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Avaliação atividade antifúngica do mirtenol frente *Candida albicans* e *Candida parapsilosis* fluconazol resistente

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AVALIAÇÃO ATIVIDADE ANTIFÚNGICA DO MIRTENOL FRENTE *Candida albicans* E *Candida parapsilosis* FLUCONAZOL RESISTENTE

Dissertação de mestrado apresentada ao Programa de Pós-Graduação em Odontologia, Centro de Ciências da Saúde Universidade Federal da Paraíba, como parte dos requisitos necessários para a obtenção do titulo de Mestre em Odontologia. Área de concentração: Ciências Odontológicas

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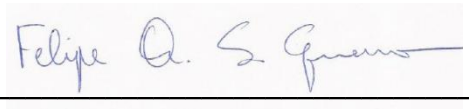

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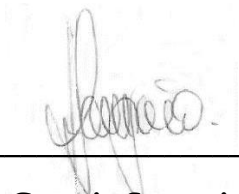

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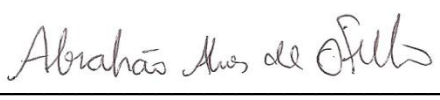

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Resumo

A candidíase ou candidíase oral é uma doença causada mais comumente pela *Candida albicans*, presentes na microbiota como comensais, é uma infecção oportunista sendo observada principalmente em pacientes imunocomprometidos. Estima-se que aproximadamente 50% da população saudável carrega espécies de *Candida* na cavidade oral. Na terapêutica das candidíases orais leves destacam-se antifúngicos azólicos ou poliênicos. Os efeitos adversos e custo do tratamento dos antifúngicos convencionais tem levado a busca de novos fármacos, uma das alternativas são os terpenos. Extraídos dos óleos essenciais são misturas complexas de vários compostos, sendo o mirtenol um exemplo. Nesse trabalho verificou-se a atividade do mirtenol contra cepas de *Candida* resistentes ao fluconazol. Foi realizada a concentração inibitória mínima, concentração fungicida mínima, além dos estudos de modulação e de associação (método *checkerboard)*, via técnica de microdiluição. Com as cepas testadas, o mirtenol demostrou a concentração inibitória mínima (CIM) e a concentração fungicida mínima (CFM) nas concentrações de 512 µg/mL, logo este possui fraca atividade antifúngica e esta tem caráter fungicida. No ensaio de modulação do mirtenol em concentração subinibitória com os principais antifúngicos houve aumento da CIM destes fármacos. Quando se associou o mirtenol com estes antifúngicos (método checkboard), observou-se resultados sinérgicos ou indiferentes com o fluconazol ou a anfotericina B, respectivamente. O mirtenol possui fraca atividade antifúngica frente as cepas ensaiadas, porém com característica fungicida, ele não aumenta a atividade do fluconazol e anfotericina B e em associação com antifúngicos convencionais demonstrou efeito sinérgico ou indiferente dependendo da cepa ensaiada.

Palavras-chave: Candidíase bucal, Produtos naturais, Monoterpenos

Abstract

Candidiasis or oral candidiasis is a disease most commonly caused by *Candida albicans*, present in the microbiota as commensals, it is an opportunistic infection being observed mainly in immunocompromised patients. It is estimated that approximately 50% of the healthy population carries *Candida* species in the oral cavity. In the treatment of mild oral candidiasis, azole or polyene antifungals are highlighted. The adverse effects and cost of conventional antifungal treatment have led to the search for new drugs, one of the alternatives being terpenes. Extracted from essential oils are complex mixtures of various compounds, myrtenol being an example. In this work, the activity of myrtenol against fluconazole resistant *Candida* strains was verified. Minimum inhibitory concentration, minimum fungicidal concentration, in addition to modulation and association studies (checkerboard method) were performed via the microdilution technique. With the strains tested, myrtenol demonstrated minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) at concentrations of 512 µg/mL, so it has weak antifungal activity and is fungicidal. In the assay of modulation of myrtenol in subinhibitory concentration with the main antifungal agents, there was an increase in the MIC of these drugs. When myrtenol was associated with these antifungal agents (checkboard method), synergistic or indifferent results were observed with fluconazole or amphotericin B, respectively. Myrtenol has weak antifungal activity against the strains tested, but with a fungicidal characteristic, it does not increase the activity of fluconazole and amphotericin B and, in association with conventional antifungal agents, it showed a synergistic or indifferent effect depending on the strain tested.

Key words: Oral candidiasis, Natural products, monoterpenes

LISTA DE ABREVIATURAS

ATCC - *American Type Culture Collection*

LM – Laboratório de Micologia

ASD – Ágar Sabouraud Dextrose

RPMI - Roswell Park Memorial Institute

DMSO – Dimetilsulfóxido

CIM - Concentração inibitória mínima

CFM - Concentração fungicida mínima

CLSI- Clinical and Laboratory Standards Institute

PM – Peso molecular

RPMI – Caldo Sabouraud Dextrose

CIF - Concentração inibitória fracionada

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1. INTRODUÇÃO

Um número crescente de infecções fúngicas tem sido relatado nas últimas décadas. Destacando-se, neste cenário, as candidíases, infecções que tem como agente etiológico as leveduras do gênero *Candida*, sendo *Candida albicans* a principal espécie isolada e causa de infecções fúngicas em humanos. A candidíase pode variar desde manifestações na mucosa vaginal e oral, até infecções sistêmicas (ALVAREZ-MORENO; CORTES; DENNING, 2018)(LAMOTH et al., 2018)(ALONSO MONGE et al., 2006).

As espécies de *Candida* estão presentes na microbiota como comensais, porém quando há um desequilíbrio na imunidade ou microbiota, pode haver manifestações agressivas(ALONSO MONGE et al., 2006). São capazes de desenvolver biofilme que, quando maduros, dificultam a ação dos fármacos, sendo necessárias altas doses o que pode tornar o tratamento perigoso, uma vez que esses pacientes já se encontram com uma baixa imunidade(NOBILE; JOHNSON, 2015).

A candidíase ou candidíase oral é uma doença causada, mais comumente pela *Candida albicans*, é uma infecção oportunista sendo observada principalmente em pacientes imunocomprometidos(FREIRE et al., 2016).

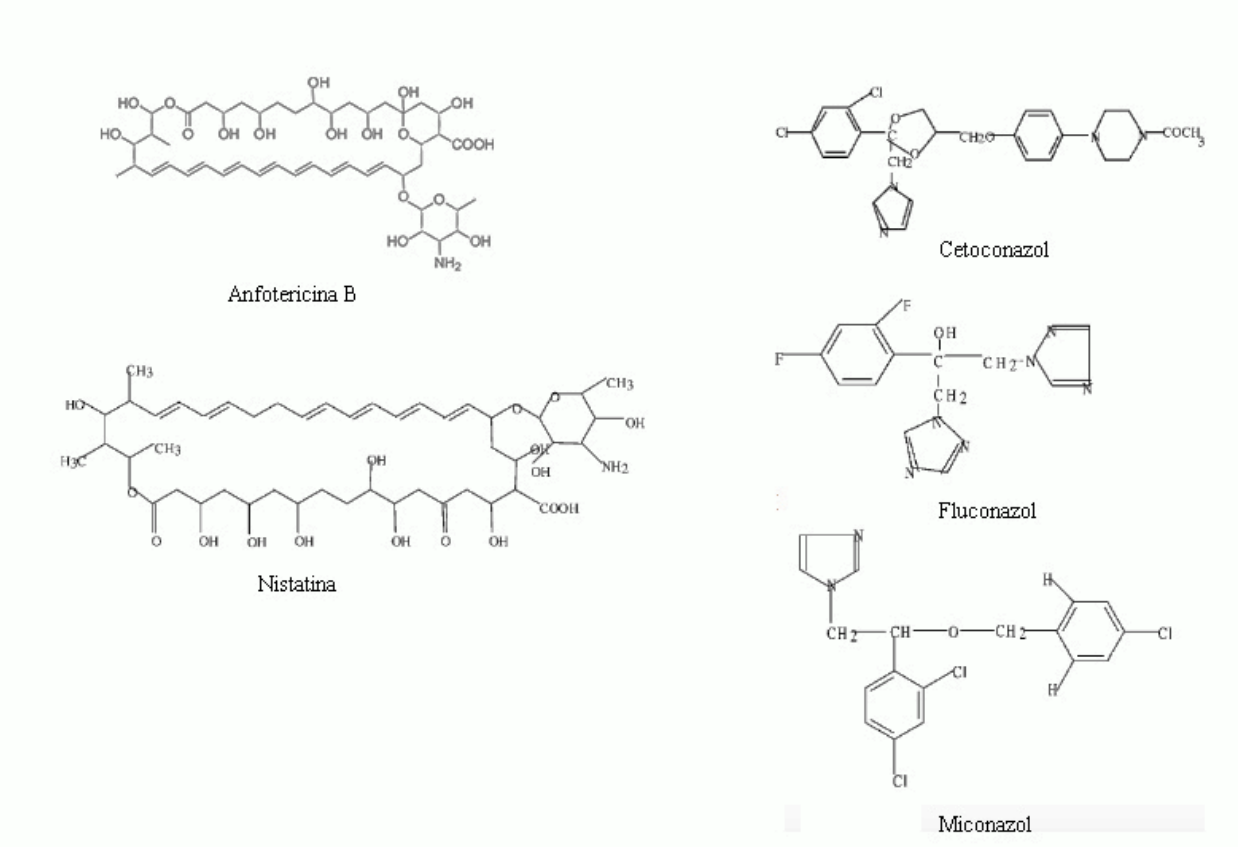
C. parapsilosis é a espécie de *Candida* mais comum depois da *C. albicans* e é encontrada em locais do corpo onde normalmente não deveriam existir microrganismos em pacientes hospitalizados. Ela é responsável por várias infecções em âmbito hospitalar por sua capacidade de formação de biofilmes em cateteres, tendo como porta de entrada principal a nutrição parenteral e por sua frequente contaminação nas mãos dos profissionais dos hospitais(TROFA; GÁCSER; NOSANCHUK, 2008).

