

***Candida albicans*: Virulence factors, pathogenesis, and ways to diagnose and control its infection**

Candida albicans: Fatores de virulência, patogênese e formas de diagnóstico e controle de sua infecção

Candida albicans: Factores de virulencia, patogénesis y formas de diagnosticar y controlar su infección

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Abstract

The objective of this study was to provide a review on *Candida albicans*, focusing on its main virulence factors, pathogenesis, and methods of diagnosis and control of infections caused by this microorganism, known as candidiasis. Its main virulence factors are adhesion, polymorphism and dimorphism, which aid in tissue invasion, phenotypic variability, tolerance to toxins, and the presence of enzymes such as proteases and phospholipases. These factors confer the fungus with the ability to colonize, establish itself, and consequently, cause infections. *C. albicans* can proliferate on the skin and mucous membranes of the oropharyngeal cavity, gastrointestinal tract, and vaginal tract. The result of this colonization is the formation of white plaques or nodules with erythematous borders in the infected area. Additionally, it may cause pain and burning or be asymptomatic. The diagnosis of candidiasis is based on the symptoms presented by the host. Cultures, histopathological examinations, blood cultures, and serum beta-glucan tests can also be used. The treatment of candidiasis is carried out with antifungals such as nystatin, clotrimazole, fluconazole, itraconazole, and amphotericin B. However, research on medicinal plant products has been conducted to provide an integrative and complementary approach to controlling this pathogen. *Thymus vulgaris* L. is a good example of this. It is a plant with various phytochemicals and recognized biological activities, including antifungal effects. Thus, this study demonstrated some morphological and pathological characteristics of *C. albicans*. It was also possible to understand how candidiasis manifests, and how it can be diagnosed and treated both conventionally and integratively.

Keywords: *Candida albicans*; Candidiasis; Diagnosis; Virulence factors.

Resumo

O objetivo deste estudo foi apresentar uma revisão sobre *Candida albicans*, acerca de seus principais fatores de virulência, patogênese e formas de diagnóstico e controle da infecção provocada pelo micro-organismo, conhecida por candidíase. Seus principais fatores de virulência são aderência, polimorfismo e dimorfismo, que auxiliam na invasão

tecidual, variabilidade fenotípica, tolerância a toxinas e enzimas como proteases e fosfolipases. Estes fatores irão conferir ao fungo capacidade para colonizar, se estabelecer e, portanto, causar infecções. *C. albicans* pode se proliferar na pele e também em mucosas da cavidade orofaríngea, trato gastrointestinal e trato vaginal. O resultado desta colonização é a formação de placas brancas ou nódulos com bordas eritematosas na região infectada. Além disso, pode apresentar dor e queimação ou ser assintomática. O diagnóstico de candidíase é dado com base nos sintomas apresentados pelo hospedeiro. Em adição, podem ser utilizados culturas, exames histopatológicos, hemoculturas e testes séricos de betaglicanos. O tratamento da candidíase é realizado com antifúngicos, como nistatina, clotrimazol, fluconazol, itraconazol e anfotericina B. Entretanto, pesquisas com produtos de plantas medicinais têm sido realizadas no intuito de trazer uma forma integrativa e complementar para o controle deste patógeno. *Thymus vulgaris* L. é um bom exemplo disto. Trata-se de um vegetal com diversos fitocompostos e reconhecidas atividades biológicas, que inclui o efeito antifúngico. Com isso, este estudo demonstrou algumas características morfológicas e patológicas de *C. albicans*. Foi possível também compreender como a candidíase se manifesta, como ela pode ser diagnosticada e tratada de forma convencional como integrativa e complementar.

Palavras-chave: *Candida albicans*; Candidíase; Diagnóstico; Fatores de virulência.

