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GUILHERME MENDES PRADO

AVALIAÇÃO DA ATIVIDADE ANTIFÚNGICA, *MOLECULAR DOCKING* E CITOTOXICIDADE DO *Cymbopogon citratus* E *Cymbopogon nardus* FRENTE A ISOLADOS CLÍNICOS DE *Candida albicans* NA FORMA PLANCTÔNICA E BIOFILME

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“Em um estado sombrio nós nos encontramos... um pouco mais de conhecimento iluminar nosso caminho pode”.

(Mestre Yoda, *Star Wars:* Episódio III)

RESUMO

O Brasil é um país rico em biodiversidade vegetal, possuindo plantas nativas ou bem adaptadas em abundância. Dentre esses vegetais alguns são utilizados pelas comunidades, como *Cymbopogon citratus* (DC.) Stapf e *Cymbopogon nardus* (L) Rendle, estes espécimes botânicos possuem no seu metabolismo secundário diversos compostos fitoquímicos com potencial antifúngico. *C. albicans* é um fungo leveduriforme causador de muitas infecções, dentre as quais as infecções relacionadas à assistência à saúde (IRAS) se destacando assim como um problema de grande impacto na saúde pública, causando especialmente as candidemias. O objetivo deste trabalho foi avaliar atividade antifúngica, citotoxicidade e *molecular docking* dos óleos essenciais de *C. citratus* (Cc) e *C. nardus* (Cn) contra isolados clínicos de *C. albicans* na forma planctônica e biofilme. Os óleos essenciais foram extraídos por hidrodestilação e os constituintes foram identificados por cromatografia gasosa juntamente com espectrometria de massa. A CIM foi determinada pelo método de microdiluição em caldo. O efeito da associação do óleo essencial com a anfotericina B foi verificado pelo teste *checkerboard*. A atividade frente ao biofilme foi determinada por meio do metabolismo com ensaio de XTT. Os ensaios de citotoxicidade foram realizados com células VERO. O *molecular docking* foi realizado para prever o modo de interação do óleo no sítio de ligação ativo da levedura. No ensaio com células planctônicas foram obtidas CIMs entre 78,12 e 156,25 µg/mL para OECC, e para OECN 312,50 e 625 µg/ml. Os resultados do *checkerboard* mostraram sinergismo entre o OECC ou OECN e a anfotericina B. O OECC inibiu atividade metabólica do biofilme em até 81%, enquanto O'CN apresentou redução de até 93%, ambos em concentração de 2x CIM. O ensaio MTT do óleo essencial de *C. citratus* mostrou citotoxicidade em concentrações testadas acima de 250 µg/ml, enquanto o do *C. nardus* não apresentou citotoxicidade até a concentração de 1.000 µg/ml. Já no *molecular docking* observou-se interação entre alguns constituintes com a proteína SAP-5. Os óleos essenciais extraídos das especies de *Cymbopogon* testados apresentaram atividade anti-*Candida* contra células planctônicas e sésseis, com potencial para aplicação posterior na terapia contra infecções causadas por *C. albicans.*

Palavras-chave: Biofilme; Candidemias; Microdiluição; Óleo Essencial; Sinergismo.

ABSTRACT

Brazil is a rich country on plant biodiversity, with native or well-adapted plants in abundance. Among these plants, some are used by communities, such as *Cymbopogon citratus* (DC). Stapf and *Cymbopogon nardus* (L) Rendle, these botanical specimens have in their secondary metabolism several phytochemical compounds with antifungal potential. *C. albicans* is a yeast-like fungus that causes many infections, among which healthcare-associated infections stand out as a problem with a great impact on public health, especially causing candidemia. The objective of this work was to evaluate antifungal activity, cytotoxicity and molecular docking of essential oils of *C. citratus* (Cc) and *C. nardus* (Cn) against clinical isolates of *C. albicans* in planktonic form and biofilm. Essential oils were extracted by hydrodistillation and constituents were identified by gas chromatography along with mass spectrometry. The MIC was determined by the broth microdilution method. The effect of the association of essential oil with amphotericin B was verified by the checkerboard test. The activity against the biofilm was determined through metabolism with XTT assay. Cytotoxicity assays were performed with VERO cells. Molecular docking was performed to predict the mode of oil interaction at the active yeast binding site. In the assay with planktonic cells MICs between 78.12 and 156.25 µg/mL were obtained for OECC, and for OECN 312.50 and 625 µg/ml. The checkerboard results showed synergism between OECC or OECN and amphotericin B. OECC inhibited biofilm metabolic activity by up to 81%, while OECN showed a reduction of up to 93%, both at a concentration of 2xMIC. The MTT assay of the essential oil of *C. citratus* showed cytotoxicity at tested concentrations above 250 µg/ml, while that of *C. nardus* did not show cytotoxicity up to a concentration of 1,000 µg/ml. In the molecular docking, interaction between some constituents and the SAP-5 protein was observed. The essential oils from the *Cymbopogon* specimens tested showed anti-*Candida* activity against planktonic and sessile cells, with potential for further application in therapy against infections caused by *C. albicans*.

Keywords: Biofilm; Candidemias; Microdilution; Essential oil; Synergism.

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LISTA DE ABREVIATURAS E SIGLAS

A3	Lanosina 14-alfa-desmetilase
AMB	Amphotericin B
ANOVA	Análise de Variância
ATCC	American type culture collection
Cc	*Cymbopogon citratus*
CIF	Concentração inibitória fracionada
CIM	Concentração inibitória mínima
CHO	Chinese hamster ovary cells
CLSI	Clinical and Laboratory Standards Institute
Cn	*Cymbopogon nardus*
FLU	Fluconazole
GC-MS	Cromatografia Gasosa Acoplada à Espectrometria de Massas
HRN	Hospital regional norte
HSCM	Hospital Santa Casa de Misericórdia
FICI	Índice de Concentração Inibitória Fracionada
IRAS	*Infecções Relacionadas à Assistência à Saúde*
LAMBIC	Laboratório de Microbiologia da Universidade Estadual Vale do Acaraú
M2	Metabolismo secundário
MEP	Methylerythritol fosfato
MTT	3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio
OE	Óleo essencial
PCR	Reação em cadeia da polimerase
PNPIC	Políticas Nacionais de Práticas Integrativas e Complementares
RPMI	*Roswell park memorial institute*
SDA	Sabouraud dextrose agar
SAP-5	Secreted aspartic proteases
SDS	Dodecilsulfato de Sódio
SFV	Soro fetal bovino
UFC	Universidade Federal do Ceará
UTI	*Unidades de Terapia Intensivas*
UVA	Universidade Estadual Vale do Acaraú
XTT	Sal tetrazole
WI38	Non-cancerous human fibroblast cell line
MRC-5	Fibroblast
HepG-2	Hepatic
RMSD	*Root mean square deviation*
ΔG	Energia livre

LISTA DE SÍMBOLOS

°C	Graus Célsius
Å	Angstrom
µL	Microlitro
µM	Micrometro
cm	Centímetro
D	Densidade
g	Grama
G	Constante gravitacional
IR	Índice de Retenção
L	litro
M	Massa molar
mg	miligramas
mL	Mililitro
mM	Milímetro
nM	Nanômetro
UFC	Unidades Formadora de Colônia

SUMÁRIO

1 INTRODUÇÃO

O Brasil é um país em desenvolvimento localizado em sua maioria na zona tropical, que apresenta uma elevada biodiversidade de espécies vegetais. Dentre essas podemos destacar plantas que possuem relevância socioeconômica para diversas comunidades devido ao seu potencial de bioprospecção para as indústrias alimentícias e farmacêuticas, assim como uso pela própria comunidade (SOUSA *et al*., 2017a).

A aplicação dessas ervas, e seus derivados, pela medicina é um ponto de relevância para saúde pública. Desse modo, a Organização Mundial da Saúde (OMS) em 1977 iniciou o processo de regularização para o uso de plantas e seus derivados, todavia apenas em 2006 com a portaria nº 971 foi que essas práticas passaram a ser regulamentadas no Brasil juntamente às práticas integrativas e complementares. Entretanto, projetos como a Farmácia Viva, criados em 1996, já eram alguns dos pontos de início para estudos de plantas medicinais no território brasileiro diretamente interligado à população (BORGES; SALES, 2018; CARNEVALE; BANDEIRA; BARROS, 2018).

A capacidade dos vegetais de sanar doenças é proveniente do metabolismo secundário (M2) dessas plantas. Este metabolismo pode produzir uma série de substâncias que variam de acordo com os espécimes vegetais e fatores ambientais, dentre os principais produtos do M2 se destacam os taninos, alcaloides, flavonoides, saponinas, cumarinas e os terpenoides. Dentre esses metabólitos os terpenoides são um dos principais constituintes dos óleos essenciais (OEs), além desse, há outro extrato de relevância, como aquoso, etanólicos e hexânico, que permite a extração de diversos metabólitos de acordo com a polaridade do composto (YANG *et al*., 2018; ISAH, 2019).

Algumas plantas se destacam pelo seu amplo acesso e uso difundido pela população e pela ciência, sendo o caso do gênero *Cymbopogon* sp., vegetal que apresenta como principais representantes as espécies *C. citratus* (DC.) Stapf e *C. nardus* (L.) Rendle, nessa ordem, nomeadas popularmente de Capim-santo e Citronela. Tais exemplares são utilizados pela comunidade principalmente pelo seu potencial calmante e repelente, respectivamente (LORENZI; MATOS, 2008; AVOSEH *et al*., 2015; GROSS *et al*., 2019). Entretanto, segundo Silva (2019), Khosravi (2018) e seus colaboradores, essas plantas também possuem um grande potencial antimicrobiano.

Tendo em vista o potencial desse gênero vegetal e o interesse das indústrias farmacêuticas para a descoberta de novos medicamentos, principalmente aqueles que possuem

atividades contra microrganismos, pode-se ressaltar aqueles que possuem propriedades farmacológicas no combate a microrganismos de difícil tratamento ou fármacos que apresentem menor toxicidade ou efeitos adversos, assim fortalecendo a pesquisa para novos fármacos a partir de plantas, como os *Cymbopogon* sp. (RANG *et al*., 2016; GALIE *et al*.,2018; WINSKA *et al*., 2019).

Assim o uso de ervas medicinais e seus derivados, se tornaram uma possível alternativa de tratamento para Infecções Relacionadas à Assistência à Saúde (IRAS), sobretudo as infecções hospitalares (nosocomiais) que se manifestam durante ou após estadias nesses ambientes, causadas por diversos tipos de microrganismos, que podem apresentar difícil terapêutica (COSTA; SILVA, 2018). Nos ambientes nosocomiais, as Unidades de Terapia Intensivas (UTI) se destacam, devido à fragilidade da saúde do paciente, aumentando o risco de IRAS, que podem desencadear custos econômicos e sociais ao hospital e/ou paciente, devido ao tempo prolongado de internação, terapia medicamentosa e riscos de morbimortalidade (SOUSA *et al*., 2017b; OLIVEIRA *et al*., 2019; ALVIM; COUTO; GAZZINELLI, 2020).

Na variedade de agentes etiológicos que podem causar infecções nosocomiais no Brasil, podemos citar os fungos, como *Candida* spp., *Cryptococcus neoformans* e *Aspergillus fumigatus*. Sendo o risco dessas infecções ampliadas pela presença de mecanismos de resistência e virulência (MURRAY; ROSENTHAL; PFALLER, 2017; BAPTISTA *et al*., 2020).

O impacto desses microrganismos para a saúde ocorre principalmente devido à perda de atividade da farmacoterapia, pela presença de mecanismos de resistência e a capacidade de formação de biofilme, expressos por genes de resistência e virulência, respectivamente (PRASAD; NAIR; BANERJEE, 2019; PEREIRA; FONTENELLE; MORAIS, 2020).

Nesses gêneros fúngicos de interesse à saúde, podemos destacar a *Candida albicans* devido a sua alta incidência na causa de IRAS. Essa levedura pode ser encontrada no meio ambiente (solo, plantas, superfícies inanimadas e água) e em animais. Também podendo fazer parte da microbiota humana normal, colonizando o trato gastrointestinal (TGI), cavidade oral, vaginal e uretra. Entretanto em pacientes imunodeprimidos pode ocasionar infecções oportunistas, como candidíase, monilíase e outras candidemias que podem evoluir para septicemia (MURRAY; ROSENTHAL; PFALLER, 2017; TONG; TANG, 2017; BAPTISTA *et al*., 2020).

A relação *C. albicans* e hospedeiros no processo infeccioso destaca-se pela capacidade de se estabelecer, colonizar, causar a doença e superar as defesas de hospedeiros, características estas diretamente ligadas com a virulência destes microrganismos. Sendo os mecanismos de virulência mais conhecidos: a aderência, polimorfismo, variabilidade fenotípica, produção de enzimas extracelulares e toxinas, esses fatores diretamente ligados à capacidade de formação do biofilme. O biofilme é uma comunidade microbiológica complexa, que possui várias etapas de formação, entre essas a fase de aderência, multiplicação e polimorfismo, formação de matriz extracelular e dispersão, proporcionando a capacidade de formação de biofilme nos mais diversos locais incluindo em dispositivos médicos como prótese, sondas e cateter, acometendo assim paciente no ambiente nosocomial, em especial em unidades de terapia intensiva (TONG; TANG, 2017; LOHSE *et al*, 2018; WALL *et al*., 2019).

Diante do exposto, a presente pesquisa se propõe analisar o potencial antifúngico e sinérgico dos óleos essenciais de plantas do gênero *Cymbopogon citratus* (Cc) e *C. nardus* (Cn) contra *C. albicans*, na forma planctônica e biofilme, isoladas de amostras coletadas em hospitais pertencentes à macrorregião de saúde de Sobral - CE, além da citotoxicidade e prováveis interações moleculares com peptidases aspárticas secretadas (SAP-5), enzima que favorece virulência fúngica.

2 REVISÃO DE LITERATURA

2.1 Plantas medicinais

O emprego de ervas para sanar malignidades não possui sua origem bem delineada, porém está interligada à biodiversidade de cada região. Tendo em vista essas características, dentre os países tropicais, o Brasil apresenta aproximadamente 45.000 espécies vegetais, o que o torna um grande detentor de uma variedade de plantas seja de origem nativa ou adaptadas pelas comunidades, além do significativo potencial de bioprospecção para novos fármacos (DUTRA *et al*., 2016; OLIVEIRA-MELO *et al*., 2019).

Além da vasta biodiversidade, outro ponto de extrema relevância para a utilização dessas plantas medicinais está relacionado ao conhecimento etnobotânico, sendo este conhecimento tradicional sobre plantas medicinais que se originaram e propagam nas comunidades e que desencadeiam um grande impacto no desenvolvimento da medicina, economia e na sociedade (ALVES, 2013; GROSS *et al*., 2019).

O conhecimento empírico é correlacionado, comumente, ao dito popular no qual afirma que por ser natural não traz malefícios, entretanto já se sabe que plantas medicinais se utilizadas de modo incorreto podem trazer riscos para a saúde do usuário. Tendo em vista essa problemática junto a possibilidade do aproveitamento desses vegetais com potencial de cura, surgiu a necessidade de implantação de políticas públicas para interligar e regular essas práticas de forma a complementar a assistência à saúde (SILVA; QUADRO; NETO, 2015; ALVES *et al*., 2018; OLIVEIRA-MELOI *et al*., 2019).