Estima-se que aproximadamente 50% da população saudável carrega espécies de *Candida* na cavidade oral, sendo que 70-80% são de *C. albicans* e *C. glabrata*. Uma pesquisa demostrou que a *C. parapsilosis* é a espécie de *Candida* mais prevalente em próteses dentárias na Argentina (RODRIGUEZ; ROSA; JEWTUCHOWICZ, 2016).

O aumento de casos de candidíase está associado a alguns fatores predisponentes do hospedeiro, tais como uso de dentaduras, xerostomia, uso prolongado de antibióticos, trauma local, desnutrição, desordens endócrinas, idade avançada, entre outros estados que geram diminuição do sistema imune individual (RODLOFF; KOCH; SCHAUMANN, 2011). Na terapêutica das candidíases orais destacam-se antifúngicos de uso tópico de

derivados azólicos, como cetoconazol, fluconazol ou miconazol e como alternativa a suspensão da nistatina ou a anfotericina B para casos de candidíase invasiva grave, ambos derivados poliênicos. (GARCIA-CUESTA; SARRION-PÉREZ; BAGÁN, 2014)(GUPTE; KULKARNI; GANGULI, 2002).

Figura 1: Estrutura química dos principais antifúngicos utilizados para tratamento dos diversos tipo de candidíases



Fonte: GUPTE; KULKARNI; GANGULI, 2002

Os azólicos, que são representados pelo fluconazol, miconazol, cetoconazol, entre outros, age na síntese do ergosterol, possuem atividade através da inibição da enzima 14α-lanosterol desmetilase e da 22Δ-desaturase. Levando ao esgotamento do ergosterol e aumento de substâncias tóxicas na célula (DELATTIN; CAMMUE; THEVISSEN, 2014).

Segundo DOCKRELL et al., 2019, o fluconazol é o medicamento de primeira escolha para tratamento de candidíase na região orofaríngea, sendo associada e menor chance de recidiva. O tratamento tópico pode ser considerado caso o fluconazol seja um obstáculo, como por exemplo em casos de alergia. Ele tem uma maior taxa de cura que o

tratamento tópico, com menores chances de recidiva e resposta em prazos mais curtos quando comparado ao itraconazol.

A anfotericina B é um polieno com amplo espectro antimicrobial. Tem mais de 50 anos de uso, mas mesmo com esse tempo, ainda é raro encontrar fungos resistentes. Tem como principal efeito colateral a nefrotoxicidade. Ainda hoje é utilizada como primeira escolha em tratamentos de candidíase invasiva e outras micoses oportunistas que podem ser fatais(HASAN et al., 2019).

A classe dos derivados poliênicos é representada pela nistatina (uso tópico) e anfotericina B (uso sistêmico). Esta classe de antifúngicos atua se ligando ao ergosterol da membrana citoplasmática do criando canais não-aquosos e aquosos. Alguns elementos presentes na célula fúngica, como o potássio, vazam por esses poros, causando uma perda de potencial e sua morte(MOHR et al., 2008). A nistatina tem como efeitos adversos mais relatos: gosto ruim e disfunções gastrointestinais como náusea, diarreia e vômito (LYU et al., 2016).

Os efeitos adversos e custo do tratamento dos antifúngicos convencionais tem sido um desafio na terapêutica destas enfermidades. Adicionalmente, observa-se um grande aumento de cepas resistentes ao fluconazol, assim gerando grande preocupação aos profissionais de saúde (DOI et al., 2016).

A resistência aos medicamentos também é um fator a ser levado em consideração no momento da prescrição. Os fungos desenvolveram quatro formas de resistência aos medicamentos(KANAFANI; PERFECT, 2008):

- Diminuição da concentração do fármaco: um sistema ativo que retira o fármaco da área de atuação, resultando numa concentração menor;
- Alteração do alvo: através de uma mutação no gene ERG11, a enzima 14α-lanosterol desmetilase é modificada e evita a ligação do fármaco a enzima;
- Regulação positiva da enzima alvo: tida como a menos eficaz, os isolados de *Candida* que tem um maior número de ERG11p estão em maior número, levando a sobrecarga do fármaco e reduzindo a eficácia para inibir a síntese do ergosterol;
- Desenvolvimento de vias de desvio: Uma mutação no gene ERG3 impede a formação de 14α-metil-3,6-diol, que causa a morte do fungo.

> O ergosterol é substituído pelo 14α-metilfecosterol deixando a membrana funcional e impedindo a ação dos azólicos.

Logo, faz-se necessário a busca de alternativas desenvolvimento de novos fármacos de maior eficácia e com menos efeitos adversos. Uma das alternativas que vem ganhando destaque nos últimos anos, como fonte de novos produtos, são plantas medicinais e seus derivados.

Uma pesquisa feita por FREIRE et al., 2016 buscou na literatura os produtos a base de plantas mais utilizados no combate às espécies de *Candida*. Nesse estudo, as plantas mais citadas foram *Melaleuca atnerfolia* (árvore-de-chá), *Allium sativum* (alho) e *Ricinus communis* (mamona), *Punica granatum* (romã), *Uncaria tomentosa* (unha de gato), *Cymbopogon citratus* (capim-limão), porém outras plantas tem potencial antifúngico.

Os produtos podem ter origem nas diversas partes da plante: folhas, flores, rizomas e frutos. Uma das substâncias extraídas das plantas que vem ganhando atenção são os óleos essenciais. Estes são extraídos por uma técnica chamada arraste a vapor ou pela prensagem do pericarpo de frutos cítricos, sendo utilizados na perfumaria, cosmética e também na indústria farmacêutica(BIZZO; ANA MARIA; REZENDE, 2009).

Os óleos essenciais são misturas complexas de vários compostos com baixo peso molecular, são altamente voláteis e capazes de gerar sabores ou aromas(TROMBETTA et al., 2005).

Os óleos essenciais podem ter de 20 a 200 componentes: hidrocarbonetos terpênicos, álcoois simples e terpênicos, aldeídos, cetonas, fenóis, ésteres, éteres, óxidos, peróxidos, furanos, ácidos orgânicos, lactonas, cumarinas e até compostos com enxofre. Os óleos essenciais também podem ser encontrados de forma sintética, estes podem ser cópias dos naturais ou composições de fantasia (SIMÕES, 2017).

Um dos componentes que vem se destacando são os terpenos. Estes são caracterizados como hidrocarbonetos insaturados, ou seja, apresentam uma ligação dupla entre os carbonos e estão agrupados em grupos de cinco carbonos(C_5H_8). Os monoterpenos tem menor massa molecular e são voláteis, sendo os principais constituintes de diversos óleos essenciais (FELIPE; BICAS, 2017).

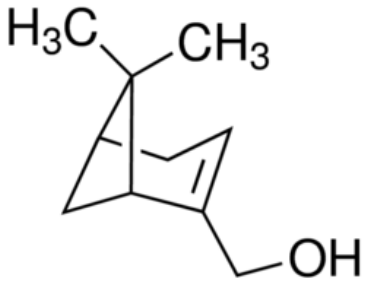
O mirtenol é o exemplo de um monoterpeno majoritário extraído do óleo essencial de algumas espécies de plantas, a exemplo da murta (*Myrthus comunis* L.) (mostrada na figura 2). A *Cyperus rotundus*, *Tanacetum vulgare* L., *Dendroctonus armandi* e a *Pinus armandii* também apresentam uma quantidade de mirtenol, porém em

menor quantidade. O mirtenol possui a fórmula $C_{10}H_{16}O$ e estrutura química ilustrada na figura 3(GOMES et al., 2017)(SELVARAJ et al., 2020).

