ANEXOS

ARTIGOS CIENTÍFICOS

Originais

- Publicado: Hidrogel contendo compostos de óleo de *Syzygium aromaticum* (cravo) são eficazes contra o crescimento de *Candida* spp.
- Aceito: Caracterização Genômica de *Klebsiella pneumoniae* Isolada de Sedimento de Manguezal do Rio Anil, Maranhão, Brasil.

Revisão

- Aceito: Aplicações antimicrobianas e regenerativas de hidrogéis carregados com óleos essenciais: uma revisão.

Capítulos de Livros

- FARAH, Emmanueli Iracema; GRACIONE FILHO, José; SOUSA, Débora Batista Pinheiro; CARVALHO NETA, Raimunda Nonata Fortes.Bioética aplicada aos comitês de ética em pesquisa humana. *In*: SOUSA, Débora Batista Pinheiro; CARVALHO NETA, Raimunda Nonata Fortes (org.). **Bioética**: uma abordagem à luz da biodiversidade e biotecnologia. São Luís: EDUFMA, 2023. p. 77-90.

REVISTA CADERNO PEDAGÓGICO – Studies Publicações Ltda.
ISSN: 1983-0882

Hydrogel-containing compounds from Syzygium aromaticum (clove) oil are effective against the growth of Candida spp.

Hidrogel contendo compostos de óleo de *Syzygium aromaticum* (cravo) são eficazes contra o crescimento de *Candida* spp.

Los hidrogele que contienen compuestos de aceite de *Syzygium aromaticum* (clavo) son eficaces contra el crecimiento de *Candida* spp.

DOI: 10.54033/cadpedv21n13-510

Originals received: 11/29/2024
Acceptance for publication: 12/20/2024

Emmanueli Iracema Farah
Doctoral student in Biodiversity and Biotechnology
Intituition: Universidade CEUMA
Address: São Luis, Maranhão, Brazil
E-mail: emmanuelifarah@gmail.com

Joveliane de Melo Monteiro
Doctoral student in Biodiversity and Biotechnology
Universidade CEUMA
Address: São Luis, Maranhão, Brazil
E-mail: jovelianemello53@gmail.com

Marliete Carvalho da Costa
Doctor in Microbiology
Intituition: Universidade CEUMA
Address: São Luis, Maranhão, Brazil
E-mail: cmarliete@yahoo.com

Pedro Agnel Dias Miranda Neto
Master in Oncology
Intituition: Universidade Federal do Maranhão
Address: São Luis, Maranhão, Brazil
E-mail: profpedroagnel@gmail.com

Luciana Protazio Dias Araujo
Master in Chemistry

Intituition: Faculdade Laboro
Address: São Luis, Maranhão, Brazil
E-mail: lucianaprotazio@hotmail.com

Bruno de Almeida Nunes
Doctor em Ciências (Biologia Molecular e Celular)
Intituition: Faculdade de Ciências da Saúde Pitágoras de Codó
Address: São Luis, Maranhão, Brazil
E-mail: nunesb.bioufma@hotmail.com

Marcelo Farias de Sousa
Biomedicine student
Intituition: Universidade CEUMA
Address: São Luis, Maranhão, Brazil
E-mail: maarceelofaariias@gmail.com

Benicio Rodrigues Sérgio
Graduated in Bachelor of Medicine
Intituition: Faculdade Ciências da Saúde Pitágoras de Codó
Address: São Luis, Maranhão, Brazil
E-mail benirodrigues@hotmail.com

Andrea de Souza Monteiro
Doctor in Microbiology
Intituition: Universidade CEUMA
Address: São Luis, Maranhão, Brazil
E-mail: andreasmont@gmail.com

ABSTRACT
Hydrogels containing essential oils are promising formulations for use with antimicrobial purposes. Therefore, this study was proposed to verify the anti-candida potential of hydrogels containing clove essential oil. For this purpose, clove oil and clove essential oil were used in the tests against the yeasts *Candida parapsilosis, Candida albicans,* and *Candida krusei*. The minimum inhibitory concentration (MIC) was determined by the microdilution method. Furthermore, the fungistatic activity was evaluated by the time-kill curve assay. The MIC assays indicated that clove essential oil presented a MIC of 32 µg/mL after 48 hours; this compound was observed to have greater efficacy than the crude oil. The kill curve assays indicate that clove essential oil was more effective, with a MIC of 64 µg/mL for all Candida strains tested after 24 and 48 hours. Hydrogel formulations were made with sodium alginate, clove oil, and clove essential oil. Physicochemical tests were then determined, and microbiological stability was determined for fungi and bacteria up to 60 days of maintenance of the hydrogel at a temperature of 30°C (±2), as recommended by the Brazilian Pharmacopoeia. The hydrogel formulations containing clove essential oil effectively controlled the *Candida* species' growth. The results suggest that clove essential oil is a promising antifungal agent against *Candida* spp—infections, especially in increasing resistance to conventional treatments.

Keywords: *Candida* species. Hydrogel. Anti-candida. Clove essential oil.

RESUMO

Os hidrogéis contendo óleos essenciais são formuações promissoras para utilizados com finalidade antimicrobiana. Sendo assim este estudo foi proposto para verificar o pontencial anti-candida de hidrogéis contendo óleo essencial de cravo. Para tanto, nos ensaios foram utilizandos óleo de cravo e óleo essencial de cravo frente as leveduras *Candida parapsilosis Candida albicans* e *Candida krusei*. A concentração inibitória mínima (MIC) foi determinada pelo método de microdiluição. Em adição, a atividade fungistática foi avaliada através de ensaio de curva de tempo morte. Os ensaios de CIM indicaram que o óleo essencial de cravo apresentou uma CIM de 32 µg/mL após 48 horas, foi observado que este composto tem uma maior eficácia em comparação com o óleo bruto. Nos ensaios de curva de morte. Os resultados indicam que o óleo essencial de cravo foi mais eficaz, com uma CIM de 64 µg/mL para todas as cepas de *Candida* testadas após 24 e 48 horas. As formulações de hidrogel foram realizadas com alginato de sódio e óleo de cravo e óleo essencial de cravo. Os testes físico-quimicos foram então determinados e a estabilidade microbiológica foi determinada para fungos e bactérias até 60 dias de manutenção do hidrogel em temperatura de 30°C (±2), conforme recomendações da Farmacopeia Brasileira. As formulações de hidrogel contendo óleo essencial de cravo foram eficazes em controlar o crescimento das espécies de *Candidas* testadas. Os resultados sugerem que o óleo essencial de cravo é um agente antifúngico promissor contra infecções por *Candida* spp., especialmente em um contexto de resistência crescente aos tratamentos convencionais.