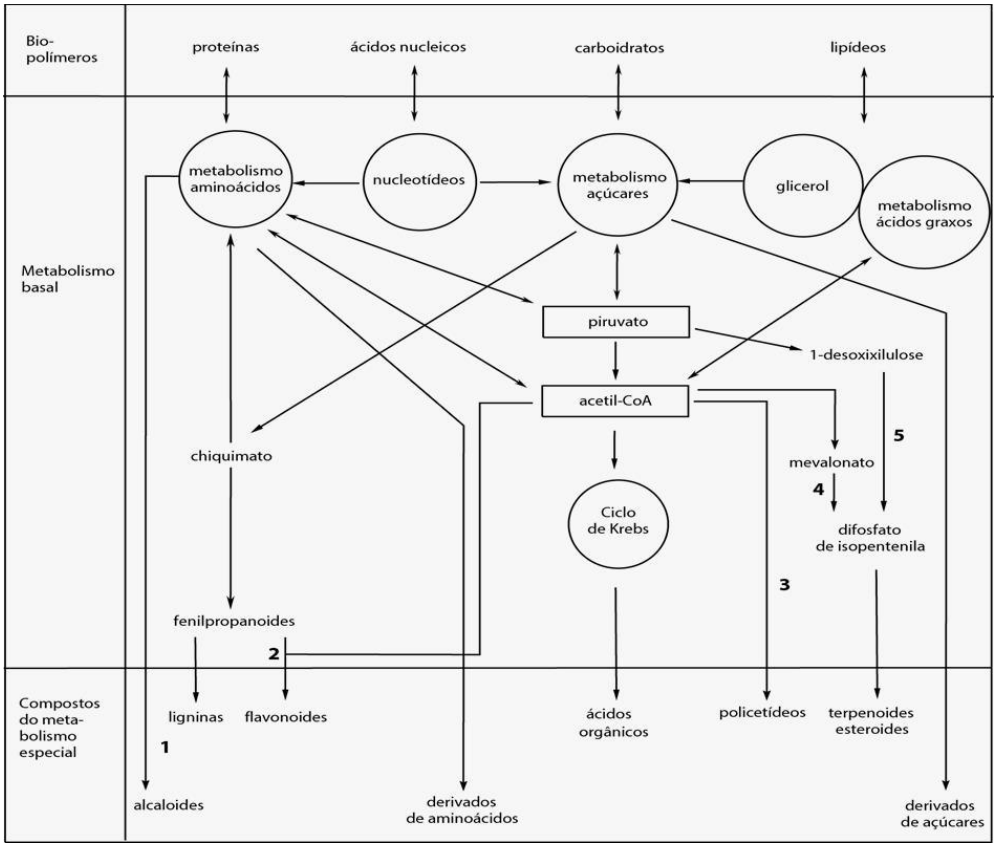
Assim, em 1977, com a 30ª Assembleia Mundial de Saúde foram instituídos as primeiras indicações para integração do uso da medicina tradicional nas políticas nacionais de saúde, e consequentemente integrando-se o uso de plantas medicinais e fitoterapia. Contudo, no Brasil, somente em 2006 com as vigências da portaria N° 971 e do decreto N° 5.813 que aprovou as Políticas Nacionais de Práticas Integrativas e Complementares (PNPIC) e as Políticas Nacionais de Plantas medicinais e fitoterápicos, respectivamente, ocorreu a regulamentação para utilização das ervas medicinais e seus derivados (BRASIL, 2006; BRASIL, 2016; BORGES; SALES, 2018; CARNEVALE; BANDEIRA; BARROS, 2018).

Entretanto, anterior a essas regulamentações governamentais, alguns projetos estaduais, como o “Farmácias Vivas” desenvolvido com apoio da Universidade Federal do Ceará pelo professor Francisco de Abreu Matos, já exerciam a atividade de catalogar as plantas

utilizadas pelas comunidades, comprovar sua eficácia, a segurança dos espécimes vegetais e a prestação de orientações para o uso correto das plantas medicinais (MATOS, 2007; CARNEVALE; BANDEIRA; BARROS, 2018).

A potencial habilidade dessas plantas de debelarem patologias é proveniente de seu metabolismo secundário, processo este que funciona como mecanismos adaptativo dos vegetais, tendo ações principais de atrair polinizadores, além de se contrapor a fitopatógenos e predadores. O M2 tem como produto uma variedade de compostos, sendo os mais conhecidos os alcaloides, flavonoides, taninos, heterosídeos, glicosídeos, terpenoides e fenilpropanoides ou fenilterpenoides, produzidos conforme as vias de biossíntese na figura 1 (YANG *et al.*, 2018; ISAH, 2019).

Figura 1 – Relação Biosintética entre o metabolismo basal (primário) e especial (secundário) e rotas de síntese para M2.



1: via dos aminoácidos; 2: via do chiquimato; 3: via do policetídeos; 4: via do ácido mevalônico; e 5: via do metileritritol fosfato (MEP).

Autoria: SIMÕES *et al*. 2017.

Dentre todas essas vias podemos destacar a do chiquimato e a do ácido mevalônico, estas principais responsáveis pela produção dos terpenoides e fenilterpenoides, metabólitos estes que apresentam uma variedade fitoquímica de compostos alcanos, alquenos lineares e com presença de heteroátomos como nitrogênio e enxofre (SIMÕES *et al*. 2017; YANG *et al*., 2018).

Para obtenção desses metabólitos vegetais pode-se utilizar de métodos extratores, como os chás (por infusão, maceração, decocção) e destilação utilizando solventes de diferentes polaridades, como a técnica de hidrodestilação, no qual permite a obtenção de óleos voláteis ou essências pelo princípio de arraste a vapor com condensação no sistema de Clevenger. No final desse procedimento obtém-se os OEs, sendo esse um tipo de extrato constituído majoritariamente por substâncias pertencentes aos grupos de terpenoides e fenilterpenoides (YANG *et al*., 2018*;* ZAYNAB *et al*., 2018).

Devido à variedade de estrutural dos constituintes dos extratos vegetais pode-se observar uma variedade de atividades biológicas, como o potencial antifúngico, antibacteriano, antiviral, anti-helmíntico, antineoplásico, anti-inflamatório, ansiolítico, anestésico e hipnótico. Dentre essas atividades o potencial antimicrobiano possui destaque principalmente devido à falta de fármacos com atividade contra alguns microrganismos (WINSKA *et al*., 2019).

Tendo em vista essa problemática de resistência à farmacoterapia antimicrobiana e o potencial medicinal das plantas, pode-se salientar algumas ervas como *Lippia sidoides* Cham., *Moringa oleifera* Lam., *Ocimum gratissimum* L., *Allium sativum* L., *Plectranthus amboinicus* (Lour.) Spreng., *Syzygium aromaticum* (L.) Mert & Perry e *Cymbopogon* sp. (LORENZI; MATOS, 2008; WINSKA *et al*., 2019; NEWMAN; CRAGG, 2020).

2.2 *Cymbopogon* sp.

Dentre todos esses vegetais pode-se apontar o gênero botânico *Cymbopogon*, pertencente à família Poaceae (antiga Gramineae) que é uma angiosperma do subgrupo das monocotiledônea, essa família é caracterizada por folhas longas de nervuras foliares paralelinérveas e cespitosas quase acaule, além desse gênero raramente apresentando inflorescências (PROCHNOW *et al*., 2018).

Trata-se de um gênero nativo do velho mundo, entretanto, pode ser facilmente cultivada em ambientes tropicais, como no Brasil, com sua utilização ocorrendo em todo território brasileiro para finalidades medicinais ou biorremediação a insetos (LORENZI; MATOS, 2008; ZAGO; MOURA, 2018).

Além do uso pela comunidade, essas plantas também são amplamente cultivadas com intuito comercial para produção de OE, sendo os principais locais de plantio a América do Sul e Central (Brasil, Argentina, Ilhas do Caribe, Guatemala, Honduras e Haiti) e Ásia (Índia, China, Sri Lanka, Java, Vietnã, Malásia, Bangladesh e Mianmar). Os OEs extraídos desses gêneros estão entre os mais produzidos no planeta, juntamente com o óleo de laranja, hortelã-pimenta e eucalipto (AVOSEH *et al*., 2015; PROCHNOW *et al.*, 2018).

Esse grupo botânico possui aproximadamente 144 espécimes, todavia podemos destacar o *C. flexuosus* (Nees) Will.Watson, *C. martini* (Roxb.) Will. Watson, *C. schoenanthus* (L.) Spreng., *C. densiflorus* (Steud.) Stapf, *C. giganteus* Chiov e especialmente o *C. citratus* e *C. winterianus* devido seu amplo emprego pela sociedade (AVOSEH *et al*., 2015).

2.2.1 *Cymbopogon citratus*

O *C. citratus* (Figura 2) devido sua ampla difusão no planeta recebe diversas nomenclaturas populares, destacando-se no Brasil com os nomes de Capim-limão, Capim-santo e Capim-cidreira. Possuindo relatos de emprego Cc pelas comunidades com finalidades contra: ansiedade leve, cólicas, analgesia, gripe, febre, colesterol alto, distúrbios intestinais e pneumonia (EKPENYONG; AKPAN; NYOH, 2015; CARREIRO *et al*., 2020).

Figura 2 – Exemplar de *C. citratus.*

Autoria: Elaborado pelo autor (2021).

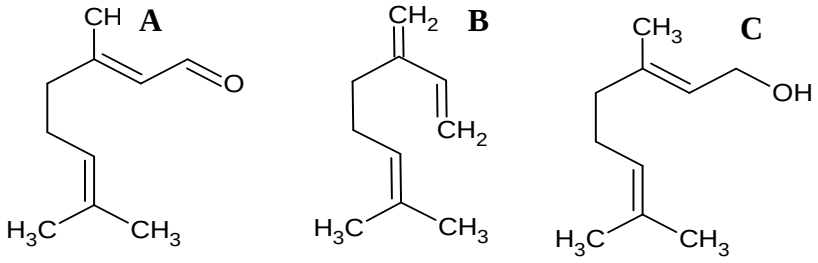
Além das finalidades citadas pelas comunidades, diversos ensaios laboratoriais permitiram constatar o potencial antioxidante e atividade antimicrobiana, sendo alguns desses microrganismos bactérias GRAM-positivas (*Streptococcus mutans, Streptococcus sobrinus, Staphylococcus aureus* e *Staphylococcus epidermidis*), bactérias gram-negativas (*Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli, Proteus vulgaris, P. mirabilis* e

Pseudomonas aeruginosa), dermatófitos (*Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum, Microsporum gypseum* e *M. canis*) (LIMA *et al*., 2018; NGUYEN *et al*., 2019 SEIBERT *et al*., 2019; SAHAL *et al*., 2020).

Outros estudos microbiológicos também observaram atividade dos extratos de Cc contra diversos espécimes *Candida*, sendo algumas a *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. orthopsilosis* e biofilme de *C. albicans* (KHOSRAVI *et al*., 2018; CÓRDOBA *et al.,* 2019).

Essa ação do Cc é proveniente do metabólito secundário que produz diversas substâncias, como taninos, flavonoides, saponinas, alcaloides, fenóis, esteroides e terpenoides. Dentre esses metabólitos pode-se destacar os monoterpenos, classificação essa dada aos tipos de terpenos que constitui majoritariamente o OE dessa planta, constituída por citral e seus isômeros, mirceno e geraniol (Figura 3) (EKPENYONG; AKPAN; DANIEL, 2014; EKPENYONG; AKPAN; NYOH, 2015; AGUILAR-ANCORI *et al*., 2018; CAVALCANTE E COSTA *et al*., 2018; NGUYEN *et al*., 2019; CARREIRO *et al*., 2020).

Figura 3 – Estrutura química da molécula de citral (A), mirceno (B) e geraniol (C).



Autoria: Adaptado de EKPENYONG, AKPAN e NYOH (2015).

Em relação à toxicidade do óleo essencial de Cc, foi constatado que existe uma alta margem de segurança, não promovendo toxicidade aguda. Em contrapartida, devido à ação hormonal do citral, o uso crônico é contraindicado para homens, pois pode gerar aumento temporário da próstata (prostatite benigna) e desencadear disfunção erétil (MATOS, 2007; EKPENYONG; AKPAN; DANIEL, 2014; LIMA *et al.,* 2017).

Deste modo, com seu mecanismo elucidado e sua ação tóxica praticamente não relatada na literatura, surge a possibilidade de empregar o OE e extratos (hidroalcoólico e aquosos) do Cc em produtos de caráter fitoterápico, como por exemplo, cosméticos e outros produtos da indústria farmacêutica e alimentícia (SILVA *et al.,* 2014).

2.2.2 *Cymbopogon nardus*

Empiricamente conhecido como Citronela ou Capim citronela o *Cymbopogon nardus* (Figura 4), antecessor geneticamente do *C. winterianus*, é utilizado por uma variedade de comunidades brasileiras, entretanto sua principal aplicação ocorre na biorremediação de insetos (SANTOS *et al*., 2019).

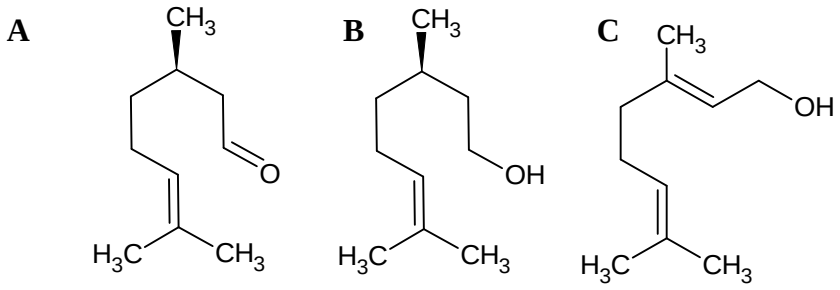
Figura 4 – Exemplar de *Cymbopogon nardus.*

Autoria: Elaborado pelo autor (2021).

Outros estudos afirmam atividade dessa planta sobre sistema nervoso e circulatório, todavia seu destaque maior ocorre no uso como inseticida contra *Culex quinquefasciatus*, larvicida e antimicrobiano (MANH *et al.*, 2020). Observou-se atividade do OE desse vegetal contra algumas Enterobacteriaceae e contra fungos do tipo leveduriformes, como *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosi* e *Candida krusei* (AHMAD; VILJOEN, 2015; SINGH; FÁTIMA; HAMEED, 2016).

Essa atividade biológica do OE ocorre devido à presença de monoterpenos, mais especificamente ao Citronelol, Citronelal e Geraniol (Figura 5), encontrados no OE de Cn, presente no território brasileiro. Já em relação à toxicidade, são escassos os estudos em mamíferos envolvendo esse vegetal, todavia existem relatos de irritação a tecidos cutâneos, principalmente ocasionada pelo óleo essencial em altas concentrações (AVOSEH *et al*., 2015; LIMA, 2018; SANTOS *et al*., 2019).

Figura 5 – Estrutura química da molécula de Citronelol (A), Citronelal (B) e Geraniol (C).



Autoria: Adaptado de AVOSEH e colaboradores (2015).

2.3 Infecções Nosocomiais

Atualmente, infecções em ambientes de nosocômios apresentam destaque dentro das IRAS, devido ser um dos principais locais de ocorrência de microrganismos resistentes aos protocolos terapêuticos. No âmbito hospitalar pode-se enfatizar as Unidades de Terapias Intensiva (UTI), principal local de tratamento a pacientes com quadros clínicos instáveis, como imunodebilitados e procedimentos invasivos comumente realizados nesses locais. Deste modo, as principais consequências são os impactos econômicos e sociais, que desencadeiam o aumento do tempo de internação e mortalidade, além do risco de desenvolvimento de microrganismos com resistência (ALVIM; COUTO; GAZZINELLI, 2020).

Esse tipo IRAS de origem nosocomial pode ter origem endógena, que é quando os microrganismos do próprio paciente desencadeiam o processo infeccioso devido a proliferação sem controle do sistema imunológico ou por mudança no sítio natural do microrganismo. Outro aparecimento das infecções hospitalares é devido a origem exógena que pode ocorrer pelas mãos de profissionais da saúde contaminados, por sondas e cateteres (SOUSA *et al*., 2017b).

Essas infecções hospitalares no Brasil podem ser causadas por diversos microrganismos, tendo destaque a família Enterobacteriaceae e Saccharomycetaceae, respectivamente, representadas pela *E. coli* e *C. albicans*. Sendo importante ressaltar o potencial perigo desses microrganismos pelo fato de comumente possuírem genes que expressam fatores de virulência e/ou resistência e assim permitindo uma maior persistência desses seres (PACZOSA; MECSAS, 2016; BRASIL; ORGANIZAÇÃO MUNDIAL DE SAÚDE, 2018).

2.4 *Candida* spp.

Dentre esses patógenos causadores de infecções hospitalares podemos destacar família Saccharomycetaceae no qual se encontram as leveduras *Candida* spp, esse gênero fúngico é composto por mais de 100 espécies, dentre essas destaca-se pela relevância clínica a *C. albicans, C. glabrata, C. parapsilosis, C. topicalis, C. krusei* e *C. auris* (MURRAY; ROSENTHAL; PFALLER, 2017; DE SOUSA *et al*., 2020).

Candida spp. são fungos leveduriformes de tamanho médio de 3 a 5 μm, que se reproduzem por brotamento ou blastoconídeos, apresentam parede celular, membrana plasmática com ergosterol (um colesterol de membrana que não é encontrado em células de mamíferos) e outras estruturas de células eucarióticas, este gênero fúngico, exceto a espécie *glabrata*, podem produzir hifas ou pseudo-hifas. Fenotipicamente esses microrganismos formam colônias brancas/leitosas, lisas e convexas, entretanto podem passar por algumas alterações devido a mutações genéticas e a alterações no microambiente (MURRAY; ROSENTHAL; PFALLER, 2017).