Figura 2: *Myrthus comunis* L., exemplo de planta com grande quantidade de mirtenol em seu óleo essencial

Fonte: ALEEM; ANIS, 2021

Figura 3: Estrutura química do mirtenol



Fonte: https://www.sigmaaldrich.com/BR/pt/product/aldrich/w343900

A etnofarmacologia de *Myrthus comunis* L. demonstra que tem ação antimicrobiana, antidiarreica, antidiabética, antiespasmódica, vasodilatadora, antiúlcera, antioxidante, anticâncer, ansiolítica, sedativo-hipnótica e anti-inflamatória, entre outras(CORDEIRO et al., 2020)(SISAY; GASHAW, 2017).

O mirtenol foi capaz de diminuir a atividade do biofilme da bactéria *Staphylococcus aureus* resistente à meticilina, atuando em vários mecanismos de virulência e sem citotoxidade observada (SELVARAJ et al., 2019). Ele foi capaz de diminuir o dano cerebral e melhorar a angiogênese em ratos com isquemia cerebral induzida(HUANG et al., 2021).

O óleo essencial da *Chamaecyparis formosensis*, que tem o mirtenol como composto majoritário, demonstrou ação antifúngica frente fitopatógenos fúngicos tais como *Trametes versicolor* e *Laetiporus sulphureus*, (WANG et al., 2005).

Assim, diante do exposto, a pesquisa de um protótipo antifúngico sobre fungos potencialmente patogênicos da cavidade oral torna-se um fator de significativo estímulo para a realização desta proposta de trabalho. Há a possibilidade de contribuir para a pesquisa científica quanto à investigação farmacológica do mirtenol frente a cepas resistentes de *Candida* spp.

2. RESULTADOS: Capítulo 1: Atividade antifúngica do mirtenol frente *Candida albicans* e *C. parapsilosis* fluconazol resistente

O manuscrito a seguir foi submetido para publicação no periódico "*Evidence-Based Complementary and Alternative Medicine*".

2.1. Introduction

Cases of fungal infections have increased in the last decade. Candidiasis is a fungal infection caused by the *Candida* species and can manifest itself in the oral cavity, vagina or systemically, and can lead to death (ALVAREZ-MORENO; CORTES; DENNING, 2018) (LAMOTH et al., 2018) (ALONSO MONGE et al., 2006).

This is an opportunistic infection, affecting mainly immunocompromised patients, with *Candida albicans* as the most frequent isolated pathogenic species (FREIRE et al., 2016).

Complex *C. parapsilosis* has been frequently isolated, being able to surpass *C. albicans* in some parts of Europe and Asia as the most frequent isolated one. It is found in the body where it should not m exist microorganisms in hospitalized patients. (TROFA; GÁCSER; NOSANCHUK, 2008).

As for the treatment of mild oral candidiasis, antifungal drugs most commonly used are derivatives azole as ketoconazole, fluconazole or miconazole or, alternatively, the polyene has the suspension of Nystatin as a choice for mild cases or amphotericin B for cases of severe invasive candidiasis and other opportunistic mycoses (GARCIA-CUESTA; SARRION-PÉREZ; BAGÁN, 2014) (GUPTE; KULKARNI; GANGULI, 2002) (HASAN et al., 2019). Associated with a lower chance of disease recurrence when compared to topical treatment, DOCKRELL et al., 2019, says that fluconazole is the drug of choice for the treatment of candidiasis in the oropharyngeal region.

The scientific search community for natural alternatives are adverse effects to drug resistance and for treatment cost (DOI et al., 2016), appearing thus an interest in Essential oils are a mixture of 20 to 200 components may be found in nature or acquired in synthetic form (TROMBETTA et al., 2005) (SIMÕES, 2017).

Among the major components of essential oils are terpenes, which are characterized as unsaturated hydrocarbons. The monoterpenes, comprise several essential oils, it has a lower molecular weight and are volatile (FELIPE; BICAS, 2017).

As an example of monoterpene, we can highlight myrtenol. This can be originally isolated from the essential oil of some plant species or acquired in synthetic form (GOMES et al., 2017) (SELVARAJ et al., 2020).

Some research has shown that the versatility of myrtenol as antimicrobial agent, this decreased the activity of the biofilm *Staphylococcus aureus* resistant to methicillin (MRSA) (Selvaraj et al., 2019). The oil essentially containing 19.49% of myrtle myrtenol was able to inhibit the growth of all strains of *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Verticillium dahliae,* which are fungi which attack many kinds of plants (YILAR; BAYAN; Onaran, 2016). In addition to these, CANNAS et al., 2014, showed that *M. communis* essential oils were efficient against *C. albicans, C. parapsilosis* and *C. tropicalis*.

Another form of use for natural products is the use as an adjuvant with known antifungal agents. This can be especially useful to decrease the antifungal dosage (FRANCISCONI et al., 2020) or to combat antifungal resistant strains (XU et al., 2019).

Given this scenario, this study aimed to evaluate the antifungal activity of myrtenol alone and in association with conventional antifungal agents.

For this work, an alternative antifungal to such a common problem today is the basis of this research.

2.2 Material and methods

2.2.1 Type of search:

This is an experimental laboratory study of a quantitative nature.

2.2.2 Research location

The antimicrobial evaluations of the test compounds were carried out in the Clinical Mycology laboratory of the Federal University of Paraíba.

2.2.3 Microorganisms

For the antifungal activity tests were used to *C. albicans* ATCC 76485 (*American Type Culture Collection*) and clinical strains of *C. albicans* LM-117, *C. albicans* LM-516, *C. albicans*

LM-587, *C. albicans* LM -6 16, as well as standard strain *C. parapsilosis* ATCC 22019, *C. parapsilosis* LM-439, *C. parapsilosis* LM-5770 , *C. parapsilosis* LM-55117 . These have their respective sensitivity profile, shown in table 1 (CLSI, 2020).

Table 1: Sensitivity profile of microorganisms

Strains		**Amphotericin B**	**Voriconazole**	**Fluconazole**
		MIC (µg/mL)		
C. albicans	**ATCC 76485**	0.25 (S)	0.125 (I)	32 (R)
	LM-117	0.25 (S)	0.062 (S)	08 (R)
	LM-516	0.25 (S)	0.125 (I)	64(R)
	LM-587	0.25 (S)	0.125 (I)	16 (R)
	LM-699	0.25 (S)	0.062 (S)	08 (R)
C. parapsilosis	**ATCC 22019**	0.25 (S)	0.125 (S)	16 (R)
	LM-439	0.50 (S)	0.50 (R)	32 (R)
	LM-546	0.50 (S)	0.50 (R)	16 (R)
	LM-5770	0.50 (S)	0.25 (I)	32 (R)
	LM- 55117	0.25 (S)	0.50 (R)	64(R)

Sensitivity profile of tested strains. (S): sensitive; (I): Intermediate sensitivity; (R): Resistant

All fungal strains belong to the mycotheca of the Laboratory for Research on Antimicrobial Activity of Natural and Synthetic Bioactive Products, of the Department of Pharmaceutical Sciences, of the Health Sciences Center, of the Federal University of Paraíba. Kindly provided by Professor Edeltrudes de Oliveira Lima.

2.2.4. Reagents used

For the maintenance of strains and tests of antifungal activity, were used the medium Sabouraud dextrose agar, RPMI 1640, L-glutamine and Roswell Park Memorial Institute (RPMI) 1640-L-glutamine (LGC BIOTECHNOLOGY/BRAZIL), prepared according to manufacturers' descriptions. Fluconazole, Tween 80% and ergosterol used during the tests were coming from Sigma-Aldrich™ Chemical Co. (St. Louis, MO, USA). Sorbitol (anhydrous D-sorbitol) was obtained from INLAB™ (São Paulo, Brazil). The myrtenol was purchased commercially from Sigma Aldrich™, with a density of 0,954g/mL. This was duly solubilized in 5% dimethyl sulfoxide (DMSO) and 2% Tween 80 emulsifier, completing the final volume with sterilized

distilled water in order to obtain an emulsion of the products at the initial concentration of 1024 µg/ mL (CLEELAND; SQUIRES, 1991).

2.2.5. Inoculum preparation

For inoculum preparation , *Candida* spp. cultures were seeded in ASD medium and incubated at 35±2°C for 24-48 h. Colonies of these cultures were suspended in a sterile 0.85% NaCl solution and adjusted according to the 0.5 McFarland standard , to obtain an inoculum of 10^6 CFU/mL (OSTROSKY et al., 2008 ; HADACEK; GREGER, 2000 ; CLEELAND; SQUIRES, 1991).

