UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE BIOCIÊNCIAS
DEPARTAMENTO DE BOTÂNICA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA VEGETAL

VIVIANE BEZERRA DA SILVA

PROSPECÇÃO FITOQUÍMICA E AVALIAÇÃO DAS ATIVIDADES ANTIMICROBIANAS DAS CASCAS DO CAULE DE *Hancornia speciosa* GOMES (APOCYNACEAE)

Recife
2025

VIVIANE BEZERRA DA SILVA

PROSPECÇÃO FITOQUÍMICA E AVALIAÇÃO DAS ATIVIDADES ANTIMICROBIANAS DAS CASCAS DO CAULE DE *Hancornia speciosa* GOMES (APOCYNACEAE)

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutora em Biologia Vegetal.
Área de concentração: Ecologia e Conservação.
Linha de pesquisa: Botânica Aplicada e Etnobotânica

Orientador: Prof. Dr. Antônio Fernando Morais de Oliveira

Coorientadora: Profª. Drª. Maria Flaviana Bezerra Morais Braga

Recife

2025

.Catalogação de Publicação na Fonte. UFPE - Biblioteca Central

Silva, Viviane Bezerra da.
Prospecção fitoquímica e avaliação das atividades antimicrobianas das cascas do caule de *Hancornia speciosa* Gomes (Apocynaceae) / Viviane Bezerra da Silva. - Recife, 2025.
130f.: il.

Tese (Doutorado) - Universidade Federal de Pernambuco, Centro de Biociências, Programa de Pós-graduação em Biologia Vegetal, 2025.
Orientação: Antônio Fernando Morais de Oliveira.
Coorientação: Maria Flaviana Bezerra Morais Braga.
Inclui referências e anexos.

1. Etnobotânica; 2. Infecções; 3. Mangabeira; 4. Planta medicinal; 5. Resistência microbiana. I. Oliveira, Antônio Fernando Morais de. II. Braga, Maria Flaviana Bezerra Morais. III. Título.

UFPE-Biblioteca Central

VIVIANE BEZERRA DA SILVA

PROSPECÇÃO FITOQUÍMICA E AVALIAÇÃO DAS ATIVIDADES ANTIMICROBIANAS DAS CASCAS DO CAULE DE *Hancornia speciosa* GOMES (APOCYNACEAE)

Tese apresentada ao Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Pernambuco como requisito parcial para obtenção do título de Doutora em Biologia Vegetal.
Área de concentração: Ecologia e Conservação.
Linha de pesquisa: Botânica Aplicada e Etnobotânica

Aprovada em: 20/02/2025

BANCA EXAMINADORA

_____
Prof. Dr. Antônio Fernando Morais de Oliveira (Orientador)
Universidade Federal de Pernambuco

_____
Profª. Drª. Laise de Holanda Cavalcanti Andrade (Examinadora Externa)
Universidade Federal de Pernambuco

_____
Profª. Drª. Cícera Datiane de Morais Oliveira Tintino (Examinadora Externa)
Universidade Regional do Cariri

_____
Profª. Drª. Eugenia Cristina Goncalves Pereira (Examinadora Externa)
Universidade Federal de Pernambuco

_____
Profª. Drª. Maria Karollyna do Nascimento Silva Leandro (Examinadora Externa)
Universidade Regional do Cariri

Dedico este trabalho de tese aos meus pais, Luiza Bezerra Felix e José Alves da Silva, por acreditarem em minha capacidade, por inspirarem meu caminho acadêmico e por compartilharem comigo cada vitória e desafio.

AGRADECIMENTOS

Em primeiro lugar, agradeço a Deus, pela dádiva da vida, pela sabedoria, e por todas as minhas conquistas pessoais e profissionais. Sou profundamente grata por Sua orientação constante, que ilumina meu caminho e me concede fé e força para seguir sempre em frente.

Agradeço à Universidade Federal de Pernambuco (UFPE) pela infraestrutura e recursos oferecidos, que foram essenciais para a realização desta pesquisa.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela concessão da bolsa e apoio financeiro para a realização da pesquisa.

Agradeço ao meu orientador Professor Dr. Antônio Fernando Morais de Oliveira, do Departamento de Botânica da UFPE por sua paciência e encorajamento ao longo do doutorado. Seu conhecimento e orientação foram fundamentais para o desenvolvimento e conclusão desta pesquisa.

Agradeço à minha coorientadora, Professora Dra. Maria Flaviana Bezerra Morais Braga, do Departamento de Ciências Biológicas da URCA. Sua orientação, carinho e conhecimento foram essenciais para o desenvolvimento deste trabalho.

Ao Programa de Pós-Graduação em Biologia Vegetal da UFPE pela oportunidade de realização do doutorado e a todos os professores pelos ensinamentos transmitidos e contributo para o meu crescimento pessoal e profissional.

Às instituições e laboratórios parceiros, expresso minha sincera gratidão pelas contribuições valiosas em diferentes etapas desta pesquisa. Dentre estes, o Laboratório de Química e Produtos Naturais da Embrapa Agroindústria Tropical (Fortaleza – CE), o Herbário Geraldo Mariz (UFPE), o Laboratório de Ecologia Aplicada e Fitoquímica (LEAF – UFPE), Laboratório de Microbiologia e Biologia Molecular (LMBM – URCA), Laboratório de Micologia Aplicada do Cariri (LMAC – URCA), Laboratório de Pesquisa em Produtos Naturais (LPPN – URCA) e ao Laboratório de Fitoquímica (IB – USP).

Aos membros da banca examinadora, pela disponibilidade de participar e pelas sugestões dadas para o aperfeiçoamento deste trabalho.

Agradeço ao meu marido, Janerson José Coelho, por seu amor, compreensão, incentivo e suporte. Agradeço também ao meu fiel amigo, Billy, cuja companhia tornaram meus dias mais leves e felizes.

Aos meus pais, José Alves da Silva e Luiza Bezerra Felix da Silva, cujo amor, apoio incondicional e incentivo foram fundamentais ao longo de minha jornada acadêmica. Dedico esta, bem como todas as minhas demais conquistas a vocês.

Quero agradecer a minha irmã Sabrina Bezerra da Silva, pela amizade, apoio e carinho, e por estar sempre torcendo pelas minhas conquistas.

Agradeço aos meus amigos pelo apoio e incentivo ao longo desta jornada. Agradeço também a todos os meus familiares por me apoiarem direta ou indiretamente.

A todos que direto ou indiretamente me doaram um pouco dos seus ensinamentos, tempo, material ou qualquer outro tipo de ajuda a minha pesquisa.

“O segredo da mangaba
Está no tempo da colheita
Na hora que fica doce
Você pode encher a cesta”
Dudé Viana

RESUMO

Hancornia speciosa Gomes, pertencente à família Apocynaceae, é uma planta nativa do Brasil, amplamente utilizada por comunidades tradicionais para o tratamento de diversas condições, incluindo infecções. Considerando o potencial terapêutico de *H. speciosa*, este estudo teve como objetivos: 1) Caracterizar quimicamente os extratos etéreo (EEHS) e metanólico (MEHS) da casca do caule de *H. speciosa;* 2) Investigar as atividades antifúngica, antivirulência e modificadora da ação do fluconazol dos extratos; 3) Avaliar as atividades antibacteriana e modificadora de fármacos de ambos os extratos contra bactérias patogênicas; 4) Avaliar a atividade tóxica dos extratos frente ao organismo modelo *Drosophila melanogaster*. Casca do caule de *H. speciosa* foi coleta em uma área de proteção ambiental da Chapada do Araripe, Ceará, Brasil. Os extratos foram preparados usando os solventes *n*-hexano, éter sulfúrico e metanol, sendo os dois últimos utilizados nas análises. Os extratos foram analisados quimicamente por cromatografia líquida acoplada à espectrometria de massas (UPLC-MS-ESI-QTOF) e por cromatografia gasosa acoplada à espectrometria de massas (GC-MS). Fenóis e flavonoides foram quantificados espectrofotometricamente. A atividade antifúngica dos extratos foi analisada tanto isoladamente quanto em combinação com o fluconazol, contra as cepas de *Candida albicans, Candida krusei* e *Candida tropicalis*, usando o método de microdiluição em caldo. Além disso, foi avaliada a capacidade dos extratos em inibir a transição morfológica para formas invasivas, como hifas e pseudohifas. A atividade antibacteriana foi avaliada por meio de testes de concentração inibitória mínima (MIC) utilizando ensaio de microdiluição seriada e de modificação de fármacos contra cepas bacterianas padrão e multirresistentes de *Escherichia coli, Pseudomonas aeruginosa* e *Staphylococcus aureus*. A toxicidade dos extratos foi avaliada em *D. melanogaster* por meio de ensaios de ingestão, com monitoramento diário da mortalidade ao longo de sete dias. Os resultados das análises fitoquímicas revelaram a presença de ácidos graxos, triterpenoides, fitosteróis, fenólicos e flavonoides em ambos os extratos. EEHS e MEHS apresentaram consideráveis concentrações de fenóis (346,4 e 340,0 mg GAE/g, respectivamente) e flavonoides (7,6 e 6,9 mg QE/g, respectivamente). Além disso, foram reportados pela primeira vez para a espécie compostos como ácido glucônico, cinchonina IIb, isômero da cinchonina Ib, isômeros de hexosídeo de laricirresinol, ácido hexacosanoico, ácido lignocérico, ácido triacontanoico e α-amirona. Quanto à atividade antifúngica, os extratos mostraram ação intrínseca contra as espécies de *Candida* e em combinação com fluconazol, potencializaram a ação deste fármaco. Adicionalmente, os extratos inibiram a transição morfológica de formas invasivas de *Candida* spp., reduzindo a formação de hifas e pseudohifas. Embora os extratos não tenham exibido atividade antibacteriana intrínseca significativa contra *E. coli, S. aureus* e *P. aeruginosa* (MIC > 512 µg/mL), eles foram capazes de modificar a atividade da gentamicina, eritromicina e norfloxacina, potencializando, em particular, o efeito da gentamicina e eritromicina contra cepas multirresistentes de *P. aeruginosa* e *E. coli*. Os testes de toxicidade em *D. melanogaster* indicaram que os extratos não são tóxicos em concentrações clinicamente relevantes, sugerindo um perfil de segurança positivo. Este estudo oferece uma validação parcial do uso etnofarmacológico de *H. speciosa* e destaca seu potencial como fonte de compostos bioativos para o desenvolvimento de novos agentes terapêuticos.

Palavras-chave: Etnobotânica; Infecções; Mangabeira; Planta medicinal; Resistência microbiana.

ABSTRACT

Hancornia speciosa Gomes, a member of the Apocynaceae family, is a plant native to Brazil and widely used by traditional communities for treating various conditions, including infections. Given the therapeutic potential of *H. speciosa*, this study aimed to 1) Chemically characterize the ethereal (EEHS) and methanolic (MEHS) extracts of the stem bark of *H. speciosa*; 2) Investigate the antifungal, antivirulence, and fluconazole-modifying activities of these extracts; 3) Assess the antibacterial and drug-modifying activities of both extracts against pathogenic bacteria; 4) Evaluate the toxicity of the extracts using the model organism *Drosophila melanogaster*. The stem bark of *H. speciosa* was collected from an environmental protection area in Chapada do Araripe, Ceará, Brazil. The extracts were prepared using n-hexane, sulfuric ether, and methanol, with the latter two used for analyses. Chemical analysis of the extracts was performed using liquid chromatography coupled with mass spectrometry (UPLC-MS-ESI-QTOF) and gas chromatography coupled with mass spectrometry (GC-MS). Phenols and flavonoids were quantified spectrophotometrically. The antifungal activity of the extracts was tested both alone and in combination with fluconazole against *Candida albicans, Candida krusei,* and *Candida tropicalis* strains using the broth microdilution method. Additionally, the ability of the extracts to inhibit the morphological transition associated with invasive forms, such as hyphae and pseudohyphae, were assessed. Antibacterial activity was evaluated through minimum inhibitory concentration (MIC) tests using serial microdilution assays and drug-modifying activity against standard and multidrug-resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The toxicity of the extracts was assessed in *D. melanogaster* through ingestion assays, with daily mortality monitoring over seven days. Phytochemical analysis revealed the presence of fatty acids, triterpenoids, phytosterols, phenolics, and flavonoids in both extracts. EEHS and MEHS contained substantial concentrations of phenols (346.4 and 340.0 mg GAE/g, respectively) and flavonoids (7.6 and 6.9 mg QE/g, respectively). Additionally, compounds such as glucuronic acid, cinchonine IIb, isomer of cinchonine Ib, isomers of lariciresinol hexoside, hexacosanoic acid, lignoceric acid, triacontanoic acid, and α-amirone were reported for the first time in this species. Regarding antifungal activity, the extracts exhibited intrinsic activity against *Candida* species and, in combination with fluconazole, enhanced drug efficacy. Furthermore, the extracts inhibited the morphological transition of invasive *Candida* forms, reducing the formation of hyphae and pseudohyphae. Although the extracts did not show significant intrinsic antibacterial activity against *E. coli*, *S. aureus*, and *P. aeruginosa* (MIC > 512 µg/mL), they were able to modify the activity of gentamicin, erythromycin, and norfloxacin, particularly enhancing the effects of gentamicin and erythromycin against multidrug-resistant strains of *P. aeruginosa* and *E. coli*. Toxicity tests in *D. melanogaster* indicated that the extracts are not toxic at clinically relevant concentrations, suggesting a favorable safety profile. This study provides partial validation of the ethnopharmacological use of *H. speciosa* and highlights its potential as a source of bioactive compounds for developing new therapeutic agents.

Keywords: Ethnobotany; Infections; Mangabeira; Medicinal plant; Microbial resistance.

LISTA DE ILUSTRAÇÕES

REFERENCIAL TEÓRICO

FIGURA 1 Riqueza e distribuição mundial das espécies de Apocynaceae..... 20

FIGURA 2 Características de *Hancornia speciosa* Gomes (Apocynaceae). (A) = Folhas; (B) = Fruto; (C) = Caule e látex; (D) = Coleta do látex..... 22

FIGURA 3 Principais mecanismos de resistência bacteriana aos antibióticos. (1) = Inativação enzimática; (2) = Efluxo do antibiótico; (3) = Alteração do sítio-alvo; (4) = Bloqueio da entrada do fármaco..... 31

ARTIGO 1 - Chemical composition, antifungal, and anti-virulence action of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) against *Candida* spp.

FIGURA 1 Map of the collection site of *Hancornia speciosa* Gomes in the Environmental Protection Area of the Chapada do Araripe, Jardim, state of Ceará, Brazil……... 39

FIGURA 2 UPLC-MS in negative ionization mode of the sulfuric ether (EEHS) (A) and methanolic (MEHS) (B) extracts of the stem bark of *Hancornia speciosa*. …… 45

FIGURA 3 Cell viability curve of *Candida* spp. strains and IC_{50} value (dashed line) of different concentrations of *Hancornia speciosa* sulfuric ether extract (EEHS), fluconazole (FCZ), and their combination (FCZ + EEHS). *Candida albicans* (4A), *Candida krusei* (4B), and *Candida tropicalis* (4C). The bars indicate the standard error of the mean (n = 3). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ compared to fluconazole (FCZ).……. 49

FIGURA 4 Cell viability curve of *Candida* spp. strains and IC_{50} values (dashed line) of different concentrations of *Hancornia speciosa* methanolic extract (MEHS), fluconazole (FCZ), and their combination (FCZ + MEHS). *Candida albicans* (5A), *Candida krusei* (5B), and *Candida tropicalis* (5C). The bars indicate the standard error of the mean (n = 3). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ compared to fluconazole (FCZ)…… …….. 50

FIGURA 5 Effect of fluconazole (FCZ), sulfuric ether extract (EEHS), and methanolic extract (MEHS) of *Hancornia speciosa* on the dimorphism of *Candida albicans* INCQS 40006. Growth control (A); FCZ (B and C); EEHS (D and E); MEHS (F and G). MC = Matrix Concentration; 100 × magnification..…..…… …….. 51

FIGURA 6 Effect of fluconazole (FCZ), sulfuric ether extract (EEHS), and methanolic extract (MEHS) of *Hancornia speciosa* on the dimorphism of *Candida krusei* INCQS 40095. Growth control (A); FCZ (B and C); EEHS (D and E); MEHS (F and G). MC = Matrix Concentration; 100 × magnification..…… …… 52

FIGURA 7 Effect of fluconazole (FCZ), sulfuric ether extract (EEHS), and methanolic extract (MEHS) of *Hancornia speciosa* on the dimorphism of *Candida tropicalis* INCQS 40042. Growth control (A); FCZ (B and C); EEHS (D and E); MEHS (F and G). MC = Matrix concentration; 100 × magnification..…… …… 53

ARTIGO 2 – Chemical composition, antibacterial potential, and toxicity of the extracts from the stem bark of *Hancornia speciosa* Gomes (Apocynaceae)

FIGURA 1 Characteristics of *Hancornia speciosa* Gomes (Apocynaceae). (A) = Leaves; (B) = Fruit; (C) = Stem and latex; (D) = Latex collection……. 72

FIGURA 2 Map of the sampling site where were collected the stem barks of *Hancornia speciosa* Gomes, Jardim city, Ceará, Brazil…….. 73

FIGURA 3 Chemical structures of the compounds identified in the extracts of the stem bark of *Hancornia speciosa* by gas chromatography-mass spectrometry…… 84

FIGURA 4 Minimum Inhibitory Concentration (MIC) of antibiotics combined with sulfuric ether (EEHS) (a) and methanolic (MEHS) (b) extracts of the stem bark of *Hancornia speciosa* against multi-resistant bacterial strains. MIC values are displayed as geometric mean. The bars represent the standard error of the mean (n = 3). **** = $p < 0.0001$…….. 87

FIGURA 5 Toxicity of sulfuric ether (EEHS) (a) and methanolic (MEHS) (b) extracts of the stem bark of *Hancornia speciosa* at different

concentrations against *Drosophila melanogaster*. * = $p < 0.05$, **** = $p < 0.0001$. Bars represent the standard error of the mean (n = 3)…….. 88

FIGURA 6 Target sites of phytochemicals, and their mechanisms of action against bacteria. 1: Inhibition of protein synthesis; 2: Inhibition of nucleic acid synthesis; 3: Damage to the cell wall; 4: Damage and rupture of the cell membrane and 5: Inhibition of the efflux pump…… 91

LISTA DE TABELAS

ARTIGO 1 - Chemical composition, antifungal, and anti-virulence action of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) against *Candida* spp.

TABELA 1 Classes of metabolites investigated in the sulfuric ether (EEHS) and methanolic (MEHS) extracts of the stem bark of *Hancornia speciosa*… 43

TABELA 2 Compounds tentatively identified in the sulfuric ether (EEHS) and methanolic (MEHS) extracts of the stem bark of *Hancornia speciosa*. … 46

TABELA 3 Half maximal inhibitory concentration (IC_{50}) values of fluconazole, the extracts of the stem bark of *Hancornia speciosa*, and their combinations against *Candida* spp. strains…….. 47

ARTIGO 2 – Chemical composition, antibacterial potential, and toxicity of the extracts from the stem bark of *Hancornia speciosa* Gomes (Apocynaceae)

TABELA 1 Resistance profile of multi-drug resistant bacteria to antibiotic drugs. Source: Laboratory of Microbiology and Molecular Biology – LMBM, Universidade Regional do Cariri – URCA (Ceará, Brazil)……. 76

TABELA 2 Chemical composition of the sulfuric ether (EEHS) and methanolic (MEHS) extracts of *Hancornia speciosa* analyzed via gas chromatography coupled to mass spectrometry……..... 79

TABELA 3 Total phenolic and flavonoid content in sulfuric ether (EEHS) and methanolic (MEHS) extracts of the stem bark of *Hancornia speciosa*… 86

LISTA DE SIGLAS

HIV - Vírus da Imunodeficiência Humana

MRSA - *Staphylococcus aureus* resistente à meticilina

OMS - Organização Mundial da Saúde

PNPIC - Política Nacional de Práticas Integrativas e Complementares

PNPMF - Política Nacional de Plantas Medicinais e Fitoterápicos

SUS - Sistema Único de Saúde

WHO - World Health Organization

SUMÁRIO

1 APRESENTAÇÃO **16**

2 REFERENCIAL TEÓRICO **18**

2.1 PLANTAS MEDICINAIS 18

2.2 APOCYNACEAE 19

2.2.1 *Hancornia speciosa* Gomes 21

2.3 RESISTÊNCIA MICROBIANA 25

2.3.1 Fungos 26

2.3.2 Bactérias 30

3 ARTIGO 1 – Chemical composition, antifungal, and anti-virulence action of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) against *Candida* spp **34**

4 ARTIGO 2 – Chemical composition, antibacterial potential, and toxicity of the extracts from the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) **69**

5 CONSIDERAÇÕES FINAIS **112**

REFERÊNCIAS **113**

ANEXO A – PRIMEIRA PÁGINA - ARTIGO 1 ..... **124**

ANEXO B – PRIMEIRA PÁGINA - ARTIGO 2 ..... **125**

ANEXO C – NORMAS PARA SUBMISSÃO DOS MANUSCRITOS AO PERIÓDICO JOURNAL OF ETHNOPHARMACOLOGY **126**

ANEXO D - REGISTRO SISGEN **127**

ANEXO E – AUTORIZAÇÃO SISBIO **128**

ANEXO F - COMPROVANTE DE DEPÓSITO E INCORPORAÇÃO DE MATERIAL VEGETAL NO HERBÁRIO UFP– GERALDO MARIZ **129**

ANEXO G - AUTORIZAÇÃO DE USO DE IMAGEM **130**

1 APRESENTAÇÃO

O uso excessivo e indiscriminado de antimicrobianos tem promovido o surgimento de microrganismos resistentes, como fungos e bactérias, resultando na ineficácia de medicamentos convencionais e no prolongamento do tratamento dos pacientes, o que, em muitos casos, pode levar ao óbito. Além disso, o desenvolvimento de novos medicamentos não consegue acompanhar a rápida evolução da resistência dos microrganismos aos fármacos antimicrobianos, agravando o problema e limitando as opções terapêuticas disponíveis.

Essa realidade é ainda mais alarmante em regiões com acesso limitado a medicamentos, como países em desenvolvimento e áreas remotas ou vulneráveis socioeconomicamente, onde as populações enfrentam dificuldades para acessar tratamentos convencionais. Nessas áreas, as plantas medicinais são uma alternativa viável e acessível, sendo amplamente utilizadas por diversas comunidades tradicionais e locais para tratar várias enfermidades.

No Brasil, o uso de plantas medicinais tem uma longa tradição, sendo uma parte essencial das práticas de saúde em muitas regiões do país. Com uma vasta biodiversidade, o país possui uma rica variedade de plantas distribuídas em diferentes fitofisionomias, muitas das quais têm propriedades terapêuticas utilizadas tanto na medicina tradicional quanto na moderna. Entre as plantas, *Hancornia speciosa* Gomes (Apocynaceae), popularmente conhecida como “mangabeira”, destaca-se por suas propriedades terapêuticas. Presente em várias regiões do Brasil, essa espécie é tradicionalmente utilizada no tratamento de diversas condições, incluindo doenças infecciosas.

Hancornia speciosa possui uma ampla variedade de compostos bioativos que contribuem para suas propriedades medicinais. Na casca do caule, por exemplo, foram encontrados flavonoides, catequinas, proantocianidinas e taninos. O látex do tronco contém flavonas, flavonóis, flavanonas, além de taninos, ácido cafeico e ácido quínico. Nos frutos, foram identificados L-bornesitol, os ácidos quínico e clorogênico, rutina, terpenos e aldeídos. As folhas apresentaram ácido protocatecuico, procianidinas dos tipos B e C, ácido cumaroilquínico, rutina, floretina, eriodictiol, quercetina, luteolina, apigenina e kaempferol.

Considerando o potencial terapêutico da casca do caule de *H. speciosa*, especialmente no tratamento de doenças infecciosas, este estudo levantou a hipótese de que os extratos da casca do caule de *H. speciosa* apresentam propriedades antimicrobianas contra microrganismos causadores de infecções, incluindo aquelas associadas a infecções nosocomiais, e que seu uso tradicional na medicina popular sugere uma baixa toxicidade. Desta forma, o objetivo deste estudo foi caracterizar a composição química dos extratos etéreo e metanólico da casca do caule

de *H. speciosa* e avaliar as suas atividades antimicrobianas e modificadoras de drogas contra as bactérias *Escherichia coli*, *Staphylococcus aureus* e *Pseudomonas aeruginosa*, bem como contra os fungos *Candida albicans*, *Candida krusei* e *Candida tropicalis*. Além disso, buscou-se investigar sua toxicidade frente ao organismo modelo *Drosophila melanogaster*.

2 REFERENCIAL TEÓRICO

2.1 PLANTAS MEDICINAIS

O uso das plantas para fins terapêuticos é uma das práticas mais antigas da humanidade, sendo parte dos conhecimentos populares de diversas culturas ao redor do mundo. Registros históricos datados de mais de 2 mil anos a.C. mostram que civilizações antigas já conheciam e utilizavam plantas para tratar doenças (Petrovska, 2012). Nas Américas, registros arqueológicos indicam que os povos ameríndios (indígenas sul-americanos) utilizavam plantas como remédio há mais de dez mil anos (Simões *et al*., 2017).

Ao longo da história, o conhecimento sobre as propriedades medicinais das plantas tem sido transmitido de geração em geração, contribuindo para o alívio de sintomas e para o tratamento de várias condições de saúde (Karahan *et al*., 2020). No Brasil, o uso das plantas medicinais é uma prática amplamente difundida nas tradições culturais e nos sistemas de saúde de diferentes grupos populacionais, como indígenas, caiçaras, quilombolas, seringueiros, rurais e outras comunidades tradicionais e locais. Esse conhecimento acumulado não apenas mantém práticas de saúde mais acessíveis e adaptadas às condições locais, mas também oferece uma base valiosa para pesquisas científicas e o desenvolvimento de novos medicamentos (Atanasov *et al*., 2015; Nascimento Magalhães *et al*., 2019; Boccolini; Boccolini, 2020).

Embora o uso de plantas medicinais seja uma prática ancestral, foi somente na década de 1970 que a Organização Mundial da Saúde (OMS) passou a reconhecer sua importância nas práticas tradicionais de saúde e seu papel na saúde global. Em 1978, a Declaração de Alma-Ata, resultado da Conferência Internacional sobre Cuidados Primários de Saúde, destacou a relevância das plantas medicinais no combate a doenças, especialmente em regiões com acesso limitado à medicina convencional, e reforçou a necessidade de políticas e regulamentações para o uso de plantas medicinais com eficácia comprovada. Ainda na mesma década, a OMS lançou o Programa de Medicina Tradicional, com o objetivo de desenvolver políticas públicas e reconhecer as plantas medicinais como um recurso valioso nas práticas de saúde (Brasil, 2015).

No Brasil, as políticas públicas sobre plantas medicinais e fitoterápicos começaram a se consolidar na década de 1980, com o Programa de Pesquisa de Plantas Medicinais, que buscava incentivar a pesquisa científica e o desenvolvimento de

medicamentos fitoterápicos baseados no uso popular de plantas (Brasil, 2006). Em 2006, foi criada a Política Nacional de Práticas Integrativas e Complementares (PNPIC), que incorporou terapias complementares ao Sistema Único de Saúde (SUS), incluindo o uso de plantas medicinais (Brasil, 2015). No mesmo ano, o Ministério da Saúde lançou a Política Nacional de Plantas Medicinais e Fitoterápicos (PNPMF) com o objetivo de garantir o acesso seguro e o uso racional desses recursos pela população. Um marco importante foi a criação das Farmácias Vivas em 2010, que organizaram a gestão, dentro do SUS, de todo o processo, desde o cultivo até a dispensação de plantas medicinais e fitoterápicos, promovendo seu uso seguro (Cherobin *et al*., 2022).

Aproximadamente 40% dos medicamentos atuais, de acordo com a OMS, são derivados do conhecimento tradicional sobre as propriedades medicinais das plantas (WHO, 2023). Cerca de 11% dos 252 medicamentos essenciais listados pela OMS são originados exclusivamente de plantas (Braga *et al*., 2021). As plantas são uma fonte rica e diversificada de compostos bioativos, o que as torna fundamentais para a saúde humana e a produção de medicamentos. Entre esses compostos, destacam-se os polifenóis, terpenoides, alcaloides e flavonoides amplamente estudados por suas propriedades medicinais (Riaz *et al*., 2023; Chaachouay; Zidane, 2024).

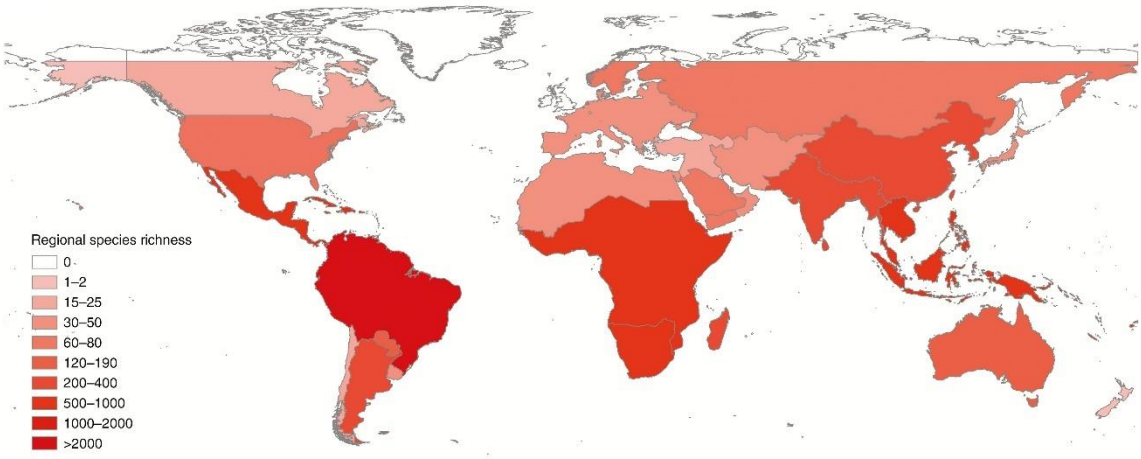
O Brasil, conhecido pela sua rica biodiversidade vegetal e herança cultural diversificada, desempenha um papel crucial na pesquisa com plantas medicinais. O país abriga uma enorme variedade de espécies vegetais distribuídas em diferentes ecossistemas, muitas das quais possuem potencial para o desenvolvimento de novos tratamentos terapêuticos. Dentro dessa diversidade, destacam-se espécies da família Apocynaceae, como *Hancornia speciosa* Gomes, *Himatanthus drasticus* (Mart.) Plumel e *Secondatia floribunda* A.DC. Estas espécies apresentam propriedades biológicas e farmacológicas promissoras, incluindo atividades antimicrobianas, anti-inflamatórias e antioxidantes. Essas propriedades são atribuídas à presença de compostos bioativos, que desempenham um papel crucial no desenvolvimento de novos tratamentos para diversas enfermidades (Dutra *et al*., 2016; Almeida *et al*., 2017; Ribeiro *et al*., 2017; Bhadane *et al*., 2018; Ribeiro *et al*., 2018; Nunes *et al*., 2022).

2.2 APOCYNACEAE

Apocynaceae Juss. pertence à classe Magnoliopsida, ordem Gentianales, e compreende cerca de 5.350 espécies distribuídas em 378 gêneros (Endress *et al*., 2018). A família é amplamente distribuída em regiões tropicais e subtropicais, com algumas

espécies adaptadas a ambientes temperados (Bhadane *et al*., 2018) (Figura 1). No Brasil, Apocynaceae está presente em todos os domínios fitogeográficos, com 103 gêneros e 993 espécies (Flora e Funga do Brasil, 2020). Taxonomicamente, a família está subdividida em cinco subfamílias: Apocynoideae, Asclepiadoideae, Periplocoideae, Rauvolfioideae e Secamonoideae (Endress *et al*., 2014; 2018).

Figura 1. Riqueza e distribuição mundial das espécies de Apocynaceae



Fonte: Ollerton *et al*. (2019). Com permissão (Creative Commons CC BY).

Os representantes de Apocynaceae compreendem árvores, arbustos, trepadeiras ou ervas, frequentemente com látex leitoso, que é uma das características distintas da família. Suas folhas são simples, geralmente inteiras e opostas, e as inflorescências são multifloras, com flores raramente solitárias. As flores, bissexuais, possuem cinco pétalas e cinco sépalas com coléteres internos, podendo assumir diferentes formas (infundibuliforme, hipocrateriforme, campanulada, urceolada ou rotácea), com ou sem corona. Os estames geralmente são cinco e o ovário é predominantemente apocárpico, com dois carpelos (raramente até 5). Os frutos variam de folículo a cápsula, baga, drupa ou sâmara, e as sementes podem ser nuas ou com diversas estruturas associadas como arilos e fimbriadas ao longo da margem (Endress *et al*., 2018).

As espécies de Apocynaceae possuem grande importância econômica, destacando-se pela produção de madeira de alta qualidade, amplamente utilizada na carpintaria, como observado no gênero *Aspidosperma* Mart. & Zucc. (Machate *et al*., 2016). Além disso, muitas espécies dessa família são populares na ornamentação, como *Allamanda cathartica* L., *Catharanthus roseus* (L.) G. Don, *Nerium oleander* L. e

Thevetia peruviana (Pers.) K. Schum. (Simões *et al*., 2017; Anand *et al*., 2020). Alguns representantes também possuem valor alimentício, como *Hancornia speciosa*, cujos frutos são apreciados no Nordeste do Brasil, consumidos tanto *in natura* quanto na preparação de diferentes receitas (Almeida *et al*., 2022). Além de aplicações econômicas e ornamentais, as plantas dessa família têm uso medicinal e na fitoterapia. Um exemplo é *Plumeria rubra* L., cujas flores ornamentais e outras partes da planta são empregadas no tratamento de asma, diabetes e problemas de pele (Bihani *et al*., 2021). No Brasil, o látex de *H. drasticus* é reconhecido por suas propriedades anti-inflamatórias, sendo utilizado no tratamento de úlceras, câncer, cicatrização e inflamações em geral (Oliveira *et al*., 2022). A casca de *H. speciosa* é usada em infusões e extratos para tratar problemas gastrointestinais, inflamações, infecções e na cicatrização de feridas (Ribeiro *et al*., 2014; Vieira; Sousa; Lemos, 2015; Penido *et al*., 2016).

Os representantes de Apocynaceae são conhecidos pela ampla diversidade de compostos químicos, incluindo alcaloides, esteróis, flavonoides, glicosídeos, lignanas, terpenos e compostos fenólicos simples, muitos dos quais apresentam importantes propriedades medicinais (Bhadane *et al*., 2018). Um exemplo notável são os alcaloides anticancerígenos vimblastina e vincristina encontrados em *Catharanthus* G. Don (Kumar *et al*., 2022). Essa diversidade química reflete na variedade de atividades biológicas e farmacológicas dos representantes da família, incluindo propriedades antioxidantes, anticancerígenas, antidiabéticas, anticonvulsivantes, antimicrobianas, anti-inflamatórias, antimaláricas, anti-HIV, cardioprotetoras, cicatrizantes, gastroprotetoras e hepatoprotetoras (Bhadane *et al*., 2018). As potencialidades biológicas e farmacológicas de Apocynaceae têm despertado interesse na comunidade científica, tanto por sua eficácia terapêutica quanto pelo potencial na descoberta de novos agentes para o tratamento de diversas doenças (Anand *et al*., 2020).

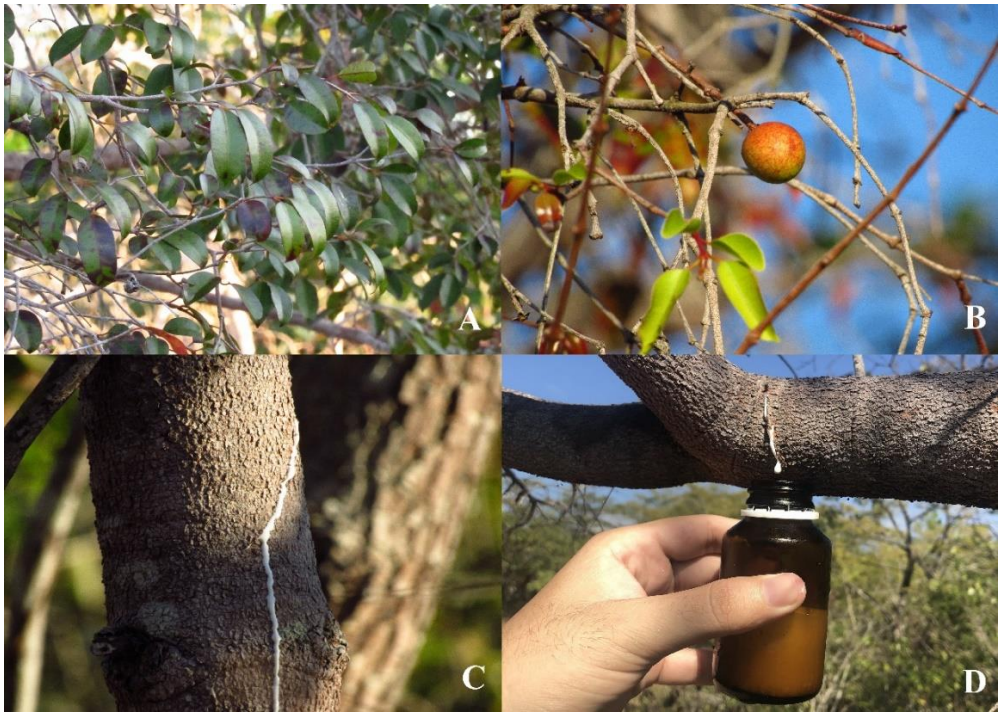
2.2.1 Hancornia speciosa Gomes

Hancornia speciosa Gomes é taxonomicamente enquadrada na classe Magnoliopsida, ordem Gentianales e família Apocynaceae. O gênero monotípico é uma homenagem ao botânico inglês Philip Hancorn, enquanto o epíteto específico *speciosa* deriva do latim e significa “bela”, “magnífica” ou “vistosa”. No Brasil, a espécie é conhecida popularmente como “mangabeira”, “mangava-mansa”, “mangaba”, “mangava” e “fruta de doente” (Ribeiro *et al*., 2014; Ribeiro *et al*., 2017; Smith, 2023).

Hancornia speciosa é uma espécie nativa não endêmica do Brasil encontrada em todas as regiões do país nos domínios fitogeográficos Amazônia, Caatinga, Cerrado e Mata Atlântica (Flora e Funga do Brasil, 2024). Sua maior incidência é observada em áreas de Cerrado, nos Tabuleiros Costeiros e baixadas litorâneas do Nordeste brasileiro (Morais *et al*., 2023). A espécie geralmente se desenvolve em solos ácidos, arenosos, de baixa fertilidade e com baixo teor de matéria orgânica (Rodrigues *et al*., 2017). Além do Brasil, sua presença é registrada na Bolívia, Paraguai e Peru (Almeida *et al*., 2022).

Morfologicamente, os indivíduos de *H. speciosa* são árvores de porte médio, variando de 4 a 10 metros de altura, que exsudam látex de coloração branca ou róseo-pálida. Suas folhas são simples, opostas, coriáceas, podendo ser pilosas ou glabras, com pecíolos curtos. Os ramos são inclinados e numerosos, apresentando coloração violácea quando jovens. As inflorescências do tipo dicásio ou cimeira contêm de 1 a 7 flores brancas, hermafroditas, e têm formato de campânula alongada (tubular). Seus frutos são bagas globosas ou ovoides, com exocarpo amarelo ou esverdeado, podendo apresentar manchas ou estrias avermelhadas (Figura 2). A polpa é carnoso-viscosa, contendo de 2 a 30 sementes discoides (Monachino, 1945; Almeida *et al*., 1998; Aguiar-Filho *et al*., 1998; Lederman *et al*., 2000; Silva *et al*., 2011; Almeida *et al*., 2022).

Figura 2. Características de *Hancornia speciosa* Gomes (Apocynaceae). (A) = Folhas; (B) = Fruto; (C) = Caule e látex; (D) = Coleta do látex.



Fonte: Silva et al., 2024.

No que diz respeito ao seu sistema reprodutivo, *H. speciosa* é uma espécie alógama, ou seja, apresenta autoincompatibilidade entre suas estruturas reprodutivas, o que impede a autofecundação. Dessa forma, a presença de polinizadores é essencial para viabilizar a fecundação cruzada e, consequentemente, a produção de frutos (Collevatti *et al*., 2016). A participação de visitantes florais diurnos e noturnos é fundamental neste processo, especialmente abelhas (Euglossini e Centridini), borboletas (Nymphalidae e Hesperiidae) e mariposas (Sphingidae). O aumento na frequência destes polinizadores resulta em uma maior taxa de frutificação, além de frutos maiores e com mais sementes (Darrault; Schlindwein, 2005).

O fruto de *H. speciosa*, conhecido como "mangaba", deriva do termo tupi-guarani "mâguaba", que significa "coisa boa de comer". Esses frutos são altamente apreciados pelo sabor agradável e podem ser consumidos *in natura* ou utilizados na preparação de sucos, doces, bolos, geleias, licores e sorvetes (Almeida *et al*., 2022). A mangaba também se destaca por seu valor nutricional, sendo considerada uma fonte de vitamina C, além de conter potássio, ferro e zinco e compostos bioativos, como carotenoides e compostos fenólicos. A coleta e comercialização da mangaba e seus derivados representam uma importante fonte de renda para muitas famílias, especialmente da região Nordeste do Brasil (Oliveira; Aloufa, 2021).