Essas leveduras podem causar uma série de patologias como candidíase, candidemias e infecções (orofaringe, esofagite, vulvovaginal, tecidos cutâneos, trato urinário, endocardite, pericardite, sistema nervoso central, abdominal e hematológica). Assim acometendo, aproximadamente, 250.000 pessoas afetadas por candidíase invasiva ao ano resultando em aproximadamente 50.000 mortes, estando entre a quarta causa de sepse nos serviços de saúde global e a sétima colocada no Brasil (MACHADO *et al*., 2021).

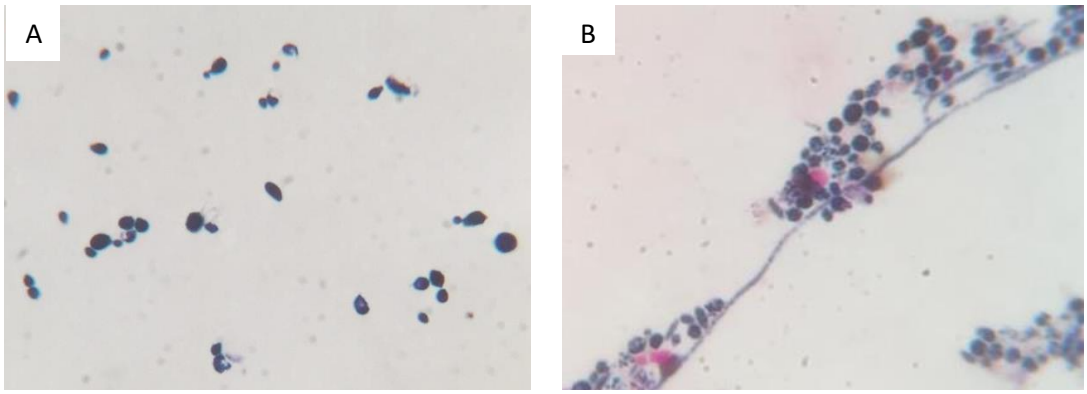
Essas leveduras podem ser agrupadas em *Candida albicans* e *Candida* não-*albicans*, sendo a de maior importância médica *C. albicans.* Espécie que é mais encontrada em isolamentos clínicos, com 90% a 100% de infecções em mucosas, 40% a 70% em infecções sanguíneas (BENHABIB *et al.*, 2020; SOUSA *et al.*, 2020).

Estas infecções por *C. albicans* ocorrem normalmente devido a diversos processos, sendo essas imunossupressões (causada por medicamentos em uso prolongado, carência de nutrientes, doenças e/ou procedimentos invasivos), desequilíbrio da microbiota normal (por uso de antibióticos de forma exacerbada e indevido) e déficit hepático (ocasionando mudanças nos mecanismos de desintoxicação gerando acúmulo de toxinas que favorece o crescimento de *C. albicans* (TONG; TANG, 2017).

As *C. albicans* (figura 6) é uma levedura, que se reproduz assexuadamente por brotamento e pode adotar três estruturas distintas: blastoconídios, hifas e pseudo-hifas. Essas

trocas morfológicas representam um fator importante para a patogênese e também estão envolvidas em diferentes estágios de desenvolvimento do biofilme, juntamente às respostas externas de estímulos ambientais, ao reconhecimento, aderência às superfícies (que pode ser biótica ou abiótica), além de secreção de enzimas (BENHABIB *et al*., 2020).

Figura 6 – Imagem de *Candida albicans* coradas com cristal violeta, na imagem "A" leveduras com blastoconídeos e "B" leveduras e hifas.



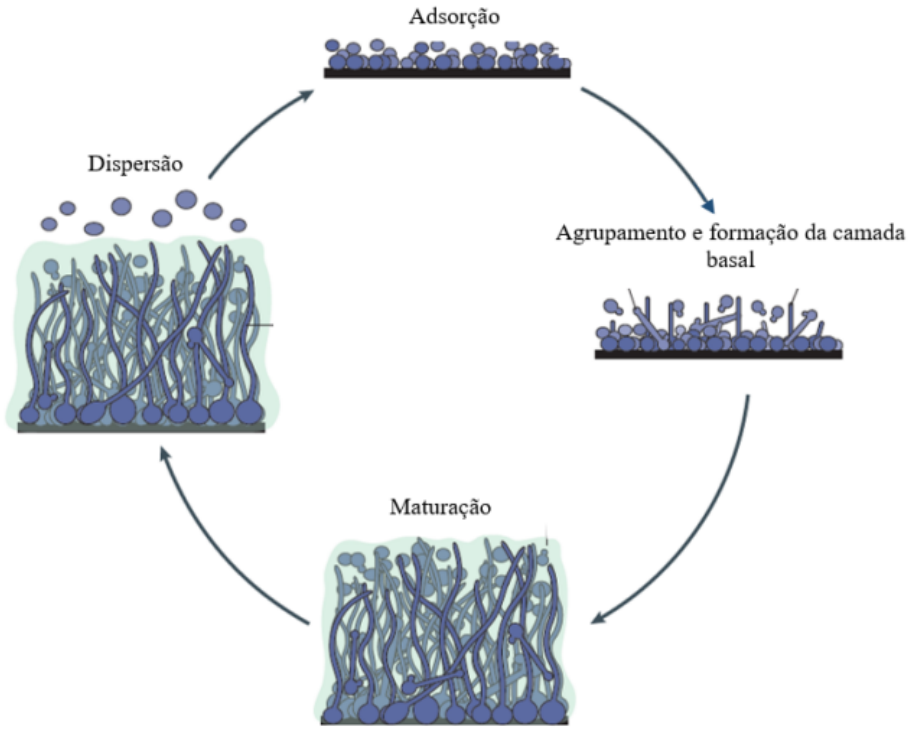
Autoria: Elaborado pelo autor (2021).

Todos esses fatores convergem na virulência dessa levedura, sendo a capacidade de formar biofilme um dos fatores de virulência correlacionado ao risco de mortalidade por esse microrganismo. Os biofilmes são um complexo microbianos fixados em uma superfície, abiótica ou não, formando uma matriz extracelular polimérica que reveste e protege a comunidade microbiana (WALL *et al*., 2019).

O processo para formação de biofilme da *Candida albicans* (figura 7) é composto por quatro etapas fundamentais, sendo adsorção; agrupamento e formação da camada basal; maturação; e dispersão. Na adsorção ou aderência ocorre a primeira etapa para o processo de formação de biofilme, nesse momento as leveduras aderem a uma superfície (abiótica ou biótica) com auxílio de intenções físico-químicas, posterior a esta etapa as leveduras se agrupam, se multiplicam, formam uma matriz extracelular, produzindo hifas e pseudo-hifas, formando assim uma camada basal. Segundo estudos in vitro, com aproximadamente 24 horas o biofilme atinge o estágio de maturação entrando em uma nova etapa de formação, momento esse que ocorre aumento da massa do biofilme pela expansão da matriz e aumento da quantidade de microcolônias microbianas. Após estas etapas ocorre a dispersão, momento esse

nos quais células fúngicas se separam do complexo e se propagam para formação de novos biofilmes (LOHSE *et al*, 2018; PEREIRA; FONTENELLE; MORAIS, 2020).

Figura 7 – Ilustração do processo de formação de biofilme de *Candida albicans*, iniciando com adsorção, agrupamento e formação da camada basal (com leveduras, pseudo-hifas e hifas), maturação e dispersão.



Autoria: Adaptado de Lohse e colaboradores (2018).

O biofilme de *C. albicans* apresenta grande relevância clínica, principalmente quando se refere a infecções sistêmicas no ambiente hospitalar, pela grande ocorrência desse tipo de biofilme em sondas e cateter. Esses complexos microbianos além de facilitarem a dispersão desses microrganismos, também conferem resistência às farmacoterapias empregadas na clínica atualmente. Este fenômeno pode ocorrer devido a matriz extracelular e toda composição física dos biofilmes (espessura, aderência e outras), aumento da expressão de bombas de efluxo, mudanças no sítio de ligação dos fármacos, sendo alguns das principais formas de resistência, proveniente da comunicação e alta variabilidade genética dessas leveduras que compõe o biofilme (LANGER *et al*., 2018; PRASAD; NAIR; BANERJEE, 2019 WALL *et al*., 2019).

Além dos fatores de virulência, como o biofilme, as *Candida* spp na forma planctônicas também desenvolveram mecanismos de resistência a fármacos, algum deles semelhante aos dos biofilmes como as bombas de efluxo ou alteração do sítio de ligação dos fármacos e outros a expressão de genes do complexo *ERG* que favorecem a resistência por aumento da produção de enzimas capazes de inativar os fármacos (ZIDA *et al*., 2017; PRASAD; NAIR; BANERJEE, 2019; PEREIRA; FONTENELLE; MORAIS, 2020).

Outra problemática está relacionada a resistência de *C. albicans* na forma planctônica ou biofilme estando diretamente relacionado ao limitado número de fármacos encontrados para terapêuticas dessas infecções, sendo os principais grupos de antibióticos antifúngicos os azóis e polienos (BASSETTI *et al*., 2018; PRASAD; NAIR; BANERJEE, 2019).

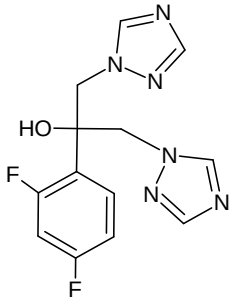
2.5 Farmacoterapia antifúngica

Algumas limitações dos antifúngicos atualmente estão relacionadas à elevada toxicidade e resistência aos fármacos presentes no mercado. Atualmente, o dilema da toxicidade para humanos é um dos maiores desafios para descoberta de novas drogas contra *C. albicans*, sendo essa toxicidade elevada devido ambas as células, humana e fúngica, serem eucarióticas (SILVA *et al.*, 2012; RANG *et al.*, 2016; BASSETTI *et al.*, 2018).

A variedade de fármacos com propriedades antifúngicas é bastante limitada, principalmente referente a levedura como *Candida* sp., causadora de infecções sistêmicas. Tais antibióticos possuem dois grupos farmacológicos de destaque os azóis fármaco sintético, representado pelo fluconazol, e os polienos fármacos de origem natural, que apresenta como representantes as anfotericina (MATTHAIOU; CHRISTODOULOPOULOU; DIMOLOPOULOS, 2015).

O fluconazol (figura 8) é um fármaco fungistático que tem ação farmacológica proveniente do núcleo de triazol que inibe a enzima A3 (lanosina 14-alfa-desmetilase) do complexo citocromo P450, consequentemente interferindo na conversão de lanosterol em ergosterol. Deste modo ocasionando uma alteração na membrana que não permite a replicação fúngica, além de inibir a conversão das leveduras para hifas ou pseudohifas (MATTHAIOU; CHRISTODOULOPOULOU; DIMOLOPOULOS, 2015; RANG *et al.*, 2016).

Figura 8 – Estrutura química do fluconazol.

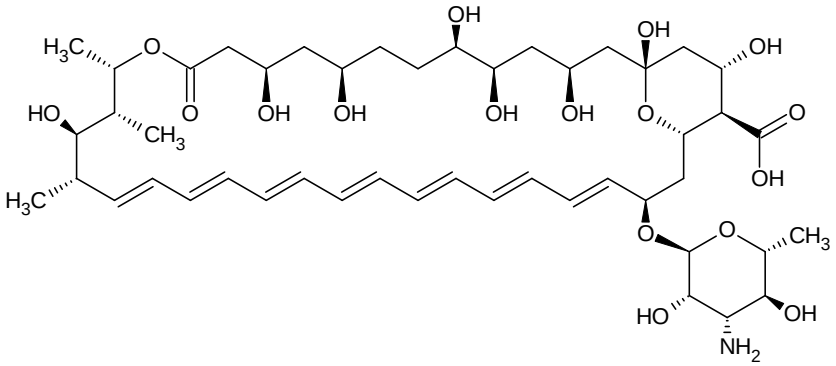


Autoria: Adaptado de National Center for Biotechnology (2021a).

Este fármaco apresenta boa absorção por via oral e intravenosa, conseguindo atingir diversos tecidos e até atravessar barreira hematoencefálica e tendo atividade contra meningites. Entretanto, como os demais antifúngicos esse fármaco pode ser tóxico ao organismo humano, causando quadros de hepatotoxicidade em alguns casos, mais raramente em comparação aos demais fármacos dessa classe (LI *et al*., 2017; BASSETTI *et al.*, 2016).

Já a anfotericina B (figura 9), é um fármaco que teve sua origem a partir de culturas de *Streptomyces*, possui moléculas grandes, próximas estruturalmente aos macrolídios. Esse fármaco tem atividade na membrana celular das *C. albicans*, tendo afinidade ao ergosterol presente nessa estrutura fúngica, formando grandes poros que consequentemente desencadeiam graves alterações no equilíbrio iônico do microrganismo o levando a morte (RANG *et al*., 2016).

Figura 9 – Estrutura química da anfotericina B.



Autoria: Adaptado de National Center for Biotechnology (2021b).

Devido ao seu amplo espectro contra fungos, essa droga é considerada padrão-ouro para infecções sistêmicas, tendo boa biodisponibilidade em tecidos diversos. Contudo, somente na via intravenosa esse fármaco apresenta absorção adequada, além disso possui lenta excreção

através dos rins, podendo se acumular nesse órgão causando lesão renal, ou seja, nefrotoxicidade (RANG *et al*., 2016; Zhou *et al*., 2017).

Atualmente, esses fármacos são as principais ferramentas na terapêutica antifúngica de forma individual ou associados a outros, como anfotericina B e fungicidina (nistatina). Porém, a carência de novas substâncias, que apresentem toxicidade reduzida, com atividade contra esses microrganismos ainda é uma necessidade, principalmente, quando observamos o surgimento de mecanismos que burlam a ação desses fármacos empregados nessas nos protocolos terapêuticas para candemias e candidíase (WINSKA *et al*, 2019).

2.6 Molecular Docking

A partir do desenvolvimento tecnológico, viu-se a possibilidade na associação entre as áreas da ciências biológicas e informática dando origem a biologia computacional e suas subáreas como bioinformática e *Molecular Docking* (Fan; Fu; Zhang, 2019). Por intermédio dessa área virtual houve o favorecimento de uma nova classificação de ensaios científicos conhecidos como *in silíco.* A partir dessa nova vertente, que teve seu início na década de 80, permitindo a realização de diversos estudos na área da genômica, protônica e farmacologia (Silva et al., 2019; Ambrósio, 2021).

Assim descartando a possibilidade de estudos utilizando de *Molecular Docking*, conhecida como ancoragem molecular, na determinação de possíveis mecanismos de interação entre uma proteína fundamental no processo fisiopatológico e a molécula química em estudo, tendo como ponto fundamental as simulações nas propriedades físico-químicas das moléculas, podendo assim mensurar um possível efeito a substância teste e/ou um provável mecanismo de ação (Ambrósio, 2021). Favorecendo assim a seleção para descoberta ou reposicionamento de potenciais fármacos, pelo custo mais acessível e menor período de tempo, quando comparado aos ensaios *in vitr*o e *in vivo*, sendo assim uma alternativa de *Virtual Screening* (Silva *et al.*, 2019; Fan; Fu; Zhang, 2019).

Tais ensaio de *Molecular Docking* podem avaliar uma série de fatores como raiz média, desvio quadrado (RMSD) e energia livre de ligação (ΔGbind), parâmetros esses relacionadas com distâncias entre os átomos doadores de proteína para avaliar a força das ligações de hidrogênio, relacionado a interação, tempo e força de ligação (Shityakov e Forster, 2014).

3 OBJETIVOS

3.1 Objetivo geral

Avaliar atividade antifúngica, citotoxicidade e *Docking molecular* dos óleos essenciais de *C. citratus* (Cc) e *C. nardus* (Cn) contra isolados clínicos de *C. albicans* na forma planctônica e biofilme.

3.2 Objetivos específicos

- Extrair óleo essencial de *Cymbopogon citratus* (Capim-santo) e *Cymbopogon nardus* (Capim-citronela);
- Indicar rendimento, densidade e composição fitoquímicos dos óleos essenciais de *C. citratus* e *C. nardus* por Cromatografia Gasosa acoplada à Espectrometria de Massas (GC-MS);
- Investigar o potencial antifúngico dos óleos essenciais de *C. citratus* e *C. nardus* em isolados clínicos de *C. albicans* de origem hospitalar, na forma planctônica e biofilme;
- Avaliar potencial sinérgico entre os OE's e Anfotericina B contra *Candida albicans*;
- Analisar citotoxicidade dos óleos essenciais;
- Determinar interação entre compostos dos OEs Cc e Cn com proteínas SAP5, por *Docking molecular*.