2.2.6. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (CFM)

The MIC was determined using the microdilution technique in a microplate containing 96 wells with a U-shaped bottom (ALAMAR™), described by the Clinical and Laboratory Standards Institute (CLSI, 2018), with some modifications. The myrtenol had their test concentrations ranging between 1024 µg/mL and 0.5 µg/mL. Fluconazole was used as a positive control in assays at concentrations ranging from 64 to 0.125 µg/mL. Tension viability controls, average sterility, DMSO and Tween 80%, solvents used to prepare myrtenol emulsion, were performed simultaneously with the assay. All are the s test s were performed are in duplicate and aseptically sealed plates were incubated at 35 ± 2°C for 24 hours to be conducted later playback.

MIC was defined as the lowest concentration of products capable of producing visible inhibition on fungal growth when compared to its control. The result was expressed by the arithmetic mean of the MICs obtained in the three tests. The antifungal activity of the products was interpreted according to the following criteria shown in table 2: (BALOUIRI; SADIKI; IBNSOUDA, 2016; SARTORATTO et al., 2004)

Table 2: Adopted antifungal expression pattern

MIC (µg/mL)	Criterion
<500	strong/great activity
600-1500	moderate activity
>1500	weak activity or inactive product

CFM was defined as the lowest concentration to inhibit visible growth on solid media. Aliquots of the wells corresponding to the MIC at higher concentrations were subcultured on Sabouraud Dextrose agar. Then the plates were incubated for 24 hours at 35°C and the reading was performed by visual observation of growth fungal in liquid environment. The CFM/CIM ratio was calculated to determine whether the substance actually had fungistatic (CFM/CIM greater than or equal to 4) or fungicidal (CFM/CIM less than 4) activity (KLEPSER et al., 1998).

2.2.7. Myrtenol modulation assay on antifungals:

The protocol used was adapted from Coutinho et al., 2009. For the evaluation of the modulation myrtenol on antifungals fluconazole, miconazole, ketoconazole and itraconazole, the MIC was assessed in the presence and absence of myrtenol against strains of *C. albicans* and *C. parapsilosis* using plates microdilution containing 96 wells with bottom shape of "U" (ALAMAR™) and in duplicate similar to the determination of the CIM, seen in item 2.2.6.

Myrtenol was homogenized in RPMI at subinhibitory concentrations (approximately MIC/8). The solutions are antifungal agents were solubilized and dilutions prepared at a concentration of 64 μg/mL and 100 μL volumes were diluted serially 1: 2 in RPMI. Each well with 100 μL of the culture medium had 10% of the fungal inoculum previously prepared as shown in item 2.2.6. The same controls used to assess the MIC were used. The plates were sealed and incubated at 35°C for 24 hours to be read (COUTINHO et al., 2009).

2.2.8. Assay checkerboard the front myrtenol to conventional antifungal the strains *C. albicans* and *C. parapsilosis*:

The method checkerboard assesses the effect of test substance in combination with one or more anti-fungal at different concentrations.

The emulsion of myrtenol and solutions of antifungal agents (amphotericin B and fluconazole) were solubilized and prepared dilutions folded concentrations (2048 and 128 μg/mL respectively) in relation to the defined initial concentration and 100 μL volumes were diluted serially 1:2 in RPMI 1640 in each well with 100 μL of the culture medium contained 10% of the fungal suspension that was previously prepared as described in item 2.2.6. Each sterile microplate was filled vertically and horizontally with the compound of myrtenol and one antifungal at a time to obtain the Fractional Inhibitory Concentration Index (FIC index). The filled plates were incubated at 28 ºC for 72 hours. The calculation of the Fractional Inhibitory Concentration (FIC) was performed to obtain a coefficient that indicated whether the association of extracts with the

antibiotic produced a synergistic (FIC ≤ 0.5), indifferent (0.5 < FIC ≤ 4.0) or antagonistic effect (FIC > 4.0) according to the formula described by MACKAY; MILNE; GOULD, 2000 which says that:

$$FIC = \frac{\text{combined MIC}}{\text{isolated MIC}}$$

2.3. Results and discussion

2.3.1. CIM and CFM

The MIC for myrtenol was 512 μg/mL, inhibiting 58.33% of the strains tested, so it has strong/great stability for antifungal activity, according to criteria by ALIGIANNIS et al., 2001. The CFM obtained was 512 μg/mL, with all strains at the same concentration (Table 1). Ergo, myrtenol showed fungicidal activity on the tested strains.

Table 1 – Results of the evaluation of the Minimum Inhibitory Concentration (MIC) and the Minimum Fungicide Concentration (MFC) of myrtenol and fluconazole against *C. albicans* and *C. parapsilosis* strains.

Microorganisms		*Myrtenol (μg/mL)*				*Flu (μg/mL)*	*Controls*	
		CIM	**CFM**		**Activity Nature**	**CIM**	**Micro**	**Ste**
Candida albicans	ATCC 76485	512	512	1	Fungicide	32	+	-
	LM-117	512	512	1	Fungicide	08	+	-
	LM-516	256	512	2	Fungicide	64	+	-
	LM-587	256	512	2	Fungicide	16	+	-
	LM-616	256	512	2	Fungicide	08	+	-
Candida parapsilosis	ATCC 22019	512	512	1	Fungicide	16	+	-
	LM-439	512	512	1	Fungicide	32	+	-
	LM-546	512	512	1	Fungicide	16	+	-
	LM-5770	256	512	2	Fungicide	32	+	-
	LM- 55117	512	512	1	Fungicide	64	+	-

Flu: fluconazole; Micro: microorganism: Ste: sterility; +: growth; -: no growth

The results of this research were lower than that observed by GRIFFIN et al., 1999 found a MIC of 720 μg/mL for myrtenol against *C. albicans* , but they were higher than that found by İŞCAN, 2017 , which had as results for two strains tested for *C. albicans* with MIC of 250 μg/mL and for *C. parapsilosis* the MIC was 500 μg/mL and 1000 μg/mL depending on the strain.

Using eugenol, a monoterpene, against *C. albicans*, SILVA et al., 2017, found a CFM of 625 μg/mL, a result superior to that found in this research. Using another terpene, geraniol, (SHARMA et al., 2018) found between 140 and 250 μg/mL of CFM against 4 different strains of *C. albicans*.

2.3.2. modulation test

The increased resistance of certain strains of *Candida* and the toxicity related to conventional antifungal agents has led to a search for alternative treatments. To combat fungal resistance or antifungal toxicity, some strategies have been adopted to minimize this problem, such as combined therapy, antivirulence agents and modulation of host immune system responses (LEE et al., 2021).

Thus, the activity of myrtenol was studied for its interference in the action of conventional drugs used in the treatment of candidiasis. Initially, it was verified whether myrtenol modulates the activity of fluconazole at subinhibitory concentrations (MIC/8). The results are shown in table 2.

Table 2 - MIC values of antifungal agents in the absence and presence of myrtenol (MIC/8) on *C. albicans* and *C. parapsilosis* strains

Microorganism		**Fluconazole (μg/ mL) (MIC)**	**Fluconazole + Myrtenol (μg/ mL) (CIM/8)**
C. albicans	ATCC - 76485	4	64
	LM-516	4	64
C. parapsilosis	ATCC - 22019	8	64
	LM-55117	4	64

As seen in table 2 the MIC values of fluconazole alone is always less than when in combination with myrtenol in four strains of *Candida*. This demonstrates that myrtenol has no modulatory potential with fluconazole. This was the first work that verified such activity.

A study by PEREIRA, 2017, demonstrated that geraniol, a monoterpene such as myrtenol, had no modulating activity with fluconazole against *C. albicans*, *C. glabrata* and *C. kusei*. Another monoterpene, citral, studied by FREIRE et al., 2017, also classified this monoterpene as a non-modifier of nystatin activity against several strains of *C. albicans*.

2.3.3. Assay *checkerboard* of myrtenol to conventional antifungal

The *checkerboard* assay was developed to quantify the effect of antimicrobial combinations on the growth of microorganisms *in vitro* (JACKSON; AGBOKE; NWOKE, 2009). The method is used to study interactions involving the antimicrobial the formation of a matrix that represents the possible combinations of two antimicrobial serially diluted. This assay is designed to assess the inhibitory effects (ELIOPOULOS; ELIOPOULOS, 1988).

VAZQUEZ, 2008, there are great advantages in combining two drugs: the activity may not be possible with just one drug; decreasing the dosage, leading to a decrease in side effects and delaying or not enabling a strain of resistant microorganisms. However, it describes possible disadvantages, such as increased costs, adverse effects of two drugs, and a false sense of security that all pathogens are safe.

In the checkerboard assay, fluconazole and amphotericin B associated with myrtenol and the fractional inhibitory concentration (FIC) of this calculated association were analyzed. The results are shown in table 3.