Palavras-chave: Espécies de *Candida.* Hidrogel. Anti-cândida. Óleo essencial de cravo..

RESUMEN

Los hidrogeles que contienen aceites esenciales son formulaciones prometedoras para su uso con fines antimicrobianos. Por lo tanto, se propuso este estudio para verificar el potencial anti-cándida de los hidrogeles que contienen aceite esencial de clavo. Para este propósito, se utilizaron aceite de clavo y aceite esencial de clavo en las pruebas contra las levaduras *Candida parapsilosis*, *Candida albicans* y *Candida krusei*. La concentración mínima inhibitoria (CMI) se determinó mediante el método de microdilución. Además, se evaluó la actividad fungistática mediante el ensayo de curva de tiempo de muerte. Los ensayos de CMI indicaron que el aceite esencial de clavo presentó una CMI de 32 µg/mL después de 48 horas; se observó que este compuesto tenía mayor eficacia que el aceite crudo. Los ensayos de curva de muerte indican que el aceite esencial de clavo fue más efectivo, con una CMI de 64 µg/mL para todas las cepas de *Candida* probadas después de 24 y 48 horas. Las formulaciones de hidrogel se elaboraron con alginato de sodio, aceite de clavo y aceite esencial de clavo. Luego se realizaron pruebas fisicoquímicas y se determinó la estabilidad microbiológica para hongos y bacterias hasta 60 días de mantenimiento del hidrogel a una temperatura de 30°C (±2), según lo recomendado por la Farmacopea Brasileña. Las formulaciones de hidrogel que contienen aceite esencial de clavo de olor controlaron eficazmente el crecimiento de las especies de Candida. Los resultados sugieren que el aceite esencial de clavo de olor es un agente antifúngico prometedor contra las infecciones por *Candida* spp., especialmente en el aumento de la resistencia a los tratamientos convencionales.

Palabras clave: Especies de Candida. Hidrogel. Anti-cándida. Aceite esencial de clavo.

1 INTRODUCTION

Infections caused by yeasts of the genus *Candida* have become a growing concern today, with a high mortality rate among critically ill patients (Rebuma et al., 2024; Araujo et al., 2024; Koleri et al., 2023). However, it is observed that some risk factors and chronic comorbidities predispose patients to developing fungal infections. Furthermore, during the SARS-Cov2 Pandemic, the development of fungal infections was observed in patients who had COVID-19, increasing the risk of death in these individuals (Araujo et al., 2024; Wu et al., 2020).

Among the most relevant species of the genus *Candida* is *Candida albicans* (Escribano et al., 2018); *Candida parapsilosis* (Daneshnia et al., 2024*); Candida tropicalis* (Megri et al., 2020), *Candida glabrata* (Zakhem et al., 2021) *Candida krusei (*Kaur et al., 2020), and the emerging *Candida auris* (Mulet et al., 2020). The species latter has raised the level of concern since high mortality rates of infected patients are indicated during episodes of candidemia (Mulet et al., 2020).

The most relevant aspect of the genus *Candida* is its increasing rate of resistance to azole antifungals, especially fluconazole (Fekkar et al., 2021). The finding in research on candidemias suggests that *C. parapsilosis* isolates carrying Y132F9 (a mutation related to fluconazole resistance) are associated with a higher patient mortality rate than seen for isolates without the mutation; these findings increase concern with the epidemiological surveillance of these clones (Corzo-Leon et al., 2021).

Resistance to antifungals commonly used in *Candida* infections is an artifact that has drawn attention to research into natural products derived from plants as a successful alternative to conventional antifungals. Therefore, essential oils are considered promising agents, as they are biocompatible with human skin and mucous membranes (Bora et al., 2023; Lamarra et al., 2020). They also have healing and anti-inflammatory properties, which denotes their importance as multifaceted pharmacological agents (Avola et al., 2020; Liao et al., 2021).

Advances in biomedical research have shown promise in producing innovative technologies that propose the formulation of hydrogels, dermal patches, and chitosan nano-emulsions incorporating essential oils to optimize medical applications (Bora et al., 2023; Nova et al., 2024; El-Naggar et al., 2023). These include the treatment of wounds with the aim of healing, however, to control skin and mucosal infections caused by pathogenic microorganisms (Bora et al., 2023; Khezri et al., 2020).

Essential oils such as clove and cinnamon are effective against *Candida albicans*; however, there is still little research on formulations for medical applications (Biernasiuk et al., 2022; da Silva Campelo et al., 2021; Tran et al., 2020). Clove oil essential has eugenol (4-allyl-2-methoxyphenol) as the primary antimicrobial constituent (Maggini et al., 2023). The eugenol has adverse modulating effects on the gene expression of the efflux pump genes *MDR1*, *MDR4*, *erg11A,* and *MedA*, associated with resistance to multiple drugs on filamentous fungi *(Sen et al., 2023).* Therefore, this study aimed to study clove essential oils and clove oil in formulating hydrogel active against the growth of *Candida albicans, Candida parapsilosis, and Candida krusei* clinical isolates.

Candidiasis is an infection caused by yeast species of the *Candida* and is typically opportunistic (Qadir et al., 2020; Alizadeh et al., 2021). Species of the genus *Candida* are part of the average human microbiota and colonize many niches, such as the gastrointestinal tract and the vaginal microbiota, presenting a commensal relationship (Chow et al., 2022). Candidiasis can occur due to an imbalance between the host and microbes, triggered by changes in the tissue barrier, due to changes in the immune response, or by impairment of the body's natural defenses (Chow et al., 2022; Gaziano et al., 2023).

More than 150 species of *Candida* have already been identified, and the *Candida albicans* species is the most frequently observed in infections (Alonso-Alonso et al., 2022). However, in recent years, the emergence of other opportunistic pathogenic species, non-albicans, has been observed, which appears to present an intrinsic resistance to treatment with azoles (Alonso-Alonso et al., 2022; Henriques, et al., 2020).

Infections caused by *Candida* species are the leading causes of morbidity and mortality and are associated with a wide variety of clinical manifestations. In general, they predominate in mucous membranes, skin tissue, and nails and, in some cases, produce systemic infections in the lungs, kidneys, heart, and central nervous system (Rautemaa-Richardson et a., 2021; Huggins et al., 2021; Strobl et 2024; Alonso-Alonso et al., 2022).