4 ARTIGOS

4.1 Artigo de Revisão

Artigo públicado (Research, Society and Development- Qualis A3)

Cymbopogon sp. from ethnobotany to antimicrobial: an integrative review

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Abstract
Considering the broad potential of the genus *Cymbopogon*, here we present a systematic literature survey on it's antimicrobial potential. This is a review, articles from the Scielo and PubMed platforms. The articles surveyed were published between 2015 and 2020, with the theme of microbiology, including ethnobotanical studies, literature reviews, *in vitro*, *in vivo*, reports of clinical trials. Works outside the selected period, duplicate articles, and those only reporting infections of plants by the microorganism were excluded. 98 studies were selected, 74% found in PubMed and 26 % in Scielo. Of this total, 21% were ethnobotanical/ethnopharmacology or literature reviews, most of them reporting the use of infusions of the species *C. citratus* (DC.) Stapf. Moreover, in 57% of the studies, survey respondents did not report antimicrobial use. In relation to 79% of the experimental studies, it was observed that 77% reported total inhibition of microbial growth, 3% indicated moderate growth inhibition, 4% low growth inhibition and 5% reported no inhibition. Among the microbial species analyzed were *Escherichia coli*, *Pseudomonas aeruginosa*, *Borrelia burgdorferi*, *Candida albicans*, *Salmonella enterica* and *Saccharomyces cerevisiae*. In addition, 5% of the articles reported antiviral activity,
5% parasitic control, 1% preventive action against contamination by mesophilic microorganisms. Although the population is not aware of the antimicrobial activity of *Cymbopogon* sp., studies have demonstrated its antimicrobial potential, thus the extracts of this genus can be an alternative for use in folk medicine as well as a source of new drugs with antimicrobial action.
Keywords: Plants; Medicinal; Phytotherapy; Ethnopharmacology; Poaceae; Lemongrass; Microbiology.

Resumo
Considerando o amplo potencial do gênero *Cymbopogon*, apresentamos aqui um levantamento sistemático da literatura sobre seu potencial antimicrobiano. Trata-se de uma revisão, artigos das plataformas Scielo e PubMed. Os artigos pesquisados foram publicados entre 2015 e 2020, com a temática da microbiologia, incluindo estudos etnobotânicos, revisões de literatura, *in vitro*, *in vivo* e relatórios de ensaios clínicos. Foram excluídos trabalhos fora do período selecionado, artigos duplicados e aqueles que relataram apenas infecções de plantas pelo microrganismo. Foram selecionados 98 estudos, 74% encontrados no PubMed e 26% no Scielo. Desse total, 21% eram etnobotânicos/etnofarmacológicos ou revisões de literatura, a maioria

relatando o uso de infusões da espécie *C. citratus* (DC.) Stapf. Além disso, em 57% dos estudos, os entrevistados não relataram uso de antimicrobianos. Em relação a 79% dos estudos experimentais, observou-se que 77% relataram inibição total do crescimento microbiano, 3% indicaram inibição moderada do crescimento, 4% baixa inibição do crescimento e 5% não relataram inibição. Entre as espécies microbianas analisadas estavam *Escherichia coli, Pseudomonas aeruginosa, Borrelia burgdorferi, Candida albicans, Salmonella enterica* e *Saccharomyces cerevisiae*. Além disso, 5% dos artigos relataram atividade antiviral, 5% controle parasitário, 1% ação preventiva contra contaminação por microrganismos mesófilos. Embora a população desconheça a atividade antimicrobiana de *Cymbopogon* sp., estudos têm demonstrado seu potencial antimicrobiano, assim os extratos deste gênero podem ser uma alternativa para uso na medicina popular bem como fonte de novos fármacos com ação antimicrobiana.
Palavras-chave: Plantas; Medicinais; Fitoterapia; Etnofarmacologia; Poaceae; Capim-limão; Microbiologia.

Resumen
Considerando el amplio potencial del género *Cymbopogon*, aquí presentamos un estudio sistemático de literatura sobre su potencial antimicrobiano. Es una revisión de artículos de las plataformas Scielo y PubMed. Los artículos investigados fueron publicados entre 2015 y 2020, con el tema de microbiología, incluyendo estudios etnobotánicos, revisiones de literatura, *in vitro*, *in vivo*, e informes de ensayos clínicos. Se excluyeron trabajos fuera del período seleccionado, artículos duplicados y aquellos que solo reportaron infecciones de plantas por microorganismos. Se seleccionaron 98 estudios, 74% encontrados en PubMed y 26% en Scielo. De este total, 21% fueron revisiones de literatura o etnobotánica/etnofarmacología, la mayoría reportando uso de infusiones de la especie *C. citratus* (DC.) Stapf. Además, en 57% de los estudios, los encuestados no informaron uso de antimicrobianos. Con relación al 79% de los estudios experimentales, se observó que 77% reportó inhibición total del crecimiento microbiano, 3% indicó inhibición moderada del crecimiento, 4% baja inhibición del crecimiento y 5% no informó inhibición. Entre las especies microbianas analizadas se encuentran *Escherichia coli, Pseudomonas aeruginosa, Borrelia burgdorferi, Candida albicans, Salmonella enterica y Saccharomyces cerevisiae*. Además, 5% de los artículos reportaron actividad antiviral, 5% control parasitario, 1% acción preventiva contra contaminación por microorganismos mesófilos. Aunque la población no conoce la actividad antimicrobiana de *Cymbopogon* sp., estudios han demostrado su potencial antimicrobiano, por que los extractos de este género pueden ser una alternativa para su uso en la medicina popular así como fuente de nuevos fármacos con acción antimicrobiana.
Palabras clave: Plantas Medicinales; Fitoterapia; Etnofarmacología; Poaceae; Lemongrass; Microbiología**.**

1. Introduction

The use of medicinal herbs preparations to treat malignancies does not have a well-established origin, but reports confirm their relevance for the development of medicine and societies. Popular knowledge is the main source of information about this practice, and this knowledge is passed down within generations of a family or community (Rodrigues et al., 2017).

Due to the use of medicinal plants in many cultures, this use has been the subject of scientific knowledge and later integrated into public health policies, especially in primary care. This occurs through the adoption of integrative and complementary practices (ICPs), including the implementation of projects such as *Farmácias Vivas* ("Living Pharmacies") in basic health units in Brazil (Brasil, 2016; Brasil, 2018; Ferreira et al., 2020).

The plants used in folk medicine need to be investigated for pharmacological activity and toxicity to ensure the safety of patients, because contrary to popular belief that natural substances will cause no harm, some plants used erroneously can be highly dangerous (Goulart et al., 2020).

Medicinal plants have several activities, such as regulation of the central nervous system, alleviation of inflammation, and maintenance of the biochemical or hormonal characteristics of the body. In addition to these uses, some plants have promising antimicrobial activity or precursor molecules of this class of drug (De Castro & Figueiredo, 2020).

Microorganisms are composed of parasites (arthropods, helminths, and protozoa), viruses, bacteria and fungi, with the ability to infect or parasitize humans and animals. Some can trigger a pathophysiological process that is treatable with a specific pharmacotherapy. However, due to the high genetic mutability of some of these microbes, a constant search for new antimicrobials is required, usually originated from molecular changes in existing drugs or secondary metabolites of plants, which are responsible for the antimicrobial action of these herbs (Oliveira et al., 2019; Sahal et al., 2020).

Some botanical genera have been widely investigated regarding their antimicrobial activity. This is the case of *Cymbopogon* Spreng., a herb belonging to the angiosperm group, being classified as a monocotyledon of the Poaceae family (Gramineae). It is an aromatic species characterized by having long leaves with parallelelineal leaf rib, and rarely present flowers (Lorenze & Matos; 2008; Nguyen et al., 2019). Considering the broad potential of the genus *Cymbopogon*, here we present a systematic survey of the literature on its antimicrobial potential.

2. Methodology

This is a cross-sectional integrative review of the literature, a search for data available on the Scielo (https://www.scielo.org) and PubMed (https://.pubmed.ncbi.nlm.ni.gov) platforms for full articles published equating as contained information, following the processes

and organized by the PRISMA protocol, following the investigative question: "*Cymbopogon* sp. does it have antimicrobial potential? (Marconi & Lakatos, 2017).

The term *Cymbopogon* was used as descriptor, adopting as inclusion criteria papers published from January 2015 to January 2020 in Portuguese, Spanish or English, with thematic area of literature review, ethnobotany, ethnopharmacology or microbiology involving *in silica, in vitro, in vivo* and clinical trials. Works outside the selected period, duplicate articles, and those where the microorganism infected only plants were excluded. Subsequently, these data were tabulated and quantified, with subsequent identification of the percentages of each variable.

3. Results and Discussion

In the study, 431 publications were analyzed, with the selection of 98 scientific works according to the inclusion and exclusion criteria. Among the selected articles, 74% (73/98) were found on the PubMed platform and 26% (25/98) in Scielo. This difference in the number of publication was possibly due to the scope of each of the platforms, PubMed has a larger number of indexed journals, from countries throughout the world, while Scielo has more restrictions on publications and its target audience is mainly from Latin American countries is restricted mainly to articles published by researchers from Latin American countries. The average annual publication was approximately 16 articles involving this theme, and the year with the highest number of publications was 2015 when considering both platforms, and 2018 when considering only PubMed.

3. 1 Ethnobotany / Reviews

Among the variety of studies, 21% (21/98) were ethnobotanical, ethnopharmacological or literature reviews. After a detailed analysis of these studies, it was found that 57% (12/21) were surveys in which community respondents did not report the use of *Cymbopogon* species to treat any infection or symptom, while in 43% (9/21) of the articles of this type, the use of plants of this genus was reported to treat pathologies caused by microorganisms, such the influenza, pneumonia, fungal diseases and parasite infections. This showed that despite the wide popular knowledge about the *Cymbopogon* genus, some communities are still not aware of its antimicrobial potential. However, studies such as Cunha

et al. (2020), Sahal et al. (2020) and Ahmad and Viljoen (2015) have demonstrated this activity against bacteria, fungi and viruses.

The main species cited was *Cymbopogon citratus* (DC.) Stapf, with 63% (19/30), considering that some studies cited more than one specimen of *Cymbopogon*. Regarding the form of preparation/extraction indicated in the studies, infusion appeared in 71% (15/21), while 29% (6/21) reported other forms of preparation (Table 1). Analysis of ethnobotanical, ethnopharmacological and literature review articles revealed that most of the studies were conducted in Africa or Brazil.

Table 1- Publications of ethnobotanical nature, ethnopharmacological and literature review, being observed biological activity, etiological agent, report of antimicrobial activity, popular name and species.

Study	**Biological activity**	**Etiological agent**	**Activity**	**Popular name**	**Species**	**Reference**
Etno	Antimycobacteria	*Mycobacterium tuberculosis*	NAR	Lemongrass	*C. giganteus*	Nguta et al. 2015
Etno	Fever/ Cramps/ Cold/Cough	Without	NAR	Lemongrass	*C. citratus*	Clement et al., 2015; Kpodar etal., 2016
Etno	Soothing/ Pneumonia/ Digestive Disorders/ Spasmolytic / Flu	Without	AR	Capim-Cidreira	*C. citratus*	Paredes et al., 2015; Bieski et al., 2015; Intriago et al., 2015; Miguéis et al., 2019; Caetano et al., 2015
Rev.	Antifungal/ Antimalarial/ Anti-inflammatory/ Antimutagenic/ Anxiolytic/Antioxidant / Hypoglycemia/ Hyperlipidemia	*Escherichia coli/ Proteus vulgaris/ Klebsiella pneumoniae/ Aspergillus* spp/ *Haemophilus influenza/ Pseudomonas aeruginosa/ Streptococcus aureus/ Streptococcus pyogenes/ Staphylococcus aureus/ Candida albicans/ Malassezia pathogenic*	AR	Lemongrass	*C. citratus*	Suroowan; Mahomoodally, 2016; Ekpenyong; Akpan; Nyoh, 2015; Donato et al., 2020

Rev.	Antifungal/ Hepatoprotective/ Antioxidant/ Antiprotozoario/ Antibacterial	Without	AR	Lemongrass	*C. parkeri/ C. olivieri/ C. citratus/ C. ambiguous/ C. flexuosus/ C. pendulus / C. proximus/ C. densiflorus*	Avoseh et al., 2015
Etno	Flu/ Stress Relief/ Inflammation/ Fever/High Cholesterol/High Blood Pressure/Indigestion	Without	AR	Capim-Santo	*C. citratus*	De Santana; Voeks; Funch, 2016
Etno	Repellent	Without	NAR	Kyayisubi	*C. citratus*	Baana; Angwech; Malinga, 2018
Etno	Antiparasitologic	Without	AR	Lemongrass	*C. citratus*	Odoh et al., 2018
Rev.	Antifungal/ Hepatoprotective/ Antioxidant/ Antiprotozoario/ Antibacterial	Without	AR	Citronela	*C. citratos/ C. winterianus*	Santos et al., 2019
Etno	Analgesia	Without	NAR	Lemongrass / Verbena	*C. citratus*	Mota et al. 2020
Etno	Colon reactivity	Without	NAR	-	*C. schoenanthus*	Djemam et al., 2020
Etno	Comforting	Without	NAR	Lemongrass	*C. citratus*	Santos; Silveira; Gomes, 2015
Rev./ Etno	Central Nervous System Disorders	Without	NAR	Capim-Cidreira/ Capim-Cidrão	*C. citratus*	Amoateng et al., 2018; Gross et al., 2019.

Rev: Review; Etno: ethnobotanical/ethnopharmacology; NAR: No Activity Report; AR: Activity Report
Source: Own authorship (2022)

C. citratus, popularly known as lemongrass or capim-santo, is one of the most common species of this genus, spread worldwide due to its adaptation to tropical conditions. It is one of the most known among the plants of the genus *Cymbopogon*, and is also widely used in folk medicine to treat gastrointestinal and central nervous system disorders (anxiolytics). However, some reports indicate its use to treat other symptoms or diseases, often correlated with infections such as the fever, pain and cough related to flu, malaria and pneumonia. Regarding

the extraction mode, infusion to make tea was the most widely disseminated in communities (Ekpenyong, Akpan & Nyoh, 2015; Baana, Angwech & Malinga, 2016; Donato et al., 2018).

3. 2 Experimental works

In 79% (77/98) most of the articles were experimental studies in which *in sílico, in vitro, in vivo* or clinical trials were performed to test the antimicrobial action of the genus *Cymbopogon*. Considering the resistance to antimicrobial therapies, a worldwide problem of complex epidemiology, these studies reported alternative therapies or sources of new molecules for the treatment of infections in humans and animals, as well as food safety (Pardo, Sati & Galas, 2018; Liu et al., 2019).

In the experimental studies, the use of eight different species of *Cymbopogon* was observed, while three studies did not report the species used. The species *C. citratus* was most cited, as well as in other types of works, followed by *C. nardus* (L.) Rendle, *C. flexuosus* (Nees) Will. Watson, *C. martini* (Roxb.) Will. Watson, *C. winterianus*, *C. schoenanthus* (L.) Spreng., *C. densiflorus* (Steud.) Stapf and *C. giganteus* Chiov. The studies of *C. citratus* reported variation in the sensitivity profile, as was the case of the species *C. nardus* and *C. martini*.

In addition, 73% (58/79) of the extracts/preparations used were essential oils (EOs), 11% (9/79) involved nanoparticles, 5% (4/79) were infusion, 8% (6/79) had other preparations and 3% (2/79) did not report the type of preparation.

Among these species reported in the studies, a variety of compounds were investigated. Citronellal, Myrcene, Citral and their chemical isomers stood out, as was the case of geraniol, a compound directly related to the antimicrobial potential of *Cymbopogon* (Trindade et al., 2015; Adukwu et al., 2016; Wu et al., 2019). Pontes et al. (2018) presumed that these compounds pass through the cell wall causing depolarization of the microorganism's plasma membrane (Miguéis et al., 2019).

This variation in the sensitivity profile may be related to seasonality, circadian cycle, soil and stress, generating changes in the synthesis of plant metabolites, which consequently can trigger different biological activities. Another factor influencing the sensitivity of microorganisms to these herbs was the mode of extraction/preparation. The main preparations found were essential oils followed by nanoparticles. The activity of these substances is already well elucidated in the literature, indicating antimicrobial potential. However, extracts that use

solvents can present the active metabolite in a smaller amount, due to the dilution of these compounds in the solvent (De Santana, Voeks & Funch, 2015; Seibert et al., 2019).