Resumen

El objetivo de este estudio fue presentar una revisión sobre *Candida albicans*, abordando sus principales factores de virulencia, patogénesis y formas de diagnóstico y control de la infección provocada por el microorganismo, conocida como candidiasis. Sus principales factores de virulencia son adherencia, polimorfismo y dimorfismo, que ayudan en la invasión tisular, variabilidad fenotípica, tolerancia a toxinas y enzimas como proteasas y fosfolipasas. Estos factores otorgarán al hongo la capacidad de colonizar, establecerse y, por lo tanto, causar infecciones. *C. albicans* puede proliferar en la piel y también en las mucosas de cavidad orofaríngea, tracto gastrointestinal y tracto vaginal. El resultado de esta colonización es la formación de placas blancas o nódulos con bordes eritematosos en la región infectada. Además, puede presentar dolor y ardor o ser asintomática. El diagnóstico de la candidiasis se realiza en función de los síntomas presentados por el huésped. Además, se pueden utilizar cultivos, exámenes histopatológicos, hemocultivos y pruebas séricas de betaglucanos. El tratamiento de la candidiasis se realiza con antifúngicos. Sin embargo, se han realizado investigaciones con productos de plantas medicinales para proporcionar un enfoque integrador y complementario para el control de este patógeno. *Thymus vulgaris* L. es un buen ejemplo de esto. Se trata de una planta con diversos fitoquímicos y reconocidas actividades biológicas, que incluyen el efecto antifúngico. Con esto, este estudio demostró algunas características morfológicas y patológicas de *C. albicans*. También fue posible comprender cómo se manifiesta la candidiasis, cómo se puede diagnosticar y tratar de manera convencional e integradora y complementaria.

Palabras clave: *Candida albicans*; Candidiasis; Diagnóstico; Factores de virulencia.

1. Introduction

Candida albicans is the most prevalent causative agent of candidiasis, an opportunistic infection, although other species also have been reported, such as *Candida tropicalis*, *Candida glabrata*, *Candida parapsilosis*, *Candida metapsilosis*, *Candida krusei*, *Candida famata*, *Candida guilliermondii* and *Candida lusitaniae*. In the context of candidiasis, an unusual increase in the fungus has been reported in the gastrointestinal, urinary and respiratory tracts. Due to the various causal factors, this disease can be considered a multifactorial syndrome. Symptoms associated with the gastrointestinal tract include bloating, gas, intestinal cramps, rectal itching and altered intestinal function. Symptoms related to the urinary tract include vaginal yeast infections and frequent bladder infections. *C. albicans* can cause vaginal infections that affect around three out of four women at least once in their lifetime, and is also responsible for deep infections related to hospital environments that result in high morbidity and mortality. This fungus can also aggravate certain chronic inflammatory bowel diseases, such as Crohn's disease and hemorrhagic colitis (Poulain, 2015). In addition, *C. albicans* has a great capacity to form biofilms, which characterizes its high virulence, and this formation is resistant to the usual antifungal therapy, the immune system and environmental factors (Gulati & Nobile, 2016).

Other general symptoms described are chronic fatigue, decreased energy and malaise (Martins *et al.*, 2014). Candidiasis can affect healthy individuals, but mainly newborns, immunodeficient patients and people taking broad-spectrum antibiotics, due to changes in their microbiota (Hani *et al.*, 2015). Variations in the immune system due to stress also alter the

resident microbiota, facilitating conditions that contribute to the excessive increase of *C. albicans* in the human body (Nobile & Johnson, 2015).

Since ancient times, the antimicrobial properties of medicinal and aromatic plants have been recognized and used in folk medicine for various purposes (Carretto *et al.*, 2013). *Thymus vulgaris* (thyme) is a plant from the Lamiaceae family, which comprises 150 genera and approximately 2,800 species distributed throughout the world. Thymol and carvacrol, phenolic components of thyme essential oil, have antimicrobial, carminative, and expectorant activities. Originally from Europe, in Brazil the plant is grown in the south and southeast and is used in cooking as a condiment and aromatic herb (Jakiemiu *et al.*, 2010). An experimental study showed that *T. vulgaris* extract was effective against biofilms, promoting high cell viability (above 50%) for all cell lines studied, including murine macrophages (RAW 264.7), human gingival fibroblasts (FMM-1), human breast carcinoma cells (MFC-7) and cervical carcinoma cells (HeLa), anti-inflammatory effect and without showing genotoxicity (de Oliveira *et al.*, 2017).

Thus, the aim of this study was presenting a review on *C. albicans* including virulence factors, pathogenesis, diagnosis, and disease control using conventional and integrative products.

2. Methodology

The present study was a narrative review (Rother, 2007). For this purpose, the studies used were obtained through searches on the PubMed platform, from the National Library of Medicine, and on the Scientific Electronic Library (SciELO).