O látex de *H. speciosa* é outro recurso valioso, extraído por cortes na casca do caule. No Brasil, este látex foi historicamente utilizado por diferentes povos para a produção de bolas de futebol e para montagem de armadilhas para a captura de pássaros (Nimuendajú, 1956; Lévi-Strauss, 1975; Smith, 2023). Durante o auge da indústria da borracha, entre a segunda metade do século XIX e o início do século XX, o látex de *H. speciosa*, conhecido no mercado internacional como "borracha de Pernambuco", era altamente valorizado (Wisniewski; Melo, 1982; Smith, 2023). Durante a Segunda Guerra Mundial, sua exploração foi intensificada para a produção de borracha, especialmente quando as forças japonesas interromperam o fornecimento de borracha natural (proveniente de *Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.) das plantações no Sudeste Asiático. Com a normalização do fornecimento de borracha natural após a guerra, a exploração comercial do látex de *H. speciosa* foi reduzida, sendo seu uso concentrado principalmente em suas propriedades medicinais (Smith, 2023).

Hancornia speciosa é listada entre as espécies nativas da flora brasileira com valor econômico atual ou potencial, sendo considerada uma prioridade para pesquisa e

conservação (Vieira; Camillo; Coradin, 2016). Apesar de sua importância econômica, é considerada uma espécie subutilizada que pode contribuir para a segurança alimentar, especialmente devido ao seu valor nutricional (FAO, 2022).

Etnofarmacologicamente, as diferentes partes de *H. speciosa* são utilizadas para o tratamento de uma ampla variedade de enfermidades como doenças da pele, doenças do sistema circulatório, doenças do sistema digestivo, doenças do sistema geniturinário, doenças do sistema respiratório, além de condições endócrinas, nutricionais e metabólicas, oculares, do sistema reprodutor feminino, neoplasma, infecções, pressão alta, dores nas costas, fraturas, hernia abdominal, distúrbios da menopausa e cicatrização de feridas (Ribeiro *et al*., 2014; Ferreira-Júnior *et al*., 2015; Macêdo *et al*., 2015; Vieira; Sousa; Lemos, 2015; Ribeiro *et al*., 2017).

A casca do caule de *H. speciosa* é tradicionalmente usada para o tratamento de úlceras estomacais, dores de estômago, gastrite, hérnia, cicatrização de feridas, câncer, infecção urinária, doenças do sistema reprodutor feminino e infecções e inflamações em geral (Ribeiro *et al*., 2014; Vieira; Sousa; Lemos, 2015; Penido *et al*., 2016). O látex da planta é empregado no tratamento de acne, verrugas, inflamações, diarreia, pancadas, bursite, tuberculose, úlceras e herpes (Silva Junior, 2004; Marinho *et al*., 2011). As folhas são utilizadas no tratamento de hipertensão e cólicas (Silva Junior, 2004; Ferrão *et al*., 2014).

A eficácia de muitas dessas aplicações populares é corroborada por pesquisas científicas. Estudos demonstraram o potencial antioxidante, gastroprotetor e antimicrobiano da casca do caule de *H. speciosa* (Costa *et al*., 2008; Moraes *et al*., 2008; Penido *et al*., 2017). O látex apresentou efeitos angiogênico, citotóxico, anti-inflamatório e genotóxico (Marinho *et al*., 2011; Almeida *et al*., 2014; Ribeiro *et al*., 2016). As folhas mostraram atividades antioxidante, antimicrobiana, anti-inflamatória, cicatrizante, vasodilatadora, anti-hipertensiva e hipoglicêmica (Ferreira *et al*., 2007; Endringer *et al*., 2009; Endringer *et al*., 2010; Pereira *et al*., 2015; Santos *et al*., 2016; Silva *et al*., 2016; Santos *et al*., 2018; Barbosa *et al*., 2019; Neto *et al*., 2020). Por sua vez, os frutos exibiram efeitos anti-inflamatório e antioxidante (Almeida *et al*., 2011; Dutra *et al*., 2017; Paula *et al*., 2018; Bitencourt *et al*., 2019; Yamashita *et al*., 2020).

Em adição às atividades biológicas e farmacológicas relatadas, a composição química de diferentes partes de *H. speciosa* tem sido objeto de estudos fitoquímicos extensivos. Na casca do caule, foram identificados flavonoides, catequinas, proantocianidinas e taninos, enquanto no látex do tronco foram encontradas flavonas,

flavonóis, flavanonas, taninos, ácido caféico e ácido quínico (Moraes *et al*., 2008; D'Abadia *et al*., 2020; Silva *et al*., 2024). Nos frutos foram detectados L-bornesitol, ácido quínico, ácido clorogênico, rutina, terpenos e aldeídos (Lima *et al*., 2015; Yamashita *et al*., 2020). Nas folhas, foram identificados ácido protocatecuico, procianidinas do tipo B e C, ácido cumaroilquínico, rutina, floretina, eriodictiol, quercetina, luteolina, apigenina e kaempferol corroborando com as atividades biológicas e farmacológicas reconhecidas (Bastos *et al*., 2017).

2.3 RESISTÊNCIA MICROBIANA

A descoberta da penicilina por Alexander Fleming em 1928, juntamente com sua aplicação clínica na década de 1940, foi um marco na história da medicina, transformando o tratamento de infecções bacterianas e salvando um grande número de vidas. Contudo, poucos anos após essa descoberta, em 1945, ao receber o Prêmio Nobel de Medicina ao lado de Howard Florey e Ernst Boris Chain, Fleming alertou sobre os riscos associados ao uso indiscriminado de antibióticos e ao surgimento de cepas bacterianas resistentes (Magalhães *et al*., 2021; Salam *et al*., 2023).

As preocupações de Fleming se mostraram relevantes com o passar do tempo, à medida em que a resistência microbiana se tornou um problema crescente, comprometendo a eficácia dos tratamentos antimicrobianos. Essa resistência ocorre quando microrganismos desenvolvem a habilidade de resistir aos efeitos de medicamentos que anteriormente eram eficazes contra eles. Embora seja mais comum em bactérias, a resistência também pode ocorrer em outros microrganismos, como fungos e protozoários (Chen *et al*., 2021; Salam *et al*., 2023). Os microrganismos resistentes são frequentemente responsáveis por infecções nosocomiais, ou seja, infecções adquiridas durante a estadia em hospitais ou unidades de saúde, impactando principalmente pacientes vulneráveis devido a condições de saúde pré-existentes ou tratamentos médicos invasivos (Khan; Baig; Mehboob, 2017).

A resistência microbiana foi considerada pela OMS uma das dez maiores ameaças à saúde pública global, tornando-se a terceira principal causa de mortes no mundo (Sharma *et al*., 2020; WHO, 2021). De acordo com a OMS, essa resistência resulta em cerca de 700 mil mortes anuais globalmente, e se não for controlada, esse número pode atingir 10 milhões até 2050 (WHO, 2019). Em 2019, estima-se que a resistência microbiana contribuiu para 4,95 milhões de mortes, sendo diretamente responsável por aproximadamente 1,27 milhão de óbitos no mundo. Os custos econômicos associados à

resistência microbiana são elevados, com estimativas indicando que, até 2050, os gastos globais diretos e indiretos poderão ultrapassar 1 trilhão de dólares, devido a tratamentos prolongados e hospitalizações (WHO, 2023a).

Apesar do desenvolvimento de novos antibióticos após a descoberta da penicilina, que ampliou as opções terapêuticas, a utilização inadequada desses medicamentos, como a automedicação e o uso profilático, tem contribuído para o aumento da resistência microbiana, tornando urgente a busca por novas alternativas (Murugaiyan *et al*., 2022). Neste cenário, pesquisadores estão explorando novas estratégias, como o uso de substâncias derivadas de recursos naturais, apresentando-se como alternativas ou complementos aos antibióticos convencionais (Abdallah *et al*., 2023; Angelini *et al*., 2024).

2.3.1 Fungos

Os fungos são organismos eucariontes e heterotróficos, podendo ser unicelulares ou multicelulares, cujas formas podem variar de leveduras a fungos filamentosos. Eles constituem um dos grupos mais diversificados da natureza, habitando diversos substratos e ambientes com diferentes condições (Naranjo-Ortiz; Gabaldón, 2019; Leite Júnior *et al*., 2020; Corbu *et al*., 2023). Até o momento, cerca de 150.000 espécies de fungos foram descritas, mas estimativas indicam que entre 2 e 13 milhões de espécies ainda não foram catalogadas (Phukhamsakda *et al*., 2022).

A parede celular dos fungos é uma estrutura composta principalmente por quitina, glucanos e proteínas. Essa parede desempenha funções como controle da permeabilidade celular, resistência, suporte estrutural e proteção contra estresse osmótico e mecânico (Garcia-Rubio *et al*., 2020). Além disso, os fungos possuem características como a capacidade de sintetizar lisina via rota biossintética do ácido α-aminoadípico e a presença de ergosterol na membrana plasmática, que ajudam a manter a integridade e fluidez dessa membrana (Rodrigues, 2018; Lu *et al*., 2023).

Os fungos desempenham funções essenciais nos ecossistemas, principalmente na decomposição da matéria orgânica, o que contribui para a ciclagem de carbono e outros nutrientes. Muitos também formam associações mutualísticas com raízes de plantas, conhecidas como micorrizas, que ampliam a capacidade de absorção de nutrientes (Fisher *et al*., 2020; Corbu *et al*., 2023). Além disso, os fungos são de grande importância na produção de antibióticos, fermentação de alimentos e bebidas, e são amplamente utilizados em biotecnologia, especialmente em processos de biorremediação (Corbu *et*

al., 2023). No entanto, o reino fúngico também inclui espécies patogênicas que podem causar infecções, desde as superficiais até as sistêmicas graves, impactando significativamente a saúde humana. Entre os principais agentes patogênicos fúngicos estão espécies dos gêneros *Aspergillus, Cryptococcus, Candida* e *Pneumocystis*, responsáveis por cerca de 90% das mortes por infecções fúngicas (Fisher *et al*., 2020; Leblanc *et al*., 2020).

Algumas espécies do gênero *Candida* representam uma séria ameaça à saúde. Embora façam parte da microbiota normal de indivíduos saudáveis, e, geralmente não causem sintomas, possuem fatores de virulência que favorecem sua patogenicidade, especialmente em hospedeiros imunocomprometidos. Entre as características incluem a capacidade de aderir a superfícies epiteliais e dispositivos médicos, a formação de biofilmes resistentes a tratamentos, a produção de enzimas hidrolíticas, como proteases e fosfolipases, e a transição fenotípica entre leveduras e hifas (dimorfismo), que contribuem para sua patogenicidade e disseminação no organismo (Arafa *et al*., 2023). Nessas condições, podem provocar desde candidíase superficial até infecções mais graves (Ciurea *et al*., 2020; Talapko *et al*., 2021). Entre as mais de 150 espécies de *Candida*, 20 são responsáveis por infecções em humanos. Essas infecções hospitalares representam cerca de 8% das infecções nosocomiais em todo o mundo e estão associadas a elevadas taxas de mortalidade (Arafa *et al*., 2023; Paz *et al*., 2023). Embora a espécie mais comum em infecções clínicas seja *C. albicans*, outras espécies não-albicans, como *C. glabrata*, *C. krusei*, *C. parapsilosis* e *C. tropicalis*, vêm se tornando mais prevalentes (Gómez-Gaviria; Mora-Montes, 2020; Arafa *et al*., 2023).

Candida albicans é um fungo comensal presente nas mucosas orais, vaginais e no trato gastrointestinal humano (Lopes; Lionakis, 2022). Sua capacidade de adaptação permite que atue como patógeno oportunista, especialmente em condições como imunossupressão, uso prolongado de antibióticos ou desequilíbrio da microbiota (Lopes; Lionakis, 2022; Jacobsen *et al*., 2023). *Candida albicans* é uma das espécies fúngicas mais frequentemente isoladas em laboratórios, estando amplamente associada a candidíase invasiva (Parambath *et al*., 2024). Uma das principais características que contribuem para sua virulência é a plasticidade morfológica, que permite a transição da forma de levedura e formas filamentosas, como hifas (células alongadas e tubulares) e pseudohifas (células alongadas ligadas à célula-mãe com constrições nas junções). Essa capacidade facilita a colonização de diferentes tecidos do hospedeiro. Além disso, *C. albicans* é conhecida por formar biofilmes, que oferecem proteção contra o sistema

imunológico do hospedeiro e tratamentos antifúngicos, e pela expressão de adesinas e invasinas, que ajudam na adesão às superfícies epiteliais e na invasão de tecidos subjacentes (Talapko *et al*., 2021; Lopes; Lionakis, 2022).

Candida krusei é outra espécie do gênero *Candida* capaz de causar infecções que vão desde as superficiais até as invasivas, caracterizando-se por sua alta taxa de mortalidade, que varia de 40 a 58%, e pela sua resistência aos tratamentos convencionais. Diferentemente da maioria das espécies do gênero, que possuem células de formato esférico ou ovoide, as células de *C. krusei* são alongadas, com aparência semelhante a grãos de arroz (Gómez-Gaviria; Mora-Montes, 2020). Assim como *C. albicans*, *C. krusei* também apresenta transição morfológica e tem a capacidade de formar biofilmes, fatores que contribuem significativamente para a sua virulência e invasão dos tecidos do hospedeiro (Gómez-Gaviria; Mora-Montes, 2020; Jamiu *et al*., 2021). As infecções causadas por *C. krusei* estão associadas a uma menor taxa de sobrevivência (53,6%) em comparação com outras espécies do gênero (Khalifa *et al*., 2021).

Candida tropicalis é uma das três espécies não-*albicans* mais frequentemente isolada, especialmente em unidades de terapia intensiva, onde está associada a elevadas taxas de mortalidade. Esta espécie é comumente detectada em infecções relacionadas a neutropenia e a malignidades hematológicas (Pfaller; Diekema, 2007; Silva *et al*., 2012; Abdel-Hamid *et al*., 2023). Sua patogenicidade é impulsionada por diferentes fatores de virulência como a produção de enzimas extracelulares, incluindo fosfolipases, hemolisinas, coagulases e proteinases, que facilitam a invasão tecidual e causam danos. Além disso, *C. tropicalis* forma biofilmes densos e resistentes, compostos por uma rede complexa de blastoconídios e uma matriz extracelular, proporcionando proteção contra terapias antifúngicas (Arastehfar *et al*., 2020; Lima *et al*., 2022). Outro fator que contribui para sua virulência é o dimorfismo celular, que permite a alternância entre formas de levedura e formas filamentosas, como hifas e pseudohifas, por meio da transição morfológica. Esse processo facilita a adesão e invasão dos tecidos, além de aumentar sua resistência aos tratamentos antifúngicos. *Candida tropicalis* também tem demonstrado resistência significativa a antifúngicos amplamente utilizados, como os azóis, tornando seu tratamento em contextos clínicos ainda mais desafiador (Keighley *et al*., 2024).

Os antifúngicos usados no tratamento das infecções por *Candida* spp. pertencem principalmente a três classes: os azóis, as equinocandinas e os polienos (Logan; Wolfe; Williamson, 2022). Os azóis são a classe de antifúngicos mais amplamente utilizada na prática clínica. Esses medicamentos são fungistáticos e agem inibindo a enzima lanosterol

14α-desmetilase do citocromo P450, codificada pelo gene Erg_{11}. Essa inibição bloqueia a síntese de ergosterol e provoca o acúmulo de esteróis tóxicos, como o 14-α-metil-3,6-diol, comprometendo a integridade da membrana celular do fungo (Bohner; Papp; Gácser, 2022; Zhu *et al*., 2023). Entre os azóis, o fluconazol é um dos mais utilizados, especialmente devido ao seu baixo custo, solubilidade e disponibilidade em várias formulações. No entanto, a natureza fungistática dos azóis, combinada com o uso inadequado ou prolongado, tem contribuído para o surgimento de microrganismos resistentes (Jamiu *et al*., 2021; Palmucci *et al*., 2024).

As equinocandinas são lipopeptídeos de origem fúngica e fazem parte de uma das classes mais recentes de antifúngicos. Elas atuam inibindo a síntese de glucano, um componente fundamental da parede celular dos fungos, por meio da inibição da enzima 1,3-β-D-glucano sintase, resultando na fragilidade celular e consequente lise celular (Jamiu *et al*., 2021; Heard; Wu; Winter, 2021). As equinocandinas apresentam baixa toxicidade em humanos devido à sua ação específica na parede celular dos fungos, estrutura ausente nas células humanas. Embora elas apresentem menos efeitos colaterais tóxicos em comparação com as outras classes de antifúngicos, seu uso clínico é limitado devido ao alto custo e à baixa biodisponibilidade oral, o que exige administração intravenosa (Szymański *et al*., 2022; Zhu *et al*., 2023).

Os polienos, por outro lado, são uma classe antiga de antifúngicos, sendo a anfotericina B seu representante mais conhecido. Eles agem ao se ligar ao ergosterol presente na membrana celular fúngica, formando poros que comprometem a integridade da membrana, desregulam a homeostase iônica e resultam em lise celular. Além disso, os polienos podem exercer atividade fungicida removendo o ergosterol das membranas lipídicas fúngicas, formando agregados fora da membrana e interferindo no funcionamento celular (Bohner; Papp; Gácser, 2022; Zhu *et al*., 2023). Embora sejam eficazes contra fungos, os polienos podem ser tóxicos para humanos devido à similaridade estrutural entre o ergosterol fúngico e o colesterol humano (Sahay *et al*., 2019; Bohner; Papp; Gácser, 2022).

Atualmente, a disponibilidade limitada de antifúngicos para o tratamento da candidíase é agravada pelo surgimento de cepas resistentes. A resistência aos antifúngicos tornou-se um desafio crescente na prática clínica, refletindo a capacidade adaptativa dos fungos e a pressão seletiva causada pelo uso inadequado ou prolongado desses medicamentos (Bohner; Papp; Gácser, 2022; Lee; Robbins; Cowen, 2023). A OMS classificou a resistência antifúngica como uma prioridade global de saúde, destacando

espécies de *Candida* como principais preocupações devido à crescente resistência e impacto nos ambientes clínicos, reforçando a necessidade de desenvolver novos tratamentos e estratégias de controle (WHO, 2022).

2.3.2 Bactérias

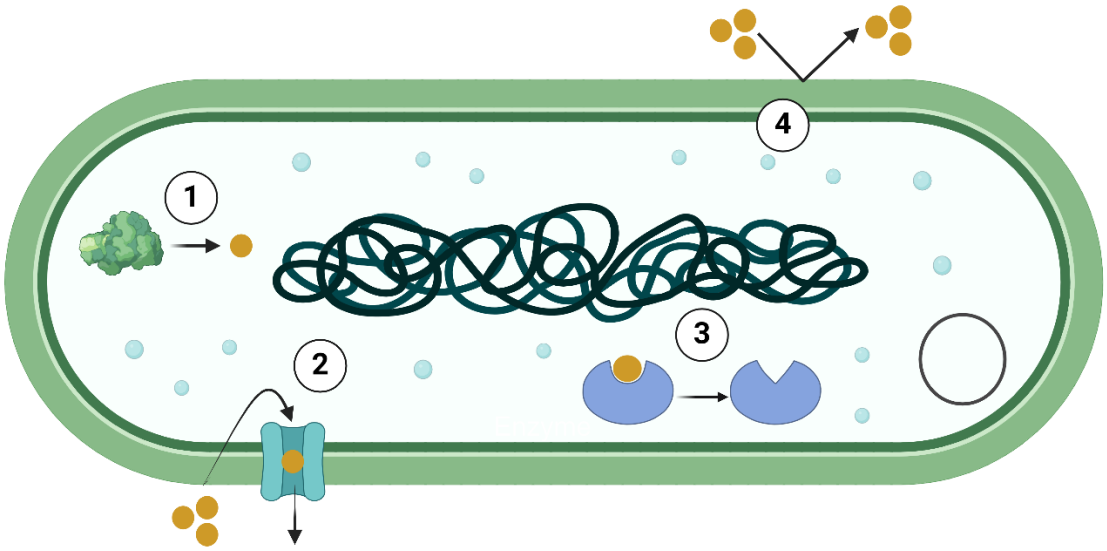
As bactérias são microrganismos unicelulares e procariontes que podem ser encontrados em praticamente todos os ambientes. Elas podem ser classificadas de acordo com seu modo de nutrição, sendo autotróficas, capazes de produzir seu próprio alimento por meio da quimiossíntese, ou heterotróficas, que obtêm nutrientes a partir da matéria orgânica presente no ambiente. Outro critério de classificação das bactérias é a estrutura de sua parede celular, dividindo-as em Gram-positivas e Gram-negativas. As primeiras possuem uma parede celular espessa, composta por várias camadas de peptidoglicano e ácidos teicóicos, enquanto as segundas apresentam uma parede celular mais fina, composta por uma ou poucas camadas de peptidoglicano, envolvidas por uma membrana externa adicional e lipopolissacarídeos inseridos nesta membrana (Waksman; Starkey, 1922; Gupta; Gupta; Singh, 2017).

As bactérias desempenham papéis cruciais em processos biológicos e ecológicos, como a decomposição de matéria orgânica, a ciclagem de nutrientes e a fixação de nitrogênio no solo (Wang; Chi; Song, 2024). Além disso, as bactérias possuem aplicações práticas em diversas áreas, incluindo a produção de alimentos, antibióticos e o tratamento de águas residuais, sendo importantes na indústria, medicina e biotecnologia. No entanto, algumas bactérias podem ser ou se tornar patogênicas, como acontece com as bactérias comensais. Normalmente inofensivas em um estado de simbiose com o hospedeiro, elas podem causar infecções e doenças quando há desequilíbrio na microbiota ou comprometimento do sistema imunológico do hospedeiro (Tshikantwa *et al*., 2018; Ali *et al*., 2023).

Bactérias patogênicas são responsáveis por uma variedade de doenças infecciosas, que vão desde infecções leves, como as de natureza cutânea, até condições graves e potencialmente fatais, como pneumonia e sepse (Soni; Sinha; Pandey, 2024). O tratamento dessas infecções enfrenta o desafio da crescente resistência aos antibióticos, que pode ocorrer por diferentes mecanismos. Esses incluem a produção de enzimas que inativam os antibióticos, a modificação dos alvos moleculares desses medicamentos, a alteração da permeabilidade da membrana celular para reduzir sua entrada, e a ativação

de bombas de efluxo que expulsam os antibióticos da célula bacteriana, entre outros (Mancuso *et al*., 2021; Muteeb *et al*., 2023) (Figura 3).

Figura 3. Principais mecanismos de resistência bacteriana aos antibióticos. (1) = Inativação enzimática; (2) = Efluxo do antibiótico; (3) = Alteração do sítio-alvo; (4) = Bloqueio da entrada do fármaco.



Fonte: Autora (2023).

A resistência bacteriana é particularmente preocupante em espécies patogênicas como *Escherichia coli*, *Pseudomonas aeruginosa* e *Staphylococcus aureus*, que estão frequentemente associadas às infecções nosocomiais. Esses microrganismos estão ligados a elevadas taxas de mortalidade e morbidade, além de prolongarem o tempo de internação e aumentarem os custos de tratamento, representando uma ameaça significativa à saúde pública (Mancuso *et al*., 2021; Murray *et al*., 2022).

Escherichia coli (Enterobacteriaceae) é uma bactéria Gram-negativa normalmente presente no intestino humano como comensal. No entanto, cepas patogênicas podem causar uma variedade de doenças, tanto intestinais, como diarreia aquosa ou sanguinolenta, síndrome hemolítico-urêmica e colite, quanto extraintestinais, como bacteremia, sepse, meningite e infecções do trato urinário. As cepas de *E. coli* podem ser classificadas em três grupos: (1) comensais, que residem normalmente no trato gastrointestinal sem causar doenças, (2) patogênicas intestinais, responsáveis por

infecções gastrointestinais diarreicas (*E. coli* enteropatogênica, *E. coli* enterotoxigênica, *E. coli* enterohemorrágica, *E. coli* enteroagregativa, *E. coli* enteroinvasiva e *E. coli* difusamente aderente), e (3) patogênicas extraintestinais, que incluem cepas uropatogênicas e associadas à meningite neonatal, que causam infecções fora do trato intestinal (Geurtsen *et al*., 2022).

O tratamento das infecções por *E. coli* tem se tornado cada vez mais desafiador devido à crescente resistência a diferentes classes de antibióticos, incluindo *β*-lactâmicos, quinolonas e aminoglicosídeos (Kakoullis *et al*., 2021). Os antibióticos *β*-lactâmicos, como penicilinas e cefalosporinas, agem inibindo a síntese da parede celular bacteriana. No entanto, a resistência a esses antibióticos ocorre principalmente pela produção de *β*-lactamases, como as de classe A (TEM e CTX-M) e as metalo-*β*-lactamases, que hidrolisam o anel *β*-lactâmico, tornando esses antibióticos ineficazes. As quinolonas, como ciprofloxacino, que inibem a enzima DNA girase, essencial para a replicação bacteriana, enfrentam resistência mediada por mutações no gene *gyrA*, responsável pela codificação da subunidade A da DNA girase, o que compromete a ação do fármaco. Já os aminoglicosídeos, como a gentamicina, que interferem na síntese proteica bacteriana ao se ligar à subunidade ribossômica 30S, perdem eficácia devido a modificações enzimáticas que inativam o antibiótico (Kakoullis *et al*., 2021; Gauba; Rahman, 2023). Esses mecanismos de resistência tornam o tratamento das infecções por *E. coli* cada vez mais complexo, muitas vezes exigindo o uso de antibióticos de última linha, como carbapenêmicos e polimixinas, que também têm apresentado aumento nos índices de resistência (Venne *et al*., 2023; Huang *et al*., 2024).

Pseudomonas aeruginosa (Pseudomonadaceae) é uma bactéria Gram-negativa amplamente encontrada em diversos ambientes, incluindo solo, água, plantas, tecido de mamíferos e ambientes hospitalares. Embora muitas vezes seja inofensiva, pode se tornar um patógeno oportunista em indivíduos imunocomprometidos (Qin *et al*., 2022; Tuon *et al*., 2022). É o quarto patógeno nosocomial mais isolado, responsável por cerca de 10% das infecções adquiridas em hospitais. As infecções associadas a *P. aeruginosa* incluem pneumonia associada à ventilação mecânica, infecções do trato urinário, feridas ou queimaduras, e septicemia. Além disso, *P. aeruginosa* é caracterizada por sua capacidade de adaptação a mudanças no ambiente, desenvolvendo resistência a antibióticos e produzindo uma variedade de fatores de virulência, o que a torna uma ameaça significativa à saúde (Qin *et al*., 2022; Bondareva *et al*., 2024; Elfadadny *et al*., 2024). A capacidade desse patógeno de formar biofilmes altamente resistentes a antibióticos é um

dos principais desafios no tratamento de infecções nosocomiais a ele associadas (Kakoullis *et al*., 2021).

O tratamento de infecções causadas por *P. aeruginosa* tem sido dificultado pela crescente resistência a antibióticos, particularmente aos *β*-lactâmicos, como cefalosporinas e carbapenêmicos. Essa resistência é atribuída a uma série de mecanismos, incluindo a produção de *β*-lactamases e a alterações na membrana externa que dificultam a entrada de antibióticos. Dentre outros mecanismos de resistência, destacam-se a produção de *β*-lactamases de classe C, como a cefalosporinase AmpC, e de carbapenemases de classe B, como as metalo-*β*-lactamases (MBLs). O aumento da resistência a esses antibióticos, somado a capacidade de adaptação de *P. aeruginosa*, torna o tratamento das infecções um desafio (Kakoullis *et al*., 2021; Gauba; Rahman, 2023). A OMS classificou *P. aeruginosa* como um patógeno de "alta prioridade" para pesquisa e desenvolvimento de novos antimicrobianos (WHO, 2024).

Staphylococcus aureus (Staphylococcaceae) é uma bactéria Gram-positiva encontrada na pele e nas mucosas humanas, como a cavidade nasal, boca e o intestino. Embora muitas vezes seja comensal, *S. aureu*s pode causar infecções leves na pele e tecidos moles até condições mais graves, como pneumonia, osteomielite, endocardite, artrite séptica, bacteremia e septicemia. (Guo *et al*., 2020; Howden *et al*., 2023). Desde a descoberta da resistência à penicilina na década de 1940, *S. aureu*s tem continuamente desenvolvido novos mecanismos de resistência. Entre eles, destacam-se a produção de *β*-lactamases e a expressão da proteína de ligação à penicilina (PBP2a), que confere resistência a quase todos os antibióticos *β*-lactâmicos. Além disso, *S. aureus* também demonstra resistência a diversos outros antibióticos amplamente utilizados, como tetraciclinas, aminoglicosídeos, macrolídeos, fluoroquinolonas, vancomicina e linezolida. Esses mecanismos incluem alterações em alvos moleculares, produção de bombas de efluxo e alteração da espessura da parede celular, reforçando sua capacidade de adaptação e dificultando o tratamento (Kakoullis *et al*., 2021; Mlynarczyk-Bonikowska *et al*., 2022).

3 ARTIGO 1 – Chemical composition, antifungal, and anti-virulence action of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) against *Candida* spp.

Artigo Publicado
Revista: *Journal of Ethnopharmacology*
ISSN: 0378-8741
Fator de Impacto: 4.8
Qualis – CAPES (2017-2020): A1 em Biodiversidade
Percentil Scopus (2024): 88%
DOI: https://doi.org/10.1016/j.jep.2023.117506

Viviane Bezerra da Silva [a,*], José Weverton Almeida-Bezerra [a], Maria Hellena Garcia Novais [b], Naiza Saraiva Farias [b], Janerson José Coelho [c], Paulo Riceli Vasconcelos Ribeiro [d], Kirley Marques Canuto [d], Henrique Douglas Melo Coutinho [e], Maria Flaviana Bezerra Morais-Braga [b], Antonio Fernando Morais de Oliveira [a]

[a] Department of Botany, Universidade Federal de Pernambuco – UFPE, Rua Professor Moraes Rego, s/n, Recife, Pernambuco, 50.670-901, Brazil
[b] Department of Biological Sciences, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105-000, Brazil
[c] Animal Science Department, Universidade Estadual do Maranhão - UEMA, São Luís, Maranhão, Brazil
[d] Multi-User Natural Products Chemistry Laboratory – LMQPN, Embrapa Agroindústria Tropical, Rua Dra. Sara Mesquita, 2270, Fortaleza, Ceará, 60511-110, Brazil
[e] Department of Biological Chemistry, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105-000, Brazil
* Corresponding author: E-mail address: viviane.silva@urca.br (V.B. Silva)

Abstract

Ethnopharmacological relevance: Hancornia speciosa Gomes is a fruit and medicinal species used for treating infectious diseases of the genitourinary system. However, its mechanism of action against microbes is still not fully understood. Infections in the genitourinary system caused by *Candida* spp. are associated with its fungal resistance and

pathogenicity. New plant-derived compounds are an alternative to fight these *Candida* infections.

Aim of the study: The objective of this study was to evaluate the anti-*Candida* effects of extracts of the stem bark of *H. speciosa*. This research investigated the chemical composition of sulfuric ether (EEHS) and methanolic (MEHS) extracts, their drug-modifying action on fluconazole, and their anti-virulence action on the morphological transition of *Candida* species.

Materials and methods: The extracts (EEHS and MEHS) of the stem bark of *H. speciosa* were chemically characterized via qualitative phytochemical screening and by liquid chromatography coupled with mass spectrometry (UPLC-MS-ESI-QTOF). The extracts were evaluated regarding their antifungal effects and fluconazole-modifying activity against *Candida albicans*, *Candida krusei*, and *Candida tropicalis* using the broth microdilution method. Additionally, the study evaluated the inhibition of fungal virulence in *Candida* species through morphological transition assays.

Results: The phytochemical screening revealed the presence of anthocyanidins, anthocyanins, aurones, catechins, chalcones, flavones, flavonols, flavanones, leucoanthocyanidins, tannins (condensed and pyrogallic), and xanthones in both extracts of the stem bark of *H. speciosa.* The UPLC-MS-ESI-QTOF analysis identified the same compounds in both extracts, predominating phenolic compounds. Some compounds were first time recorded in this species: gluconic acid, cinchonain IIb, cinchonain Ib isomer, and lariciresinol hexoside isomers. Most of the intrinsic antifungal activity was observed for the MEHS against *C. krusei* (IC_{50}: 58.41 μg/mL). At subinhibitory concentrations (MC/8), the EEHS enhanced the action of fluconazole against all *Candida* strains. The MEHS exhibited greater efficacy than fluconazole inhibiting *C. krusei* growth. The EEHS completely inhibited hyphae appearance and reduced pseudohyphae formation in *C. albicans*.

Conclusion: The stem bark of *H. speciosa* is a rich source of bioactive compounds, especially phenolic. Phenolic compounds can have important roles in fighting infectious diseases of the genitourinary system, such as candidiasis. The extracts of *H. speciosa* improved the action of the drug fluconazole against *Candida* species, inhibited hyphae appearance, and reduced pseudohyphae formation. The results of this study can support the development of new therapeutics against resistant strains of *Candida*.

Keywords: Cinchonain; Ethnomedicine; Flavonoids; Hyphae; Phenolic compounds.

1. Introduction

The growing prevalence of fungal infections caused by the *Candida* genus is an ongoing challenge for the human health system (Li et al., 2018). These microorganisms are naturally found in the microbiota of the human body, inhabiting gastrointestinal and genitourinary tracts, skin, and mucous membranes (Eksi et al., 2022; Saracino et al., 2022). However, under circumstances that modify or stress the balance of the microbiota, there is the proliferation and dissemination of *Candida* yeasts, leading to a broad range of infections, varying from skin infections to invasive candidiasis (bloodstream infection) (Talapko et al., 2021; Valand and Girija, 2021).

Candida albicans is recognized as the predominant species associated with fungal infections, nevertheless, recent reports indicate an increase in the incidence of infections caused by non-albicans *Candida*, such as *C. tropicalis* and *C. krusei* (World Health Organization, 2020, 2022). Several factors contributed to this scenario, including intensive and inappropriate use of broad-spectrum antibiotics, immunosuppressive treatments, and prolonged hospital stays (Çoban et al., 2023; Srivastava et al., 2018). These factors led to antifungal resistance and the pathogenicity of *Candida* species, making the treatment of infections more challenging (Poissy et al., 2022).

The pathogenicity of *Candida* spp. is influenced by different virulence mechanisms, including the ability to form biofilms, secretion of extracellular enzymes, the capacity of adhesion and invasion into host cells, and the ability to undergo morphological transition from yeast to filamentous forms such as hyphae and pseudohyphae (Gizinska et al., 2019; Melo et al., 2019). The morphological transition observed in some *Candida* species is particularly worrying, as this trait allows the yeast to assume an invasive form, significantly enhancing its capacity to cause severe and disseminated infections (Das et al., 2019; Kornitzer, 2019). The presence of hyphae and pseudohyphae in some *Candida* species enables them to penetrate tissue and organs, making these infections a constant health challenge, worsened by the limited number of available antifungal classes to fight these infections (Capoci et al., 2019; Khan et al., 2021; Li et al., 2020).

Studies have been carried out to discover new natural or synthetic substances that can inhibit the proliferation of different pathogens with minimum side effects, as well as compounds that can improve the activity of standard antifungal drugs (Costa et al., 2021; Feitosa et al., 2022; Vaou et al., 2021). Among the natural products, those derived from

medicinal plants are of great interest to the pharmaceutical industry, due to their great antimicrobial potential (Martins et al., 2015; Ugboko et al., 2020). In recent years, there have been significant advances regarding the research of the antifungal potential of natural products (e.g., oils and plant extracts) (Lima et al., 2022; Gao et al., 2023; Almeida-Bezerra et al., 2023; Sampaio et al., 2023). Natural products can have several advantages compared to synthetic antifungal drugs, they generally are safer, display high biodegradability, and have low toxicity for humans (Ju et al., 2022; Osonga et al., 2022). Furthermore, several natural products have demonstrated a broad diversity of active compounds against different fungi strains (Zheng et al., 2023; Sasidharan et al., 2023).

The antifungal properties of plant extracts have been confirmed by a variety of studies, demonstrating their effectiveness in inhibiting the growth of different pathogenic fungi (Lima et al., 2022; Gao et al., 2023; Almeida-Bezerra et al., 2023). These studies also revealed the mechanisms of action against fungi, especially their interference in essential metabolic processes and inhibiting the formation of structures associated with fungal pathogenicity and virulence (e.g., hyphae) (Ju et al., 2022; Wu et al., 2022; Sasidharan et al., 2023). These findings emphasized the importance of medicinal plants and their compounds as promising sources for developing new antifungal drugs. (Sasidharan et al., 2023).

According to the review published by Bhadane et al. (2018), species belonging to the Apocynaceae family have been widely used in herbal medicine. Apocynaceae species are widely recognized for having diverse bioactive compounds, including alkaloids, terpenoids, flavonoids, simple phenols, hydrocarbons, and lactones (Bhadane et al., 2018). Among the species of this family, some have shown medicinal properties as anticonvulsant, anticancer, antimalarial, gastroprotective, antitussive, antipyretic, antiviral, and anti-inflammatory (Anand et al., 2020). The success of the therapeutics based on the use of Apocynaceae species, for example, led to the development of antitumor drugs of great clinical importance, such as vincristine and vinblastine (Almagro et al., 2015).

Among the Apocynaceae species, *Hancornia speciosa* Gomes, a fruit species known in Brazil as “mangabeira”, stands out in herbal medicine in the treatment of diseases associated with the genitourinary and digestive systems, such as general infections, diarrhea, dysentery, urinary infection, and fungal diseases (Cruz et al., 2021; D’Abadia et al., 2020; Albuquerque and Meiado et al., 2015; Nunes et al., 2022). The medicinal properties of this species can be attributed to its chemical composition, and

several organs of this plant were identified as a source of bioactive compounds, such as caffeic acid, quinic acid, catechins, proanthocyanidins, tannins, flavones, flavonols, and flavanones (Bastos et al., 2017; D'Abadia et al., 2020; Moraes et al., 2008).

Considering the pharmacological potential of *H. speciosa* described in ethnomedicinal reports, particularly in the context of treating infectious diseases associated with the genitourinary system, the objective of this study was to investigate the chemical composition and *in vitro* effects of the stem bark extracts of *H. speciosa* against *Candida* species and also their drug-modifying action on fluconazole. Additionally, this study aimed to investigate the effects of these extracts on the inhibition of the morphological transition in *Candida* species.

2. Materials and methods

2.1. Collection of plant material

The stem bark of *H. speciosa* was collected in September 2021 in the plateau known as Chapada do Araripe, in an Environmental Protection Area (EPA) located in the municipality of Jardim (state of Ceará, Brazil), under the coordinates 7º29′02.4″S and 39º16′51.9″W, at an altitude of 920 m above sea level (Fig. 1). An exsiccate of the species was deposited at the UFP Herbarium - Geraldo Mariz of the Universidade Federal de Pernambuco – UFPE, under registration number #88,947. This study was approved by the Biodiversity Authorization and Information System (SISBio) under registration number 80293–1 and the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number A535238.

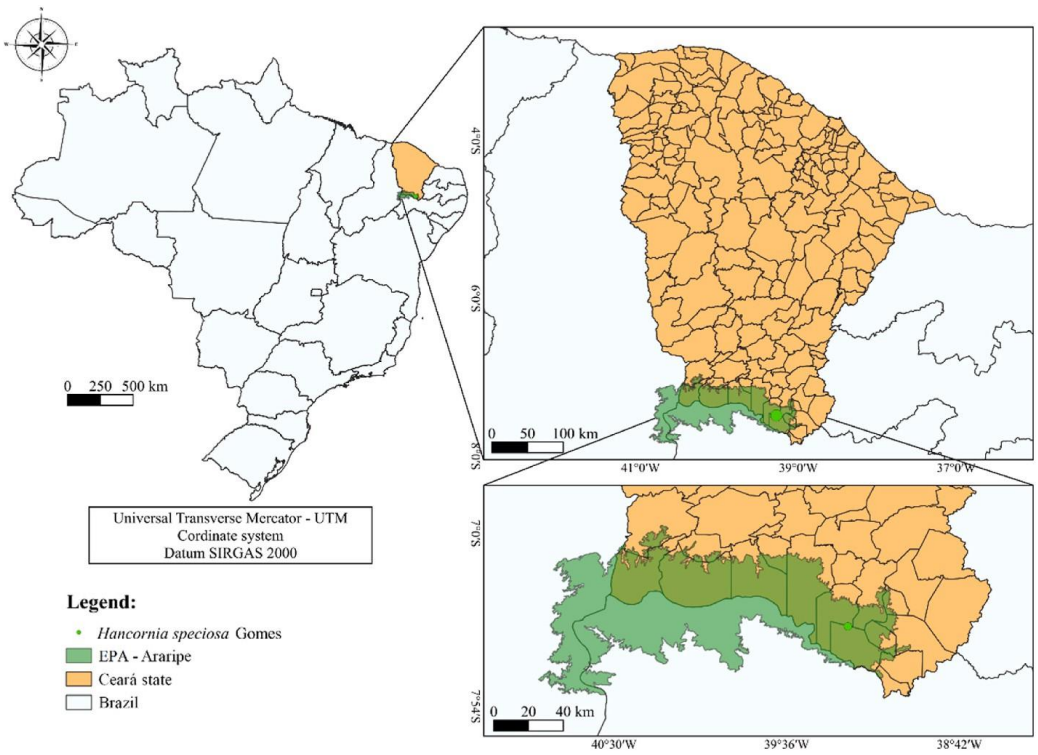


Fig. 1. Map of the collection site of *Hancornia speciosa* Gomes in the Environmental Protection Area of the Chapada do Araripe, Jardim, state of Ceará, Brazil.