In the analyzed studies, tests were reported in strains of 52 genera and 98 distinct species. Of this total number of species, 63% (60/96) were bacteria, 29% (28/96) fungi (filamentous or yeast), 4% (4/96) viruses and 4% (4/96 parasites (arthropods, helminths and protozoa).

In the analysis of the results of articles that evaluated antifungal and antibacterial action, 77% (60/78) reported total inhibition of growth, 3% (2/78) moderate inhibition, 4% (3/78) low growth inhibition and 5% (4/78) no microbial inhibition. Some of these were strains of *Escherichia coli* (with antimicrobial resistance), *Pseudomonas aeruginosa*, *Borrelia burgdorferi*, *Candida albicans* (with antimicrobial resistance), *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Saccharomyces cerevisiae*. In addition, 5% (4/78) reported antiviral activity, 5% (4/78) parasitic control and 1% (1/78) preventive action against contamination by mesophilic microorganisms.

Regarding the evaluation of plant extracts of *Cymbopogon* species, a variety of tests were performed, the main ones being the microdilution or disk diffusion tests, to determine the Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Minimum Concentration of Biofilm Eradication, Fractional Inhibitory Concentration Index (FICI), and exposure with observation and cell culture.

The broth microdilution MIC test stood out in this review as the most used method, probably because it is a method recommended by the Clinical and Laboratory Standards Institute (CLSI) to determine drug inhibition concentrations against anaerobic bacteria growth, yeasts and filamentous fungi. This method was adapted according to the tested substances, for example involving concentration of the tested substances or their solubilization process. In relation to solubilization of the test compound, it was observed that in the case of essential oils, due to their apolar nature, a substance is required to solubilize them in the culture medium, such as Tween 20 or 80 and Dimethyl sulfoxide (DMSO). These substances are used in the lowest practical concentrations due to toxicity (Balouiri et al., 2016; Costa, Moura & Millezi, 2019).

The disk diffusion method was reported to have good applicability due to its ease of conduction and low cost. However, it is not as precise as microdilution, because it depends on the diffusion of the bioactive substance in the medium and on the macro growth of the strains

for the interpretation of the results, while microdilution test results can be read by absorbance and consequently produce more accurate inhibition results (Balouiri et al., 2016).

These methods are widely applied to extracts, especially essential oils derived as nanoparticles, in bioprospecting in the fight against various microorganisms. *In vitro* trials have been conducted in recent years to ascertain the antifungal potential against strains of *Candida albicans, Candida tropicalis, Candida krusei, Candida parapsilosis, Cryptococcus grubii, Penicillium corylophilum, Trichophyton rubrum, Trichophyton mentagrophytes, Microsporum gypseum* and *Microsporum canis*, pathogens that are sometimes neglected despite their clinical relevance. This is especially true of *Candida* sp., which has potential to develop biofilms and cause opportunistic infections in debilitated patients, especially in hospitals. In particular, it is the most common microorganism found in patients with oral cancer, whose incidence has been increasing significantly among young people and women, with 350,000 – 400,000 cases per year (De Toleto et al., 2016; Dias et al., 2017; Almeida et al., 2016; Gholizadeh et al., 2016; Khosravi et al., 2018; Da Silva Gündel et al., 2018; Córdoba et al., 2019; Ji et al., 2019; Silva et al., 2019; Sahal, et al., 2020).

Growth inhibition activity was also observed in gram-positive bacteria, such as *Staphylococcus epidermidis, Staphylococcus aureus, Enterococcus faecalis* and *Streptococcus mutans*, as well as against gram-negative bacteria, especially *E. coli, P. aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii* and *Proteus mirabilis*, showing a variation in relation to the results of the MIC and highlighting that non-fermenting bacteria, *P. aeruginosa* and *A. baumannii*, where less inhibition was seen (Scazzocchio et al., 2016; Chaftar et al., 2015; Leite et al., 2016; Souza et al., 2016; Rossi et al., 2017; De Silva et al., 2017; Luís et al., 2017; Bermúdez-Vásquez, Granados-Chinchilla, Molina, 2019; Costa, Moura, Millezi, 2019; Oliveira et al., 2019; Cunha et al., 2020).

In addition to studies showing inhibitory action against fungi and bacteria, some studies have investigated antiviral action of the essential oil of the species *C. citratus*, applied as a nanogel, against Herpes simplex viruses. The EOs of *citratus* and *nardus* were tested against the Mastadenovirus HAdV-5 and were found to have antiviral activity in both studies, with *C. citratus* also being effective in Murine norovirus 1 (MNV-1) and Dengue virus type 2 DENV-2 models. However, only a few studies have been published in recent years investigating the antiviral potential and action mechanism of these plants (Kim et al, 2017; Almeida et al., 2018; Chiamenti et al., 2019; Rosmalena et al., 2019).

Another group of microorganisms against which substances obtained from *Cymbopogon* sp. were studied was protozoa, namely *Plasmodium* sp., *Pediculus capitis*, *Giardia lamblia* and *Haemonchus contortus.* These studies showed the potential for prevention or control of growth of the populations of these parasites. However, only a few studies have been published, despite the potential of this plant (Macedo et al., 2015; Chukwuocha et al., 2016; Limoncu et al., 2017; Méabed, Abou-Sreea & Roby, 2018; Macedo et al., 2019).

There is strong global concern about multidrug resistance to conventional antibiotics. Thus, in 2019 the World Health Organization included antimicrobial resistance as one of the top ten global health threats (WHO, 2019). The findings of this review showed good effectiveness of *Cymbopogon* sp. against microorganisms, so it is important to conduct further studies to analyze the effectiveness of essential oils and their derivatives against drug-resistant microorganisms.

Singh, Fatima and Hameed (2016) reported citronella's potential against *C. albicans* in daily cleaning of dentures. Another use of the EOs is treatment of water and food, as indicated by Chaftar et al. (2015) and Boeira et al. (2018), who compared the effectiveness of different oils and extracts for control of crop pests and food rot.

Another way to use volatile oils of this botanical genus is with the addition of metallic nanoparticles, such as silver, or nanoemulsions, to enhance the biological activity of essential oils by preserving their constituents until contact with microorganisms, thus triggering more effective responses. This can be a strategy for use of natural products against resistant microorganisms (Bansod et al., 2015; Liakos et al., 2016; Rossi et al., 2017; Da Silva Gündel et al., 2018; Seibert et al., 2019; Cherian et al., 2020).

4. Conclusion

This integrative review of *Cymbopogon* allows us to conclude that the PubMed platform presented the largest number of publications during the period studied and in both platforms, the largest number of publications occurred in 2015. In the case of ethnobotany, ethnopharmacology and literature review articles, the interviewees were generally unaware of *Cymbopogon's* potential to treat infections.

The highest percentage of publications were experimental studies, of which more than half involving *Cymbopogon* species found antimicrobial potential against infectious agents, with *C. citratus* being the most studied species.

The great antimicrobial potential of medicinal plants of the genus *Cymbopogon* was revealed, thus recommending greater bioprospecting for the discovery of new drugs to combat microorganisms, with or without resistance to current pharmacotherapeutics. Therefore, further studies are needed to test secondary metabolites, their molecular variations and synergism with other drugs for application against multidrug-resistant microorganisms.

Cymbopogon derived EOs also have strong potential for the development of drugs with action against bacteria and fungi, as well as for food preservation. However, more studies still need to be carried out to evaluate the use of volatile oils against multidrug-resistant viruses, parasites and microorganisms due to their relevance in human and veterinary medicine. In addition, studies with nanoparticles and nanoemulsions have shown better microbiological performance compared to free EOs, thus being a possible source for the development of new pharmaceutical products. Furthermore, it is necessary to carry out characterization and isolation studies of EOs as well as toxicity studies to ensure repeatability and feasibility in the use of these substances.

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4.2 Artigo experimental

Artigo a submeter (Archives of Microbiology – Fator de Impacto 2.667(2021)/QualisB2)

Antifungal, molecular docking and cytotoxic effect of the essential oil of *Cymbopogon citratus* (DC) Stapf. and *Cymbopogon nardus* (L.) Rendle against *Candida albicans*

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Abstract
Brazil is blessed with an enormous plant biodiversity, where herbs of the genus *Cymbopogon* especially, *C. citratus* and *C. nardus*, broad antimicrobial potential. Candidemias caused by *Candida albicans* are highly prevalent in immunosuppressed patients and are also associated with infections caused by biofilms on medical devices. The aim of this work was to evaluate the antimicrobial potential of *C. citratus* (EOCC) and *C. nardus* (EOCN) essential oils against *C. albicans* in planktonic and biofilm forms. The essential oils were obtained by hydrodistillation and chemical composition was assessed by GC-FID and GC-MS. The Minimal Inibitory Concentration (MIC) was determined by the broth microdilution method and the effect of the mixture of essential oils and amphotericin B was evaluated by the checkerboard test. Biofilm activity was determined through metabolism with XTT assay. Cytotoxicity assays were performed with VERO cells. In addition, molecular docking was performed to predict the effect of oil interaction at the yeast active binding site. Results showed antimicrobial activity of essential oils against *C. albicans* planktonic cells, with MIC values ranged between 156.25 and 78.12 µg/mL for EOCC, and 625 and 312.50 µg/mL for EOCN. The checkerboard assay showed synergism between EOCC or EOCN and amphotericin B. Essential oils inhibited biofilm metabolic activity by up to 81% and 93%, for EOCC and EOCN, respetively. MTT

assays showed cytotoxicity at concentrations above 250 µg/mL for EOCC, while for EOCN it wasnot observed cytotoxicity up to a concentration of 1,000 µg/mL. Moreover, molecular docking showed interaction between some constituents of EOCC and EOCN with the SAP-5 protein. Results point out that essential oils of *Cymbopogon* spp. studied showed anti-*Candida* activity against planktonic and sessile cells, with potential for later application in therapy against infections caused by *C. albicans.*
Keywords: Biofilm; Candidemias; Essential oil; Synergism; *Cymbopogon*.

Introduction

Countries with tropical ecosystems, such as Brazil, have great potential for bioprospecting of native and cultivated plants. Among these plants, species of the *Cymbopogon* genus, belonging to the Poaceae family, have approximately 144 known varieties (Avoseh *et al*., 2015; Aguilar *et al*., 2019). This genus is characterized by having aromatic monocots, whose main representatives are *C. citratus* (DC.) Stapf and *C. nardus* (L.) Rendle, in that order, popularly named Capim-Santo and Citronela. These species are used by the community mainly for it's anxiolytic and insect repellent potential, respectively (Avoseh *et al*., 2015; Prochnow *et al*., 2018; Carreiro *et al*., 2020). Being therapeutic potential of their metabolism products of these plants, through secondary mevalonic acid, experimental studies support the possibility of using phytocomplexes, such as essential oils, for antimicrobial activity against viruses, bacteria, protozoa and fungi. Thus, the use of *Cymbogopon* species as medicinal plant and its pharmacological products are promissors as alternative therapeutic to clinically invasive infections and with high antimicrobial resistance (Sousa *et al*., 2020; Kaur *et al*., 2021; Prado *et al*., 2022).

The use of medicinal plants for integrative and complementary health practices (ICHP) is a therapeutical alternative, alone or combined with conventional drugs, for the treatment of invasive infections (Borges and Sales, 2018). This is particularly the case of treatment protocols that use drugs with high toxicity, such as antifungals of the azole and polyene classes, which reportedly are hepatotoxic and nephrotoxic, respectively (Bassetti *et al*., 2018).

Among the etiological agents that can cause opportunistic infections are species of the genus *Candida*. These yeasts are responsible for 400,00 cases of invasive candidiasis per year, resulting in 46 to 75% mortality in the world in 2022, being the fourth leading cause of sepsis in global health services, and the seventh in Brazil (Brown *et al*., 2012; Pappas *et al*., 2018; Machado *et al*., 2021). They can be grouped into *Candida albicans* and non-*albicans Candida*, the most prevalent being *C. albicans*. It is the species found in clinical isolates, causing 90% to 100% of mucosal infections and 40% to 70% of blood infections (Boucherit-Otmani *et al*.,

2021; Sousa *et al.*, 2020). Risk groups for *Candida* spp. are patients with acquired immunodeficiency syndromes (AIDS), diabetes and with immunosuppressive medication (Tong and Tang, 2017; Pappas *et al*., 2018).

C. albicans stands out for its ability to establish, colonize and cause disease by overcoming host defenses, characteristics that are directly linked to the virulence of these microorganisms. The best-known virulence mechanisms are adherence, polymorphism, phenotypic variability, and production of extracellular enzymes and toxins. All these factors are directly linked to biofilm formation capacity (Tong and Tang, 2017; Wall *et al*., 2019).

Biofilms are complex microbiological communities that have, among their many characteristics, the ability to adhere, multiply and form extracellular matrices and disperse, along with polymorphism. These mechanisms enable biofilms to develop on the most diverse surfaces, including medical devices such as prostheses, probes and catheters. Biofilm formation on these devices particularly affects immunocompromised patients, especially those in intensive care units (Lohse *et al*., 2018).

Thus, the aim of this study was to evaluate the cytotoxicity and antifungal activity of essential oils (EOs) of *C. citratus* and *C. nardus*, against clinical isolates and wild-type strains of *C. albicans* in planktonic and sessile forms. In addition, we evaluated the synergistic potential of the EOs with amphotericin B as pilot study to reduce doses of the standard drug, with subsequent determination of the interaction of the major EOs compounds with SAP5 adhesion protein by molecular docking.

Material and methods

Plant material

The leaves of *C. citratus* and *C. nardus* were collected in the morning, from plants cultivated in the municipality of Sobral, Ceará (Brazil), located at coordinates 3°42'07"S 40°21'53"W. Voucher specimens were identified and deposited with the Professor Francisco de Abreu Matos Herbarium (HUVA) at Vale do Acaraú State University, registered as exsiccate number 18614 and 20807, respectively.

Extraction and chemical characterization of essential oil

Fresh leaves of *C. citratus* and *C. nardus* were macerated and subjected to hydrodistillation for 2 h in a modified Clevenger apparatus. After extraction, the yield and relative density of essential oils were determined

The chemical analysis of essential oils (EOs) was carried out according to Arantes *et al*. (2019), by gas chromatography. GC-FID analyses were performed *with a* Shimadzu Nexis GC-2030 gas chromatograph and flame ionization detector *(*GC-FID*)* equipped with an AOC-20i plus autoinjector (HERCULES Lab, Univ Évora, Portugal), with dimensions of 30 m x 0.25 mm i.d. and film thickness of 0.50 µm, and a Zebron ZB-5HT Inferno™ fused-silica non-polar capillary column (Phenomenex, USA), using the LabSolutions software version 5.92 (Shimadzu Corporation). GC-MS analyses were performed with a GC-MS-QP2010 Series (Shimadzu) gas chromatograph, fitted with Zebron ZB-5HT Inferno™ non-polar fused-silica (30 m × 0.25 mm i.d., film thickness 0.50 µm), interfaced with a detector model Polaris Q (E. I. quadrupole). Compounds were identified by their retention indices (RI) and their mass spectra of the NIST11 (National Institute of Standards and Technology) library. Retention indices were determined by interpolation relative to the C8–C22 n-alkanes retention times and compared with those of authentic samples, from the laboratory database and with literature data (Babushok *et al*., 2011; Vieira *et al*., 2013; Arantes *et al*., 2019; Pandur *et al*., 2022).

For biological assays, the *C. citratus* essential oil (EOCC) and *C. nardus* essential oil (EOCN) were solubilized in RPMI-1640 medium, supplemented with L-glutamine (Sigma-Aldrich, St. Louis, MO, USA) and Tween® 80 at 0.1% (Nascimento *et al*., 2007).

Yeast strains

The standard *C. albicans* strains was obtained from the *American Type Culture Collection* (ATCC90028). Clinical isolates of *C. albicans* (LABMIC 0102, LABMIC 0104, LABMIC 0125 and LABMIC 0127) (Table 2) were were provided by the Santa Casa de Misericordia Hospital, Sobral (Ceará, Brazil) and Norte Regional Hospital (Ceará, Brazil). Strains phenotypic and molecular identifications were performed by CHROMagar (CHROMagar *Candida*, France), a Vitek 2 system (BioMerieux Vitek, Hazelwood, France) and PCR (Cellco Biotech, Brazil). This study was authorized by the Research Ethics Committee of Acaraú Valley State University under reference number 4.0633.262.

