing for continued and sustained release of the drug, which is critical to maintaining effective levels of the drug over a long period of time. This sustained release pattern reduces the frequency of drug administration and decreases the amount of fluctuation between peak and trough levels of the drug; consequently, this improves treatment outcomes. The bioadhesiveness of the nanopatch also provides increased amount of time for the patch to remain on the surface of the skin before being lost to external influences like sweat or friction, therefore, improving localized retention of the drug.

The integrated delivery system aims to overcome significant limitations of traditional topical formulations-incomplete skin penetration, rapid clearance of pharmaceutical substances from the body, and inadequate retaining power at the site of application. The integrated delivery system provides a new platform that enables more efficient and compliant patient therapeutic options by integrating liposomal nanocarrier technology with the bioadhesive patch technology.

Overall, thymol-encapsulated liposomal nanopatches containing lipid-wax provide an innovative, safe, and effective method for the treatment of superficial fungal infections. Future studies focused on supporting the clinical validation of these formulations, conducting long-term stability studies, and developing scalable manufacturing processes are critical next steps leading to the practical application of these formulations in dermatology.

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