The pathogenicity of yeast is determined by genetic factors, which will only be expressed when subjected to favorable conditions (Czajka et al., 2023; Gunaratnam et al., 2021). Factors such as the *Candida* strain adhesion capacity are considered one of the most important virulence factors (Gunaratnam et al., 2021). Another essential characteristic of pathogenicity is dimorphism, which facilitates the penetration and dissemination of the microorganism throughout the host's body. In the yeast form, it can be quickly disseminated throughout the organism, and in the hyphal form, it can invade tissues and form biofilms (Gunaratnam et al., 2021). An aspect related to the recurrence of infections and the development of antifungal resistance is the ability of *Candida* species to form biofilms on biotic or abiotic surfaces (Kaur et al., 2023). Biofilm organization provides advantages to yeast survival and increases resistance to conventional antifungals (Ramos et al., 2020).

Essential oils are mixtures of volatile and aromatic compounds, represented mainly by monoterpenes and sesquiterpenes and, in lower concentrations, aldehydes, alcohols, ethers, and phenols (Paulino et al., 2022). Due to their diverse nature, essential oils can be extracted from different parts of plants, such as flowers, leaves, seeds, and even bark, so EOs can be extracted using various techniques (Chebbac et al., 2022).

Essential oils (EO) are composed of various chemical substances, significantly reducing the development of resistance to these molecules (Iseppi et al., 2021). This unique characteristic of EOs is due to their complex composition, allowing them to simultaneously act on several cellular targets. The cell membrane microbial is one of the first places to be affected by EOs (Iseppi et al., 2021). The lipophilic substances present in EOs can integrate into the cell membrane, altering its proper functioning. This integration can lead to membrane permeability changes, affecting cellular homeostasis and resulting in microbial death. In addition to affecting the cell membrane, EOs can directly interfere with the function of various enzymes and proteins within the cell. These interferences can inhibit crucial enzymatic processes, impairing the cell's ability to perform its normal functions (Chebbac et al., 2022). Another point of impact of EOs is the metabolism of fatty acids. They can interfere with fatty acid metabolic pathways, altering the production and storage of energy within the cell. These metabolic changes can significantly affect cell growth and survival (Iseppi et al., 2021).

EOs act by causing damage to the cytoplasmic membrane of bacteria, such as *Vibrio vulnificus*, *Escherichia* coli 0157:H7, and *Salmonella enterica* subsp enterica serovar Typhi, leading to a decrease in the synthesis of adenosine triphosphate (ATP), causing significant energy losses (Turgis et al., 2009; Luo et al., 2022). Research also shows increased levels of reactive oxygen species (ROS); in turn, these compounds cause lipid peroxidation of microbial cell membranes, thus reducing the permeability and integrity of cell membranes(Kong et al., 2022).

Syzygium aromaticum (L.) Merr. Commonly called clove, it is a tree belonging to the *Myrtaceae* family and is a promising medicinal plant for its antimicrobial use. clove essential oil (EO) is made up of several compounds. The main component of EO is eugenol (4-Allyl-2-Methoxyphenol), representing 50% of its composition. In turn, eugenyl acetate, β-caryophyllene, and α-humulene remain in the EO composition (Buru et al., 2022).

Eugenol is a phenylpropanoid derived from synthesizing the amino acid phenylalanine (Nisar et al., 2021). It has an intense taste and smell, being the most volatile molecule in the constitution of clove EO. Due to its volatile nature, it is proposed for the use of EO in emulsified systems, nano-emulsions, or conventional emulsions, and or its incorporation in hydrogel formulations, which increases the stability and permanence of clove essential oils on surface films, has slow-release when applied to the surface of the skin and mucous membranes (da Silva et al., 2021; Gandhi et al., 2021)

I faced an increase in the number of reports of resistance to available synthetic antifungals presented by *Candida* species. There has been an increasing number of studies on natural products that seek better results in treating patients and present themselves as potential therapeutic alternatives. Clove essential oil (*Syzygium aromaticum*), possessing established antimicrobial and anti-inflammatory properties, is one of the products exhibiting promising therapeutic potential (Haro-González et al., 2021).

Clove essential oil, known for its antimicrobial and analgesic properties, can be incorporated into an emulsion to facilitate topical application and increase its stability. Clove essential oils, often used in perfumes and skin care products due to their pleasant aroma and relaxing properties, can be formulated into hydrogel to improve their even dispersion in cosmetic products. Hydrogels are used in wound dressings because they help maintain a moist environment, promoting healing and relieving pain (Hidalgo et al., 2024). Hydrogels are also effective in removing necrotic tissue and preventing infections. When incorporated with healing and antimicrobial components, hydrogel formulations become interesting from a pharmacological point of view.

However, few hydrogels with clove essential oil are currently being explored, and the physicochemical study of formulations with botanical components is not being done.

3 METHODOLOGY

Microorganisms

The *Candida* spp clinical isolates and ATCCs belong to the Applied Microbiology Laboratory at the CEUMA University. The yeast cultures are stored in inclined tubes containing Sabouraud agar medium (Difco) at 4°C and cryopreserved in vials at -80°C in Sabouraud broth (Difco) médium supplemented with 20% glycerol.

Determination of minimum Inhibitory concentration (MIC)

The microdilution method determined the minimum inhibitory concentration (MIC) using a 96-well plate in increasing concentrations. The stock solutions containing clove essential oil (CEO), clove oil (fix) (CO), and eugenol will be diluted and transferred to the first well; the following will contain a serial dilution of the compounds to obtain a concentration between 100 and 0.1 mg/mL.

The suspension of strains *C. parapsilosis* ATCC 22019 and *C. albicans* ATCC 10231, *C. krusei* ATCC 6258, *C. albicans* CA1, *C. parapsilosis* CA3, and *C. krusei* CA4, previously prepared at a concentration of $1x10^6$ cells/mL will be adjusted in RPMI 1640 medium (Sigma -Aldrich) so that it contains $1x10^3$ cells/mL. It was then added to the wells. Negative controls containing only RPMI medium and solutions were included. The positive growth control was composed of fungal inoculum and RPMI. The plates were incubated at 37 °C for 48 h.

The Minimum Inhibitory Concentration (MIC) was defined as the lowest compound concentration that inhibits growth compared to the control. Three independent experiments were performed in duplicate.

Time-kill-curve assay

The fungistatic and fungicid activity of clove essential oil (CEO) and clove oil (CO) was evaluated using clinical strains *C. albicans* CA1, *C. parapsilosis* CA3, and *C. krusei* CA4. The time-kill curve of these yeasts was also assessed. For the tests, the compounds were diluted at concentrations of 16 µg/mL, 32 µg/mL, 64 µg/mL and 128 µg/mL. The compounds were added together with a fungal suspension at a concentration of 10^6 cells/ml in RPMI 1640 medium (without phenol red, Sigma-Aldrich) in wells of a 96-well polystyrene plate, and a final volume of 200 µL in each well; the plate will be incubated an oven at 37 ºC. To determine cell viability, an aliquot of 100 μL will be taken from each well at intervals of 0, 6, 12, 24, 36, 48, and 72 hours and subjected to serial dilution in 900 microliters (1:10) in 0.9% saline. After dilution, a 20 μL aliquot of the dilution was added to Saboraud agar medium surfaces for colony counting according to the exposure time/concentration of the compounds. Yeast colony count data were expressed in Log CFU/mL. All tests were performed in triplicate.