Inoculum preparation for antifungal susceptibility tests

The inoculum was prepared from cultures maintained in the laboratory, on Sabouraud dextrose agar (SDA) (Difco, Detroit, MI). Cells were cultured for 24 h at 35±2 ºC. Yeast colonies were transferred to tubes containing sterile PBS to obtain suspensions with turbidity equivalent to 0.5 on the McFarland scale (c. 10^6 CFU per ml). These suspensions were then diluted 1:2,000 with RPMI-1640 medium supplemented with L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), to obtain concentration $2x10^2$ CFU per ml, in agreement with the directions of the Clinical and Laboratory Standards Institute (CLSI) M27-A3 standard (CLSI, 2008).

Broth microdilution method

The minimum inhibitory concentration (MIC) of yeast growth was determined by broth microdilution with 96-well plates, in accordance with the CLSI M27-A3 (CLSI, 2008). The EOCC and EOCN was tested in the concentration range of 2.44-2,500 μg/ml. Then, 100 μL of inoculum was added to 100 μl of test solution. Amphotericin B (AMB) and fluconazole (FLC) were used as standard drug controls in the ranges of 16 – 0.015μg/ml and 64 - 0.0625 μg/ml, respectively. The MIC was defined as the lowest oil concentration that caused 100% inhibition of visible fungal growth, according Fontenelle *et al*., (2007, 2008).

Checkerboard assay

To determine the modulatory effect, we used the two clinical isolates that showed the lowest MIC in the broth microdilution tests (LABMIC 0102 and LABMIC 0105), as well as the ATCC90028 strain. For this analysis, the antifungal drug of choice was amphotericin B. Despite being effective, this drug has a strong nephrotoxic effect, so the use of lower concentrations would also reduce this effect.

Initially, 50 μL of RPMI-1640 was added to each will of a 96-well plate and then 50 μL of EOCC and EOCN was added. Serial dilutions of the EOCC end EOCN were performed in the concentration range from MIC to MIC/10. Subsequently, 50 μL of different concentrations of AMB was added to each of the lines. Cells treated only with EOCC individually or AMB alone, at their respective MIC values (Table 2), along with untreated fungal suspensions were used as controls. The plates were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration at which no visual growth (absence of turbidity) was observed. The FICI was calculated by the sum of FICo + FICa, where O represents EOCC and amphotericin B. In turn, FICo was calculated as MICo combined/MICo alone, while FICa was calculated as MICa

combined/MICa alone. Synergism was defined as FICI ≤ 0.5, while no interaction was recorded when 0.5 < FICI ≤ 4.0, and antagonism when FICI >4.0 (White *et al.*, 1996; Rosato *et al.*, 2008).

Biofilm assay

The susceptibility of *Candida* sp. biofilm by *C. citratus* and *C. nardus* was performed according to Gonçalves *et al*. (2017). Briefly, 100 µL of inoculum was transferred to each well of a 96-well microplate. Then, 100 µL of RPMI-140 supplemented with *C. citratus* or *C. nardus* essential oil were added twice the MIC value for each microorganism. The microplate was then incubated for 48h at 37°C. Wells containing only culture medium without inoculum or with inoculum and unsupplemented RPMI-1640 were used as controls and wells with RPMI-1640 supplemented with Amphoterician B 2 µg/mL were used as a positive control. After incubation, the biofilm activity was by tetrazole salt (XTT).

Determination of metabolism by XTT/Menadione

After the incubation period, the biofilm was washed three times with saline solution (0.85%; pH=7.00) to remove planktonic cells. To determine the metabolic activity, 100 µL of XTT-menadione solution (1 µL 1mM menadione in 10 mL of 0.5g/L XTT) was added to the wells of each plate and incubated in the dark for 2h at 37ºC. After this period, the supernatant solution was transferred to a new plate and read with optical density measured at 490nm (Gonçalves *et al*. 2017).

Cytotoxicity assay

The evaluation of cytotoxic activity the EOCC and EOCN was performed using the viability method of 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyl-tetrazolium bromide (MTT), described by Mosmann *et al*. (1983) with modifications. The objective was to analyze the mitochondrial activity of viable cells (Nery *et al*., 2014). Mammalian Vero cells (epithelial cells from the kidneys of African green monkeys) from the Rio de Janeiro (Brazil) Cell Bank (no. 0245) were used. The cells ($2x10^5$ cells/ml) were cultured in Leibovitz medium (Cultilab, SP, Brazil) supplemented with 10% fetal bovine serum and solution content of streptomycin (20 mg/ml), penicillin (10,000 U/ml) and AMB (1mg/ml). The EOCC and EOCN was tested in the concentration range of 1,000 to 31.25 µg/ml.

After formation of the monolayer cells, 48 h post-incubation, the medium was removed and the essential oils diluted in the predefined concentrations (31.25, 62.5, 125, 250, 500 and 1,000 µg/ml) were added. The plates were incubated for 7 days, after which the supernatant was removed from the wells, followed by addition of 100 µL of L-15 medium supplemented with 2% fetal bovine serum + 10 µL of the MTT solution. The experiments were performed in triplicates. After 4 h, the supernatant was discarded and 100 µL of DMSO was added, and the absorbance was read in a spectrophotometer at 540 nm (Mosmann *et al*., 1983). The cytotoxicity percentage was calculated as [(A/B)/x100)], where A and B are the absorbances of untreated control and treated cells, respectively. Cytotoxic concentrations of 50% (CC50) were used.

Molecular docking

Preparation of ligands

The PubChem repository (https://pubchem.ncbi.nlm.nih.gov/) was used to obtain the tridimensional structures of the ligands Citronellal (7794), Citronellol (8842), Geraniol (637566), Elemol (92138), α-cadinene (10398656), Caryophyllene oxide (1742210), eugenol (3314), γ-cadinene (15094), Geranial (638011), Geranyl acetate (1549026), Germacrene D (5317570), Isopulegol (170833), Limonene (22311), Linalol (6549), linalyl acetate (8294), terpinyl acetate (538936), β-myrcene (348293176), neral (643779), neryl acetate (1549025), as well the control ligands: amphotericin B (5280965), and fluconazole (3365). The low energy conformers were optimized with MMFF94 (Merck Molecular Force Field 94) and the steepest descent algorithm with cycles of five interactions through MarvinSketch™ (https://chemaxon.com/products/marvin) (Csizmadia, 2019; Chemaxon, 2019) and Avogadro ™ (http://avogadro.cc/) codes (Hanwell *et al*., 2012).

Anchoring procedure

The Protein Data Bank repository was utilized to obtain the target macromolecule denominated “Secreted aspartic proteinase (Sap) 5 from *Candida albicans*” (PDB ID: 2QZX), confirmed by X-ray diffraction (R-Value Free: 0.275 and R-Value Work: 0.224), deposited with a resolution of 2.50 Å and classified as a hydrolase enzyme. The target preparation

removed water molecules, followed by addition of polar hydrogens and Gasteiger charges (Yan *et al*., 2014) through AutoDock Tools (Huey *et al*., 2012).

With a grid box configured at the centers x, y and z equal to 19,613 Å, 19.76 Å and 44,435 Å, respectively, fitted with Cartesian dimensions x = 64 Å, y = 58 Å and z = 108 Å, involving the entire surface of the protein, the AutoDock Vina software (Trott and Olson, 2010) was used to perform molecular docking simulations with fifty independent simulations for each ligand.

To choose the best ligand, two criteria were used. The first was the root mean square deviation (RMSD), a validation criterion of simulations realized with ideal parameters until 2.0 Å (Yusuf *et al*., 2010). The second criterion utilized was the free energy of binding (ΔG_{bind}), which is considered ideal when values are lower than or equal to -6.0 kcal/mol (Shityakov and Förster, 2014). The parameters proposed by Imberty *et al*. (1991) were used with the distances between the donor atoms with protein to evaluate the strength of the hydrogen bonds. The distances between hydrogen bonds of 2.5 Å to 3.1 Å, 3.1 Å to 3.55 Å, and greater than 3.55 Å, are classified as strong, moderate and weak, respectively.

Statistical analysis

The biofilm assay data were submitted to one-way analysis of variance (ANOVA), followed by the Tukey multiple comparison test using GraphPad® Prism version 5. 4 (GraphPad Software, San Diego, California, USA). Statistical significance was set as $p < 0.05$.

Results and discussion

Characterization of essential oils

The oil extracted from the leaves of *C. citratus* was hyaline and yellowish, with a citrus aroma, with a relative density of 0.832 ±0.038 g/ml and a yield of 0.399 ±0.103%. Obtained yield extraction was different from that found by Santos *et al.* (2009) and Domingues and Paiva (2021), who obtained yields of 0.66% to 1.15%. The essential oil of *C. nardus* was clear, colorless and with a citronella aroma. Yield extraction and relative density were 0.71% and 0.88 g/mL, respectively. The yield obtained was lower than that found by Sawadogo *et al.* (2022) who reported values of 1.37% for EOCN. These differences in productivity may be related eith to the age of plants and environmental factors such as soil type, moisture content,

seasonality and season of the year in which the plant was collected (Santos *et al*., 2009; Kaur *et al*., 2021).

The chemical composition of essential oils is shown in Table 1. There were identified 22 compounds for *C. citratos* essential oil (98 %) and 14 compounds for *C. nardus* essential oil (97 %). Chemical profile of both essential oils showed a high oxygenated monoterpene content with 95 % for the EOCC and 91 % for the oil for EOCN, respectively. The major constituents were neral (36.13 %) and geranial (48.47 %) for the essential oil of *C. citratos* and citronellal (44.44 %), citronellol (16.97 %) and geraniol (26.55 %) for the oil of *C. nardus.*

Studies carried out with essential oils of *Cymbopogon* species have shown that these oils have unique chemical properties rich in oxygenated monoterpenes with significant inter/intra-species differences (Jin et al., 2022). The outcomes for the essential oil of *C. citratus* agree with the bibliography that suggest that this essential oil is rich in neral (31–45%) and geranial (27-55%), with a predominance of geranial in the Brazilian species (Barbosa et al., 2008; da Silva et al., 2020; Jin et al., 2022; Paiva et al., 2022; Sawadogo et al., 2022; Sharma & Kaur, 2022). They were also found in the essential oil of *C. citratus* collected and studied by Saboia *et al*. (2022) on the Maranhão State (Brazil) and correlated with the therapeutic potential of this plant. Among these compounds, citral and your isomers (Oxygenated monoterpenes) were present in the highest percentages in the EOCC.

The chemical profile of *C. nardus* is according to the other Brazilian *C. nardus* essential oils described in bibliography, with some seasonal and geographical differences , in different periods and locations in Brazil, were citronellal (28 - 50%), geraniol (17 - 34%) and citronellol (11 - 25%) (Aguiar et al., 2014; Andrade, Cardoso, Batista, Mallet, & Machado, 2012; Castro, Perini, Santos, & Leal, 2010; da Silva et al., 2020; Gaspar de Toledo et al., 2020; Guandalini Cunha et al., 2020; Kaur, Bhardwaj, & Kaur, 2021; Pontes et al., 2019; Trindade, de Araujo Oliveira, de Castro, & de Oliveira Lima, 2015). Guandalini Cunha et al. (2020) and da Silva et al. (2020) also detected the presence of nerol (>10%) in the essential oil of *C. nardus* from Araçatuba, São Paulo, and Seropédia, Rio de Janeiro (Brazil), respectively, while Gaspar de Toledo et al. (2020) observed the presence of geranial (13%) and neral (10%) in the essential oil of *C. nardus* from Araraquara, São Paulo (Brazil). In the analysed essential oil of *C. nardus* it was observed the presence of geranial (0.4%) but that neral and nerol were absent. These findings show that the phytochemical profile of EOCN and EOCC is stable in major

components, with variation in the concentration of their major and minor components regardless of the period and place where the plant was collected (Avoseh *et al*., 2015).

Table 1 Chemical composition of the essential oil of *C. citratus* and *C. nardus* identified by GC-MS.

Class	Compound	*RI $_{Exp.}$	RI $_{bib.}$	*C. citratus*	*C. nardus*
				Area (%) Mean$_{\pm SD}$	**Area (%) Mean$_{\pm SD}$**
HM	β-Myrcene	990	990 [a]	2.17 ± 0.05	__
HM	Limonene	1024	1026 [a]	__	1.82 ± 0.02
HM	α-Terpinolene	1078	1076 [b]	0.23 ± 0.01	__
OM	Linalool	1096	1095 [a]	0.71 ± 0.01	0.53 ± 0.01
OM	*trans*-Pinocarveol	1134	1134 [a]	0.16 ± 0.01	__
OM	Isopulegol	1144	1144 [a]	0.12 ± 0.02	0.92 ± 0.01
OM	Menthone	1151	1150 [a]	0.12 ± 0.01	__
OM	Citronellal	1155	1153 [d]	__	**44.44 ± 0.15**
OM	Borneol	1163	1162 [a]	0.46 ± 0.01	__
OM	4-Terpineol	1175	1174 [a]	0.11 ± 0.01	__
OM	α-Terpineol	1181	1190 [d]	0.72 ± 0.03	__
OM	Myrtenal	1191	1192 [d]	0.14 ± 0.01	__
OM	Citronellol	1230	1228 [d]	__	**16.97 ± 0.09**
OM	Neral (Citral-b)	1241	1242 [c]	**36.13 ± 0.18**	__
OM	Geraniol	1257	1255 [d]	2.11 ± 0.03	**26.55 ± 0.12**
OM	Geranial (Citral-a)	1273	1274 [c]	**48.47 ± 0.19**	0.35 ± 0.01
OM	Thymol	1293	1294 [a]	0.12 ± 0.01	__
OM	Myrtenyl acetate	1330	1329 [d]	0.31 ± 0.01	__
OM	Terpinyl acetate	1338	1347 [d]	1.86 ± 0.01	0.19 ± 0.01
OM	Eugenol	1363	1358 [d]	0.78 ± 0.03	0.85 ± 0.02
HS	α-Copaene	1366	1369 [b]	0.43 ± 0.07	__
OM	Neryl acetate	1374	1363 [d]	2.40 ± 0.02	__
OM	Geranyl acetate	1382	1380 [d]	0.29 ± 0.01	0.41 ± 0.02
HS	β-Caryophyllene	1427	1417 [a]	0.17 ± 0.01	__
HS	D-Germacrene	1478	1481 [d]	__	0.33 ± 0.01
HS	γ-Cadinene	1511	1515 [c]	__	0.10 ± 0.01
OS	Elemol	1548	1548 [d]	__	3.25 ± 0.03
OS	Caryophyllene oxide	1582	1582 [a]	0.11 ± 0.01	__
OS	α-Cadinol	1653	1652 [d]	__	0.63 ± 0.01
Total identified				98.12 ± 0.74	97.47 ± 0.53

*RI $_{Exp.}$ - Retention index relative to C8–C22 *n*-alkanes on the Zebron ZB-5HT Inferno™ apolar column.

[a] Retention index reported by Arantes *et al*. (2019).

[b] Retention indices reported by Videira *et al*. (2013).

[c] Retention indices reported by Pandur *et al*. (2022).

[d] Retention indices reported by Babushok *et al*. (2011).

Minimum inhibitory concentration

For EOCC and EOCN, the antifungal potential was observed by the broth microdilution method, with MIC values in the range between 156.25 and 78.12 µg/ml and 625 to 312.5 µg/mL, respectively, against *C. albicans*, type strain and clinical isolates (Table 2). *Cymbopogon* essential oils slowed antifungal activity against *C. albicans*, with EOCC showing the highest anti-*Candida* activity.

Table 2 Antifungal activity of essential oil of *C. citratus* and *C. nardus* against *C. albicans.*

C. albicans strains*	Source	*Cymbopogon citratus*	*Cymbopogon nardus*
		MIC (µg/mL)	MIC (µg/mL)
LABMIC 0102	Hemoculture	78.12	312.50
LABMIC 0104	Tracheal aspirate	78.12	312.50
LABMIC 0105	Hemoculture	78.12	312.50
LABMIC 0125	Diabetic foot	156.25	625.00
LABMIC 0127	Diabetic foot	78.12	625.00
ATCC 90028	ATCC, EUA	156.25	625.00

*The values of MICs of controls AMB and FLU were 1 µg/mL and 0.5 - 0.25 µg/mL, respectively.

The results of the antifungal activity observed by the broth microdilution method indicate that essential oils have components with antimicrobial activity, corroborating the antimicrobial activity against *Candida* spp. for monoterpenes components reported for other plants, such as *Mentha arvensis* L., *Mentha pulegium* L., *Ocimum basilicum* L. (Zabka *et al*., 2014; Rhimi *et al*., 2022). Other studies with *Cymbopogon* species reported antifungal activity of *Candida* spp. from different origins, with a MIC of 1.25 to 562 µg/mL (Trindade *et al*., 2015; Kandimalla *et al*., 2016; Toledo *et al*., 2016; Domingues and Paiva, 2021; Rhimi *et al*., 2022).

