UNIVERSIDADE FEDERAL DO CEARÁ – UFC
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIAS DA SAÚDE

AMANDA DE LIMA BARROS

AVALIAÇÃO DA ATIVIDADE ANTIFÚNGICA, *MOLECULAR DOCKING* E CITOTOXICIDADE DO ÓLEO ESSENCIAL DE *Vitex gardneriana* Shauer FRENTE CEPAS DE *Candida albicans*

SOBRAL
2025

AMANDA DE LIMA BARROS

AVALIAÇÃO DA ATIVIDADE ANTIFÚNGICA, *MOLECULAR DOCKING* E CITOTOXICIDADE DO ÓLEO ESSENCIAL DE *Vitex gardneriana* Shauer FRENTE CEPAS DE *Candida albicans*

Dissertação apresentada ao Mestrado em Ciências da Saúde da Universidade Federal do Ceará (UFC) como requisito parcial para obtenção do Título de Mestre em Ciências da Saúde. Área de concentração: Biologia molecular e de micro-organismos.

Orientadora: Profa. Dra. Raquel Oliveira dos Santos Fontenelle.
Coorientadora: Profa. Dra. Andréa Maria Neves.

SOBRAL

2025

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Sistema de Bibliotecas
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

L696a Lima Barros, Amanda.
Avaliação da atividade antifúngica, molecular docking e citotoxicidade do óleo essencial de Vitex gardneriana Schauer frente cepas de Candida albicans / Amanda Lima Barros. – 2025.
80 f. : il. color.

Dissertação (mestrado) – Universidade Federal do Ceará, Campus de Sobral, Programa de Pós-Graduação em Ciências da Saúde, Sobral, 2025.
Orientação: Profa. Dra. Raquel Oliveira dos Santos Fontenelle.
Coorientação: Profa. Dra. Andréa Maria Neves.

1. Bioinformática; 2. Sinergismo; 3. Microdiluição; 4. Óleo Essencial; 5. Candidemias. I. Título.
CDD 610

AMANDA DE LIMA BARROS

AVALIAÇÃO DA ATIVIDADE ANTIFÚNGICA, *MOLECULAR DOCKING* E CITOTOXICIDADE DO ÓLEO ESSENCIAL DE *Vitex gardneriana* Shauer FRENTE CEPAS DE *Candida albicans*

Dissertação apresentada ao Mestrado em Ciências da Saúde da Universidade Federal do Ceará (UFC) como requisito parcial para obtenção do Título de Mestre em Ciências da Saúde. Área de concentração: Biologia molecular e de micro-organismos.

Aprovada em: 30/07/2025

BANCA EXAMINADORA

Prof. Dra. Raquel Oliveira dos Santos Fontenelle - Orientadora
Universidade Estadual Vale do Acaraú – (UVA)

Prof. Dr. Francisco César Barroso Barbosa – 1º Examinador
Universidade Federal do Ceará – (UFC)

Dra. Francisca Lidiane Linhares de Aguiar
Universidade Estadual Vale do Acaraú – (UVA)

A Deus por seu amor e dissernimento consedidos. Aos meus pais, Ângela Maria Maciel de Lima Barros (*In memoriam*) e Manoel Lucilanio Barros por todo amor e doação. A minha filha Yasmin Barros Boriz por ser luz em minha vida.

Dedico.

AGRADECIMENTOS

Toda honra e glória a Deus Pai todo poderoso por todo seu infinito amor que me amparou e a Nossa Senhora de Fátima por sua piedosa intercessão. Agradeço incansavelmente a minha família. À minha amada Yasmin Barros Boriz, minha filhinha, agradeço por ser meu ponto de paz, amor e doçura. Meu amor por você me dá força e coragem. A minha amada mãe, Ângela Maria Maciel (*In memoriam*), por sempre ter me incentivado a estudar, por ser minha maior inspiração, fonte inesgotável de amor, cuidado e dedicação. Suas orações foram ouvidas, nosso sonho está se realizando. Ao meu amado pai, Manoel Lucilanio Barros, seus conselhos, incentivo constante e todo amor dedicado a mim foram fundamentais para que eu me mantivesse firme no percurso. A minha irmã, Alana Maria de Lima Barros, agradeço por todo incentivo, zelo e por ser inspiração.

Agradeço imensamente a profa. Dra. Raquel Oliveira dos Santos Fontenelle por todo apoio, conselhos, cuidado, dedicação e acolhimento, especialmente nos momentos mais desafiadores. Sua orientação foi um fator determinante para o sucesso desta etapa e para além dela. À Dra. Andrea Maria Neves, agradeço por sua coorientação, disponibilidade em estar sempre pronta para auxiliar, sanar dúvidas e direcionar para o melhor percurso. Sua calma, firmeza e cuidado foram essenciais.

Ao prof. Dr. Francisco César Barroso Barbosa por sua disponibilidade e atenção. À Dra. Francisca Lidiane Linhares por todo apoio, direcionamento, orientações e auxílio no desenvolvimento das pesquisas científicas. Suas contribuições foram importantíssimas.

Ao Programa de Pós-Graduação em Ciências da Saúde agradeço pela oportunidade de acesso à educação e desenvolvimento de pesquisas criteriosas e potencialmente transformadoras. Aos professores que com dedicação permitiram que esta experiência fosse linda e enriquecedora.

Aos meus colegas de mestrado e de laboratório (Laboratório de Microbiologia – LABMIC) agradeço por serem ombro amigo nos momentos desafiadores tornando o processo mais leve. Em especial agradeço aos amigos, Prof. Dr. Elnatan Bezerra, Eduarda Loiola, Ingrid Fernandes, Matheus Rocha, Emmanuel Marinho e Márcia Marinhoque pelo apoio e colaboração.

“Quanto mais eu estudo a natureza, mais me maravilho com a obra do Criador.”

(Louis Pasteur)

RESUMO

As infecções causadas por *Candida albicans*, fungo leveduriforme de importância clínica têm se tornado um problema crescente de saúde pública, especialmente devido ao aumento da resistência aos antifúngicos disponíveis. Frente a esse cenário, torna-se urgente a busca por alternativas terapêuticas mais eficazes, acessíveis e com menos efeitos adversos. O Brasil, por sua vez, possui ampla biodiversidade vegetal, incluindo espécies utilizadas por comunidades tradicionais, como *Vitex gardneriana* Schauer, planta aromática e medicinal com reconhecidas propriedades antimicrobianas, antioxidantes e larvicidas. Embora promissora, essa espécie ainda carece de estudos frente a cepas clínicas de *C. albicans*. Diante do exposto, propõe-se com esta pesquisa analisar o potencial antifúngico, sinérgico e composição química do óleo essencial obtido da espécie botânica *V. gardneriana* frente a cepas clínicas isoladas de amostras provenientes do Hospital Santa Casa de Misericórdia de Sobral – CE, bem como, obter o perfil de citotoxicidade e determinar a interação dos constituintes do óleo da *V. gardneriana* com proteínas do patógeno *C. albicans*. A atividade antifúngica foi avaliada por microdiluição em caldo, apresentando uma CIM de 5 mg/mL para todas as cepas. Um efeito sinérgico foi observado quando combinado com Anfotericina B, reduzindo a concentração inibitória do OE em mais de 30 vezes. A atividade antioxidante (método DPPH) apresentou um IC50 de 85,86 mg/mL, enquanto os ensaios hemolíticos em eritrócitos humanos apresentaram um IC50 de 20 mg/mL. O *molecular docking* revelou maior afinidade dos compostos de EO com Sap5 (-7,3 kcal/mol) do que com Als3 (-6,2 kcal/mol), com alguns ligantes (p. ex., 1-epi-cubenol, óxido de cariofileno) ligando-se a regiões diferentes da Anfotericina B. O óleo essencial da *Vitex gardneriana* Shauer (OEVG) mostrou-se como um importante achado para a ciência, pois, apresentou atividade antifúngica, efeito sinérgico com Anfotericina B, baixa citotoxicidade e atividade antioxidante. Além disso, os dados encontrados sugerem um possível efeito sinérgico dos ligantes no perfil de controle da Anfotericina B. Desse modo, é essencial a realização de ensaios *In vivo* e clínicos a fim de confirmar a eficácia e a segurança farmacológica dos compostos analisados.

Palavras-Chave: Bioinformática; Sinergismo; Microdiluição; Óleo Essencial; Candidemias.

ABSTRACT

Infections caused by Candida albicans, a clinically important yeast-like fungus, have become a growing public health problem, particularly due to increasing resistance to available antifungals. Given this scenario, the search for more effective, affordable, and less adverse therapeutic alternatives is urgently needed. Brazil, in turn, boasts a broad plant biodiversity, including species used by traditional communities, such as Vitex gardneriana Schauer, an aromatic and medicinal plant with recognized antimicrobial, antioxidant, and larvicidal properties. Although promising, this species still lacks studies against clinical strains of C. albicans. Given the above, this study aims to analyze the antifungal and synergistic potential and chemical composition of the essential oil obtained from the botanical species V. gardneriana against clinical strains isolated from samples from the Santa Casa de Misericordia Hospital in Sobral, Ceará. It also aims to obtain the cytotoxicity profile and determine the interaction of the constituents of V. gardneriana oil with proteins from the pathogen C. albicans. Antifungal activity was assessed by broth microdilution, yielding an MIC of 5 mg/mL for all strains. A synergistic effect was observed when combined with amphotericin B, reducing the inhibitory concentration of the essential oil by more than 30-fold. Antioxidant activity (DPPH method) showed an IC50 of
85.86 mg/mL, while hemolytic assays in human erythrocytes showed an IC50 of 20 mg/mL. *Molecular docking* revealed greater affinity of EO compounds with Sap5 (-7.3 kcal/mol) than with Als3 (-6.2 kcal/mol), with some ligands (e.g., 1-epi-cubenol, caryophyllene oxide) binding to different regions of Amphotericin B. The essential oil of Vitex gardneriana Shauer (OEVG) proved to be an important finding for science, as it presented antifungal activity, a synergistic effect with Amphotericin B, low cytotoxicity, and antioxidant activity. Furthermore, the data found suggest a possible synergistic effect of the ligands on the control profile of Amphotericin
B. Therefore, it is essential to carry out in vivo and clinical trials in order to confirm the efficacy and pharmacological safety of the compounds analyzed.

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

LISTA DE FIGURAS

RIVISÃO DE LITERATURA

ARTIGO DE REVISÃO

ARTIGO EXPERIMENTAL

LISTA DE TABELAS

ARTIGO DE REVISÃO

ARTIGO EXPERIMENTAL

LISTA DE ABREVIATURAS E SIGLAS

Als3	*Agglutinin-like sequence*
AMB	Anfotericina B
ATCC	*American type culture collection*
CFM	Concentração fungicida mínima
CIF	Concentração inibitória fracionada
CIM	Concentração inibitória mínima
CLSI	*Clinical and Laboratory Standards Institute*
DPPH	2,2-difenil-1-picril-hidrazila
FAEX	Fazenda Experimental da Universidade Estadual Vale do Acaraú
GC-MS	Cromatografia Gasosa acoplada à Espectrometria de Massas
HUVA	Herbário da Universidade Estadual Vale do Acaraú
ICIF	Índice de Concentração Inibitória Fracionada
IV%	Índice de varredura em porcentagem
IC_{50}	Concentração inibitória em 50%
LABMIC	Laboratório de Microbiologia da Universidade Estadual Vale do Acaraú
LGB	*Lamarkian Genetic Algorithm*
MMFF	*Merck Molecular Force Field*
OE	Óleo essencial
OEVG	Óleo essencial de *Vitex gardneriana*
PCR	Reação em cadeia da polimerase
PDB	*Protein Data bank*
RMSD	*Root mean square deviation*
RPMI	*Roswell park memorial institute*

SAP-5 *Secreted aspartic proteases*

SDA Sabouraud dextrose ágar

UFC Universidade Federal do Ceará

UNILAB Universidade da Integração Internacional da Lusofonia Afro-Brasileira

UVA Universidade Estadual Vale do Acaraú

WHO *World Health Organization*

LISTA DE SÍMBOLOS

°C	Grau célsius
%	Porcentagem
Å	Angstrom
µL	Microlitro
µM	Micrometro
cm	Centímetro
M	Massa Molar
mg	Miligrama
ml	Mililitro
mm	Milimetro
UFC	Unidade Formadora de Colõnia
Kg	Kilograma
S	Sul
W	Oeste
p/p	Peso do óleo essencial/peso da materia vegetal seca
v/v	Volume soluto/volume solução

SUMÁRIO

1 INTRODUÇÃO

As plantas medicinais são amplamente utilizadas por comunidades em todo o mundo para fins terapêuticos, inclusive no tratamento de infecções. Essa prática ancestral é especialmente comum no Brasil, onde fatores como a vasta biodiversidade vegetal e a valorização dos saberes populares, transmitidos entre gerações, favorecem o uso tradicional desses recursos (Ribeiro *et al*., 2018). A partir da investigação desses conhecimentos empíricos, pesquisas científicas vêm sendo conduzidas, revelando compostos naturais com potencial terapêutico frente a infecções microbianas (Monteiro *et al*., 2015; Morais *et al*., 2020; Silva *et al*., 2022).

Dentre as infecções microbianas, destaca-se o crescimento expressivo das infecções fúngicas, impulsionado, entre outros fatores, pelo uso prolongado e em altas doses de antifúngicos convencionais. Esse cenário favorece o surgimento de cepas resistentes, reduzindo as chances de sucesso do tratamento. Diante disso, torna-se urgente a busca por alternativas terapêuticas mais eficazes e com menos efeitos adversos, sendo os produtos de origem natural, como os derivados de plantas medicinais, uma das opções mais promissoras (Talapko *et al*., 2021; Carrillo-Muñoz *et al*., 2014).

A levedura *Candida albicans* é reconhecida como o principal agente etiológico de infecções fúngicas nosocomiais, sendo responsável por aproximadamente 70% dos casos em âmbito global (Talapko *et al*., 2021). Índivíduos imunocomprometidos, como pacientes com AIDS, transplantados ou com neoplasias hematológicas, apresentam maior suscetibilidade a essas infecções oportunistas (Carrillo-Muñoz *et al*., 2014). Diante desse contexto, cresce o interesse em estratégias terapêuticas alternativas. A combinação de fármacos com óleos essenciais tem se destacado por potencializar a atividade antimicrobiana e reduzir a probabilidade de desenvolvimento de resistência microbiana (Sztukowska *et al*., 2018).

No campo da bioprospecção de produtos naturais, a bioinformática estrutural tem se revelado uma ferramenta essencial. Técnicas como o *molecular docking* permitem prever a interação de compostos bioativos com alvos moleculares específicos, auxiliando na triagem e no reposicionamento de moléculas com potencial terapêutico. Utilizando bases de dados e softwares como o AutoDock Vina, é possível estimar a afinidade de compostos presentes em óleos essenciais com proteínas de patógenos, viabilizando a identificação de candidatos promissores de forma rápida e com menor custo (Huey, *et al*., 2012).

A flora da Caatinga é reconhecida por sua riqueza em metabólitos secundários e princípios ativos, sendo diversas espécies desse bioma alvo de estudos científicos com foco em sua aplicação medicinal. Esses compostos vegetais atuam como mecanismos de defesa e são

influenciados por fatores como genótipo da espécie, estratégia para atrair dispersores, localização geográfica, sazonalidade, ritmo circadiano, estresse hídrico e proteção contra exposição a herbivoria (Souza *et al*., 2022).

Nesse contexto, o gênero *Vitex*, o maior da família *Lamiaceae*, com cerca de 250 espécies — reúne arbustos e pequenas árvores amplamente distribuídas em regiões tropicais. No semiárido brasileiro, destaca-se *Vitex gardneriana* Schauer, uma espécie nativa frequentemente encontrada próxima a cursos d'água e tradicionalmente utilizada na medicina popular para o alívio de inflamações e dores. Estudos recentes demonstram que *V. gardneriana* apresenta atividades antimicrobiana, antioxidante e larvicida, reforçando seu potencial como fonte de novos agentes terapêuticos (Barreto; Almeida; Filho, 2021; Morais *et al*., 2020; Silva *et al*., 2019).

Diante do exposto, propõe-se com esta pesquisa analisar o potencial antifúngico, sinérgico e composição química do óleo essencial obtido da espécie botânica *V. gardneriana* frente a cepas clínicas isoladas de amostras provenientes do Hospital Santa Casa de Misericórdia de Sobral – CE, bem como, obter o perfil de citotoxicidade e determinar a interação dos constituintes do óleo da *V. gardneriana* com proteínas do patógeno *C. albicans*.

2 REVISÃO DE LITERATURA

2.1 Plantas Medicinais

O uso de plantas medicinais representa uma das práticas terapêuticas mais antigas da humanidade, sendo adotado por diferentes culturas para o tratamento e prevenção de enfermidades. No Brasil, esse conhecimento é fortemente enraizado na tradição popular e nas práticas de comunidades rurais, indígenas e quilombolas, que se utilizam de diferentes espécies vegetais com finalidades medicinais, transmitindo esse saber por gerações (Ribeiro *et al*., 2018). A diversidade de biomas, resulta em uma flora rica e variada, tornando o Brasil uma das maiores reservas de biodiversidade do mundo (Pereira, *ei al*., 2025).

A ampla distribuição geográfica das espécies medicinais no território brasileiro e as espécies introduzidas pelas comunidades possibilitam a existência de distintos perfis fitoquímicos. Conferindo diferentes composições químicas e metabólitos secundários, os quais são produzidos pelas plantas como forma de defesa contra predadores, estresse ambiental ou patógenos. Tais compostos apresentam relevante potencial farmacológico, incluindo atividades antimicrobiana, anti-inflamatória, antioxidante, antiparasitária e antitumoral (Pereira, *et al*., 2025; Yang, *et al*., 2018).

A diversidade fitoquímica encontrada nas plantas brasileiras inclui sesquiterpeos, alcaloides, flavonoides, terpenoides, taninos, cumarinas, e saponinas, muitos dos quais vêm sendo explorados como protótipos para o desenvolvimento de novos medicamentos (Yang *et al*., 2018). Essas substâncias, quando isoladas, purificadas ou utilizadas em extratos e óleos, demonstram eficácia em diferentes modelos experimentais, tornando-se alvos da bioprospecção farmacêutica (Hernandez *et* al., 2025). Ressalta-se que, além do valor terapêutico, o cultivo e aproveitamento de espécies medicinais também podem promover desenvolvimento econômico sustentável e fortalecimento das práticas integrativas no Sistema Único de Saúde (SUS) (Brasil, 2017).

A regulamentação do uso de plantas medicinais no Brasil ocorreu apenas em 2006, com a promulgação da Portaria nº 971/2006 e do Decreto nº 5.813/2006, que instituíram, respectivamente, a Política Nacional de Práticas Integrativas e Complementares (PNPIC) e a Política Nacional de Plantas Medicinais e Fitoterápicos. Tais políticas estimularam a integração das práticas de atenção primária do SUS, a pesquisa científica, a formação profissional e a valorização da biodiversidade nacional como fonte estratégica para o desenvolvimento de novos fitoterápicos (Brasil, 2006).

Neste contexto, a Caatinga, bioma exclusivamente brasileiro, também tem despertado a

atenção da comunidade científica. Apesar de historicamente negligenciada em comparação a outros biomas, sua flora possui uma grande variedade de espécies adaptadas às condições adversas do semiárido, muitas das quais produzem metabólitos secundários com alto valor biotecnológico, os quais podem ser encontrados nos óleos essenciais produzidos pelas espécies botânicas (Sá-Filho *et al*., 2022). A resiliência dessas plantas frente ao estresse hídrico e à radiação intensa favorece a síntese de compostos com propriedades antioxidantes e antimicrobianas, características desejáveis na busca por novos agentes terapêuticos.

2.2 *Vitex* sp.

Entre as famílias botânicas com representatividade no semiárido nordestino, destaca-se a *Lamiaceae*, cujos gêneros apresentam ampla distribuição e uso medicinal consolidado. O gênero *Vitex* desponta como um dos mais promissores em termos de potencial farmacológico. Com espécies distribuídas majoritariamente em regiões tropicais e subtropicais, frequentemente empregados na medicina tradicional para o tratamento de inflamações, infecções, distúrbios hormonais e dores (Das *et al*., 2022; Islam *et al*., 2024).

As espécies desse gênero incluem, predominantemente, arbustos e árvores de médio porte, caracterizadas por folhas compostas palmadas, inflorescências terminais vistosas e frutos geralmente drupáceos (Zaki *et al*., 2020). Tradicionalmente, diversas espécies de *Vitex* têm sido utilizadas na medicina popular em diferentes culturas, especialmente no tratamento de desordens inflamatórias, doenças infecciosas, febres, dores reumáticas, disfunções hormonais e como agentes sedativos (Das *et al*., 2022).

A literatura científica vem corroborando o uso tradicional de espécies do gênero *Vitex*, demonstrando uma variedade de propriedades farmacológicas associadas à presença de metabólitos secundários com elevada atividade biológica. Entre os principais compostos isolados destacam-se os sesquiterpenos presente nos óleos essenciais (Morais *et al*., 2020).

Dentre as espécies mais estudadas internacionalmente, *Vitex agnus-castus* é amplamente utilizada por seus efeitos sobre o sistema endócrino feminino, sendo utilizada no tratamento da síndrome pré-menstrual, distúrbios menstruais e infertilidade. Já *Vitex negundo*, comum no sul e sudeste asiático, é explorada por suas propriedades anti-inflamatórias, analgésicas e antissépticas (Mendes *et al*., 2022; Smrity *et al*., *2019*).