Hydrogel preparation

The hydrogel formula was made with 2% and 4% sodium alginate (Sigma Aldrich), Twen 20% (Sigma Aldrich), 2% clove essential oil (CEO), crude clove oil (fix) (CO), and eugenol (Sigma-Aldrich) and essential oil (doTerra), in a final volume of 100 ml of formulation. After formulation, about 5 milligrams of the gel were added to perforated circular wells of about 4 mm in diameter in Sabouraud agar containing inocula of *C. albicans* CA1, *C.parapsilosis* CA3, and *C . krusei CA4* at a density of 0.2 OD in a spectrophotometer at 600 nm. After adding the gel to the wells, the plates were incubated for 48 hours at 37ºC, and the inhibition zones were then determined in cm. All assays were performed in triplicate.

Physical-chemical characterization: spreadability test

The spreadability of the cosmetic gel was determined by inserting equivalent to 1g between glass plates on a graph paper scale, with a glass plate equivalent to approximately 500 g placed on the sample. After 1 minute, the covered surface was measured using a graduated ruler, measuring the diameter in two opposite positions, and these values were calculated using the average diameter formula. The spreadability (Ei), determined at 25°C, was calculated using equation 2:

$$S = \frac{MxL}{T}$$

Where, \
S-Spreadability, g.cm/s
M-Weight put on the upper glass
L-Length of glass slide
T-Time for spreading gel in sec.

pH assessment

The pH of topical gel formulations was assessed using pH strips and a digital pH meter.

Stability of microbiological contamination

As recommended in the Brazilian Pharmacopoeia, 5th edition, the microbiological evaluation was carried out by counting total viable microorganisms (bacteria, fungi, and yeasts) on plates, using Sabouraud-dextrose agar and casein-soy agar (TSA-TrypticSoy Agar, Difico), respectively. The plates containing Sabouraud-dextrose were incubated in an oven at 28°C ± 2°C, and the TSA plates were incubated in an oven at a temperature of 35°C ± 2°C, both for 24 hours. At the end of the incubation period, colony counts were performed on the plates where growth occurred, reported in CFU/mL.

4 RESULTS AND DISCUSSIONS

A total of 3 strains of *Candida* spp. as *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, and three clinical strains were analyzed for susceptibility to clove oil and clove essential (Table 2). Candidiasis, a fungal infection caused by multiple *Candida* species, presents a substantial global health challenge owing to escalating resistance to conventional antifungal agents.

REVISTA CADERNO PEDAGÓGICO – Studies Publicações Ltda.
ISSN: 1983-0882

Table 1- Minimum inhibitory concentration of clove essential oil and clove oil against *Candida* spp

Compounds	strains	Minimum inhibitory concentration	
		MIC 24h	MIC 48h
		Concentration (µg/mL)	
Clove oil	C. *krusei* ATCC 6258	128	64
	C. albicans ATCC 10231	128	64
	C. parapsilosis ATCC 22019	128	64
	C. albicans CA1	128	64
	C. parapsilosis CA3	128	64
	C. krusei CA4	128	64
Clove essential oil	C. *krusei* ATCC 6258	64	32
	C. albicans ATCC 10231	64	32
	C. parapsilosis ATCC 22019	64	32
	C. albicans CA1	64	32
	C. parapsilosis CA3	64	32
	C. krusei CA4	64	32
Anf. B	C. *krusei* ATCC 6258	64	64
	C. albicans ATCC 10231	64	64
	C. parapsilosis ATCC 22019	64	64
	C. albicans CA1	64	64
	C. parapsilosis CA3	64	64
	C . krusei CA4	64	64

Natural compounds offer promising alternatives; clove oil, in particular, exhibits potent antifungal properties. Originating from *Syzygium aromaticum*, clove oil has undergone extensive research recently due to its diverse biological activities, including efficacy against *Candida* species. In this study, it was possible to observe an antimicrobial effect of clove oil and clove essential oil against *C. albicans* CA1, *C. parapsilosis* CA3, and *C. krusei* CA4. Similar results were observed in studies conducted by other authors, which tested efficiency in *Candida albicans* and *Candida* spp. not-albicans, with MICs verified in concentrations of 0.5–2 and 0.25–2 mg/mL, respectively (Biernasiuk et al., 2022).

REVISTA CADERNO PEDAGÓGICO – Studies Publicações Ltda.
ISSN: 1983-0882

Time-kill-curve assay in clinical isolates

The results indicate that the essential oil of clove was more effective, with a MIC of 64 μg/mL for all *Candida* strains tested after 24 and 48 hours. In contrast, crude oil showed an MIC of 128 μg/mL for most species after 24 hours, with some species reducing to 64 μg/mL after 48 hours (Figures 1, 2, and 3). These data suggest that the essential oil clove has more powerful active components or, in higher concentrations, can inhibit *Candida* spp growth more effectively.

Time-kill curve assays significantly reduced the cell population of *C. albicans* CA1, *C. parapsilosis* CA3, and *C. krusei* CA4 strains when clove essential oil was used at 128 μg/mL. The results showed that after 6 hours of exposure, significant death of the yeast population occurs. At a concentration of 64 μg/mL, a 60% drop in cell viability was observed after 36 hours of testing, indicating that the cells of this subpopulation had not adapted to the toxic effects of clove essential oil. Significant reductions in cell viability were observed for *C. krusei* CA4 and *C. parapsilosis* CA3 at a concentration of 64 μg/mL when compared with the control and with the lowest concentrations used in the time kill-curve assay.