Paiva et al. (2022), in a study carried out on the antimicrobial action of the essential oil of *C. citratus* (84.53% citral and 13.76% myrcene), observed that both showed antifungal activity against 193 strains of *Candida albicans* isolated from the oral cavity. In another study, Boukhatem, Ferhat, Kameli, Saidi, and Kebir (2014) observed that the essential oil of *C. citratus* from Algeria (42.2% geranial, 31.5% neral, and 7.5% β-myrcene) showed antifungal activity (disk diffusion assay) against 15 isolates of yeast and filamentous fungi strains, with zone of growth inhibition of 15-90mm of diameter (40 uL of essential oil) for the 8 strains of *Candida* spp. Previous studies carried out with citral (*cis*-isomer geranial and *trans*-isomer neral) - the major component of the essential oil of *C. citratus* - concluded that this compound has significant antifungal activity against *Candida* spp(Leite, Bezerra, de Sousa, Guerra, &

Lima Ede, 2014; Silva Cde, Guterres, Weisheimer, & Schapoval, 2008; Zore, Thakre, Jadhav, & Karuppayil, 2011).

Toledo et al. (2020), in another study using essential oil of *C. nardus* (27.34 percent citronellal, 23.21 percent geraniol, 13.37 percent geranial, 12.49 percent citronellol, and 10.31 percent neral), observed the sensitivity of two *C. albicans* strains, with MIC values ≥ 500 µg/mL, suggesting that the antifungal activity of the essential oil of *C. nardus* is greater the higher its citronellal content. Additionally, Singh, Fatima, and Hameed (2016) and Saibabu, Singh, Ansari, Fatima, and Hameed (2017) observed the strong antifungal activity of citronellal against *C. albicans* strains, attributing its anticandidal mechanism: i) interference in membrane homeostasis, increasing fungal hypersensitivity to membrane disturbing agents, reducing ergosterol levels and decreasing glucose-induced H+ extrusion; ii) the induction of oxidative and genotoxic stress through an increase in the production of reactive oxygen species; iii) inhibition of the virulent attributes of the transition from yeast to hyphae and biofilm formation; as well as iv) the reduction of cell adherence to the polystyrene surface and to human oral epithelial cells.

The differences in minimal inhibitory concentration between studies are associated with the seasonal variation in the composition of volatile oils. EUCAST establishes cut-off points, compliance ranges for MICs that indicate resistance or susceptibility. According to these indices, the strains studied here are sensitive to both amphotericin B and fluconazole, while the essential oil showed variation of only two concentrations between strains.

All clinical isolates were sensitive to amphotericin B and fluconazole. For natural products, there are no cutoff values that indicate resistance or sensitivity of the isolates, however, concentrations in which the natural compound has a MIC lower than or equal to 1,000µg/mL indicate sensitivity of the microorganism to the product of plant origin (Toledo *et al*., 2016).

This antifungal potential presented by these essential oils of the genus *Cympopogon*, rich in oxygenated monoterpenes, has the potential to interfere with replication, fixation, production of hyphae or fluidity of fungal cell walls, possibly interfering through the interference of these compounds with the fluidity of the *Candida albicans* membrane (Almeida; Almeida; Gherardi, 2020; Shaban *et al*., 2020).

Checkerboard Essay

The results obtained from the checkerboard test to determine the effects of the combination of amphotericin B and essential oils are shown in Table 3, with fractional inhibitory concentration (FICI) indices between 0.16 and 0.31 for samples tested with EOCC and reduction of the MIC of the EOCC together with AMB by up to 8 times, indicating a synergistic effect according to the standards proposed by Rosato *et al*. (2008). As for the combination between EOCN and amphotericin B, synergistic and additive activity was observed, presenting FICI between 0.50 and 0.37, reducing the concentration with inhibitory activity of amphotericin B by up to 4 times and of the essential oil by up to 8 times the value of the MIC of the compound.

Table 3 Evaluation of the synergistic effect of EOCC or OECN and AMB against *C. albicans*

Strains	*Cymbopogon citratus*		Amphotericin B		**FICI**	**Effect**
	MIC µg/mL (Individual)	MIC µg/mL (Combined)	MIC µg/mL (Individual)	MIC µg/mL (Combined)		
LABMIC0102	78.12	9.77	1	0.125	0.25	Synergistic
LABMIC0105	78.12	2.44	1	0.125	0.16	Synergistic
ATCC 90028	156.25	9.77	1	0.250	0.31	Synergistic
	Cymbopogon nardus		Amphotericin B			
	MIC µg/mL (Individual)	MIC µg/mL (Combined)	MIC µg/mL (Individual)	MIC µg/mL (Combined)		
LABMIC0102	312.5	78.125	1	0.25	0.5	Additive
LABMIC0105	312.5	39.0625	1	0.25	0.375	Synergistic
ATCC 90028	625	78.125	1	0.25	0.375	Synergistic

FICI: Fractional Inhibitory Concentration Index Synergism was defined as FICI ≤ 0.5, while no interaction was annotated when 0.5 < FICI ≤ 4.0, and antagonism when FICI >4.

This study is promising, considering that in the literature we found few studies trying the EOCC or EOCN, however in the study by Silva *et al*. (2019) testing only citronellal, one of the compounds that can be isolated from the EOCN, and amphotericin B found only an indifferent effect with an ATCC76615 and a clinical isolate of *Candida albicans* tested. In the test carried out by Silva *et al*. (2020) and Khan *et al*. (2012), who used citronellol and geraniol, respectively, in combination with amphotericin B *Candidas* spp., observed a synergistic effect between these substances against *C. albicans*. Thus, shown potential for use in conjunction with these substances as well as in essential oil.

Thus, shown the possibility of using essential oils, as well as the phytocompounds isolated from this phytocomplex, together with standard drugs such as amphotericin B, a drug

of great relevance for the treatment of candidiasis and hospital candidemias, but with high toxicity.

Biofilm Assays (XTT/Menadione)

In the XTT assay, it evaluates the metabolic activity of reducing the dehydrogenase enzymes presents in the mitochondrial electron transport system, consequently evaluating the metabolism of the sessile cells that make up the biofilm (SILVA *et al*., 2008).

Thus, after carrying out the test with EOCC, a metabolic reduction of 58 to 81% was observed, while for the EOCN it reduced the metabolic activity of the biofilm by 83.66 to 93.4% (Figure 01), results that demonstrate a significant reduction in metabolism obtained with concentrations of 2 times the MIC for a clinical isolate (LABMIC 0102) and ATCC 90028, a result close to those found by Khan *et al*. (2012) and Silva *et al*. (2020) who observed anti-biofilm activity at concentrations of 360 to 125 µg/mL with *Cymbopogon* sp. This reduction is correlated to the phytocomponents of the EOCN, which according to Singh, Fátima and Hameed (2016) can act on membrane homeostasis and consequently leaving it more vulnerable and inhibiting aspects related to virulence such as the transition from yeast to hypha.

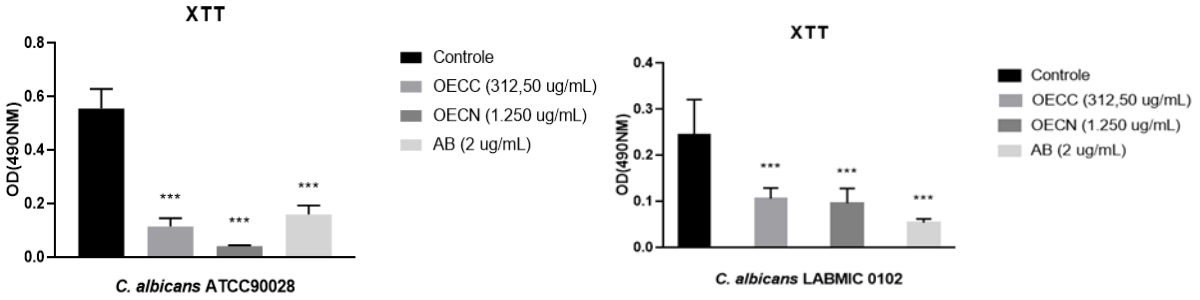


Fig. 1 Effect of essential oil from *C. citratus* and *C. nardus* on mature biofilms of *C. albicans* strains. Data are expressed as mean and standard deviation of XTT absorbance values normalized with those of the drug-free growth control ($P<0.05$). *Significant reduction of biofilm biomass compared to negative control.

Cytotoxicity Assay

The MTT assay with the essential oil of *C. citratus* showed cytotoxicity at concentrations tested above 250 µg/mL, while *C. nardus* did not observe toxicity up to the concentration of 1,000 µg/mL, as reported in Table 4, comparing to concentrations of the *C. citratus* essential oil. For the MIC cytotoxicity, note that both essential oils have anti-*Candida* potential with low toxicity. MTT (Sigma-Aldrich, USA) is a yellow salt that, when metabolized

by mitochondrial dehydrogenase enzymes present in viable cells, is converted into insoluble crystals of formazan (purple color), allowing indirect quantification of the percentage of cell viability (Mosmann *et al*. al., 1983).

Table 4 Cytotoxicity assay of essential oils of *C. citratus* and *C. nardus* in vero cells.

Concentration (µg/mL)	1000	500	250	125	62.5	31.25	C-
%Viability (*C. citratus*)	38.40	29.63	51.88	75.03	92.73	64.79	100
%Viability (*C. nardus*)	90.28	90.28	97.32	105.17	113.57	93.33	100

C-: untreated cells

In assays with HaCaT cells (Koba *et al*., 2009), Chinese hamster ovary cells (CHO) and the non-cancerous human fibroblast cell line (WI38) showed that EOCC presented cytotoxicity at concentrations above 100 µg/mL, since for EOCN in CHO and WI38 cells determined low toxicity for EOCN and its major components (Kpoviessi *et al*., 2014).

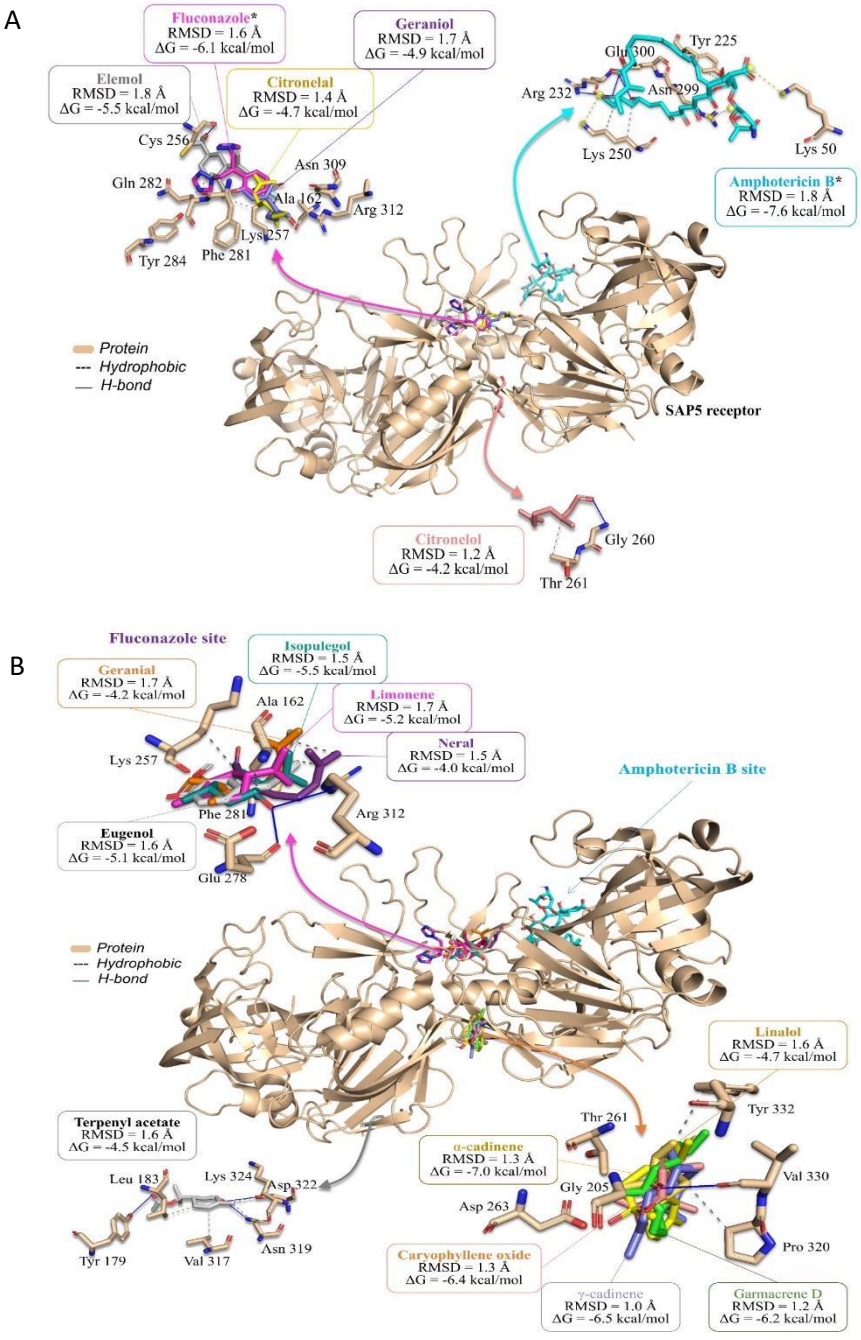
However, a study with EOCC of Silva *et al*. (2020) obtained toxicity results close to 500 µg/mL in fresh human erythrocytes, a result close to that of the present study. Regarding the EOCN and citronellal Toledo *et al*. (2016), it was observed that it had low cytotoxicity at the values tested up to 100 µg/mL for MRC-5 (fibroblast) and HepG-2 (hepatic), among these being less cytotoxic to liver cells.

Such toxicity of essential oils is related to terpene compounds and the ability of these substances to interfere with the fluidity of membranes, with toxicity depending on the dose. Therefore, these natural compounds can be used for therapy with careful consideration of the level of toxicity by monitoring and supervision (Ortega-Cuadros *et al*., 2018; Popova *et al*., 2019).

Molecular Docking

The molecular docking simulations were performed to understand the possible mechanism action of essential oils components against *Candida albicans*. SAP5 is a protease from the SAPS family (Secreted Aspartic Proteases) that has been associated with virulence characteristics in *C. albicans*. The main function of these enzymes is to degrade proteins, but they play an important role in biofilm formation. It is recognized that *C. albicans* biofilms secrete more SAPs than planktonic cells. Therefore, here we analyze the interaction of this protein with the EOCC and EOCN compounds (Min *et al*., 2013).

The results of ΔG (free energy of binding) and RMSD values of ligands with the SAP5 protein can be observed between figure 2 for OECN constituents and EOCC components, while the types of interactions are listed in table 5.



C

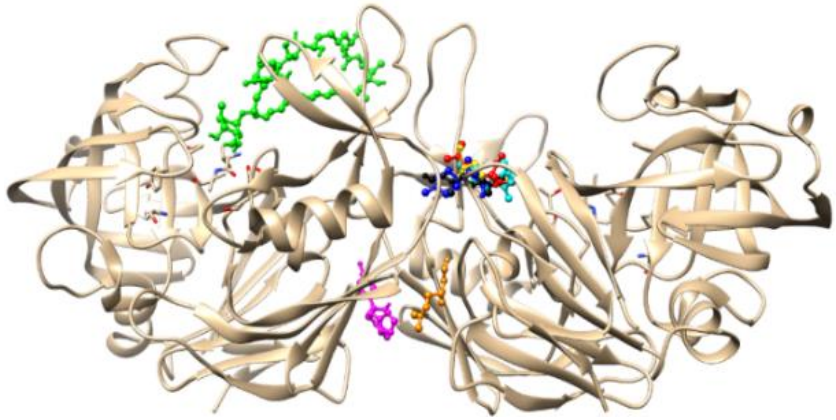


Fig 2. In image A, observe the binding sites between Citronelal, Elemol and Geraniol ligands; In image B it represents the binding sites between α-cadinene, caryophyllene oxide, eugenol, γ-cadinene, geraniol, germacrene D, isopulegol, limonene, linalool, Neral, terpenyl acetate (minor ligands); and in image C, linalyl acetate (red), terpenyl acetate (blue), geranial (black), β-myrcene (yellow), neral (orange) and neryl acetate (cyan). All represent binding with the SAP5 protein and with the control ligands Amphotericin B and Fluconazole*.

Table 5 Interactions with distances (Å) between the analyzed EOCC and EOCN binding constituents and SAP5 protein amino acid residues.