Table 3 - Determination of the Fractional Inhibitory Concentration Index (FIC) of the association between myrtenol with amphotericin B and fluconazole on *C. albicans and C. parapsilosis* strains

Microorganism		**FIC Fluconazole and Myrtenol**	**FIC Amphotericin B and Myrtenol**
C. albicans	ATCC - 76485	1	0.125
	LM-516	1	4
C. parapsilosis	ATCC - 22019	0.25	0.625
	LM-55117	1	4

The results demonstrate that there is a synergistic effect of the association of fluconazole and myrtenol, observed in the strain *C. parapsilosis* ATCC-22019, since the FIC was 0.25 and indifferent in all other strains. The same synergistic effect was observed when myrtenol was associated with amphotericin B in *C. albicans* ATCC - 76485 strains, which has an FIC of 0.125 and an indifferent effect in the other strains, as the FIC was always greater than 0.5. Being the first study described in the literature with myrtenol.

This result is corroborated by SILVA et al., 2020, who studied the monoterpene citronellol in two optical isomers, against strains of *C. albicans* and *C. tropicalis*. With *C. albicans*, the result in three strains shows: two strains with synergistic result and one strain with indifferent result in combination with amphotericin B.

In this study, the result is partially similar to what DINIZ-NETO, 2018 found when he associated geraniol with amphotericin B against strains of *C. albicans* and *C. tropicalis*. Found an indifferent effect for all worked strains.

2.4. Conclusion

- ✓ Myrtenol has strong antifungal activity against the tested strains
- ✓ The activity is fungicidal
- ✓ Myrtenol does not potentiate the activity of fluconazole and amphotericin B, antifungals chosen in the research.
- ✓ The association of myrtenol and conventional antifungal agents showed a synergistic or indifferent effect depending on the strain tested.

2.5. References

ALEEM, M.; ANIS, M. **Therapeutic potential of Habb-ul-Aas (Myrtus communis Linn.) with Unani Perspective and Modern Pharmacology: A review**. *Journal of Pharmacognosy and Phytochemistry*, [s.l.], v. 10, nº 1, p. 910–923, 2021. ISSN: 23498234, DOI: 10.22271/phyto.2021.v10.i1m.13452.

ALIGIANNIS, N. et al. **Composition and antimicrobial activity of the essential oils of two Origanum species**. *Journal of Agricultural and Food Chemistry*, [s.l.], v. 49, nº 9, p. 4168–4170, 2001. ISSN: 00218561, DOI: 10.1021/jf001494m.

ALONSO MONGE, R. et al. **The MAP kinase signal transduction network in Candida albicans**. *Microbiology*, [s.l.], v. 152, nº 4, p. 905–912, 2006. ISSN: 13500872, DOI: 10.1099/mic.0.28616-0.

ALVAREZ-MORENO, C. A.; CORTES, J. A.; DENNING, D. W. **Burden of fungal infections in Colombia**. *Journal of Fungi*, [s.l.], v. 4, nº 2, p. 1–13, 2018. ISBN: 5713165000, ISSN: 2309608X, DOI: 10.3390/jof4020041.

BALOUIRI, M.; SADIKI, M.; IBNSOUDA, S. K. **Methods for in vitro evaluating antimicrobial activity: A review**. *Journal of Pharmaceutical Analysis*, [s.l.], v. 6, nº 2, p. 71–79, 2016. ISSN: 20951779, DOI: 10.1016/j.jpha.2015.11.005.

BEJESHK, M. A. et al. **Anti-inflammatory and anti-remodeling effects of myrtenol in the lungs of asthmatic rats: Histopathological and biochemical findings**. *Allergologia et Immunopathologia*, [s.l.], v. 47, nº 2, p. 185–193, 2019. ISSN: 15781267, DOI: 10.1016/j.aller.2018.09.003.

BHATIA, S. P. et al. **Fragrance material review on myrtenol**. *Food and Chemical Toxicology*, [s.l.], v. 46, nº 11 SUPPL., p. S237–S240, 2008. ISSN: 02786915, DOI: 10.1016/j.fct.2008.06.091.

BIZZO, H. R.; ANA MARIA, C. H.; REZENDE, C. M. **Oleos essenciais no brasil: aspectos gerais, desenvolvimento e perspectivas**. *Quimica Nova*, [s.l.], v. 32, nº 3, p. 588–594, 2009. ISSN: 16787064, DOI: 10.1590/S0100-40422009000300005.

CORDEIRO, L. et al. **Antibacterial and antibiofilm activity of myrtenol against Staphylococcus aureus**. *Pharmaceuticals*, [s.l.], v. 13, nº 6, p. 1–12, 2020. ISSN: 14248247, DOI: 10.3390/ph13060133.

COUTINHO, H. D. M. et al. **Herbal therapy associated with antibiotic therapy: Potentiation of the antibiotic activity against methicillin - Resistant Staphylococcus aureus by Turnera ulmifolia L**. *BMC Complementary and Alternative Medicine*, [s.l.], v. 9, p. 1–4, 2009. ISSN: 14726882, DOI: 10.1186/1472-6882-9-13.

DELATTIN, N.; CAMMUE, B. P.; THEVISSEN, K. **Reactive oxygen species-inducing antifungal agents and their activity against fungal biofilms**. *Future Medicinal Chemistry*, [s.l.], v. 6, nº 1, p. 77–90, 2014. ISSN: 17568919, DOI: 10.4155/fmc.13.189.

DOCKRELL, D. et al. **British <scp>HIV</scp> Association guidelines on the management of opportunistic infection in people living with <scp>HIV</scp> : The clinical management of Candidiasis 2019**. *HIV Medicine*, [s.l.], v. 20, nº S8, p. 2–24, 2019. ISSN: 1464-2662, DOI: 10.1111/hiv.12806.

DOI, A. M. et al. **Epidemiology and microbiologic characterization of nosocomial candidemia from a Brazilian national surveillance program**. *PLoS ONE*, [s.l.], v. 11, nº 1, p. 1–9, 2016. ISSN: 19326203, DOI: 10.1371/journal.pone.0146909.

ELIOPOULOS, G. M.; ELIOPOULOS, C. T. **Antibiotic combinations: should they be tested?** *Clinical Microbiology Reviews*, [s.l.], v. 1, nº 2, p. 139–56, 1988. ISSN: 0893-8512, DOI: 10.1128/cmr.1.2.139.

FELIPE, L. O.; BICAS, J. L. **Terpenos: compostos majoritários de óleos essenciais**. *Química e Sociedade*, [s.l.], v. 39, nº 2, p. 120–130, 2017. ISSN: 01048899.

FRANCISCONI, R. S. et al. **Terpinen-4-ol and nystatin co-loaded precursor of liquid crystalline system for topical treatment of oral candidiasis**. *Scientific Reports*, [s.l.], v. 10, nº 1, p. 1–12, 2020. ISBN: 0123456789, ISSN: 20452322, DOI: 10.1038/s41598-020-70085-z.

FREIRE, J. C. P. et al. **Atividade antifúngica de fitoterápicos sobre espécies de Candida: uma revisão de literatura**. *ARCHIVES OF HEALTH INVESTIGATION*, [s.l.], v. 5, nº 6, p. 537–546, 2016. ISSN: 2317-3009, DOI: 10.21270/archi.v5i6.1790.

_____. **Antifungal Activity of Essential Oils against Candida albicans Strains Isolated from Users of Dental Prostheses**. *Evidence-based Complementary and Alternative Medicine*, [s.l.], v. 2017, 2017. ISSN: 17414288, DOI: 10.1155/2017/7158756.

GARCIA-CUESTA, C.; SARRION-PÉREZ, M. G.; BAGÁN, J. V. **Current treatment of oral candidiasis: A literature review**. *Journal of Clinical and Experimental Dentistry*, [s.l.], v. 6, nº 5, p. e576–e582, 2014. ISSN: 19895488, DOI: 10.4317/jced.51798.

GOMES, B. S. et al. **Anti-inflammatory effect of the monoterpene myrtenol is dependent on the direct modulation of neutrophil migration and oxidative stress**. *Chemico-Biological Interactions*, [s.l.], v. 273, p. 73–81, 2017. ISSN: 18727786, DOI: 10.1016/j.cbi.2017.05.019.

GRIFFIN, S. G. et al. **The role of structure and molecular properties of terpenoids in determining their antimicrobial activity**. *Flavour and Fragrance Journal*, [s.l.], v. 14, nº 5, p. 322–332, 1999. ISSN: 08825734, DOI: 10.1002/(SICI)1099-1026(199909/10)14:5<322::AID-FFJ837>3.0.CO;2-4.

GUPTE, M.; KULKARNI, P.; GANGULI, B. N. **Antifungal antibiotics**. *Applied Microbiology and Biotechnology*, [s.l.], v. 58, nº 1, p. 46–57, 2002. ISSN: 01757598, DOI: 10.1007/s002530100822.