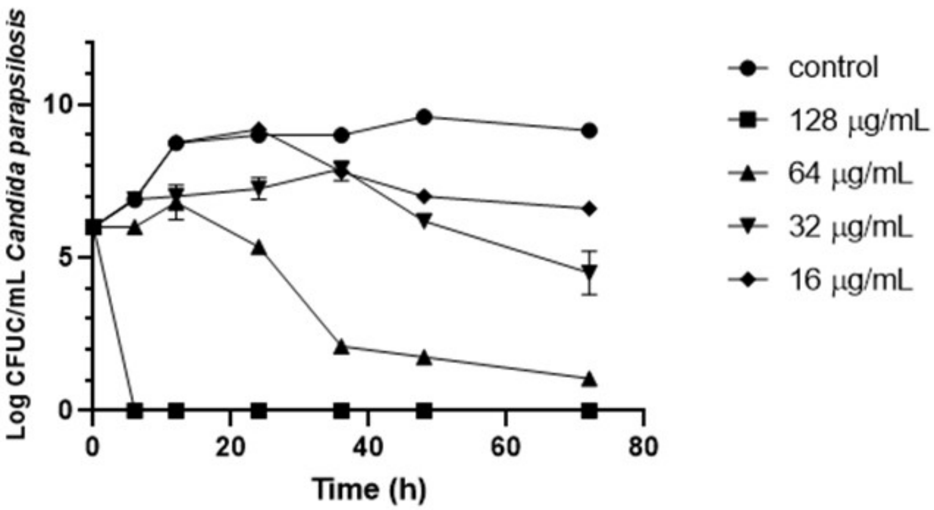

Figure 1-Time kill curve of *Canida albicans* CA1 against clove essential oil for 72 hours; count in LogCFU/mL (concentrations used 128 μg/mL, 64 μg/mL, 32 μg/mL, and 16 μg/mL)

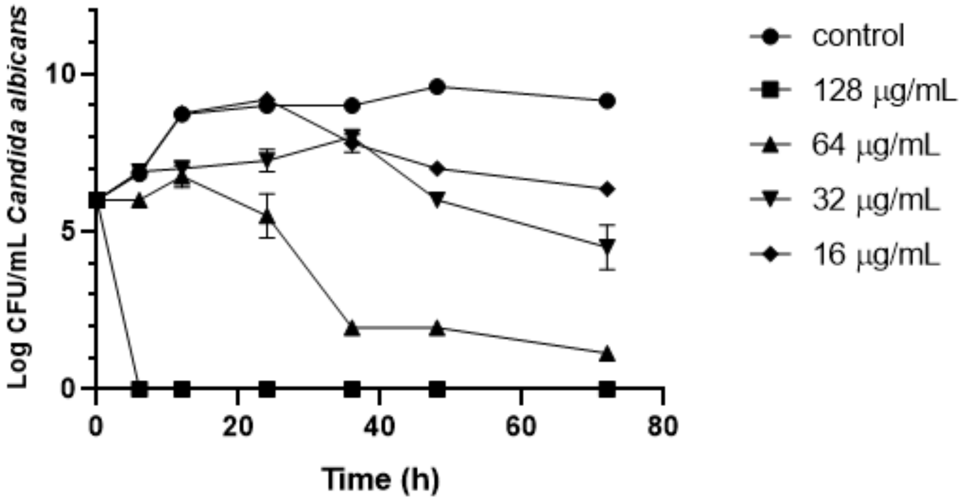

Figure 2- Time kill curve of *Candida parapsilosis* CA3 when treated with clove essential oil for 72 hours; count in LogUFC/mL (concentrations used 128 µg/mL, 64 µg/mL, 32 µg/mL, and 16 µg/mL)

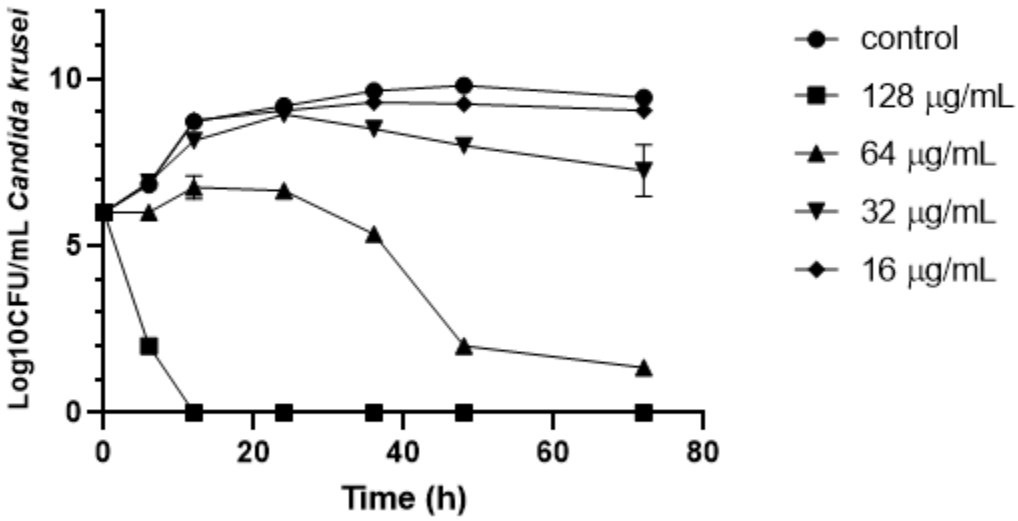

Figure 3- Time kill curve of *Candida krusei* CA4 when treated with clove essential oil for 72 hours; count in LogUFC/mL (concentrations used 128 µg/mL, 64 µg/mL, 32 µg/mL, and 16 µg/mL).

The time-kill-curve tests provide a more detailed view of the dynamics of inhibition of the growth of *Candida* spp—Clove essential oil hair. At a concentration of 128 µg/mL, there was a significant reduction in the cellular population of *C. albicans* CA1, *C. parapsilosis* CA3, and *C. krusei* CA4 after just 6 hours of exposure. These results indicate the rapid and effective action of the essential oil. Additionally, at 64 µg/mL, a 60% cell survival rate was observed after 36 hours. They indicated that cells did not develop resistance to the essential oil's toxic effects.

Hydrogel ointment formulation trials and anti-candida activity tests

The hydrogel formulation was analyzed for anti-candida activity, using strains of *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019 and *C. albicans* ATCC 10231, *C. albicans* CA1, *C. parapsilosis* CA3, and *C.krusei* CA4. The method used was agar diffusion. The formula was made with 4% sodium alginate, 10 grams of propylene glycol, 2% surfactant, 2% crude, and essential clove oil in a final volume of 100 ml of formulation. The results of the activity tests are described in the table below:

The anti-*Candida* activity of a hydrogel formulation incorporating raw clove and clove essential oil was assessed. Results showed effectiveness against diverse *Candida* strains. Agar diffusion tests exhibited pronounced inhibition halos, confirming the formulation's ability to suppress *Candida* spp. proliferation.

The in vitro antimicrobial efficacy of the formulation was substantially augmented, as indicated by a 4 Log_{10}CFU/mL decrease in viable cell counts of *C. albicans* CA1, *C. parapsilosis* CA3, and *C. krusei* CA4 within a 4-hour assay period. Similarly, the therapeutic efficacy of essential oil against *C. albicans* was demonstrated in the presence of *Mentha longifolia* (EOML) essential oil in a hydrogel formulation containing carbopol 940 (Nidhi et al., 2023).