No Brasil, apesar do potencial fitoterápico promissor, o gênero ainda é pouco explorado cientificamente em comparação com outras regiões do mundo. No entanto, *V. gardneriana* vêm sendo alvo de investigações recentes, sobretudo por apresentar ações farmacológicas relevantes e por estar inserida em contextos tradicionais de uso popular (Silva *et al*., 2019; Vale *et al*.,

2019).

2.2.1 *Vitex gardneriana* Schauer

A espécie *V. gardneriana* Schauer, popularmente conhecida como Jaramataia, é endêmica do Nordeste brasileiro e adaptada às margens de rios e áreas úmidas da Caatinga, tem sido utilizada popularmente para o alívio de dores e processos inflamatórios. O uso tradicional de *V. gardneriana* pela população está associado ao tratamento de processos inflamatórios, dores musculares e contusões, sendo geralmente aplicada na forma de infusão ou decocção das folhas. Essas práticas populares, transmitidas oralmente, estimulam o interesse da comunidade científica na investigação dos potenciais farmacológicos da espécie (Morais *et al*., 2020; Silva *et al*., 2019).

Figura 1 – Exemplar de *Vitex gardneriana* Shauer.

Fonte: Elaborado pela autora (2024).

Considerando a crescente demanda por compostos naturais com ação antifúngica e menor toxicidade, especialmente frente a microrganismos de importância médica, como por exemplo, *C. albicans*, torna-se essencial aprofundar o conhecimento científico sobre essa espécie e seus constituintes bioativos (Almeida *et al*., 2025).

O óleo essencial (OE) extraído das folhas de *V. gardneriana* apresenta elevado teor de sesquiterpenos, mais especificamente o trans-calameneno, o 6,9-guaiadieno e o óxido de cariofileno. Essas substâncias têm sido associadas a uma ampla gama de atividades biológicas, incluindo propriedades antifúngicas, anti-inflamatórias e antioxidantes (Pereira *et al*., 2018).

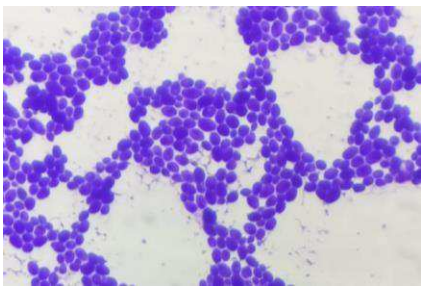
Figura 2 – Óleo essencial extraído das folhas de *V. gadneriana*

Fonte: Elaborado pela autora (2024).

2.3 *Candida albicans*

A levedura *C. albicans* (figura 3) , pertencente ao reino Fungi, filo Ascomycota, classe Saccharomycetes e família Saccharomycetaceae, é o principal agente causador de infecções fúngicas oportunistas em seres humanos (Campos *et al.,* 2020). Esse microrganismo é polimórfico, apresentando-se como blastoconídios ovalados (3–5 µm), pseudo-hifas ou hifas verdadeiras, uma estratégia adaptativa fundamental para sua disseminação e patogenicidade (Francimat *et al*., 2021). A parede celular é rica em quitina, β-glucanas e manoproteínas, já o ergosterol presente na membrana é um alvo frequente de fármacos antifúngicos, fator que confere a estes fungos resistência e capacidade de evadir a resposta imune do hospedeiro (Schaefer *et al*., 2021).

Figura 3 – Imagem do fungo *Candida albicans* coradas com cristal violeta



Fonte: Elaborado pela autora (2025).

Mais de 100 espécies compõem o gênero *Candida*, com destaque clínico às patógenas *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* e *C. auris*, sendo que *C. albicans* é a mais isolada, respondendo por 90–100% das infecções mucocutâneas e por 40-70%

das candidemias (Campos *et al*., 2020). Anualmente, estima-se que cerca de 250 mil casos de candidíase invasiva resultam em 50 mil óbitos globais, figurando entre as principais causas de sepse hospitalar (Schaefer *et al*., 2021).

A virulência de *C. albicans* resulta de diversos mecanismos que atuam sinergicamente para garantir sua adesão, invasão e evasão do sistema imune. Um dos principais fatores envolvidos nestes processos é a transição morfológica entre levedura e hifa, fundamental para a invasão tecidual e formação de biofilmes. Outro importante fator é a produção de adesinas com destaque para a glicoproteína Als3, essencial para a fixação em células epiteliais e substratos abióticos. Além disso, ela atua como invasina, promovendo a internalização do fungo em células humanas (Sztukowska *et al*., 2018).

Outro fator relevante é a secreção de enzimas hidrolíticas, como as aspartil proteases, especialmente a Sap5 ((*Secreted aspartyl proteinase 5*), que degrada proteínas do hospedeiro, facilita a invasão e favorece a captação de nutrientes como ferro. Complementando esse arsenal de virulência, a candidalysina, uma toxina peptídica produzida durante a fase, provoca lise celular e inflamação, sendo associada a formas graves de candidíase. Esses elementos tornam *C. albicans* um modelo relevante na pesquisa de infecções fúngicas (Gholam *et al*., 2022).

A morfologia, regulação genética, fatores de virulência e mecanismos de resistência de *C. albicans* fundamentam a escolha desta espécie como alvo deste estudo. Em especial, a avaliação da ação antifúngica do óleo essencial de *V. gardneriana*, seu potencial sinérgico com fármacos convencionais, a análise de citotoxicidade e estudos *in silico* por *molecular docking*, se apresentam como abordagens integradas promissoras para enfrentar eficazmente essa levedura patogênica de alta importância médica.

2.4 Terapia antifúngica

No panorama social vigente, uma das principais limitações no tratamento das infecções fúngicas, especialmente aquelas causadas por *C. albicans*, está relacionada à toxicidade dos antifúngicos disponíveis e ao aumento da resistência aos fármacos convencionais. Esta levedura se reproduz assexuadamente por brotamento e apresenta morfologia polimórfica, podendo assumir três formas distintas: blastoconídios, hifas e pseudo-hifas. (Nazarro *et al*., 2017; Powers *et al*., 2019; Pereira *et al*., 2021; Scalabrin *et al*., 2021).

A elevada toxicidade decorre, em grande parte, da semelhança estrutural entre células fúngicas e humanas, uma vez que ambas são eucarióticas, o que restringe a seletividade das drogas e amplia o risco de efeitos colaterais (Pereira *et al*.,2021; Silva *et al*., 2024).

Além disso, o número de classes farmacológicas com atividade efetiva contra leveduras é bastante restrito, o que favorece o surgimento de cepas multirresistentes. Dentre os principais antifúngicos disponíveis, destaca-se a Anfotericina B, um fármaco pertencente à classe dos poliênicos, cuja ação consiste em danificar a membrana celular dos fungos. Esse composto é obtido a partir de bactérias do gênero *Streptomyces* e é considerado o padrão-ouro no tratamento de infecções fúngicas sistêmicas graves. Sua ação ocorre por meio da ligação ao ergosterol presente na membrana fúngica, promovendo a formação de poros que culminam na morte celular. Apesar de sua elevada eficácia, o uso clínico da Anfotericina B é limitado devido à sua nefrotoxicidade e absorção adequada apenas por meio de administração intravenosa, uma vez que apresenta baixa absorção oral (Zhou *et al*., 2017)

Nesse contexto, ferramentas de bioinformática estrutural como o *molecular docking* vêm sendo amplamente utilizadas na bioprospecção de óleos essenciais cujos constituintes podem atuar sobre alvos moleculares específicos do patógeno com a finalidade de compreender os mecanismos de interação entre tais compostos e proteínas-chave de *C. albicans*,

2.5 *Molecular docking*

Na pesquisa por novos agentes antifúngicos, a aplicação de ferramentas *in silico* tem se consolidado como uma abordagem estratégica para otimizar o processo de triagem de compostos bioativos. Dentre essas ferramentas, destaca-se o *molecular docking*, uma técnica de modelagem computacional amplamente utilizada na bioprospecção de fármacos por prever a interação entre moléculas de interesse (como os constituintes de óleos essenciais) e alvos moleculares específicos (como proteínas relacionadas à virulência de patógenos) (Bastos *et al*., 2023; Silva *et al*., 2019).

O *molecular docking* em sido explorado para avaliar a afinidade de compostos naturais com proteínas essenciais à sobrevivência e patogenicidade de espécies como *C. albicans*, possibilitando identificar interações relevantes para o desenvolvimento de agentes terapêuticos com ação antifúngica (Bastos *et al*., 2023). De acordo com Bouamrane *et al*. (2022) essa técnica permite estimar a energia livre de ligação, predizer a orientação dos ligantes no sítio ativo da proteína e, consequentemente, inferir o potencial de inibição da atividade biológica da enzima ou estrutura-alvo.

Entre os principais alvos moleculares de *C. albicans*, destacam-se a proteína transmembranar Als3 (*Agglutinin-like sequence 3*) e a enzima Sap5 ambas fortemente associadas à virulência fúngica. Als3 é uma glicoproteína de adesão presente na superfície da hifa, essencial para a fixação da levedura em células epiteliais humanas, biofilmes e superfícies

abióticas, além de estar envolvida na invasão celular (Charpak-amikam *et al*., 2022). Já a proteína Sap5 pertence à família das aspartil proteases secretadas (SAPs), responsáveis pela degradação de proteínas do hospedeiro, facilitando a penetração tecidual, evasão da resposta imune e obtenção de nutrientes (Dhanasekaran *et al*., 2024).

Neste estudo, o *molecular docking* foi utilizado como ferramenta para avaliar a interação entre os principais constituintes do óleo essencial de *V. gardneriana* Schauer e proteínas de interesse relacionadas à virulência de *C. albicans.* Está pesquisa visa compreender os mecanismos de ação em nível molecular e contribuir para a identificação de potenciais alvos terapêuticos, consolidando o uso de técnicas computacionais como aliadas na descoberta de novos agentes antifúngicos derivados de produtos naturais.

3 RELEVÂNCIA E JUSTIFICATIVA

Diante dos numerosos eventos de doenças infecciosas registrados nos últimos anos e a redução de registro de novos fármacos é essencial pesquisar por novos produtos naturais que apresentem bioprospecção. Nesse aspecto, plantas medicinais tiveram suas propriedades antimicrobianas, antioxidantes, larvicidas, acaricida, antitumoral, antiviral e cicatrizante (Ribeiro *et al*., 2018; Silva *et al*., 2019; Morais *et al*., 2020; Barreto; Almeida; Filho, 2021; Silva *et al*., 2021) reconhecidas cientificamente demonstrando que investigar os costumes populares pode ser importante para descobrir compostos bioativos com potencial antifúngico.

Ademais, a Caatinga possui grande diversidade de plantas aromáticas, das quais os óleos obtidos têm diversas propriedades de interesse farmacêutico. Estudos preliminares mostraram efeito sinérgico do óleo essencial de *V. gardneriana* Schauer combinado com a droga padrão Anfotericina B.

Portanto, o estudo acerca da investigação química, citotoxicidade, bem como sua ação antifúngica frente a cepas clínicas de *C. albicans* e a realização de estudos de *molecular docking* para um melhor entendimento da interação entre compostos presentes no óleo essencial da espécie vegetal *V. gardneriana* com proteínas do patógeno *C. albicans*, visa colaborar para a geração de conhecimentos científicos, a fim de apresentar uma pesquisa com dados preliminares que possa subsidiar estudos posteriores na busca por novos fármacos com potencial antifúngico e reduzida citotoxicidade.

4. OBJETIVOS

4.1 Objetivo geral

Analisar o potencial antifúngico, sinérgico e composição química do óleo essencial obtido da espécie botânica *V. gardneriana* frente a cepas clínicas isoladas de amostras provenientes do Hospital Santa Casa de Misericórdia de Sobral – CE, bem como, obter o perfil de citotoxicidade e determinar a interação dos constituintes do óleo da V. gardneriana com proteínas do patógeno C. albicans.

4.2 Objetivos específicos

- Identificar a composição química do OEVG;
- Determinar a atividade antifúngica e sinérgica do óleo essencial combinado com Anfotericina B;
- Estabelecer a interação entre compostos presentes no OEVG com proteínas do patógeno *C. albicans*, por molecular *docking*;
- Investigar a citotoxicidade do óleo essencial de *V. gardneriana* em células hemolíticas;
- Contribuir para a valorização e sistematização do conhecimento tradicional e apresentar resultados preliminares que trarão informações importantes para a condução de pesquisas futuras.

5 ARTIGOS

5.1 Artigo de revisão

Artigo de revisão submetido (Hoehnea – Qualis B1)

The genus *Vitex* as a Source of Antimicrobial Agents[1]

Amanda de Lima Barros[a*], **Andréa Maria Neves**[b*], **Elnatan Bezerra de Souza**[b*], **Raquel Oliveira dos Santos Fontenelle**[b*]

The genus *Vitex* as a Source of Antimicrobial Agents

Amanda de Lima Barros
amandabarrosbio@gmail.com
https://orcid.org/0000-0002-0683-7122
Andréa Maria Neves
andreamarianeves@gmail.com
https://orcid.org/0000-0002-7388-1844
Elnatan Bezerra de Souza
Elnatan_souza@uvanet.br
https://orcid.org/0000-0002-5222-4378
Raquel Oliveira dos Santos Fontenelle
raquelbios@yahoo.com.br
https://orcid.org/0000-0002-8865-5954

1. Part of the master's dissertation
2. [a] Master's student in Health Sciences, Federal University of Ceará, Av. Commander Maurocélio Rocha Pontes, 100, 62042-250, Sobral-CE, Brazil;
2. [b] Department of Agricultural and Biological Sciences, Vale do Acaraú State University, Av. Padre Francisco Sadoc de Araújo, 850 - Alto da Brasília, 62010-295 Sobral-CE, Brazil;
3. Corresponding author: amandabarrosbio@gmail.com

The Genus *Vitex* as a Source of Antimicrobial Agents

ABSTRACT
The genus *Vitex* Tour. Ex L. (*Lamiaceae*) stands out in the health sector due to the pharmacological potential of the phytochemicals present in its species. This study aimed to gather information on the antimicrobial potential of the species of the genus, presenting in general terms the ethnopharmacological studies, popular use, the most commonly used plant parts, extracts, and constituents. This is a bibliographic survey based on the popular use of species of the genus *Vitex* and their proven biological activities (*in vitro*). The eight species discussed here are widely distributed in various parts of the world and have antibacterial and antifungal activity against standard and clinical strains. It was observed that the species studied presented great diversity in terms of the chemical profile obtained from the bark, leaves, and fruits, and it was possible to identify important phytochemicals. Thus, the genus *Vitex* demonstrates potential for future pharmacological studies.
Keywords: antibacterial activity, antifungal, microbiological.

RESUMO
O gênero *Vitex* Tour. Ex L. (*Lamiaceae*) destaca-se no setor da saúde devido ao potencial farmacológico dos fitoquímicos presentes em suas espécies. Este estudo teve como objetivo reunir informações sobre o potencial antimicrobiano das espécies do gênero, apresentando em linhas gerais os estudos etnofarmacológicos, o uso popular, as partes vegetais, extratos e constituintes mais utilizados. Trata-se de um levantamento bibliográfico baseado no uso popular de espécies do gênero *Vitex* e suas atividades biológicas comprovadas (*in vitro*). As oito espécies aqui discutidas são amplamente distribuídas em diversas partes do mundo e apresentam atividade antibacteriana e antifúngica contra cepas padrãos e clínicas. Observou-se que as espécies estudadas apresentaram grande diversidade quanto ao perfil químico obtido a partir da casca, folhas e frutos, sendo possível identificar fitoquímicos importantes. Assim, o gênero *Vitex* demonstra potencial para futuros estudos farmacológicos.
Palavras-chave: atividade antibacteriana, antifúngica, microbiológica.

Introduction

In a scenario where microorganisms of medical importance are gradually showing resistance to conventional drugs and causing several problems in public health (Nogueira and Silva, 2021), researchers around the world have invested in the development of new research aimed at finding substances with antimicrobial activity from natural products (Ahmad, *et al*., 2021). Thus, based on reports of popular applications, science seeks to understand which compounds have biological activities (Ebani and Mancianti, 2020).

In this context, many plant species that are traditionally used as antibacterial and antifungal agents in natural medicine represent promising sources of raw material in the search for new phytopharmaceuticals that can be used for these therapeutic applications, taking into account that the molecular diversity of natural products is much higher than that derived from synthetic products, in addition to their low toxicity, lower cost, and fewer side effects. According to the World Health Organization (WHO), more than 80% of the world's population depends on traditional medicine to meet their main health needs (Kowalski *et al*., 2020; Ghavam *et al*., 2020; Neves *et al*., 2019).

Lamiaceae (*Labiatae Juss*.) comprises approximately 236 genera and about 7,300 species, mostly occurring in environments with high altitude and latitude, tropical or subtropical climate, and open vegetation formations (Karpinki, 2020; Ebadollahi, 2020). In Brazil, 46 genera and 524 species are recognized, with six genera and 343 species being endemic (Soares, Pastore, & Jardim, 2019). *Lamiaceae* are plants that occur in the form of trees, shrubs, subshrubs, and herbs, most of which are odoriferous. A striking characteristic is the presence of essential oils that give the species of this family properties that arouse pharmacological interest (Spréa *et al*., 2022; Michel *et al*., 2020).

Vitex agnus-castus is one of the most numerous genera of *Lamiaceae*. Ex L., which is composed of approximately 250 species with wide distribution, among which are trees and

shrubs in rupicolous and terrestrial substrates, with compound digitate or compound unifoliate leaves and distributed in tropics and subtropics, generally near rivers (Das *et al*., 2022; Islam *et al*., 2024). In folk medicine, *Vitex* species have been traditionally used for analgesic, anti-inflammatory, antimicrobial, and insecticidal purposes, as well as treating dermatitis and controlling the menstrual cycle. Its bioactive properties are found mainly in its leaves but also in its stems, fruits, and seeds (Kamal *et al*., 2022; Salleh and Zaki, 2020).

The *Vitex* genus has gained attention in the health sector due to the pharmacological potential contributed by the phytochemicals present in its species. They can be promoted as a variety of supplements to help improve and treat different types of diseases, or even be used as complementary medicine along with other conventional treatments. Thus aroused the interest of many researchers in discovering new pharmacologically active compounds in species of this genus (Kamal *et al.*, 2022).

Studies with medicinal plants of the genus *Vitex* have shown that substances found in essential oils and extracts obtained from these species have therapeutic potential for the treatment of various diseases. Given the therapeutic and scientific importance of the genus *Vitex*, this article aimed to conduct a bibliographic survey on the antimicrobial potential of the main species of the genus, presenting the ethnopharmacological studies, as well as the popular use, in addition to the most commonly used plant parts, their extracts and constituents.

Methodology

This is a bibliographic survey of articles based on the popular use of species of the *Vitex* genus, as well as their proven biological activities (*in vitro*). The databases used for the review were: Capes (http://www.periodicos.capes.gov), Scielo (http://www.scielo.org), Science Direct (http://www.sciencedirect.com), Google Scholar (http://www.scholar.google.com), and Scopus (www.scopus.com). In the period from March to June of this year, the research obtained through the descriptors in health sciences, *Vitex* genus, chemical compounds, antibacterial, and

antifungal in the languages: Portuguese and English was addressed. After reading the abstracts, the articles related to the research object were selected and analyzed in full, while those that were duplicated or that were distant from the research object were excluded from the study.

Results and discussion

Vitex negundo L.

Popularly known as the Chinese chaste tree, it has five digitated leaves with, generally, three lanceolate leaflets and a trunk with reddish-brown bark. It is widely distributed in South and Southeast Asia, Sri Lanka, India, and Pakistan, in various habitats (Sol *et al.*, 2020). It is commonly used in folk medicine due to its therapeutic potential, extracted from various parts of the plant, from the root to the seeds. This species is traditionally used to treat respiratory and ocular diseases, inflammation, vermifuge, depressed lactation, leucoderma, and enlarged spleen, among other applications (Khan *et al.*, 2024; Smrity *et al.*, 2019).

The antibacterial activity was evaluated using the agar diffusion technique. The methanolic extract of the leaves was effective against *Streptococcus pneumoniae, Staphylococcus aureus, Clostridium* sp.*, Bacillus subtilis*, with a maximum inhibition zone of 29 mm, and *Pseudomonas aeruginosa, Salmonella* sp.*, Klebsiella pneumoniae*, and *Escherichia coli* with a maximum inhibition zone of 26 mm (Giri and Das, 2018). The methanolic extract obtained from the bark of *V. negundo* showed antibacterial activity against *Bacillus subtilis* (1.562 mg/mL) and *Staphylococcus aureus* (0.245 mg/mL) (Koirala *et al.*, 2020).

The microorganisms *Candida albicans* and *Trichophyton rubrum* were sensitive to the essential oil of *V. negundo* seeds in a broth microdilution assay, presenting Minimum Inhibitory Concentration (MIC) of 4.0 µg/ml and 32 µg/ml, respectively (Ai *et al.*, 2014). According to Zareshahrabadi *et al.* (2023), the essential oil obtained from the leaves, fruits, and flowers was effective in inhibiting the biofilm of *C. albicans*, while the essential oil from the fruit was more

efficient in inhibiting the growth of the yeasts *C. glabrata, C. dubliniensis, C. krusei, C. tropicalis, and C. parapsilosis* with MICs in the range of 4-16 µg/ml.