HADACEK, F.; GREGER, H. **Testing of antifungal natural products: Methodologies, comparability of results and assay choice**. *Phytochemical Analysis*, [s.l.], v. 11, nº 3, p. 137–147, 2000. ISSN: 09580344, DOI: 10.1002/(SICI)1099-1565(200005/06)11:3<137::AID-PCA514>3.0.CO;2-I.

HASAN, F. et al. **Topical Amphotericin B formulas: Promising new application**. *International Journal of Medical Science and Current Research (IJMSCR) International Journal of Medical Science and Current Research*, [s.l.], v. 2, nº 4, p. 2209–2862, 2019.

HUANG, S. et al. **Myrtenol improves brain damage and promotes angiogenesis in rats with cerebral infarction by activating the ERK1/2 signalling pathway**. *Pharmaceutical Biology*, [s.l.], v. 59, nº 1, p. 584–593, 2021. ISSN: 17445116, DOI: 10.1080/13880209.2021.1917626.

İŞCAN, G. **Antibacterial and anticandidal activities of common essential oil constituents**. *Records of Natural Products*, [s.l.], v. 11, nº 4, p. 374–388, 2017. ISSN: 13076167.

JACKSON, C.; AGBOKE, A.; NWOKE, V. **In vitro evaluation of antimicrobial activity of combinations of nystatin and Euphorbia hirta leaf extract against Candida albicans by the checkerboard method**. *Journal of Medicinal Plants Research*, [s.l.], v. 3, nº 9, p. 666–669, 2009. ISBN: 2348035499, ISSN: 19960875.

KANAFANI, Z. A.; PERFECT, J. R. **Resistance to antifungal agents: Mechanisms and clinical impact**. *Clinical Infectious Diseases*, [s.l.], v. 46, nº 1, p. 120–128, 2008. ISSN: 10584838, DOI: 10.1086/524071.

KLEPSER, M. E. et al. **Evaluation of endpoints for antifungal susceptibility determinations with LY303366**. *Antimicrobial Agents and Chemotherapy*, [s.l.], v. 42, nº 6, p. 1387–1391, 1998. ISSN: 00664804, DOI: 10.1128/aac.42.6.1387.

LAMOTH, F. et al. **Changes in the epidemiological landscape of invasive candidiasis**. *Journal of Antimicrobial Chemotherapy*, [s.l.], v. 73, nº January, p. i4–i13, 2018. ISSN: 14602091, DOI: 10.1093/jac/dkx444.

LEE, Y. et al. **Antifungal Drug Resistance: Molecular Mechanisms in Candida albicans and beyond**. *Chemical Reviews*, [s.l.], v. 121, nº 6, p. 3390–3411, 2021. ISSN: 15206890, DOI: 10.1021/acs.chemrev.0c00199.

LYU, X. et al. **Efficacy of nystatin for the treatment of oral candidiasis: A systematic review and meta-analysis**. *Drug Design, Development and Therapy*, [s.l.], v. 10, p. 1161–1171, 2016. ISSN: 11778881, DOI: 10.2147/DDDT.S100795.

MACKAY, M. L.; MILNE, K.; GOULD, I. M. **Comparison of methods for assessing synergic antibiotic interactions**. *International Journal of Antimicrobial Agents*, [s.l.], v. 15, nº 2, p. 125–129, 2000. ISSN: 09248579, DOI: 10.1016/S0924-8579(00)00149-7.

MOHR, J. et al. **Current options in antifungal pharmacotherapy**. *Pharmacotherapy*, [s.l.], v. 28, nº 5 PART 1, p. 614–645, 2008. ISSN: 02770008, DOI: 10.1592/phco.28.5.614.

MOREIRA, M. R. C. et al. **Anxiolytic-like effects and mechanism of (-)-myrtenol: A monoterpene alcohol**. *Neuroscience Letters*, [s.l.], v. 579, p. 119–124, 2014. ISSN: 18727972, DOI: 10.1016/j.neulet.2014.07.007.

NOBILE, C. J.; JOHNSON, A. D. **Candida albicans Biofilms and Human Disease**. *Annual Review of Microbiology*, [s.l.], v. 69, nº 1, p. 71–92, 2015. ISSN: 15453251, DOI: 10.1146/annurev-micro-091014-104330.

OSTROSKY, E. A. et al. **Métodos para avaliação da atividade antimicrobiana e determinação da Concentração Mínima Inibitória (CMI) de plantas medicinais**. *Revista Brasileira de Farmacognosia*, [s.l.], v. 18, nº 2, p. 301–307, 2008. ISSN: 0102-695X, DOI: 10.1590/s0102-695x2008000200026.

RODLOFF, A.; KOCH, D.; SCHAUMANN, R. **Epidemiology and antifungal resistance in invasive candidiasis**. *European Journal of Medical Research*, [s.l.], v. 16, nº 4, p. 187, 2011. ISSN: 2047-783X, DOI: 10.1186/2047-783X-16-4-187.

RODRIGUEZ, L.; ROSA, A.; JEWTUCHOWICZ, V. **Candida parapsilosis Sensu Stricto: A Common Colonizing in Oral Mucosa of Argentine Subjects**. *Journal of Emerging Infectious Diseases*, [s.l.], v. 01, nº 04, p. 1–8, 2016. ISSN: 24724998, DOI: 10.4172/2472-4998.1000118.

SARTORATTO, A. et al. **Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil**. *Brazilian Journal of Microbiology*, [s.l.], v. 35, nº 4, p. 275–280, 2004. ISSN: 16784405, DOI: 10.1590/S1517-83822004000300001.

SELVARAJ, A. et al. **Myrtenol Attenuates MRSA Biofilm and Virulence by Suppressing sarA Expression Dynamism**. *Frontiers in Microbiology*, [s.l.], v. 10, nº September, p. 1–15, 2019. ISSN: 1664302X, DOI: 10.3389/fmicb.2019.02027.

_____. **Antibiofilm and antivirulence efficacy of myrtenol enhances the antibiotic susceptibility of Acinetobacter baumannii**. *Scientific Reports*, [s.l.], v. 10, nº 1, p. 1–14, 2020. ISBN: 4159802079, ISSN: 20452322, DOI: 10.1038/s41598-020-79128-x.

SHARMA, Y. et al. **Anti-Candida activity of geraniol: Effect on hydrolytic enzyme secretion and biofilm formation**. *Journal of Pure and Applied Microbiology*, [s.l.], v.

12, nº 3, p. 1337–1349, 2018. ISSN: 2581690X, DOI: 10.22207/JPAM.12.3.36.

SILVA, D. et al. **Combination with Amphotericin B against Candida Spp.** [s.l.], 2020.

SILVA, I. et al. **Antifungal Activity of Eugenol and its Association with Nystatin on Candida albicans**. *Pesquisa Brasileira em Odontopediatria e Clínica Integrada*, [s.l.], v. 17, nº 1, p. 1–8, 2017. ISSN: 15190501, DOI: 10.4034/PBOCI.2017.171.16.

SISAY, M.; GASHAW, T. **Ethnobotanical, Ethnopharmacological, and Phytochemical Studies of Myrtus communis Linn: A Popular Herb in Unani System of Medicine**. *Journal of Evidence-Based Complementary and Alternative Medicine*, [s.l.], v. 22, nº 4, p. 1035–1043, 2017. ISBN: 2156587217718, ISSN: 21565872, DOI: 10.1177/2156587217718958.

TROFA, D.; GÁCSER, A.; NOSANCHUK, J. D. **Candida parapsilosis, an emerging fungal pathogen**. *Clinical Microbiology Reviews*, [s.l.], v. 21, nº 4, p. 606–625, 2008. ISSN: 08938512, DOI: 10.1128/CMR.00013-08.

TROMBETTA, D. et al. **Mechanisms of antibacterial action of three monoterpenes**. *Antimicrobial Agents and Chemotherapy*, [s.l.], v. 49, nº 6, p. 2474–2478, 2005. ISSN: 00664804, DOI: 10.1128/AAC.49.6.2474-2478.2005.

VAZQUEZ, J. A. **Combination antifungal therapy for mold infections: Much ado about nothing?** *Clinical Infectious Diseases*, [s.l.], v. 46, nº 12, p. 1889–1901, 2008. ISSN: 10584838, DOI: 10.1086/588475.

WANG, S. Y. et al. **Chemical composition and antifungal activity of essential oil isolated from Chamaecyparis formosensis Matsum. wood**. *Holzforschung*, [s.l.], v. 59, nº 3, p. 295–299, 2005. ISSN: 00183830, DOI: 10.1515/HF.2005.049.

XU, J. et al. **Eucalyptal D enhances the antifungal effect of fluconazole on fluconazole-resistant candida albicans by competitively inhibiting efflux pump**. *Frontiers in Cellular and Infection Microbiology*, [s.l.], v. 9, nº JUN, p. 1–10, 2019. ISSN: 22352988, DOI: 10.3389/fcimb.2019.00211.