Table 2- Determination of growth inhibition halos of *Candida krusei* ATCC 6258, *Candida parapsilosis* ATCC 22019 and *Candida albicans* ATCC 10231, *Candida albicans* CA1, *Candida parapsilosis* CA3 and *Candida krusei* CA4 in triplicate tests of the hydrogel ointment using crude clove oil and clove essential oil (values are attributed to inhibition of the formulation).

Hydrogel clove oil		
Candida albicans ATCC 10231	*Candida krusei* ATCC 6258	*Candida parapsilosis* ATCC 22019
2,0 cm (±0,3)	**2,5 cm (±0,1)**	**2,0 cm (±0,2)**

Hydrogel essential oil		
Candida albicans ATCC 10231	*Candida krusei* ATCC 6258	*Candida parapsilosis* ATCC 22019
2,9 cm (±0,3)	**3,2 cm (±0,3)**	**2,9 cm (±0,2)**

Hydrogel clove oil		
Candida albicans CA1	*Candida krusei CA3*	*Candida parapsilosis CA4*
2,2 cm (±0,1)	**2,5 cm (±0,1)**	**2,5 cm (±0,2)**

Hydrogel clove essential oil		
Candida albicans CA1	*Candida krusei CA3*	*Candida parapsilosis CA4*
2,9 cm (±0,3)	**3,1 cm (±0,2)**	**3,0 cm (±0,2)**

Preliminary Stability Test (EP)

Hydrogel samples (4% alginate + clove essential oil) were analyzed for fourteen days in the preliminary stability test, being alternated daily between two different temperatures: freezer 10 ± 2ºC and oven 45 ± 2ºC (Table 3). During this monitoring period, it showed no changes in organoleptic characteristics (appearance, color, and odor), remaining stable, in addition to spreadability, whose values were not statistically significantly different, thus being characterized as unchanged. The hydrogel presented considerable changes within the standard regarding the physical-chemical characteristics (density and pH). The analysis was conducted for 60 days (from 10 to 10 days, T0 to T6).

Table 3: Organoleptic and physicochemical characteristics of the hydrogel containing clove essential oil

Time	**Organoleptic and physicochemical characteristics**					
	Appearance	**Color**	**Odor**	**pH**	**Densidade g/mL**	Spallability (g.cm/s)
T0	N	N	N	5.3	0.990	39
T1	N	N	N	4.9	0.889	39
T2	N	N	N	5.5	0.820	39
T3	N	N	N	4.7	0.778	39
T4	N	N	N	5.1	0.889	39
T5	N	N	N	5.0	0.624	38
T6	N	N	N	4.9	0.656	38

Test preliminary stability tests indicate that the hydrogel formulation maintains its organoleptic characteristics (appearance, color, and odor) and stability for 60 days, even when subjected to temperature variations. Furthermore, the formulation showed excellent microbiological stability, with no contamination detected after 60 days of packaging. These promising results suggest that the hydrogel formulation may be a viable clinical option.

Reports have shown that adding essential oils to a hydrogel reduces the required doses of essential oils while retaining their antioxidant-antimicrobial activity (Sánchez-González et al., 2010). They are hydrogenated from alginate, combined with essential oils, as they have great potential in various biomedical applications, including wound healing, inhibition of opportunistic micro-organisms, as well as their administration to combat oral candidiasis, among others.A hydrogel containing clove essential oil may optimize skin penetration and retention, enhancing therapeutic effectiveness

5 CONCLUSION

The study demonstrates that clove essential oil is highly effective against several species of *Candida*, with a consistently low MIC and rapid action in reducing cell viability. The hydrogel formulation containing essential clove oil was highly influential, suggesting potential clinical use as an antifungal treatment. These are important for developing new therapies against infections by *Candida* spp., especially in the context of increasing resistance to conventional therapies. Clove essential oil, particularly in hydrogel formulations, shows great potential as an effective antifungal treatment against *Candida* infections. This study is relevant to the general public as it offers a promising alternative to traditional antifungal therapies facing increasing resistance. This could lead to more effective and accessible treatments for fungal infections, significantly benefiting public health.

ACKNOWLEDGEMENTS

The field activity of this study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; Edital PDPG 32/2022 Conselho and Fundação do Amparo a Pesquisa do Estado do Maranhão (FAPEMA).

REFERENCES

Alizadeh, V., Hashemi, S. J., Rafat, Z., Daie-Ghazvini, R., Rezaie, S., Golipoor, Z., & Sharafkhah, M. (2021). Isolation of *Candida* species on human mucosal surfaces. *New Zealand Journal of Medical Laboratory Science*, *75*(1), 33-37..

Alonso-Alonso, C., Larburu-Aristizabal, I., Iglesias-Gaspar, M. T., Urreta-Barallobre, I., & Emparanza-Knorr, J. I. (2022). Incidence and factors associated with *Candida* infection in neonatal diaper dermatitis. *Journal of Neonatal Nursing*, *28*(3), 177-181.

Araujo, J. M., de Almeida Junior, J. N., Magri, M. M. C., Costa, S. F., & Guimarães, T. (2024). Epidemiological Assessment and Risk Factors for Mortality of Bloodstream Infections by Candida sp. and the Impact of the COVID-19 Pandemic Era. *Journal of Fungi*, *10*(4), 268.

Avola, R., Granata, G., Geraci, C., Napoli, E., Graziano, A. C. E., & Cardile, V. (2020). Oregano (*Origanum vulgare* L.) essential oil provides anti-inflammatory activity and facilitates wound healing in a human keratinocytes cell model. *Food and Chemical Toxicology*, *144*, 111586.

Biernasiuk, A., Baj, T., & Malm, A. (2022). Clove essential oil and its main constituent, eugenol, as potential natural antifungals against *Candida* spp. alone or in combination with other antimycotics due to synergistic interactions. *Molecules*, *28*(1), 215.

Bora, L., Iftode, A., Muț, A. M., Vlaia, L. L., Olteanu, G. E., Muntean, D., ... & Danciu, C. (2023). Stability Profile and Clinical Evaluation of an Innovative Hydrogel Containing Polymeric Micelles as Drug Delivery Systems with Oregano Essential Oil against Fibroepithelial Polyps. *Pharmaceuticals*, *16*(7), 980.

Buru, A. S., Neela, V. K., Mohandas, K., & Pichika, M. R. (2022). Microarray analysis of the genomic effect of eugenol on methicillin-resistant *Staphylococcus aureus*. *Molecules*, *27*(10), 3249.

Chebbac, K., Ghneim, H. K., El Moussaoui, A., Bourhia, M., El Barnossi, A., Benziane Ouaritini, Z., ... & Guemmouh, R. (2022). Antioxidant and antimicrobial activities of chemically-characterized essential oil from Artemisia aragonensis Lam. against drug-resistant microbes. *Molecules*, *27*(3), 1136.