Gas chromatography-mass spectrometry analysis revealed monoolein as the major compound present in the methanolic extract obtained from the bark of *V. negundo* (Koirala *et al.*, 2020). Phytochemical screening detected the presence of primary and secondary metabolites and the following major chemical constituents: vitedoin A (1), 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde (2), vitexdoin F (3), detetrahydroconidendrin (4), and vitexdoin E (5) (Giri and Das, 2018).

Vitex gardneriana Shauer

This species, popularly known as jaramataia or jerematai, is medium-sized and can reach about 6 meters in height. Has lilac flowers and simple, opposite, and lanceolate leaves. It is endemic to Brazil, with its distribution restricted to the Caatinga Phytogeographic Domain, especially in the Northeast region. In folk medicine, its leaves, bark, and fruits are used in the form of infusion to treat bronchitis, flu, and sinusitis, and as an analgesic and anti-inflammatory agent (Morais *et al.*, 2020).

Studies reveal that the essential oil extracted from the leaves has antibacterial capacity against planktonic cells and biofilms of multidrug-resistant strains of *S. aureus* and *P. aeruginosa* with a MIC of 0.31% v/v (Vale *et al.*, 2019). According to Pereira *et al.* (2020), the essential oil, when associated with gentamicin, demonstrated promising antimicrobial activity against the bacterial strains *S. aureus* (32 µg/mL) and *Escherichia coli* (1024 µg/mL).

The aforementioned study also investigated antifungal activity and reported a potential to reduce the growth of *C. albicans* and *C. tropicalis* by approximately 70% and 30%, respectively (Vale *et al.*, 2019). In a trial that investigated the influence of the circadian rhythm on the antifungal activity of the essential oil from the leaves of *V. gardeneriana* from the sample

obtained at 8 am, Brasília time, it was shown to be more efficient against clinical strains of *T. rubrum* in a test with a MIC of 0.1 to 0.3 mg/mL (Pereira *et al.*, 2018).

The ethanolic extract obtained from the fruit presented secondary metabolites in its phytochemical composition, such as tannins, alkaloids, and terpenes, corroborating a possible antioxidant potential also present in the fruit, since the association between one or more of these constituents is indicative of oxidative inhibition (Silva *et al.*, 2022).

Regarding the chemical composition of this species, it was observed that this may vary according to the part of the plant collected, as well as environmental and climatic factors. The major compounds commonly found were cis-Calamenene, 6,9-Guaidiene, and caryophyllene oxide (Silva *et al.*, 2019).

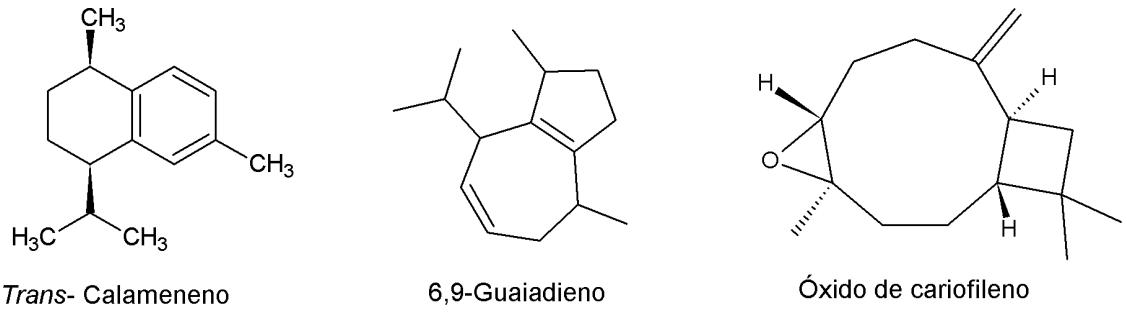


Figure 1 The major chemical constituents of the essential oil from the leaves of *V. gardneriana*. Trans-calamenene (A), 6,9-guaiadiene (B), caryophyllene oxide (C) (Adaptado Silva *et al.*, 2019).

Vitex agnus-castus L.

Commonly known as chaste tree or monk's pepper, it is a large shrub or small tree that can measure up to 3 meters in height, its leaves are compound and palmate, blue flowers and dark purple globose drupe-type fruits, it is distributed in Central Asia, Southern Europe and the Mediterranean region, although it is not an endemic species, it has been recorded in the northeast and southeast of Brazil in the Phytogeographic Domains of Caatinga and Atlantic Forest (Soares *et al.*, 2019). Ethnobotanical research involving V. agnus-castus reveals that this species can help in the treatment of syndromes and issues related to menstruation, in addition to being used as an antidiabetic, antispasmodic, and for relieving stomach pain and digestive

problems (Rocha *et al.*, 2017; Zhelev *et al.*, 2022).

Studies carried out with the essential oil obtained from the fruits of *V. agnus-castus* grown in Bulgaria show antibacterial effect by exhibiting zone of inhibition against strains of Salmonella abony NCTC 6017 (11.15±0.05 mm), *S. aureus* ATCC 6538 (11.25±0.05 mm), and B. subtilis ATCC 6633 (12.03±0.02 mm) (Zhelev *et al.*, 2022). The essential oil from the leaves, collected in northern Brazil, demonstrated promising activity against cariogenic microorganisms, presenting the respective MIC values for the study strains *Streptococcus mutans* ATCC 25175 (15.6 μg/mL), *S. mitis* ATCC 49456 (31.25 μg/mL), *S. sanguinis* ATCC 10556 (200 μg/mL), *S. sobrinus* ATCC 33478 (125 μg/mL), *S. salivarus* ATCC 25975 (200 μg/mL), *Lactobacillus casei* ATCC 11578 (15.6 μg/mL), *Enterococcus faecalis* ATCC 4082 (200 μg/mL) (Gonçalves, *et al.*, 2017).

In a study that isolated *C. albicans* samples from 40 patients with vulvovaginal candidiasis and subjected them to broth microdilution using alcoholic and aqueous extracts obtained from the leaves of *V. agnus-castus*, collected in Iran, it obtained antifungal potential against the aforementioned clinical isolates, with MICs in the range of 7.81–15.62 μg/mL and 15.62–62.5 μg/mL, respectively (Keikha *et al.*, 2018). The ethanolic (Et), aqueous (Aq) and methanolic (Me) extracts obtained from the leaves collected in the city of Dammam, Saudi Arabia, and subjected to the agar diffusion method, showed antifungal activity with a Zone of inhibition in mm described as follows: *C. tropicalis (*7.50 ± 0.50 Et); (5.67 ± 0.29 Aq) and (5.33 ± 0.29 Me), *C. albicans* (5.83 ± 0.29 Et); (5.00 ± 0.50 Aq) and (5.00 ± 0.50 Me) and *C. ciferrii* (4.33 ± 0.58 Et); (3.33 ± 0.29 Aq) and (3.33 ± 0.29 Me), respectively (Farokhzad *et al.* 2023).

The major metabolite identified in *V. agnus-castus* by gas chromatography analysis in the essential oils obtained from its leaves (18.27%), flowers (17.16%), and seeds (14.92%) was 1,8-cineole (Habbab *et al.*, 2016). The oil obtained from the fruit presented linoleic acid (71.5%) as its main compound, followed by oleic acid (14.0%) (Zhelev *et al.*, 2022).

Vitex trifolia L.

Lagundi or Neer Nochi, as it is popularly known, is a large, deciduous shrub native to regions from East Africa to French Polynesia and is commonly found on the banks of water bodies. In Brazil, it occurs as a non-native species, with records in the Amazon, Caatinga, and Cerrado. It has compound leaves, usually with 3 leaflets, and purple flowers. Its leaves are consumed fresh to improve memory, to treat fever, headache, diarrhea, and fungal and bacterial infections. Meanwhile, the oil obtained from its leaves is used in massages to relieve muscle pain (Parkhe *et al.*, 2019).

The methanolic extract of *V. trifolia* was shown to be efficient in its ability to inhibit the formation of *P. aeruginosa* biofilms with a minimum inhibition of 50 µg/ml and a maximum inhibition of 100 µg/ml (Mary *et al.*, 2015). Its antibacterial activity was also evaluated by the disk diffusion method against *B. subtilis*, presenting an inhibition zone of 0.5 cm in the acetone extract and 0.75 cm in the ethanol extract. For *E. coli*, the values obtained were 0.15 cm in acetone extract, 0.25 cm in ethanol extract, and 0.35 cm in aqueous extract (Mary *et al.*, 2014).

The essential oil obtained from the leaves of *V. trifolia* and subjected to broth microdilution showed antifungal activity against standard strains of *C. albicans* (ATCC 90029 – ATCC 1162) and *C. krusei* (ATCC 6258) with MICs of 62.5, 7.8, and 1.9 µl/ml, respectively (Devi *et al.*, 2014).

Gas Chromatography coupled with Mass Spectrometry analysis identified the presence of 50 constituents, the main ones being: 1R-α-pinene, Ocimene, 3-carene, toluene, p-ethyl-, eucalyptol, and 3-methoxy5-methylphenol (Devi *et al.*, 2014). The qualitative analysis of phytochemicals in the leaves of *V. trifolia* revealed the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, and terpenoids (Mary *et al.*, 2014).

Vitex pinnata L.

Laban or Kulimpapa, as it is commonly known, is a small tree with blue flowers and purple fruits (Goh *et al.*, 2017). Its native distribution was described in tropical Asia, and it can also be found in some regions of the African continent. Ethnobotanical studies report the use of tea in decoction of roots and bark for the treatment of stomach pain, fever, and muscle pain, while the leaves are prepared as a poultice for wound healing (Shafie *et al.*, 2020).

The Kirby-Bauer test with paper discs soaked in methanolic extract of V. pinnata leaves collected in England showed inhibitory activity on the growth of S. mutans ATCC 31987 with ANOVA test significance of 0.000 < 0.05 (Nuraskin *et al.*, 2019). The ethyl acetate extract obtained from *V. pinnata* leaves collected in Brunei showed antibacterial activity against strains of *S. aureus* (ATCC-29213), *B. subtilis* (ATCC-11774), *E. coli* (ATCC-11775), and *P. aeruginosa* (ATCC-27853) the values referring to the inhibition halo expressed as mean ± standard deviation found were 10.2 ± 0.3, 9.3 ± 0.5, 11.6 ± 0.2 and 6.2 ± 0.5 mm, respectively(Shafie *et al.*, 2020). The lipophilic extract of its bark showed potential to inhibit the growth of *Mycobacterium tuberculosis* with a MIC of 32.5 µl/ml through the in vitro test using the Alamar Blue microplate (Abdelbaset *et al.*, 2023).

The ethanolic extract obtained from the bark of a specimen of *V. pinnata* collected in Sri Lanka allowed the discovery of a new iridoid glycoside called pinnatoside, which showed antifungal activity against *C. albicans* with a MIC of 16 µl/ml (Kamal *et al.*, 2022).

When investigating the phytochemical composition using the gas chromatography method associated with mass chromatography, the presence of two steroids: ß-sitosterol and stigmasterol, and three flavonoids: 5-hydroxy-3,7,4'-trimethoxyflavone, 5-hydroxy-7,4'-dimethoxy-flavone, and 5-hydroxy-3,3',4',7-tetramethoxyflavone was diagnosed (Kamal *et al.*, 2017).

Vitex doniana Sweet

Commonly known as African tarumã or black plum (*V. doniana*), this is a robust tree

with fine hairs on its young branches and elongated, greenish flowers with purple petals. This species is native to the Cerrado region, but is often cultivated due to its sweet-tasting fruit. Its leaves are traditionally used in folk medicine to treat people suffering from dysentery, rheumatic pain, inflammatory disorders, respiratory diseases, and toothache, among others (Jean *et al.*, 2019).

The fractional ethanol extracts of the leaves obtained in Nigeria were subjected to microbiological sensitivity testing and were shown to be effective against nine clinical isolates of *S. typhi*, presenting bactericidal action with an activity of 150-300 µg/mL (Noel *et al.*, 2022). Another test, using the broth microdilution technique, was carried out with the ethanolic extract obtained from the fruits of *V. doniana*, collected in Benin, and showed promising MIC results against *Proteus mirabilis* (1.25 µg/mL), *Micrococcus luteus* (0.312 µg/mL), and *P. vulgaris* (1.25 µg/mL) (Dah-Nouvlessounon *et al.*, 2023). The literature reports antifungal activity of ethanol extract obtained from *V. doniana* leaves against Fusarium verticilliodes and *Aspergillus tamarii* with inhibition diameters of 17.75±0.35 mm and 16.00±0.00 mm. When subjected to broth microdilution, the ethanol extract showed activity against *C. albicans* with a MIC of 1.25 µg/mL (Dah-Nouvlessounon *et al.*, 2023).

When analyzing the chemical composition of extracts from the stem bark and leaves, the presence of bioactive components, alkaloids, glycosides, tannins, polyphenols, anthroquinone, saponins, and flavonoids was observed (Ali *et al.*, 2017). Through gas chromatography, a study carried out in Nigeria analyzing the methanolic extract of *V. doniana* leaves showed the presence of forty-three phytochemicals, with the highest concentration being linalool (7.89%) and β-Bisabolene (7.22%), in addition to the presence of trace elements (Manganese, iron and zinc) (Sani *et al.*, 2019). Analysis by Gas Chromatography Coupled to Mass Spectrometry of the fruit of *V. doniana* revealed the presence of trimethylsilyl sugar derivatives such as: 1, 3, 4, 5, 6-pentakis-O-(trimethylsilyl); 3, 4, 5-tris-O-(trimethylsilyl)-

pentose; Methyl 2, 3, 5-tris-O-(trimethylsilyl); Pentakis-O-(trimethylsilyl); Methyl 2, 3, 5, 6-tetrakis-O-(trimethylsilyl); 1, 2, 3, 4, 6-pentakis-O-trimethylsilyl); Methyl 2,3-bis-O-trimethylsilyl; 1, 2, 3, 5-tetrakis-O-(trimethylsilyl).

Vitex rivularis Gürke

Popularly known as Mevassa, the species *V. rivularis* is a large, leafy tree that can reach up to 12 meters in height. It has elongated leaves with 5 to 7 leaflets and long petioles. Its flowers are small and white and purple in color. It is found natively in the region from West Africa to Angola, in the humid tropical biome regions. In folk medicine, its leaves are used as an infusion to treat skin and nail mycoses (Zaki *et al.*, 2020).

Regarding bacterial activity, the methanolic extract of *V. rivularis* leaves showed inhibitory potential by the agar dilution method against standard strains of *E. coli* (ATCC 25922), *B. subtilis* (ATCC 6638), *S. aureus* (ATCC 59232), *P. mirabilis* (ATCC 15290), and *P. aeruginosa* (ATCC 15442) with MICs ranging from 0.25-0.50 mg/mL (Idowu *et al.*, 2022).

An assay using the broth microdilution method showed antifungal activity of methanolic extracts of leaves (EMF) and seeds (SEM) with MIC of 15 ± 0.00 mg/ml (EMF) and 7.50 ± 0.00 (SEM) for *C. albicans*, respectively, and 7.50 ± 0.00 mg/ml for both extracts against *C. neoformans* (Marguerite *et al.*, 2020).

Phytochemical screening performed using leaves and seeds of *V. rivularis* showed bioactive constituents such as phenols, tannins, flavonoids, anthraquinones, and glycosides, in addition to saponins and alkaloids found only in the leaves and triterpenes present only in the seeds (Marguerite *et al.*, 2020). The chemical characterization of the essential oil obtained from the aerial parts of *V. rivularis* showed the presence of germacrene D (20.6%); -curcumene (9.7%); ar-turmeric (5.5%); -copaene (6.4 vs. 5.0%) and -caryophyllene (6.6%) (Cabral *et al.*, 2009).

Vitex mollis Kunth

Native to Central America, the southern United States, and Mexico, this species is popularly known as uvalamo, and is characterized by being a large tree that can measure approximately 15 meters in height, with trifoliate leaves, white, blue, or purple flowers, and purple fruits. Its popular use has been described to treat conditions such as diarrhea, fever, abdominal cramps, pain, and scorpion stings (Montes-Ávila *et al.*, 2022).

The disk diffusion test performed with methanolic extract of *V. mollis* leaves collected in Mexico showed antibacterial activity against standard *S. aureus* strains (ATCC 25923, ATCC 29213, and ATCC 43300) with inhibition diameters of 37±0.15, 4.65±0.14, and 6.31±0.25 mm, respectively. Regarding the clinical isolates of bovine S. aureus, 5.47±0.38 for MRSA1 and 5.73±0.25 for MRSA2, and for the *S. aureus* strains isolated from rabbits, the result obtained was 6.10±0.17 for SOSA1 and 6.80±0.20 for SOSA2 (Medina *et al.*, 2017).

The methanolic extract obtained from the trunk of *V. mollis* in Mexico by the broth microdilution method showed antifungal activity for the species *F. verticillioides* (29.8 μg/mL), *F. oxysporum* (52.9 μg/mL), and *F. tapsinum* (109.0 μg/mL). The aqueous extract of the trunk presented an MIC of 51.3 μg/mL for *F. verticillioides* and 85.1 μg/mL for *F. oxysporum* (Valencia-Botin *et al.*, 2018).

The methanolic, aqueous, acetone, and hexane extracts obtained from the leaves and stems of *V. mollis*, subjected to phytochemical analysis, revealed the presence of phenols, flavonoids, triterpenes, and coumarins (Morales-Del-Rio *et al.*, 2015; Valencia-Botin *et al.*, 2018).

The antibacterial and antifungal activities, medicinal properties, and chemical compounds found in the studied Vitex species are listed in Tables 1, 2, 3, and 4, respectively.

Table 1 - *In vitro* antibacterial activity studies in species of the genus *Vitex*

Plant species	Part of the plant	Extract	Microorganism species	References
	Leaves	Methanolic	*Streptococcus pneumoniae*	Giri e Das, 2018
	Leaves	Methanolic	*Staphylococcus aureus*	Giri et al., 2018; (Koirala *et al.*, 2020
V. negundo L	Leaves	Methanolic	*Clostridium*	Giri e Das, 2018
	Leaves	Methanolic	*Bacillus subtilis*	Giri et al., 2018; Koirala *et al.*, 2020
	Leaves	Methanolic	*Pseudomonas aeruginosa*	Giri e Das, 2018
	Leaves	Methanolic	*Salmonella* sp	Giri e Das, 2018
	Leaves	Methanolic	*Klebsiella pneumoniae*	Giri e Das, 2018
	Leaves	Methanolic	*Escherichia coli*	Giri e Das, 2018
V. gardneriana Shauer	Leaves	oil	*Staphylococcus aureus*	Vale *et al*., 2019; Macedo *et al.* 2019
	Leaves	oil	*Pseudomonas aeruginosa*	Vale *et al*., 2019
	Leaves	5-hydroxy-3,7,4'-trimethoxyflavone	*Escherichia coli*	Macedo *et al.* 2019
	Seed	oil	*Escherichia coli*	Hábbab, *et al.,* 2016
	Seed	oil	*Klebsiella pneumoniae*	Habbab, *et al.,* 2016
V. agnus castus L.	Seed	oil	*Pseudomonas aeruginosa*	Habbab, *et al.,* 2016
	Leaves	oil	*Streptococcus mutans*	Goncalves *et al.,* 2017
	Leaves	oil	*Lactobacillus casei*	Goncalves *et al.,* 2017
	Leaves	oil	*Estreptococo mitis*	Goncalves *et al.,* 2017
V. trifolia L.	Leaves	Methanolic	*Pseudomonas aeruginosa*	Mary *et al*., 2015
	Leaves	Methanolic	*Bacillus subtilis*	Mary *et al*., 2015
	Leaves	Methanolic	*Escherichia coli*	Mary *et al*., 2014
V. pinnata L	Leaves	Methanolic	*Streptococcus mutans*	Nuraskin *et al*., 2019
	Cascas	Lipophilic	*Mycobacterium tuberculosis*	Abdelbaset *et al*., 2023
V. doniana Sweet	Leaves	Ethanolic	*Salmonella typhi*	Dawang *et al*., 2022
	Leaves	Ethanolic	*Staphylococcus lentus*	Dah-Nouvlessounon *et al.,* 2023
	Leaves	Ethanolic	*Staphylococcus haemolyticus*	Dah-Nouvlessounon *et al.,* 2023
V. rivularis Gürke	Leaves	Methanolic	*Staphylococcus aureus*	Idowu *et al*., 2022
V. rivularis Gürke	Leaves	Methanolic	*Staphylococcus aureus*	Idowu *et al*., 2022
	Leaves	Methanolic	*Escherichia coli*	Idowu *et al*., 2022
	Leaves	Methanolic	*Bacillus subtilis*	Idowu *et al*., 2022
	Leaves	Methanolic	*Pseudomonas aeruginosa*	Idowu *et al*., 2022
	Leaves	Methanolic	*Proteus mirabilis*	Idowu *et al*., 2022
V. mollis Kunth	Leaves	Methanolic	*Staphylococcus aureus*	Medina *et al*., 2017