2.2. Preparation of the extracts

To produce the extracts of the stem bark, we followed the methodology described by Rodrigues et al. (2022). Around 950 g of the stem bark of *H. speciosa* was dried at 45 ºC for seven days. Following, the stem bark was ground and subjected to an exhaustive extraction using n-hexane for 72 h, aiming to remove compounds with low polarity. After filtration, a reextraction was carried out using sulfuric ether, and subsequently with methanol for an equal period. At the end of the process, the sulfuric ether (EEHS) and methanolic (MEHS) extracts were stored in an amber flask at room temperature. They were kept stored until the chemical analyses and bioactivity tests. The n-hexane extract was not used in this study.

2.3. Phytochemical analysis

2.3.1. Qualitative chemical prospecting

For the qualitative evaluation of the presence of secondary metabolites, we followed the methodology described by Matos (2009), which is used for the detection of alkaloids, anthocyanidins, anthocyanins, aurones, catechins, chalcones, flavones, flavonols, flavanones, leucoanthocyanidins, tannins (condensed and pyrogallic), and

xanthones. The presence of each metabolite was verified by the colorimetric alteration of the extracts and the formation of precipitates after the addition of specific reagents.

A quantity of 300 mg of each extract of *H. speciosa* was diluted in 30 mL of ethanol (70%). Subsequently, 3 mL of each resulting solution was dispensed into eight bottles, totaling sixteen. A specific reagent was added to each bottle to reveal the chemical classes present in the solutions. The identification of phenols and tannins was carried out using ferric chloride ($FeCl_3$). The screening of anthocyanidins, anthocyanins, flavonoids, leucoanthocyanidins, catechins, and flavanones was carried out by adding 1% hydrochloric acid (HCl) and 5% sodium hydroxide (NaOH). For the alkaloid identification test, acetic acid (5%), 10% ammonium hydroxide (NH_4OH), chloroform, sodium hydroxide, and Dragendorff's reagent were used (Matos, 2009).

2.3.2 *Ultra-performance liquid chromatography coupled to quadrupole/ time of flight mass spectrometry system (UPLC-MS-ESI-QTOF)*

The analysis of the extracts was performed using an Acquity UPLC (Waters, USA) system coupled to a mass system (Q-TOF, Waters) according to the method previously described by Carvalho et al. (2019) with some modifications. A Waters Acquity BEH C18 column (150 mm × 2.1 mm, 1.7 μm) was used at 40 ◦C for the separation. An injection volume of 5-μL aliquot of each extract was subjected to an exploratory gradient with the mobile phase composed of deionized water (A) and acetonitrile (B), both containing formic acid (0.1% v/v).

The extracts were subjected to the exploratory gradient as follows: 2–100% B (22.0 min), 100% B (22.1–25.0 min), and 2% B (26.0–30.0 min), with a flow rate of 0.3 mL/min. The ionization was performed using an electrospray ionization source in negative mode (ESI^-), acquired at the range of 110–1180 Da. The optimized instrumental parameters were: desolvation temperature at 350 ºC, capillary voltage at 3.2 kV, source temperature at 120 ◦C, cone voltage at 15 V, and desolvation gas flow at 500 L/h. The software MassLynx 4.1 (Waters MS Technologies, Manchester, UK) was used to assign exact masses, as well as the molecular formula of the compounds. Data were analyzed via comparison with those described in the literature regarding the species of the Apocynaceae family. Peak identification was determined by the *m/z* values.

2.4 Antifungal activity

2.4.1 Strains, culture media, drugs, reagents, and solution preparation

For the anti-*Candida* assays, three standard yeast strains were used: *Candida albicans* - CA INCQS 40006, *Candida krusei* - CK INCQS 40095, and *Candida tropicalis* - CT INCQS 40042, from the Culture Collection of the National Institute of Quality Control in Health (INCQS) of the Fundação Oswaldo Cruz (Manguinhos, Rio de Janeiro, Brazil).

The culture media Sabouraud Dextrose Broth (SDB) (Himedia®) and Sabouraud Dextrose Agar (SDA) (Difco®) were used in the antifungal assays, both prepared according to the manufacturer's recommendations. For the morphological transition assays, Potato Dextrose Agar (PDA) – Becton Dickinson & Co. USA, bacteriological agar - ISOFAR, and Yeast Peptone Dextrose (YPD) - Difco® media were used, also prepared according to the manufacturer's instructions. All media were diluted in distilled water and sterilized in a vertical autoclave (121 ºC for 15 min).

Regarding the drugs, the antifungal Fluconazole (Flucolcid®) was used as the positive control in the antifungal assays. The drug was initially diluted in dimethylsulfoxide (DMSO) and subsequently adjusted to varied concentrations from 4 to 2048 μg/mL. The extracts of *H. speciosa* were prepared following the same process. Nevertheless, in the test of combined activity, the products were solubilized in DMSO, and diluted to reach a fixed concentration of MC/8, where MC represented the matrix concentration of the evaluated products equivalent to 2048 μg/mL (Lima et al., 2022).

The fungal strains inoculums used in the microdilution tests were prepared according to the 0.5 McFarland turbidity standard in a saline solution. Subsequently, the concentration was adjusted to 0.5 × 103 CFU in the SDB medium (CLSI, 2017). For the morphological transition assays, the yeasts were reactivated in a YPD medium enriched with 5% sterile Fetal Bovine Serum - Laborclin® and then transferred to a Petri dish containing SDA. For these tests, a depleted PDA culture medium was used to stimulate the formation of hyphae and pseudohyphae, in which Tween 80 P.S. - Dinâmica® (Indaiatuba, São Paulo, Brazil) was added. In this medium, the extracts were added at varied concentrations of MC/2 (1024 μg/mL) and MC/4 (512 μg/mL).

2.4.2 Determination of the half-maximal inhibitory concentration (IC_{50})

For the cell viability assay, it was used the method of microdilution in broth. Flat-bottomed 96-well plates (Kasvi) received 100 μL of SDB containing the fungal inoculum

(0.5 x 10^3 CFU). Subsequently, 100 µL of the extracts or fluconazole were added until reaching the final concentrations ranging from 2 to 1.024 µg/mL (CLSI, 2017). The last two wells of the plates were kept for growth control (11th) and sterility evaluation (12th). In addition to these plates, extract dilution controls were prepared simultaneously, with the inoculum replaced by saline solution. All plates were incubated in a microbial incubator at a constant temperature of 37 ◦C for 24 h. After this incubation period, the plates were subjected to spectrophotometric reading at a wavelength of 630 nm (ELISA, Thermo Plate®) (Morais-Braga et al., 2016).

2.4.3 Minimum fungicidal concentration (MFC)

A quantity of 10 µL was removed from the solution of each well in the plates of the antifungal assay, except for the sterile control. This solution was transferred to Petri dishes containing SDA medium, which was distributed according to a guide card fixed below the plate. These plates were incubated for 24 h at 37 ºC, aiming to analyze the presence or absence of *Candida* colonies (Fonseca et al., 2022). The lowest concentration with no growth of *Candida* spp. was considered the MFC of the extract.

2.4.4 Modifying activity

The extracts of *H. speciosa* (EEHS and MEHS) were evaluated at subinhibitory concentrations (MC/8) to verify if they were able to modify the activity of fluconazole, the essay followed the methodology described by Lima et al. (2022) with some modifications. SDB medium (Himedia®) containing *H. speciosa* extracts at their sub-inhibitory concentrations was added to the plates. Subsequently, fluconazole was added at concentrations ranging from 2 to 1024 µg/mL. The controls of the extract dilution in combination with fluconazole were also performed. After 24 h of growth at 37 ◦C, spectrophotometric readings were performed at a wavelength of 630 nm (ELISA, Thermo Plate®). The results were used to obtain a cell viability curve (Morais-Braga et al., 2016).

2.4.5. Evaluation of fungal virulence inhibition

The evaluation of fungal virulence inhibition was performed according to Carneiro et al. (2019). To observe the yeasts, slides were prepared in sterile micromorphological chambers. A volume of 3 mL of depleted PDA medium was added to the slides containing MC/2 and MC/4 concentrations of the extracts of *H. speciosa*. In the chambers, aliquots of *Candida* spp. were added in two parallel streaks on the solid

medium. Finally, the slides were covered with a sterile coverslip. Subsequently, these slides were placed in a humid chamber and brought to a microbial incubator at 37 ◦C for 24 h. A growth control for the morphological transition of *Candida* spp. was performed, as well as a positive control (fluconazole). At the end of the incubation period, the slides were examined and photographed under an L-2000I-TRINO/6633 optical microscope (Bioval, São Paulo, Brazil).

2.5. Statistical analysis

The antifungal assays were performed in quadruplicate. The means and standard errors of the mean (±SEM) were determined for each assay. A one-way analysis of variance (ANOVA) followed by Tukey's test with 95% confidence was performed. The *p* values were classified as $p < 0.0001$ to 0.001 (*** = extremely significant), 0.001 to 0.01 (** = highly significant), 0.01 to 0.05 (* = significant), and $p > 0.05$ (ns = not significant). To determine the half-maximal inhibitory concentration (IC_{50}) of the antifungal assays, a non-linear regression analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, USA).

3. Results

3.1 Chemical composition

The qualitative analysis of the chemical composition of *H. speciosa* extracts using colorimetric assays and precipitation tests showed the presence of different classes of phenolic compounds in both extracts. However, the EEHS showed no alkaloids in its composition, which may be associated with the polarity of the solvent used (Table 1).

Table 1

Classes of metabolites investigated in the sulfuric ether (EEHS) and methanolic (MEHS) extracts of the stem bark of *Hancornia speciosa*.

Classes	**Extracts**	
	EEHS	**MEHS**
Alkaloids	-	+
Anthocyanins and Anthocyanidins	+	+
Catechins	+	+
Chalcones and Aurones	+	+
Phenols	+	+

Flavones, Flavonols, and Xanthones	+	+
Flavanones	+	+
Flavononols	+	+
Leucoanthocyanidins	+	+
Condensed tannins	+	+
Hydrolyzable tannins	+	+

+ presence; - absence

The chromatograms of the EEHS and MEHS extracts acquired by UPLC-MS are shown in Fig. 2 (A and B, respectively). Based on the analysis of the mass fragments and information from the literature, a total of twelve compounds were identified in both extracts. The compounds identified are listed in Table 2, respectively, where is provided information regarding the elution order, molecular formula, error, and major fragments (MS/MS) (Supplementary Table 1).

In both extracts, we characterized the presence of gluconic acid (peak 1, *m/z*: 195), quinic acid (peak 2, *m/z*: 191), vanillic acid (peak 3, *m/z*: 167), chlorogenic acid (peak 4, *m/z*: 353), procyanidin B dimer (peak 5, *m/z*: 577), catechin (peak 6, *m/z*: 289), procyanidin B trimer (peak 7, *m/z*: 865), epicatechin (peak 8, *m/z*: 289), cinchonain IIb (peak 9, *m/z*: 739), lariciresinol hexoside isomers (peaks 10 and 11, *m/z*: 521), phloretin (peak 11, *m/z*: 273), and cinchonain Ib isomers (peaks 12, 14 and 15, *m/z*: 451).

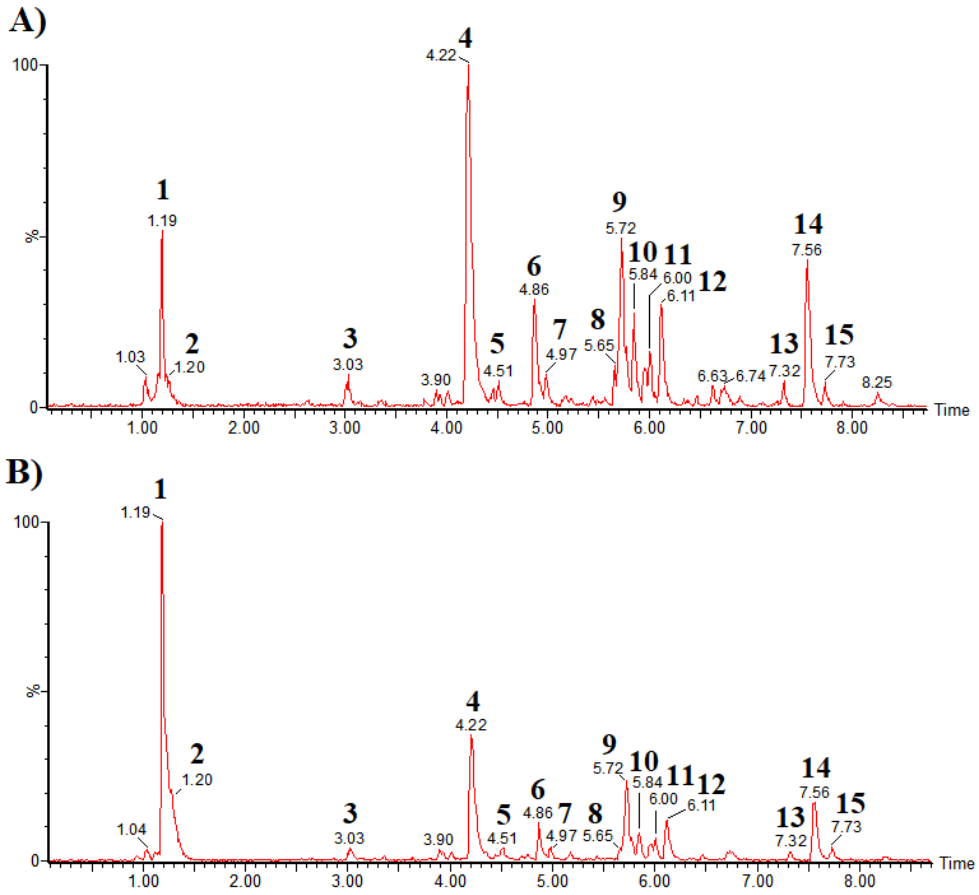


Fig. 2. UPLC-MS in negative ionization mode of the sulfuric ether (EEHS) (A) and methanolic (MEHS) (B) extracts of the stem bark of *Hancornia speciosa*.

Table 2. Compounds tentatively identified in the sulfuric ether (EEHS) and methanolic (MEHS) extracts of the stem bark of *Hancornia speciosa*.

Peak N°	Rt min	$[M-H]^-$ Observed (EEHS)	$[M-H]^-$ Observed (MEHS)	$[M-H]^-$ Calculated	Product Ions (MS/MS) (intensity %)	Empirical Formula	ppm (error)	Compound	References
1	1.19	195.0478	195.0499	195.0505	-	$C_6H_{12}O_7$	-3,1	Gluconic acid	(Bashir et al., 2021)
2	1.20	191.0530	191.0558	191.0556	-	$C_7H_{11}O_6$	-1.0	Quinic acid	(Bastos et al., 2017; Pereira et al., 2015)
3	3.03	167.0353	167.0343	167.0344	152 (32)	$C_8H_7O_4$	-0.6	Vanillic acid	(Huang et al., 2019)
4	4.22	353.0860	353.0877	353.0873	191 (100)	$C_{16}H_{17}O_9$	1.1	Chlorogenic acid	(Bashir et al., 2021; Bastos et al., 2017; Corrêa et al., 2023)
5	4.51	577.1344	577.1360	577.1346	451 (10), 425 (22), 407 (80), 289 (100)	$C_{30}H_{25}O_{12}$	2.4	Procyanidin B dimer	(Bastos et al., 2017)
6	4.86	289.0695	289.0711	289.0711	245 (20)	$C_{15}H_{13}O_6$	0.0	Catechin	(Bastos et al., 2017; Corrêa et al., 2023)
7	4.97	865.1989	865.1990	865.1980	577 (10), 289 (50)	$C_{45}H_{37}O_{18}$	1.2	Procyanidin B trimer	(Bastos et al., 2017)
8	5.65	289.0692	289.0698	289.0711	125 (20)	$C_{15}H_{13}O_6$	-4.8	Epicatechin	(Sinan et al., 2021)
9	5.72	739.1678	739.1653	739.1663	587 (32), 449 (21), 289 (36)	$C_{39}H_{31}O_{15}$	-1.4	Cinchonain IIb	(Boléa et al., 2019)
10	5.84	521.1994	521.2025	521.2023	359 (40)	$C_{26}H_{33}O_{11}$	0.4	Lariciresinol hexoside isomer	(Morreel et al., 2014)
11	6.00	521.2047	521.2028	521.2023	359 (32)	$C_{26}H_{33}O_{11}$	1.0	Lariciresinol hexoside isomer	(Morreel et al., 2014)
12	6.11	451.1009	451.1016	451.1029	341 (100), 217 (30), 189 (18)	$C_{24}H_{19}O_9$	-2,9	Cinchonain Ib isomer	(Dall'Acqua et al., 2021; Santos et al., 2016)
13	7.32	273.0758	273.0740	273.0763	167 (24)	$C_{15}H_{13}O_5$	-7.3	Phloretin	(Bastos et al., 2017; Gudžinskaitė et al., 2020)
14	7.56	451.1015	451.1041	451.1029	341 (100), 217 (25), 189 (14)	$C_{24}H_{19}O_9$	2.7	Cinchonain Ib isomer	(Dall'Acqua et al., 2021; Santos et al., 2016)
15	7.73	451.1045	451.1039	451.1029	341 (100), 217 (34), 189 (20)	$C_{24}H_{19}O_9$	2.2	Cinchonain Ib isomer	(Dall'Acqua et al., 2021; Santos et al., 2016)

3.2 Antifungal and drug-modifying activity

The Minimum Fungicidal Concentration (MFC) showed results greater than 1024 μg/mL for the substances tested (stem bark extracts and fluconazole). The antifungal and drug-modifying activities of the extracts of *H. speciosa* are shown in Fig. 3. In general, the sulfuric ether extracts of *H. speciosa* (EEHS) showed antifungal activity against the tested strains, where *C. krusei* was the most susceptible (Fig. 3B), and *C. tropicalis* the most resistant (Fig. 3C). At sub-inhibitory concentrations (MC/8), EEHS was able to intensify the action of fluconazole against all strains. The antifungal activity was more intense against *C. albicans* (Fig. 3A) and *C. tropicalis* (Fig. 3C).

The methanolic extract of *H. speciosa* (MEHS) also showed an anti-*Candida* effect against all strains evaluated (Fig. 4), being more active against *C. albicans* (Fig. 4A) and *C. krusei* (Fig. 4B), and less active against *C. tropicalis* (Fig. 4C). It is worth noting that this extract was more active than fluconazole against *C. krusei* at the concentration range from 4 to 64 μg/mL (Fig. 4B). Similarly, to the EEHS, the MEHS in combination with fluconazole increased the antifungal activity against *C. albicans* (Fig. 4A) and *C. tropicalis* (Fig. 4C).

The results observed for the antifungal activity and drug-modifying effects of the extracts can be partially explained by the IC_{50} values shown in Table 3. It is noticeable the high antifungal activity of the MEHS compared to the EEHS. The IC_{50} value of this extract against *C. krusei* (58.41 μg/mL) was two times lower than the IC_{50} verified for fluconazole (125.2 μg/mL). The susceptibility of *C. albicans* to the MEHS was also noted, as its IC_{50} was 80.61 μg/mL, considered a low concentration. Regarding the modifying activity, the IC_{50} values showed that both extracts were able to enhance the action of fluconazole against all strains, with the MEHS being the most effective.

Table 3

Half maximal inhibitory concentration (IC_{50}) values of fluconazole, the extracts of the stem bark of *Hancornia speciosa*, and their combinations against *Candida* spp. strains.

Treatments	**Strains/IC_{50} (μg/mL)**		
	CA INCQS 40006	**CK INCQS 40095**	**CT INCQS 40042**
Fluconazole (FCZ)	16.07	125.2	4.775
EEHS	628	137.9	1889

Treatments	Strains/IC_{50} (µg/mL)		
	CA INCQS 40006	**CK INCQS 40095**	**CT INCQS 40042**
FCZ + EEHS	2.836	25.66	1.267
MEHS	80.61	58.41	288
FCZ + MEHS	2.264	11.07	0.3945

EEHS = sulfuric ether extract, MEHS = methanolic extract, CA = *Candida albicans*, CK = *Candida krusei*, and CT = *Candida tropicalis*. Values in bold denote lower IC_{50} values compared to fluconazole.

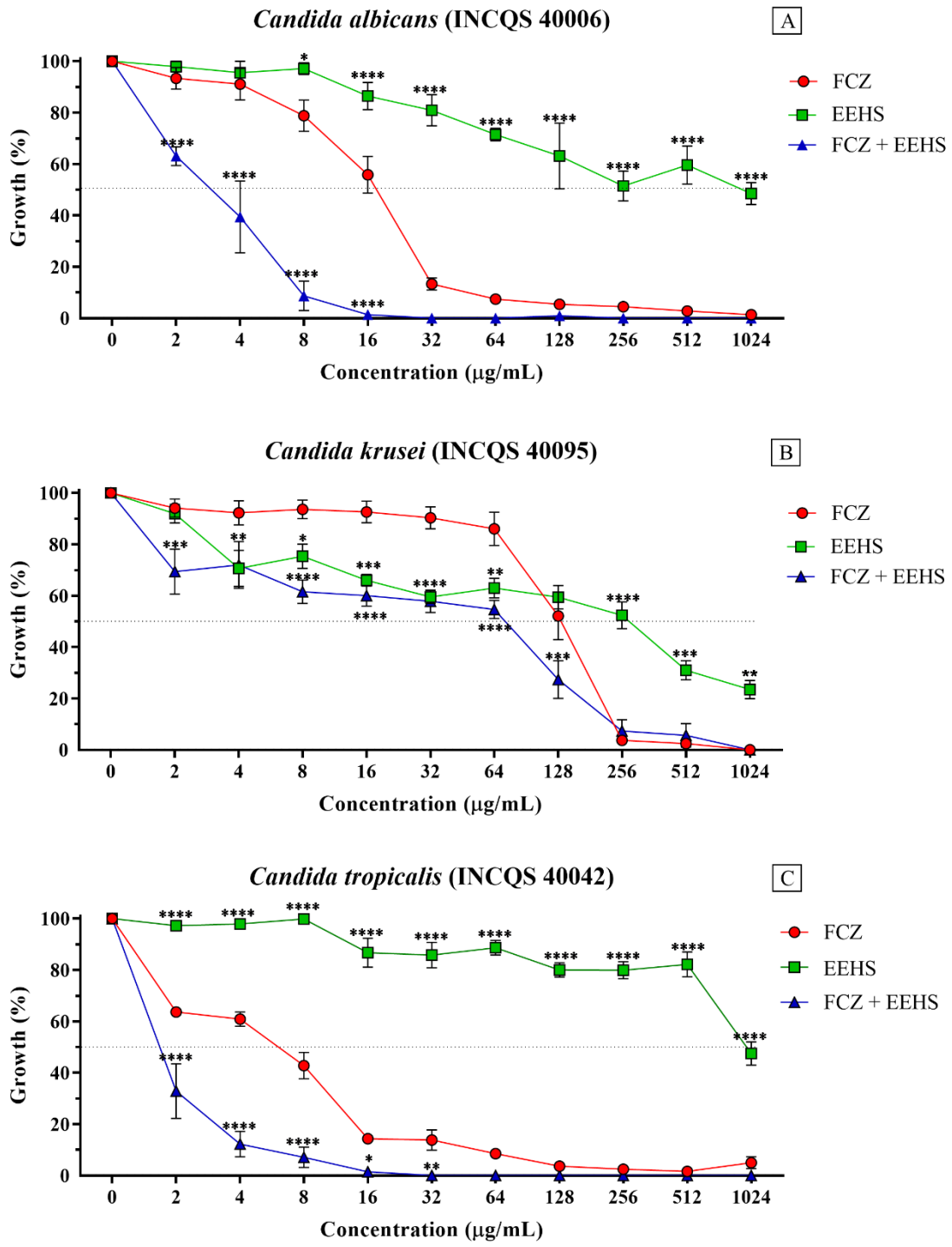


Fig. 3. Cell viability curve of *Candida* spp. strains and IC_{50} value (dashed line) of different concentrations of *Hancornia speciosa* sulfuric ether extract (EEHS), fluconazole (FCZ), and their combination (FCZ + EEHS). *Candida albicans* (4A), *Candida krusei* (4B), and *Candida tropicalis* (4C). The bars indicate the

standard error of the mean (n = 3). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ compared to fluconazole (FCZ).

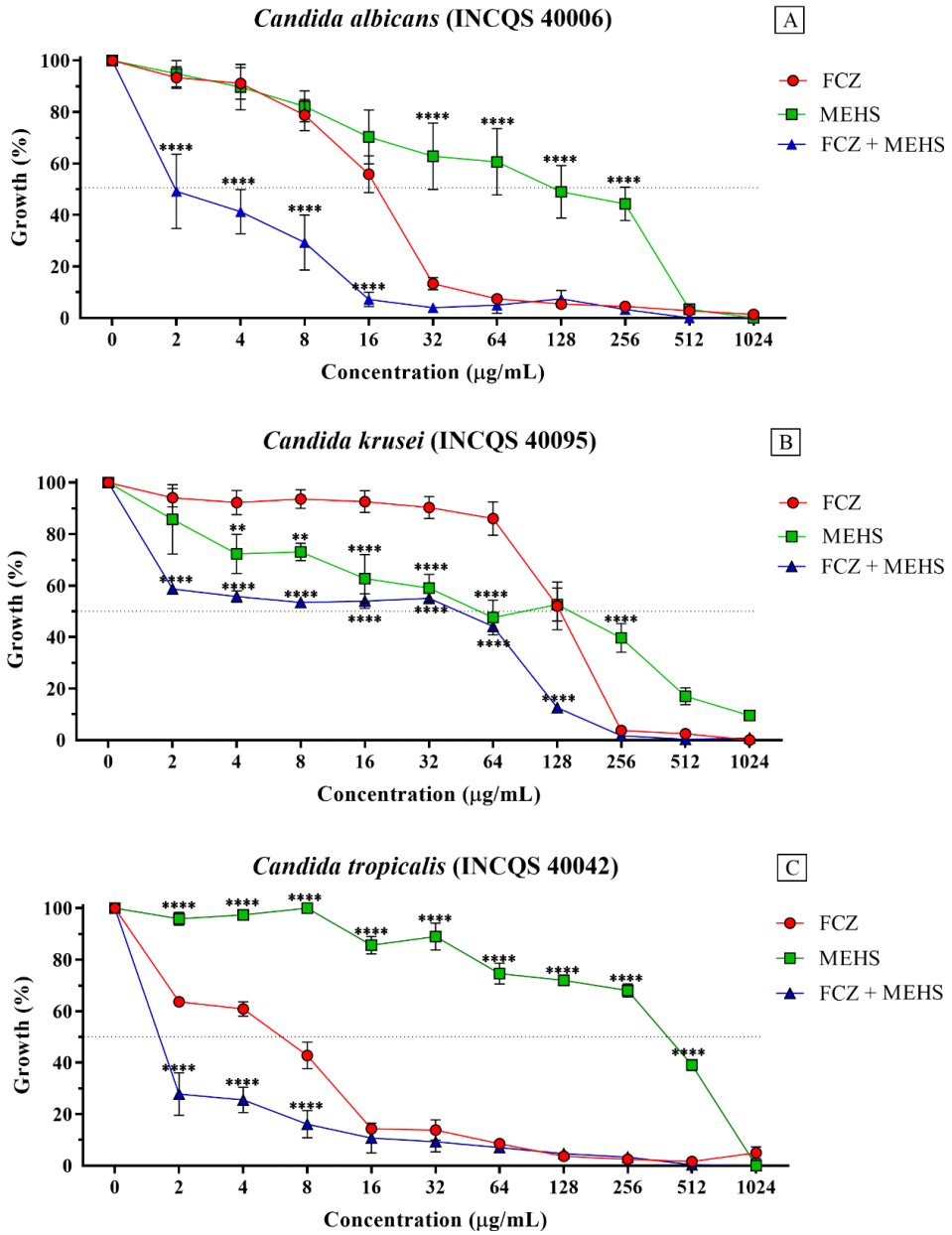


Fig. 4. Cell viability curve of *Candida* spp. strains and IC_{50} values (dashed line) of different concentrations of *Hancornia speciosa* methanolic extract (MEHS), fluconazole (FCZ), and their combination (FCZ + MEHS). *Candida albicans* (5A), *Candida krusei* (5B), and *Candida tropicalis* (5C). The bars indicate the standard error of the mean (n = 3). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$ compared to fluconazole (FCZ).

3.3 Inhibition of fungal virulence

In addition to the anti-*Candida* effect, it was possible to observe that the extracts of *H. speciosa* were able to reduce and inhibit the virulence of some *Candida* strains. In Fig. 5, for example, it is noticed that EEHS (Fig. 5D and E) completely inhibited the formation of filaments in *C. albicans* in both concentrations evaluated. These findings were similar to those verified for fluconazole (Fig. 5B and C). On the other hand, the MEHS (Fig. 5F and G) did not completely inhibit the formation of hyphae and pseudohyphae. However, when compared to the growth control (Fig. 5A), it was able to reduce the appearance of hyphae.

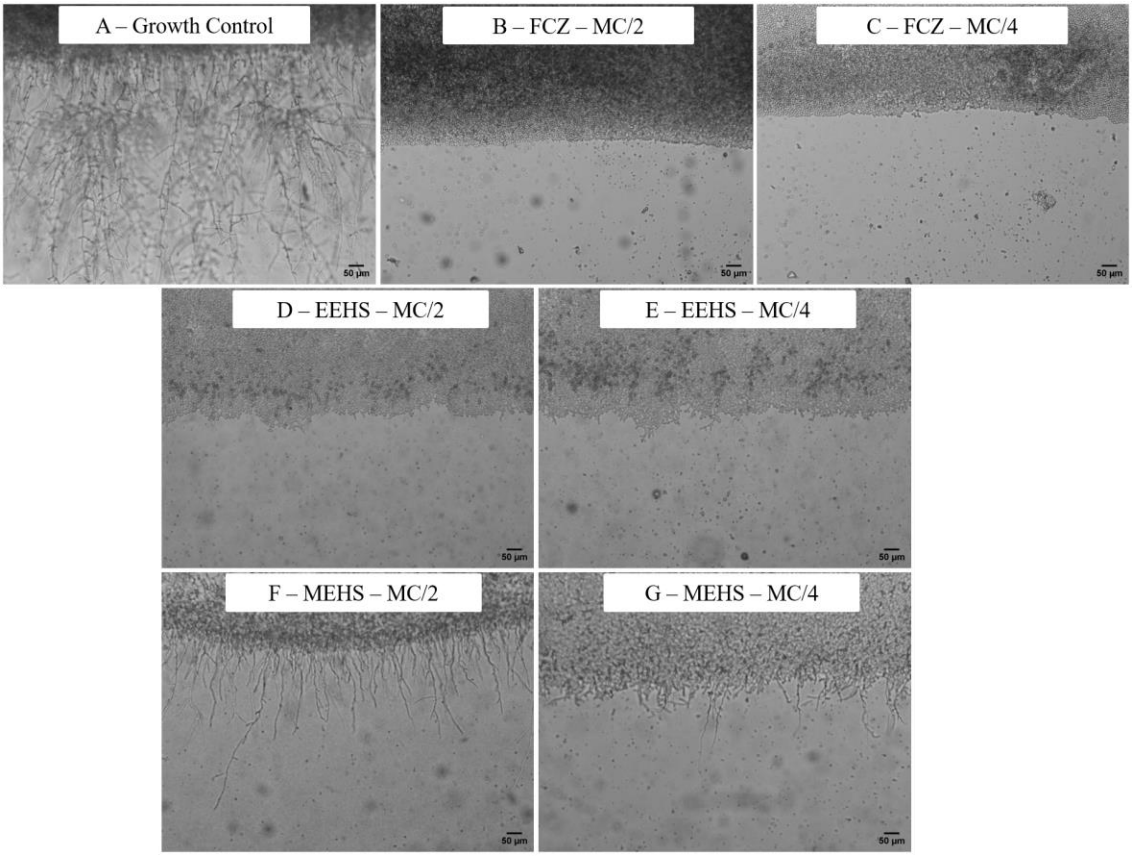


Fig. 5. Effect of fluconazole (FCZ), sulfuric ether extract (EEHS), and methanolic extract (MEHS) of *Hancornia speciosa* on the dimorphism of *Candida albicans* INCQS 40006. Growth control (A); FCZ (B and C); EEHS (D and E); MEHS (F and G). MC = Matrix Concentration; 100 × magnification.

Fig. 6 shows that the positive control (fluconazole) was able to inhibit the formation of filamentous structures of *C. krusei*. However, the *H. speciosa* extracts were not able to inhibit this

virulence factor compared to the growth control (Fig. 6A). For the *C. tropicalis* strain (Fig. 7), although the extracts did not inhibit the morphological transition as observed for fluconazole (Fig. 7B and C), they were able to reduce the quantity and size of the filamentous structures. This effect was mostly observed for the EEHS MC/2 (Fig. 7D).

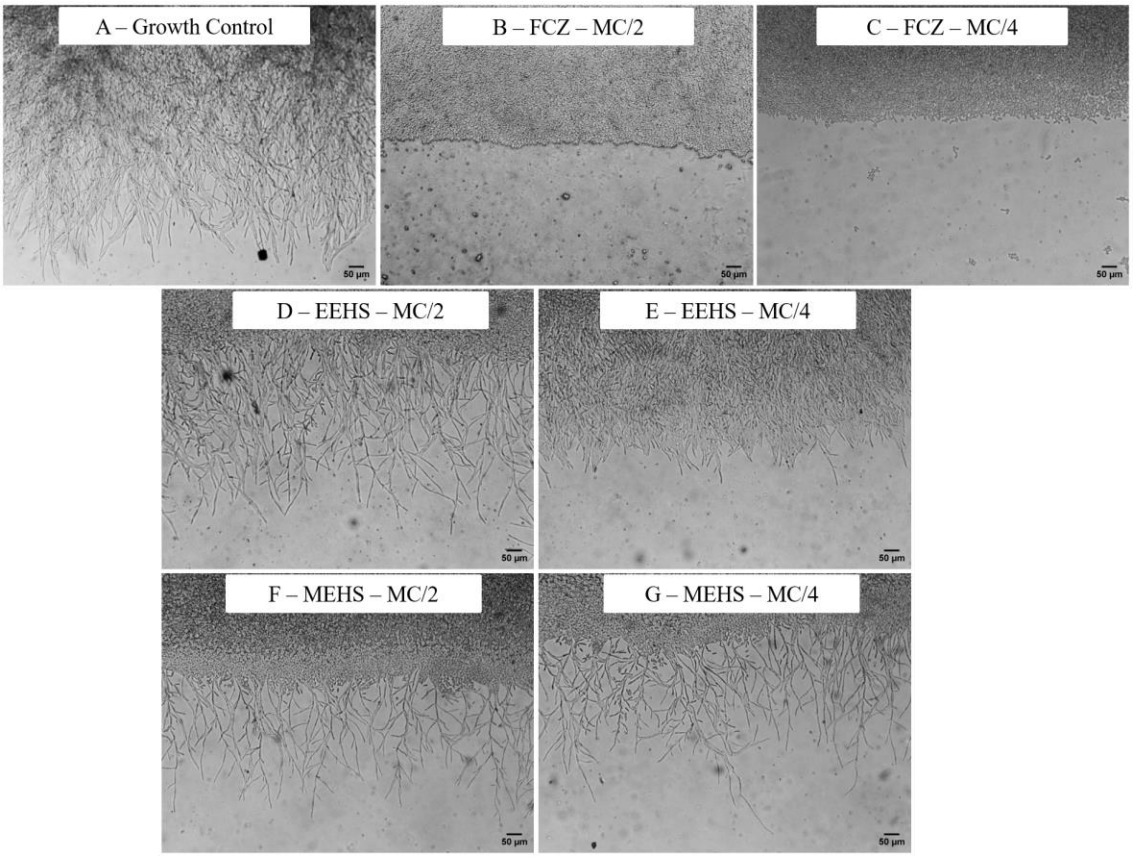


Fig. 6. Effect of fluconazole (FCZ), sulfuric ether extract (EEHS), and methanolic extract (MEHS) of *Hancornia speciosa* on the dimorphism of *Candida krusei* INCQS 40095. Growth control (A); FCZ (B and C); EEHS (D and E); MEHS (F and G). MC = Matrix Concentration; 100 × magnification.

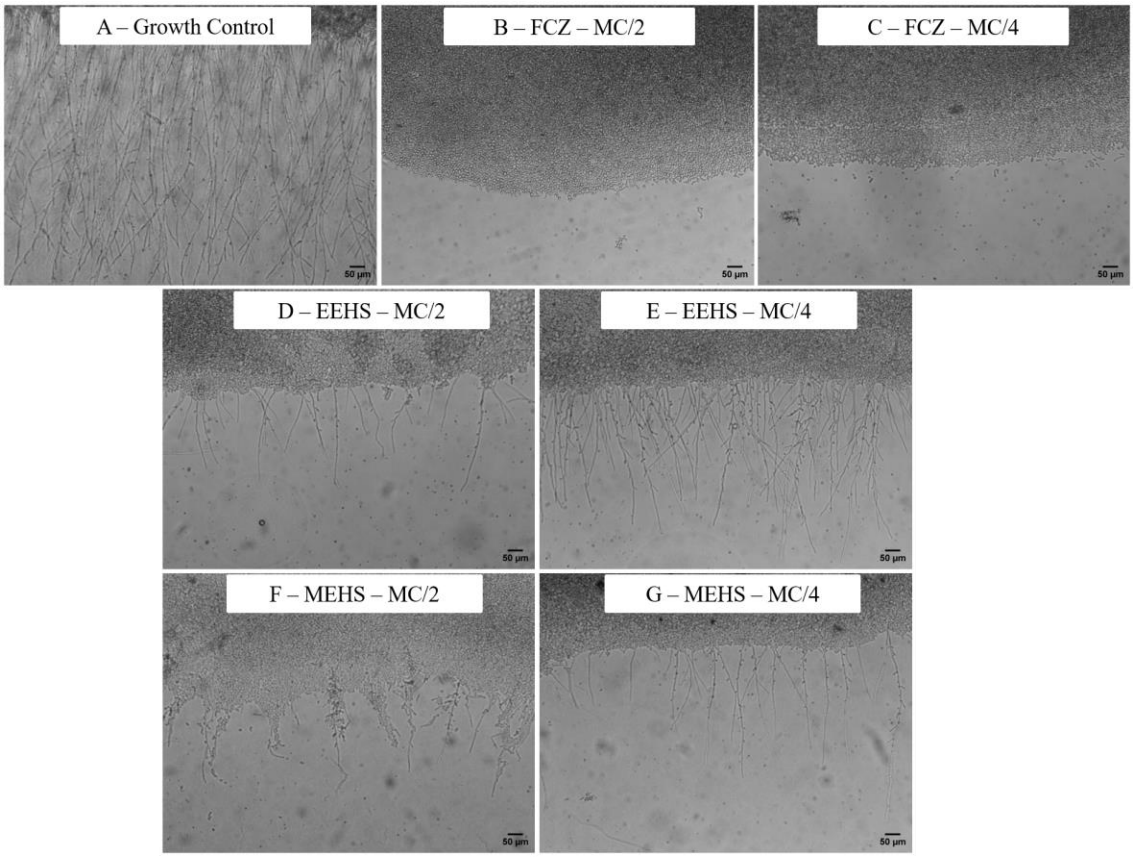


Fig. 7. Effect of fluconazole (FCZ), sulfuric ether extract (EEHS), and methanolic extract (MEHS) of *Hancornia speciosa* on the dimorphism of *Candida tropicalis* INCQS 40042. Growth control (A); FCZ (B and C); EEHS (D and E); MEHS (F and G). MC = Matrix concentration; 100 × magnification.

4. Discussion

From an ethnopharmacological perspective, *H. speciosa* has been used in the treatment of infectious diseases associated with the genitourinary system (Cruz et al., 2021; Albuquerque and Meiado, 2015; Nunes et al., 2022). Chemically, the leaves and fruits of *H. speciosa* are the most well-studied organs of this plant, displaying several compounds such as phenolic acids, flavonoids, terpenes, and fatty acids (Almeida et al., 2022; Nunes et al., 2022; Leite et al., 2020; Sousa et al., 2022). Regarding the bark of *H. speciosa*, which was the focus of our study, the research is still incipient. In a previous study, phenolic compounds such as flavonols, catechin, rutin, and tannins were the main identified (Leite et al., 2020). According to these authors, the bark of *H. speciosa* has high levels of phenols followed by flavonoids, and tannins are found in minor quantities.

Our findings evidenced that the stem bark of *H. speciosa* contains phenolic compounds, such as quinic acid, vanillic acid, chlorogenic acid, and catechin. These compounds were

previously reported in the leaves and fruits of this species (Bitencourt et al., 2019; Santos et al., 2016; Leite et al., 2020). In addition, our research identified other substances, such as procyanidin B, epicatechin, and phloretin, a dihydrochalcone flavonoid, which had already been found in the leaves of *H. speciosa* (Almeida et al., 2022; Bastos et al., 2017). These compounds are known for their bioactive properties, including antimicrobial, antioxidant, and anti-inflammatory action (Almeida et al., 2022; Bitencourt et al., 2019; Santos et al., 2016).