Compound	Residue	AA	Distance (Å)	Interaction
Caryophyllene oxide	205B	Gly	2.80	Hydrogen bonds
Caryophyllene oxide	261B	Thr	3.30	Hydrophobic
Caryophyllene oxide	330A	Val	3.72	Hydrophobic
Citronelal	162A	Ala	3.78	Hydrophobic
Citronelal	257B	Lys	3.52	Hydrophobic
Citronelal	281A	Phe	3.70	Hydrophobic
Citronelal	312A	Arg	2.77	Hydrogen bonds
Citronelol	260B	Gly	2.78	Hydrogen bonds
Citronelol	261B	Thr	3.82	Hydrophobic
Elemol	256A	Cys	2.83	Hydrogen bonds
Elemol	257A	Lys	3.71	Hydrophobic
Elemol	282A	Gln	3.36	Hydrophobic
Eugenol	257B	Lys	3.75	Hydrophobic
Eugenol	281A	Phe	3.79	Hydrophobic
Geranial	281A	Phe	3.71	Hydrophobic
Geranial	162 A	Ala	3.99	Hydrophobic
Geranial	281 A	Phe	3.73	Hydrophobic
Geranial	297 A	Arg	3.53	Hydrophobic
Geranial	598 B	Lys	3.65	Hydrophobic
Geraniol	257B	Lys	4.00	Hydrophobic
Geraniol	281A	Phe	3.74	Hydrophobic
Geraniol	282A	Gln	2.38	Hydrogen bonds

Geraniol	309A	Asn	3.74	Hydrophobic
Germacrene D	261B	Thr	3.11	Hydrophobic
Germacrene D	330A	Val	3.77	Hydrophobic
Isopulegol	257B	Lys	3.79	Hydrophobic
Isopulegol	278A	Glu	1.84	Hydrogen bonds
Isopulegol	281A	Phe	3.75	Hydrophobic
Isopulegol	312A	Arg	2.53	Hydrogen bonds
Limonene	162A	Ala	3.72	Hydrophobic
Limonene	257B	Lys	3.56	Hydrophobic
Linalol	329A	Pro	3.77	Hydrophobic
Linalol	330A	Val	1.95	Hydrogen bonds
Linalol	332A	Tyr	3.58	Hydrophobic
Linalyl acetate	162 A	Ala	3.95	Hydrophobic
Linalyl acetate	222 A	Thr	3.73	Hydrophobic
Linalyl acetate	281A	Phe	3.51	Hydrophobic
Linalyl acetate	598B	Lys	3.64	Hydrophobic
Linalyl acetate	297A	Arg	5.14	Salt bridges
Neral	162A	Ala	3.68	Hydrophobic
Neral	257B	Lys	3.64	Hydrophobic
Neral	281A	Phe	3.85	Hydrophobic
Neral	329A	Pro	3.85	Hydrophobic
Neral	330A	Val	3.85	Hydrophobic
Neral	602B	Thr	3.86	Hydrophobic
Neral	601B	Gly	2.73	Hydrogen bonds
Terpenyl acetate	179B	Tyr	3.06	Hydrogen bonds
Terpenyl acetate	183B	Leu	3.90	Hydrophobic
Terpenyl acetate	317B	Val	3.73	Hydrophobic
Terpenyl acetate	319B	Asn	2.85	Hydrogen bonds
Terpenyl acetate	322B	Asp	3.17	Hydrogen bonds
Terpenyl acetate	324B	Lys	3.87	Hydrophobic
Terpenyl acetate	598B	Lys	3.57	Hydrophobic
Terpenyl acetate	312A	Arg	2.46	Hydrogen bonds
α-cadinene	261B	Thr	3.47	Hydrophobic
α-cadinene	329A	Pro	3.57	Hydrophobic
α-cadinene	330A	Val	3.61	Hydrophobic
α-cadinene	332A	Tyr	3.77	Hydrophobic
β-Myrcene	222A	Thr	3.81	Hydrophobic
β-Myrcene	223A	Ile	3.91	Hydrophobic
β-Myrcene	297A	Arg	3.79	Hydrophobic
β-Myrcene	309A	Asn	3.82	Hydrophobic
β-Myrcene	598B	Lys	3.82	Hydrophobic
γ-cadinene	329A	Pro	3.66	Hydrophobic
Amphotericin B*	289A	Lys	3.78	Hydrophobic
Amphotericin B*	393B	Trp	3.46	Hydrophobic
Amphotericin B*	622B	Phe	3.64	Hydrophobic
Amphotericin B*	627B	Thr	3.52	Hydrophobic

Amphotericin B*	394B	Arg	2.08	Hydrogen bonds
Amphotericin B*	566B	Tyr	2.50	Hydrogen bonds
Amphotericin B*	586B	Asp	2.20	Hydrogen bonds
Amphotericin B*	587B	Ser	2.96	Hydrogen bonds
Amphotericin B*	588B	Ala	2.71	Hydrogen bonds
Amphotericin B*	625B	Tyr	3.10	Hydrogen bonds
Amphotericin B*	627B	Thr	2.87	Hydrogen bonds
Amphotericin B*	50A	Lys	4.57	Salt bridges
Amphotericin B*	225A	Tyr	3.61	Hydrophobic
Amphotericin B*	250A	Lys	3.84	Hydrophobic
Amphotericin B*	250A	Lys	4.27	Salt bridges
Amphotericin B*	232A	Arg	3.20	Hydrogen bonds
Amphotericin B*	299A	Arg	5.22	Salt bridges
Amphotericin B*	300A	Glu	2.23	Hydrogen bonds
Fluconazole*	671B	Val	3.44	Hydrophobic
Fluconazole*	202A	Asn	2.57	Hydrogen bonds
Fluconazole*	205A	Gly	3.20	Hydrogen bonds
Fluconazole*	261A	Thr	2.93	Hydrogen bonds
Fluconazole*	522B	Ser	2.40	Hydrogen bonds
Fluconazole*	619B	Glu	3.23	Halogen bonds
Fluconazole*	257B	Lys	3.93	Hydrophobic
Fluconazole*	284A	Tyr	3.03	Hydrogen bonds

*Comparative ligands used in molecular docking testing.

Among the major ligands identified in the chromatogram, it was possible to observe that ligands linalyl acetate, geranial, β-myrcene, Neral, terpinyl acetate, neryl acetate, Citronelal, Elemol and Geraniol occupied the comparative binding site to Fluconazole. However, the ligands showed free energy values outside the ideality spectrum, that is, -6.0 kcal/mol (Shityakov and Förster, 2014), in relation to Fluconazole itself (-6.1 kcal/mol).

The ligands showed interactions in common with the Lys 257 residues, with calculated distances of 3.52 Å, 3.71 Å and 3.47 Å for Citronelal, Elemol and Geraniol ligands (respectively), which characterize interactions of moderate strength (Imberty; Hardman; Carver, 1991), as well as occurs with the comparative Fluconazole (3.51 Å), where the interactions are predominantly hydrophobic. The linalyl acetate ligand exhibited four hydrophobic interactions, with residues Ala 162 A (3.95 Å), Thr 222 A (3.73 Å), Phe 281 A (3.51 Å and 3.49 Å), Lys 598 B (3.64 Å) and a bridge salt with the residue Arg 297 A (5.14 Å). The terpinyl acetate molecule registered a strong hydrogen bond and two hydrophobic interactions with the residue Arg 312 A (2.46 Å) and Lys 598 B (3.57 Å and 3.48 Å), respectively. The ligands geranial and β-myrcene registered only hydrophobic interactions with residues Ala 162 A (3.99 Å), Phe 281 A (3.73 Å), Arg 297 A (3.53 Å) and Lys 598 B (3. 65 Å)

and Thr 222 A (3.81 Å), Ile 223 A (3.91 Å), Arg 297 A (3.79 Å), Asn 309 A (3.82 Å), Lys 598 B (3, 82 Å and 3.71 Å), respectively. Four hydrophobic interactions and one strong hydrogen bond were observed for the Neral ligand with Pro 329 A (3.85 Å and 3.73 Å), Val 330 A (3.85 Å), Thr 602 B (3.86 Å) and Gly 601 B (2.73 Å) residues, respectively. The amphotericin B ligand recorded five hydrophobic interactions and a moderate hydrogen bond with Lys 289 A (3.78 Å), Trp 393 B (3.46 Å), Phe 622 B (3.64 Å and 3.47 Å), Thr 627 B (3.52 Å) and Tyr Residues 625 B (3.10 Å), respectively. Furthermore, seven strong hydrogen bonds were observed for the same ligand, with Arg 394 B (2.08 Å), Tyr 566 B (2.67 Å, 2.27 Å and 2.50 Å), Asp 586 B (2.20 Å), Ser 587 B (2.96 Å Å), Ala 588 B (2.71 Å) and Thr 627 B (2.87 Å). Regarding the fluconazole ligand, this molecule exhibited a hydrophobic interaction and a halogen bond with Val 671 B (3.44 Å) and Glu 619 B (3.23 Å), respectively. In addition, five hydrogen bonds were recorded (four strong and one moderate), with residues Asn 202 A (2.57 Å), Ser 522 B (2.40 Å), Thr 261 A (3.02 Å and 2 .93 Å) and Gly 205 A (3.20 Å), respectively. Therefore, the interactions observed between the analyzed ligands with the SAP5 protein explain the ΔGbind values observed in Table 5.

The strong hydrogen bonds observed in terpinyl acetate-SAP5 and fluconazole-SAP5 complexes may contribute about 2.63 to 14.33 kcal/mol of free binding energy (Steed and Atwood, 2022) because the intermolecular force is the strongest and most influential in molecular recognition (Dong and Davis, 2020). Furthermore, Figure 2 shows the binding site between the ligands analyzed with the SAP5 protein. On the other hand, none of the phytochemicals showed affinity for the catalytic site of the ligand Amphotericin B, which showed excellent affinity with the receptor in these tests (-7.6 kcal/mol). In addition, RMSD values lower than 2.0 Å infer that the ligands have a free energy within the reliability standard, but that they have low affinity with the SAP5 receptor.

In relation to simulations for the minor ligands identified in the chromatogram, it was possible to observe that the ligands Eugenol, Isopulegol and Limonene showed low affinity with the SAP5 receptor and occupy the same catalytic site of the comparative ligand Fluconazole, where the free energy values are higher to -6.0 kcal/mol, although they are within the mean squared deviation range, ie RMSD < 2.0 Å. Furthermore, the compounds showed interactions in common with the Phe 281 residue (except for Limonene), with distances in the order of 3.79 Å and 3.75 Å for the ligands Eugenol and Isopulegol (respectively), which characterize interactions of moderate strength (Table 5).

Furthermore, it is possible to point out that the α-cadinene, Caryophyllene oxide, and Germacrene D ligands occupy an allosteric site with hydrophobic interactions in common with the residues of Thr 261B, where distances > 3.11 Å characterize interactions of moderate strength, and with the residue of Val 330A, where distances > 3.55 Å characterize weak interactions (Table 5), including the possibility of an H-bond interaction between the ligand Caryophyllene oxido, by the presence of an epoxide group, and the residue of Gly 205B, where the distance on the order of 2.80 Å characterizes a strong hydrogen interaction (Table 5). It is noteworthy that the compounds showed, at the same time, excellent affinity energy with the SAP5 receptor, that is, with ΔG values lower than -6.0 kcal/mol, as well as RMSD values that guarantee the statistical reliability of the test (RMSD < 2.0 Å), indicating that they can act as SAP5 modulators by synergism associated with the ligand Fluconazole (Figure 2).

Conclusion

Essential oils of *Cymbopogon citratus* and *Cymbopogon nardus* are rich in oxygenated monoterpenes with a higher content of geranial and neral for EOCC and citronellal, geraniol and citronellol for EOCN. Phytochemical substances which are associated with the antifungal properties of these oils from *Cymbopogon* spp. Both essential oils have antimicrobial against *Candida albicans* strains, in planktonic and biofilm form, in non-toxic concentrations. In addition to presenting synergistic potential when combined with amphotericin B, thus raising the possibility of formulations that use both substances together to a more promising therapy, with less toxicity to the patient.

Regarding to the study of molecular docking, we observed a low interaction between the major constituents of both essential oils with the SAP-5 enzyme, however the minor components of EOCN, such as α-Cadinene, Caryophyllen oxide and Germacrene D, approved a better front to the SAP5, it showed interaction in different binding sites of the standard drugs, thus supporting a synergistic effect.

Thus, *C. citratus* and *C. nardus* have potential against *C. albicans* in planktonic form, carefully or together with amphotericin B, and at concentrations of 2x the MIC, they have inhibitory potential to the *Candida* biofilm. Nevertheless, there is a need for tests to determine molecular dynamics, verifying the stability of the formation of complexes between these ligands and the SAP5 receptor, and further studies are needed to understand the mechanism of action of this essential oil, through preclinical and clinical trials for greater safety and

measurement of the effectiveness of possible therapies and subsequent production of pharmaceutical formulations.

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5 CONCLUSÕES

- O óleo essencial de *Cymbopogon citratus* e *Cymbopogon nardus* apresentou rendimento e densidade compatível com a literatura, ambos apresentando monoterpenos oxigenados como constituintes majoritários;
- Foi observado que OECC e OECN apresentaram atividade anti-*Candid*a na forma planctônica e biofilme. Além de efeito sinérgico com Anfotericina B contra os microrganismos testados;
- Em relação aos ensaios de citotoxicidade se determinou que o óleo de *C. nardus* e *C. citratus* apresentaram toxicidade acima das concentrações inibitórias mínimas;
- Já na determinação de interação de constituintes dos óleos essenciais e a proteína SAP-5, observou-se interação intermolecular, contudo ainda necessitando de estudos para determinar a estabilidade entre as ligações observadas;
- Constatou-se que ambos OE's apresentam potencial de bioprospecção e aproveitamento farmacológico. Contudo, ainda existe a necessidade de ensaios em modelos animais e clínicos para assegurar uso seguro e eficaz dessas substâncias e seus derivados.

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ANEXO A – Artigo publicado

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Cymbopogon sp. from ethnobotany to antimicrobial: an integrative review

Cymbopogon sp. da etnobotânica ao antimicrobiano: uma revisão integrativa

Cymbopogon sp. de la etnobotánica a los antimicrobianos: una revisión integrative

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Abstract

Considering the broad potential of the genus *Cymbopogon*, here we present a systematic literature survey on it's antimicrobial potential. This is a review, articles from the Scielo and PubMed platforms. The articles surveyed were published between 2015 and 2020, with the theme of microbiology, including ethnobotanical studies, literature reviews, *in vitro*, *in vivo*, reports of clinical trials. Works outside the selected period, duplicate articles, and those only reporting infections of plants by the microorganism were excluded. 98 studies were selected, 74% found in PubMed and 26% in Scielo. Of this total, 21% were ethnobotanical/ethnopharmacology or literature reviews, most of them reporting the use of infusions of the species *C. citratus* (DC.) Stapf. Moreover, in 57% of the studies, survey respondents did not report antimicrobial use. In relation to 79% of the experimental studies, it was observed that 77% reported total inhibition of microbial growth, 3% indicated moderate growth inhibition, 4% low growth inhibition and 5% reported no inhibition. Among the microbial species analyzed were *Escherichia coli*, *Pseudomonas aeruginosa*, *Borrelia burgdorferi*, *Candida albicans*, *Salmonella enterica* and *Saccharomyces cerevisiae*. In addition, 5% of the articles reported antiviral activity, 5% parasitic control, 1% preventive action against contamination by mesophilic microorganisms. Although the population is not aware of the antimicrobial activity of *Cymbopogon* sp., studies have demonstrated its antimicrobial potential, thus the extracts of this genus can be an alternative for use in folk medicine as well as a source of new drugs with antimicrobial action.

Keywords: Plants; Medicinal; Phytotherapy; Ethnopharmacology; Poaceae; Lemongrass; Microbiology.

Resumo

Considerando o amplo potencial do gênero *Cymbopogon*, apresentamos aqui um levantamento sistemático da literatura sobre seu potencial antimicrobiano. Trata-se de uma revisão, artigos das plataformas Scielo e PubMed. Os artigos pesquisados foram publicados entre 2015 e 2020, com a temática da microbiologia, incluindo estudos etnobotânicos, revisões de literatura, *in vitro*, *in vivo*, relatórios de ensaios clínicos. Foram excluídos trabalhos fora do período selecionado, artigos duplicados e aqueles que relatavam apenas infecções de plantas pelo microrganismo. Foram selecionados 98 estudos, 74% encontrados no PubMed e 26% no Scielo. Desse total, 21% eram