Chow, E. W., Pang, L. M., & Wang, Y. (2024). The impact of the host microbiota on Candida albicans infection. *Current Opinion in Microbiology*, *80*, 102507. Swidergall, M., & LeibundGut-Landmann, S. (2022). Immunosurveillance of *Candida albicans* commensalism by the adaptive immune system. *Mucosal immunology*, *15*(5), 829-836.

Corzo-Leon, D. E., Peacock, M., Rodriguez-Zulueta, P., Salazar-Tamayo, G. J., & MacCallum, D. M. (2021). General hospital outbreak of invasive candidiasis due to azole-resistant Candida parapsilosis associated with an Erg11 Y132F mutation. *Medical mycology*, *59*(7), 664-671.

Czajka, K. M., Venkataraman, K., Brabant-Kirwan, D., Santi, S. A., Verschoor, C., Appanna, V. D., ... & Tharmalingam, S. (2023). Molecular Mechanisms Associated with Antifungal Resistance in Pathogenic Candida Species. *Cells*, *12*(22), 2655.

da Silva Campelo, M., Melo, E. O., Arrais, S. P., do Nascimento, F. B. S. A., Gramosa, N. V., de Aguiar Soares, S., ... & Ricardo, N. M. P. S. (2021). Clove essential oil encapsulated on nanocarrier based on polysaccharide: A strategy for the treatment of vaginal candidiasis. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *610*, 125732.

Daneshnia, F., Floyd, D. J., Ryan, A. P., Ghahfarokhy, P. M., Ebadati, A., Jusuf, S., ... & Mansour, M. K. (2024). Evaluation of outbreak persistence caused by multidrug-resistant and echinocandin-resistant *Candida parapsilosis* using multidimensional experimental and epidemiological approaches. *Emerging Microbes & Infections*, *13*(1), 2322655.)

El-Naggar, M. E., Abdelgawad, A. M., Abdel-Sattar, R., Gibriel, A. A., & Hemdan, B. A. (2023). Potential antimicrobial and antibiofilm efficacy of essential oil nanoemulsion loaded polycaprolactone nanofibrous dermal patches. *European*

Elsherif, W. M., & Shrief, L. M. T. A. (2021). Effects of three essential oils and their nano-emulsions on Listeria monocytogenes and *Shigella flexneri* in Egyptian Talaga cheese. *International Journal of Food Microbiology*, *355*, 109334.

Escribano, P., Sánchez-Carrillo, C., Muñoz, P., Bouza, E., & Guinea, J. (2018). Reduction in percentage of clusters of *Candida albicans* and *Candida parapsilosis* causing candidemia in a general hospital in Madrid, Spain. *Journal of clinical microbiology*, *56*(7), 10-1128.

Fekkar, A., Blaize, M., Bouglé, A., Normand, A. C., Raoelina, A., Kornblum, D., & Imbert, S. (2021). Hospital outbreak of fluconazole-resistant *Candida parapsilosis:* arguments for clonal transmission and long-term persistence. *Antimicrobial agents and chemotherapy*, *65*(5), 10-1128.

Gandhi, J., Suthar, D., Patel, H., Shelat, P., & Parejiya, P. (2021). Development and characterization of microemulsion based topical gel of essential oil of clove (Syzygium aromaticum) for superficial fungal infections. *Advances in Traditional Medicine*, *21*, 519-534.

Gaziano, R., Sabbatini, S., & Monari, C. (2023). The interplay between *Candida albicans*, vaginal mucosa, host Immunity and resident microbiota in health and disease: An overview and future perspectives. *Microorganisms*, *11*(5), 1211.

Gunaratnam, G., Dudek, J., Jung, P., Becker, S. L., Jacobs, K., Bischoff, M., & Hannig, M. (2021). Quantification of the adhesion strength of *Candida albicans* to tooth enamel. *Microorganisms*, *9*(11), 2213.

Henriques, M., & Williams, D. (2020). Pathogenesis and Virulence of *Candida albicans* and *Candida glabrata*. *Pathogens*, *9*(9), 752.

Haro-González, J. N., Castillo-Herrera, G. A., Martínez-Velázquez, M., & Espinosa-Andrews, H. (2021). Clove essential oil (Syzygium aromaticum L. Myrtaceae): Extraction, chemical composition, food applications, and essential bioactivity for human health. *Molecules*, *26*(21), 6387.

Huggins, J. P., Hohmann, S., & David, M. Z. (2021, February). *Candida* infective endocarditis: a retrospective study of patient characteristics and risk factors for death in 703 United States cases, 2015–2019. In *Open Forum Infectious Diseases* (Vol. 8, No. 2, p. ofaa628). US: Oxford University Press.

Iseppi, R.; Mariani, M.; Condò, C.; Sabia, C.; Messi, P. Essential Oils: A Natural Weapon against Antibiotic-Resistant Bacteria Responsible for Nosocomial Infections. *Antibiotics* **2021**, *10*, 417.

Jahromi, M., Niakousari, M., & Golmakani, M. T. (2022). Fabrication and characterization of pectin films incorporated with clove essential oil emulsions stabilized by modified sodium caseinate. *Food Packaging and Shelf Life*, *32*, 100835.

Kaur, J., & Nobile, C. J. (2023). Antifungal drug-resistance mechanisms in Candida biofilms. *Current opinion in microbiology*, *71*, 102237. Alonso, V. P. P., Lemos, J. G., & do Nascimento, M. D. S. (2023). Yeast biofilms on abiotic surfaces: adhesion factors and control methods. *International journal of food microbiology*, *400*, 110265.

Kaur, H., Shankarnarayana, S. A., Hallur, V., Muralidharan, J., Biswal, M., Ghosh, A. K., ... & Rudramurthy, S. M. (2020). Prolonged outbreak of *Candida krusei* candidemia in paediatric ward of tertiary care hospital. *Mycopathologia*, *185*, 257-268.

Kong, A. S. Y., Maran, S., Yap, P. S. X., Lim, S. H. E., Yang, S. K., Cheng, W. H., ... & Lai, K. S. (2022). Anti-and pro-oxidant properties of essential oils against antimicrobial resistance. *Antioxidants*, *11*(9), 1819.

Khezri, K., Farahpour, M. R., & Rad, S. M. (2020). Efficacy of Mentha pulegium essential oil encapsulated into nanostructured lipid carriers as an in vitro antibacterial and infected wound healing agent. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *589*, 124414.

Koleri, J., Petkar, H. M., Rahman S. Al Soub, H. A., & Rahman S. AlMaslamani, M. A. (2023). Candida auris Blood stream infection-a descriptive study from Qatar. *BMC infectious diseases*, *23*(1), 513.