It is important to mention that our study revealed the presence of compounds that had not been previously described in the species, such as gluconic acid, cinchonain IIb, cinchonain Ib isomer, and lariciresinol hexoside isomers, indicating the existence of a variety of bioactive compounds in the stem bark of *H. speciosa*. These compounds may play an important role in the biological activities attributed to the stem bark, including antifungal and drug-enhancing activity. Previous studies showed that phenolic compounds such as cinchonain exhibited promising biological activities, including antioxidant properties (Ao et al., 2011; Resende et al., 2011), and antibacterial and antifungal activities against pathogens (Ming et al., 2002; Pizzolatti et al., 2002).

The gluconic acid found in the extracts is an organic acid widely used in the pharmaceutical, food, and chemical industries because of its low toxicity and capacity to form water-soluble complexes (Vandenberghe et al., 2018). Its presence in the stem bark of *H. speciosa* suggests that this species could be a potential source of this compound, which has low toxicity and can be used in several industrial applications, including biological activity. The identification of lariciresinol hexoside isomer is also relevant, as lignan compounds such as lariciresinol have been associated with biological activities, including antioxidant (Bajpai et al., 2017a), antibacterial (Bajpai et al., 2017b), and anticancer activity (Ma et al., 2016). Therefore, the presence of this specific isomer may contribute to the understanding of the medicinal properties attributed to the stem bark of *H. speciosa*.

Plant extracts represent a rich and diverse source of bioactive compounds. They can display the ability to inhibit the growth and replication of a variety of pathogenic fungi, such as *Candida* spp. (Lima et al., 2022; Almeida-Bezerra et al., 2023). Studies on plant products have been seeking to address the mechanisms involved in antifungal activity and how the bioactive compounds found in these products act on the vital processes of fungi (Gao et al., 2023; Ju et al., 2022; Almeida-Bezerra et al., 2023). The main mechanisms of action of these plant products against fungi are based on the inhibition of cell wall formation, induced damage to the plasmatic membrane and

genetic material, inhibition of biofilm formation, and reduction of hyphae growth, among others (Wu et al., 2022; Almeida-Bezerra et al., 2023). Due to the wide variety of compounds found in plant extracts, the antifungal activities of these products generally are not due to a single isolated mechanism but the result of combined effects (Wu et al., 2022).

The anti-*Candida* activity observed can be associated with the chemical composition of the stem bark since there are reports that flavonoids have antifungal properties (Nguyen et al., 2021; Seleem et al., 2017). Flavonoids can act through various mechanisms, for example, disrupting the plasma membrane, inhibiting cell wall formation, inducing mitochondrial dysfunction, inhibiting cell division, inhibiting efflux pumps, and inhibiting RNA/DNA and protein synthesis (Al Aboody and Mickymaray, 2020).

In addition to flavonoids, chlorogenic acid was identified in *H. speciosa* extracts, which showed antifungal properties against fluconazole-resistant *Candida* spp. According to some reports, chlorogenic acid causes the reduction in cell viability, increases mitochondrial depolarization potential, increases the production of reactive oxygen species (ROS), induces potassium efflux, DNA fragmentation, and externalization of phosphatidylserine, indicating an apoptotic process in *Candida* spp. yeasts (Silva et al., 2022; Yun and Lee, 2017). This process occurs because chlorogenic acid induces the activation of proteases, such as caspases, which are considered apoptotic markers (Yun and Lee, 2017).

In addition to its antifungal effect, chlorogenic acid was capable of intensifying the activity of fluconazole against multi-resistant strains of *C. albicans*, *C. krusei*, *C. bovina*, and *C. parapsilosis*, reducing the MIC up to 99.99% (Rhimi et al., 2020). The results of our study demonstrate that extracts of *H. speciosa*, in which chlorogenic acid was found, potentiated the action of fluconazole. This synergism is promising, considering that both drugs can act simultaneously on different targets, making it more challenging for strains to develop drug resistance (Al Aboody and Mickymaray, 2020).

The extracts of *H. speciosa* were able to reduce the growth and inhibit the virulence of *Candida* spp., confirming its traditional use in herbal medicine to treat genitourinary system infections. Although the extracts did not show greater activity than fluconazole, they can be an alternative for the development of new antifungal agents, as the anti-*Candida* activities occurred at clinically relevant concentrations (Houghton et al., 2007; Panontin et al., 2021). The *in vitro* antifungal results demonstrated in this study are promising and can contribute to the development

of new products based on this natural resource, which is low-cost, culturally accepted, and found throughout a large part of the Brazilian territory (Alves et al., 2021; Dutra et al., 2016; Süntar, 2020).

Regarding the inhibition of *Candida* spp. virulence, only the sulfuric ether extract (EEHS) was able to inhibit the morphological transition of *C. albicans*. Although the compositions of these extracts were similar, differences between compound concentrations could result in distinct effects on *Candida* species (Lima et al., 2022). This finding is particularly relevant since the morphological transition is associated with the pathogenicity of *C. albicans*. In this phase, *C. albicans* display the ability to adhere to surfaces and form biofilms, making the treatment more challenging (Cassone and Cauda, 2012; Chen et al., 2020).

Liu et al. (2021) demonstrated that phloretin, which is present in *H. speciosa* extracts, suppressed the pathogenicity and virulence of *C. albicans*, inhibiting biofilm formation and suppressing yeast-to-hypha transition, via regulation of hypha-associated genes. Saito et al. (2013) also evidenced that catechin inhibited the morphological transition of *C. albicans* by suppressing the intracellular signal transduction. The ability of the EEHS to inhibit this transition suggests an important therapeutic potential for controlling fungal infections caused by *C. albicans* (Bu et al., 2022). Additional tests with *H. speciosa* extracts are necessary to evaluate their pharmacokinetics and pharmacodynamics, including acute, subacute, and chronic toxicity, to ensure their safety. Furthermore, chemical stability tests of the extracts should be performed to understand the degradation of the active principles, ensuring their efficacy.

5. Conclusions

Our investigation demonstrated that the stem bark of *Hancornia speciosa* is an important source of bioactive compounds that can support the treatment of infectious diseases associated with the genitourinary system, such as candidiasis. Such potential may be associated with the presence of phenolic substances, known for their broad spectrum of activities. In addition, the presence of compounds not previously described for this species, such as gluconic acid, cinchonain IIb, cinchonain Ib isomer, and lariciresinol hexoside isomer, reveals the existence of a variety of bioactive compounds in the stem bark with potential to be explored in medical therapies. The extracts were also able to potentiate the activity of the drug fluconazole against multi-resistant microorganisms, which is important in the battle against microbial resistance. Further studies

should be carried out to identify and elucidate the mechanisms involved in the observed antifungal activities.

Funding

This study was partially funded by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financial Code 001.

CRediT authorship contribution statement

Viviane Bezerra da Silva: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **José Weverton Almeida-Bezerra**: Formal analysis, Investigation. **Maria Hellena Garcia Novais**: Investigation. **Naiza Saraiva Farias**: Investigation. **Janerson José Coelho**: Formal analysis. **Paulo Riceli Vasconcelos Ribeiro:** Software. **Kirley Marques Canuto:** Software. **Henrique Douglas Melo Coutinho:** Investigation. **Maria Flaviana Bezerra Morais-Braga:** Supervision. **Antonio Fernando Morais de Oliveira:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The first author thanks CAPES for the doctoral scholarship. The last author thanks the National Council for Scientific and Technological Development (CNPq - Brazil) for the research fellowship (310177/2022- 7).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi. org/10.1016/j.jep.2023.117506.

References

Al Aboody, M.S., Mickymaray, S., 2020. Anti-fungal efficacy and mechanisms of flavonoids. Antibiotics 9, 45. https://doi.org/10.3390/antibiotics9020045

Albuquerque, U.P., Meiado, M.V., 2015. Sociobiodiversidade na Chapada do Araripe, 3ª. ed., NUPEEA, Recife.

Almagro, L., Fernández-Pérez, F., Pedreño, M.A., 2015. Indole alkaloids from *Catharanthus roseus*: bioproduction and their effect on human health. Molecules 20 (2), 2973–3000. https://doi.org/10.3390/molecules20022973

Almeida, F.L.C., de Oliveira, E.N.A., Almeida, E.C., de Souza, W.F.C., Silva, M.O., de Melo, A.M., Castro, M.P.J., Bullo, G.T., Luna, L.C., Prata, A.S., 2022. *Hancornia speciosa*: An overview focused on phytochemical properties, recent achievements, applications, and future perspectives. Int. J. Gastron. Food Sci. 29, 100561. https://doi.org/10.1016/j.ijgfs.2022.100561

Almeida-Bezerra, J. W., da Cruz, R. P., Pereira, R. L. S., da Silva, V. B., de Sousa, D. D. O. B., Neto, J. X. D. S., Souza, L.A.L., Araújo, N.M.S., Silva, R.G.G., Lucetti, D.L., Coutinho, H.D.M., Morais-Braga, M.F.B., Oliveira, A. F. M., 2023. *Caryocar coriaceum* fruits as a potential alternative to combat fungal and bacterial infections: In vitro evaluation of methanolic extracts. Microb. Pathog. 106203. https://doi.org/10.1016/j.micpath.2023.106203

Alves, D. da N., Ferreira, A.R., Duarte, A.B.S., Melo, A.K.V., de Sousa, D.P., Castro, R.D. de, 2021. Breakpoints for the classification of anti-*Candida* compounds in antifungal screening. Biomed Res. Int. 2021, 1-8. https://doi.org/10.1155/2021/6653311

Anand, U., Nandy, S., Mundhra, A., Das, N., Pandey, D.K., Dey, A., 2020. A review on antimicrobial botanicals, phytochemicals and natural resistance modifying agents from Apocynaceae family: Possible therapeutic approaches against multidrug resistance in pathogenic microorganisms. Drug Resist. Updat. 51, 100695. https://doi.org/10.1016/j.drup.2020.100695

Ao, C., Higa, T., Khanh, T.D., Upadhyay, A., Tawata, S., 2011. Antioxidant phenolic compounds from *Smilax sebeana* Miq. LWT - Food Sci. Technol. 44 (7), 1681–1686. https://doi.org/10.1016/j.lwt.2011.02.001

Bajpai, V.K., Alam, M.B., Quan, K.T., Kwon, K.-R., Ju, M.-K., Choi, H.-J., Lee, J.S., Yoon, J.-I., Majumder, R., Rather, I.A., 2017a. Antioxidant efficacy and the upregulation of Nrf2-mediated HO-1 expression by (+)-lariciresinol, a lignan isolated from *Rubia philippinensis*, through the activation of p38. Sci. Rep. 7, 46035. https://doi.org/10.1038/srep46035

Bajpai, V.K., Shukla, S., Paek, W.K., Lim, J., Kumar, Pradeep, Kumar, Pankaj, Na, M., 2017b. Efficacy of (+)-Lariciresinol to control bacterial growth of *Staphylococcus aureus* and *Escherichia coli* O157: H7. Front. Microbiol. 8, 804. https://doi.org/10.3389/fmicb.2017.00804

Bashir, K., Naz, S., Farooq, U., Wahid, F., Shah, A.J., McCauley, E.P., Crews, P., Khan, T., 2021. Assessing the ethnobotanical potential of *Carissa opaca* berries by merging outcomes from metabolomics profiling, enzyme assays, and in silico docking studies. Food Chem. 363, 130259. https://doi.org/10.1016/j.foodchem.2021.130259

Bastos, K.X., Dias, C.N., Nascimento, Y.M., Da Silva, M.S., Langassner, S.M.Z., Wessjohann, L.A., Tavares, J.F., 2017. Identification of phenolic compounds from *Hancornia speciosa* (Apocynaceae) leaves by uhplc orbitrap-hrms. Molecules 22 (1), 143. https://doi.org/10.3390/molecules22010143

Bhadane, B.S., Patil, M.P., Maheshwari, V.L., Patil, R.H., 2018. Ethnopharmacology, phytochemistry, and biotechnological advances of family Apocynaceae: A review. Phytother. Res. 32, 1181–1210. https://doi.org/10.1002/ptr.6066

Bitencourt, M.A.O., Torres-Rêgo, M., de Souza Lima, M.C.J., Furtado, A.A., de Azevedo, E.P., do Egito, E.S.T., da Silva-Júnior, A.A., Zucolotto, S.M., de Freitas Fernandes-Pedrosa, M., 2019. Protective effect of aqueous extract, fractions and phenolic compounds of *Hancornia speciosa* fruits on the inflammatory damage in the lungs of mice induced by *Tityus serrulatus* envenomation. Toxicon 164, 1–9. https://doi.org/10.1016/j.toxicon.2019.03.018

Boléa, G., Ginies, C., Vallier, M.-J., Dufour, C., 2019. Lipid protection by polyphenol-rich apple matrices is modulated by pH and pepsin in in vitro gastric digestion. Food Funct. 10, 3942–3954. https://doi.org/10.1039/C9FO00705A

Bu, Q.-R., Bao, M.-Y., Yang, Y., Wang, T.-M., Wang, C.-Z., 2022. Targeting Virulence Factors of *Candida albicans* with Natural Products. Foods 11, 2951.

Capoci, I.R.G., Sakita, K.M., Faria, D.R., Rodrigues-Vendramini, F.A.V., Arita, G.S., de Oliveira, A.G., Felipe, M.S., Maigret, B., Bonfim-Mendonça, P. de S., Kioshima, E.S., Svidzinski, T.I.E., 2019. Two New 1,3,4-Oxadiazoles With Effective Antifungal Activity Against *Candida albicans*. Front. Microbiol. 10, 2130. https://doi.org/10.3389/fmicb.2019.02130

Carneiro, J.N.P., da Cruz, R.P., da Silva, J.C.P., Rocha, J.E., de Freitas, T.S., Sales, D.L., Bezerra, C.F., de Oliveira Almeida, W., da Costa, J.G.M., da Silva, L.E., 2019. *Piper diospyrifolium* Kunth.: Chemical analysis and antimicrobial (intrinsic and combined) activities. Microb. Pathogen. 136, 103700. https://doi.org/10.1016/j.micpath.2019.103700

Carvalho, K.R, Zocolo, G.J., Pereira, R.C.A., Martins, F.I.C.C., Ribeiro, P.R.V., de Brito, E.S., Silveira, E.R., Canuto, K.M., 2019. Development of a UPLC-ESI-MS method for simultaneous determination of flavonoids and diterpenes in *Egletes viscosa* (L.) Less herbal products. J. Pharm. Biomed. Anal. 166, 155–163. https://doi.org/10.1016/j.jpba.2019.01.008

Cassone, A., Cauda, R., 2012. *Candida* and candidiasis in HIV-infected patients: where commensalism, opportunistic behavior and frank pathogenicity lose their borders. AIDS 26 (12), 1457–1472. https://doi.org/10.1097/QAD.0b013e3283536ba8

Chen, H., Zhou, X., Ren, B., Cheng, L., 2020. The regulation of hyphae growth in *Candida albicans*. Virulence 11, 337–348. https://doi.org/10.1080/21505594.2020.1748930

CLSI, 2017. Reference Method for Broth Dilution Antifungal susceptibility testing of yeast. 4th ed. CLSI standard M27 Wayne, PA: Clinical and Laboratory Standards Institute.

Çoban, Y., Köker, A., Tunçer, G.Ö., Akbaş, Y., Aydın, S., Kara, T.T., 2023. Retrospective evaluation of *Candida* infections in pediatric intensive care units. Pediatr. Int. 4 (1), 42-46. https://doi.org/10.59213/TP.2023.37450

Corrêa, P.G., Moura, L.G.S., Amaral, A.C.F., Souza, F.C.A., Aguiar, J.P.L., Aleluia, R.L., Silva, J.R.A., 2023. Chemical and nutritional characterization of *Ambelania duckei* (Apocynaceae) an unexplored fruit from the Amazon region. Food Res. Int. 163, 112290. https://doi.org/10.1016/j.foodres.2022.112290

Costa, A.R., Almeida-Bezerra, J.W., da Silva, T.G., Pereira, P.S., de Oliveira Borba, E.F., Braga, A.L., Fonseca, V.J.A., de Menezes, S.A., da Silva, F.S.H, Fernandes, P.A.S., de Oliveira,

M.G., de Oliveira, T.J.S., Tavares, A.B., de Brito, E.S., Ribeiro, P.R.V., dos Santos, L.T., dos Santos, A.T.L., Morais-Braga, M.F.B., Sampaio, R.S.L., da Cruz, R.P., Duarte, A.E., Barros, L.M., 2021. Phytochemical profile and anti-*Candida* and cytotoxic potential of *Anacardium occidentale* L. (cashew tree). Biocatal. Agric. Biotechnol. 37, 102192. https://doi.org/10.1016/j.bcab.2021.102192

Cruz, R.P. da, Almeida-Bezerra, J.W., Menezes, S.A. de, Silva, V.B. da, Santos, L.T. dos, Morais-Braga, M.F.B., Moraes, J.L. de, 2021. Ethnopharmacology of the angiosperms of Chapada of Araripe located in Northeast of Brazil. J. Environ. Anal. Prog. 6 (4), 326–351. https://doi.org/10.24221/jeap.6.4.2021.4272.326-351

D'Abadia, P.L., Bailão, E.F.L.C., Lino Júnior, R.S., Oliveira, M.G., Silva, V.B., Oliveira, L. a. R., Conceição, E.C., Melo-Reis, P.R., Borges, L.L., Gonçalves, P.J., Almeida, L.M., 2020. *Hancornia speciosa* serum fraction latex stimulates the angiogenesis and extracellular matrix remodeling processes. An. Acad. Bras. Ciênc. 92 (2), e20190107. https://doi.org/10.1590/0001-3765202020190107

Dall'Acqua, S., Ak, G., Sinan, K.I., Elbasan, F., Ferrarese, I., Sut, S., Yıldıztugay, E., Peron, G., Schievano, E., Nancy Picot-Allain, M.C., Mahomoodally, M.F., Zengin, G., 2021. *Hypericum triquetrifolium* and *H. neurocalycinum* as Sources of Antioxidants and Multi-Target Bioactive Compounds: A Comprehensive Characterization Combining In Vitro Bioassays and Integrated NMR and LC-MS Characterization by Using a Multivariate Approach. Front. Pharmacol. 12, 660735. https://doi.org/10.3389/fphar.2021.660735

Das, S., Bhuyan, R., Bagchi, A., Saha, T., 2019. Network analysis of hyphae forming proteins in *Candida albicans* identifies important proteins responsible for pathovirulence in the organism. Heliyon 5, e01916. https://doi.org/10.1016/j.heliyon.2019.e01916

Dutra, R.C., Campos, M.M., Santos, A.R.S., Calixto, J.B., 2016. Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. Pharmacol. Res. 112, 4–29. https://doi.org/10.1016/j.phrs.2016.01.021

Eksi, F., Hassan, B.A., Ugur, B.K., Yildiz, H., Erinmez, M., Ganidagli, S., 2022. An epidemiologic analysis of *Candida* spp. urinary infections in intensive care unit. Rev. epidemiol. Control. Infec. 80–86. https://doi.org/10.17058/reci.v12i2.17026

Feitosa, B.F., de Alcântara, C.M., de Lima, A.B.S., Silva, A.S., Araújo, A. dos S., Cavalcanti, M.T., Mori, E., Araújo, I.M., de Farias, P.A.M., Wilairatana, P., Coutinho, H.D.M., 2022.

Bioactive Natural Products for Chemical Control of Microorganisms: Scientific Prospecting (2001–2021) and Systematic Review. Molecules 27 (18), 5917. https://doi.org/10.3390/molecules27185917

Fonseca, V.J.A., Braga, A.L., de Almeida, R.S., da Silva, T.G., da Silva, J.C.P., de Lima, L.F., dos Santos, M.H.C., Silva, R.R.S., Teixeira, C.S., Coutinho, H.D.M., Morais-Braga, M.F.B., 2022. Lectins ConA and ConM extracted from *Canavalia ensiformis* (L.) DC and *Canavalia rosea* (Sw.) DC inhibit planktonic *Candida albicans* and *Candida tropicalis.* Arch. Microbiol. 204, 346. https://doi.org/10.1007/s00203-022-02959-x

Gao, Q., Qi, J., Tan, Y., Ju, J., 2023. Antifungal mechanism of *Angelica sinensis* essential oil against *Penicillium roqueforti* and its application in extending the shelf life of bread. Int. J. Food Microbiol. 110427. https://doi.org/10.1016/j.ijfoodmicro.2023.110427

Gizińska, M., Staniszewska, M., Ochal, Z., 2019. Novel Sulfones with Antifungal Properties: Antifungal Activities and Interactions with *Candida* spp. Virulence Factors. Mini-Rev. Med. Chem. 19 (1), 12–21. https://doi.org/10.2174/1389557518666180924121209

Gudžinskaitė, I., Stackevičienė, E., Liaudanskas, M., Zymonė, K., Žvikas, V., Viškelis, J., Urbštaitė, R., Janulis, V., 2020. Variability in the Qualitative and Quantitative Composition and Content of Phenolic Compounds in the Fruit of Introduced American Cranberry (*Vaccinium macrocarpon* Aiton). Plants 9, 1379. https://doi.org/10.3390/plants9101379

Houghton, P.J., Howes, M.-J., Lee, C.C., Steventon, G., 2007. Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant. J. Ethnopharmacol. 110 (3), 391–400. https://doi.org/10.1016/j.jep.2007.01.032

Huang, Y., Adeleye, A.S., Zhao, L., Minakova, A.S., Anumol, T., Keller, A.A., 2019. Antioxidant response of cucumber (*Cucumis sativus*) exposed to nano copper pesticide: Quantitative determination via LC-MS/MS. Food Chem. 270, 47–52. https://doi.org/10.1016/j.foodchem.2018.07.069

Ju, J., Guo, Y., Cheng, Y., Yaoc, W., 2022. Analysis of the synergistic antifungal mechanism of small molecular combinations of essential oils at the molecular level. Ind. Crops. Prod. 188, 115612. https://doi.org/10.1016/j.indcrop.2022.115612

Khan, F., Bamunuarachchi, N.I., Tabassum, N., Jo, D.-M., Khan, M.M., Kim, Y.-M., 2021. Suppression of hyphal formation and virulence of *Candida albicans* by natural and

synthetic compounds. Biofouling 37, 626–655. https://doi.org/10.1080/08927014.2021.1948538

Kornitzer, D., 2019. Regulation of *Candida albicans* hyphal morphogenesis by endogenous signals. J. Fungi 5 (1), 21. https://doi.org/10.3390/jof5010021

Leite, S.P., Adami, T.B., Bjerk, T.R., Dos, M.R., Souza, R., Cardos, C.A.L., Krause, L.C., Caramão, E.B., 2020. Ultrasonic assisted extraction of bioactive compounds from different parts of *Hancornia Speciosa* Gomes. J. Med. Plants Res. 14, 300–308. https://doi.org/10.5897/JMPR2020.6944

Li, Y., Shan, M., Li, S., Wang, Y., Yang, H., Chen, Y., Gu, B., Zhu, Z., 2020. Teasaponin suppresses *Candida albicans* filamentation by reducing the level of intracellular cAMP. Ann. Transl. Med. 8, 175. https://doi.org/10.21037/atm.2020.01.124

Li, Y., Sun, L., Lu, C., Gong, Y., Li, M., Sun, S., 2018. Promising Antifungal Targets Against *Candida albicans* Based on Ion Homeostasis. Front. Cell. Infect. Microbiol. 8. https://doi.org/10.3389/fcimb.2018.00286

Lima, L.F., Andrade-Pinheiro, J.C., Freitas, M.A., da Silva, A.I., Fonseca, V.J.A., da Silva, T.G., da Silva, J.C.P., de Lima, R.H., Sales, D.L., Neves, R.P., de Brito, E.S., Ribeiro, P.R.V., Canuto, K.M., Coutinho, H.D.M., Siyadatpanah, A., Kim, B., Morais-Braga, M.F.B., 2022. Anti-*Candida* Properties of *Gossypium hirsutum* L.: Enhancement of Fungal Growth, Biofilm Production and Antifungal Resistance. Pharmaceutics 14, 698. https://doi.org/10.3390/pharmaceutics14040698

Liu, N., Zhang, N., Zhang, S., Zhang, L., Liu, Q., 2021. Phloretin inhibited the pathogenicity and virulence factors against *Candida albicans*. Bioengineered 12, 2420–2431. https://doi.org/10.1080/21655979.2021.1933824

Ma, Z.-J., Wang, X.-X., Su, G., Yang, J.-J., Zhu, Y.-J., Wu, Y.-W., Li, J., Lu, L., Zeng, L., Pei, H.-X., 2016. Proteomic analysis of apoptosis induction by lariciresinol in human HepG2 cells. Chem. Biol. Interact. 256, 209–219. https://doi.org/10.1016/j.cbi.2016.07.011

Martins, N., Barros, L., Henriques, M., Silva, S., Ferreira, I.C.F.R., 2015. Activity of phenolic compounds from plant origin against *Candida* species. Ind. Crops Prod. 74, 648–670. https://doi.org/10.1016/j.indcrop.2015.05.067

Matos, F.J.A., 2009. Introdução à Fitoquímica Experimental, 3ª. ed, UFC, Fortaleza.

Melo, A.P.V., Zuza-Alves, D.L., da Silva-Rocha, W.P., de Souza, L.B.F.C., Francisco, E.C., Melo, A.S.A., Chaves, G.M., 2019. Virulence factors of *Candida* spp. obtained from blood cultures of patients with candidemia attended at tertiary hospitals in Northeast Brazil. J. Mycol. Med. 29 (2), 132–139. https://doi.org/10.1016/j.mycmed.2019.02.002

Ming, D.S., López, A., Hillhouse, B.J., French, C.J., Hudson, J.B., Towers, G.H.N., 2002. Bioactive Constituents from *Iryanthera megistophylla*. J. Nat. Prod. 65, 1412–1416. https://doi.org/10.1021/np020169l

Moraes, T. de M., Rodrigues, C.M., Kushima, H., Bauab, T.M., Villegas, W., Pellizzon, C.H., Brito, A.R.M.S., Hiruma-Lima, C.A., 2008. *Hancornia speciosa*: indications of gastroprotective, healing and anti-*Helicobacter pylori* actions. J. Ethnopharmacol. 120 (2), 161–168. https://doi.org/10.1016/j.jep.2008.08.001

Morais-Braga, M.F.B., Sales, D.L., Carneiro, J.N.P., Machado, A.J.T., dos Santos, A.T.L., de Freitas, M.A., Martins, G.M. de A.B., Leite, N.F., de Matos, Y.M.L.S., Tintino, S.R., Souza, D.S.L., Menezes, I.R.A., Ribeiro-Filho, J., Costa, J.G.M., Coutinho, H.D.M., 2016. *Psidium guajava* L. and *Psidium brownianum* Mart ex DC.: Chemical composition and anti - *Candida* effect in association with fluconazole. Microb. Pathog. 95, 200–207. https://doi.org/10.1016/j.micpath.2016.04.013

Morreel, K., Saeys, Y., Dima, O., Lu, F., Van de Peer, Y., Vanholme, R., Ralph, J., Vanholme, B., Boerjan, W., 2014. Systematic structural characterization of metabolites in *Arabidopsis* via candidate substrate-product pair networks. Plant Cell 26, 929–945. https://doi.org/10.1105/tpc.113.122242

Nguyen, W., Grigori, L., Just, E., Santos, C., Seleem, D., 2021. The in vivo anti-*Candida* albicans activity of flavonoids. J. Oral. Biosci. 63, 120–128. https://doi.org/10.1016/j.job.2021.03.004

Nunes, V.V., Silva-Mann, R., Souza, J.L., Calazans, C.C., 2022. Pharmaceutical, food potential, and molecular data of *Hancornia speciosa* Gomes: a systematic review. Genet. Resour. Crop. Evol. 69, 525–543. https://doi.org/10.1007/s10722-021-01319-w

Osonga, F.J., Eshun, G., Kalra, S., Yazgan, I., Sakhaee, L., Ontman, R., Jiang, S., Sadik, O.A., 2022. Influence of Particle Size and Shapes on the Antifungal Activities of Greener Nanostructured Copper against *Penicillium italicum*. ACS Agric. Sci. Technol. 2 (1), 42-56. https://doi.org/10.1021/acsagscitech.1c00102

Panontin, J.F., Neres, R.P., Fernandes, R. de M.N., Scapin, E., Seibert, C.S., 2021. Chemical characterization and toxicological analyses of hydroalcoholic extracts from the stem and leaves of mangabeira (*Hancornia speciosa* Gomes) as a guide for the development of green cosmetics. J. Med. Plants Res. 15 (8), 366–379. https://doi.org/10.5897/JMPR2021.7099

Pereira, A.C., Pereira, A.B.D., Moreira, C.C.L., Botion, L.M., Lemos, V.S., Braga, F.C., Cortes, S.F., 2015. *Hancornia speciosa* Gomes (Apocynaceae) as a potential anti-diabetic drug. J. Ethnopharmacol. 161, 30–35. https://doi.org/10.1016/j.jep.2014.11.050

Pizzolatti, M.G., Venson, A.F., Smânia, A., Smânia, E. de F.A., Braz-Filho, R., 2002. Two epimeric flavalignans from *Trichilia catigua* (Meliaceae) with antimicrobial activity. Z. Naturforsch. C. J. Biosci. 57, 483–488. https://doi.org/10.1515/znc-2002-5-614

Poissy, J., Rouzé, A., Cornu, M., Nseir, S., Sendid, B., 2022. The Changing Landscape of Invasive Fungal Infections in ICUs: A Need for Risk Stratification to Better Target Antifungal Drugs and the Threat of Resistance. J. Fungi. 8 (9), 946. https://doi.org/10.3390/jof8090946

Resende, F.O., Rodrigues-Filho, E., Luftmann, H., Petereit, F., Mello, J.C.P. de, 2011. Phenylpropanoid substituted flavan-3-ols from *Trichilia catigua* and their in vitro antioxidative activity. J. Braz. Chem. Soc. 22, 2087–2093. https://doi.org/10.1590/S0103-50532011001100010

Rhimi, W., Aneke, C.I., Annoscia, G., Otranto, D., Boekhout, T., Cafarchia, C., 2020. Effect of chlorogenic and gallic acids combined with azoles on antifungal susceptibility and virulence of multidrug-resistant *Candida* spp. and Malassezia furfur isolates. Med. Mycol. 58, 1091–1101. https://doi.org/10.1093/mmy/myaa010

Rodrigues, F.C., dos Santos, A.T.L., da Cruz, R.P., Almeida-Bezerra, J.W., Coutinho, H.D.M., Ribeiro, P.R.V., de Brito, E.S., Morais-Braga, M.F.B., de Oliveira, A.F.M., 2022. Antimicrobial activity, modulatory effect and phytochemical analysis of *Sida galheirensis* Ulbr. (Malvaceae). S. Afr. J. Bot. 147, 286–293. https://doi.org/10.1016/j.sajb.2022.01.021

Saito, H., Tamura, M., Imai, K., Ishigami, T., Ochiai, K., 2013. Catechin inhibits *Candida albicans* dimorphism by disrupting Cek1 phosphorylation and cAMP synthesis. Microb. Pathog. 56, 16-20. https://doi.org/10.1016/j.micpath.2013.01.002

Sampaio, R.S.L., Pereira, R.L.S., Coutinho, H.D.M., Almeida-Bezerra, J.W., Morais-Braga, M.F.B., Santana, M.S., Silva, M.E.P., Santos, A.T.L., Fonseca, V.J.A., Costa, A.R., Silva, V.B., Rodrigues, F.C., Bezerra, J.J.L., Raposo, A., Lima, J.P.M., Barros, L. M. (2023).

Chemical composition and antimicrobial potential of *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. and *Syagrus cearensis N*oblick (Arecaceae). Microb. Pathog. 180, 106147. https://doi.org/10.1016/j.micpath.2023.106147

Santos, U.P., Campos, J.F., Torquato, H.F.V., Paredes-Gamero, E.J., Carollo, C.A., Estevinho, L.M., Souza, K. de P., Santos, E.L. dos, 2016. Antioxidant, Antimicrobial and Cytotoxic Properties as Well as the Phenolic Content of the Extract from *Hancornia speciosa* Gomes. PLoS One 11, e0167531. https://doi.org/10.1371/journal.pone.0167531

Saracino, I.M., Foschi, C., Pavoni, M., Spigarelli, R., Valerii, M.C., Spisni, E., 2022. Antifungal Activity of Natural Compounds vs. *Candida* spp.: A Mixture of Cinnamaldehyde and Eugenol Shows Promising In Vitro Results. Antibiotics 11, 73. https://doi.org/10.3390/antibiotics11010073

Sasidharan, S., Nishanth, K. S., Nair, H., 2023. A semi purified hydroalcoholic fraction from *Caesalpinia bonduc* seeds causes ergosterol biosynthesis inhibition in Candida albicans resulting in cell membrane damage. Front. Pharmacol., 14, 1189241. https://doi.org/10.3389/fphar.2023.1189241

Seleem, D., Pardi, V., Murata, R.M., 2017. Review of flavonoids: A diverse group of natural compounds with anti-*Candida albicans* activity in vitro. Arch. Oral Biol. 76, 76–83. https://doi.org/10.1016/j.archoralbio.2016.08.030

Silva, C.R., Sá, L.G. do A.V., dos Santos, E.V., Ferreira, T.L., Coutinho, T. do N.P., Moreira, L.E.A., Campos, R.S., de Andrade, C.R., da Silva, W.M.B., Carneiro, I.S., Silva, J., Silva, J., dos Santos, H.S., Marinho, E.S., Cavalcanti, B.C., de Moraes, M.O., Júnior, H.V.N., Andrade Neto, J.B., 2022. Evaluation of the antifungal effect of chlorogenic acid against strains of *Candida* spp. resistant to fluconazole: apoptosis induction and *in silico* analysis of the possible mechanisms of action. J. Med. Microbiol. 71 (5). https://doi.org/10.1099/jmm.0.001526

Sinan, K.I., Dall'Acqua, S., Ferrarese, I., Mollica, A., Stefanucci, A., Glamočlija, J., Sokovic, M., Nenadić, M., Aktumsek, A., Zengin, G., 2021. LC-MS Based Analysis and Biological Properties of *Pseudocedrela kotschyi* (Schweinf.) Harms Extracts: A Valuable Source of Antioxidant, Antifungal, and Antibacterial Compounds. Antioxidants 10, 1570. https://doi.org/10.3390/antiox10101570

Sousa, E.O. de, Costa, M. do S., Oliveira-Tintino, C.D.M., Nonato, C. de F.A., Pinheiro, J.C.A., Coutinho, H.D.M., Menezes, I.R.A. de, Costa, J.G.M., 2022. Chemical Composition of the Fixed Oil of *Harconia speciosa* and Modulation of the Antibiotic Activity against Non-Resistant and MDR Bacterial Strains. Separations 9, 249. https://doi.org/10.3390/separations9090249

Srivastava, V., Singla, R.K., Dubey, A.K., 2018. Emerging Virulence, Drug Resistance and Future Anti-fungal Drugs for *Candida* Pathogens. Curr. Top. Med. Chem. 18 (9), 759–778. https://doi.org/10.2174/1568026618666180528121707

Süntar, I., 2020. Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. Phytochem. Rev. 19, 1199–1209. https://doi.org/10.1007/s11101-019-09629-9

Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., Škrlec, I., 2021. *Candida albicans*-The Virulence Factors and Clinical Manifestations of Infection. J. Fungi 7, 79. https://doi.org/10.3390/jof7020079

Ugboko, H.U., Nwinyi, O.C., Oranusi, S.U., Fatoki, T.H., Omonhinmin, C.A., 2020. Antimicrobial Importance of Medicinal Plants in Nigeria. Sci. World J. 2020, 7059323. https://doi.org/10.1155/2020/7059323

Valand, N., Girija, U.V., 2021. *Candida* Pathogenicity and Interplay with the Immune System. Adv. Exp. Med. Biol. 1313, 241–272. https://doi.org/10.1007/978-3-030-67452-6_11

Vandenberghe, L.P.S., Karp, S.G., de Oliveira, P.Z., de Carvalho, J.C., Rodrigues, C., Soccol, C.R., 2018. Chapter 18 - Solid-State Fermentation for the Production of Organic Acids, in: Pandey, A., Larroche, C., Soccol, Carlos Ricardo (Eds.), Current Developments in Biotechnology and Bioengineering. Elsevier, pp. 415–434. https://doi.org/10.1016/B978-0-444-63990-5.00018-9

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 (10), 2041. https://doi.org/10.3390/microorganisms9102041

World Health Organization (WHO) - First meeting of the WHO antifungal expert group on identifying priority fungal pathogens, 2020. https://www.who.int/publications-detail-redirect/9789240006355 (accessed 4 February, 2023).

World Health Organization (WHO) - WHO fungal priority pathogens list to guide research, development and public health action, 2022. https://www.who.int/publications-detail-redirect/9789240060241 (accessed 4 February, 2023).

Wu, H., Zhao, F., Li, Q., Huang, J., Ju, J., 2022. Antifungal mechanism of essential oil against foodborne fungi and its application in the preservation of baked food. Crit. Rev. Food Sci. Nutr., 1-13. https://doi.org/10.1080/10408398.2022.2124950

Yun, J., Lee, D.G., 2017. Role of potassium channels in chlorogenic acid-induced apoptotic volume decrease and cell cycle arrest in *Candida albicans.* Biochim. Biophys. Acta, Gen. Subj. 1861, 585–592. https://doi.org/10.1016/j.bbagen.2016.12.026

Zheng, L., Guo, H., Zhu, M., Xie, L., Jin, J., Korma, S. A., Jin, Q., Wang, X., Cacciotti, I., 2023. Intrinsic properties and extrinsic factors of food matrix system affecting the effectiveness of essential oils in foods: a comprehensive review. Crit. Rev. Food Sci. Nutr., 1-34. https://doi.org/10.1080/10408398.2023.2184767

4 ARTIGO 2 – Chemical composition, antibacterial potential, and toxicity of the extracts from the stem bark of *Hancornia speciosa* Gomes (Apocynaceae)

Artigo Publicado

Revista: *Journal of Ethnopharmacology*

ISSN: 0378-8741

Fator de Impacto: 4.8

Qualis – CAPES (2017-2020): A1 em Biodiversidade

Percentil Scopus (2024): 88%

DOI: https://doi.org/10.1016/j.jep.2024.118631

Viviane Bezerra da Silva [a,*], José Weverton Almeida-Bezerra [b], Raimundo Luiz Silva Pereira [b,] Bruno Melo de Alcântara [b], Cláudia Maria Furlan [c], Janerson José Coelho [d], Henrique Douglas Melo Coutinho [e], Maria Flaviana Bezerra Morais-Braga [b], Antonio Fernando Morais de Oliveira[a]

[a] Departamento de Botânica, Universidade Federal de Pernambuco – UFPE, Rua Professor Moraes Rego, s/n, Recife, Pernambuco, 50.670–901, Brazil

[b] Departamento de Ciências Biológicas, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105–000, Brazil

[c] Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, São Paulo, 05508–090, Brazil

[d] Universidade Estadual do Ceará - UECE, Faculdade de Educação, Ciências e Letras dos Inhamuns – CECITEC, Tauá, 63660-000, Ceará, Brazil

[e] Departamento de Química Biológica, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105–000, Brazil

Abstract

Ethnopharmacological relevance: Hancornia speciosa is a medicinal plant popularly used to treat different medical issues, including infectious diseases. Exploring the therapeutic potentialities of the extracts from medicinal plants combined with conventional antibiotic drugs is a promising horizon, especially considering the rising microbial resistance.

Aim of the study: This study aimed to characterize the chemical composition of the ethereal (EEHS) and methanolic (MEHS) extracts of the stem bark of *H. speciosa*, and also evaluate their antibacterial and drug-modifying activity, and toxicity.

Materials and methods: The extracts were characterized by gas chromatography coupled to mass spectrometry (GC–MS). Additionally, total phenol and flavonoid contents were determined. The antibacterial and antibiotic-modifying activity was evaluated against strains of *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* using the serial microdilution method, obtaining the minimum inhibitory concentration (MIC). The toxicity assay was carried out using the *Drosophila melanogaster* model.

Results: Thirty compounds were identified in the extracts of the stem bark of *H. speciosa*, with triterpenoids being predominant in both extracts. Additionally, fatty alcohols, carbohydrates, fatty acids, phenolic acids, and phytosterols were identified in both extracts. EEHS and MEHS extracts had considerable phenol contents (346.4 and 340.0 mg GAE/g, respectively). Flavonoids were detected in a lower proportion (7.6 and 6.9 mg QE/g, respectively). *H. speciosa* extracts did not display intrinsic antibacterial activity against the bacterial strains evaluated, however, they were capable of modifying the activity of gentamicin, erythromycin, and norfloxacin. EEHS increased the efficacy of norfloxacin against *E. coli* and *S. aureus*, reducing MIC values by 50%. MEHS potentiated the action of gentamicin against all bacterial strains, especially against *E. coli*. The extracts did not display toxicity at clinically relevant concentrations against *D. melanogaster*.

Conclusion: The stem bark of *H. speciosa* was considered a rich source of bioactive compounds. Our findings evidenced the therapeutic potential of *H. speciosa* extracts for the development of new pharmaceutical therapeutics against bacteria. Although the extracts did not exhibit intrinsic antibacterial activity, they enhanced the efficacy of commercial antibiotic drugs and were non-toxic at clinically relevant concentrations. Future studies are needed to elucidate the mechanisms of action of these extracts, ensuring their safety and efficacy.