For article searches, the following keywords were used: (i) *Candida*, (ii) *Candida albicans*, (iii) candidiasis, (iv) diagnosis, (v) pathogenesis, (vi) treatment, (vii) virulence factors, (viii) alternative treatments, and (ix) *Thymus vulgaris*. It is important to note that these keywords were related to the main subject of this study, which was *Candida albicans*.

The studies were selected according to the following criteria: (i) complete articles; (ii) articles published from 2000 onwards; and (iii) articles in the English language.

3. Results and Discussion

3.1 The genus *Candida*

The genus *Candida*, although there are approximately 200 species such as *C. albicans*, *C. parapsilosis*, *Candida tropicalis*, *C. glabrata*, *C. krusei*, *C. guilliermondii* and *C. lusitaniae*, constitutes the main group of yeasts that cause opportunistic infections in humans, *C. albicans* is the most prevalent causative agent of candidiasis, an opportunistic infection due to the diversity of causative factors, this disease can be considered a multifactorial syndrome. Its onset can be related to an inadequate lifestyle, as well as constitutional factors such as gender, age, race and family history, as well as environmental factors such as a sedentary lifestyle, stress, smoking, alcoholism, unhealthy diet and obesity, exposure to high levels of estrogens (contraceptives, pregnancy and hormone replacement), uncontrolled diabetes mellitus, the use of topical and systemic antibiotics and inadequate hygiene habits (Colombo *et al.*, 2013).

3.2 *Candida albicans*

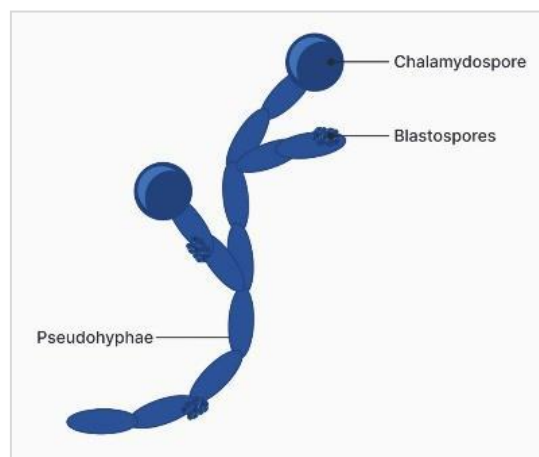
C. albicans is among the most prevalent fungal species in the human microbiota, asymptotically colonizing healthy individuals. The large increase in the incidence of fungal infections in recent decades is mainly correlated with immunosuppressed patients such as AIDS patients, those undergoing chemotherapy, organ transplants and device implants, causing a variety of mucosal infections, including oral and vaginal candidiasis, which can be found in around three out of four women at least once in their lifetime (Wang & Yan, 2015). In addition, it is one of the leading causes of bloodstream infections

in hospital settings in the United States, with an attributable mortality rate of 35 to 60% resulting in high morbidity and mortality (Kadosh, 2019).

3.2.1 Morphology

C. albicans is a fungal species in the forms of yeasts, blastoconidia, pseudohyphae and hyphae, being a polymorphic organism that undergoes morphological transition between them. According to Figure 1, under normal growth conditions, round-shaped spores with thick cell walls called chlamydoconidia can form, which is why the term polymorphism has been replaced by dimorphism, since there are other cell forms between yeast and hyphae (Villar *et al.*, 2004).

Figure 1 - Morphology of *Candida albicans*.



Source: Authors.

Thus, in macroscopic terms, *C. albicans* is characterized by its moist colonial morphology, with a specific, creamy smell, with a yellowish-white, smooth or rough coloration in *Sabouraud* agar culture, with the formation of a germ tube with fermentative capacity and an acidic pH favorable to its proliferation of between 2.5 and 7.5. Microscopically, it has an ovoid shape of the blastoconidium type or elongated, spherical, with a diameter of 3 to 5 μm and when stained with the gram technique they are purple in color indicating gram-positive, there are numerous epidemiological peculiarities in different species of *Candida* for this reason the need for identification at the species level associated with diseases is justified, especially for choosing the best therapeutic approach to be taken (Lu *et al.*, 2014).