Table 2 – *In vitro* antifungal activity studies in species of the genus *Vitex*

Plant species	Part of the plant	Extract	Microorganism species	References
V. negundo L.	Seed	oil	*Candida albicans*	Ai *et al*., 2014
	Seed	oil	*trichophyton rubrum*	Ai *et al*., 2014
	Fruits	oil	*Candida glabrata*	Zareshahrabadi *et al.* 2023
	Fruits	oil	*Candida dubliniensis*	Zareshahrabadi *et al.* 2023
	Fruits	oil	*Candida Krusei*	Zareshahrabadi *et al.* 2023
	Fruits	oil	*Candida tropicalis*	Zareshahrabadi *et al.* 2023
	Fruits	oil	*Candida parapsilosis*	Zareshahrabadi *et al.* 2023
V. gardneriana Shauer	Leaves	oil	*Candida albicans*	Vale *et al*., 2019
	Leaves	oil	*Candida tropicallis*	Vale *et al*., 2019
	Leaves	oil	*Trichophyton rubrum*	Pereira *et al*., 2017
V. agnus castus L.	Leaves	Alcoholic and aqueous	*Candida albicans*	Keikha *et al*., 2018
	Leaves	Ethanolic, methanolic and aqueous	*Candida tropicalis*	Farokhzad *et al.* 2023
	Leaves	Ethanolic, methanolic and aqueous	*Candida albicans*	Farokhzad *et al.* 2023
V. agnus castus L.	Leaves	Ethanolic, methanolic, and aqueous	*Candida Ciferrii*	Farokhzad *et al.* 2023
V. trifolia L.	Leaves	Oil	*Candida albicans*	Devi *et al*., 2014
	Leaves	Oil	*Candida Krusei*	Devi *et al*., 2014

V. pinnata L.	Bark	Ethanolic	*Candida albicans*	Kamal *et al*., 2022
V. doniana Sweet	Leaves	Ethanolic	*Fusarium verticilliodes*	Dah-Nouvlessounon *et al.,* 2023
	Leaves	Ethanolic	*Aspergillus tamarii*	Dah-Nouvlessounon *et al.,* 2023
V. rivularis Gürke	Leaves	Methanolic	*Candida albicans*	Marguerite *et al*., 2020
	Leaves	Methanolic	*Cryptococcus neoformans*	Marguerite *et al*., 2020
V. mollis Kunth	Stem	Methanolic	*Fusarium verticillioides*	Valencia-Botin *et al*., 2018
V. mollis Kunth	Stem	Methanolic	*Fusarium oxysporum*	Valencia-Botin *et al*., 2018

Table 3 – Species of the genus *Vitex* with medicinal properties

Plant species	Popular name	Part used	Preparation	Indication	References
V. Negundo L.	Chinese chaste tree	Leaves, flowers, and fruits	Infusion	Respiratory diseases, eye diseases, inflammations, deworming, deprived lactation, leucoderma, enlarged spleen	Khan, *et al.,* 2024; Smrity, *et al*., 2019
V. agnus ca stus L.	monk's pepper	Leaves	Infusion	Menstrual syndromes and issues, antidiabetic, antispasmodic, and for relief of stomach pain and digestive problems	Zhelev, *et al*., 2022; Rocha, *et al.*, 2017
V. trifolia L.	Lagundi ou Neer Nochi	Leaves	*In natura, infusion* and oil	Memory, as antifebrile, headache, asthma, and diarrhea. relief of muscle pain	Parkhe *et al*., 2019
V. pinnata L.	Laban ou Kulimpapa	Roots and barks	Decoction and poultice	Stomach aches, fever and muscle pain, wound healing	Shafie *et al*., 2020
V. doniana Sweet	African Tarumã or Black Plum	Leaves and bark	Infusion	Dysentery, rheumatic pains, inflammatory disorders, respiratory diseases, toothache	Jean *et al.,* 2019
V. agnus castus L.	monk's pepper	Leaves	Infusion	Menstrual syndromes and issues, antidiabetic, antispasmodic and for relief of stomach pain and digestive problems	Zhelev, *et al*., 2022; Rocha, *et al.*, 2017
V. rivularis Gürke	_____	Leaves	Infusion	Micoses de pele e unhaSkin and nail mycoses	Zaki *et al*., 2020
V. mollis Kunth	Uvalamo	Leaves	Infusion	Diarrhea, fever, abdominal cramps, pain and scorpion stings	Montes-Ávila *et al.*, 2022

Table 4 - *Vitex* species and their main compounds found in the composition of essential oils and extracts

Vitex species	Major compounds	Product	References
V. Negundo L.	Carbohydrates, proteins, amino acids, steroids, cardiac glycosides, anthraquinone glycosides, saponins, flavonoids, tannins, and phenolic compounds	Aqueous extract of leaves	Pawar e Kamble, 2017
V. gardneriana Shauer	Cis-Calamenene, 6,9-Guaidenene, and Caryophyllene Oxide	Leaf oil	Pereira *et al*., 2018
V. agnus castus L.	1,8-cineole, linoleic acid and oleic acid	Leaf oil	Habbab, *et al.,* 2016
V. trifólia L.	1R-α-pinene, Ocimene, 3-carene, Toluene, p-ethyl, Eucalyptol and 3-methoxy5-methylphenol	Leaf oil	Devi *et al*., 2014
V. pinnata L.	1R-α-pinene, Ocimene, 3-carene, Toluene, p-ethyl, Eucalyptol and 3-methoxy5-methylphenol	Leaf oil	Kamal *et al*., 2016
V. doniana Sweet	1, 3, 4, 5, 6-pentakis-O-(trimethylsilyl), 3,4,5-tris-O-(trimethylsilyl)-pentose, Methyl2,3,5-tris-O-(trimethylsilyl), Pentakis-O-(trimethylsilyl), Methyl2,3,5,6-tetrakis-O-(trimethylsilyl), 1,2,3,4,6- pentakis-O-trimethylsilyl), Methyl2,3-bis-O trimethylsilyl and 1,2,3,5-tetrakis-O-(trimethylsilyl).	Leaf oil	Chinyere *et al.,* 2021
V. rivularis Gürke	Germacrene D, curcumene, ar-curcuma, Copaene and Caryophyllene	Leaf oil	Cabral *et al*., 2009
V, mollis Kunth	Phenols, flavonoids, triterpenes and coumarins	Methanolic extract of the stem	Valencia-Botin *et al*., 2018

This manuscript has not been published, either in whole or in part, and is not submitted to any other journal.

Mechanism of action of essential oils and plant extracts against pathogens

Antimicrobial drugs derived from microbial or chemical products play important roles in the fight against pathogens. However, with widespread use worldwide, they have resulted in

widespread resistance and side effects. Therefore, a sharp increase in the proportion and number of bacteria and fungi resistant to various chemical antimicrobial agents has been observed in recent decades (Liang; Huang; Ma, 2022).

Medicinal and aromatic plants contain various types of chemical constituents that

have antimicrobial properties. These constituents are synthesized by plants to protect them from the action of microbial pathogens. The antimicrobial properties of essential oils and plant extracts depend primarily on the variation in their chemical constituents, the quantity of the main individual compounds, and the volatile nature of their components. These chemical compounds are secreted through a series of molecular interactions under specific biotic and abiotic stress conditions. Each compound may exhibit a different mechanism of action against microorganisms (Gonelimali *et al.*, 2018; Swami; Akhatar; Sinniah, 2016).

In general, the mechanism of action of essential oils is mediated by a series of biochemical reactions in the microbial cell. Several mechanisms have been described to explain the antibacterial activity of essential oils. These metabolites can affect both the outer envelope of the bacterial cell and the cytoplasm. The typical hydrophobicity of essential oils is responsible for the disruption of bacterial structures, which leads to increased permeability due to the inability to separate these metabolites from the bacterial cell membrane. Other mechanisms include degradation of the cell wall, damage to the cytoplasmic membrane, coagulation of the cytoplasm, damage to membrane proteins, increased permeability leading to leakage of cell contents, reduction of the proton motive force, and reduction of the intracellular ATP pool (Swami; Akhatar; Sinniah, 2016; Nazzaro *et al.*, 2013)

The antifungal actions of essential oils are similar to the antibacterial mechanisms explained above. Exposure to essential oils generally leads to coagulation of cellular components due to irreversible damage to the cell membrane. In yeast cells, essential oils establish a membrane potential across the cell membrane and disrupt ATP production, which

leads to damage to the cell membrane. Essential oils have the ability to penetrate and disrupt fungal cell walls and cytoplasmic membranes through a permeabilization process, which leads to the disintegration of mitochondrial membranes. This is caused by changes in electron flow within the electron transport system (ETS) pathway. This process can damage the lipids, proteins, and nucleic acid content of cells infected by fungal pathogens. Essential oils can disrupt the depolarization of mitochondrial membranes by affecting ion channels, especially Ca2+ ions, proton pumps, and ATP pools, and thus decrease the membrane potential. This change in membrane fluidity can cause electrolyte leakage and hinder cytochrome C pathways, protein metabolism, and calcium ion concentrations. Therefore, permeabilization of the inner and outer mitochondrial membranes causes apoptosis or cell necrosis, leading to cell death (Swami; Akhatar; Sinniah, 2016).

The antifungal actions of essential oils are similar to the antibacterial mechanisms explained above. Generally, exposure to essential oils leads to coagulation of cellular components due to irreversible damage to the cell membrane. In yeast cells, essential oils establish a membrane potential across the cell membrane and disrupt ATP production, which leads to damage to the cell membrane. Essential oils have the ability to penetrate and disrupt the fungal cell wall and cytoplasmic membranes through a process of permeabilization, which leads to the disintegration of mitochondrial membranes. This is caused by changes in the flow of electrons within the electron transport system (ETS) pathway. This process can damage the lipids, proteins, and nucleic acid content of cells infected by fungal pathogens. Essential oils can disrupt the depolarization of mitochondrial membranes by affecting ion channels, especially Ca2+ ions, proton pumps, and ATP pools, and thus decrease the membrane potential. This change in membrane fluidity can cause electrolyte leakage and hinder cytochrome C pathways, protein metabolism, and calcium ion concentrations. Therefore, permeabilization of the inner and outer mitochondrial membranes causes apoptosis or cell necrosis, leading to cell death

(Swami; Akhatar; Sinniah, 2016).

In addition, the efficacy of bioactive plant extracts contains complex mixtures of solubilized plant compounds, and their synergistic action can produce an enhanced effect. The microbial cell can be affected by these phytochemical constituents in several ways. In general, the main target site of the active compounds is the cytoplasmic membrane, affecting its structure and integrity, permeability, or functionality (Vaou *et al.*, 2021).

Final Considerations

The literature research showed that *Vitex* species have diverse chemical compositions and prove their antimicrobial activity, with several reports of their applicability in traditional medicine being recorded. Among these, we can mention their popular use in the treatment of respiratory and ocular diseases, bruises, digestive problems, diabetes, headaches, wound healing, toothache, inflammatory disorders, mycoses, and menstrual cramps, among others.

As for the microbiological tests observed, it is possible to state that all the species studied have relevant antibacterial and antifungal potential due to the presence of important phytochemicals obtained from oils and extracts of the bark, leaves, or fruit. However, the literature lacks further studies based on the phytochemicals found to provide more information regarding the major compounds of these species.

Acknowledgements

We would like to thank Vale do Acaraú State University and the Federal University of Ceará for providing access to the journal portals via institutional email.

Contributions of the authors

Amanda de Lima Barros

Contribution to the conception and design of the study; Contribution to the collection of data; Contribution to the analysis and interpretation of data; Contribution to the preparation of the manuscript.

Andréa Maria Neves

Contribution to the conception and design of the study; Contribution to the analysis and interpretation of data; Contribution to the review of the manuscript.

Elnatan Bezerra de Souza

Contribution to project administration; Contribution to data analysis and interpretation; Contribution to manuscript review.

Raquel Oliveira dos Santos Fontenelle

Substantial contribution to the analysis and interpretation of data; Contribution to the review of the manuscript and general supervision.

Conflict of interest

There is no conflict of interest in the writing and dissemination of this manuscript

Cited literature

Abdelbaset, S., Barakat, H., El-Shahawi, M., Ghaith, T. 2023. GC-MS profiling of *Vitex pinnata* bark lipophilic extract and screening of its anti-TB and cytotoxic activities. Natural Product Research, 37, 2718–2724. [Crossref]

Ahmad, I., Ahmed, F., Khan, M., Hussain, F. 2021**.** Environmental antimicrobial resistance and its drivers: a potential threat to public health. Journal of Global Antimicrobial Resistance, 27, 101–111. [Crossref]

Ai, H.W., Zhang, L., Yang, L. 2014. Antifungal properties and chemical analysis of essential oil from *Vitex negundo* seeds. British Journal of Pharmaceutical Research, 4, 541–548. [Link]

Ali, M., Aminu, F., Ibrahim, I.S. 2017. In-vitro assessment of antibacterial activity and phytochemical screening of *Vitex doniana* on clinical isolate of *Salmonella typhi*. IN-VITRO, 3, 9-16.

Bakr, R. O., El-Fayoumi, R.A., Abdel-Aziz, A.S. 2020**.** Antimicrobial activity of *Vitex agnus-castus* essential oil and *molecular docking* study of its major constituents. Journal of Essential Oil Bearing Plants, 23, 184–193. [Crossref]

Balasubramani, S., Ramesh, S., Srinivasan, V. 2017**.** Development of nanoemulsion from *Vitex negundo* L. essential oil and its efficacy in antioxidant, antimicrobial, and larvicidal activities (*Aedes aegypti* L.). Environmental Science and Pollution Research, 24, 15125–15133. [Link]

Cabral, C., Denny, R., Gomes, S., 2009**.** Composition and antifungal activity of the essential oil from Cameroonian *Vitex rivularis* Gürke. Natural Product Research, 23, 1478–1484. [Crossref]

Chinyere, I., Julius, I. U., Samuel, O. E. 2021**.** Determination of the flavoring components in *Vitex doniana* fruit following hydrodistillation extraction. American Journal of Food and Nutrition, 9, 69-75. [Link]

Cho, H., Lee, J., Han, M. 2023**.** Antioxidant Activity of *Vitex rotundifolia* Seeds and Phytochemical Analysis Using HPLC-PDA. Natural Product Sciences, 29, 1–9. [Crossref]
Dah-Nouvlessounon, D., Edorh, P., Kpanou, T. 2023**.** Phytochemical Analysis, Antioxidant and Antimicrobial Properties of *Vitex doniana* Sweet Leaves and Fruits Extracts. American Journal of Biochemistry, 13, 14–24. [Crossref]
Das, N., Poudel, B., Mishra, R. 2022. Traditional uses, phytochemistry, and pharmacology of genus *Vitex* (*Lamiaceae*). Phytotherapy Research, 36, 571–671. [Crossref]
Devi, W.R. & Singh, C.B. 2014. Chemical composition, anti-dermatophytic activity, antioxidant, and total phenolic content within the essential oil of *Vitex trifolia*. International Journal of Phytocosmetics and Natural Ingredients, 1, 5. [Link]
Ebadollahi, A., Ziaee, M., Palla, F. 2020**.** Essential oils extracted from different species of the *Lamiaceae* plant family as prospective bioagents against several detrimental pests. Molecules, 25, 1556. [Crossref]
Farokhzad, F., Asgar, M., Heidari, M. 2023**.** Phytochemical, Antioxidant and Antifungal Investigations of *Vitex agnus-castus* Leaf Essential Oil. BioControl in Plant Protection, 10, 67–80. [Link]
Ghavam, M., Tavasoli, A., Saeed, A. 2020. Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of *Salvia hydrangea* DC. Ex Benth. Scientific Reports, 10, 15647. [Crossref]
Giri Prasad, T. & Das, V. 2018. Evaluation of phytochemical and antimicrobial activities of Vitex negundo L. leaf extracts. International Journal of Research and Analytical Reviews, 5, 888–892. [Link]
Goh, P.Y.M., Basri, A.M., Yasin, H., Taha, H., Ahmad, N. 2017. Ethnobotanical review and pharmacological properties of selected medicinal plants in Brunei Darussalam: *Litsea elliptica, Dillenia suffruticosa, Dillenia excelsa, Aidia racemosa, Vitex pinnata,* and *Senna alata*. Asian Pacific Journal of Tropical Biomedicine, 7, 173–180. [Crossref]
Goncalves, R., Ayres, V.F., Carvalho, C.E., Souza, M.G., Guimaraes, A.C., Correa, G.M., Martins, C.H.G., Takera, R., Silva, E.O., Crotti, A.E.M. 2017. Chemical composition and antibacterial activity of the essential oil of *Vitex agnus-castus* L. (*Lamiaceae*). Annals of the Brazilian Academy of Sciences, 89, 2825–2832. [Crossref]
Gonelimali, F.D., Lin, J., Miao, W., Xuan, J., Charles, F., Chen, M., Hatab, S.R. 2018. Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Frontiers in Microbiology, 9, 1639. [Crossref]
Habbab, A., Sekkoum, K., Belboukhari, N., Cheriti, A., Y. Aboul-Enein, H. 2016. Essential oil chemical composition of *Vitex agnus-castus* L. from Southern-West Algeria and its antimicrobial activity. Current Bioactive Compounds, 12, 51–60. [Link]
Idowu, P. A., Samuel, B. B., Mbachu, C. C. 2022. Bioassay-guided isolation of an antibacterial flavone and cytotoxicity of the leaf extracts of *Vitex rivularis* Gurke (Lamiaceae). Nigerian Journal of Pharmaceutical Research, 18, 35–41. [Crossref]
Islam, Z., Caldeira, G. I., Caniça, M., Islam, N., Silva, O. 2024. Vitex Genus as a Source of Antimicrobial Agents. Plants, 13, 401.[Crossref]
Jean, B. M., Nâg-Tiero, M. R., Hermann, O. Y., Germaine, N. O. 2019. Traditional uses, phytochemistry, and pharmacology review of 2 Vitex: diversifolia and doniana. International Journal of Research and Development in Pharmacy & Life Sciences, 8, 29–36. [Crossref]
Kamal, N., Clements, C., Gray, A.I., Edrada-Ebel, R., 2016. Anti-infective activities of secondary metabolites from *Vitex pinnata*. Journal of Applied Pharmaceutical Science, 6, 102–106. [Crossref]
Karpinski, T.M. 2020**.** Essential oils of *Lamiaceae* family plants as antifungals.

Biomolecules, 10, 103. [Crossref]
Keikha, N., Shafaghat, M., Mousavia, S.M., Moudi, M., Keshavarzi, F. 2018. Antifungal effects of ethanolic and aqueous extracts of Vitex agnus-castus against vaginal isolates of *Candida albicans*. Current Medical Mycology, 4, 1. [Crossref]
Khan, S. Z., Hazrat, A., Khan, F. A., Yahya, M., Rahim, G., Mukhtiar, M., Khan, M. A., Ullah, H. 2024. Assessment of Phytochemical Profiling and Therapeutic Potential of the Ethanolic Extract of *Vitex negundo* (L.). Sarhad Journal of Agriculture, 40, 395–406. [Crossref]
Koirala, N., Dhakal, C., Munankarmi, N.N., Ali, S. W., Hameed, A., Martins, N., Sharifi-Rad, J., Imran, M., Arif, A.M., Hanif, M.S., Basnyat, R.C., Salehi, B. 2020. *Vitex negundo* Linn.: phytochemical composition, nutritional analysis, and antioxidant and antimicrobial activity. Cellular and Molecular Biology, 66, 1–7. [Crossref]
Leitão, S.G., Santos, T.C.D., Delle Monache, F., Matheus, M.E., Fernandes, P.D., Marinho, B.G. 2011. Phytochemical profile and analgesic evaluation of *Vitex cymosa* leaf extracts. Brazilian Journal of Pharmacognosy, 21, 874–883. [Crossref]
Liang, J., Huang, X., Ma, G. 2022. Antimicrobial activities and mechanisms of extract and components of herbs in East Asia. RSC Advances, 12, 29197–29213. [Crossref]
Macedo, I., da Silva, J.H., Silva, P.T., Cruz, B.G., Vale, J.P., Santos, H.S., Bandeira, P.N., Souza, E.B., Xavier, M.R., Coutinho, H.D.M., Braz-Filho, R., Teixeira, A.M.R. 2019. Structural and microbiological characterization of 5-hydroxy-3, 7, 4′-trimethoxyflavone: a flavonoid isolated from *Vitex gardneriana* Schauer leaves. Microbial Drug Resistance, 25, 434–438. [Crossref]
Mancianti, F. & Ebani, V.V. 2020. Biological Activity of Essential Oils. Molecules, 25, 678. [Crossref]
Simo, M., Chen, Z., Siwe, G., Majoumouo, S., Donati, M., Toghueo, R., Jazet, P., Sameza, M., Fekam, F., Froldi, G. 2020. Antifungal, Antioxidant Activity and Phytochemical Analysis of Extracts from *Vitex rivularis* (*Lamiaceae*). Research & Reviews: A Journal of Pharmacognosy, 1, 23–48. [Link]
Mary, R.N.I., Banu, N. 2015. Screening of antibiofilm and anti-quorum sensing potential of *Vitex trifolia* in *Pseudomonas aeruginosa*. Int. J. Pharm. Pharm. Sci., 7, 242–245. [Link]
Mary, R.N.I., Meenashree, B., Vasanthi, V. J. 2014. Screening of antibacterial activity and qualitative and quantitative analysis of phytochemicals in *Vitex trifolia*. Int. J. Curr. Microbiol. Appl. Sci., 3, 425–431. [Link]
Medina, M.F.E., Alaba, P.A., Estrada-Zuñiga, M.E., Velázquez-Ordoñez, V., Barbabosa-Pliego, A., Salem, M.Z.M., Alonso-Fresán, M.U., Camacho-Díaz, L.M., Salem, A.Z. 2017 Anti-staphylococcal properties of four plant extracts against sensitive and multi-resistant bacterial strains isolated from cattle and rabbits. Microbial pathogenesis 113, 286–294. [Crossref]
Michel, J., Abd Rani, N. Z., Husain, K. 2020. A review on the potential use of medicinal plants from Asteraceae and *Lamiaceae* plant families in cardiovascular diseases. Frontiers in Pharmacology 11, 852. [Crossref]
Montes-Ávila, J., López-Angulo, G., Duarte-de-la-Peña, G., Díaz-Camacho, S.P., Osuna-Galindo, V.C., López-Valenzuela, J.Á., Delgado-Vargas, F. 2022. Antioxidant, antibacterial, and antiparasitic activities of green nanoparticles synthesized from water-soluble melanins of fruits. Bionanociência 12, 228–240. [Crossref]
Morais, S.M., Alves, D.R., Frota, L.S., Pinheiro, S.D.O.P., Silva, A.C., Silva, W.M.B. 2020. Atividades antioxidantes e anticolinesterásicas do extrato das folhas de jaramataia (*Vitex gardneriana* Schauer). Rev. Bras. Desenvolvimento 6, 28802–28810. [Crossref]
Morales-Del-Rio, J. A., Gutiérrez-Lomelí, M., Robles-García, M.A., Aguilar, J.A.,