Keywords: Antimicrobial; Ethnomedicine; Mangabeira; Medicinal plants; Therapeutic potential.

1. Introduction

Antibiotics were discovered at the beginning of the 20th century, and since then, they have been widely used for treating and preventing different types of infectious diseases (Hutchings et

al., 2019; Ding et al., 2020). However, the intensive and inappropriate use of these drugs has contributed to selecting resistant microorganisms. The appearance of resistant strains is currently outpacing the development of new antimicrobial drugs (Dadgostar, 2019; Udaondo and Matilla, 2020). Microbial resistance reduces the effectiveness of antibiotics, and it is considered a serious threat to the medical systems. Consequently, treating infectious diseases becomes more difficult and costly, affecting the quality of life of the patients under treatment, and in many cases leading to death (Dadgostar, 2019; Morel et al., 2020). According to the World Health Organization (WHO - World Health Organization, 2019), 700,000 deaths are caused by pathogenic microorganisms annually, and the majority of these cases are in underdeveloped and developing countries. Furthermore, it is estimated that by 2050 the world could reach 10 million deaths per year associated with microbial resistance if effective actions are not taken in time. Among these actions, the discovery of new compounds capable of intensifying the activity of commercial antimicrobial drugs has a prominent space (Tagliabue and Rappuoli, 2018; Trotter et al., 2019).

Nosocomial microorganisms such as pathogenic bacteria are known for their capacity to develop microbial resistance. These bacteria are associated with infections acquired in hospital environments, affecting around 10% of the patients and resulting in extra costs for the public health system (Khan et al., 2015; Edwardson and Cairns, 2019). Among the main nosocomial bacteria, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* stand out globally. These bacterial species are known to cause opportunistic infections, due to their strong resistance, pathogenicity, and virulence. Additionally, these bacteria increase the morbidity and mortality of infected hosts (Hoang et al., 2018; Horn et al., 2018; Khalil et al., 2018). The development of new alternative treatments, including the discovery of new antimicrobial substances has become an urgent need, bringing attention to the use of compounds from medicinal plants (Ueda et al., 2023).

Throughout history, medicinal plants have demonstrated important roles in therapeutics, providing evidence of their effectiveness, which has been passed from generation to generation. The accessibility, low cost, and cultural acceptability make medicinal plants a viable option for treating several infectious diseases, especially in poor and non-developed countries (Hamid et al., 2023). Several medicinal plant species showed great potential against multidrug-resistant bacterial strains (Araújo et al., 2022; Pontes et al., 2022; Almeida-Bezerra et al., 2023). The use of phenolic compounds from plants with strong antimicrobial capacity has been reported as an alternative to the development of new antibacterial agents (Ueda et al., 2023).

Hancornia speciosa Gomes (Apocynaceae) (Fig. 1), commonly known as “mangabeira”, is a recognized medicinal species. Ethnopharmacologically, the stem bark of this species is used to treat a variety of medical conditions, including stomach ulcers, stomach pain, diarrhea, dysentery, gastritis, hernia, wound healing, cancer, urinary tract infections, female reproductive system diseases, inflammation and general infections (Ribeiro et al., 2014; Albuquerque and Meiado, 2015; Vieira et al., 2015; Penido et al., 2016; Ribeiro et al., 2017). The therapeutic and healing properties of *H. speciosa* stem bark can be attributed to various compounds found in the species, such as flavonoids, catechins, proanthocyanidins, and tannins (Moraes et al., 2008; D’Abadia et al., 2020; Almeida et al., 2022; Silva et al., 2024). Considering the therapeutic potential of *H. speciosa* stem bark described in ethnomedicinal studies, especially in the treatment of infectious diseases, and due to the increasing resistance of pathogenic bacteria to commercial antimicrobial drugs, there is a need for new compounds with antimicrobial properties. Therefore, it is highly relevant to explore the antimicrobial potential of *H. speciosa* against these types of pathogenic bacterial strains, including those associated with nosocomial infections.

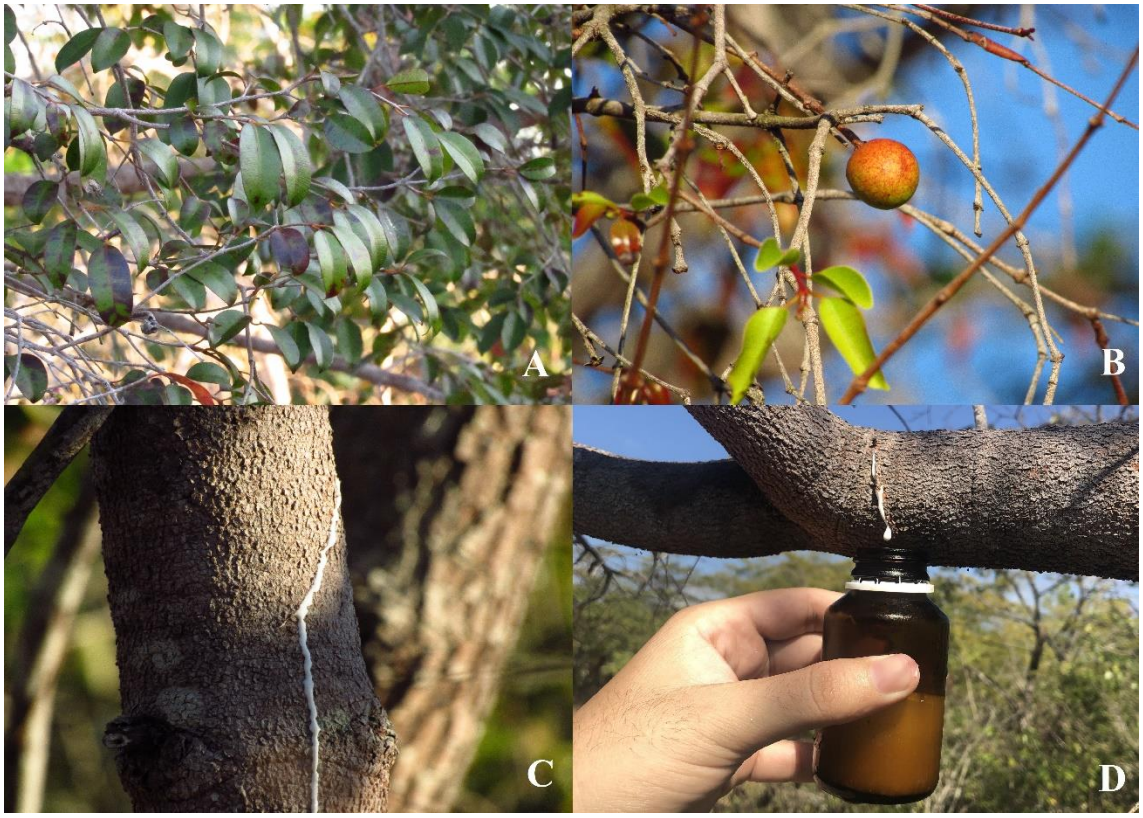


Fig. 1. Characteristics of *Hancornia speciosa* Gomes (Apocynaceae). (A) = Leaves; (B) = Fruit; (C) = Stem and latex; (D) = Latex collection.

This study hypothesizes that the extracts of the stem bark of *H. speciosa* have antimicrobial effects against bacteria causing infections. The objective of this study was to characterize the chemical composition of sulfuric ether and methanolic extracts from the stem bark of *H. speciosa* and verify their antibacterial and antibiotic-modifying activity against multi-resistant bacterial strains (*E. coli*, *P. aeruginosa,* and *S. aureus*). Additionally, the toxicity of these extracts was tested on *Drosophila melanogaster*.

2. Materials and methods

2.1. Collection of botanic material

The samples of stem barks of *H. speciosa* were collected in the municipality of Jardim, state of Ceará, Brazil, in the Environmental Protection Area of Chapada do Araripe, at 920 m altitude, under the coordinates 7◦ 29′02.4″S and 39◦ 16′51.9″W (Fig. 2). The exsiccate of the species was deposited in the Herbarium UFP – Geraldo Mariz under registration number #88,947.

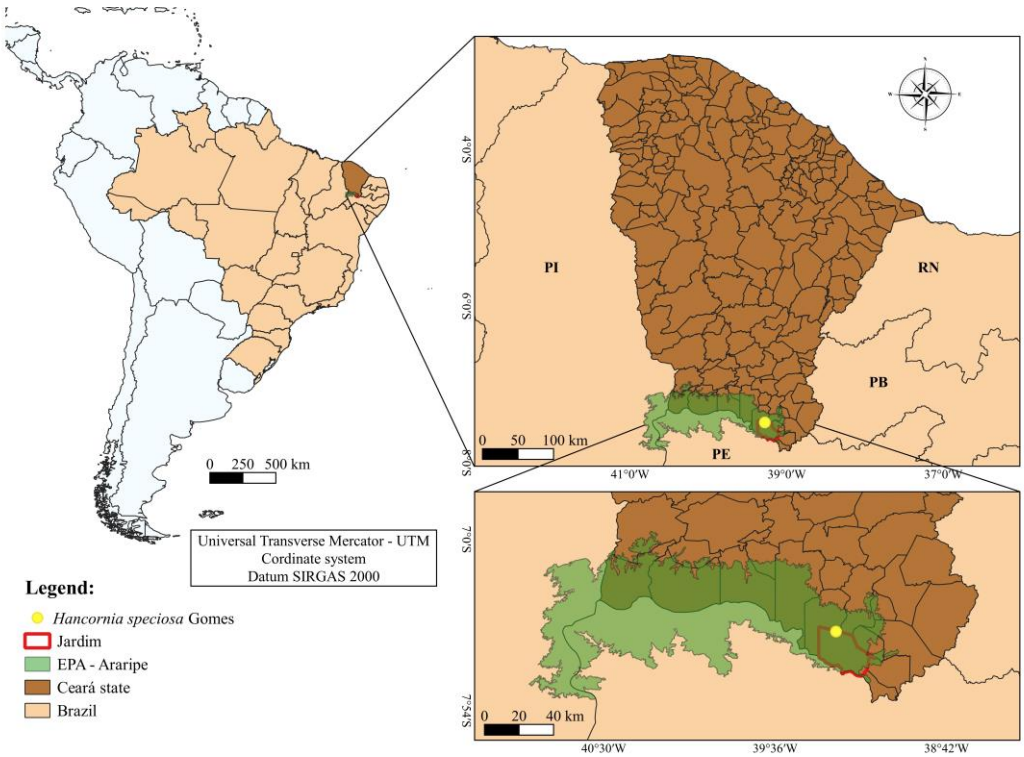


Fig. 2. Map of the sampling site where were collected the stem barks of *Hancornia speciosa* Gomes, Jardim city, Ceará, Brazil.

2.2. Preparation of extracts

A quantity of 950 g of dehydrated *H. speciosa* stem bark was crushed and subjected to exhaustive extraction using *n*-hexane for 72 h at room temperature to remove low-polarity substances. After the removal of the residues, two sequential extractions were performed using sulfuric ether and methanolic solvents, for 72 h each. After each step, the samples were filtered and the solvents were removed using a rotary evaporator (Silva et al., 2024). At the end of this process, the sulfuric ether and methanolic extracts of *H. speciosa* (EEHS and MEHS, respectively) were stored in an amber flask at room temperature. These extracts were kept stored until the chemical analysis and biological activity tests.

2.3. Phytochemical analysis

2.3.1. Gas chromatography coupled to mass spectrometry (GC–MS)

Before the analysis, the extracts were derivatized using 50 μL of pyridine, and 50 μL of N, O–Bis(trimethylsilyl)trifluoroacetamide (BSTFA), for 1 h at 70 ºC. After derivatization, 1 μL of each sample was injected into a gas chromatography system (GC 6850 Network, Agilent) coupled to a mass spectrometer (MSD VL 5975C, Agilent), equipped with an Agilent HP5-MS column (30 m, 0.25 mm, 0.25 μm). Helium was used as attraction gas under a flow of 1 mL min^{-1}. The temperatures of the injector, quadrupole, and ion source were set to 300 ºC, 150 ºC, and 230 ºC, respectively. Ionization was performed by electron impact at 70 eV, with a recorded mass range from 50 to 600 m/z at a rate of 2.66 $scans^{-1}$. The column temperature was set up to the initial temperature of 100 ºC maintained for 5 min, followed by an increase of 5 ºC per minute until reaching 320 ºC (final temperature). The total analysis period took 49 min.

The spectral data of each extract were processed (peak alignment, deconvolution, and calculation of the Linear Retention Index (LRI)), along with compound identification using the Global Natural Product Social Molecular Networking (GNPS), which generated an information table containing retention time, peak area, molecular ion and possible identification suggestion for each compound. A minimum cosine index of 0.30 and an LRI window of 30 were established for

compound identification. Compounds with a cosine index above the minimum but without LRI matching were grouped into the same class (according to GNPS) (Sala-Carvalho et al., 2022).

2.3.2. Total phenols and flavonoids

The Folin-Ciocalteu method was used to determine the total phenol contents (Singleton et al., 1999), with some modifications. Initially, an ethanolic solution (1 mg/mL) of *H. speciosa* extracts was added to a volumetric flask, together with 250 µL of Folin-Ciocalteu reagent and 3 mL of distilled water. After stirring for 30 s, 1 mL of 15% sodium carbonate (Na_2CO_3) was added. After 2 h, the absorbance was measured on a spectrophotometer at 760 nm (SmartSpec Plus, Bio-Rad, USA). The results obtained expressed the total phenolic content in µg of gallic acid equivalents per mg of *H. speciosa* extract (GAE/mg).

To quantify total flavonoids it was used the method described by Woisky and Salatino (1998), with some adaptations. The *H. speciosa* extracts were diluted in distilled water until they reached a concentration of 1 mg/mL. Then, 3 mL of methanol and 1 mL of aluminum chloride (5% $AlCl_3$) were added to each of the extracts. After standing for 30 min, the absorbance was measured at 425 nm using a spectrophotometer (SmartSpec Plus, Bio-Rad, USA). It was compared to a blank sample solely containing methanol. Total flavonoid content was determined using a quercetin standard curve (Sigma-Aldrich®). The results obtained express the total flavonoid content in µg of quercetin equivalents per mg of *H. speciosa* extract (QE/mg).

2.4. Antibacterial activity

2.4.1. Strains, culture medium, drugs, reagents, and preparation of solutions

Standard bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853, and *Staphylococcus aureus* ATCC 22923), and multidrug-resistant strains (*Escherichia coli* 06, *Pseudomonas aeruginosa* 24, and *Staphylococcus aureus* 10) were used in the tests. The resistance profile of multi-drug resistant bacteria to antibacterial drugs is described in the antibiogram (Table 1). The bacterial strains were inoculated in Petri dishes containing Heart Infusion Agar (HIA), and incubated for 24 h in a microbiological incubator at a constant temperature of 37 ºC. After the bacterial growth, they were collected and diluted in sterile saline solution (0.9%) until reaching a turbidity value of 0.5 at the McFarland scale ($1x10^8$ CFU/mL). From this solution, 150 µL of the

inoculum was removed and added to a Brain Heart Infusion (BHI) solution (10%), for use in the antibacterial and drug-modifying assays.

The extracts of *H. speciosa* were diluted in dimethylsulfoxide (DMSO, Merck, Darmstadt, Germany) to reach an initial concentration of 20 mg/ mL. Following, the extracts were diluted in sterile distilled water to reach an initial concentration of 1024 μg/mL. The antibacterial drugs norfloxacin, gentamicin, and erythromycin (Sigma-Aldrich, St. Louis, Missouri, USA) were directly diluted in sterile distilled water reaching a concentration of 1024 μg/mL.

Table 1

Resistance profile of multi-drug resistant bacteria to antibiotic drugs. Source: Laboratory of Microbiology and Molecular Biology – LMBM, Universidade Regional do Cariri – URCA (Ceará, Brazil).

Bacteria	**Origin**	**Drug resistance**
Escherichia coli 06	Urine culture	Cefalothin, cephalexin, cefadroxil, ceftriaxone, cefepime, ampicillin-sulbactam, amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, cefepime.
Pseudomonas aeruginosa 24	Uroculture	Amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, cefepime.
Staphylococcus aureus 10	Rectal smear	Cefadroxil, cefalexin, cephalothin, oxacillin, penicillin, ampicillin, amoxicillin, moxifloxacin, ciprofloxacin, levofloxacin, ampicillin-sulbactam, amoxicillin/clavulanic acid, erythromycin, clarithromycin, azithromycin, clindamycin.

2.4.2. Minimum inhibitory concentration – MIC

To determine the minimum inhibitory concentration, 96–well flat-bottom plates (KASVI®, São José dos Pinhais, PR, Brazil) were initially filled with 100 μL of BHI solution + 10% of the inoculum each well. Subsequently, serial dilution (1:1 v/v) was performed with the extracts or standard drugs at decreasing concentrations (512 – 0.5 μg/mL). After dilution, the plates were kept in a microbial incubator at 37 °C. After 24 hours, a 20 μL solution of 0.01% resazurin (Sigma–Aldrich, St. Louis, Missouri, USA) was added to each well to allow redox reactions. A color

change indicated bacterial growth. The MIC was considered the well with the lowest concentration that inhibited bacterial growth (Fernandes et al., 2022).

2.4.3. Drug-enhancing activity

After determining the MIC, the drug–enhancing experiment was carried out based on the methodology described by Coutinho et al. (2008). Initially, the extracts were evaluated at sub-inhibitory concentrations (MIC/8). A BHI solution containing 10% of the inoculum and the sub-inhibitory concentration of the extracts was prepared. An aliquot of 100 μL was distributed into the wells of the plates in a uniform pattern. Subsequently, a serial dilution (1:1 v/v) was performed using standard antibacterial drugs at decreasing concentrations (512–0.5 μg/mL). These plates were incubated in a microbial incubator at 37 ºC. At the end of the growth period, the plates were read by adding 20 μL of an aqueous solution of resazurin.

2.5. Toxicity analysis

2.5.1. Toxicity assay on Drosophila melanogaster

For toxicity assessment, it was used the ingestion method with *Drosophila melanogaster* as the standard organism (Costa et al., 2020), with some modifications. Adult *D. melanogaster* flies (males and females) aged 6 days were transferred to 300 mL glass flasks (20 flies per flask) (Supplementary Fig. 1). At the bottom of the flasks were placed the basal diet of the flies with the addition of *H. speciosa* extracts at different concentrations (1, 10, and 100 mg/g) for the treated groups, and distilled water for the control group. The choice for these concentrations followed established practices in the literature, which employ logarithmic intervals for efficient coverage of the concentrations (Rand, 2010; Rajan and Perrimon, 2011; Cunha et al., 2015). They were kept under 12-h light cycle (light/dark), at 25 °C ± 1 °C, and 60% relative humidity, for 7 days. The number of dead individuals was counted daily until the end of the experiment (7 days).

2.6. Statistical analysis

All experiments were performed in triplicates, and the means with their respective standard errors (±SEM) were specific to each test. A one-way analysis of variance (ANOVA) was performed, followed by Tukey's test at a 95% confidence level. Significance values were

categorized as $p < 0.0001$ (**** = extremely significant), 0.0001 to 0.001 (*** = extremely significant), 0.001 to 0.01 (** = very significant), 0 .01 to 0.05 (* = significant) and $p > 0.05$ (ns = not significant). The statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, United States).

3. Results

3.1. Chemical composition

The GC-MS analysis revealed the presence of 30 compounds in the extracts of *H. speciosa* (Table 2 and Fig. 3). Among these compounds, 19 were identified in both extracts (EEHS and MEHS), while nine were found only in the EEHS extract, and the other two exclusively in the MEHS extract. In the EEHS extract, triterpenoids were the predominant compounds (42.07%), followed by fatty acids (6.29%), and carbohydrates (5.17%). The fractions of phytosteroids, alcohols, phenolic acids, and other compounds were also detected, with 2.49%, 0.58%, 0.39%, and 4.38%, respectively. In the MEHS extract, triterpenoids were also identified, but found in smaller quantities (8.70%), followed by fatty acids (4.29%), phytosteroids (2.65%), and alcohols (2.43%). Carbohydrates, phenolic acids, and other compounds were found in relatively small amounts in the total extract composition (1.23%, 0.75%, and 0.98%, respectively).

Table 2

Chemical composition of the sulfuric ether (EEHS) and methanolic (MEHS) extracts of *Hancornia speciosa* analyzed via gas chromatography coupled to mass spectrometry.

Class / Compound	Retention time (min)	Area (%)	Molecular formula	Molecular weight (g/mol)	Biological properties	Extract
Fatty alcohol						
n–Octacosanol **(1)**	44.977 / 44.967	0.30 / 0.95	$C_{28}H_{58}O$	410.80	**Antibacterial** (Sengupta et al., 2018); **Larvicidal /Insecticidal** (Zavala-Sánchez et al., 2020); **Antifungal** (Shehata et al., 2024)	EEHS / MEHS
n–Tetradecanol **(2)**	23.979 / 23.975	0.28 / 1.19	$C_{14}H_{30}O$	214.39	**Antibacterial** (Verde et al., 2017)	EEHS / MEHS
n–Triacontanol **(3)**	47.393	0.29	$C_{30}H_{62}O$	438.80	**Antioxidant/Antibacterial** (Ali et al., 2022)	MEHS
Carbohydrate						
D–Psicofuranose **(4)**	24.924	0.53	$C_6H_{12}O_6$	180.16	-	EEHS
Fructofuranose **(5)**	24.777 / 24.768	0.51 / 0.36	$C_6H_{12}O_6$	180.16	-	EEHS / MEHS
Galactopyranose **(6)**	26.558	0.48	$C_6H_{12}O_6$	180.16	-	EEHS
Galactose **(7)**	24.578	0,29	$C_6H_{12}O_6$	180.16	-	EEHS
Glucopyranose **(8)**	26.381	2.35	$C_6H_{12}O_6$	180.16	-	EEHS
Glucose **(9)**	28.085	1.64	$C_6H_{12}O_6$	180.16	-	EEHS
Maltose **(10)**	39.608 / 39.595	0.37 / 0.87	$C_{12}H_{22}O_{11}$	342.30	-	EEHS / MEHS
Fatty acid						
Elaidic acid (18:1–*trans*) **(11)**	30.626	0.32	$C_{20}H_{38}O_2$	310.5	**Larvicidal**	EEHS

					(Perumalsamy et al., 2015); **Cytotoxic activity** (Zha et al., 2021)	
Hexacosanoic acid (C26:0) **(12)**	43.650 / 43.639	0.53/ 0.66	$C_{26}H_{52}O_2$	396.7	**Antifungal** (Singh and Singh, 2003); **Antibacterial** (Rehan et al., 2020)	EEHS / MEHS
Lignoceric acid (C24:0) **(13)**	41.010 / 40.997	1.13 / 0.55	$C_{24}H_{48}O_2$	368.6	-	EEHS / MEHS
Triacontanoic acid (C30:0) **(14)**	48.499	0.91	$C_{30}H_{60}O_2$	452.8	-	EEHS
Palmitoleic acid (C16:1) **(15)**	28.554 / 28.545	1.08 / 1.32	$C_{16}H_{30}O_2$	254.41	**Cytotoxic** (Yamasaki et al., 2003) **Antibacterial** (Watanabe et al., 2021; Wang et al. 2022)	EEHS / MEHS
Oleic acid (C18:1–*cis*) **(16)**	31.521 / 31.511	1.43 / 1.01	$C_{18}H_{34}O_2$	282.5	**Antibacterial** (Dilika et al., 2000); **Larvicidal** (Rahuman et al., 2008); **Cytotoxic** (Permyakov et al., 2012); **Antifungal** (Walters et al., 2004; Verma et al., 2014)	EEHS / MEHS
Linolenic acid (C18:3) **(17)**	31.419 / 31.414	0.47 / 0.40	$C_{18}H_{30}O_2$	278.4	**Cytotoxic** (Vartak et al., 2000); **Antifungal** (Waters et al., 2004); **Antibacterial** (Obonyo et al., 2012; Jung et al., 2015)	EEHS / MEHS
Behenic acid (C22:0) **(18)**	38.184 / 38.174	0.42 / 0.35	$C_{22}H_{44}O_2$	340.6	**Larvicidal** (Wuillda et al., 2019); **Antibacterial** (Ravi et al., 2024)	EEHS / MEHS

Phenolic acid						
Gallic acid **(19)**	27.364 / 27.345	0.18 / 0.40	$C_7H_6O_5$	170.12	**Cytotoxic** (Alves et al., 2016; Jiang et al., 2022); **Larvicidal** (Punia et al., 2021); **Antifungal** (Liberato et al., 2022); **Antibacterial** (Keyvani-Ghamsari et al., 2023)	EEHS / MEHS
Protocatechoic acid **(20)**	24.699 / 24.649	0.21 / 0.35	$C_7H_6O_4$	154.12	**Cytotoxic** (Babich et al., 2002); **Larvicidal** (Daniel et al., 2020); **Antibacterial** (Liu et al., 2005; Fifere et al., 2022)	EEHS / MEHS
Phytosterol						
Campesterol **(21)**	46.533 / 46.532	1.99 / 1.50	$C_{28}H_{48}O$	400.7	**Cytotoxic** (O'Callaghan et al., 2013); **Antifungal** (Choi et al., 2017); **Antibacterial** (Silva et al., 2023)	EEHS / MEHS
y–Sitosterol **(22)**	47.456 / 47.562	0.50 / 1.15	$C_{29}H_{50}O$	414.7	**Antifungal** (Mbambo et al., 2012); **Cytotoxic** (Sirikhansaeng et al., 2017); **Larvicidal** (Mishra et al., 2020); **Antibacterial** (Luhata and Usuki, 2021)	EEHS / MEHS
Triterpenoid						

α–Amyrin **(23)**	47.749	1.93	$C_{30}H_{50}O$	426.7	**Antifungal** (Johann et al., 2007); **Larvicidal** Kuppusamy et al., 2009); **Antibacterial** (Díaz-Ruiz et al., 2012)	MEHS
α-Amyrone **(24)**	47.661 / 47.667	8.94 / 0.73	$C_{30}H_{48}O$	424.7	-	EEHS / MEHS
Friedelan–3–one **(25)**	49.728 / 49.667	3.51 / 2.58	$C_{30}H_{50}O$	426.7	**Antibacterial / Antifungal** (Ichiko et al. 2016; Okafor et al., 2022); **Cytotoxic** (Radi et al., 2023)	EEHS / MEHS
Lupeol **(26)**	48.357	25.52	$C_{30}H_{50}O$	426.7	**Cytotoxic** (Chaturvedi et al., 2008; Akwu et al., 2020); **Larvicidal** (Nobsathian et al., 2018); **Antibacterial** (Rosandy et al., 2021); **Antifungal** (Javed et al., 2021)	EEHS
Ursolic acid **(27)**	50.765 / 50.731	4.10 / 3.46	$C_{30}H_{48}O_3$	456.7	**Cytotoxic** (Ma et al., 2005); **Antifungal** (Shaik et al., 2016); **Antibacterial** (Nascimento et al., 2014; Sycz et al., 2022); **Larvicidal** (Kamatchi et al., 2023)	EEHS / MEHS
Others						
Glucuronic acid **(28)**	28.284	2.07	$C_6H_{10}O_7$	194.14	**Antibacterial** (Ansari et al., 2019)	EEHS

Shikimic acid **(29)**	25.802 / 25.814	0.64 / 0.41	$C_7H_{10}O_5$	174.15	**Antibacterial** (Bai et al., 2015); **Antifungal** (Batory; Rotsztejn, 2022); **Cytotoxic** (Meghdadi et al., 2024)	EEHS / MEHS
Trans–5–*O*–Caffeoyl–D–quinic acid **(30)**	45.422 / 45.403	1.67 / 0.57	$C_{16}H_{18}O_9$	354.31	**Antibacterial** (Aires et al., 2017)	EEHS / MEHS

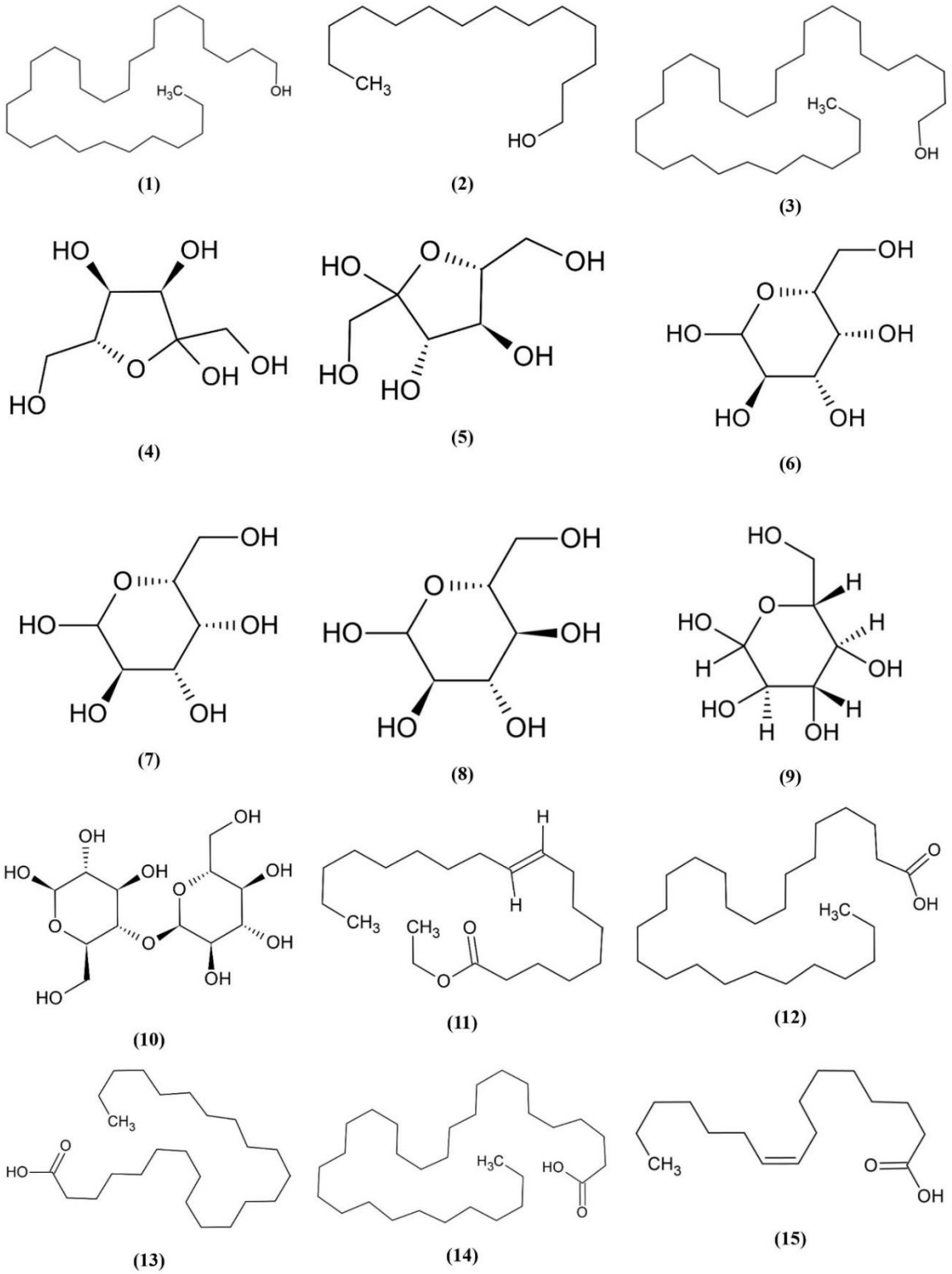


Fig. 3. Chemical structures of the compounds identified in the extracts of the stem bark of *Hancornia speciosa* by gas chromatography-mass spectrometry.

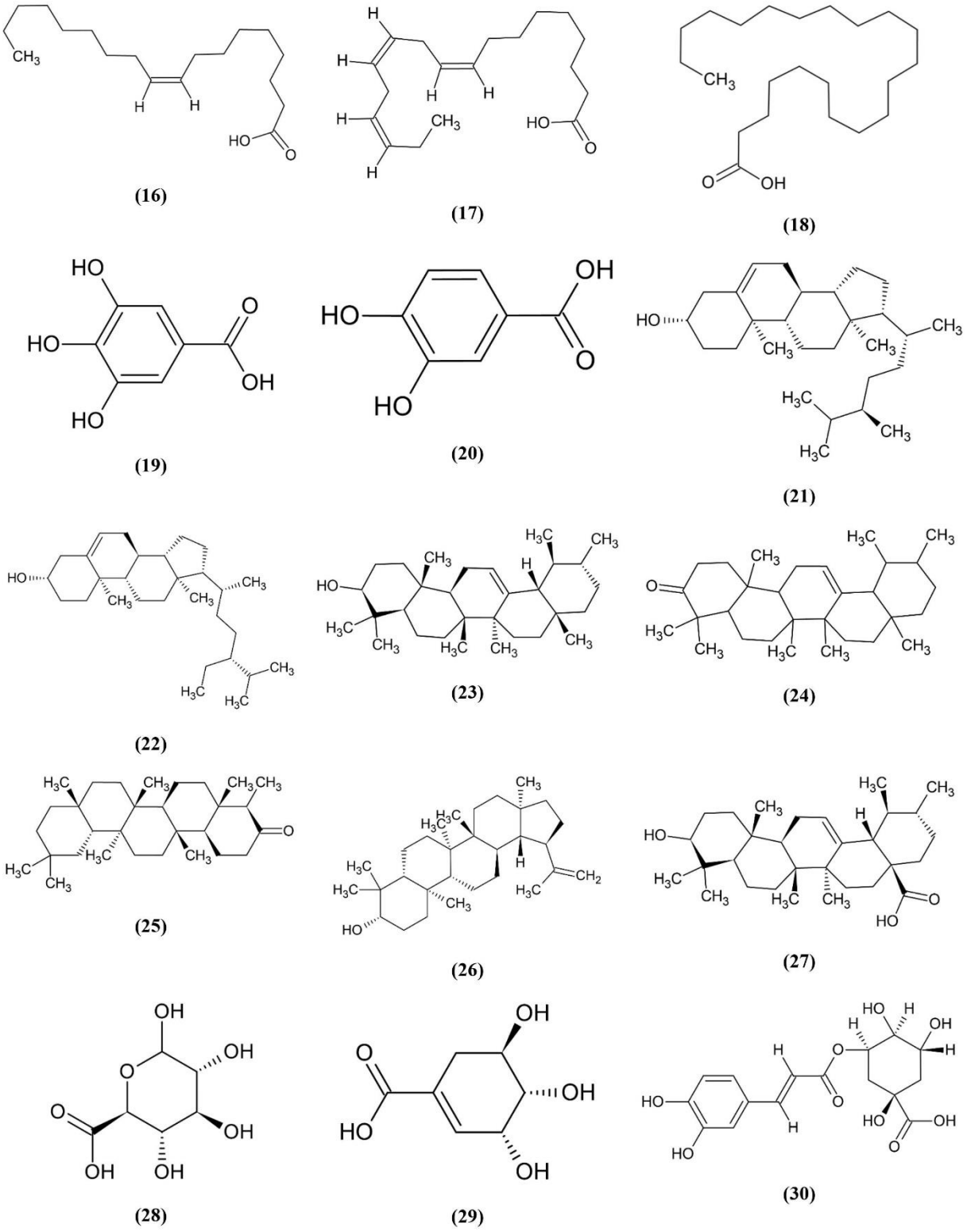


Fig. 3. (*continued*).

The total phenolic and flavonoid contents found in the stem bark extracts (EEHS and MEHS) of *H. speciosa* can be observed in Table 3. For EEHS and MEHS, total phenolic contents ranged from 340.0 to 346.4 mg GAE/g per extract, respectively, and did not differ

statistically ($p > 0.05$). The total flavonoids in the extracts were comparable ($p > 0.05$) (Table 3).

Table 3

Total phenolic and flavonoid content in sulfuric ether (EEHS) and methanolic (MEHS) extracts of the stem bark of *Hancornia speciosa.*

Extract	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
EEHS	346.4 ± 17.8	7.6 ± 0.3
MEHS	340.0 ± 45.4	6.9 ± 1.0

± Standard error (n = 3). GAE = gallic acid equivalent, QE = quercetin equivalent.

3.2. Antibacterial and drug-modifying activity

Although *H. speciosa* is used in herbal medicine for treating infections, its extracts did not show intrinsic antibacterial effects against standard and multi-resistant strains of *E. coli*, *P. aeruginosa,* and *S. aureus* (MIC >512 μg/mL). On the other hand, *H. speciosa* extracts were able to intensify the antibacterial activity of gentamicin and erythromycin against multi-resistant strains of *P. aeruginosa* and *E. coli* ($p < 0.0001$). The EEHS produced a negative effect on the action of two drugs, reducing their effects against *S. aureus*. In this specific case, EEHS significantly ($p < 0.0001$) increased MIC values from 25.39 μg/mL to 101.59 μg/mL for gentamicin; and from 2 μg/mL to 406.37 μg/mL for erythromycin. Regarding the drug norfloxacin, the EEHS extract increased its action against *E. coli* and *S. aureus*, reducing their MIC values by 50% (Fig. 4a).

The MEHS extract of *H. speciosa* potentialized the effect of all multi-resistant strains, notably against *E. coli*, where the MIC value was reduced by more than 50% ($p < 0.001$). When combined with erythromycin, MEHS significantly ($p < 0.0001$) reduced the MIC value of this drug from 128 μg/mL to 25.39 μg/mL against *P. aeruginosa*. On the other hand, this combination increased the MIC value from 2 μg/mL to 80 μg/ mL against *S. aureus*. The combination of MEHS extract with norfloxacin made *E. coli* and *S. aureus* strains more susceptible to the drug ($p < 0.0001$), however, it displayed an antagonistic effect against *P. aeruginosa* (Fig. 4b).

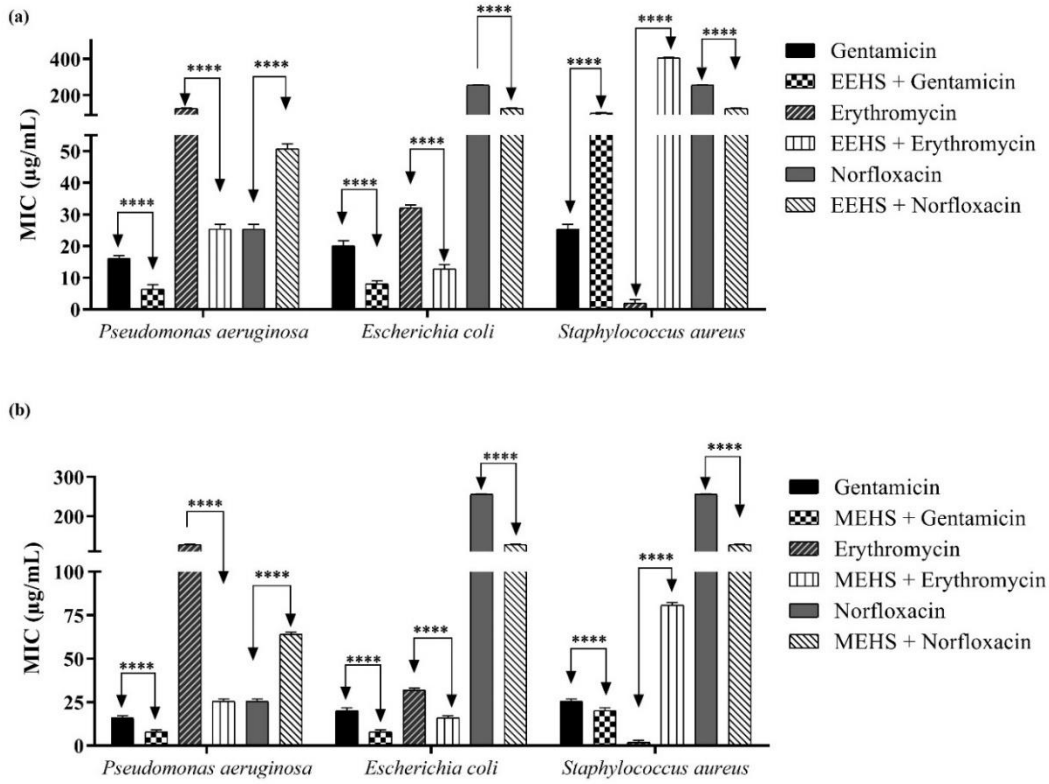


Fig. 4. Minimum Inhibitory Concentration (MIC) of antibiotics combined with sulfuric ether (EEHS) (a) and methanolic (MEHS) (b) extracts of the stem bark of *Hancornia speciosa* against multi-resistant bacterial strains. MIC values are displayed as geometric mean. The bars represent the standard error of the mean (n = 3). **** = $p < 0.0001$.

3.3. Toxicity

3.3.1. In vivo toxicity assay against Drosophila melanogaster

In the toxicity test against *D. melanogaster*, EEHS extract did not show toxic effects at clinically relevant concentrations, indicating its safety up to 10 mg/g. However, the extract showed toxicity at a dosage of 100 mg/ g from the second day of exposure, leading to a mortality rate of 20%, and reaching 28.3% at the end of the 7-day toxicity assay (Fig. 5a). On the other hand, MEHS extract did not show toxicity in any concentration tested over 7 days period, when compared with the control group ($p > 0.05$) (Fig. 5b).