Lamarra, J., Bucci, P., Giannuzzi, L., Montanari, J., Rivero, S., & Pinotti, A. (2020). Biomaterial-based dressings as vehicle for chitosan-encapsulated cabreuva essential oil: Cytotoxicity and regenerative activity. *Reactive and Functional Polymers*,*156*, 104728.

Liao, J., Xie, X., Wang, W., Gao, Y., Cai, Y., Peng, J., ... & Wang, L. (2021). Anti-inflammatory activity of essential oil from leaves of Blumea balsamifera (L.) DC through inhibiting TLR4/NF-kB signaling pathways and NLRP3 inflammasome activation in LPS-induced RAW264. 7 macrophage cells. *Journal of essential oil bearing plants*, *24*(2), 160-176.

Luo, K., Zhao, P., He, Y., Kang, S., Shen, C., Wang, S., ... & Shi, C. (2022). Antibacterial effect of oregano essential oil against Vibrio vulnificus and its mechanism. *Foods*, *11*(3), 403.

Maggini, V., Semenzato, G., Gallo, E., Nunziata, A., Fani, R., & Firenzuoli, F. (2024). Atividade antimicrobiana do óleo essencial de *Syzygium aromatum* no tratamento da saúde humana. *Moléculas* , *29* (5), 999.

Megri, Y., Arastehfar, A., Boekhout, T., Daneshnia, F., Hörtnagl, C., Sartori, B., ... & Hamrioui, B. (2020). *Candida tropicalis* is the most prevalent yeast species causing candidemia in Algeria: the urgent need for antifungal stewardship and infection control measures. *Antimicrobial Resistance & Infection Control*, *9*, 1-10.

Mulet Bayona, J. V., Tormo Palop, N., Salvador García, C., Herrero Rodríguez, P., Abril López de Medrano, V., Ferrer Gómez, C., & Gimeno Cardona, C. (2020). Characteristics and management of candidaemia episodes in an established *Candida auris* outbreak. *Antibiotics*, *9*(9), 558.

Nidhi, P., Dev, K., Negi, P., & Sourirajan, A. (2023). Development and evaluation of hydrogel formulation comprising essential oil of Mentha longifolia L. for oral candidiasis. *Advances in Traditional Medicine*, *23*(3), 777-787.

Nisar et al., 2021. Nisar, M. F., Khadim, M., Rafiq, M., Chen, J., Yang, Y., & Wan, C. C. (2021). Pharmacological properties and health benefits of eugenol: A comprehensive review. *Oxidative medicine and cellular longevity*, *2021*(1), 2497354.

Nova, B. G. V., dos Santos Silva, L., da Silva Andrade, M., de Santana, A. V. S., da Silva, L. C. T., Sá, G. C., ... & Abreu, A. G. (2024). The essential oil of *Melaleuca alternifolia* incorporated into hydrogel induces antimicrobial and anti-inflammatory effects on infected wounds by *Staphylococcus aureus*. *Biomedicine & Pharmacotherapy*, *173*, 116389.).

Paulino, B. N., Silva, G. N., Araújo, F. F., Néri-Numa, I. A., Pastore, G. M., Bicas, J. L., & Molina, G. (2022). Beyond natural aromas: The bioactive and technological potential of monoterpenes. *Trends in Food Science & Technology*, *128*, 188-201.

Qadir, M. I., & Asif, H. (2020). An overview to candidiasis-a Candida infection. *International Journal of Advanced Research in Microbiology and Immunology*, *2*(1), 31-33. Species of the genus *Candida* are part of the normal human microbiota and colonize many niches, such as the gastrointestinal tract and the vaginal microbiota, presenting a commensal relationship

Ramos, L. S., Mello, T. P., Branquinha, M. H., & Santos, A. L. (2020). Biofilm formed by *Candida haemulonii* species complex: Structural analysis and extracellular matrix composition. *Journal of Fungi*, *6*(2), 46.

Rautemaa-Richardson, R., & Richardson, M. D. (2021). Systemic fungal infections. *Medicine*, *49*(12), 760-765.

Rebuma, T., Parmar, R. B., Parmar, B. C., & Pal, M. (2024). Emerging fungal pathogens pose a growing threat to global health. *Journal of Advances in Microbiology Research*, *5*(2), 01-05.

Sánchez-González L, González-Martínez C, Chiralt A, Cháfer M. Physical and antimicrobial properties of chitosan–tea tree essential oil composite films. J Food Eng. 2010;98:443–52.

Sen, P., Gupta, L., Vijay, M., Vermani Sarin, M., Shankar, J., Hameed, S., & Vijayaraghavan, P. (2023). 4-Allyl-2-methoxyphenol modulates the expression of genes involved in efflux pump, biofilm formation and sterol biosynthesis in azole resistant Aspergillus fumigatus. *Frontiers in Cellular and Infection Microbiology*, *13*, 1103957.

Shen, Y., Ni, Z. J., Thakur, K., Zhang, J. G., Hu, F., & Wei, Z. J. (2021). Preparation and characterization of clove essential oil loaded nanoemulsion and pickering emulsion activated pullulan-gelatin based edible film. *International journal of biological macromolecules*, *181*, 528-539.
Sen, P., Gupta, L., Vijay, M., Vermani Sarin, M., Shankar, J., Hameed, S., & Vijayaraghavan, P. (2023). 4-Allyl-2-methoxyphenol modulates the expression of genes involved in efflux pump, biofilm formation and sterol biosynthesis in azole resistant Aspergillus fumigatus. *Frontiers in Cellular and Infection Microbiology*, *13*, 1103957.

REVISTA CADERNO PEDAGÓGICO – Studies Publicações Ltda.
ISSN: 1983-0882

Strobl, B., Miranda, S., Lassnig, C., Schmidhofer, K., Kjartansdottir, H., Vogl, C., ... & Kuchler, K. (2024). Lack of TYK2 Signaling Enhances Host Resistance to *Candida albicans* Skin Infection.

Tran, H. N., Graham, L., & Adukwu, E. C. (2020). In vitro antifungal activity of Cinnamomum zeylanicum bark and leaf essential oils against *Candida albicans* and *Candida auris*. *Applied Microbiology and Biotechnology*, *104*, 8911-8924.

Turgis M, Han J, Caillet S, Lacroix M. Antimicrobial activity of mustard essential oil against *Escherichia coli O157:H7 and Salmonella typhi Food Control. 2009;20:1073-1079.*

Wu, Z.; McGoogan, J.M. Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases from the Chinese Center for Disease Control and Prevention. JAMA 2020, 323, 1239–1242.