Lugo-Cervantes, E., Guerrero-Medina, P.J., Ruiz-Cruz, S., Cinco-Moroyoqui, F.J., Wong-Corral, F.J., Del-Toro-Sánchez, C.L. 2015. Anti-Inflammatory Activity and Changes in Antioxidant Properties of Leaf and Stem Extracts from *Vitex mollis* Kunth during In Vitro Digestion. Evid.-Based Complement. Evidence-Based Complementary and Alternative Medicine 349235. [Crossref]
Nazzaro, F., Fratianni, F., De Martino, L., Coppola, R., De Feo, V. 2013. Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6, 1451–1474. [Crossref]
Neves, A.M., Silva, H.S., Sousa, E.B., Fontenelle, R.O.S., Silva, A.C., Morais, S.M., 2019. Perfil fitoquímico e avaliação da atividade antifúngica da fração hexânica de *Mitracarpus baturitensis* (Rubiaceae). Essentia-Revista de Cultura, Ciência e Tecnologia da UVA. [Link]
Noel, D., Wain, J., Lar, P. 2022. Use of *Vitex doniana* (black plum) and Abutilon hirtum (Florida keys) extracts as an integral part of phytomedicine in tackling multidrug-resistant Salmonella. Journal of Infection in Developing Countries 16, 1323–1328. [Crossref]
Nuraskin, C.A., Idroes, R.M., Soraya, C., Djufri. 2019. Activities inhibition methanol extract of Laban leaf (*Vitex pinnata*) on the growth of bacteria *S. mutans* ATCC 31987. In IOP Conference Series: Materials Science and Engineering 509, 012008. [Link]
Parkhe, G. &Bharti, D.; 2019. Phytochemical investigation and determination of total phenols and flavonoid concentration in the leaves extract of *Vitex trifolia* Linn. Journal of Drug Delivery & Therapeutics 9, 705–707. [Crossref]
Pawar, S. & Kamble, V. 2017. Phytochemical screening, elemental and functional group analysis of *Vitex negundo* L. leaves. International Journal of Pharmacy and Pharmaceutical Sciences 9(6), 226-230. [Link]
Pereira, E.J.P., Silva, H.C., Holanda, C.L., de Menezes, J.E.S.A., Siqueira, S.M.C., Rodrigues, THS, ... & Santos, HS. 2018. Chemical composition, cytotoxicity, and larvicidal activity against *Aedes aegypti* of essential oils from *Vitex gardneriana* Schauer. Boletín Latinoamericano y del Caribe de Plantas Medicinais y Aromáticas 17, 217–225. [Link]
Pereira, R.L.S., Freitas, T.S.D., Freitas, P.R., Araújo, A.C.J.D., Campina, F.F., Fidelis, K.R., Vale, J.P.C., Barreto, A.C.H., Coutinho, H.D.M., Bandeira, P.N., Teixeira, A.M.R., Santos, H.S.D. 2020. Effects of seasonality on antibacterial and antibiotic-potentiating activity against multidrug-resistant strains of *Escherichia coli* and *Staphylococcus aureus* and ATR-FTIR spectra of essential oils from *Vitex gardneriana* leaves. Current microbiology 77, 3969–3977. [Crossref]
Rocha, T.T., Tavares-Martins, A.C.C., Lucas, F.C.A. 2017. Traditional populations in environmentally protected areas: an ethnobotanical study in the Soure Marine Extractive Reserve of Brazil. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 16, 410–427. [Link]
Sanni, M., Olajide, J. E., Omattah, G.O., 2019. Chemical constituents and radical scavenging activity of methanol leaf extract of Vitex doniana. FUW Trends in Science and Technology Journal 4, 179–183. [Link]
Shafie, N.A., Suhaili, N.A., Taha, H., Ahmad, N. 2020. Evaluation of antioxidant, antibacterial, and wound healing activities of *Vitex pinnata*. F1000Research 9, 187. [Crossref]
Silva, B.F., Martins, S.S., Martins, M.G.Q., Pereira, A.M.G., Vale, J.P.C., Santos, H.S., Rodrigues, T.H.S., Evaristo, F.F.V. 2022. Composição química, polifenóis totais, atividade antioxidante e citotóxica do extrato etanólico de frutos da *Vitex gardneriana* Schauer. Pesquisa, Sociedade e Desenvolvimento 11, e52311427265. [Crossref]
Silva, L.O.P. & Nogueira, J.M.R. 2021**.** Resistência bacteriana: potencial de plantas medicinais como alternativa para antimicrobianos. RBAC. (Online) 53, 21–27. [Link]

Silva, P.T., Silva, P.T., Santos, H.S., Teixeira, A.M.R., Bandeira, P.N., Holanda, C.L., Vale, J.P.C., Pereira, E.J.P., Menezes, J.E.S.A., Rodrigues, T.H.S., Souza, E.B., Silva, H.C., Santiago, G.M.P. 2019. Seasonal variation in the chemical composition and larvicidal activity against *Aedes aegypti* of essential oils from *Vitex gardneriana* Schauer. S. Afr. J. Bot. 124, 329–332. [Crossref]
Smrity, K.K., Sultana, S., Hassan, M.A., Hossain, M.L. 2019. Medicinal activity of *Vitex negundo* L. (Family: *Lamiaceae*) leaves extract: Assessment of phytochemical and pharmacological properties. J. Pharmacogn. Phytochem 8, 3571–3575. [Link]
Soares, A.S., Pastore, J.F.B., Jardim, J.G. 2019. *Lamiaceae* no Rio Grande do Norte, Brasil. Rodriguésia 70, e03312017. [Crossref]
Spréa, R. M., Caleja, C., Pinela, J., Finimundy, T.C., Calhelha, R.C., Kostić, M., ... & Barros, L. 2022. Comparative study on the phenolic composition and in vitro bioactivity of medicinal and aromatic plants from the *Lamiaceae* family. Food Research International 157, 111875. [Crossref]
Sundaresan, S., Kumar, L.V., Gopinathan, R.N. 2020. Phytochemical profiling and biological studies of *Vitex altissima* (L) leaves collected from South Kerala. In: AIP Conf. Proc. 2287 [Crossref]
Swamy, M.K., Akhtar, M.S., Sinniah, U.R. 2016. Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Complementary and Alternative Medicine 2016, 3012462. [Crossref]
Udeani, T.K., Ugwu, L.O., Nnadi, C.O., Okwuosa, C.N. 2021. Evaluation of anti-microbial activity and proximate composition of alkaloids from *Vitex doniana* Seed. Dhaka University Journal of Pharmaceutical Sciences 20, 81–86. [Crossref]
Vale, J.P.C., Ribeiro, L.H.F., Vasconcelos, M.A., Sá-Firmino, N.C., Pereira, A.L., Nascimento, M.F., Rodrigues, T.H.S., Silva, P.T., Sousa, K.C., Silva, R.B., Neto, L.G.N., Saker-Sampaio, S., Bandeira, P.N., Santos, S.S., Souza, E.B., Teixeira, E.H. 2019. Chemical composition, antioxidant, antimicrobial, and antibiofilm activities of Vitex gardneriana Schauer leaves' essential oil. Microbial pathogenesis 135, 103608. [Crossref]
Valencia-Botin, A.J., Gutierrez-Lomeli, M., Morales-Del-Rio, J.A., Guerrero-Medina, P.J., Robles-Garcia, M.A., Ruiz-Cruz, S., Toro-Sánchez, C.L.D. 2018. Inhibitory effect of *Vitex mollis* Kunth extracts against bacteria and *Fusarium* species of human and agricultural importance. Revista fitotecnia mexicana 41, 353–363. [Crossref]
Vaou, N.; Stavropoulou, E.; Voidarou, C., Tsigalou, C., Bezirtzoglou, E. 2021. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms 9, 2041. [Crossref]
Zaki, F.L.M. & Salleh, W.M.N.H.W. 2020. Essential oils and biological activities of the genus *Vitex* (*Lamiaceae*)–A review. Natural Volatiles and Essential Oils 7, 1–9. [Crossref]
Zareshahrabadi, Z., Saharkhiz, M.J., Izadpanah, M., Iraji, A., Emaminia, M., Motealeh, M., Khodadadi, H., Zomorodian, K. 2023. Chemical composition and antifungal and antibiofilm effects of *Vitex pseudo-negundo* essential oil against pathogenic fungal strains. Evidence-Based Complementary and Alternative Medicine 3423440. [Crossref]
Zhelev, I., Petkova, Z., Kostova, I., Damyanova, S., Stoyanova, A., Dimitrova-Dyulgerova, I., Sayed, AA. 2022**.** Chemical composition and antimicrobial activity of essential oil of fruits from *Vitex agnus-castus* L., growing in two regions in Bulgaria. Plants 11, 896. [Crossref]
Zulkifli, L., Basri, M.H., Syukur, A. 2021. Antibacterial activity of *Vitex trifolia* methanol extract against pathogenic bacteria. In Journal of Physics: Conference Series 1793, 012060. [Link]

6.2 Artigo experimental

Artigo experimental submetido (Anais da Academia Brasileira de Ciências – QUALIS A2)

Antifungal, *molecular docking,* antioxidant and cytotoxic effect of the essential oil of *Vitex gardneriana* Schauer against *Candida albicans*

Amanda Lima Barros[1]; Francisca Lidiane Linhares de Aguiar[2*], Andréa Maria Neves[2], Rita Ingrid Sousa Fernandes[2], Matheus Nunes da Rocha[3], Emmanuel Silva Marinho[3], Márcia Machado Marinho[3], Raquel Oliveira dos Santos Fontenelle[1,3]

[1] Master's in Health Sciences, Medical School, Federal University of Ceará, Av. Cmte. Maurocélio Rocha Pontes, 100 - Jocely Dantas de Andrade Torres, Sobral- CE, 62042-250, Brazil;
[2] Department of Agricultural and Biological Sciences, State University Vale of Acarau, Av. Padre Francisco Sadoc de Araujo, 850 – Alto da Brasilia, Sobral- CE, 62010-295, Brazil;
[3] Center for Science and Technology, Postgraduate Program in Natural Sciences, State University of Ceará, Av. Dr. Silas Munguba, 1700 - Itaperi, Fortaleza - CE, 60714-903.

*Corresponding author: Francisca Lidiane Linhares de Aguiar. E-mail: lidianelinhares@yahoo.com.br

Orcid of the authors
Amanda Lima Barros: 0000-0002-0683-7122
Francisca Lidiane Linhares de Aguiar: 0000-0003-1733-2119
Andréa Maria Neves: 0000-0002-7388-1844
Rita Ingrid Sousa Fernandes: 0009-0005-0442-5596
Emmanuel Silva Marinho: 0000-0002-4774-8775
Márcia Machado Marinho: 0000-0002-7640-2220
Matheus Nunes da Rocha: 0000-0002-7640-2220
Raquel Oliveira dos Santos Fontenelle: 0000-0002-8865-5954

Vitex gardneriana antifungal effect against yeast.

MICROBIOLOGIA

Abstract

Vitex gardneriana Shauer, popularly known as "Jaramataia," is widely used in folk medicine for its anti-inflammatory and analgesic properties. This study aimed to determine the chemical composition and evaluate the antifungal, antioxidant, and cytotoxic effects of the essential oil (EO) from its leaves. The EO was obtained via hydrodistillation and analyzed by gas chromatography-mass spectrometry, identifying 16 components, with trans-calamenene (30.28%) and 6,9-guaiadiene (14.06%) as major compounds. Antifungal activity was assessed by broth microdilution, showing a MIC of 5 mg/mL against standard and clinical strains. A synergistic effect was observed when combined with Amphotericin B, significantly reducing

the inhibitory concentration of OEVG. Antioxidant activity (DPPH method) showed an IC_{50} of 85.86 mg/mL, while hemolytic assays on human erythrocytes showed an IC_{50} of 20 mg/mL. *Molecular docking* revealed stronger affinity of EO compounds to Sap5 (-7.3 kcal/mol) than Als3 (Agglutinin-like sequence 3) (-6.2 kcal/mol), with some ligands (e.g., 1-epi-Cubenol, caryophyllene oxide) binding to different regions than Amphotericin B. These findings suggest that V. gardneriana OE has promising antifungal properties, especially when used in combination with existing antifungals.

Keywords: Als3, candidiasis, hydrodistillation, Jaramataia, Sap5.

Introduction

Essential oils consist of a mixture of aromatic and volatile compounds that can be obtained from various parts of plants, mainly from leaves and flowers. Plant-derived essential oils have demonstrated promising antimicrobial therapeutic effects, which are usually accompanied by reduced side effects. For this reason, they are traditionally used as antibacterial and antifungal agents in natural medicine, and the interest in plant-derived bioactives can be explained mainly by the emergence of strains resistant to conventional antimicrobials (Ghavam *et al*., 2020).

The spread of antimicrobial resistance is an emerging global threat to human health, associated with high mortality rates, lack of effective surveillance systems, and few available alternative treatment options (Stevenson *et al*., 2022). However, the global problem of antimicrobial resistance is of particular concern in developing countries, where species resistant to traditional antifungal agents have increased significantly, and infectious diseases are highly prevalent, with cost constraints that prevent the widespread application of newer and more expensive agents (Bastos *et al*., 2023; Vale *et al*., 2019).

Several pathogens are associated with a high level of resistance to conventional antifungals, including species of the genus *Candida*. In recent years, opportunistic fungal infections caused by *C. albicans* have increased significantly. These yeasts can be responsible for severe clinical manifestations, ranging from localized superficial mucocutaneous disorders to invasive diseases involving multiple organ systems and potentially fatal (Talapko *et al*., 2021). The pathogenicity of *C. albicans* is attributed to many determining factors, including the production of enzymes such as the adhesin ALs3 and secreted aspartic protease SAP5 (Silva *et al*., 2022).

Secreted aspartic proteases (SAPs) produced by *Candida* spp. are classified into 10

types, with SAP5 being the most important, since this enzyme can degenerate proteins, facilitating invasion into the host's epithelial tissue. These enzymes have high specificity against substrates present in human proteins belonging to the immune system. Thus, they can bypass the innate immune system. In addition to the genes that express SAPs, another group of genes that is also important in the virulence and pathogenicity of *Candida* species are the ALS, which consist of a group of genes ranging from ALS1 to ALS8, of which ALS3 is the main enzyme of this family and has the greatest effect on adhesion to *C. albicans* species. In addition, ALS3 facilitates the formation of biofilms, in addition to being able to invade epithelial and endothelial tissues, causing endocytosis of host cells and binding to ferritin to obtain iron. These are important mechanisms for the development and resistance to infection (Silva *et al*., 2022).

Therefore, many researchers are focusing on combination therapies with natural antimicrobial agents, such as essential oils. In general, combination therapies have been frequently used to improve biological efficiency; decrease the effective dose to reduce costs and adverse side effects; increase the spectrum of activity, and prevent drug resistance through the multiplicity and structural and functional diversity of bioactive compounds present in the mixture, thus benefiting patients (Benamar-Aissa; Gourine; Yousfi, 2024). Natural compounds derived from plants are considered fundamental sources of new chemical diversity, representing an alternative to synthetic drugs, as potential new drugs against resistant pathogens, due to their significant pharmacological and toxicological properties (Neves *et al*.; 2021).

The genus *Vitex* belongs to the *Lamiaceae* family, with approximately 250 species, and is mainly composed of shrubs, with the bioindicator tree *Vitex gardneriana* Schauer, popularly known as “Jaramataia”, standing out in northeastern Brazil. This plant is commonly used in folk medicine as an anti-inflammatory and analgesic. Scientific studies have reported that this species has antimicrobial, antioxidant and larvicidal activity (Morais *et al*., 2020; Silva *et al*., 2019; Barreto; Almeida; Filho, 2021).

In view of the above, the objective of this work was to determine the chemical composition, evaluate the antioxidant activity of the essential oil obtained from the leaves of *V. gardneriana*, its antifungal potential and its combined effect with Amphotericin B, as well as the computational study of the mechanism of action of the main compounds isolated from the essential oil against the enzymes ALs3 and Sap5 of *C. albicans* and the cytotoxicity profile, in order to promote both knowledge about this species, as well as enhance the discovery of new phytopharmaceuticals to combat resistant fungi.

Materials and methods

Plant material

Fresh leaves of *V. gardneriana* (10.0 kg) were collected at the Experimental Farm of the Vale do Acaraú State University at coordinates 03° 57′ 7″ S 40° 18′ 24.2″ W) (FAEX-UVA), located 11 km from the city of Sobral, Ceará, Brazil. The botanical identification of the species was performed at the Francisco José de Abreu Matos Herbarium (HUVA), State University of Vale do Acaraú -UVA, by Professor Dr. Elnatan Bezerra de Souza, and the voucher was deposited under the registry number HUVA 26174.

Essential oil extraction

Fresh leaves (3.6 kg) of *V. gardneriana* were subjected to hydrodistillation in a Clevenger-type apparatus for 2 h to obtain the essential oil. The yield (p/p) was determined by the ratio between the mass of the oil obtained and the mass of the fresh plant material used in the extraction. After filtering and drying with anhydrous sodium sulfate, the isolated oil was stored in an amber bottle and kept refrigerated for later analysis (Silva *et al*., 2022).

Gas chromatography coupled to mass spectrometry (GC-MS)

The chemical analysis of the constituents of the essential oil of *V. gardneriana* leaves (OEVG) was performed by gas chromatography combined with mass spectrometry (GCMS) in an Agilent model GC-7890B/MSD-5977A (quadrupole) apparatus, with electron impact at 70 eV and HP-5MS methylpolysiloxane column (30 m x 0.25 mm x 0.25 μm, Agilent). Helium was used as carrier gas with a flow rate of 1.00 mL. min-1, with injector temperature of 250 °C, detector temperature of 150 °C, transfer line temperature of 280 °C. With an initial chromatographic oven temperature of 70 °C, and a heating ramp of 4 °C.min-1 up to 180 °C and an increase of 10 °C/min-1 up to 250 °C at the end of the run (34.5 min). The identification of the compounds was performed by analyzing the fragmentation patterns displayed in the mass spectra with those present in the database provided by the equipment (NIST version 2.0 of 2012 - 243,893 compounds) and literature data (Adams, 2017).

***In vitro* antifungal assay**

Microorganisms

In vitro sensitivity tests were performed with clinical isolates of *C. albicans* obtained from patients admitted to the Santa Casa de Misericordia Hospital in Sobral, Ceará. These strains were identified by chromogenic, PCR, and vitek methods and then stored in Sabouraud Dextrose Agar culture medium at the Microbiology Laboratory of the Universidade Estadual Vale do Acaraú (LABMIC-UVA). In this study, a total of 03 strains of *C. albicans* were used, 02 strains from clinical isolates: LABMIC 0102 (blood culture), LABMIC 0105 (blood culture), and *C. albicans* ATCC 90028 used as a standard strain for the analyses.

Preparation of inoculum for antifungal susceptibility testing

In the preparation of the inoculum, fragments of *C. albicans* in Sabouroud Dextrose Agar culture medium were transferred to tubes containing 5 mL of saline solution to obtain concentrations equivalent to 5 x 10^4 UFC/mL on the McFarland scale. The suspension was obtained by diluting 1:100 followed by 1:20 of the standard suspension for *C. albicans* with RPMI 1640 medium with L-glutamine, without sodium bicarbonate, to obtain inoculum concentrations of 2.5–5 x 10^3 UFC mL-1 for *C. albicans*.

Broth Microdilution Assay

Antifungal activity was determined by the broth microdilution method according to the M27-A3 guidelines for yeasts, according to the Clinical and Laboratory Standards Institute (CLSI M27-A3, 2008) protocol, with modifications for natural products. The Minimum Inhibitory Concentration (MIC) was determined according to Fontenelle *et al*., (2008) and the CLSI guidelines document (2008). OEVG (10 mg/mL) was diluted with 945 µL of RPMI (Roswell Park Memorial Institute) 1640, 50 µL of DMSO and 5 µL of tween and homogenized in a vortex mixer. The microdilution test was performed in 96-well microdilution plates, in which OEVG was tested at concentrations ranging from 0.002 to 2.5 mg/mL. Amphotericin B (AMB) was prepared and placed at concentrations ranging from 16 µg/mL to 0.125 µg/mL. Finally, 100 µL of the inoculum was added to the plate wells, and this entire procedure was performed in duplicate. The plates were incubated at 37°C, and visual reading was performed after 48 hours. The MIC is defined as the lowest concentration of the sample capable of inhibiting 100% of visible fungal growth.