3.2.2 Virulence factors

Virulence factors of *C. albicans* have long been associated with its ability to undergo a morphological transition from its yeast forms that present characteristics of single oval cells to filaments with elongated cells being connected end to end, these filaments play an important role in the establishment of biofilm, violation of endothelial cells and macrophages, invasion of epithelial cell beds and contact detection called tigmotropism. The virulence factors increase the effectiveness in the development of infections depending on the stage in places such as mucosa or systemic, these processes are favored by the imbalance between parasite and host. The main known virulence factors of *C. albicans* are adherence, polymorphism/dimorphism that aid tissue invasion, phenotypic variability, thermotolerance to toxins and enzymes such as proteases and phospholipases, all of which confer the fungus' ability to colonize and establish infections (Colombo *et al.*, 2013).

3.2.3 Filamentation

Virulence by filamentation is due to the formation of mycelia and the antigenic variability of their surface and mycelial shape, which favors adherence and makes phagocytosis by the immune system more difficult. When phagocytosed, yeast cells produce hyphae and secrete proteases along the hyphae that kill the phagocytic cells. These hyphae have a greater potential to adhere to human epithelial cells than blastoconidia, and in this format are considered more invasive and pathogenic. Evidence points to an association between filaments and pathogenesis which has shown that a strain of *C. albicans* is deleted for HGC1, encoding a cyclin-related protein important for the growth of these hyphae (Brito *et al.*, 2009).

3.2.4 Biofilm

Biofilms go through four stages until they reach their mature and developed form: adhesion, initiation (also known as proliferation), maturation and dispersion. Biofilms in *C. albicans* are highly structured, containing yeast-like cells, hyphal cells and pseudohyphal cells, plus the extracellular matrix that surrounds them. This biofilm acts as a reservoir of resistant cells that can spread, multiply and seed infections. Once it has matured, it disperses yeast-predominant cells that sprout and disperse their hyphae, spreading into the bloodstream and affected areas, causing large resistant infections. These hyphae observed in the mature biofilm are also used to distinguish *C. albicans* from other species such as *C. parapsilosis*, which have smaller and shorter hyphae contributing to lower resistance (Lohse *et al.*, 2018).

In addition to forming biofilm on host surfaces such as mucous membranes, epithelial cells and parenchymal organs, it is also present on implanted medical devices such as catheters, pacemakers, heart valves and joint prostheses and dentures, considering that most of the target audience for these implants are immunocompromised people where fungal infection is becoming more prevalent every day. Existing drugs against planktonic *C. albicans* are largely ineffective against *C. albicans* cells in biofilms and when administered in large doses, the side effects on patients are very high, making conventional treatment less efficient and risky (Lohse *et al.*, 2018).

3.3 Pathogenesis

Candidiasis caused by the fungus *C. albicans* has relevant clinical manifestations in the vast majority of patients. It is a commensal fungus that colonizes the oropharyngeal cavity, gastrointestinal tract, vaginal tract and skin (Mukaremera *et al.*, 2017). There are several clinical manifestations arising from candidiasis in these areas, which can range from superficial mucocutaneous disorders to serious infections affecting various organs. Oral candidiasis is the most common fungal infection in the oral cavity (Lewis & Williams, 2017) and is caused by *Candida* species. It is the transition from normal to pathogenic flora that allows the opportunistic infection to take hold, as there is an overgrowth of *Candida*. It is a disease with wide-ranging clinical manifestations, so it is divided into primary candidosis (in which only the oral cavity and perioral areas are affected) and secondary candidosis (in which the infection occurs as part of a systemic disease (Baumgardner *et al.*, 2019). The manifestations in the oral cavity can be variable, with the acute pseudomembranous form (called thrush by the population) being the most common.

The lesions resulting from this infection are white plaques or nodules with erythematous borders, and can also be asymptomatic or present pain and burning. In the gastrointestinal tract, infections can arise from dysregulation of the resident microbiota due to immune dysfunction and through damage to the intestinal mucus barrier, with esophageal candidiasis being the most common infectious esophagitis caused by fungi (Allert *et al.*, 2018). Among the most common clinical manifestations are acute odynophagia, dysphagia and costochondritis. On the skin, *C. albicans* is responsible for around 80% of fungal infections. Symptoms may appear such as: Erythema, erosions, superficial skin and mucous membrane lesions, itching and

burning. It should be noted that the severity of the lesions depends on the site of the body affected (Bologna *et al.*, 2018). and other previous factors (in immunocompromised people, the fungal infection can evolve and become fatal).