YILAR, M.; BAYAN, Y.; ONARAN, A. **Chemical composition and antifungal effects of vitex agnus-castus L. and myrtus communis L. plants**. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, [s.l.], v. 44, nº 2, p. 466–471, 2016. ISSN: 18424309, DOI: 10.15835/nbha44210399.

3. CONSIDERAÇÕES FINAIS

Este estudo permitiu o início de pesquisa com o mirtenol para suas potenciais atividades antifúngicas sobre as espécies de *Candida*.

O mirtenol tem outros usos na indústria cosmética(BHATIA et al., 2008), possuindo também atividade documentada como ansiolítica(MOREIRA et al., 2014), antibiótica(CORDEIRO et al., 2020), anti-inflamatória(BEJESHK et al., 2019) e antifúngica(İŞCAN, 2017). O mecanismo de ação antifúngica ainda é desconhecido.

Seria ideal que mais testes fossem realizados para o uso *in vivo* para determinação da eficiência antifúngica.

REFERÊNCIAS

ALEEM, M.; ANIS, M. **Therapeutic potential of Habb-ul-Aas (Myrtus communis Linn.) with Unani Perspective and Modern Pharmacology: A review**. *Journal of Pharmacognosy and Phytochemistry*, [s.l.], v. 10, nº 1, p. 910–923, 2021. ISSN: 23498234, DOI: 10.22271/phyto.2021.v10.i1m.13452.

ALIGIANNIS, N. et al. **Composition and antimicrobial activity of the essential oils of two Origanum species**. *Journal of Agricultural and Food Chemistry*, [s.l.], v. 49, nº 9, p. 4168–4170, 2001. ISSN: 00218561, DOI: 10.1021/jf001494m.

ALONSO MONGE, R. et al. **The MAP kinase signal transduction network in Candida albicans**. *Microbiology*, [s.l.], v. 152, nº 4, p. 905–912, 2006. ISSN: 13500872, DOI: 10.1099/mic.0.28616-0.

ALVAREZ-MORENO, C. A.; CORTES, J. A.; DENNING, D. W. **Burden of fungal infections in Colombia**. *Journal of Fungi*, [s.l.], v. 4, nº 2, p. 1–13, 2018. ISBN: 5713165000, ISSN: 2309608X, DOI: 10.3390/jof4020041.

BALOUIRI, M.; SADIKI, M.; IBNSOUDA, S. K. **Methods for in vitro evaluating antimicrobial activity: A review**. *Journal of Pharmaceutical Analysis*, [s.l.], v. 6, nº 2, p. 71–79, 2016. ISSN: 20951779, DOI: 10.1016/j.jpha.2015.11.005.

BEJESHK, M. A. et al. **Anti-inflammatory and anti-remodeling effects of myrtenol in the lungs of asthmatic rats: Histopathological and biochemical findings**. *Allergologia et Immunopathologia*, [s.l.], v. 47, nº 2, p. 185–193, 2019. ISSN: 15781267, DOI: 10.1016/j.aller.2018.09.003.

BHATIA, S. P. et al. **Fragrance material review on myrtenol**. *Food and Chemical Toxicology*, [s.l.], v. 46, nº 11 SUPPL., p. S237–S240, 2008. ISSN: 02786915, DOI: 10.1016/j.fct.2008.06.091.

BIZZO, H. R.; ANA MARIA, C. H.; REZENDE, C. M. **Oleos essenciais no brasil: aspectos gerais, desenvolvimento e perspectivas**. *Quimica Nova*, [s.l.], v. 32, nº 3, p. 588–594, 2009. ISSN: 16787064, DOI: 10.1590/S0100-40422009000300005.

CORDEIRO, L. et al. **Antibacterial and antibiofilm activity of myrtenol against Staphylococcus aureus**. *Pharmaceuticals*, [s.l.], v. 13, nº 6, p. 1–12, 2020. ISSN:

14248247, DOI: 10.3390/ph13060133.

COUTINHO, H. D. M. et al. **Herbal therapy associated with antibiotic therapy: Potentiation of the antibiotic activity against methicillin - Resistant Staphylococcus aureus by Turnera ulmifolia L**. *BMC Complementary and Alternative Medicine*, [s.l.], v. 9, p. 1–4, 2009. ISSN: 14726882, DOI: 10.1186/1472-6882-9-13.

DELATTIN, N.; CAMMUE, B. P.; THEVISSEN, K. **Reactive oxygen species-inducing antifungal agents and their activity against fungal biofilms**. *Future Medicinal Chemistry*, [s.l.], v. 6, nº 1, p. 77–90, 2014. ISSN: 17568919, DOI: 10.4155/fmc.13.189.

DOCKRELL, D. et al. **British <scp>HIV</scp> Association guidelines on the management of opportunistic infection in people living with <scp>HIV</scp> : The clinical management of Candidiasis 2019**. *HIV Medicine*, [s.l.], v. 20, nº S8, p. 2–24, 2019. ISSN: 1464-2662, DOI: 10.1111/hiv.12806.

DOI, A. M. et al. **Epidemiology and microbiologic characterization of nosocomial candidemia from a Brazilian national surveillance program**. *PLoS ONE*, [s.l.], v. 11, nº 1, p. 1–9, 2016. ISSN: 19326203, DOI: 10.1371/journal.pone.0146909.

ELIOPOULOS, G. M.; ELIOPOULOS, C. T. **Antibiotic combinations: should they be tested?** *Clinical Microbiology Reviews*, [s.l.], v. 1, nº 2, p. 139–56, 1988. ISSN: 0893-8512, DOI: 10.1128/cmr.1.2.139.

FELIPE, L. O.; BICAS, J. L. **Terpenos: compostos majoritários de óleos essenciais**. *Química e Sociedade*, [s.l.], v. 39, nº 2, p. 120–130, 2017. ISSN: 01048899.

FRANCISCONI, R. S. et al. **Terpinen-4-ol and nystatin co-loaded precursor of liquid crystalline system for topical treatment of oral candidiasis**. *Scientific Reports*, [s.l.], v. 10, nº 1, p. 1–12, 2020. ISBN: 0123456789, ISSN: 20452322, DOI: 10.1038/s41598-020-70085-z.

FREIRE, J. C. P. et al. **Atividade antifúngica de fitoterápicos sobre espécies de Candida: uma revisão de literatura**. *ARCHIVES OF HEALTH INVESTIGATION*, [s.l.], v. 5, nº 6, p. 537–546, 2016. ISSN: 2317-3009, DOI: 10.21270/archi.v5i6.1790.

_____. **Antifungal Activity of Essential Oils against Candida albicans Strains Isolated from Users of Dental Prostheses**. *Evidence-based Complementary and*

Alternative Medicine, [s.l.], v. 2017, 2017. ISSN: 17414288, DOI: 10.1155/2017/7158756.

GARCIA-CUESTA, C.; SARRION-PÉREZ, M. G.; BAGÁN, J. V. **Current treatment of oral candidiasis: A literature review**. *Journal of Clinical and Experimental Dentistry*, [s.l.], v. 6, nº 5, p. e576–e582, 2014. ISSN: 19895488, DOI: 10.4317/jced.51798.

GOMES, B. S. et al. **Anti-inflammatory effect of the monoterpene myrtenol is dependent on the direct modulation of neutrophil migration and oxidative stress**. *Chemico-Biological Interactions*, [s.l.], v. 273, p. 73–81, 2017. ISSN: 18727786, DOI: 10.1016/j.cbi.2017.05.019.

GRIFFIN, S. G. et al. **The role of structure and molecular properties of terpenoids in determining their antimicrobial activity**. *Flavour and Fragrance Journal*, [s.l.], v. 14, nº 5, p. 322–332, 1999. ISSN: 08825734, DOI: 10.1002/(SICI)1099-1026(199909/10)14:5<322::AID-FFJ837>3.0.CO;2-4.

GUPTE, M.; KULKARNI, P.; GANGULI, B. N. **Antifungal antibiotics**. *Applied Microbiology and Biotechnology*, [s.l.], v. 58, nº 1, p. 46–57, 2002. ISSN: 01757598, DOI: 10.1007/s002530100822.

HADACEK, F.; GREGER, H. **Testing of antifungal natural products: Methodologies, comparability of results and assay choice**. *Phytochemical Analysis*, [s.l.], v. 11, nº 3, p. 137–147, 2000. ISSN: 09580344, DOI: 10.1002/(SICI)1099-1565(200005/06)11:3<137::AID-PCA514>3.0.CO;2-I.

HASAN, F. et al. **Topical Amphotericin B formulas: Promising new application**. *International Journal of Medical Science and Current Research (IJMSCR) International Journal of Medical Science and Current Research*, [s.l.], v. 2, nº 4, p. 2209–2862, 2019.