Microdilution checkboard assay

The synergistic activity between OEVG and Amphotericin B was determined by the checkboard technique using all strains of *C. albicans*. Initially, 50 µL of RPMI-1640 was added to all wells of the 96-well microdilution plate. Then, 50 µL of each OEVG dilution was added in vertical orientation, with concentrations ranging from 5 mg/mL to 0.03 mg/mL. In horizontal orientation, 50 µL of Amphotericin B (standard antifungal) were placed at concentrations ranging from 16 µg/mL to 0.125 µg/mL, then 100 µL of *C. albicans* suspension (2.5–5 x 103 UFC mL-1) were added to all wells and incubated at 37ºC for 24h. The assays were performed in triplicate. The drug interaction was verified by calculating the Fractional Inhibitory Concentration Index (FICI). The FCI was calculated by adding the Fractional Inhibitory Concentration (FIC) for each of the compounds tested, being defined as the addition of the Minimum Inhibitory Concentration (MIC) values of each drug in the combination divided by the MIC of the drug alone. The ICIF was interpreted as indicative of a synergistic effect at values ≤ 0.5, an indifferent effect at values > 0.5 or ≤4.0, and an antagonistic effect at values >

4.0 (Johnson *et al*., 2004).

Molecular Docking

Ligand and Receptor Preparation for Docking Studies

The chemical structures of the molecules 1-epi-Cubenol (CID519857), 6,9-guaiadiene (CID527113), caryophyllene oxide (CID1742210), epi-alpha-cadinol (CID160799) and trans-calamenene (CID6429022) were obtained from the PubChem repository (https://pubchem.ncbi.nlm.nih.gov/), the lowest energy conformers were saved at physiological pH using the MarvinSketch software (Csizmadia, 1775) and optimized using the Avogadro software (Hanwell *et al*., 2012), configured to use a steepest descent algorithm with cycles of 50 interactions, applying the MMFF94 force field (Merck Molecular Force Field) (Halgren, 1196; Neto *et al*., 2021).

To investigate the mechanism of action of the ligands against *C. albicans*, the structures of the targets Als3 and Sap5 were obtained from the Protein Data Bank repository (https://www.rcsb.org/) (Berman *et al*., 2003), PDBs ID: 4LEB (Lin et al., 2014) and 2QZX (Borelli *et al*., 2008). In the preparation of the targets, residues were removed, polar hydrogens, Kollman charges and Gasteiger charges (Yan *et al*., 2014) were added using the Autodocktools™ software (Huey *et al*., 2012).

***Molecular Docking* Simulation and Data Output**

The *molecular docking* simulations were performed using the software AutodockVina (Trott and Olson *et al*., 2010), Lamarkian Genetic Algorithm (LGA), Exhaustiveness 64 (Marinho *et al*., 2020) and grid box involving the entire structure of the protein targets using the axes (-5,780 x, 2,958 y, -13,744 z) and size (108 x, 122 y, 96 z) against Als3, axes (22,230 x, 17,900 y, 42,750 z) and size (72 x, 76 y, 118 z) against Sap5. Fifty simulations were performed, generating 20 poses per simulation and as a criterion for selecting the best pose, the statistical parameter RMSD (Root Mean Square Deviation) was used with values up to 2.0 Å (Yusuf *et al*., 2008) and affinity energy lower than -6.0 kcal/mol (Shityakov and Forsten, 2014; Silva *et al*., 2022). To obtain comparative data, simulations were performed with the Amphotericin B control.

Visualization of Binding Modes and Receptor–Ligand Interactions

Data were analyzed using UCSF Chimera™ (Pettersen *et al*., 2004), Pymol (Delano, 2020), and Discovery Studio Visualizer™ viewer (Biovia, 2016) software. The Protein-Ligand Interaction Profiler (PLIP) server (Salentin *et al*., 2015; Adasme *et al*., 2021) was used to visualize molecular interactions and hydrogen bonds.

Hemolytic Activity

The hemolytic activity of OEVG was determined in human cells (Opinion Number: 6,662,155) centrifuged for 10 min and distributed in 96-well plates. After plasma removal, the pellet containing red blood cells was washed five times with PBS and then resuspended in PBS to obtain an 8% (v/v) suspension. Then, a 100 µL aliquot of this suspension was added to different microcentrifuge tubes containing 100 µL of 2x serial dilutions of essential oil, ranging from 0.005 to 2.5 mg/mL. The final concentrations were 4% (v/v) of erythrocyte suspension and the essential oil concentration range was 0.01-5.0 mg/mL. The resulting suspensions were incubated in a refrigerated shaker for 60 min at 37 °C. After the incubation time, the samples were centrifuged for 2 min and the supernatants were transferred to 96-well plates for reading by absorbance at 540 nm using a Bio tek Synergy HT multiplate reader. As positive and negative controls, Triton X-100 at 1% and 4% (v/v) of RBCs in PBS without essential oil (untreated), respectively, was used. The test was performed in triplicate and the percentage of hemolysis was calculated using the following expression:(Abs540nm-treated-Abs540nmuntreated)/(Abs540nm1%TritonX-100-Abs540nmuntreated) x 100 (Ahmad *et al*., 2010).

Antioxidant assay

The antioxidant activity of OEVG was determined by the 2,2´-diphenyl-1-picrylhydrazyl (DPPH) radical reduction method, according to the methodology modified by Brand-Williams *et al*. (1995), Sanchez-Moreno *et al*. (1998). Ethanolic solutions of the samples were prepared at concentrations of 5.0; 2.5; 1.25; 0.62; 0.31 and 0.15 mg mL-1. 100 µL of OEVG with 2.4 mL of DPPH (0.04 mg/mL-1) were used. For the control tube, 100 µL of ethanol with 2.4 mL of DPPH were used in triplicate. The reaction medium was kept in the absence of light for 30 minutes, and then the reading was performed at a wavelength of 515 nm. The DPPH radical scavenging activity of the extracts at different concentrations was calculated by expressing the scanning index in percentage (IV%): IV% = (DPPH Abs - Sample Abs / DPPH Abs) x 100. The effective concentration to inhibit 50% of the DPPH free radical (EC50) was obtained using Excel 2019 Software, where, from the values of the final sample concentrations and the scanning index (IV%), scatter plots were generated whose straight-line equations were used to obtain the mean and standard deviation values. For comparison purposes, a calibration curve was constructed with different percentages of the flavonoid quercetin, which has high antioxidant activity against DPPH free radicals.

Results and discussion

Chemical composition of *V. Gardnerian* essential oil

The essential oil from *V. gardneriana* leaves analyzed by GCMS showed a brownish

color with a yield of 0.025%. Sixteen constituents and their respective contents (in %) were identified, which are shown in Table 1.

Table 1. Results of the chemical composition of the essential oil of *V. gardneriana.*

Compound	IA_{calc}[1]	IA_{lit}[2]	Relative area (%)
α-copaene	1376	1374	1.47
β-caryophyllene	1419	1417	0.65
6,9-guaiadiene	1443	1442	14.06
epi-cubebol	1495	1493	0.96
γ-cadinene	1516	1513	0.97
trans-calamenene	1524	1521	30.28
α-calacorene	1543	1544	3.02
β-calacorene	1564	1564	1.25
caryophyllene oxide	1583	1582	6.64
1-epi-cubenol	1628	1627	5.16
epi-α-cadinol	1643	1638	4.64
α-muurolol	1647	1644	1.28
α-cadinol	1655	1652	2.74
cis-calamenen-10-ol	1661	1660	1.71
trans-calamenen-10-ol	1663	1668	1.27
cadalene	1675	1675	1.91

1Calculated arithmetic index.

2Arithmetic index of literature (Van den Dool and Kratz, 1969).

The essential oil presented a high content of sesquiterpenes, with the major constituent being trans-calamenene (30.28%), followed by 6,9-guaiadiene (14.06%), caryophyllene oxide (6.64%), 1-epi-cubenol (5.16%) and epi-α-cadinol (4.64%) (Table e figure 1). Sesquiterpenes are secondary metabolites, belonging to the terpene class that present great diversity of chemical structure, and are composed of 15 carbons, formed by three isoprene units (Hernandez-Leon et al., 2021).

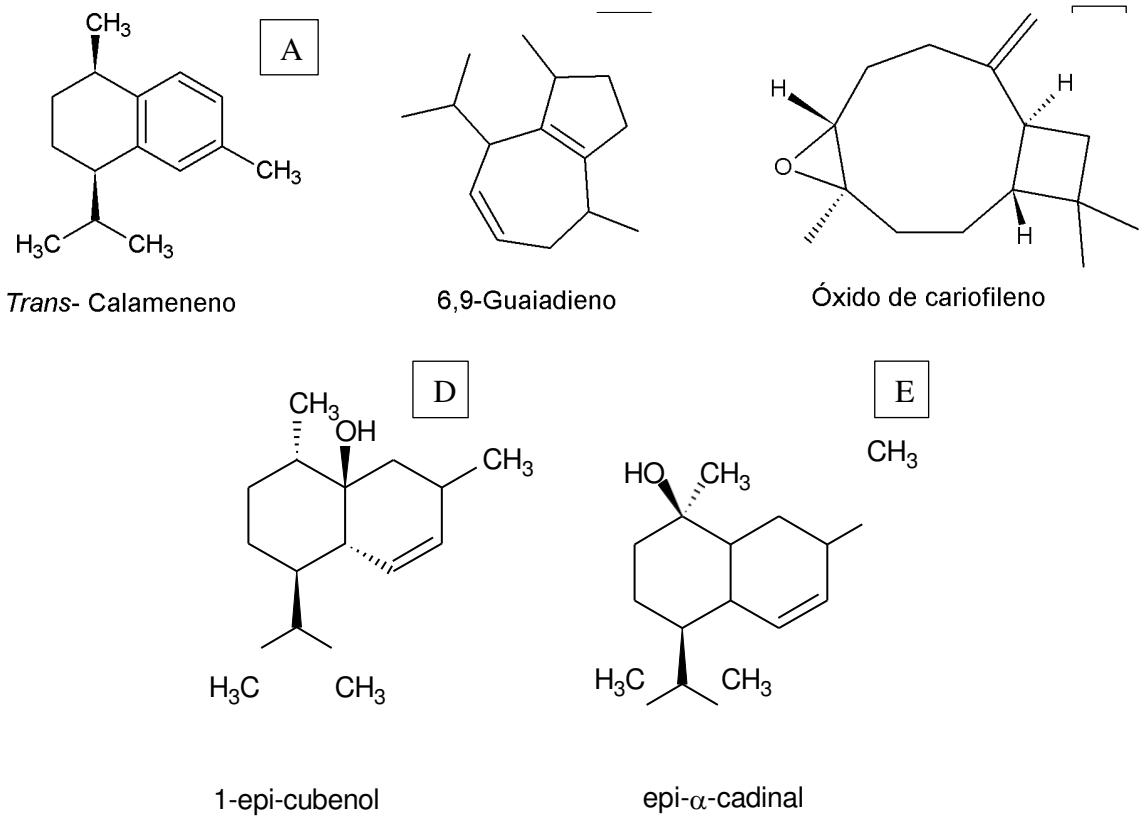


Figure 1. Majority constituents. trans-calamenene (A), 6,9-guaiadiene (B), caryophyllene oxide (C), 1-epi-cubenol (D) and epi-α-cadinol (E).

The chemical composition of the essential oil of *V. gardneriana* showed a high content of sesquiterpenes, the same result recorded by Vale *et al*., 2019; Pereira *et al*., 2018. However, these same authors identified cis-calamene as the major constituent (29.7%) in the essential oil of *V. gardneriana* leaves and significant amounts of 6,9-guaiadiene (14.5%), caryophyllene oxide (14.0%) and 1-epi-cubenol (3.2%). Among these compounds, only 6,9-guaiadiene, caryophyllene oxide and 1-epi-cubenol were found in the present study. Plant metabolism is affected by climatic conditions, resulting in the production of different substances in different proportions. Furthermore, in oil plants, oil yield and chemical composition vary considerably due to intrinsic (sexual, seasonal, ontogenetic and genetic variations) and extrinsic (ecological and environmental aspects) factors, which act with varying degrees of intensity, therefore mediating the quantity and nature of the substances produced. This result is in agreement with the literature on the chemical composition of essential oils related to the genus *Vitex*, which is characterized by the predominant presence of sesquiterpenes, including 6,9-guaidiene, a terpenoid, which can be a good chemosystematic biomarker for the genus (Vale *et al*., 2019; Silva *et al*., 2019; Filho *et al*., 2017; Asdadi *et al*., 2015).

Figure 2 shows the chromatogram of the OEVG, indicating the major compounds identified. The sesquiterpene trans-calamenene was identified as the predominant compound,

followed by 6,9-guaiadiene, caryophyllene oxide, 1-epi-cubenol and epi-α-cadinol.

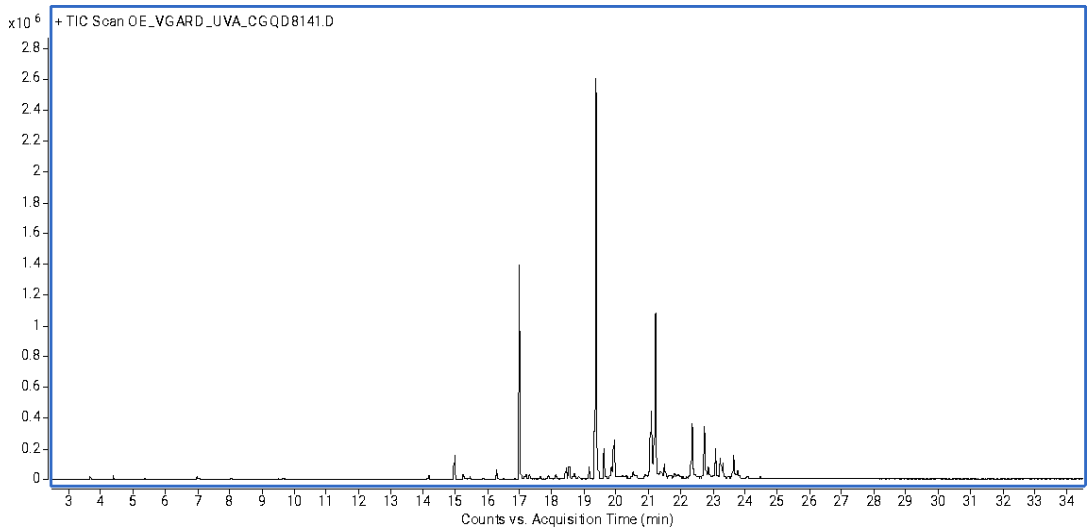


Figure 2. Chromatogram obtained by GC-MS for analysis of the chemical composition of the essential oil of *V. gardineriana.*

Antifungal activity

Regarding the antifungal assays, OEVG showed inhibitory activity against the control strain and against all clinical isolates of *C. albicans* with a MIC of 5,0 mg/mL. All tested strains of *C. albicans* were also sensitive to Amphotericin B at a concentration of 1 µg/mL (Table 02).

Table 2 - Minimum inhibitory concentration (MIC) of OEVG against *C. albicans* cells.

Strains	Origin	MIC (µg/ml)
ATCC 90028	ATCC	5,000
LABMIC 0102	blood culture	5,000
LABMIC 0105	blood culture	5,000

Source: LABMIC: Microbiology Laboratory

The antifungal activity of essential oils is related to their hydrophobic property, so that, when coming into contact with the fungal cell, the components of this mixture interact with the different fungal organelles, such as mitochondria and plasma membrane, thus altering their permeability and consequently causing structural disorders, which can promote the exposure of the cell contents, including the nucleus, causing cell death (Neves *et al*., 2019).

In the literature, some studies show anti-*Candida* properties for essential oils from

different species of the genus *Vitex*, which proves to be a genus of great relevance for the isolation and identification of the compounds responsible for this activity (Zareshahrabadi *et al*., 2023; Asdadi *et al*., 2015; Ai *et al*., 2013). Vale *et al*., (2019) demonstrated that the essential oil obtained from the leaves of *V. gardneriana* was able to reduce the growth of *C. albicans* and *C. tropicalis* at all concentrations tested. The essential oil showed approximately 70% to 9% and 30% to 3% growth reduction in *C. albicans* and *C. tropicalis*, respectively. The essential oil from the leaves of *V. agnus-castus* showed antifungal activity against *C. albicans, C. tropicalis, C. parapsilosis* and *C. dubliniensis* with MIC of 3.1 µL/mL and MFC of 6.3 µL/mL for all species studied, while for *C. krusei* the MIC was 12.5 µL/mL and MFC of 12.5 µL/mL (Katiraee *et al*., 2015). Also, for this species, Maltas *et al*., (2010) observed antifungal activity of the methanolic extract against *C. albicans* by the disk diffusion method (11 mm at 100 mg/ml-1) and broth microdilution method, with MIC of 0.39 mg/ml-1.

It is worth mentioning that this antifungal activity presented by OEVG may be associated with its secondary metabolites, such as sesquiterpenes, including caryophyllene oxide, which has already been reported in previous studies as having antifungal activity. It is worth remembering that essential oils are complex mixtures of several compounds, therefore, it is difficult to attribute their biological activity to a particular constituent, since a synergistic phenomenon is also possible between several components of the essential oil, including those that are present in small concentrations (Barros *et al*., 2023; Asdadi *et al*., 2015). Therefore, the antifungal property of OEVG in the present study can be attributed to its main compounds and their synergistic effects.

Checkerboard assay

The interaction assays between OEVG and Amphotericin B (AmB) on *C. albicans* strains ATCC 90028, LABMIC 0102 and LABMIC 0105 are shown in Table 3.

Table 3 - Evaluation of the synergistic effect of OEVG associated with AMB against *C. albicans*

	OEVG		Amphotericin B		ICIF	Effect
C. albicans	MIC (µg/mL) Individual	MIC (µg/mL) combined	CIM (µg/mL) Individual	CIM (µg/mL) combined		
ATCC 90028	5,000	150	1	0.12	0,.5	Synergic

LABMIC 0102	5,000	150	1	0.12	0.15	Synergic
LABMIC 0105	5,000	310	1	0.25	0.31	Synergic

ICIF: Fractional Inhibitory Concentration Index was defined as synergistic when ICIF ≤ 0.5, indifferent effect in values > 0.5 or ≤ 4.0, and antagonistic effect in values > 4.0.

Combinations of natural compounds with conventional antifungals can improve or facilitate the interaction of an antimicrobial agent with its target within the pathogen and thus prevent the emergence of resistance. Such a strategy can reduce toxicity, since lower concentrations of both agents can be used, providing a more effective alternative against fungal infections (Blanc *et al*., 2023; Ayaz *et al*., 2019). Furthermore, such combinations show an increase in the spectrum of activity and biological efficiency (Benamar-Aissa; Gourine; Yousfi, 2024).

Thus, according to the results found, the combinations of OEVG and AmB provided very pronounced synergistic effects in relation to all *Candida* strains tested, with ICIF values between 0.15 and 0.31 µg/mL (Table 3). Thus, the combined therapeutic effect of OEVG with AmB was greater than their individual effects, showing a significant reduction in the MIC values of the essential oil against all strains, as well as a promising reduction in the MIC of AmB for the *Candida* strains tested by up to 8 times.

In the literature, no studies were found on the synergistic potential of Amphotericin B with the essential oil of *V. gardneriana* against clinical strains of *C. albicans*, this being the first report of a study of synergistic activity for the referred species. However, modulatory activity tests carried out with the essential oil of *V. gardneriana* leaves combined with Cetoconazole showed that there was a significant reduction in the MIC value of the essential oil against the dermatophyte *Trichophyton rubrum*, as well as a significant reduction in the MIC of Ketoconazole from 0.1 to 0.5 for the T. rubrum strain (Pereira *et al*., 2018).

The results of the modulatory activity showed that the combined use of the natural product (OEVG) and AmB reduced the MIC of this antifungal agent from 1 µg/mL to 0.12 and 0.25 µg/mL. This result indicates that these natural products have an effect on *C. albicans* strains and a possible mechanism for the inhibition of microbial growth may be the hydrophobic nature of the constituents of the natural products, which can act on the plasma membrane, making it more permeable to antifungal agents and, thus, affecting the biochemical apparatus of the respiratory and energy production chain (Nogueira Sobrinho *et al*., 2020).

Molecular Docking

No estudo de *molecular docking* foram observados valores de RMSD (Root Mean Square Deviation) na ordem de 0.631 e 1.974 Å (Tabela 4) e energia de afinidade na ordem de -8.1 a -5.4 kcal/mol (Figura 3). O redocking dos inibidores co-cristalizados frente a Als3 (Hepta-Thr) e Sap5 (PepA) mostrou valores de RMSD na ordem de 1.998 Å e 1.610 Å e energia de afinidade de -6.2 kcal/mol e -7.3 kcal/mol respectivamente. A análise dos padrões de interação mostrou que os compostos avaliados apresentaram Hydrogen Bonds, interações Hydrophobic frente a Als3; Hydrogen Bonds, interações Hydrophobic e Salt Bridge frente a Sap5 (Table 4).