3.4 Diagnosis

The diagnosis of candidiasis is generally based on the patient's symptoms, as *Candida* infections have typical symptoms. In addition, fungal cultures, histopathological examinations, blood cultures and serum beta-glycan tests can be carried out to confirm the diagnosis. As it is a commensal, just an increase in the amount of *Candida spp* in the vagina, mouth, feces, skin or urine is not enough to indicate an infection, so there needs to be a corresponding clinical lesion, as well as histological evidence showing tissue invasion (Pappas *et al.*, 2016). It should be noted that if a culture is carried out and specimens are found in previously sterile areas, such as blood, cerebrospinal fluid and the pericardium, systemic therapy must be started. If invasive candidiasis is suspected, serum beta-glycan tests can be carried out, as a positive result indicates a high probability of invasive candidiasis.

3.5 Treatment

Treatment is carried out with antifungal medication. Candidiasis affecting the skin, vagina and mouth is treated with topical application of antifungal medication to the affected area. The most commonly used drugs are nystatin with zinc oxide and clotrimazole. The doctor may also choose to prescribe fluconazole orally (as there are no creams or ointments) in clinically stable patients. In infections of the gastrointestinal tract, such as esophageal infections, therapy is based on the use of oral fluconazole or itraconazole (Pappas *et al.*, 2016). Drugs such as anidulafungin, caspofungin, micafungin (echinocandins) or amphotericin B administered intravenously, voriconazole administered orally or intravenously, isavuconazole administered orally and posaconazole administered orally are used in more severe cases of invasive candidiasis in which the first-choice drugs have not been effective. In general, candidiasis requires treatment over a period of 14 to 21 days.

3.6 Alternative treatments

Conventional pharmacological treatment varies according to the site of infection. For example, when it affects the vulva and vagina, the most commonly used antifungal drugs are from the azoles class, which includes imidazoles (butoconazole, clotrimazole, miconazole and ketoconazole), triazoles (fluconazole and terconazole), which act by inhibiting the synthesis of ergosterol present in the fungal cell, and the class of polyenes (amphotericin B and nystatin), which alter the permeability of the fungal cell membrane (Costa *et al.*, 2003). The first-line drug for *C. albicans* endocarditis is amphotericin B, often followed by fluconazole because of frequent relapses (Ellis *et al.*, 2001; Bezerra *et al.*, 2020).

Although the treatment of candidiasis with antifungal drugs is highly effective, their use is associated with adverse reactions, such as changes in taste, gastrointestinal and allergic symptoms (Bakhshi *et al.*, 2012). In addition, studies have shown the resistance of *Candida spp.* to antifungal drugs due to their frequent and long-lasting use (Maubon *et al.*, 2014; Bailly *et al.*, 2016), which is common among denture wearers (Bailly *et al.*, 2016; Lewis & Williams, 2017; Gad & Fouda, 2020).

In this sense, more and more studies are being carried out on the application of herbal medicines as an alternative to fungal diseases. The most studied plants are *Melaleuca atherfolia* (tea tree), *Allium sativum* (garlic) and *Ricinus communis* (castor bean) (Pereira *et al.*, 2005). The antifungal activity observed in the *Punicaceae* family, in a study by Vasconcelos *et al.* (2003), may be related to the presence of flavonoid glycoside tannins, components that have antimicrobial and anti-inflammatory properties.

3.6.1 Thyme (*Thymus vulgaris*)

Thymus vulgaris is an aromatic and medicinal plant. The genus *Thymus* has 928 recognized species in Europe, North America, South America, Asia and Australia (Micucci *et al.*, 2020). A study of *T. vulgaris* grown in Romania has been described in the literature in order to understand the chemical and antimicrobial characteristics of the plant's essential oil. There, it is known for its expectorant, antitussive, antibroncholytic, antispasmodic, anthelmintic, carminative and diuretic properties. According to this study, the essential oil has thymol, terpinen and p-cymene as its main substances, respectively (Borugă *et al.*, 2014).