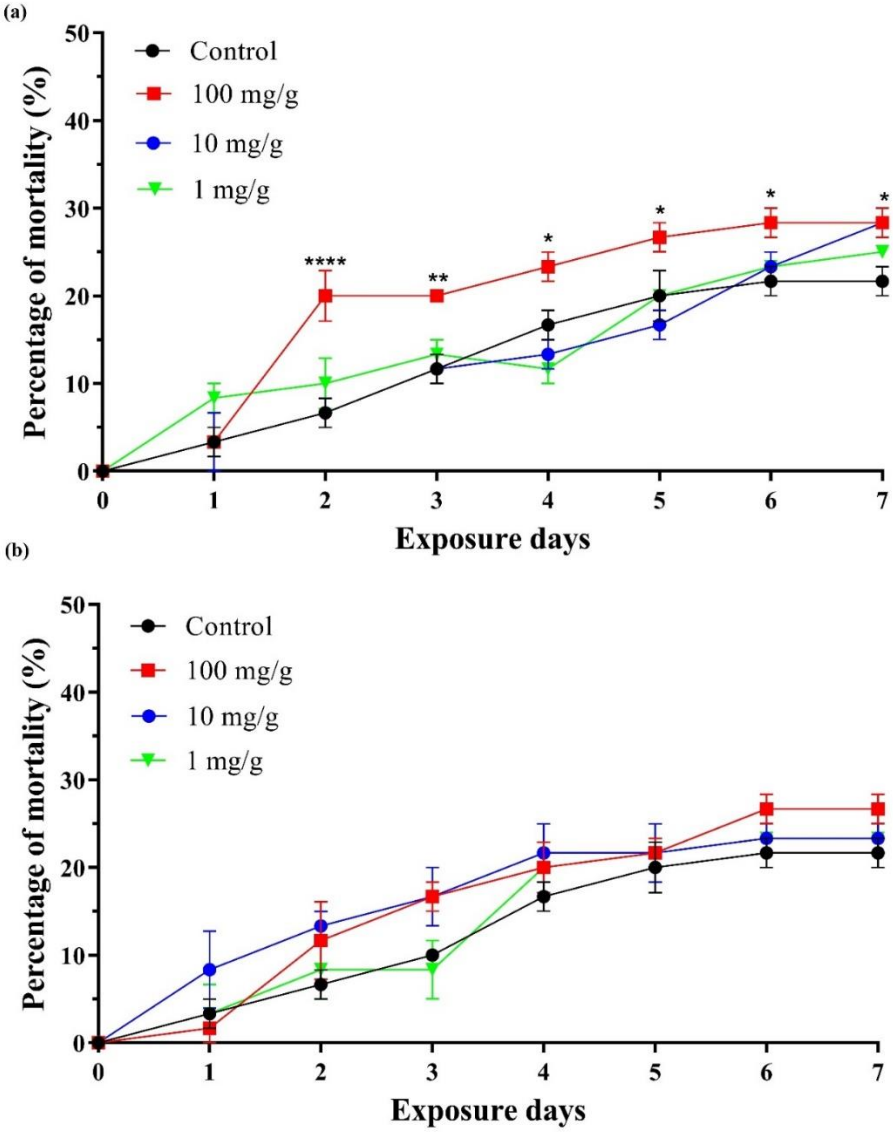


Fig. 5. Toxicity of sulfuric ether (EEHS) (a) and methanolic (MEHS) (b) extracts of the stem bark of *Hancornia speciosa* at different concentrations against *Drosophila melanogaster*. * = $p < 0.05$, **** = $p < 0.0001$. Bars represent the standard error of the mean (n = 3).

4. Discussion

The ethnopharmacological knowledge has been a valuable source for the discovery of promising candidates to be used in the development of new antimicrobial agents, as well as for the identification of natural compounds that can enhance the activity of conventional antibiotic drugs (Calixto, 2019; Chaachouay and Zidane, 2024). In the present study, we investigated two extracts from the stem bark *H. speciosa*, a plant commonly used in Brazilian herbal medicine for treating various infectious diseases. According to the literature, chemical constituents of high and medium polarity from *H. speciosa* exhibit remarkable bioactivity (Barbosa et al., 2019;

Almeida et al., 2022). Additionally, methanol has been shown in other studies to be effective in extracting a wide range of phytochemical compounds with different polarities (Anoor et al., 2022; Zarrinmehr et al., 2022; Riyadi et al., 2023). Based on this evidence, our study focused on two highly polar extracts: sulfuric ether and methanolic.

In a recent publication by our research group, we used liquid chromatography (UPLC–QTOF–MS/MS) to identify the chemical composition of the ethereal and methanolic extracts of the stem bark of *H. speciosa* (Silva et al., 2024). In this analysis, we mainly identified catechin, chlorogenic acid, epicatechin, procyanidin B dimer, procyanidin B trimer, vanillic acid, quinic acid, and phloretin, cinchonain IIb, lariciresinol hexoside isomers, cinchonain Ib isomers, and gluconic acid. In the current study, using GC–MS we identified fatty alcohols, carbohydrates, fatty acids, phenolic acids, phytosterols, and triterpenoids. These diverse findings highlight the importance of using different methods to investigate the compounds present in plant extracts.

Our current findings corroborate previous studies that also identified phytocompounds such as carbohydrates, fatty acids, phenolic acids, and phytosterols in *H. speciosa* (Santos et al., 2018; Silva and Jorge, 2020; Silva et al., 2024). It is worth noting that some of the metabolites identified in this study, such as hexacosanoic acid, lignoceric acid, triacontanoic acid, and α-amyrone, were identified for the first time in *H. speciosa*. Furthermore, the literature demonstrates that several compounds found in the extracts, such as linolenic acid, behenic acid, gallic acid, lupeol, and ursolic acid, have antibacterial activity (Jung et al., 2015; Sycz et al., 2022; Dwivedi et al., 2024).

Our study also evaluated the total phenolic and flavonoid content in the extracts. These constituents can be an indicator of the pharmacological and biological potential of plant extracts (Angeloni et al., 2021). According to the literature, phenolic and flavonoid compounds exhibit a wide range of activities, such as anti-inflammatory, antioxidant, antiulcer, anticarcinogenic, and antibacterial (Goławska et al., 2023; Sun and Shahrajabian, 2023). Our results showed that EEHS and MEHS extracts had considerable total phenol contents, higher than the findings reported by Panontin et al. (2022) which investigated the leaves of the same species, using a different solvent and extraction method. It is important to emphasize that the contents of phenols and flavonoids can vary depending on soil and climate conditions, site and time of collection, part of the plant extracted, the solvent and method used for extraction (Martins et al., 2016; Adhikari et al., 2020; Valencia et al., 2023).

Regarding antibacterial activity, despite ethnopharmacological records that mention the use of *H. speciosa* in the treatment of infections (Ribeiro et al., 2017; Cruz et al., 2021), our

findings showed that the extracts (EEHS and MEHS) from the stem bark did not show an intrinsic antibacterial effect, at clinically relevant concentrations, displaying a MIC > 512 μg/mL (Houghton et al., 2007). On the other hand, Santos et al. (2016) reported that the ethanolic extract of *H. speciosa* leaves displayed antibacterial activity against different bacterial strains, including *S. aureus*.

Our results demonstrated that the extracts from the stem bark of *H. speciosa* enhanced the activity of three commercial antibiotic drugs, a promising alternative for improving antibacterial therapy. The evaluation of the enhancing activity of antibiotics in microbiological assays aims to determine the ability of other compounds to increase or intensify the therapeutic effects of the drugs. The identification of compounds that enhance the activity of antibiotics is crucial to optimizing treatments, enabling the reduction of the antibiotic dose and minimizing potential adverse effects (Coutinho et al., 2008; Confessor et al., 2024). These assays generally involve biological models or *in vitro* tests, in which the compound or product is combined with the antibiotic in different concentrations. Synergistic or additive effects of these drug-enhancing compounds can be compared with the results obtained based on the isolated effect of the antibiotic drugs (Carneiro et al., 2019; Confessor et al., 2024).

Multi-resistant bacteria can develop mechanisms to modify the structure of the antibiotics (e.g., aminoglycoside class), reducing or nullifying their action (Garneau-Tsodikova and Labby, 2016). In our study, extracts from the stem bark of *H. speciosa* enhanced the activity of gentamicin, an aminoglycoside antibiotic. Although we cannot assure that the flavonoids found in the extracts are responsible for inhibiting enzymes related to bacterial resistance, Górniak et al. (2019) suggested that these compounds can interact with acetyltransferases, nucleotidyltransferases, and phosphotransferases, inhibiting their action.

It is worth mentioning that the combinations of the stem bark extracts of *H. speciosa* with different antibiotics intensified the action of all drugs against the *E. coli* strain. This bacteria species is one of the main causes of infection in the urinary tract and bloodstream in humans on a global scale (Čurová et al., 2020). *Escherichia coli* is commonly found in the intestinal tract and is normally harmless, being considered a commensal bacterium (Pakbin et al., 2021). However, these microorganisms can acquire resistance genes, resulting in failures in the treatment of intestinal and extraintestinal infections that can lead to health risks including morbidity and mortality (Pokharel et al., 2023). This reinforces the importance of implementing strategies to solve the problem of antimicrobial resistance (Eisinger et al., 2023).

The intensification of the antibacterial effect against *E. coli* observed in our study may be related to the presence of phenolic compounds in the extracts of the stem bark of *H. speciosa*.

Different findings corroborated the antibacterial activity of these compounds against *E. coli* (Miklasińska-Majdanik et al., 2018; Ecevit et al., 2022; Lobiuc et al., 2023). Among the mechanisms of action include the inhibition of biofilm formation (Bernal-Mercado et al., 2018; Tian et al., 2022), increase in membrane permeability, and rupture of the bacteria (Hao et al., 2021; Tian et al., 2022). According to recent findings (Bernal-Mercado et al., 2018; Hao et al., 2021; Tian et al., 2022), gallic acid and protocatechuic acid, phenolic compounds identified in our extracts, demonstrated these effects. It is believed that the synergy between the compounds present in the extracts and the antibiotics contributed to increasing antibacterial activity against *E. coli*, fighting the bacteria more effectively.

According to the literature, the combination of conventional antibiotic drugs and natural substances such as plant extracts has shown significant antibacterial activities (Araújo et al., 2022; Pontes et al., 2022; Almeida-Bezerra et al., 2023). Some phytochemicals present in the plant extracts can act through different mechanisms of action against bacteria, which include inhibition of the biosynthesis of nucleic acid and proteins, damage to the bacterial membrane and cell wall, inactivation of the efflux pump, and inhibition of virulence mechanisms of the bacteria (e.g., ability to form biofilms) (Fig. 6) (Ayaz et al., 2019; Khameneh et al., 2019; Górniak et al., 2019; Dassanayake et al., 2021; Mahamud et al., 2022).

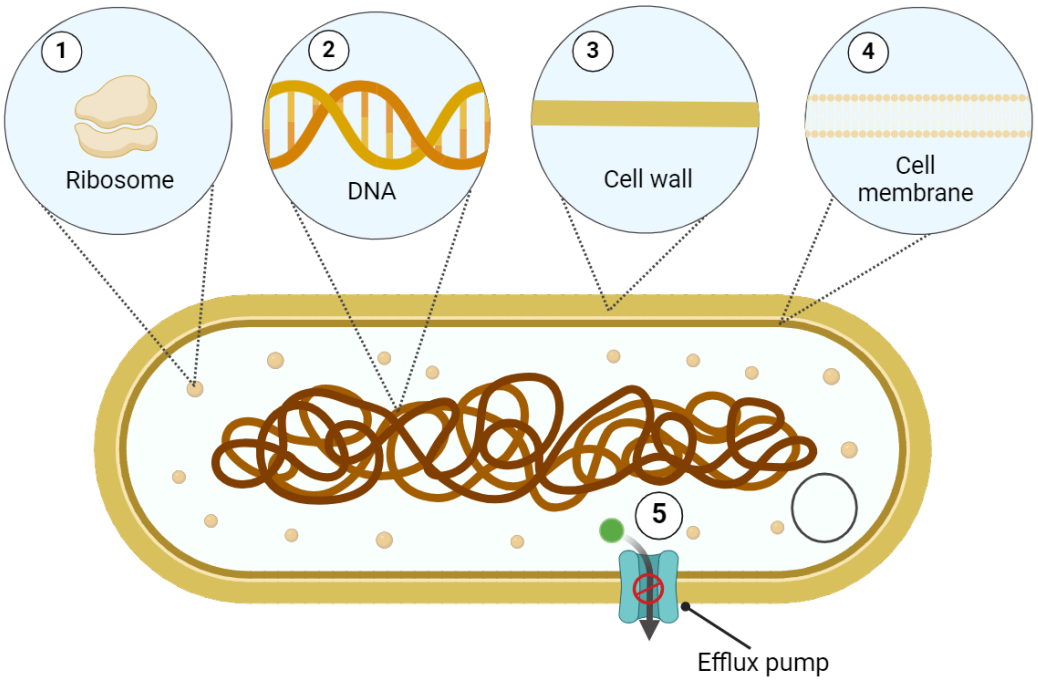


Fig. 6. Target sites of phytochemicals, and their mechanisms of action against bacteria. 1: Inhibition of protein synthesis; 2: Inhibition of nucleic acid synthesis; 3: Damage to the cell wall; 4: Damage and rupture of the cell membrane and 5: Inhibition of the efflux pump.

The compounds present in the stem bark extracts of *H. speciosa* can operate through diverse and complex mechanisms to produce an antibacterial effect, impacting various aspects of the bacterial cell. Fatty acids, for example, can destabilize cell membranes, interrupt the electron transport chain, uncouple oxidative phosphorylation, inhibit enzymatic activities of the membranes, and reduce nutrient absorption (Yoon et al., 2018). Flavonoids, on the other hand, can interact with the bacterial plasma membrane, increasing its permeability to the drug and inhibiting bacterial growth even at low concentrations (Cushnie and Lamb, 2011; Górniak et al., 2019; Farhadi et al., 2019).

Triterpenoids have lipophilic properties, which facilitate interaction with the bacterial cell wall. These compounds can interfere with the biosynthesis of the cell wall and inhibit virulence mechanisms, such as the formation of biofilms. Furthermore, triterpenoids can penetrate the bacterial cell directly impacting protein synthesis, DNA replication and repair (Ibrahim et al., 2019). The lipophilicity is also crucial to the antibacterial potential of phenolic acids. Their lipophilic nature allows them to cross the bacterial cell membrane, resulting in acidification of the cytoplasm and disruption of the membrane structure. This can induce protein denaturation and increase membrane permeability, allowing ions such as potassium (K^+) to outflow from the cell (Lobiuc et al., 2023).

According to Miklasińska-Majdanik et al. (2018) and Kauffmann and Castro (2023), phenolic compounds can negatively affect bacteria through different mechanisms, such as reducing biofilm formation, disturbing membrane integrity and inhibiting virulence factors. Phenolic compounds present in *H. speciosa* extracts may be associated with the intensification of the observed antibiotic activity. An example of this is eriodictyol, identified in previous studies with these extracts (Silva et al., 2024). This compound has been recognized as one of the most powerful antimicrobials of plant origin (Khameneh et al., 2019). According to Wang et al. (2021), eriodictyol acts by inhibiting different virulence mechanisms of the Sortase A enzyme, including the formation of biofilms of methicillin-resistant *S. aureus*. Furthermore, studies indicate that phloretin, also present in *H. speciosa* extracts (Silva et al., 2024), inhibits the Sortase B enzyme, essential for *S. aureus* infection (Wang et al., 2018), and inhibits the expression of toxin-related genes and signaling systems in *E. coli* (Lee et al., 2011).

The intensification of antibacterial activity observed in this study was probably the result of the synergistic action of the antibiotic with different secondary metabolites present in the extracts. The diversity of mechanisms of action of these compounds may have contributed to the intensification of antibacterial activity by acting on multiple targets and different bacterial resistance mechanisms (Ayaz et al., 2019).

The possible antagonistic effect observed by the association of stem bark extracts of *H. speciosa* with norfloxacin against *P. aeruginosa*, and with gentamicin and erythromycin against *S. aureus* can be attributed to the interference on the sites of action of these antibiotics, as well as the possible chelation with these drugs (Bezerra et al., 2017). Direct interference on sites of action reduces antibiotic effectiveness, while chelation forms stable complexes, decreasing the availability and effectiveness of the antibiotics. These mechanisms can compromise the action of antibiotics, contributing to the occurrence of antagonistic effects of the plant extracts (Oliveira et al., 2017).

In our study, in addition to the evaluation of the antibacterial activity, we also investigated the toxicity of *H. speciosa* extracts against *D. melanogaster*, a viable model organism commonly used to assess the toxic activities of natural products (Supplementary Table 1). *Drosophila melanogaster* is genetically significant because it has about 60% homologous genes with humans, with fewer redundant genes. Additionally, around 75% of human disease-associated genes have counterpart homologs in flies (Ugur et al., 2016). Furthermore, it is a low-cost and highly sensitive model, offering an efficient alternative to traditional animal models in toxicological assays, thus circumventing associated ethical challenges (Cunha et al., 2015; Coutinho et al., 2018; Costa et al., 2020). In our study, the stem bark extracts of *H. speciosa* did not cause *in vivo* toxicity in *D. melanogaster* at clinically relevant concentrations (≤ 10 mg/g). The absence of toxicity against *Allium cepa* and *Artemia salina* was also verified by Panontin et al. (2021) using the hydroethanol extract from the stem of *H. speciosa*.

The drug-modifying action of the extracts *H. speciosa* and the absence of toxicity effects observed in the present study, qualify this plant for future research on antibiotic development and therapy. However, it is necessary to carry out additional pre-clinical studies to evaluate the mechanisms involved in the pharmacokinetics and pharmacodynamics of *H. speciosa* extracts, which is essential for understanding their efficacy and safety before considering their introduction as a new pharmaceutical product.

5. Conclusion

The findings of this study partially support the ethnopharmacological practices associated with the use of *H. speciosa* in the treatment of infections. Although *H. speciosa* extracts did not show intrinsic antibacterial activity at clinically relevant concentrations against the bacterial strains (*E. coli*, *P. aeruginosa,* and *S. aureus*), they demonstrated the ability to enhance the antibacterial effect of commercial antibiotic drugs (gentamicin, erythromycin, and

norfloxacin). Furthermore, the extracts did not show toxicity at clinically relevant concentrations in tests with *D. melanogaster*.

These results evidenced the potentialities of stem bark *H. speciosa* as a source of bioactive compounds for the development of new therapeutic agents. The ability of these extracts to intensify the action of antibiotics brings new strategies for the development of therapeutics against bacterial infections, especially, under the current increasing of bacterial resistance to antibiotics. However, it is important to mention that additional research is needed to better understand the therapeutic potential and mechanisms underlying the properties of *H. speciosa* extracts. The identification and characterization of the bioactive compounds found in the extracts, the investigation of their activity against other pathogens, and the evaluation of their safety and efficacy in more complex animal models, are crucial steps for the development of new antibiotics based on *H. speciosa*.

Ethical standards

This study was approved by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen-Brazil) under the registration number A535238, and by the Sistema de Autorização e Informação em Biodiversidade (SISBio-Brazil) under the registration number 80293-1.

Funding

This study was partially funded by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financial Code 001.

Data statement

Data will be made available on request.

CRediT authorship contribution statement

Viviane Bezerra da Silva: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **José Weverton Almeida-Bezerra:** Investigation, Formal analysis. **Raimundo Luiz Silva Pereira:** Investigation. **Bruno Melo de Alcântara:** Software. **Cláudia Maria Furlan:** Software. **Janerson José Coelho:** Formal analysis. **Henrique Douglas Melo Coutinho:** Project adminis- tration. **Maria Flaviana Bezerra Morais-Braga:** Supervision. **Antonio Fernando Morais de Oliveira:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Abbreviations

BHI – Brain Heart Infusion

CFU/mL – Colony Forming Units per Milliliter

DMSO – Dimethylsulfoxide

EEHS – Sulfuric Ether Extract of *Hancornia speciosa*

GAE – Gallic Acid Equivalents

GC–MS - Gas Chromatography coupled to Mass Spectrometry

HIA – Heart Infusion Agar

MEHS - Methanolic Extract of *Hancornia speciosa*

MIC – Minimum Inhibitory Concentration

ns – Not Significant

QE – Quercetin Equivalents

SEM - Standard Error of the Mean

UPLC–QTOF–MS/MS – Ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2024.118631.

References

Adhikari, L., Kotiyal, R., Pandey, M., Bharkatiya, M., Sematy, A., Semalty, M., 2020. Effect of geographical location and type of extract on total phenol/flavon contents and antioxidant activity of different fruits extracts of *Withania somnifera*. Curr. Drug Discov. Technol. 17, 92–99. https://doi.org/10.2174/1570163815666180807100456.

Aires, A., Dias, C., Carvalho, R., Saavedra, M.J., 2017. Analysis of glycosylated flavonoids extracted from sweet-cherry stems, as antibacterial agents against pathogenic *Escherichia coli* isolates. Acta Biochim. Pol. 64, 265–271. https://doi.org/10.18388/ABP.2016_1374.

Akwu, N., Naidoo, Y., Singh, M., Thimmegowda, S.C., Nundkumar, N., Lin, J., 2020. Isolation of lupeol from grewia lasiocarpa stem bark: antibacterial, antioxidant, and cytotoxicity activities. Biodiversitas 21, 5684–5690. https://doi.org/10.13057/biodiv/d211213.

Albuquerque, U.P. de, Meiado, M.V., 2015. In: Sociobiodiversidade Na Chapada Do Araripe, first ed. NUPEEA, Recife.

Ali, J.S., Riaz, N., Mannan, A., Tabassum, S., Zia, M., 2022. Antioxidative-, antimicrobial-, enzyme inhibition-, and cytotoxicity-based fractionation and isolation of active components from *Monotheca buxifolia* (Falc.) A. DC. Stem extracts. ACS Omega 7, 3407–3423. https://doi.org/10.1021/acsomega.1c05647.

Almeida, F.L.C., Oliveira, E.N.A., Almeida, E.C., Souza, W.F.C., Silva, M.O., Melo, A.M., Castro, M.P.J., Bullo, G.T., Luna, L.C., Prata, A.S., Forte, M.B.S., 2022. *Hancornia speciosa*: an overview focused on phytochemical properties, recent achievements, applications, and future perspectives. Int. J. Gastron. Food Sci. 29, 100561. https://doi.org/10.1016/j.ijgfs.2022.100561.

Almeida-Bezerra, J.W., Cruz, R.P., Pereira, R.L.S., Silva, V.B., Sousa, D.O.B., Neto, J.X.S., Souza, L.A.L., Araújo, N.M.S., Silva, R.G.G., Lucetti, D.L., Coutinho, H.D.M., Morais-Braga, M.F.B., Oliveira, A.F.M., 2023. *Caryocar coriaceum* fruits as a potential alternative to combat fungal and bacterial infections: in vitro evaluation of methanolic extracts. Microb. Pathog. 181, 106203 https://doi.org/10.1016/j.micpath.2023.106203.

Alves, A.D.C.S., Mainardes, R.M., Khalil, N.M., 2016. Nanoencapsulation of gallic acid and evaluation of its cytotoxicity and antioxidant activity. Mater. Sci. Eng. C 60, 126–134. https://doi.org/10.1016/j.msec.2015.11.014.

Angeloni, S., Spinozzi, E., Maggi, F., Sagratini, G., Caprioli, G., Borsetta, G., Ak, G., Sinan, K.I., Zengin, G., Arpini, S., Mombelli, G., Ricciutelli, M., 2021. Phytochemical profile and biological activities of crude and purified *Leonurus cardiaca* extracts. Plants 10, 195. https://doi.org/10.3390/plants10020195.

Anoor, P.K., Yadav, A.N., Rajkumar, K., Kande, R., Tripura, C., Naik, K.S., 2022. Methanol extraction revealed anticancer compounds quinic acid, 2(5h)-furanone and phytol in *Andrographis paniculata*. Mol. Clin. Oncol. 17 https://doi.org/10.3892/mco.2022.2584.

Ansari, F., Pourjafar, H., Kangari, A., Homayouni, A., 2019. Evaluation of the glucuronic acid production and antibacterial properties of kombucha black tea. Curr. Pharmaceut. Biotechnol. 20, 985–990. https://doi.org/10.2174/1389201020666190717100958.

Araújo, N.J.S., Silva, A.R.P., Costa, M.S., Freitas, T.S., Barbosa Filho, J.M., Matos, Y.M.L. S., Morais-Braga, M.F.B., Pereira Junior, F.N., Silva, C.A.P., Souza, E.O., Ribeiro, P.R.V., Lacerda, B.C.V.G., Andrade, E.M., Coutinho, H.D.M., Andrade-Pinheiro, J.C., 2022. Chemical characterization UPLC-ESI-QToF-MSE, antibacterial and antibiofilm potential of *Sarcomphalus joazeiro* (MART.) Hauenschild. Food Biosci. 50, 102066 https://doi.org/10.1016/j.fbio.2022.102066.

Ayaz, M., Ullah, F., Sadiq, A., Ullah, F., Ovais, M., Ahmed, J., Devkota, H.P., 2019. Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. Chem. Biol. Interact. 308, 294–303. https://doi.org/10.1016/j.cbi.2019.05.050.

Babich, H., Sedletcaia, A., Kenigsberg, B., 2002. In vitro cytotoxicity of protocatechuic acid to cultured human cells from oral tissue: involvement in oxidative stress. Pharmacol. Toxicol. 91, 245–253. https://doi.org/10.1034/j.1600-0773.2002.910505.x.

Bai, J., Wu, Y., Liu, X., Zhong, K., Huang, Y., Gao, H., 2015. Antibacterial activity of shikimic acid from pine needles of *Cedrus deodara* against *Staphylococcus aureus* through damage to cell membrane. Int. J. Mol. Sci. 16, 27145–27155. https://doi.org/10.3390/ijms161126015.

Barbosa, A.M., Santos, K.S., Borges, G.R., Muniz, A.V.C.S., Mendonça, F.M.R., Pinheiro, M.S., Franceschi, E., Dariva, C., Padilha, F.F., 2019. Separation of antibacterial biocompounds from *Hancornia speciosa* leaves by a sequential process of pressurized liquid extraction. Sep. Purif. Technol. 222, 390–395. https://doi.org/10.1016/j.seppur.2019.04.022.

Batory, M., Rotsztejn, H., 2022. Shikimic acid in the light of current knowledge. J. Cosmet. Dermatol. 21, 501–505. https://doi.org/10.1111/jocd.14136.

Bernal-Mercado, A.T., Vazquez-Armenta, F.J., Tapia-Rodriguez, M.R., Islas-Osuna, M.A., Mata-Haro, V., Gonzalez-Aguilar, G.A., Lopez-Zavala, A.A., Ayala-Zavala, J.F., 2018. Comparison of single and combined use of catechin, protocatechuic, and vanillic acids as antioxidant and antibacterial agents against Uropathogenic *Escherichia coli* at planktonic and biofilm levels. Molecules 23, 2803–2813. https://doi.org/10.3390/molecules23112813.

Bezerra, C.F., Camilo, C.J., Silva, M.K.N., Freitas, T.S., Ribeiro-Filho, J., Coutinho, H.D. M., 2017. Vanillin selectively modulates the action of antibiotics against resistant bacteria. Microb. Pathog. 113, 265–268. https://doi.org/10.1016/j.micpath.2017.10.052.

Calixto, J.B., 2019. The role of natural products in modern drug discovery. An. Acad. Bras. Cienc. 91, 1–7. https://doi.org/10.1590/0001-3765201920190105.

Carneiro, J.N.P., da Cruz, R.P., Silva, J.P.C., Rocha, J.E., Freitas, T.S., Sales, D.L., Bezerra, C.F., Almeida Martins, W.O., Costa, J.G.M., Silva, L.E., Amaral, W., Rebelo, R.A., Begnini, I.M., Coutinho, H.D.M., Morais-Braga, M.F.B., 2019. *Piper diospyrifolium* Kunth.: chemical analysis and antimicrobial (intrinsic and combined) activities. Microb. Pathog. 136, 103700 https://doi.org/10.1016/j.micpath.2019.103700.

Chaachouay, N., Zidane, L., 2024. Plant-derived natural products: a source for drug discovery and development. drugs and drug candidates 3, 184–207. https://doi.org/10.3390/ddc3010011.

Chaturvedi, P.K., Bhui, K., Shukla, Y., 2008. Lupeol: connotations for chemoprevention. Cancer Lett. 263, 1–13. https://doi.org/10.1016/j.canlet.2008.01.047.

Choi, N.H., Jang, J.Y., Choi, G.J., Choi, Y.H., Jang, K.S., Nguyen, V.T., Min, B.S., Le Dang, Q., Kim, J.C., 2017. Antifungal activity of sterols and dipsacus saponins isolated from *Dipsacus asper* roots against phytopathogenic fungi. Pestic. Biochem. Physiol. 141, 103–108. https://doi.org/10.1016/j.pestbp.2016.12.006.

Confessor, M.V.A., Agreles, M.A.A., Campos, L.A.A., Neto, A.F.S., Borges, J.C., Martins, R.M., Scavuzzi, A.M.L., Lopes, A.C.S., Kretzschmar, E.A.M., Cavalcanti, I.M. F., 2024. Olive oil nanoemulsion containing curcumin: antimicrobial agent against multidrug-resistant bacteria. Appl. Microbiol. Biotechnol. 108 https://doi.org/10.1007/s00253-024-13057-x.

Costa, A.R., Silva, J.R.L., Oliveira, T.J.S., Silva, T.G., Pereira, P.S., Borba, E.F.O., Brito, E. S., Ribeiro, P.R.V., Almeida-Bezerra, J.W., Júnior, J.T.C., Menezes, I.R.A., Kamdem, J.P., Duarte, A.E., Barros, L.M., 2020. Phytochemical profile of *Anacardium occidentale* L. (cashew tree) and the cytotoxic and toxicological evaluation of its bark and leaf extracts. South Afr. J. Bot. 135, 355–364. https://doi.org/10.1016/j.sajb.2020.09.017.

Coutinho, H.D.M., Costa, J.G.M., Lima, E.O., Falcão-Silva, V.S., Siqueira-Júnior, J.P., 2008. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. And chlorpromazine. Chemotherapy 54, 328–330. https://doi.org/10.1159/000151267.

Coutinho, H.D.M., Oliveira-Tintino, C.D. de M., Tintino, S.R., Pereira, L.S.P., Freitas, T.S. de, Silva, M.A.P. da, Franco, J.L., Cunha, F.A.B., Costa, J.G.M., Menezes, I.R.A. de, Boligon, A.A., Rocha, J.B.T. da, Rocha, M.I., Santos, J.F.S., 2018. Toxicity against *Drosophila melanogaster* and antiedematogenic and antimicrobial activities of *Alternanthera brasiliana* (L.) Kuntze (Amaranthaceae). Environ. Sci. Pollut. Res. Int. 25, 10353–10361. https://doi.org/10.1007/s11356-017-9366-x.

Cruz, R.R., Almeida-Bezerra, J.W., Menezes, S.A., Silva, V.B., Santos, L.T., Morais- Braga, M.F.B., de Moraes, J.L., 2021. Ethnopharmacology of the angiosperms of Chapada of Araripe located in northeast of Brazil. JEAP 6, 326–351. https://doi.org/10.24221/jeap.6.4.2021.4272.326-351.

Cunha, F.A.B., Wallau, G.L., Pinho, A.I., Nunes, M.E.M., Leite, N.F., Tintino, S.R., Costa, G.M., Athayde, M.L., Boligon, A.A., Coutinho, H.D.M., Pereira, A.B., Posser, T., Franco, J.L., 2015. *Eugenia uniflora* leaves essential oil induces toxicity in *Drosophila melanogaster*: involvement of oxidative stress mechanisms. Toxicol. Res. 4, 634–644. https://doi.org/10.1039/c4tx00162a.

Čurová, K., Slebodníková, R., Kmeťová, M., Hrabovský, V., Maruniak, M., Liptáková, E., Siegfried, L., 2020. Virulence, phylogenetic background and antimicrobial resistance in *Escherichia coli* associated with extraintestinal infections. J Infect Public Heal 13, 1537–1543. https://doi.org/10.1016/j.jiph.2020.06.032.

Cushnie, T.P.T., Lamb, A.J., 2011. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimicrob. Agents 38, 99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014.

D'Abadia, P.L., Bailão, E.F.L.C., Júnior, R.S.L., Oliveira, M.G., Silva, V.B., Oliveira, L.A.R., Conceição, E.C., Melo-Reis, P.R., Borges, L.L., Gonçalves, P.J., Almeida, L.M., 2020. *Hancornia speciosa* serum fraction latex stimulates the angiogenesis and extracellular matrix remodeling processes. An. Acad. Bras. Cienc. 92 https://doi.org/10.1590/0001-3765202020190107.

Dadgostar, P., 2019. Antimicrobial resistance: implications and costs. Infect. Drug Resist. 12, 3903–3910. https://doi.org/10.2147/idr.s234610.

Daniel, I.J., Innocent, E., Sempombe, J., Mugoyela, V., Fossen, T., 2020. Isolation and characterization of larvicidal phenolic acids from *Kotschya thymodora* leaves. J. Appl. Sci. Environ. Manag. 24, 1483–1488. https://doi.org/10.4314/jasem.v24i8.26.

Dassanayake, M.K., Khoo, T.-J., An, J., 2021. Antibiotic resistance modifying ability of phytoextracts in anthrax biological agent *Bacillus anthracis* and emerging superbugs: a

review of synergistic mechanisms. Ann. Clin. Microbiol. Antimicrob. 20 https://doi.org/10.1186/s12941-021-00485-0.

Díaz-Ruiz, G., Hernández-Vázquez, L., Luna, H., Del Carmen Wacher-Rodarte, M., Navarro-Ocanã, A., 2012. Growth inhibition of streptococcus from the oral cavity by α-amyrin esters. Molecules 17, 12603–12611. https://doi.org/10.3390/molecules171112603.

Dilika, F., Bremner, P.D., Meyer, J.J.M., 2000. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia 71, 450–452. https://doi.org/10.1016/S0367-326X(00)00150-7.

Ding, Y., Li, Z., Xu, C., Qin, W., Wu, Q., Wang, X., Cheng, X., Li, L., Huang, W., 2020. Fluorogenic probes/inhibitors of β-lactamase and their applications in drug-resistant bacteria. Angew. Chem. 60, 24–40. https://doi.org/10.1002/anie.202006635.

Dwivedi, G.R., Pathak, N., Tiwari, N., Negi, A.S., Kumar, A., Pal, A., Sharma, A., Darokar, M.P., 2024. Synergistic antibacterial activity of gallic acid based chalcone indl 2 by inhibiting efflux pump transporters. Chem. Biodiversity 21. https://doi.org/10.1002/cbdv.202301820.

Ecevit, K., Barros, A.A., Silva, J.M., Reis, R.L., 2022. Preventing microbial infections with natural phenolic compounds. Future Pharmacol 2, 460–498. https://doi.org/10.3390/futurepharmacol2040030.

Edwardson, S., Cairns, C., 2019. Nosocomial infections in the ICU. Anaesth. Intensive Care 20, 14–18. https://doi.org/10.1016/j.mpaic.2018.11.004.

Eisinger, R.W., Williams, M.P., Choe, S.H., Krofah, E., 2023. A call to action-stopping antimicrobial resistance. JAC-Antimicrob. Resist. 5 https://doi.org/10.1093/jacamr/dlac142.

Farhadi, F., Khameneh, B., Iranshahi, M., Iranshahy, M., 2018. Antibacterial activity of flavonoids and their structure–activity relationship: an update review. Phytother Res. 33, 13–40. https://doi.org/10.1002/ptr.6208.

Fernandes, P.A.S., Pereira, R.L.S., dos Santos, A.T.L., Coutinho, H.D.M., Morais-Braga, M. F.B., da Silva, V.B., Costa, A.R., Generino, M.E.M., de Oliveira, M.G., de Menezes, S. A., dos Santos, L.T., Siyadatpanah, A., Wilairatana, P., Portela, T.M.A., Gonçalo, M. A.B.F., Almeida-Bezerra, J.W., 2022. Phytochemical analysis, antibacterial activity and modulating effect of essential Oil from *Syzygium cumini* (L.) Skeels. Molecules 27, 3281. https://doi.org/10.3390/molecules27103281.

Fifere, A., Turin-Moleavin, I.A., Rosca, I., 2022. Does protocatechuic acid affect the activity of commonly used antibiotics and antifungals? Life 12, 1010. https://doi.org/10.3390/life12071010.

Garneau-Tsodikova, S., Labby, K.J., 2016. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. MedChemComm 7, 11–27. https://doi.org/10.1039/c5md00344j.

Goławska, S., Łukasik, I., Chojnacki, A.A., Chrzanowski, G., 2023. Flavonoids and phenolic acids content in cultivation and wild collection of european cranberry bush *Viburnum opulus* L. Molecules 28, 2285. https://doi.org/10.3390 molecules28052285.

Górniak, I., Bartoszewski, R., Króliczewski, J., 2019. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochemistry Rev. 18, 241–272. https://doi.org/10.1007/s11101-018-9591-z.

Hamid, L., Alsayari, A., Tak, H., Mir, S.A., Ali, M., Wahab, S., Bader, G.N., 2023. An Insight into the global problem of gastrointestinal helminth infections amongst livestock: does nanotechnology provide an alternative? Agriculture 13, 1359. https://doi.org/10.3390/agriculture13071359.

Hao, J., Lei, Y., Gan, Z., Zhao, W., Shi, J., Jia, C., Sun, A., 2021. Synergetic inactivation mechanism of protocatechuic acid and high hydrostatic pressure against *Escherichia coli* O157. Foods 10. https://doi.org/10.3390/foods10123053.

Hoang, S., Georget, A., Asselineau, J., Venier, A.G., Leroyer, C., Rogues, A.M., Thiébaut, R., 2018. Risk factors for colonization and infection by *Pseudomonas aeruginosa* in patients hospitalized in intensive care units in France. PLoS One 13, e0193300. https://doi.org/10.1371/journal.pone.0193300.

Horn, J., Stelzner, K., Rudel, T., Fraunholz, M., 2018. Inside job: *Staphylococcus aureus* host-pathogen interactions. Int J. Med. Microbiol. 308, 607–624. https://doi.org/10.1016/j.ijmm.2017.11.009.

Houghton, P.J., Howes, M.-J., Lee, C.C., Steventon, G., 2007. Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant. J. Ethnopharmacol. 110, 391–400. https://doi.org/10.1016/j.jep.2007.01.032.

Hutchings, M.I., Truman, A.W., Wilkinson, B., 2019. Antibiotics: past, present and future. Curr. Opin. Microbiol. 51, 72–80. https://doi.org/10.1016/j.mib.2019.10.008.

Ibrahim, H.A., Elgindi, M.R., Ibrahim, R.R., El-Hosari, D.G., 2019. Antibacterial activities of triterpenoidal compounds isolated from *Calothamnus quadrifidus* leaves. BMC Compl. Alternative Med. 19 https://doi.org/10.1186/s12906-019-2512-x.

Ichiko, C.O., Terrumun, A.T.A., John, O.I., John, V.A., 2016. In vitro antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* LHerit, ex Dc. Afr. J. Biotechnol. 15, 531–538. https://doi.org/10.5897/ajb2015.15091.

Javed, S., Mahmood, Z., Khan, K.M., Sarker, S.D., Javaid, A., Khan, I.H., Shoaib, A., 2021. Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. Sci. Rep. 11, 8417. https://doi.org/10.1038/s41598-021-87725-7.

Jiang, Y., Pei, J., Zheng, Y., Miao, Y., Duan, B., Huang, L., 2022. Gallic acid: a potential anti-cancer agent. Chin. J. Integr. Med. 28, 661–671.

Johann, S., Soldi, C., Lyon, J.P., Pizzolatti, M.G., Resende, M.A., 2007. Antifungal activity of the amyrin derivatives and in vitro inhibition of *Candida albic*ans adhesion to human epithelial cells. Lett. Appl. Microbiol. 45, 148–153. https://doi.org/10.1111/j.1472-765X.2007.02162.x.

Jung, S.W., Thamphiwatana, S., Zhang, L., Obonyo, M., 2015. Mechanism of antibacterial activity of liposomal linolenic acid against *Helicobacter pylori*. PLoS One 10, e0116519. https://doi.org/10.1371/journal.pone.0116519.

Kamatchi, P.A.C., Maheswaran, R., Sivanandhan, S., Ignacimuthu, S., Balakrishna, K., Reegan, A.D., Arivoli, S., 2023. Bioefficacy of ursolic acid and its derivatives isolated from *Catharanthus roseus* (L) G. Don leaf against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi* larvae. Environ. Sci. Pollut. Res. 30, 69321–69329. https://doi.org/10.1007/s11356-023-27253-1.

Kauffmann, A.C., Castro, V.S., 2023. Phenolic compounds in bacterial inactivation: a perspective from Brazil. Antibiotics 12, 645–645. https://doi.org/10.3390/antibiotics12040645.

Keyvani-Ghamsari, S., Rahimi, M., Khorsandi, K., 2023. An update on the potential mechanism of gallic acid as an antibacterial and anticancer agent. Food Sci. Nutr. 11, 5856–5872. https://doi.org/10.1002/fsn3.3615.