Table 4. RMSD, amino acids residues, distances and interaction types of the compounds against the *C. albicans* receptors

Als3/Ligand	RMSD (Å)	Residue	Interaction	Distance (Å)
1-epi-Cubenol	1.802	Phe-60A	Hydrophobic	3.67
		Thr-65A	Hydrophobic	3.74
		Pro-174A	Hydrophobic	3.79
6,9-Guaiadiene	0.631	Ser-125A	Hydrophobic	3.75
		Leu-128A	Hydrophobic	3.35
		Leu-128A	Hydrophobic	3.79
		Glu-129A	Hydrophobic	3.89
		Lys-132A	Hydrophobic	3.82
		Ser-229A	Hydrophobic	3.62
		Ser-229A	Hydrophobic	3.93
Caryophyllene oxide	1.974	Lys-59A	Hydrophobic	3.56
		Phe-60A	Hydrophobic	3.67
		Phe-81A	Hydrophobic	3.78
		Pro-174A	Hydrophobic	3.64
		Pro-174A	Hydrophobic	3.67
		Ala-83A	H-Bond	2.34
Epi-alpha-Cadinol	1.866	Lys-59A	Hydrophobic	3.70
		Thr-65A	Hydrophobic	3.97
		Pro-174A	Hydrophobic	3.41

		Pro-174A	Hydrophobic	3.94
		Thr-62A	H-Bond	2.10
Trans-Calamenene	1.067	Val-161A	Hydrophobic	3.68
		Val-161A	Hydrophobic	3.96
		Leu-167A	Hydrophobic	3.63
		Leu-293A	Hydrophobic	3.70
		Leu-293A	Hydrophobic	3.72
		Trp-295A	Hydrophobic	3.70
Amphotericin B	1.477	Phe-87A	Hydrophobic	3.80
		Glu-86A	H-Bond	3.38
		Ser-125A	H-Bond	3.20
		Asn-212A	H-Bond	2.21
		Val-215A	H-Bond	2.33
		Ser-230A	H-Bond	3.17

Sap5/Ligand	**RMSD (Å)**	**Residue**	**Interaction**	**Distance (Å)**
1-epi-Cubenol	1.851	Ile-12B	Hydrophobic	3.62
		Ile-30B	Hydrophobic	3.49
		Trp-51B	Hydrophobic	3.63
		Tyr-84B	Hydrophobic	3.79
		Arg-120B	Hydrophobic	3.46
		Ile-123B	Hydrophobic	3.60
		Ile-12B	H-Bond	2.54
6,9-Guaiadiene	1.339	Ile-12B	Hydrophobic	3.99
		Trp-51B	Hydrophobic	3.52
		Ala-119B	Hydrophobic	3.75
		Ile-123B	Hydrophobic	3.74
		Thr-221B	Hydrophobic	3.75
Caryophyllene oxide	1.647	Phe-128B	Hydrophobic	3.46
		Ile-142B	Hydrophobic	3.62
		Leu-320B	Hydrophobic	3.81
Epi-alpha-Cadinol	1.325	Thr-261B	Hydrophobic	3.63
		Val-330A	Hydrophobic	3.87
Trans-Calamenene	1.016	Thr-261B	Hydrophobic	3.59

		Pro-329A	Hydrophobic	3.62
		Val-330A	Hydrophobic	3.75
Amphotericin B	1.880	His-8B	Hydrophobic	3.94
		Asn-160B	H-Bond	2.90
		Glu-163B	H-Bond	1.98
		Glu-163B	H-Bond	1.99
		Ser-165B	H-Bond	3.00
		Ser-165B	H-Bond	3.26
		Thr-166B	H-Bond	1.94
		Thr-166B	H-Bond	2.53
		Arg-204A	H-Bond	3.57
		Asp-255A	H-Bond	3.32
		Asp-255A	H-Bond	3.54
		Arg-204A	Salt Bridge	5.23

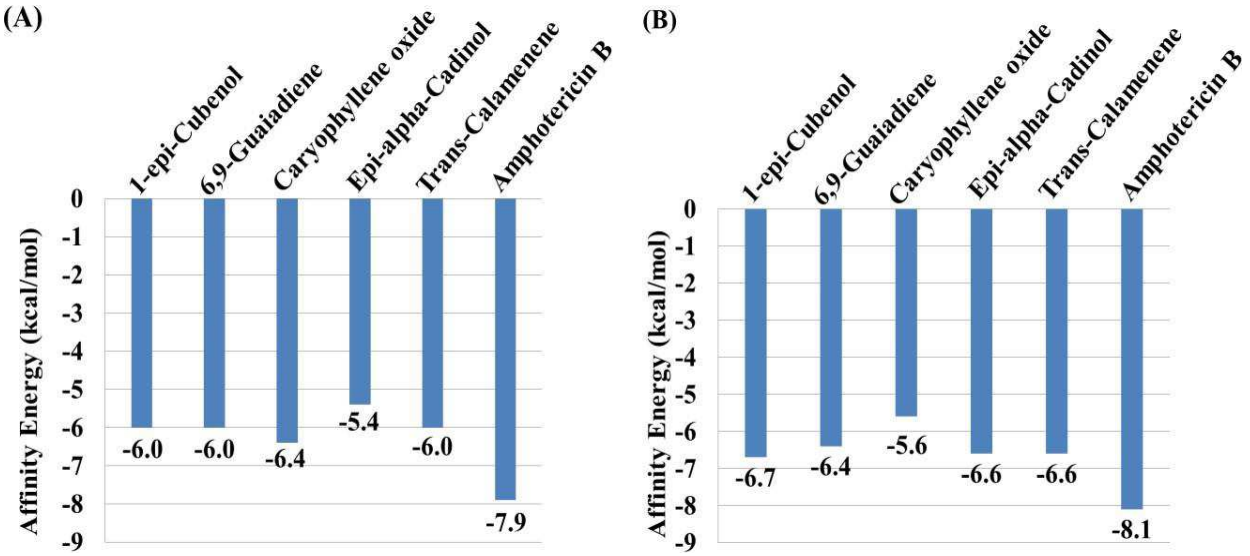


Figure 3: Affinity energy (kcal/mol) of the compounds against Als3 (A) and Sap5 (B)

The *molecular docking* study showed that, with the exception of caryophyllene oxide, the compounds presented better affinity energy values against Sap5, indicating that they have more affinity for Sap5 than for Als3 (Figure 3). We also observed that the control Amphotericin B presented the best affinity energy values against Als3 and Sap5 compared to the evaluated

compounds.

The interaction profile of the ligands was compared to the interaction profile of the control Amphotericin B. Detailed analysis of the interactions against Als3 showed that 1-epi-Cubenol, caryophyllene oxide, epi-alpha-cadinol and trans-calamenene interact in a region different from the binding site of the control. We also observed that 6,9-Guaiadiene binds close to the binding site of Amphotericin B, presenting a common interaction with the amino acid residue Ser-125A (Figure 4.C).

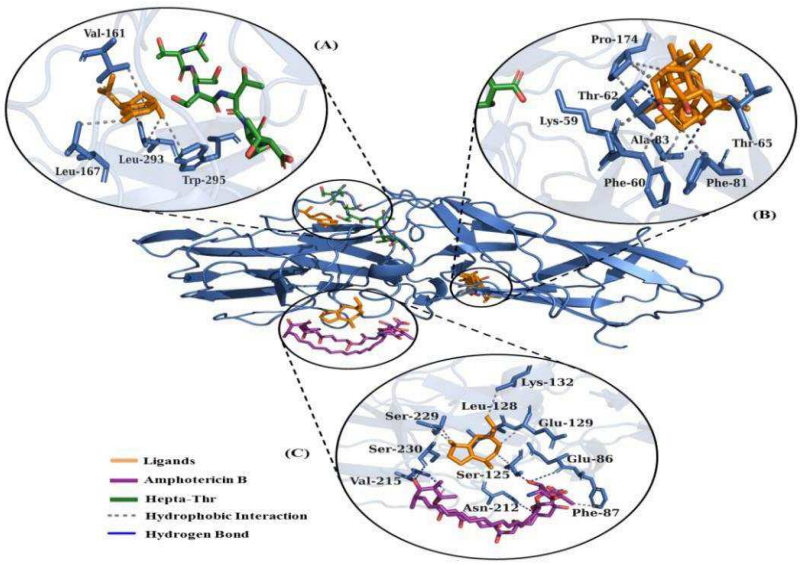


Figure 4. Binding sites against Als3. Trans-calamenene and Hepta-Thr (inhibitor co-crystallised) (A), 1-epi-Cubenol, caryophyllene oxide, epi-alpha-cadinol (B), 6,9-Guaiadiene and Amphotericin B (C).

The binding site of the co-crystallized hepta-Thr inhibitor is formed by residues Ala-19, Tyr-21, Asn-22, Gly-27, Thr-28, Lys-59, Thr-61, Ala-116, Tyr-166, Thr-168, Ser-170, Val-172, Arg-294, Trp-295, Thr-296, Tyr-298. Comparing to the hepta-Thr/Als3 complex, we observed that the compounds caryophyllene oxide, epi-alpha-cadinol and trans-calamenene bind close to the binding site of the co-crystallized inhibitor, having in common interactions with residues Lys-59A (caryophyllene oxide and epi-alpha-cadinol) (Figure 4. B) and Trp-295A (trans-calamenene) (Figure 3. A), indicating an effect similar to hepta-Thr. We also observed that 1-epi-Cubenol bound to the same region as caryophyllene oxide, epi-alpha-cadinol; however, no significant interactions were observed against Als3 (Table 4).

Against Sap5, we observed that the compounds bind to different regions of the Amphotericin B binding site (Figure 5. D), suggesting a possible synergistic effect with the control used. The binding site of the co-crystallized PepA inhibitor on chains A and B of Sap5 is formed by residues Ile-12, Asp-32, Gly-34, Ser-35, Lys-83, Tyr-84, Gly-85, Asp-86, Ile-123, Gly-220, Thr-221, Thr-222, Ile-223 and Ile-305. Data analysis showed that epi-alpha-cadinol,

trans-calamenene (Figure 5. B) and caryophyllene oxide (Figure 5. C) bind to different regions of the binding site region of the co-crystallized inhibitor, suggesting a possible synergistic effect with PepA. It also showed that 1-epi-Cubenol and 6,9-Guaiadiene bind to chain B, in the same region of the PepA binding site, having in common interactions with residues Ile-12B, Tyr-84B, Ile-123B (1-epi-Cubenol); Ile-12B, Ile-123B and Thr-221B (6,9-Guaiadiene), indicating that they have similar action to the co-crystallized inhibitor (Figure 5. A).

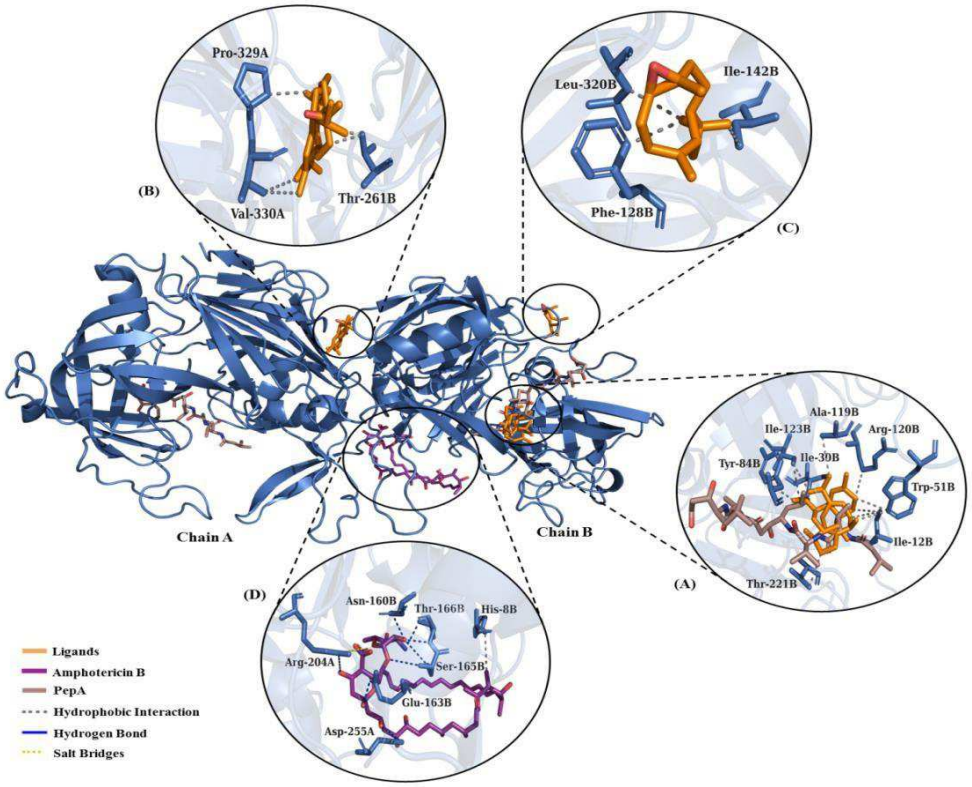


Figure 5. Binding sites against Sap5.1-epi-Cubenol, 6,9-Guaiadiene and PepA (inhibitor co-crystallised) in the B chain (A); epi-alpha-cadinol and trans-calamenene (B); caryophyllene oxide (C) and Amphotericin B (D).

The expansion of models for in silico analysis using biological macromolecules deposited in databases has contributed significantly to the evaluation of hypotheses in the field of pharmacology, as well as in the investigation of correlation with biological information, constituting a favorable scenario for the application of computational systems for predicting binding affinity with target proteins, in addition to studies in the area of drug development and design. Among the tools used, *molecular docking* stands out in microbiology for its ability to prospect protein targets in microorganisms, in addition to molecules with high affinity (Silva *et al*., 2022).

No *molecular docking* studies were found in the literature with the essential oil of *V. gardneriana* or even with its main compounds. However, it has been reported in the literature that sesquiterpene compounds suppress enzymes, such as SAP (Azevedo *et al*., 2016). The

molecular docking results show that the compound trans-calamenene interacts with residues of the binding site of the Als3/hepta-Thr complex and the ligands 1-epi-Cubenol and 6,9-Guaiadiene interact with residues of the binding site of the Sap5/PepA complex, indicating that they have similar action to the hepta-Thr and PepA inhibitors co-crystallized in Als3 and Sap5, respectively. This study provides preliminary information on the best candidates that showed interactions with some catalytic residues of the target enzymes. Consequently, the proposed chemicals can be used as a guide for the development of a lead compound for drug discovery for candidiasis in future research. These compounds may hold the key to the future discovery of a new drug or the development of existing drugs through chemical structural change.

Hemolytic activity

Tests to determine the *in vitro* hemolytic activity of OEVG were performed at concentrations of 0.01-5.0 mg/mL. Based on the results observed, OEVG demonstrated an IC50 of 20 mg/mL (Figure 6). The hemolytic action of a natural product or medicine can occur through several mechanisms, from the dissolution or increase in the permeability of cell membranes to complete cell lysis. The methods for determining hemolytic activity *in vitro* consist of verifying possible damage caused by substances present in essential oils to erythrocyte membranes, which, when lysed, release hemoglobin (Pereira *et al*., 2018). There are records in the literature on hemolytic activity for the species studied, in which the essential oil of *V. gardneriana* did not show cytotoxicity (IC50 > 2.50 mg/mL) by the hemolysis assay (Pereira *et al*., 2018).

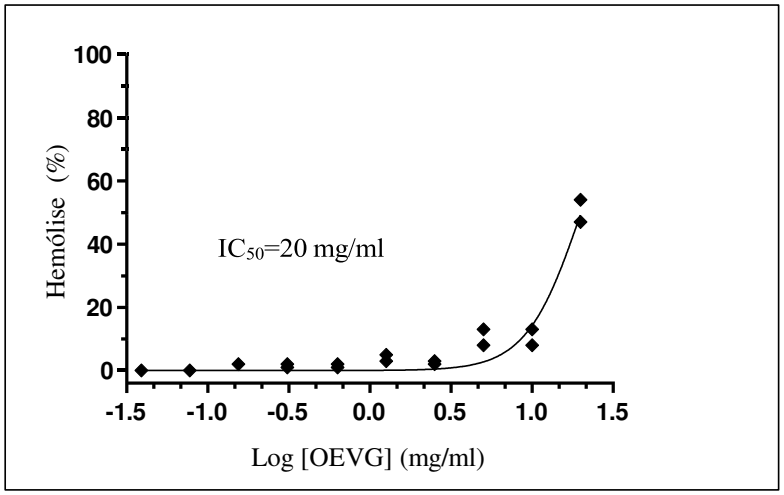


Figure 6. Hemolytic activity of *V. gardneriana* Schauer essential oil.

Antioxidant Assay

The antioxidant potential of *V. gardneriana* essential oil was quantified as the concentration required to inhibit 50% of the DPPH radical (EC50) (Table 5).

Table 5 - Results of the antioxidant activity (DPPH) assay of *V. gardneriana* leaf essential oil

SAMPLE	DPPH CE_{50} ($\mu g.mL^{-1}$)	Quercetina
OEFVG	85,86 ± 0,00	1,03 ± 0,00

All analyses were performed in triplicate and results were expressed as mean ± standard deviation.

The results showed that the EC50 value for OEVG was 85.86 ± 0.00 mg/mL. The low antioxidant activity demonstrated by the essential oil of *V. gardneriana* leaves can be explained by the low concentration of compounds with this potential. Similar to our results, previous studies revealed that OEVG also showed low antioxidant activity (Vale *et al*., 2019).

Conclusion

In vitro tests showed that OEVG has potential against standard and clinical strains of the species *C. albicans*. This activity may be associated with secondary metabolites found in its composition, such as caryophyllene oxide, which has been reported in previous studies for its antifungal activity. The data found suggest a possible synergistic effect of the ligands on the Amphotericin B control profile. It was found that 1-epi-Cubenol has a common interaction with the amino acid residue Ser-125A. The *molecular docking* performed showed greater affinity with SAP5 than with Als3.

The preliminary information reported in this study presents the interactions obtained with some catalytic residues of the target enzymes, as well as the microbiological activity found, chemical composition and cytotoxicity profile of OEVG. Thus, these reported findings require further studies in order to better understand how OEVG behaves *in vivo* assays, and thus, enable the development of a compound that will enable the discovery of drugs for candidiasis in future research.

Acknowledgments
The authors thank to FUNCAP, from the state government, for financial support.

Conflicts of interest
The authors declare no conflicts of interest.

Ethical approval
Opinion Number: 6,662,155 regarding the cytotoxicity test with human blood cells

Author Contribution

ALB: Data curation, formal analysis, investigation and original draft preparation. FLLA: Data curation, investigation, methodology, visualization and writing - review & editing. AMN: Investigation, supervision, writing- review & editing. RISF: Data curation and investigation. MNR: Data curation and investigation. MM: Formal analysis, investigation, methodology and writing - review & editing. ESM: Investigation, resources, supervision, validation. ROSF: Methodology, project administration, resources, supervision and writing - review & editing. All authors have read and approved the submission of the manuscript.

References

ADAMS R P. 2017. Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Gruver, TX USA: Texensis Publishing 4: 46-52. http://www.juniperus.org/uploads/2/2/6/3/22639912/bk4frontisbnpreface-contents5thedonline2017.pdf

AHMAD A, KHAN A, MANZOOR N, & KHAN L A. 2010. Evolution of ergosterol biosynthesis inhibitors as fungicidal against Candida. Microbi. Pathogenes 48(1): 35-41. https://doi.org/10.1016/j.micpath.2009.10.001

AI H W, KANG YX, CAO Y, & ZHENG CJ. 2014. Antifungal Properties and Chemical Analysis of Essential Oil from *Vitex negundo* Seeds. Journal of Pharmaceutical Research International 4:541-548. https://doi.org/10.9734/BJPR/2014/7079

ASDADI A, HAMDOUCH A, OUKACHA A, MOUTAJ R, GHARBY S, HARHAR H, M. HADEK EL, CHEBLI B & HASSANI L I. 2015. Study on chemical analysis, antioxidant and in vitro antifungal activities of essential oil from wild *Vitex agnus-castus* L. seeds growing in area of Argan Tree of Morocco against clinical strains of *Candida* responsible for nosocomial infections. Journal de mycologie medicale 25(4): 118-e127. https://doi.org/10.1016/j.mycmed.2015.10.005

MUHAMMAD AYAZ M A, FARHAT ULLAH FU, ABDUL SADIQ AS, FARMAN ULLAH FU, MUHAMMAD OVAIS MO, JAWAD AHMED JA, & DEVKOTA HP. 2019. Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance.Chemico-Biological Interactions 308: 294-303. https://doi.org/10.1016/j.cbi.2019.05.050

AZEVEDO MM, ALMEIDA CA, CHAVES FC, RODRIGUES IA, BIZZO HR, ALVIANO CS, ALVIANO DS. 2016. 7-hydroxycalamenene Effects on Secreted Aspartic Proteases Activity and Biofilm Formation of Candida spp. Pharmacogn Mag 12(45):36-40. PMID: 27019560; PMCID: PMC4787334. https://doi.org/10.4103/0973-1296.176022

BARROS DB ET AL. 2023. Antifungal activity of terpenes isolated from the Brazilian Caatinga: a review. Brazilian Journal of Biology, *83*, e270966. https://doi.org/10.1590/1519-6984.270966

BASTOS RC, ALVES EF, NEVES AM, VASCONCELOS CFM, BRITO EHS & FONTENELLE ROS. 2023. Molecular diagnosis compared to conventional diagnosis for rapid detection of resistant strains of Candida spp. Research Society Dev 12(4):e14112441088. https://doi.org/10.33448/rsd-v12i4.41088

BENAMAR-AISSA B, GOURINE N, OUINTEN M & YOUSFI M. 2024. Synergistic effects of essential oils and phenolic extracts on antimicrobial activities using blends of Artemisia campestris, Artemisia herba alba, and Citrus aurantium. Biomol Concepts 15(1):20220040. https://doi.org/10.1515/bmc-2022-0040

BLANC AR, SORTINO MA, BUTASSI E & SVETAZ LA. 2023. Synergistic effects of Thymus vulgaris essential oil in combination with antifungal agents and inhibition of

virulence factors of Candida albicans. Phytomed Plus 3(4):100481. https://doi.org/10.1016/j.phyplu.2023.100481

BRAND-WILLIAMS W, CUVELIER ME & BERSET CLWT. 1995. Use of a free radical method to evaluate antioxidant activity. LWT Food Sci Technol 28(1):25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

PA W. 2002. Reference method for broth dilution antifungal susceptibility testing of yeasts, approved standard. CLSI Document M27-A2.