HUANG, S. et al. **Myrtenol improves brain damage and promotes angiogenesis in rats with cerebral infarction by activating the ERK1/2 signalling pathway**. *Pharmaceutical Biology*, [s.l.], v. 59, nº 1, p. 584–593, 2021. ISSN: 17445116, DOI: 10.1080/13880209.2021.1917626.

İŞCAN, G. **Antibacterial and anticandidal activities of common essential oil constituents**. *Records of Natural Products*, [s.l.], v. 11, nº 4, p. 374–388, 2017. ISSN:

13076167.

JACKSON, C.; AGBOKE, A.; NWOKE, V. **In vitro evaluation of antimicrobial activity of combinations of nystatin and Euphorbia hirta leaf extract against Candida albicans by the checkerboard method**. *Journal of Medicinal Plants Research*, [s.l.], v. 3, nº 9, p. 666–669, 2009. ISBN: 2348035499, ISSN: 19960875.

KANAFANI, Z. A.; PERFECT, J. R. **Resistance to antifungal agents: Mechanisms and clinical impact**. *Clinical Infectious Diseases*, [s.l.], v. 46, nº 1, p. 120–128, 2008. ISSN: 10584838, DOI: 10.1086/524071.

KLEPSER, M. E. et al. **Evaluation of endpoints for antifungal susceptibility determinations with LY303366**. *Antimicrobial Agents and Chemotherapy*, [s.l.], v. 42, nº 6, p. 1387–1391, 1998. ISSN: 00664804, DOI: 10.1128/aac.42.6.1387.

LAMOTH, F. et al. **Changes in the epidemiological landscape of invasive candidiasis**. *Journal of Antimicrobial Chemotherapy*, [s.l.], v. 73, nº January, p. i4–i13, 2018. ISSN: 14602091, DOI: 10.1093/jac/dkx444.

LEE, Y. et al. **Antifungal Drug Resistance: Molecular Mechanisms in Candida albicans and beyond**. *Chemical Reviews*, [s.l.], v. 121, nº 6, p. 3390–3411, 2021. ISSN: 15206890, DOI: 10.1021/acs.chemrev.0c00199.

LYU, X. et al. **Efficacy of nystatin for the treatment of oral candidiasis: A systematic review and meta-analysis**. *Drug Design, Development and Therapy*, [s.l.], v. 10, p. 1161–1171, 2016. ISSN: 11778881, DOI: 10.2147/DDDT.S100795.

MACKAY, M. L.; MILNE, K.; GOULD, I. M. **Comparison of methods for assessing synergic antibiotic interactions**. *International Journal of Antimicrobial Agents*, [s.l.], v. 15, nº 2, p. 125–129, 2000. ISSN: 09248579, DOI: 10.1016/S0924-8579(00)00149-7.

MOHR, J. et al. **Current options in antifungal pharmacotherapy**. *Pharmacotherapy*, [s.l.], v. 28, nº 5 PART 1, p. 614–645, 2008. ISSN: 02770008, DOI: 10.1592/phco.28.5.614.

MOREIRA, M. R. C. et al. **Anxiolytic-like effects and mechanism of (-)-myrtenol: A monoterpene alcohol**. *Neuroscience Letters*, [s.l.], v. 579, p. 119–124, 2014. ISSN: 18727972, DOI: 10.1016/j.neulet.2014.07.007.

NOBILE, C. J.; JOHNSON, A. D. **Candida albicans Biofilms and Human Disease**.

Annual Review of Microbiology, [s.l.], v. 69, nº 1, p. 71–92, 2015. ISSN: 15453251, DOI: 10.1146/annurev-micro-091014-104330.

OSTROSKY, E. A. et al. **Métodos para avaliação da atividade antimicrobiana e determinação da Concentração Mínima Inibitória (CMI) de plantas medicinais**. *Revista Brasileira de Farmacognosia*, [s.l.], v. 18, nº 2, p. 301–307, 2008. ISSN: 0102-695X, DOI: 10.1590/s0102-695x2008000200026.

RODLOFF, A.; KOCH, D.; SCHAUMANN, R. **Epidemiology and antifungal resistance in invasive candidiasis**. *European Journal of Medical Research*, [s.l.], v. 16, nº 4, p. 187, 2011. ISSN: 2047-783X, DOI: 10.1186/2047-783X-16-4-187.

RODRIGUEZ, L.; ROSA, A.; JEWTUCHOWICZ, V. **Candida parapsilosis Sensu Stricto: A Common Colonizing in Oral Mucosa of Argentine Subjects**. *Journal of Emerging Infectious Diseases*, [s.l.], v. 01, nº 04, p. 1–8, 2016. ISSN: 24724998, DOI: 10.4172/2472-4998.1000118.

SARTORATTO, A. et al. **Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil**. *Brazilian Journal of Microbiology*, [s.l.], v. 35, nº 4, p. 275–280, 2004. ISSN: 16784405, DOI: 10.1590/S1517-83822004000300001.

SELVARAJ, A. et al. **Myrtenol Attenuates MRSA Biofilm and Virulence by Suppressing sarA Expression Dynamism**. *Frontiers in Microbiology*, [s.l.], v. 10, nº September, p. 1–15, 2019. ISSN: 1664302X, DOI: 10.3389/fmicb.2019.02027.

_____. **Antibiofilm and antivirulence efficacy of myrtenol enhances the antibiotic susceptibility of Acinetobacter baumannii**. *Scientific Reports*, [s.l.], v. 10, nº 1, p. 1–14, 2020. ISBN: 4159802079, ISSN: 20452322, DOI: 10.1038/s41598-020-79128-x.

SHARMA, Y. et al. **Anti-Candida activity of geraniol: Effect on hydrolytic enzyme secretion and biofilm formation**. *Journal of Pure and Applied Microbiology*, [s.l.], v. 12, nº 3, p. 1337–1349, 2018. ISSN: 2581690X, DOI: 10.22207/JPAM.12.3.36.

SILVA, D. et al. **Combination with Amphotericin B against Candida Spp.** [s.l.], 2020.

SILVA, I. et al. **Antifungal Activity of Eugenol and its Association with Nystatin on Candida albicans**. *Pesquisa Brasileira em Odontopediatria e Clínica Integrada*, [s.l.], v. 17, nº 1, p. 1–8, 2017. ISSN: 15190501, DOI: 10.4034/PBOCI.2017.171.16.

SISAY, M.; GASHAW, T. **Ethnobotanical, Ethnopharmacological, and Phytochemical Studies of Myrtus communis Linn: A Popular Herb in Unani System of Medicine**. *Journal of Evidence-Based Complementary and Alternative Medicine*, [s.l.], v. 22, nº 4, p. 1035–1043, 2017. ISBN: 2156587217718, ISSN: 21565872, DOI: 10.1177/2156587217718958.

TROFA, D.; GÁCSER, A.; NOSANCHUK, J. D. **Candida parapsilosis, an emerging fungal pathogen**. *Clinical Microbiology Reviews*, [s.l.], v. 21, nº 4, p. 606–625, 2008. ISSN: 08938512, DOI: 10.1128/CMR.00013-08.

TROMBETTA, D. et al. **Mechanisms of antibacterial action of three monoterpenes**. *Antimicrobial Agents and Chemotherapy*, [s.l.], v. 49, nº 6, p. 2474–2478, 2005. ISSN: 00664804, DOI: 10.1128/AAC.49.6.2474-2478.2005.

VAZQUEZ, J. A. **Combination antifungal therapy for mold infections: Much ado about nothing?** *Clinical Infectious Diseases*, [s.l.], v. 46, nº 12, p. 1889–1901, 2008. ISSN: 10584838, DOI: 10.1086/588475.

WANG, S. Y. et al. **Chemical composition and antifungal activity of essential oil isolated from Chamaecyparis formosensis Matsum. wood**. *Holzforschung*, [s.l.], v. 59, nº 3, p. 295–299, 2005. ISSN: 00183830, DOI: 10.1515/HF.2005.049.

XU, J. et al. **Eucalyptal D enhances the antifungal effect of fluconazole on fluconazole-resistant candida albicans by competitively inhibiting efflux pump**. *Frontiers in Cellular and Infection Microbiology*, [s.l.], v. 9, nº JUN, p. 1–10, 2019. ISSN: 22352988, DOI: 10.3389/fcimb.2019.00211.

YILAR, M.; BAYAN, Y.; ONARAN, A. **Chemical composition and antifungal effects of vitex agnus-castus L. and myrtus communis L. plants**. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, [s.l.], v. 44, nº 2, p. 466–471, 2016. ISSN: 18424309, DOI: 10.15835/nbha44210399.

ANEXO

PRINT DE SUBMISSÃO DO ARTIGO A REVISTA