Khalil, I.A., Troeger, C., Blacker, B.F., Rao, P.C., Brown, A., Atherly, D.E., Brewer, T.G., Engmann, C.M., Houpt, E.R., Kang, G., Kotloff, K.L., Levine, M.M., Luby, S.P., MacLennan, C.A., Pan, W.K., Pavlinac, P.B., Platts-Mills, J.A., Qadri, F., Riddle, M.S., Ryan, E.T., Shoultz, D.A., Steele, D., Walson, J.L., Sanders, J.W., Mokdad, A.H., Murray, C.J.L., Hay, S.I., Reiner, R.C., 2018. Morbidity and mortality due to shigella and enterotoxigenic *Escherichia coli* diarrhoea: the global burden of disease study

1990–2016. Lancet Infect. Dis. 18, 1229–1240. https://doi.org/10.1016/s1473-3099(18)30475-4.

Khameneh, B., Iranshahy, M., Soheili, V., Bazzaz, B.S.F., 2019. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob. Resist. Infect. Control 8, 1–28. https://doi.org/10.1186/s13756-019-0559-6.

Khan, H.A., Ahmad, A., Mehboob, R., 2015. Nosocomial infections and their control strategies. Asian Pac. J. Trop. Biomed. 5, 509–514. https://doi.org/10.1016/j.apjtb.2015.05.001.

Kuppusamy, C., Murugan, K., Arul, N., Yasodha, P., 2009. Larvicidal and insect growth regulator effect of α-amyrin acetate from *Catharanthus roseus* Linn against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Entomol. Res. 39, 78–83. https://doi.org/10.1111/j.1748-5967.2009.00196.x.

Lee, J.-H., Regmi, S.C., Kim, J.-A., Cho, M.H., Yun, H., Lee, C.-S., Lee, J., 2011. Apple flavonoid phloretin inhibits *Escherichia coli* O157:H7 biofilm formation and ameliorates colon inflammation in rats. Infect. Immun. 79, 4819–4827. https://doi.org/10.1128/iai.05580-11.

Liberato, I., Lino, L.A., Souza, J.K.D., Neto, J.B.A., Sá, L.G.A.V., Cabral, V.P.F., Silva, C. R., Cavalcanti, B.C., Moraes, M.O., Freire, V.N., Júnior, H.V.N., Andrade, C.R., 2022. Gallic acid leads to cell death of *Candida albicans* by the apoptosis mechanism. Future Microbiol. 17, 599–606. https://doi.org/10.2217/fmb-2021-0139.

Liu, K. Sen, Tsao, S.M., Yin, M.C., 2005. *In vitro* antibacterial activity of roselle calyx and protocatechuic acid. Phyther. Res. 19, 942–945. https://doi.org/10.1002/ptr.1760.

Lobiuc, A., Pavˇal, N.-E., Mangalagiu, I.I., Gheorghiţaˇ, R., Teliban, G.-C., Mantu, D.A., Stoleru, V., 2023. Future antimicrobials: natural and functionalized phenolics. Molecules 28, 1114–1114. https://doi.org/10.3390/molecules28031114.

Luhata, L.P., Usuki, T., 2021. Antibacterial activity of β-sitosterol isolated from the leaves of *Odontonema strictum* (Acanthaceae). Bioorg. Med. Chem. Lett. 48, 128248.

Ma, C.M., Cai, S.Q., Cui, J.R., Wang, R.Q., Tu, P.F., Hattori, M., Daneshtalab, M., 2005. The cytotoxic activity of ursolic acid derivatives. Eur. J. Med. Chem. 40, 582–589. https://doi.org/10.1016/j.ejmech.2005.01.001.

Mahamud, A.G.M.S.U., Nahar, S., Ashrafudoulla, M.A., Park, S.H., Ha, S.-D., 2022. Insights into antibiofilm mechanisms of phytochemicals: prospects in the food industry. Crit. Rev. Food Sci. Nutr. 64, 1–28. https://doi.org/10.1080/10408398.2022.2119201.

Martins, N., Barros, L., Ferreira, I.C.F.R., 2016. In vivo antioxidant activity of phenolic compounds: facts and gaps. Trends Food Sci. Technol. 48, 1–12. https://doi.org/10.1016/j.tifs.2015.11.008.

Mbambo, B., Odhav, B., Mohanlall, V., 2012. Antifungal activity of stigmasterol, sitosterol and ergosterol from *Bulbine natalensis* Baker (Asphodelaceae). J. Med. Plants Res. 6, 5135–5141. https://doi.org/10.5897/jmpr12.151.

Meghdadi, P., Bamoharram, F.F., Karimi, E., Ghasemi, E., 2024. Shikimic acid nanoformulations: a comprehensive inquiry into anticancer potential and apoptotic induction on A2058 skin cancer cells. Bionanoscience 1–8. https://doi.org/10.1007/s12668-024-01490-1.

Miklasińska-Majdanik, M., Kępa, M., Wojtyczka, R.D., Idzik, D., Wąsik, T.J., 2018. Phenolic compounds diminish antibiotic resistance of *Staphylococcus aureus* clinical strains. Int. J. Environ. Res. Publ. Health 15, 2321–2321. https://doi.org/10.3390/ijerph15102321.

Mishra, M., Sharma, A., Dagar, V.S., Kumar, S., 2020. Effects of B-sitosterol on growth, development and midgut enzymes of *Helicoverpa armigera* Hübner. Arch. Biol. Sci. 72, 271–278. https://doi.org/10.2298/ABS200308021M.

Moraes, T. de M., Rodrigues, C.M., Kushima, H., Bauab, T.M., Villegas, W., Pellizzon, C. H., Brito, A.R.M.S., Hiruma-Lima, C.A., 2008. *Hancornia speciosa*: indications of gastroprotective, healing and anti-*Helicobacter pylori* actions. J. Ethnopharmacol. 120, 161–168. https://doi.org/10.1016/j.jep.2008.08.001.

Morel, C.M., Alm, R.A., Årdal, C., Bandera, A., Bruno, G.M., Carrara, E., Colombo, G.L., de Kraker, M.E.A., Essack, S., Frost, I., Gonzalez-Zorn, B., Goossens, H., Guardabassi, L., Harbarth, S., Jørgensen, P.S., Kanj, S.S., Kostyanev, T., Laxminarayan, R., Leonard, F., Hara, G.L., Mendelson, M., Mikulska, M., Mutters, N. T., Outterson, K., Rodriguez Banõ, J., Tacconelli, E., Scudeller, L., 2020. A one health framework to estimate the cost of antimicrobial resistance. Antimicrob. Resist. Infect. Control 9, 1–14. https://doi.org/10.1186/s13756-020-00822-6.

Nascimento, P.G.G., Lemos, T.L.G., Bizerra, A.M.C., Arriaga, A.M.C., Ferreira, D.A., Santiago, G.M.P., Braz-Filho, R., Costa, J.G.M., 2014. Antibacterial and antioxidant activities of ursolic acid and derivatives. Molecules 19, 1317–1327. https://doi.org/10.3390/molecules19011317.

Nobsathian, S., Bullangpoti, V., Kumrungsee, N., Wongsa, N., Ruttanakum, D., 2018. Larvicidal effect of compounds isolated from *Maerua siamensis* (Capparidaceae)

against *Aedes aegypti* (Diptera: Culicidae) larvae. Chem. Biol. Technol. Agric. 5, 1–7. https://doi.org/10.1186/s40538-018-0120-5.

O'Callaghan, Y., Kenny, O., O'Connell, N.M., Maguire, A.R., McCarthy, F.O., O'Brien, N. M., 2013. Synthesis and assessment of the relative toxicity of the oxidised derivatives of campesterol and dihydrobrassicasterol in U937 and HepG2 cells. Biochimie 95, 496–503. https://doi.org/10.1016/j.biochi.2012.04.019.

Obonyo, M., Zhang, Li, Thamphiwatana, S., Pornpattananangkul, D., Fu, V., Zhang, Liangfang, 2012. Antibacterial activities of liposomal linolenic acids against antibiotic-resistant *Helicobacter pylori*. Mol. Pharm. 9, 2677–2685. https://doi.org/10.1021/mp300243w.

Okafor, G.C.O., Oyewale, A.O., Habila, J.D., Akpemi, M.A., 2022. Isolation, characterization, and assessment of the *in vitro* antibacterial and antifungal properties of methanol extracts and friedelan-3-one from *Uapaca ambanjensis* (Leandri). J. Appl. Sci. Environ. Manag. 26, 1479–1486. https://doi.org/10.4314/jasem.v26i9.4.

Oliveira, F.S., Freitas, T.S., da Cruz, R.P., Costa, M.S., Pereira, R.L.S., Quintans-Júnior, L. J., Andrade, T.A., Menezes, P.D.P., de Sousa, B.M.H., Nunes, P.S., Serafini, M.R., de Menezes, I.R.A., Araújo, A.A.S., Coutinho, H.D.M., 2017. Evaluation of the antibacterial and modulatory potential of α-bisabolol, β-cyclodextrin and α-bisabolol/β-cyclodextrin complex. Biomed. Pharmacother. 92, 1111–1118. https://doi.org/10.1016/j.biopha.2017.06.020.

Pakbin, B., Brück, W.M., Rossen, J.W.A., 2021. Virulence factors of enteric pathogenic *Escherichia coli*: a review. Int. J. Mol. Sci. 22, 9922. https://doi.org/10.3390/ijms22189922.

Panontin, J.F., Barbosa, R.D.S., Isaac, V., Seibert, C.S., Scapin, E., 2022. Chemical composition, antioxidant activity and development of a facial serum formulation from the extract of *Hancornia speciosa*. Nat. Prod. Res. 36, 6121–6125. https://doi.org/10.1080/14786419.2022.2053968.

Panontin, J.F., Neres, R.P., Fernandes, R.M.N., Scapin, E., Seibert, C.S., 2021. Chemical characterization and toxicological analyses of hydroalcoholic extracts from the stem and leaves of mangabeira (*Hancornia speciosa* Gomes) as a guide for the development of green cosmetics. J. Med. Plants Res. 15, 366–379. https://doi.org/10.5897/JMPR2021.7099.

Penido, A.B., Morais, S.M., Ribeiro, A.B., Silva, A.Z., 2016. Ethnobotanical study of medicinal plants in imperatriz, state of maranhão, northeastern Brazil. Acta Amazonica 46, 345–354. https://doi.org/10.1590/1809-4392201600584.

Permyakov, S.E., Knyazeva, E.L., Khasanova, L.M., Fadeev, R.S., Zhadan, A.P., Roche-Hakansson, H., Håkansson, A.P., Akatov, V.S., Permyakov, E.A., 2012. Oleic acid is a key cytotoxic component of HAMLET-like complexes. Biol. Chem. 393, 85–92. https://doi.org/10.1515/BC-2011-230.

Perumalsamy, H., Jang, M.J., Kim, J.R., Kadarkarai, M., Ahn, Y.J., 2015. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. Parasit. vectors 8. https://doi.org/10.1186/s13071-015-0848-8.

Pokharel, P., Dhakal, S., Dozois, C.M., 2023. The diversity of *Escherichia coli* pathotypes and vaccination strategies against this versatile bacterial pathogen. Microorganisms 11, 344–344. https://doi.org/10.3390/microorganisms11020344.

Pontes, M.C., Cavalcante, N.B., Leal, A.E.B.P., de Oliveira, A.P., Coutinho, H.D.M., de Menezes, I.R.A., Delange, D.M., Turatti, I.C.C., de Oliveira, G.G., Neto, F.C., Tomaz, J.C., Lopes, N.P., Almeida, J.R.G.S., 2022. Chemical constituents and antibacterial activity of *Bromelia laciniosa* (Bromeliaceae): identification and structural characterization. Phytomed. Plus. 2, 100215 https://doi.org/10.1016/j.phyplu.2022.100215.

Punia, A., Chauhan, N.S., Singh, D., Kesavan, A.K., Kaur, S., Sohal, S.K., 2021. Effect of gallic acid on the larvae of *Spodoptera litura* and its parasitoid Bracon hebetor. Sci. Rep. 11, 531. https://doi.org/10.1038/s41598-020-80232-1.

Radi, M.H., El-Shiekh, R.A., El-Halawany, A.M., Abdel-Sattar, E., 2023. Friedelin and 3β-friedelinol: pharmacological activities. Rev. Bras. Farmacogn. 33, 886–900.

Rahuman, A.A., Venkatesan, P., Gopalakrishnan, G., 2008. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. Parasitol. Res. 103, 1383–1390. https://doi.org/10.1007/s00436-008-1146-6.

Rajan, A., Perrimon, N., 2011. *Drosophila* as a model for interorgan communication: lessons from studies on energy homeostasis. Dev. Cell 21, 29–31. https://doi.org/10.1016/j.devcel.2011.06.034.

Rand, M.D., 2010. Drosophotoxicology: the growing potential for *Drosophila* in neurotoxicology. Neurotoxicol. Teratol. 32, 74–83. https://doi.org/10.1016/j.ntt.2009.06.004.

Ravi, L., Ajith Kumar, K., Shree Kumari, G.R., Mathew, J., Harshitha, S., Panda, M., Shivani, S., Paul, A., Chandana, T.S., Anil, A., Megha, J.K., Mukherjee, T., Bhattacharjee, S., Raveendran Nair, M., Subhanjan, V., Mohanasrinivasan, V., Jain, P., 2024. Behenic

Acid as a multi-target inhibiting antibacterial phytochemical against *Vibrio parahaemolyticus* and *Aeromonas hydrophila* for effective management of aquaculture infections: an *in-silico, in-vitro* & *in-vivo* experimentation. J. Biomol. Struct. Dyn. 1–16. https://doi.org/10.1080/07391102.2024.2317988.

Rehan, M.S., Ansari, F.A., Singh, O., 2020. Isolation, identification, antibacterial activity and docking of fatty acid and fatty alcohol from *Rumex dentatus* leaf Extract. Int. J. Pharmaceut. Sci. Rev. Res. 64, 7–11. https://doi.org/10.47583/ijpsrr.2020.v64i01.002.

Ribeiro, D.A., de Oliveira, L.G.S., de Macêdo, D.G., de Menezes, I.R.A., da Costa, J.G.M., da Silva, M.A.P., Lacerda, S.R., de, A., Souza, M.M., 2014. Promising medicinal plants for bioprospection in a Cerrado area of Chapada do Araripe, Northeastern Brazil. J. Ethnopharmacol. 155, 1522–1533. https://doi.org/10.1016/j.jep.2014.07.042.

Ribeiro, R.V., Bieski, I.G.C., Balogun, S.O., de, O., Martins, D.T., 2017. Ethnobotanical study of medicinal plants used by ribeirinhos in the north araguaia microregion, mato grosso, Brazil. J. Ethnopharmacol. 205, 69–102. https://doi.org/10.1016/j.jep.2017.04.023.

Riyadi, P.H., Susanto, E., Anggo, A.D., Arifin, M.H., Rizki, L., 2023. Effect of methanol solvent concentration on the extraction of bioactive compounds using ultrasonic-assisted extraction (UAE) from Spirulina platensis. Food Res. 7, 59–66. https://doi.org/10.26656/fr.2017.7(s3).9.

Rosandy, A.R., Ishak, S.S.O., Sabri, N.A., Ahmad, W.Y.W., Al Muqarrabun, L.M.R., 2021. Antibacterial activity of lupeol from the bark of *Dehaasia cuneate* (Lauraceae). Curr. Res. Biosci. Biotechnol. 2, 145–148. https://doi.org/10.5614/crbb.2021.2.2/bofy6724.

Sala-Carvalho, W.R., Montessi-Amaral, F.P., Esposito, M.P., Campestrini, R., Rossi, M., Peralta, D.F., Furlan, C.M., 2022. Metabolome of *Ceratodon purpureus* (Hedw.) Brid., a cosmopolitan moss: the influence of seasonality. Planta 255, 50–77. https://doi.org/10.1007/s00425-022-03857-8.

Santos, U.P. dos, Tolentino, G.S., Morais, J.S., Souza, K.P., Estevinho, L.M., Dos Santos, E. L., 2018. Physicochemical characterization, microbiological quality and safety, and pharmacological potential of *Hancornia speciosa* Gomes. Oxid. Med. Cell. Longev. 1–17. https://doi.org/10.1155/2018/2976985.

Santos, U.P., Campos, J.F., Torquato, H.F.V., Paredes-Gamero, E.J., Carollo, C.A., Estevinho, L.M., Souza, K.P., Santos, E.L., 2016. Antioxidant, antimicrobial and cytotoxic properties as well as the phenolic content of the extract from *Hancornia speciosa* Gomes. PLoS One 11, e0167531. https://doi.org/10.1371/journal.pone.0167531.

Sengupta, S., Nandi, I., Bhattacharyya, D.K., Ghosh, M., 2018. Anti-oxidant and Anti- bacterial properties of 1-octacosanol isolated from rice bran wax. J Plant Biochem. Physiol. 6, 206. https://doi.org/10.4172/2329-9029.1000206.

Shaik, A.B., Ahil, S.B., Govardhanam, R., Senthi, M., Khan, R., Sojitra, R., Kumar, S., Srinivas, A., 2016. Antifungal effect and protective role of ursolic acid and three phenolic derivatives in the management of sorghum grain mold under field conditions. Chem. Biodivers. 13, 1158–1164. https://doi.org/10.1002/cbdv.201500515.

Shehata, A.I., Rasheed, M., Rafiq, H., Khalid, N., Rafique, A., Alhoshy, M., Habib, Y.J., El Basuini, M.F., 2024. Multi-functional application of octacosanol as a feed additive in animal and aquaculture: a review. J. Anim. Physiol. Anim. Nutr. https://doi.org/10.1111/jpn.14002.

Silva, A.C., Jorge, N., 2020. Potential of mangaba (*Hancornia speciosa*), mango (*Mangifera indica* L.), and papaya (*Carica papaya* L.) seeds as sources of bioactive compounds. Rev. Ceres 67, 439–447. https://doi.org/10.1590/0034-737x202067060003.

Silva, F.E.F., das Chagas Lima Pinto, F., Loiola Pessoa, O.D., Marques da Fonseca, A., Martins da Costa, J.G., Pinheiro Santiago, G.M., 2023. Campesterol semi-synthetic derivatives as potential antibacterial: in vitro and *in silico* Evaluation. Chem. Biodivers. 20, e202300536 https://doi.org/10.1002/cbdv.202300536.

Silva, V.B., Almeida-Bezerra, J.W., Novais, M.H.G., Farias, N.S., Coelho, J.J., Ribeiro, P. R.V., Canuto, K.M., Coutinho, H.D.M., Morais-Braga, M.F.B., de Oliveira, A.F.M., 2024. Chemical composition, antifungal, and anti-virulence action of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) against *Candida* spp. J. Ethnopharmacol. 321, 117506 https://doi.org/10.1016/j.jep.2023.117506.

Singh, B., Singh, S., 2003. Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. Phyther. Res. 17, 814–816. https://doi.org/10.1002/ptr.1202.

Singleton, V.L., Orthofer, R., Lamuela-Raventós, R., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 152–178. Academic Press.

Sirikhansaeng, P., Tanee, T., Sudmoon, R., Chaveerach, A., 2017. Major phytochemical as γ - sitosterol disclosing and toxicity testing in Lagerstroemia species. Evid. base Compl. Alternative Med. 2017, 7209851 https://doi.org/10.1155/2017/7209851.

Sun, W., Shahrajabian, M.H., 2023. Therapeutic potential of phenolic compounds in medicinal plants—natural health products for human health. Molecules 28, 1845. https://doi.org/10.3390/molecules28041845.

Sycz, Z., Wojnicz, D., Tichaczek-Goska, D., 2022. Does secondary plant metabolite ursolic acid exhibit antibacterial activity against Uropathogenic *Escherichia coli* living in single- and multispecies biofilms? Pharmaceutics 14, 1691. https://doi.org/10.3390/pharmaceutics14081691.

Tagliabue, A., Rappuoli, R., 2018. Changing priorities in vaccinology: antibiotic resistance moving to the top. Front. Immunol. 9, 1068. https://doi.org/10.3389/fimmu.2018.01068.

Tian, Q., Wei, S., Zheng, S., Xu, S., Liu, M., Bo, R., Li, J., 2022. Bactericidal activity of gallic acid against multi-drug resistance *Escherichia coli*. Microb. Pathog. 173, 105824 https://doi.org/10.1016/j.micpath.2022.105824.

Trotter, A.J., Aydin, A., Strinden, M.J., O'Grady, J., 2019. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. Curr. Opin. Microbiol. 51, 39–45. https://doi.org/10.1016/j.mib.2019.03.001.

Udaondo, Z., Matilla, M.A., 2020. Mining for novel antibiotics in the age of antimicrobial resistance. Microb. Biotechnol. 13, 1702–1704. https://doi.org/10.1111/1751-7915.13662.

Ueda, J.M., Milho, C., Heleno, S.A., Soria-Lopez, A., Carpena, M., Alves, M.J., Pires, T., Prieto, M.A., Simal-Gandara, J., Calhelha, R.C., Ferreira, I.C.F.R., Barros, L., 2023. Emerging strategies to combat methicillin-resistant *Staphylococcus aureus* (MRSA): natural agents with high potential. Curr. Pharmaceut. Des. 29, 837–851. https://doi.org/10.2174/1381612829666230410095155.

Ugur, B., Chen, K., Bellen, H.J., 2016. *Drosophila* tools and assays for the study of human diseases. Dis. Model. Mech. 9, 235–244. https://doi.org/10.1242/dmm.023762.

Valencia, D., Aguilar-González, D.I., Ortega-García, J., Godoy-Hernández, G., Leyva- Peralta, M.A., Moo-Huchín, V.M., Aarland, R.C., Quintero-Vargas, J., Mendoza- Espinoza, J.A., Zarza-García, A.L., 2023. Phytochemical Profile, Antioxidant and antiproliferative activity from leaves and seeds of *Bixa orellana* L. from the Yucatán peninsula, Mexico. Phcog. Mag. 19, 482–490. https://doi.org/10.1177/09731296231158492.

Vartak, S., Robbins, M.E.C., Spector, A.A., 2000. The selective cytotoxicity of γ-linolenic acid (GLA) is associated with increased oxidative stress. Adv. Exp. Med. Biol. 469, 493–498. https://doi.org/10.1007/978-1-4615-4793-8_72.

Verde, K., Eun, J., Kim, M.J., Kang, M.-J., Jang, A.-R., Kim, J.R., Kim, S., Chang, K.-T., Hong, J.J., Parque, J.H., 2017. Inhibitory effect of 1-tetradecanol on *Helicobacter pylori*-induced production of interleukin-8 and vascular endothelial growth factor in gastric

epithelial cells. Mol. Med. Rep. 16, 9573–9578. https://doi.org/10.3892/mmr.2017.7793.

Verma, S., Bhardwaj, A., Vij, M., Bajpai, P., Goutam, N., Kumar, L., 2014. Oleic acid vesicles: a new approach for topical delivery of antifungal agent. Artif. Cells, Nanomed. Biotechnol. 42, 95–101. https://doi.org/10.3109/21691401.2013.794351.

Vieira, L.S., Sousa, R.S., Lemos, J.R., 2015. Plantas medicinais conhecidas por especialistas locais de uma comunidade rural maranhense. Rev. Bras. Plantas Med. 17, 1061–1068. https://doi.org/10.1590/1983-084x/15_009.

Walters, D., Raynor, L., Mitchell, A., Walker, R., Walker, K., 2004. Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia 157, 87–90.

Wang, F., Guo, Y., Cao, Y., Zhang, C., 2022. In vitro antibacterial activity of palmitoleic acid isolated from filamentous microalga *Tribonema minus* against fish pathogen *Streptococcus agalactiae*. J. Ocean Univ. China 21, 1615–1621. https://doi.org/10.1007/s11802-022-5047-6.

Wang, G., Gao, Y., Wang, H., Wang, J., Niu, X., 2018. Phloretin reduces cell injury and inflammation mediated by *Staphylococcus aureus* via targeting sortase B and the molecular mechanism. Appl. Microbiol. Biotechnol. 102, 10665–10674. https://doi.org/10.1007/s00253-018-9376-8.

Wang, L., Li, Q., Li, J., Jing, S., Jin, Y., Yang, L., Yu, H., Wang, D., Wang, T., Wang, L., 2021. Eriodictyol as a potential candidate inhibitor of Sortase A protects mice from methicillin-resistant *Staphylococcus aureus*-induced pneumonia. Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.635710.

Watanabe, T., Yano, S., Kawai, T., Jinbo, Y., Nonomura, Y., 2021. Selective antibacterial activity of palmitoleic acid in emulsions and other formulations. J. Surfactants Deterg. 24, 973–979. https://doi.org/10.1002/jsde.12529.

WHO - World Health Organization, 2019. New Report Calls for Urgent Action to Avert Antimicrobial Resistance Crisis. https://www.who.int/news/item/29-04-2019-ne w-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis. (Accessed 3 May 2024).

Woisky, R.G., Salatino, A., 1998. Analysis of propolis: some parameters and procedures for chemical quality control. J. Apicult. Res. 37, 99–105. https://doi.org/10.1080/00218839.1998.11100961.

Wuillda, A.D.S.J., Martins, R.C.C., Costa, F.D.N., 2019. Larvicidal activity of secondary plant metabolites in aedes aegypti control: an overview of the previous 6 years. Nat. Prod. Commun. 14, 1934578X19862893 https://doi.org/10.1177/1934578X19862893.

Yamasaki, M., Chujo, H., Nou, S., Tachibana, H., Yamada, K., 2003. Alleviation of the cytotoxic activity induced by trans10, cis12-conjugated linoleic acid in rat hepatoma dRLh-84 cells by oleic or palmitoleic acid. Cancer Lett. 196, 187–196. https://doi.org/10.1016/S0304-3835(03)00215-5.

Yoon, B.K., Jackman, J.A., Valle-González, E.R., Cho, N.-J., 2018. Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. Int. J. Mol. Sci. 19, 1114. https://doi.org/10.3390/ijms19041114.

Zarrinmehr, M.J., Daneshvar, E., Nigam, S., Gopinath, K.P., Biswas, J.K., Kwon, E.E., Wang, H., Farhadian, O., Bhatnagar, A., 2022. The effect of solvents polarity and extraction conditions on the microalgal lipids yield, fatty acids profile, and biodiesel properties. Bioresour. Technol. 344, 126303–126303. https://doi.org/10.1016/j.biortech.2021.126303.

Zavala-Sánchez, M.A., Rodríguez-Chávez, J.L., Figueroa-Brito, R., Quintana-López, C.M., Bah, M.M., Campos-Guillén, J., Bustos-Martínez, J.A., Zamora-Avella, D., Ramos-López, M.A., 2020. Bioactivity of 1-octacosanol from *Senna crotalarioides* (Fabaceae: Caesalpinioideae) to control *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Fla. Entomol. 102, 731–731. https://doi.org/10.1653/024.102.0410.

Zha, S., Ueno, M., Liang, Y., Okada, S., Oda, T., Ishibashi, F., 2021. Induction of apoptotic cell death in human leukemia u937 cells by c18 hydroxy unsaturated fatty acid isolated from red alga *Tricleocarpa jejuensis*. Mar. Drugs 19, 138-138. https://doi.org/10.3390/md19030138.

5 CONSIDERAÇÕES FINAIS

Este estudo revelou que as indicações terapêuticas do uso etnofarmacológico de *H. speciosa* por comunidades tradicionais e locais no tratamento de infecções foram parcialmente confirmadas pelos resultados encontrados. Na avaliação antifúngica, os extratos etéreo e metanólico de *H. speciosa* demonstraram atividade antifúngica intrínseca contra espécies de *Candida.* Além disso, em concentrações subinibitórias, potencializaram a ação do fluconazol contra todas as cepas testadas. Os extratos também foram capazes de inibir a transição morfológica para formas invasivas, como hifas e pseudohifas, que é um importante mecanismo de virulência. No que se refere à atividade antibacteriana, embora os extratos não tenham demonstrado ação antibacteriana direta contra *E. coli*, *S. aureus* e *P. aeruginosa*, eles modificaram a eficácia dos antibióticos comerciais, como gentamicina, eritromicina e norfloxacina, ampliando seus efeitos.

Os testes de toxicidade realizados em *D. melanogaster* indicaram que os extratos não são tóxicos em concentrações clinicamente relevantes, sugerindo um perfil de segurança favorável. O estudo também revelou que os extratos de *H. speciosa* possuem uma composição química rica, incluindo ácidos graxos, fitosteróis, triterpenoides, fenóis e flavonoides. Além disso, foram identificados pela primeira vez na espécie compostos como ácido glucônico, cinchonina IIb, isômero da cinchonina Ib, isômeros de hexosídeo de laricirresinol, ácido hexacosanoico, ácido lignocérico, ácido triacontanoico e α-amirona.

Dado o crescente interesse na pesquisa de plantas medicinais como estratégia para a descoberta de novas opções terapêuticas, os resultados deste estudo destacam o potencial de *H. speciosa* como uma rica fonte de compostos bioativos, reforçando sua relevância na busca por novos agentes terapêuticos.

REFERÊNCIAS

ABDALLAH, Emad M. *et al*. Back to Nature: Medicinal plants as promising sources for antibacterial drugs in the post-antibiotic era. **Plants**, v. 12, n. 17, p. 3077, 2023.

ABDEL-HAMID, Rasha M. *et al*. The impact of increasing non-*albicans Candida* trends on diagnostics in immunocompromised patients. **Brazilian Journal of Microbiology**, v. 54, n. 4, p. 2879-2892, 2023.

AGUIAR-FILHO, Severino Pessoa de; BOSCO, João; ARAÚJO, Ivaldo Antonio de. **A mangabeira (*Hancornia speciosa*): domesticação e técnicas de cultivo.** João Pessoa: EMEPA-PB, 1998.

ALI, Asghar *et al*. Microbial biofilms: applications, clinical consequences, and alternative therapies. **Microorganisms**, v. 11, n. 8, p. 1934, 2023.

ALMEIDA, Francisco Lucas Chaves *et al*. *Hancornia speciosa*: An overview focused on phytochemical properties, recent achievements, applications, and future perspectives. **International Journal of Gastronomy and Food Science**, v. 29, p. 100561, 2022.

ALMEIDA, Luciane Madureira *et al*. *Hancornia speciosa* latex for biomedical applications: physical and chemical properties, biocompatibility assessment and angiogenic activity. **Journal of Materials** Science: Materials in Medicine, v. 25, p. 2153-2162, 2014.

ALMEIDA, Maria Mozarina Beserra *et al*. Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. **Food Research International**, v. 44, n. 7, p. 2155-2159, 2011.

ALMEIDA, Semíramis Pedrosa de; PROENÇA, Carolyn Elinore; SANO, Sueli Matiko; RIBEIRO, José Felipe. **Cerrado**: espécies vegetais úteis. Planaltina: EMBRAPA-CPAC, 1998.

ALMEIDA, Sheyla Cristina Xenofonte de et al. Himatanthus drasticus: a chemical and pharmacological review of this medicinal species, commonly found in the Brazilian Northeastern region. **Revista Brasileira de Farmacognosia**, v. 27, n. 6, p. 788-793, 2017.

ANAND, Uttpal *et al*. A review on antimicrobial botanicals, phytochemicals and natural resistance modifying agents from Apocynaceae family: Possible therapeutic approaches against multidrug resistance in pathogenic microorganisms. **Drug Resistance Updates**, v. 51, p. 100695, 2020.

ANGELINI, Paola. Plant-Derived Antimicrobials and Their Crucial Role in Combating Antimicrobial Resistance. **Antibiotics**, v. 13, n. 8, p. 746, 2024.

ARAFA, Sara H. *et al*. Candida diagnostic techniques: a review. **Journal of Umm Al-Qura University for Applied Sciences**, v. 9, n. 3, p. 360-377, 2023.

ARASTEHFAR, Amir *et al*. Antifungal susceptibility, genotyping, resistance mechanism, and clinical profile of *Candida tropicalis* blood isolates. **Medical Mycology**, v. 58, n. 6, p. 766-773, 2020.

ATANASOV, Atanas G. *et al*. Discovery and resupply of pharmacologically active plant-derived natural products: A review. **Biotechnology advances**, v. 33, n. 8, p. 1582-1614, 2015.

BARBOSA, Andriele M. *et al*. Separation of antibacterial biocompounds from *Hancornia speciosa* leaves by a sequential process of pressurized liquid extraction. **Separation and Purification Technology**, v. 222, p. 390-395, 2019.

BASTOS, K.X. *et al*. Identification of phenolic compounds from *Hancornia speciosa* (Apocynaceae) leaves by UHPLC orbitrap-HRMS. **Molecules**, v.22, n.1, p.143, 2017.

BHADANE, Bhushan S. *et al*. Ethnopharmacology, phytochemistry, and biotechnological advances of family Apocynaceae: A review. **Phytotherapy research**, v. 32, n. 7, p. 1181-1210, 2018.

BIHANI, Tanay. *Plumeria rubra* L.–A review on its ethnopharmacological, morphological, phytochemical, pharmacological and toxicological studies. **Journal of Ethnopharmacology**, v. 264, p. 113291, 2021.

BITENCOURT, Mariana Angélica Oliveira *et al*. Protective effect of aqueous extract, fractions and phenolic compounds of *Hancornia speciosa* fruits on the inflammatory damage in the lungs of mice induced by *Tityus serrulatus* envenomation. **Toxicon**, v. 164, p. 1-9, 2019.

BOCCOLINI, Patricia de Moraes Mello; BOCCOLINI, Cristiano Siqueira. Prevalence of complementary and alternative medicine (CAM) use in Brazil. **BMC complementary medicine and therapies**, v. 20, p. 1-10, 2020.

BOHNER, Flora; PAPP, Csaba; GÁCSER, Attila. The effect of antifungal resistance development on the virulence of *Candida* species. **FEMS Yeast Research**, v. 22, n. 1, p. foac019, 2022.

BONDAREVA, Natalia E. *et al*. Study of the antibacterial activity of superhydrophilic and superhydrophobic copper substrates against multi-drug-resistant hospital-acquired *Pseudomonas aeruginosa* isolates. **International Journal of Molecular Sciences**, v. 25, n. 2, p. 779, 2024.

BRAGA, Fernão Castro. Brazilian traditional medicine: Historical basis, features and potentialities for pharmaceutical development. **Journal of Traditional Chinese Medical Sciences**, v. 8, p. S44-S50, 2021.

BRASIL. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Básica. **Política nacional de práticas integrativas e complementares no SUS**: atitude de ampliação de acesso. 2. ed. Brasília: Ministério da Saúde, 2015.

BRASIL. Ministério da Saúde. Secretaria de Ciência, Tecnologia e Insumos Estratégicos. Departamento de Assistência Farmacêutica. **A fitoterapia no SUS e o Programa de Pesquisa de Plantas Medicinais da Central de Medicamentos**. Brasília: Ministério da Saúde, 2006.

CHAACHOUAY, Noureddine; ZIDANE, Lahcen. Plant-derived natural products: a source for drug discovery and development. **Drugs and Drug Candidates**, v. 3, n. 1, p. 184-207, 2024.

CHEN, Shanshan *et al*. Transcriptomic responses of foodborne pathogens to the food matrix. **Current Opinion in Food Science**, v. 42, p. 23-30, 2021.

CHEROBIN, Fabiane *et al*. Plantas medicinais e políticas públicas de saúde: novos olhares sobre antigas práticas. **Physis: Revista de Saúde Coletiva**, v. 32, p. e320306, 2022.

CIUREA, Cristina Nicoleta *et al*. *Candida* and candidiasis—opportunism versus pathogenicity: a review of the virulence traits. **Microorganisms**, v. 8, n. 6, p. 857, 2020.

COLLEVATTI, Rosane G. *et al*. Gene flow among *Hancornia speciosa* (Apocynaceae) varieties and hybrid fitness. **Tree Genetics & Genomes**, v. 12, p. 1-12, 2016.

CORBU, Viorica Maria *et al*. Current insights in fungal importance—a comprehensive review. **Microorganisms**, v. 11, n. 6, p. 1384, 2023.

COSTA, Elenir da Silva *et al*. Antimicrobial activity of some medicinal plants of the Cerrado, Brazil. **Phytotherapy Research**, v. 22, n. 5, p. 705-707, 2008.

D'ABADIA, P.L. *et al*. *Hancornia speciosa* serum fraction latex stimulates the angiogenesis and extracellular matrix remodeling processes. **An. Acad. Bras. Cienc**., v.92, n.2, 2020.

DARRAULT, Reisla O.; SCHLINDWEIN, Clemens. Limited fruit production in *Hancornia speciosa* (Apocynaceae) and pollination by nocturnal and diurnal insects 1. **Biotropica: The Journal of Biology and Conservation**, v. 37, n. 3, p. 381-388, 2005.

DUTRA, Rafael C. *et al*. Medicinal plants in Brazil: Pharmacological studies, drug discovery, challenges and perspectives. **Pharmacological research**, v. 112, p. 4-29, 2016.

DUTRA, Rodrigo Luiz Targino *et al*. Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions. **Food Research International**, v. 100, p. 650-657, 2017.

ELFADADNY, Ahmed *et al*. Antimicrobial resistance of *Pseudomonas aeruginosa*: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. **Frontiers in Microbiology**, v. 15, p. 1374466, 2024.

ENDRESS, Mary E. *et al*. Apocynaceae. In: KADEREIT, Joachim Walter; BITTRICH, Volker (Ed.). **The families and genera of vascular plants**. 15. ed. Berlin: Springer, 2018.

ENDRESS, Mary E.; LIEDE-SCHUMANN, Sigrid; MEVE, Ulrich. An updated classification for Apocynaceae. **Phytotaxa**, v. 159, n. 3, p. 175-194, 2014.

ENDRINGER, Denise C. *et al*. Evaluation of Brazilian plants on cancer chemoprevention targets in vitro. **Phytotherapy Research**, v. 24, n. 6, p. 928-933, 2010.

ENDRINGER, Denise C.; PEZZUTO, John M.; BRAGA, Fernão C. NF-κB inhibitory activity of cyclitols isolated from Hancornia speciosa. **Phytomedicine**, v. 16, n. 11, p. 1064-1069, 2009.

FAO - FOOD AND AGRICULTURE ORGANIZATION. **Food Biodiversity 2022**. Disponível em: <https://www.fao.org/infoods/infoods/food-biodiversity/en/>. Acesso em: 9 set. 2023.

FERRÃO, Bruno Henrique *et al*. Importância do conhecimento tradicional no uso de plantas medicinais em Buritis, MG, Brasil. **Ciência e Natura**, v. 36, p. 321-334, 2014.

FERREIRA, Herick Campos *et al*. Endothelium-dependent vasodilation induced by *Hancornia speciosa* in rat superior mesenteric artery. **Phytomedicine**, v. 14, n. 7-8, p. 473-478, 2007.

FERREIRA-JÚNIOR, Washington Soares *et al*. Check-list das plantas medicinais na Chapada do Araripe. In: ALBUQUERQUE, Ulysses Paulino; MEIADO, Marcos Vinícius (org.). **Sociobiodiversidade na Chapada do Araripe**. 1. ed. Recife: NUPEEA, 2015. p. 431-450.

FISHER, Matthew C. *et al*. Threats posed by the fungal kingdom to humans, wildlife, and agriculture. **MBio**, v. 11, n. 3, p. 10.1128/mbio. 00449-20, 2020.

FLORA E FUNGA DO BRASIL (2020 em construção). **Apocynaceae in Flora e Funga do Brasil**. Jardim Botânico do Rio de Janeiro. Disponível em: <https://floradobrasil.jbrj.gov.br/FB48>. Acesso em: 26 fev. 2024.

FLORA E FUNGA DO BRASIL (2020 em construção). ***Hancornia* in Flora e Funga do Brasil.** Jardim Botânico do Rio de Janeiro. Disponível em: <https://floradobrasil.jbrj.gov.br/FB15558>. Acesso em: 26 fev. 2024.

GARCIA-RUBIO, Rocio *et al*. The fungal cell wall: *Candida*, *Cryptococcus*, and *Aspergillus* species. **Frontiers in microbiology**, v. 10, p. 492056, 2020.

GAUBA, Anusha; RAHMAN, Khondaker Miraz. Evaluation of antibiotic resistance mechanisms in gram-negative bacteria. **Antibiotics**, v. 12, n. 11, p. 1590, 2023.

GEURTSEN, Jeroen *et al*. Genomics and pathotypes of the many faces of *Escherichia coli*. **FEMS microbiology reviews**, v. 46, n. 6, p. fuac031, 2022.

GÓMEZ-GAVIRIA, Manuela; MORA-MONTES, Hector M. Current aspects in the biology, pathogeny, and treatment of *Candida krusei*, a neglected fungal pathogen. **Infection and drug resistance**, p. 1673-1689, 2020.

GUO, Yunlei *et al*. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. **Frontiers in cellular and infection microbiology**, v. 10, p. 107, 2020.

GUPTA, Ankit; GUPTA, Rasna; SINGH, Ram Lakhan. Microbes and environment. **Principles and applications of environmental biotechnology for a sustainable future**, v. 1, p. 43-84, 2017.

HEARD, Stephanie C.; WU, Guangwei; WINTER, Jaclyn M. Antifungal natural products. **Current opinion in biotechnology**, v. 69, p. 232-241, 2021.

HOWDEN, Benjamin P. *et al*. *Staphylococcus aureus* host interactions and adaptation. **Nature Reviews Microbiology**, v. 21, n. 6, p. 380-395, 2023.

HUANG, Jiewen *et al.* Carbapenem-resistant Escherichia coli exhibit diverse spatiotemporal epidemiological characteristics across the globe. **Communications Biology**, v. 7, n. 1, p. 51, 2024.

JACOBSEN, Ilse D. The role of host and fungal factors in the commensal-to-pathogen transition of *Candida albicans*. **Current Clinical Microbiology Reports**, v. 10, n. 2, p. 55-65, 2023.