SENA-FILHO JG ET AL. 2017. Volatile metabolomic composition of *Vitex* species: chemodiversity insights and acaricidal activity. Front Plant Sci 8:1931. https://doi.org/10.3389/fpls.2017.01931

FONTENELLE ROS, MORAIS SM, BRITO EHS, BRILHANTE RSN, CORDEIRO RA, NASCIMENTO NRF ET AL. 2008. Antifungal activity of essential oils of Croton species from the Brazilian Caatinga biome. J Appl Microbiol 104(5):1383–1390. https://doi.org/10.1111/j.1365-2672.2007.03707.x

GHAVAM M, MANCA ML, MANCONI M & BACCHETTA G. 2020. Chemical composition and antimicrobial activity of essential oils obtained from leaves and flowers of Salvia hydrangea DC. ex Benth. Sci Rep 10(1):15647. https://doi.org/10.1038/s41598-020-73193-y

HERNANDEZ-LEON A, MORENO-PÉREZ GF, MARTÍNEZ-GORDILLO M, AGUIRRE-HERNÁNDEZ E, VALLE-DORADO MG, DÍAZ-REVAL MI & GONZÁLEZ-TRUJANO ME. 2021. *Lamiaceae* in Mexican species, a great but scarcely explored source of secondary metabolites with potential pharmacological effects in pain relief. Molecules 26(24):7632. https://doi.org/10.3390/molecules26247632

JOHNSON MD, MACDOUGALL C, OSTROSKY-ZEICHNER L, PERFECT JR & REX JH. 2004. Combination antifungal therapy. Antimicrob Agents Chemother 48(3):693–715. https://doi.org/10.1128/aac.48.3.693-715.2004

KATIRAEE F, MAHMOUDI R, TAHAPOUR K, HAMIDIAN G & EMAMI SJ. 2015. Biological properties of *Vitex agnus-castus* essential oil (phytochemical component, antioxidant and antifungal activity). Biotechnol Health Sci 2(2):e26797. https://www.researchgate.net/publication/278381569

MALTAS E, UYSAL A, YILDIZ S & DURAK Y. 2010. Evaluation of antioxidant and antimicrobial activity of *Vitex agnus castus* L. Fresenius Environ Bull. https://www.researchgate.net/publication/256394211

NEVES AM, SANTOS SILVA H, SOUSA EB, FONTENELLE ROS, SILVA AC & MORAIS SM. 2019. Perfil fitoquímico e avaliação da atividade antifúngica da fração hexânica de Mitracarpus baturitensis (Rubiaceae). Essentia Rev Cult Ciênc Tecnol UVA. https://essentia.uvanet.br/index.php/ESSENTIA/article/view/241

NEVES AM, FONTENELLE ROS, LOPES FS, MENDES JFS, RODRIGUES ALM, MARINHO M, MARINHO ES & MORAIS SM. 2021. Perfil fenólico, atividade antioxidante e antifúngica de extratos de quatro plantas medicinais da família Anacardiaceae. Pesqui Soc Desenvolv 8:e44510817421. https://doi.org/10.33448/rsd-v10i8.17421

NOGUEIRA SOBRINHO AC ET AL. 2020. Antifungal and antioxidant activities of Vernonia chalybaea Mart. ex DC. essential oil and their major constituent β-caryophyllene. Braz Arch Biol Technol 63:e20190177. https://doi.org/10.1590/1678-4324-2020190177

PEREIRA EJP, SILVA HC, HOLANDA CL, MENEZES JESA, SIQUEIRA SMC, RODRIGUES THS, FONTENELLE ROS, VALE JPC, SILVA PT, SANTIAGO GP & SANTOS HS. 2018. Composição química, citotoxicidade e atividade larvicida contra Aedes aegypti de óleos essenciais de *Vitex gardneriana* Schauer. Bol Latinoam Caribe Plant Med

Aromat 17:302–309. https://www.researchgate.net/publication/325286592

PEREIRA EJP ET AL. 2018. Circadian rhythm, and antimicrobial and anticholinesterase activities of essential oils from *Vitex gardneriana*. Nat Prod Commun 13(5):1934578X1801300528. https://doi.org/10.1177/1934578X1801300528

SÁNCHEZ-MORENO C, LARRAURI JA & SAURA-CALIXTO F. 1998. A procedure to measure the antiradical efficiency of polyphenols. J Sci Food Agric 76(2):270–276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-

DA SILVA BF, SOUSA MARTINS S, QUEIROZ MARTINS MG, PEREIRA AMG, VALE JPC, SANTOS HS ET AL. 2022. Composição química, polifenóis totais, atividade antioxidante e citotóxica do extrato etanólico de frutos da *Vitex gardneriana* Schauer. Research Society Dev 11(4):e52311427265. https://doi.org/10.33448/rsd-v11i4.27265

DA SILVA CR, MOURA FLD, ALVES FILHO ALA, SOUSA CAMPOS R, FARIAS CABRAL VP, SÁ LGDAV ET AL. 2022. Evaluation of interactions of silibinin with the proteins ALS3 and SAP5 against Candida albicans. J Health Biol Sci 10(1):1–6. https://doi.org/10.12662/2317-3076jhbs.v10i1.4239.p1-6.2022

SILVA PT ET AL. 2019. Seasonal variation in the chemical composition and larvicidal activity against Aedes aegypti of essential oils from *Vitex gardneriana* Schauer. S Afr J Bot 124:329–332. https://doi.org/10.1016/j.sajb.2019.04.036

STEVENSON EM, GAZE WH, GOW NA, HART A, SCHMIDT W, USHER J, WARRIS A, WILKINSON H & MURRAY AK. 2022. Antifungal exposure and resistance development: defining minimal selective antifungal concentrations and testing methodologies. Front Fungal Biol 3:918717. https://doi.org/10.3389/ffunb.2022.918717

TALAPKO J, JUZBASIC M, MATIJEVIC T, PUSTIJANAC E, BEKIC S, KOTRIS I & SKRLEC I. 2021. Candida albicans—the virulence factors and clinical manifestations of infection. J Fungi 7(2):79. https://doi.org/10.3390/jof7020079

VAN DEN DOOL H & KRATZ PD. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J Chromatogr. https://doi.org/10.1016/S0021-9673(01)80947-X

VALE JPC ET AL. 2019. Chemical composition, antioxidant, antimicrobial and antibiofilm activities of *Vitex gardneriana* Schauer leaves's essential oil. Microb Pathog 135:103608. https://doi.org/10.1016/j.micpath.2019.103608

ZARESHAHRABADI Z, SAHARKHIZ MJ, IZADPANAH M, IRAJI A, EMAMINIA M, MOTEALEH M, KHODADADI H, ZOMORODIAN K. 2023. Chemical composition and antifungal and antibiofilm effects of *Vitex pseudo-negundo* essential oil against pathogenic fungal strains. Evid Based Complement Alternat Med 2023:3423440. https://doi.org/10.1155/2023/3423440

6 CONCLUSÃO

Dentre as espécies do gênero *Vitex*, destacam-se *V. agnus-castus* e *V. negundo* por serem amplamente estudadas na literatura científica; entretanto, no Brasil, a espécie *V. gardneriana* é notável por sua ampla distribuição e uso tradicional por comunidades locais. O potencial de bioprospecção do óleo essencial de *V. gardneriana* Schauer (OEVG) pode estar relacionado à presença de sesquiterpenos como constituintes majoritários em sua composição química. Os resultados obtidos demonstraram que o OEVG apresentou maior eficácia antifúngica frente a cepas padrão e clínicas de *Candida albicans* quando combinado com a Anfotericina B, evidenciando um efeito sinérgico entre os compostos. Além disso, o OEVG não apresentou citotoxicidade em células humanas, o que reforça sua segurança potencial e o posiciona como uma alternativa promissora no desenvolvimento de novos agentes antifúngicos. No estudo de *molecular docking*, foram observadas afinidades energéticas relevantes, especialmente frente à proteína Sap5, com destaque para os compostos *epi-α-cadinol*, *trans-calameneno* e óxido de cariofileno. A análise dos perfis de interação revelou que esses compostos estabelecem ligações específicas, como *hydrogen bonds*, interações hidrofóbicas e *salt bridges*, sugerindo mecanismos de ação distintos e, em alguns casos, semelhantes aos dos inibidores co-cristalizados hepta-Thr e PepA. Esses achados indicam que o OEVG possui potencial biotecnológico e terapêutico, podendo contribuir no desenvolvimento de novos fitofármacos. No entanto, são necessários ensaios *In vivo* e clínicos a fim de confirmar a eficácia e a segurança farmacológica dos compostos analisados.

REFERÊNCIAS

ALMEIDA, Bianca Leal *et al*. Candidemia em Pacientes de UTI: Quais São os Verdadeiros Fatores de Mudança para a Sobrevivência?. **Journal of Fungi** , v. 11, n. 2, p. 152, 2025.

BARRETO, Ighor C.; DE ALMEIDA, Anderson S.; SENA FILHO, José G. Taxonomic Insights and Its Type Cyclization Correlation of Volatile Sesquiterpenes in *Vitex* Species and Potential Source Insecticidal Compounds: A Review. **Molecules**, v. 26, n. 21, p. 6405, 2021. https://doi.org/10.3390/molecules26216405

BOUAMRANE, S. *et al*. 3D-QSAR, *molecular docking*, molecular dynamic simulation, and ADMET study of bioactive compounds against *candida albicans*. **Moroccan Journal of Chemistry**, v. 10, n. 3, p. J. Chem. 10 N° 3 (2022) 523-541, 2022. https://doi.org/10.48317/IMIST.PRSM/morjchem-v10i3.33141

BRASIL. Decreto nº 5.813, de 22 de junho de 2006. Aprova a Política Nacional de Plantas Medicinais e Fitoterápicos. Diário Oficial da União, Brasília, DF, 22 jun. 2006.

BRASIL. Ministério da Saúde. Portaria nº 971, de 3 de maio de 2006. Aprova a Política Nacional de Práticas Integrativas e Complementares (PNPIC) no Sistema Único de Saúde (SUS). Diário Oficial da União, seção 1, 4 de maio de 2006**.**

CAMPOS, Tábata *et al*. Avaliação do comportamento de leveduras do gênero *Candida* a fármacos antifúngicos. **Ciência & Inovação**, v. 5, n. 1, 2020.

Carrillo-Muñoz, Afonso Javier, *et al*., Combination antifungal therapy; a strategy for the management of invasive fungal infections. **Revista Española de Quimioterapia. Madrid: Sociedad Española de Quimioterapia**, vol. 27, no. 3, p. 141-158. ISSN 0214-3429. https://doi.org/10.3390/jof7020079

DAS, Niranjan et al. Traditional uses, phytochemistry, and pharmacology of genus Vitex (*Lamiaceae*). **Phytotherapy Research**, v. 36, n. 2, p. 571-671, 2022. **https://doi.org/10.1002/ptr.7330**

FRANCIMAT, Lílian Pinheiro et al. Eficiência na desinfecção de alginato contaminado com *C. albicans* utilizando-se ácido peracético e alecrim-pimenta Antimicrobial effect of Lippia sidoides and peracetic acid on *C. albicans* biofilm formed on alginate impression. **Brazilian Journal of Health Review**, v. 4, n. 5, p. 18601-18611, 2021. https://doi.org/10.34119/bjhrv4n5-013

HERNANDEZ, Anselmo Enrique Ferrer *et al*. Estudo fitoquímico das plantas do gênero *solanum* no brasil e sua importância como fonte de matéria prima esteroidal. **Biotecnologia e biodiversidade: novas perspectivas em pesquisa-volume 2**. Editora Científica Digital, 2025. p. 75-90. https://doi.org/**10.37885/241017895**

MENDES, Ceci *et al*. Narrative review of *Vitex agnus-castus* in symptoms in Gynecology. **Revista da Associação Médica Brasileira**, v. 68, p. 716-719, 2022. https://doi.org/10.1590/1806-9282.20220174

MONTEIRO, L. C. C. F. *et al*. Atividade antioxidante, teor de fenóis e atividade larvicida frente ao *Aedes aegypti* de Vitex gardneriana Schauer. **Blucher Chemistry Proceedings**, v. 3, n. 1, p. 292-300, 2015.

MORAIS, Selene Maia *et al*. Atividades antioxidantes e anticolinesterásicas do extrato das folhas de Jaramataia (*Vitex gardneriana* Schauer). **Brazilian Journal of Development**, v. 6, n. 5, p. 28802-28810, 2020. https://doi.org/10.34117/bjdv6n5-358

MORAIS, Selene Maia *et al*. Atividades antioxidantes e anticolinesterásicas do extrato das

folhas de Jaramataia (*Vitex gardneriana* Schauer). **Brazilian Journal of Development**, v. 6, n. 5, p. 28802-28810, 2020. https://doi.org/10.34117/bjdv6n5-358

NAZZARO, Filomena *et al*. Essential oils and antifungal activity. **Pharmaceuticals**, v. 10, n. 4, p. 86, 2017. https://doi.org/10.3390/ph10040086

PEREIRA, Andréia Mara; REYDON, Bastiaan Philip; DA SILVEIRA, José Maria Ferreira Jardim. Bioprospecção, biodiversidade e conhecimentos tradicionais: uma proposta de modelo institucional para o Brasil. **ARACÊ**, v. 7, n. 4, p. 20222-20243, 2025. https://doi.org/10.56238/arev7n4-263

PEREIRA, R. *et al*. Biofilm of *Candida albicans*: formation, regulation and resistance. **Journal of applied microbiology**, v. 131, n. 1, p. 11-22, 2021. https://doi.org/10.1111/jam.14949

POWERS, Chelsea N. *et al*. Bigger data approach to analysis of essential oils and their antifungal activity against *Aspergillus niger, Candida albicans*, and *Cryptococcus neoformans*. **Molecules**, v. 24, n. 16, p. 2868, 2019. https://doi.org/10.3390/molecules24162868

RIBEIRO, Victor Pena *et al*. Brazilian medicinal plants with corroborated anti inflammatory activities: a review. Pharmaceutical biology, v. 56, n. 1, p. 253-268, 2018.https://doi.org/10.1080/13880209.2018.1454480

SCALABRIN, Andreia Cristine *et al*. Utilização do óleo essencial de TEA TREE (*Melaleuca alternifolia* Cheel) em mulheres com candidíase vulvovaginal: uma revisão sistemática. **Produtos Naturais e Suas Aplicações: da comunidade para o laboratório**, v. 1, p. 181-200, 2021. https://doi.org/10.37885/210303597

SCHAEFER, C. C. S. *et al*. *Candida auris*: características clínicas de uma levedura nosocomial emergente resistente a medicamentos/*Candida auris*: características clínicas de uma levedura nosocomial emergente resistente a medicamentos. **Revista Brasileira de Desenvolvimento**, v. 8, n. 5, p. 37062-37077, 2022. https://doi.org/10.34119/bjhrv4n5-013

SIILVA, Isamara Ferreira *et al*. Atividades biológicas de plantas medicinais utilizadas na Medicina Veterinária no Brasil entre 2000 e 2020: Uma revisão de literatura. **Research, Society and Development**, v. 10, n. 8, p. e46710817223-e46710817223, 2021. https://doi.org/10.33448/rsd-v10i8.17223

SILVA, Benise Ferreira *et al*. Composição química, polifenóis totais, atividade antioxidante e citotóxica do extrato etanólico de frutos da *Vitex gardneriana* Schauer. **Research, Society and Development**, v. 11, n. 4, p. e52311427265-e52311427265, 2022. https://doi.org/10.33448/rsd-v11i4.27265

SILVA, Diego Romario *et al*. Abordagens in silico para triagem de alvos moleculares em *Candida albicans*: Uma visão proteômica da descoberta e desenvolvimento de fármacos. **European Journal of Pharmacology** , v. 842, p. 64-69, 2019. https://doi.org/10.1016/j.ejphar.2018.10.016

SILVA, P. T. *et al*. Seasonal variation in the chemical composition and larvicidal activity against Aedes aegypti of essential oils from *Vitex gardneriana* Schauer. **South African Journal of Botany**, v. 124, p. 329-332, 2019. https://doi.org/10.1016/j.sajb.2019.04.036

SOUZA, Ana Valéria Vieira de *et al*. Bioprospecção de plantas da Caatinga com potencial para produção de fitomedicamentos, **fiocruz**, 2020. https://doi.org/10.32712/2446- 4775.2022.1283

SZTUKOWSKA, Maryta N. *et al.* Community development between *Porphyromonas*

gingivalis and *Candida albicans* mediated by InlJ and Als3. **MBio**, v. 9, n. 2, p. 10.1128/mbio. 00202-18, 2018. https://doi.org/10.1128/mbio.00202-18

TALAPKO, Jasminka *et al. Candida albicans* - the virulence factors and clinical manifestations of infection. **Journal of Fungi**, v. 7, n. 2, p. 79, 2021. https://doi.org/10.3390/jof7020079

YANG, L. *et al*. Response of plant secondary metabolites to environmental factors. **Molecules**, v.4, n. 23, p. 762, 2018. https://doi.org/10.3390/molecules23040762

ZHOU, L. *et al*. Preparation, characterization, and evaluation of amphotericin B-loaded MPEG-PCL-g-PEI micelles for local treatment of oral *Candida albicans.* International Journal of Nanomedicine, v. 12, p. 4269-4283, 2017. https://doi.org/10.2147/IJN.S124264

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ATIVIDADE ANTIMICROBIANA E HEMOLÍTICA DE PRODUTOS NATURAIS DA REGIÃO CAATINGA (BIOMA)

Pesquisador: ANTONIA NADIA BRITO DOS SANTOS

Área Temática:

Versão: 2

CAAE: 74431123.6.0000.5053

Instituição Proponente: Universidade Estadual Vale do Acaraú - UVA

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.662.155

Apresentação do Projeto:

A pesquisa é de caráter quantitativo. Visa fazer análise in vitro de uma pequena amostra do sangue humno quanto a toxicidade do óleo essencial de C. sativum (coentro). A CL50 (concentração letal) será calculada utilizado no graph prism, bem como os gráficos apresentando a porcentagem de atividade hemolítica encontrada para os óleos essenciais

Objetivo da Pesquisa:

Objetivo Primário:

• Investigar a constituição química, atividade antimicrobiana e a citotoxicidade de óleos essenciais de plantas da caatinga contra microorganismos patógenos.

Objetivo Secundário:

• Coletar, extrair e realizar a caracterização química dos óleos essenciais;• Avaliar a atividade antimicrobiana;

• Determinar a citoxicidade dos óleos essenciais em eritrócitos humanos.

Avaliação dos Riscos e Benefícios:

Há uma boa discussão relacionada aos possíveis riscos e benefícios envolvidos no desenvolvimento da pesquisa

Endereço: Av Comandante Maurocélio Rocha Ponte, 150
Bairro: Derby **CEP:** 62.041-040
UF: CE **Município:** SOBRAL
Telefone: (88)3677-4255 **Fax:** (88)3677-4242 **E-mail:** cep_uva@uvanet.br

Comentários e Considerações sobre a Pesquisa:

Pesquisa importante quanto ao desenvolvimento de novos fármacos e medicamentos para uso médico/farmacêutico.

Considerações sobre os Termos de apresentação obrigatória:

Adequados

Recomendações:

Não se aplicam

Conclusões ou Pendências e Lista de Inadequações:

Não se aplicam

Considerações Finais a critério do CEP:

Aprovado por este comitê.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇOES_BASICAS_DO_PROJETO_2215134.pdf	08/11/2023 16:55:29		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.docx	08/11/2023 16:54:17	ANTONIA NADIA BRITO DOS SANTOS	Aceito
Outros	LATTES.pdf	19/09/2023 19:32:27	ANTONIA NADIA BRITO DOS	Aceito
Declaração de Pesquisadores	COMPROMISSO.docx	19/09/2023 19:31:39	ANTONIA NADIA BRITO DOS	Aceito
Declaração de Instituição e Infraestrutura	Anuencia.pdf	19/09/2023 19:29:31	ANTONIA NADIA BRITO DOS SANTOS	Aceito
Projeto Detalhado / Brochura Investigador	DOCPROJETO2.pdf	19/09/2023 19:29:06	ANTONIA NADIA BRITO DOS SANTOS	Aceito
Folha de Rosto	FOLHAROSTO.pdf	19/09/2023 19:27:35	ANTONIA NADIA BRITO DOS	Aceito

Situação do Parecer:

Aprovado

Endereço: Av Comandante Maurocélio Rocha Ponte, 150
Bairro: Derby **CEP:** 62.041-040
UF: CE **Município:** SOBRAL
Telefone: (88)3677-4255 **Fax:** (88)3677-4242 **E-mail:** cep_uva@uvanet.br

Necessita Apreciação da CONEP:
Não

SOBRAL, 21 de Fevereiro de 2024

Assinado por:
Eroteíde Leite de Pinho
(Coordenador(a))

Endereço: Av Comandante Maurocélio Rocha Ponte, 150
Bairro: Derby **CEP:** 62.041-040
UF: CE **Município:** SOBRAL
Telefone: (88)3677-4255 **Fax:** (88)3677-4242 **E-mail:** cep_uva@uvanet.br