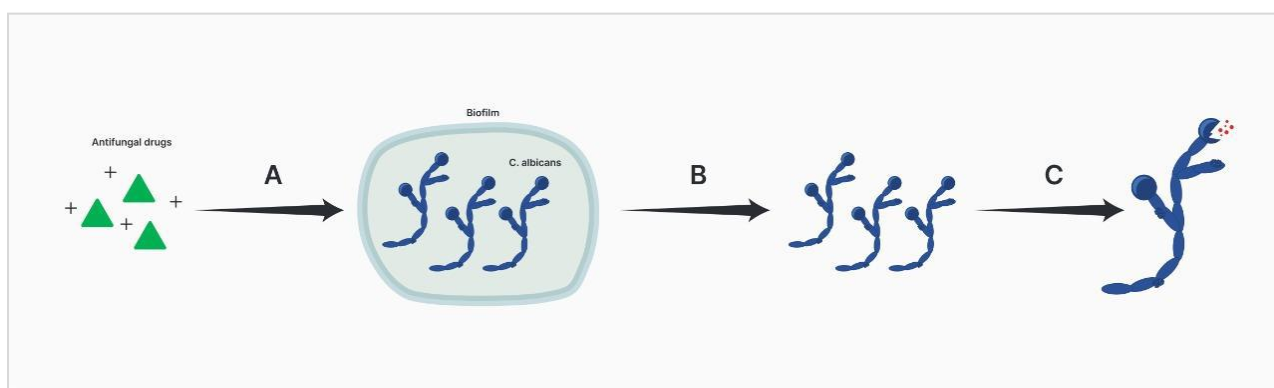
Thymol and thyme essential oil have long been used in traditional medicine, especially in the treatment of upper respiratory system ailments, due to their expectorant, antiviral and antiseptic properties. New studies have documented other properties inherent to these compounds, such as antibiofilm, antifungal, antileishmanial, antiviral and anticancer properties. In addition, new presentations containing these substances, such as nanocapsules, have been produced to extend their use (Kowalczyk *et al.*, 2020). The essential oil in its solid state shows antimicrobial characteristics against pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *C. albicans*, in a similar way to the oil in its liquid state, but without activity against commensal strains (Micucci *et al.*, 2020).

The role of biofilm in the virulence and pathogenicity of *Candida sp.* consists of increasing the level of resistance and protection against the host's defense system (Cavalheiro & Teixeira, 2018). Thus, the inhibition or eradication of pre-formed pathogenic biofilm by anti-infective agents is considered an effective approach to fight infections (Jafri & Ahmad, 2020).

The cell membrane of fungi contains ergosterol. Ergosterol is essential for their growth and proper functioning. Thymol's probable antifungal mechanism is its ability to act on the metabolism of fatty acids, including ergosterol. It is able to reduce the extracellular polymeric matrix by increasing reactive oxygen species and oxidative stress. Thymol treatment has been shown to decrease ergosterol in *Candida* membranes, disrupting and causing enzymatic disturbances to the membrane, as well as extensive damage and cell death (Figure 2). A study demonstrated the antifungal activity of *T. vulgaris* essential oil against, among other pathogens, *Candida* spp. (*C. albicans*, *C. glabrata*, *C. kefyr* and *C. parapsilosis*), compared to amphotericin, at concentrations between 0.5 and 10mg/ml (Al-Shahrani *et al.*, 2017).

The antifungal activity of thyme extract is probably explained by its hydrophobic property, which can bind to the fungal plasma membrane and affect its proliferation by interfering with membrane permeability or inhibiting spore germination and cellular respiration (Mohammadi *et al.*, 2019).

Figure 2 - Effects of *T. vulgaris* L. on *Candida albicans* infection. (A) Essential oil and thymol potentiate antifungal action. (B) Essential oil and thymol inhibit biofilm formation. The interaction of thymol with the cell envelope leads to rupture of the cell membrane, causing leakage of the intracellular content and consequently cell death (C).



Source: Authors.

4. Conclusion

This study demonstrated morphological and pathological characteristics of *C. albicans*. Furthermore, it was possible to understand how candidiasis manifests, how it can be diagnosed and treated both conventionally and integratively with medicinal plant products.

Future studies, for our research group, involve analyses of other *Candida* species, including those that have shown a greater capacity to deactivate antifungals. Additionally, studying more species of medicinal plants with the potential to eliminate these fungi.

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