JAMIU, A. T. *et al*. Update on *Candida krusei*, a potential multidrug-resistant pathogen. **Medical mycology**, v. 59, n. 1, p. 14-30, 2021.

KAKOULLIS, Loukas *et al*. Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. **Antibiotics**, v. 10, n. 4, p. 415, 2021.

KARAHAN, Faruk *et al*. Heavy metal levels and mineral nutrient status in different parts of various medicinal plants collected from eastern Mediterranean region of Turkey. **Biological Trace Element Research**, v. 197, p. 316-329, 2020.

KEIGHLEY, Caitlin *et al*. *Candida tropicalis*—A systematic review to inform the World Health Organization of a fungal priority pathogens list. **Medical Mycology**, v. 62, n. 6, 2024.

KHALIFA, Hazim O. *et al*. Prevalence of antifungal resistance, genetic basis of acquired azole and echinocandin resistance, and genotyping of *Candida krusei* recovered from an international collection. **Antimicrobial Agents and Chemotherapy**, v. 66, n. 2, p. e01856-21, 2022.

KHAN, Hassan Ahmed; BAIG, Fatima Kanwal; MEHBOOB, Riffat. Nosocomial infections: Epidemiology, prevention, control and surveillance. **Asian Pacific Journal of Tropical Biomedicine**, v. 7, n. 5, p. 478-482, 2017.

KUMAR, Sunil; SINGH, Bikarma; SINGH, Ramesh. *Catharanthus roseus* (L.) G. Don: A review of its ethnobotany, phytochemistry, ethnopharmacology and toxicities. **Journal of Ethnopharmacology**, v. 284, p. 114647, 2022.

LEBLANC, Emmanuelle V. *et al*. Structure-guided approaches to targeting stress responses in human fungal pathogens. **Journal of Biological Chemistry**, v. 295, n. 42, p. 14458-14472, 2020.

LEDERMAN, Ildo Eliezer *et al*. Mangaba (Hancornia speciosa Gomes). **Mangaba (*Hancornia speciosa* Gomes)**. Jaboticabal: FUNEP, 2000.

LEE, Yunjin; ROBBINS, Nicole; COWEN, Leah E. Molecular mechanisms governing antifungal drug resistance. **Antimicrobials and Resistance**, v. 1, n. 1, p. 5, 2023.

LEITE JÚNIOR, D. P. *et al*. The rise of fungi: evidence on the Global Scale. Old Known Silences or Mysterious Threats to the Planet. **Microbiology Research Journal International**, v. 30, n. 10, p. 18-49, 2020.

LÉVI-STRAUSS, Claude. **The Raw and the Cooked: Introduction to a Science of Mythology: I.** New York: Harper Colophon Books, 1975.

LIMA, Juliana Pinto de *et al*. The antioxidative potential and volatile constituents of mangaba fruit over the storage period. **Scientia Horticulturae**, v. 194, p. 1-6, 2015.

LIMA, Ricardo *et al*. The emerging threat antifungal-resistant *Candida tropicalis* in humans, animals, and environment. **Frontiers in Fungal Biology**, v. 3, p. 957021, 2022.

LNIMUENDAJÚ, Curt. Os Apinayé. **Boletim do Museu Paraense Emílio Goeldi**, v. 12, p. 1-150, 1956.

LOGAN, Ashley; WOLFE, Amanda; WILLIAMSON, John C. Antifungal resistance and the role of new therapeutic agents. **Current Infectious Disease Reports**, v. 24, n. 9, p. 105-116, 2022.

LOPES, José Pedro; LIONAKIS, Michail S. Pathogenesis and virulence of *Candida* albicans. **Virulence**, v. 13, n. 1, p. 89-121, 2022.

LU, Hengqian *et al*. Roles of the fungal-specific lysine biosynthetic pathway in the nematode-trapping fungus *Arthrobotrys oligospora* identified through metabolomics analyses. **Journal of Fungi**, v. 9, n. 2, p. 206, 2023.

MACÊDO, Delmacia G. *et al*. Práticas terapêuticas tradicionais: uso e conhecimento de plantas do cerrado no estado de Pernambuco (Nordeste do Brasil). **Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromática**s, v. 14, n. 6, p. 491-508, 2015.

MACHATE, David Johane; ALVES, Flávio Macedo; FARINACCIO, Maria Ana. Aspidosperma (Apocynaceae) in Mato Grosso do Sul State, Brazil. **Rodriguésia**, v. 67, p. 1011-1024, 2016.

MAGALHÃES, Catarina *et al*. To give or not to give antibiotics is not the only question. **The Lancet Infectious Diseases**, v. 21, n. 7, p. e191-e201, 2021.

MANCUSO, Giuseppe *et al*. Bacterial antibiotic resistance: the most critical pathogens. **Pathogens**, v. 10, n. 10, p. 1310, 2021.

MARINHO, Diogo Guimarães *et al*. The latex obtained from *Hancornia speciosa* Gomes possesses anti-inflammatory activity. **Journal of Ethnopharmacology**, v. 135, n. 2, p. 530-537, 2011.

MLYNARCZYK-BONIKOWSKA, Beata *et al*. Molecular mechanisms of drug resistance in Staphylococcus aureus. **International journal of molecular sciences**, v. 23, n. 15, p. 8088, 2022.

MONACHINO, Joseph. A revision of *Hancornia* (Apocynaceae). **Lilloa**, p. 19-48, 1945.

MORAES, Thiago de Mello *et al*. *Hancornia speciosa*: Indications of gastroprotective, healing and anti-*Helicobacter pylori* actions. **Journal of Ethnopharmacology**, v. 120, n. 2, p. 161-168, 2008.

MORAIS, Gabriela Corrêa; RESENDE, Rafael Tassinari; CHAVES, Lázaro José. Reproductive patterns, morpho-agronomic variability and selection for breeding *Hancornia speciosa* Gomes (Apocynaceae). **Genetic Resources and Crop Evolution**, v. 71, n. 5, p. 2173-2188, 2024.

MURRAY, Christopher JL *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. **The lancet**, v. 399, n. 10325, p. 629-655, 2022.

MURUGAIYAN, Jayaseelan *et al*. Progress in alternative strategies to combat antimicrobial resistance: Focus on antibiotics. **Antibiotics**, v. 11, n. 2, p. 200, 2022.

MUTEEB, Ghazala *et al*. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. **Pharmaceuticals**, v. 16, n. 11, p. 1615, 2023.

NARANJO-ORTIZ, Miguel A.; GABALDÓN, Toni. Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. **Biological Reviews**, v. 94, n. 6, p. 2101-2137, 2019.

NASCIMENTO MAGALHÃES, Karla *et al*. Medicinal plants of the Caatinga, northeastern Brazil: Ethnopharmacopeia (1980–1990) of the late professor Francisco José de Abreu Matos. **Journal of ethnopharmacology**, v. 237, p. 314-353, 2019.

NETO, Leila S. *et al*. A treatment with a boiled aqueous extract of *Hancornia speciosa* Gomes leaves improves the metabolic status of streptozotocin-induced diabetic rats. **BMC complementary medicine and therapies**, v. 20, n. 1, p. 1-8, 2020.

NUNES, Valdinete Vieira et al. Pharmaceutical, food potential, and molecular data of *Hancornia speciosa* Gomes: a systematic review. **Genetic Resources and Crop Evolution**, v. 69, n. 2, p. 525-543, 2022.

OLIVEIRA, Kívia Soares de; ALOUFA, Magdi Ahmed Ibrahim. Knowledge, use, and management of magaba (*Hancornia speciosa* Gomes) by extrativist communities on the coast of Rio Grande do Norte, Northeast Brazil. **Acta Botanica Brasilica**, v. 35, n. 2, p. 276-289, 2021.

OLIVEIRA, Maraiza Gregorio de *et al*. Revisão da literatura científica de *Himatanthus drasticus* (Mart.) Plumel. **Research, Society and Development**, v. 11, n. 11, p. e531111133849-e531111133849, 2022.

OLLERTON, Jeff *et al*. The diversity and evolution of pollination systems in large plant clades: Apocynaceae as a case study. **Annals of Botany**, v. 123, n. 2, p. 311-325, 2019.

PALMUCCI, Julia R. *et al*. A ketogenic diet enhances fluconazole efficacy in murine models of systemic fungal infection. **Mbio**, v. 15, n. 5, p. e00649-24, 2024.

PARAMBATH, Sarika *et al*. *Candida albicans*—A systematic review to inform the World Health Organization Fungal Priority Pathogens List. **Medical Mycology**, v. 62, n. 6, 2024.

PAULA, Ladyslene Christhyns *et al*. Influence of preservation methods on the bioactivity of mangaba (*Hancornia speciosa* Gomes) from the Brazilian savannah**. Food Science and Technology**, v. 39, p. 403-409, 2018.

PAZ, Ignacio Uriel Macias *et al*. *Candida albicans* el principal hongo patógeno oportunista en humanos. **Revista argentina de microbiología**, v. 55, n. 2, p. 12-12, 2023.

PENIDO, Alexandre Batista *et al*. Ethnobotanical study of medicinal plants in Imperatriz, State of Maranhão, Northeastern Brazil. **Acta Amazonica**, v. 46, p. 345-354, 2016.

PENIDO, Alexandre Batista *et al*. Medicinal plants from northeastern Brazil against Alzheimer's disease. **Evidence-Based Complementary and Alternative Medicine**, v. 2017, 2017.

PEREIRA, Aline C. *et al*. *Hancornia speciosa* Gomes (Apocynaceae) as a potential anti-diabetic drug. **Journal of Ethnopharmacology**, v. 161, p. 30-35, 2015.

PETROVSKA, Biljana Bauer. Historical review of medicinal plants' usage. **Pharmacognosy reviews**, v. 6, n. 11, p. 1, 2012.

PFALLER, Michael A.; DIEKEMA, Daniel J.. Epidemiology of invasive candidiasis: a persistent public health problem. **Clinical microbiology reviews**, v. 20, n. 1, p. 133-163, 2007.

PHUKHAMSAKDA, Chayanard *et al*. The numbers of fungi: Contributions from traditional taxonomic studies and challenges of metabarcoding. **Fungal diversity**, v. 114, n. 1, p. 327-386, 2022.

QIN, Shugang *et al*. *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. **Signal transduction and targeted therapy**, v. 7, n. 1, p. 199, 2022.

RIAZ, Muhammad *et al*. Phytobioactive compounds as therapeutic agents for human diseases: A review. **Food Science & Nutrition**, v. 11, n. 6, p. 2500-2529, 2023.

RIBEIRO, Daiany Alves *et al*. Promising medicinal plants for bioprospection in a Cerrado area of Chapada do Araripe, Northeastern Brazil. **Journal of Ethnopharmacology**, v. 155, n. 3, p. 1522-1533, 2014.

RIBEIRO, Daiany Alves *et al.* Chemical profile and antimicrobial activity of Secondatia floribunda A. DC (Apocynaceae). **Asian Pacific Journal of Tropical Biomedicine**, v. 7, n. 8, p. 739-749, 2017.

RIBEIRO, Reginaldo Vicente *et al*. Ethnobotanical study of medicinal plants used by Ribeirinhos in the North Araguaia microregion, Mato Grosso, Brazil. **Journal of ethnopharmacology**, v. 205, p. 69-102, 2017.

RIBEIRO, T. P. *et al*. Evaluation of cytotoxicity and genotoxicity of *Hancornia speciosa* latex in *Allium cepa* root model. **Brazilian Journal of Biology**, v. 76, p. 245-249, 2016.

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.

RODRIGUES, Arthur Almeida *et al*. Aluminum influence on *Hancornia speciosa* seedling emergence, nutrient accumulation, growth and root anatomy. **Flora**, v. 236, p. 9-14, 2017.

RODRIGUES, Marcio. The multifunctional fungal ergosterol. **MBio**, v. 9, n. 5, p. 1-5, 2018.

SAHAY, Pranita *et al*. Pharmacologic therapy of mycotic keratitis. **Survey of ophthalmology**, v. 64, n. 3, p. 380-400, 2019.

SALAM, Md Abdus *et al*. Antimicrobial resistance: a growing serious threat for global public health. **Healthcare**, v. 11, n. 13, p. 1-20, 2023.

SANTOS, Uilson Pereira dos *et al*. Antioxidant, antimicrobial and cytotoxic properties as well as the phenolic content of the extract from *Hancornia speciosa* Gomes. **PLoS One**, v. 11, n. 12, p. e0167531, 2016.

SANTOS, Uilson Pereira dos *et al*. Physicochemical characterization, microbiological quality and safety, and pharmacological potential of *Hancornia speciosa* Gomes. **Oxidative Medicine and Cellular Longevity**, v. 2018, 2018.

SHARMA, Aditi *et al*. Antimicrobial terpenoids as a potential substitute in overcoming antimicrobial resistance. **Current Drug Targets**, v. 21, n. 14, p. 1476-1494, 2020.

SILVA JUNIOR, Josué Francisco. A cultura da mangaba. **Revista Brasileira de Fruticultura**, v. 26, 2004.

SILVA, Ana Veruska C. da *et al*. Divergência genética entre acessos de mangabeira (*Hancornia speciosa* Gomes). **Revista Brasileira de Ciências Agrárias**, v. 6, n. 4, p. 572-578, 2011.

SILVA, Grazielle C. *et al*. Potent antihypertensive effect of *Hancornia speciosa* leaves extract. **Phytomedicine**, v. 23, n. 2, p. 214-219, 2016.

SILVA, Sónia *et al*. *Candida glabrata, Candida parapsilosis* and *Candida tropicalis*: biology, epidemiology, pathogenicity and antifungal resistance. **FEMS microbiology reviews**, v. 36, n. 2, p. 288-305, 2012.

SILVA, Viviane Bezerra et al. Chemical composition, antibacterial potential, and toxicity of the extracts from the stem bark of *Hancornia speciosa* Gomes (Apocynaceae). **Journal of Ethnopharmacology**, v. 335, p. 118631, 2024.

SIMÕES, C.M.O. *et al*. **Farmacognosia**: do produto natural ao medicamento. 6.ed. Porto Alegre: Artmed, 2017.

SMITH, Nigel. Amazon fruits: an ethnobotanical journey. **Springer Nature**, v. 1, p. 127-175, 2023.

SONI, Jyoti; SINHA, Sristi; PANDEY, Rajesh. Understanding bacterial pathogenicity: a closer look at the journey of harmful microbes. **Frontiers in Microbiology**, v. 15, p. 1370818, 2024.

SZYMAŃSKI, Mateusz *et al*. Echinocandins–structure, mechanism of action and use in antifungal therapy. **Journal of enzyme inhibition and medicinal chemistry**, v. 37, n. 1, p. 876-894, 2022.

TALAPKO, Jasminka *et al*. *Candida albicans*—the virulence factors and clinical manifestations of infection. **Journal of Fungi**, v. 7, n. 2, p. 79, 2021.

TSHIKANTWA, Tiroyaone Shimane *et al*. Current trends and potential applications of microbial interactions for human welfare. **Frontiers in microbiology**, v. 9, p. 1156, 2018.

TUON, Felipe Francisco *et al*. Pathogenesis of the *Pseudomonas aeruginosa* biofilm: a review. **Pathogens**, v. 11, n. 3, p. 300, 2022.

VENNE, Danielle M. *et al.* Review and analysis of the overlapping threats of carbapenem and polymyxin resistant E. coli and Klebsiella in Africa. **Antimicrobial Resistance & Infection Control**, v. 12, n. 1, p. 29, 2023.

VIEIRA, Larissa dos Santos; SOUSA, Rosemary da Silva; LEMOS, Jesus Rodrigues. Plantas medicinais conhecidas por especialistas locais de uma comunidade rural maranhense. **Revista brasileira de plantas medicinais,** v. 17, p. 1061-1068, 2015.

VIEIRA, Roberto Fontes; CAMILLO, Julcéia; CORADIN, Lidio. Espécies nativas da flora brasileira de valor econômico atual ou potencial: plantas para o futuro: região Centro-Oeste. **Série Biodiversidade**, v. 44, 2016.

WAKSMAN, Selman A.; STARKEY, Robert L. Carbon assimilation and respiration of autotrophic bacteria. **Proceedings of the Society for Experimental Biology and Medicine**, v. 20, n. 1, p. 9-14, 1922.

WANG, Xueling; CHI, Yongkuan; SONG, Shuzhen. Important soil microbiota's effects on plants and soils: a comprehensive 30-year systematic literature review. **Frontiers in Microbiology**, v. 15, p. 1347745, 2024.

WHO - WORLD HEALTH ORGANIZATION. **10 global health issues to track in 2021**. Disponível em: <https://www.who.int/news-room/spotlight/10-global-health-issues-to-track-in-2021>. Acesso em: 13 ago. 2023.

WHO - WORLD HEALTH ORGANIZATION. **No time to Wait: Securing the future from drug-resistant infections 2019**. Disponível em: <https://www.who.int/publications/i/item/no-time-to-wait-securing-the-future-from-drug-resistant-infections>. Acesso em: 13 ago. 2024.

WHO - WORLD HEALTH ORGANIZATION. **Antimicrobial resistance. 2023a** Disponível em: <https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance>. Acesso em: 13 ago. 2023.

WHO - WORLD HEALTH ORGANIZATION. **Traditional medicine has a long history of contributing to conventional medicine and continues to hold promise**. **2023.** Disponível em: <https://www.who.int/news-room/feature-stories/detail/traditional-medicine-has-a-long-history-of-contributing-to-conventional-medicine-and-continues-to-hold-promise>. Acesso em: 9 set. 2023.

WHO - WORLD HEALTH ORGANIZATION. **WHO bacterial priority pathogens list, 2024**. Disponível em: <https://www.who.int/publications/i/item/9789240093461>. Acesso em: 9 set. 2024.

WHO - WORLD HEALTH ORGANIZATION. **WHO fungal priority pathogens list to guide research, development and public health action**. **2022**. Disponível em: <https://www.who.int/publications/i/item/9789240060241>. Acesso em: 29 ago. 2023.

WISNIEWSKI, Alfonso; MELO, Elio Francisco Marques de. **Borrachas Naturais Brasileiras, III: Borracha de Mangabeira**. Belém: EMBRAPA, Centro de Pesquisa Agropecuária do Trópico Úmido, 1982.

YAMASHITA, Fabiana de Oliveira *et al*. Mangaba (*Hancornia speciosa* Gomes) fruit juice decreases acute pulmonary edema induced by *Tityus serrulatus* venom: Potential application for auxiliary treatment of scorpion stings. **Toxicon**, v. 179, p. 42-52, 2020.

ZHU, Ping *et al*. New antifungal strategies: drug combination and co-delivery. **Advanced Drug Delivery Reviews**, v. 198, p. 114874, 2023.

ANEXO A

PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NO PERIÓDICO ***JOURNAL OF ETHNOPHARMACOLOGY*** (ARTIGO 1)

Disponível em: https://doi.org/10.1016/j.jep.2023.117506

Journal of Ethnopharmacology 321 (2024) 117506

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Chemical composition, antifungal, and anti-virulence action of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) against *Candida* spp.

Viviane Bezerra da Silva [a,*], José Weverton Almeida-Bezerra [a], Maria Hellena Garcia Novais [b], Naiza Saraiva Farias [b], Janerson José Coelho [c], Paulo Riceli Vasconcelos Ribeiro [d], Kirley Marques Canuto [d], Henrique Douglas Melo Coutinho [e], Maria Flaviana Bezerra Morais-Braga [b], Antonio Fernando Morais de Oliveira [a]

[a] Department of Botany, Universidade Federal de Pernambuco – UFPE, Rua Professor Moraes Rego, s/n, Recife, Pernambuco, 50.670-901, Brazil
[b] Department of Biological Sciences, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105-000, Brazil
[c] Animal Science Department, Universidade Estadual do Maranhão - UEMA, São Luís, Maranhão, Brazil
[d] Multi-User Natural Products Chemistry Laboratory – LMQPN, Embrapa Agroindustria Tropical, Rua Dra. Sara Mesquita, 2270, Fortaleza, Ceará, 60511-110, Brazil
[e] Department of Biological Chemistry, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105-000, Brazil

ARTICLE INFO

Handling Editor: V Kuete

Keywords:
Cinchonain
Ethnomedicine
Flavonoids
Hyphae
Phenolic compounds

ABSTRACT

Ethnopharmacological relevance: *Hancornia speciosa* Gomes is a fruit and medicinal species used for treating infectious diseases of the genitourinary system. However, its mechanism of action against microbes is still not fully understood. Infections in the genitourinary system caused by *Candida* spp. are associated with its fungal resistance and pathogenicity. New plant-derived compounds are an alternative to fight these *Candida* infections.
Aim of the study: The objective of this study was to evaluate the anti-*Candida* effects of extracts of the stem bark of *H. speciosa*. This research investigated the chemical composition of sulfuric ether (EEHS) and methanolic (MEHS) extracts, their drug-modifying action on fluconazole, and their anti-virulence action on the morphological transition of *Candida* species.
Materials and methods: The extracts (EEHS and MEHS) of the stem bark of *H. speciosa* were chemically characterized via qualitative phytochemical screening and by liquid chromatography coupled with mass spectrometry (UPLC-MS-ESI-QTOF). The extracts were evaluated regarding their antifungal effects and fluconazole-modifying activity against *Candida albicans*, *Candida krusei*, and *Candida tropicalis* using the broth microdilution method. Additionally, the study evaluated the inhibition of fungal virulence in *Candida* species through morphological transition assays.
Results: The phytochemical screening revealed the presence of anthocyanidins, anthocyanins, aurones, catechins, chalcones, flavones, flavonols, flavanones, leucoanthocyanidins, tannins (condensed and pyrogallic), and xanthones in both extracts of the stem bark of *H. speciosa*. The UPLC-MS-ESI-QTOF analysis identified the same compounds in both extracts, predominating phenolic compounds. Some compounds were first time recorded in this species: gluconic acid, cinchonain IIb, cinchonain Ib isomer, and lariciresinol hexoside isomers. Most of the intrinsic antifungal activity was observed for the MEHS against *C. krusei* (IC_{50}: 58.41 μg/mL). At subinhibitory concentrations (MC/8), the EEHS enhanced the action of fluconazole against all *Candida* strains. The MEHS exhibited greater efficacy than fluconazole inhibiting *C. krusei* growth. The EEHS completely inhibited hyphae appearance and reduced pseudohyphae formation in *C. albicans*.
Conclusion: The stem bark of *H. speciosa* is a rich source of bioactive compounds, especially phenolic. Phenolic compounds can have important roles in fighting infectious diseases of the genitourinary system, such as candidiasis. The extracts of *H. speciosa* improved the action of the drug fluconazole against *Candida* species, inhibited hyphae appearance, and reduced pseudohyphae formation. The results of this study can support the development of new therapeutics against resistant strains of *Candida*.

* Corresponding author.
E-mail address: viviane.silva@urca.br (V.B. Silva).

https://doi.org/10.1016/j.jep.2023.117506
Received 10 October 2023; Received in revised form 17 November 2023; Accepted 22 November 2023
Available online 25 November 2023

ANEXO B

PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NO PERIÓDICO ***JOURNAL OF ETHNOPHARMACOLOGY*** (ARTIGO 2)

Disponível em: https://doi.org/10.1016/j.jep.2024.118631

Journal of Ethnopharmacology 335 (2024) 118631

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Chemical composition, antibacterial potential, and toxicity of the extracts from the stem bark of *Hancornia speciosa* Gomes (Apocynaceae)

Viviane Bezerra da Silva [a,*], José Weverton Almeida-Bezerra [b], Raimundo Luiz Silva Pereira [b], Bruno Melo de Alcântara [b], Cláudia Maria Furlan [c], Janerson José Coelho [d], Henrique Douglas Melo Coutinho [e], Maria Flaviana Bezerra Morais-Braga [b], Antonio Fernando Morais de Oliveira [a]

[a] Departamento de Botânica, Universidade Federal de Pernambuco – UFPE, Rua Professor Moraes Rego, s/n, Recife, Pernambuco, 50.670–901, Brazil
[b] Departamento de Ciências Biológicas, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105–000, Brazil
[c] Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, São Paulo, 05508–090, Brazil
[d] Universidade Estadual do Ceará - UECE, Faculdade de Educação, Ciências e Letras dos Inhamuns – CECITEC, Tauá, 63660-000, Ceará, Brazil
[e] Departamento de Química Biológica, Universidade Regional do Cariri – URCA, Rua Cel. Antônio Luiz, 1161, Crato, Ceará, 63.105–000, Brazil

ARTICLE INFO

Handling Editor: V Kuete

Keywords:
Antimicrobial
Ethnomedicine
Mangabeira
Medicinal plants
Therapeutic potential

ABSTRACT

Ethnopharmacological relevance: Hancornia speciosa is a medicinal plant popularly used to treat different medical issues, including infectious diseases. Exploring the therapeutic potentialities of the extracts from medicinal plants combined with conventional antibiotic drugs is a promising horizon, especially considering the rising microbial resistance.

Aim of the study: This study aimed to characterize the chemical composition of the ethereal (EEHS) and methanolic (MEHS) extracts of the stem bark of *H. speciosa*, and also evaluate their antibacterial and drug-modifying activity, and toxicity.

Materials and methods: The extracts were characterized by gas chromatography coupled to mass spectrometry (GC–MS). Additionally, total phenol and flavonoid contents were determined. The antibacterial and antibiotic-modifying activity was evaluated against strains of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* using the serial microdilution method, obtaining the minimum inhibitory concentration (MIC). The toxicity assay was carried out using the *Drosophila melanogaster* model.

Results: Thirty compounds were identified in the extracts of the stem bark of *H. speciosa*, with triterpenoids being predominant in both extracts. Additionally, fatty alcohols, carbohydrates, fatty acids, phenolic acids, and phytosterols were identified in both extracts. EEHS and MEHS extracts had considerable phenol contents (346.4 and 340.0 mg GAE/g, respectively). Flavonoids were detected in a lower proportion (7.6 and 6.9 mg QE/g, respectively). *H. speciosa* extracts did not display intrinsic antibacterial activity against the bacterial strains evaluated, however, they were capable of modifying the activity of gentamicin, erythromycin, and norfloxacin. EEHS increased the efficacy of norfloxacin against *E. coli* and *S. aureus*, reducing MIC values by 50%. MEHS potentiated the action of gentamicin against all bacterial strains, especially against *E. coli*. The extracts did not display toxicity at clinically relevant concentrations against *D. melanogaster*.

Conclusion: The stem bark of *H. speciosa* was considered a rich source of bioactive compounds. Our findings evidenced the therapeutic potential of *H. speciosa* extracts for the development of new pharmaceutical therapeutics against bacteria. Although the extracts did not exhibit intrinsic antibacterial activity, they enhanced the efficacy of commercial antibiotic drugs and were non-toxic at clinically relevant concentrations. Future studies are needed to elucidate the mechanisms of action of these extracts, ensuring their safety and efficacy.

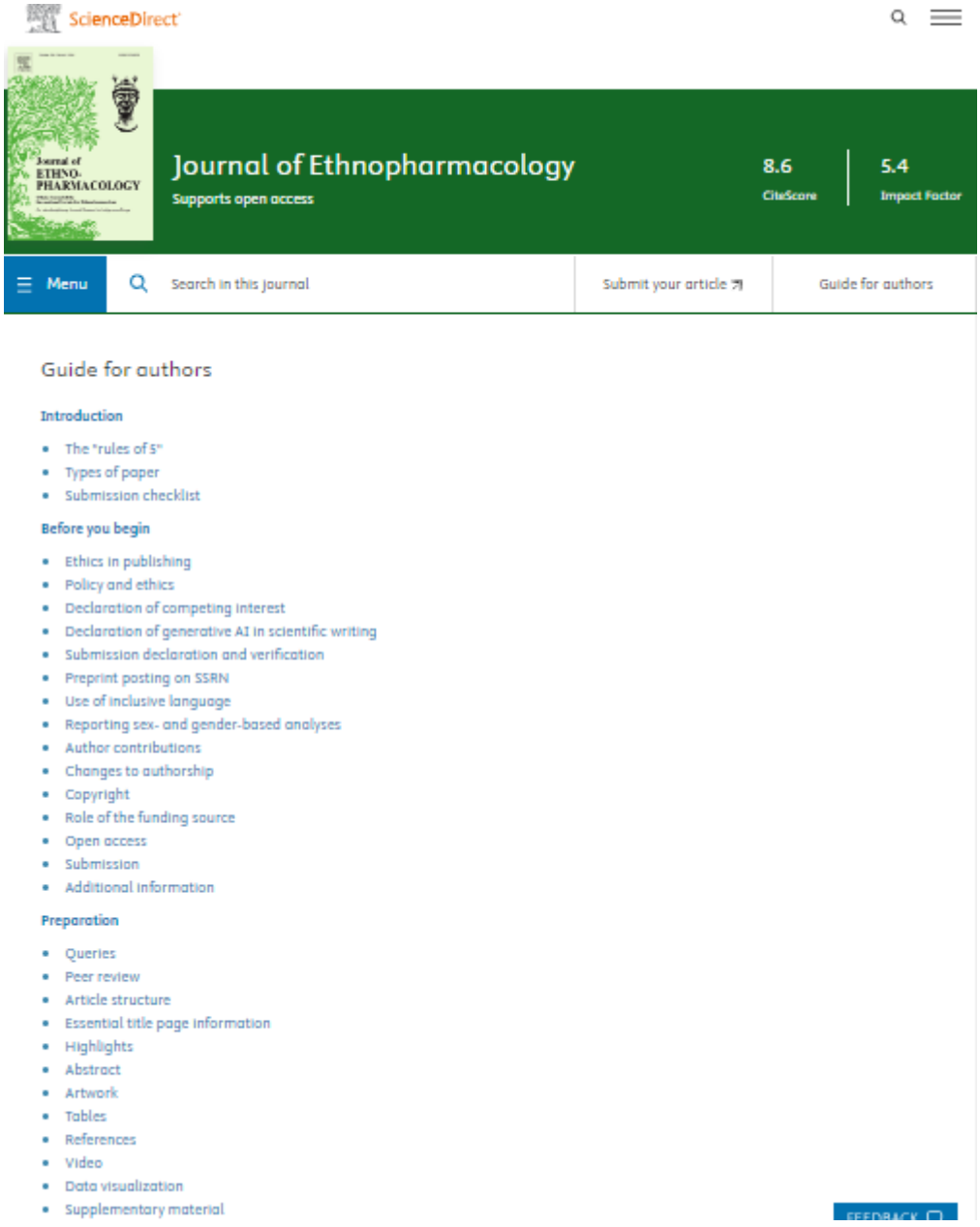
* Corresponding author.
E-mail address: viviane.silva@urca.br (V.B. Silva).

https://doi.org/10.1016/j.jep.2024.118631
Received 5 June 2024; Received in revised form 15 July 2024; Accepted 24 July 2024
Available online 25 July 2024

ANEXO C

NORMAS PARA SUBMISSÃO DOS MANUSCRITOS AO PERIÓDICO ***JOURNAL OF ETHNOPHARMACOLOGY***

Disponível em: https://www.sciencedirect.com/journal/journal-of-ethnopharmacology/publish/guide-for-authors



ANEXO D

CERTIDÃO DE CADASTRO EMITIDA PELO SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso

Cadastro nº A535238

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**A535238**
Usuário:	**Viviane Bezerra da Silva**
CPF/CNPJ:	**068.023.213-35**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa e Desenvolvimento Tecnológico**

Espécie

Hancornia speciosa

Título da Atividade:	**PROSPECÇÃO FITOQUÍMICA E AVALIAÇÃO DAS ATIVIDADES ANTIMICROBIANA, ANTIPARASITÁRIA E TOXICOLÓGICA DO LÁTEX E CASCAS DO CAULE DE Hancornia speciosa Gomes (APOCYNACEAE)**

Equipe

Viviane Bezerra da Silva	**Universidade Federal de Pernambuco - UFPE**

Data do Cadastro:	**03/11/2021 12:28:58**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **12:29** de **03/11/2021**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN

ANEXO E

LICENÇA DE COLETA DE MATERIAL BOTÂNICO PARA ATIVIDADES COM FINALIDADE CIENTÍFICA NO SISTEMA DE AUTORIZAÇÃO E INFORMAÇÃO EM BIODIVERSIDADE - SISBIO

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 80293-1	Data da Emissão: 13/10/2021 18:25:49	Data da Revalidação*: 13/10/2022
De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.		

Dados do titular

Nome: Viviane Bezerra da Silva	CPF: 068.023.213-35
Título do Projeto: PROSPECÇÃO FITOQUÍMICA E AVALIAÇÃO DAS ATIVIDADES ANTIMICROBIANA, ANTIPARASITÁRIA E TOXICOLÓGICA DO LÁTEX E CASCAS DO CAULE DE Hancornia speciosa Gomes (APOCYNACEAE)	
Nome da Instituição: Universidade Federal de Pernambuco - UFPE	CNPJ: 24.134.488/0001-08

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Coleta de látex e cascas do caule	10/2021	01/2022

Observações e ressalvas

1	Deve-se observar as as recomendações de prevenção contra a COVID-19 das autoridades sanitárias locais e das Unidades de Conservação a serem acessadas.
2	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros).
3	Esta autorização NÃO libera o uso da substância com potencial agrotóxico e/ou inseticida e NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de atender às exigências e obter as autorizações previstas em outros instrumentos legais relativos ao registro de agrotóxicos (Lei nº 7.802, de 11 de julho de 1989, Decreto nº 4.074, de 4 de janeiro de 2002, entre outros)
4	O titular de autorização ou de licença permanente, assim como os membros de sua equipe, quando da violação da legislação vigente, ou quando da inadequação, omissão ou falsa descrição de informações relevantes que subsidiaram a expedição do ato, poderá, mediante decisão motivada, ter a autorização ou licença suspensa ou revogada pelo ICMBio, nos termos da legislação brasileira em vigor.
5	Este documento somente poderá ser utilizado para os fins previstos na Instrução Normativa ICMBio nº 03/2014 ou na Instrução Normativa ICMBio n° 10/2010, no que especifica esta Autorização, não podendo ser utilizado para fins comerciais, industriais ou esportivos. O material biológico coletado deverá ser utilizado para atividades científicas ou didáticas no âmbito do ensino superior.
6	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
7	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.
8	O titular de licença ou autorização e os membros da sua equipe deverão optar por métodos de coleta e instrumentos de captura direcionados, sempre que possível, ao grupo taxonômico de interesse, evitando a morte ou dano significativo a outros grupos; e empregar esforço de coleta ou captura que não comprometa a viabilidade de populações do grupo taxonômico de interesse em condição in situ.
9	Esta autorização NÃO exime o pesquisador titular e os membros de sua equipe da necessidade de obter as anuências previstas em outros instrumentos legais, bem como do consentimento do responsável pela área, pública ou privada, onde será realizada a atividade, inclusive do órgão gestor de terra indígena (FUNAI), da unidade de conservação estadual, distrital ou municipal, ou do proprietário, arrendatário, posseiro ou morador de área dentro dos limites de unidade de conservação federal cujo processo de regularização fundiária encontra-se em curso.
10	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.

Este documento foi expedido com base na Instrução Normativa nº 03/2014. Através do código de autenticação abaixo, qualquer cidadão poderá verificar a autenticidade ou regularidade deste documento, por meio da página do Sisbio/ICMBio na Internet (www.icmbio.gov.br/sisbio).

Código de autenticação: 0802930120211013 **Página 1/4**

ANEXO F

COMPROVANTE DE DEPÓSITO E INCORPORAÇÃO DE MATERIAL VEGETAL NO HERBÁRIO UFP– GERALDO MARIZ

URCA webMail

José Weverton Almeida Bezrrra - Aluno Curso de Biologia <weverton.almeida@urca.br>

Números tombamentos UFP.../Re: Solicitação - Número Herbário

4 mensagens

Marlene Barbosa <marlenealencar@yahoo.com.br> 26 de abril de 2022 às 07:43
Responder a: Marlene Barbosa <marlenealencar@yahoo.com.br>
Para: José Weverton Almeida Bezerra <weverton.almeida@urca.br>

Bom dia, Weverton!
Abaixo os números de tombamentos:
UFP 88.948 - Caryocar coriaceum (pequizeiro)
UFP 88.947 - Hancornia speciosa (mangabeira).

Abraços,

Em segunda-feira, 25 de abril de 2022 18:50:41 GMT-3, Marlene Barbosa <marlenealencar@yahoo.com.br> escreveu:

Boa noite Weverton!
Graças a Deus estou bem; votos de que você também esteja.
Amanhã quando chegar à UFPE escreverei informando os números de tombamentos pois somente agora tive acesso a sua mensagem.
Tudo de bom!
Abraços,

Em segunda-feira, 25 de abril de 2022 13:39:11 BRT, José Weverton Almeida Bezerra <weverton.almeida@urca.br> escreveu:

Bom dia profa Dra Marlene Alencar, tudo bem com a senhora?
O motivo de meu e-mail é a solicitação do número de herbário de duas plantas que foram depositadas no dia 20 de abril pela doutoranda Felicidade Caroline, sendo elas *Caryocar coriaceum* e *Hancornia speciosa*. Desde já agradeço a colaboração.
Forte abraço

--

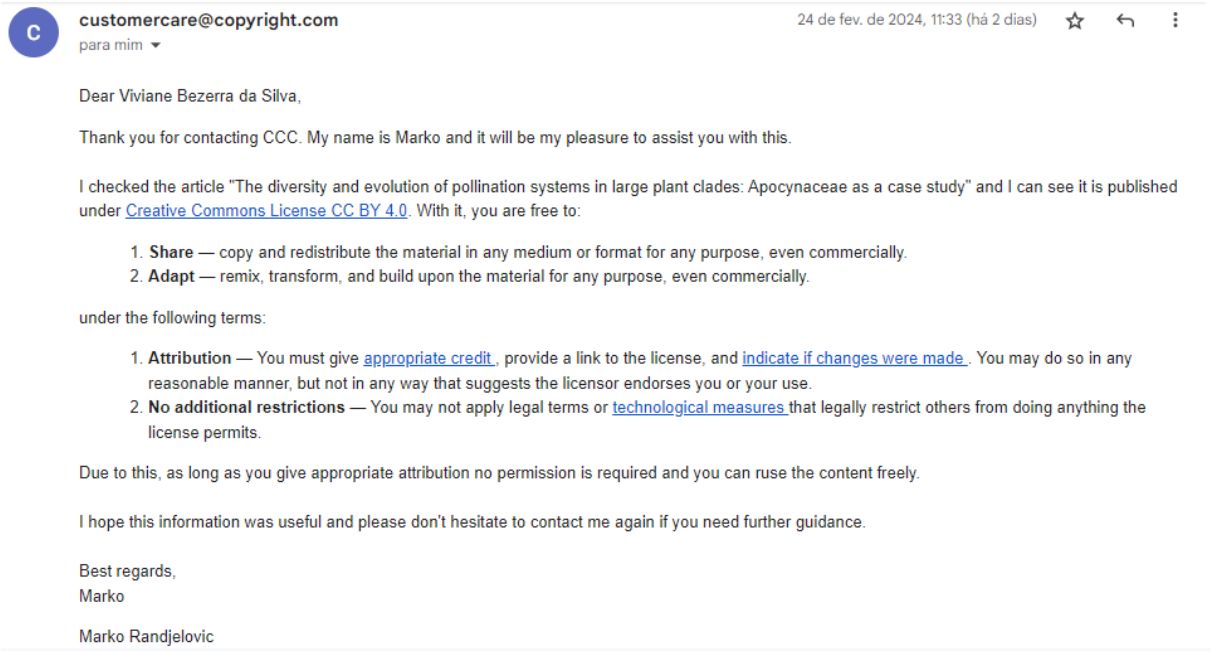
Prof. José Weverton Almeida Bezerra
Doutorando no Programa de Pós-Graduação em Biologia Vegetal - UFPE
Mestre em Biologia Vegetal - UFPE
Especialista em Microbiologia - FAVENI
Graduado em Ciências Biológicas - URCA
Universidade Regional do Cariri- URCA
Lattes
Orcid: 0000-0002-0966-9750

José Weverton Almeida Bezerra <weverton.almeida@urca.br> 26 de abril de 2022 às 08:50
Para: Marlene Barbosa <marlenealencar@yahoo.com.br>

Bom dia professora, muito obrigado. Tudo de bom para a senhora.
Forte abraço.
[Texto das mensagens anteriores oculto]

ANEXO G

COMPROVANTE DE AUTORIZAÇÃO DE USO DA FIGURA 1 DO REFERENCIAL TEÓRICO. OLLERTON ET AL., (2019)



customercare@copyright.com 24 de fev. de 2024, 11:33 (há 2 dias)

para mim

Dear Viviane Bezerra da Silva,

Thank you for contacting CCC. My name is Marko and it will be my pleasure to assist you with this.

I checked the article "The diversity and evolution of pollination systems in large plant clades: Apocynaceae as a case study" and I can see it is published under Creative Commons License CC BY 4.0. With it, you are free to:

1. **Share** — copy and redistribute the material in any medium or format for any purpose, even commercially.
2. **Adapt** — remix, transform, and build upon the material for any purpose, even commercially.

under the following terms:

1. **Attribution** — You must give appropriate credit , provide a link to the license, and indicate if changes were made . You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.
2. **No additional restrictions** — You may not apply legal terms or technological measures that legally restrict others from doing anything the license permits.

Due to this, as long as you give appropriate attribution no permission is required and you can ruse the content freely.

I hope this information was useful and please don't hesitate to contact me again if you need further guidance.

Best regards,
Marko

Marko Randjelovic