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

FELICIDADE CAROLINE RODRIGUES

COMPOSIÇÃO QUÍMICA E ATIVIDADE ANTIMICROBIANA DE *Cordiera myrciifolia* (K. SCHUM.) C.H. PERSS. & DELPRETE E *Tocoyena formosa* (CHAM. & SCHLTDL.) K. SCHUM. (RUBIACEAE)

Recife
2024

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Tese apresentada ao Programa de pós-graduação em Biologia Vegetal da Universidade Federal de Pernambuco como requisito como requisito para o 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

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Aos meus avós, Lusanira e Luiz, por acreditarem na mudança de vida através da educação. Dedico.

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Ninguém nasce feito, é experimentando-nos no mundo que nós nos fazemos.

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RESUMO

A busca por compostos bioativos para o tratamento de infecções tem crescido devido as altas taxas de resistência microbiana. Rubiaceae é conhecida por sua diversidade química associada ao seu potencial farmacológico, principalmente para o tratamento de doenças infecciosas, ainda assim, algumas espécies dessa família permanecem sem dados sobre suas propriedades antimicrobiano. Nesse sentido, o objetivo desse trabalho foi caracterizar quimicamente os extratos foliares de *Cordiera myrciifolia* (K. Schum.) C.H. Perss. & Delprete E *Tocoyena formosa* (Cham. & Schltdl.) K. Schum. e avaliar a atividade antimicrobiana e efeito em conjunto com fármacos. Os extratos de ambas as espécies foram analisados por métodos cromatográficos e espectrométricos. Para os estudos de atividade antibacteriana, os extratos etanólico (EECM) e hexânico (HEMC) de *C. myrciifolia* e de *T. formosa* (EETF e HETF) foram avaliados por meio do teste de microdiluição em caldo contra bactérias padrão e multirresistentes de *Escherichia coli*, *Pseudomonas aeruginosa* e *Staphylococcus aureus*. Quanto à atividade antifúngica, os extratos foram avaliados de modo isolado e combinados com o Fluconazol contra as cepas de *Candida* spp. Além disso, o efeito na inibição de virulência de *Candida* spp. também foi avaliado. Quimicamente, o HECM é composto por ácidos graxos, terpenos, alcanos, entre outros, com o lupeol sendo o composto majoritário. No EECM, foi observada a presença de variados compostos, com destaque para a presença de flavonoides. Nos extratos HETF e EETF foram identificados como compostos majoritários o Nonacosano e o ácido ursólico, respectivamente. O HECM demonstrou atividade bacteriana contra as cepas padrão de *E. coli* e *S. aureus*, com concentrações de 8 e 16 µg/mL, respectivamente. Apenas os efeitos da Norfloxacina e Imipenem foram potencializadas pelos extratos para as bactérias multirresistentes de *E. coli* e *S. aureus*, respectivamente. Quanto ao EECM, não foi observada atividade isolada, mas quando em combinação com Norfloxacina a atividade foi potencializada contra *E. coli*, *P. aeruginosa* e *S. aureus*. Em relação aos extratos de *T. formosa*, foi possível observar atividade antibacteriana isolada do HETF para as cepas padrões de *E. coli* e *S. aureus*, com concentrações de 128 e 256 µg/mL. Para o EETF foi observada atividade apenas para *E. coli*, com 256 µg/mL. Relativo à atividade potencializadora de HETF essa foi observada apenas para *S. aureus*, quando associado ao Imipenem. Para as cepas de *P. aeruginosa* e *E. coli*, Norfloxacina foi potencializada pela combinação com EETF, enquanto para a cepa de *S. aureus* a única potencialização foi entre Imipenem e EETF. Ambos os extratos de *C. myrciifolia* apresentaram resultados significativos contra *Candida* spp., com concentrações variando de 36,6 a 129,1 µg/mL. A atividade antivirulência também foi observada para a cepa de *Candida tropicalis*. Quanto a atividade anti-*Candida* de *T. formosa*, ambos os extratos demonstraram efeitos moderados para *Candida*, variando as concentrações entre 57,0 e 378 µg/mL. A atividade antivirulência também foi observada para a cepa de *C. tropicalis*. Esses resultados corroboram com o potencial previsto em Rubiaceae, indicando que essas duas espécies podem ser promissoras no desenvolvimento de formulações terapêuticas para o tratamento de infecções fúngicas e bacterianas.

Palavras-chave: Resistência bacteriana; Compostos bioativos; Potencialização de fármacos; Candidíase.

ABSTRACT

The search for bioactive compounds for the treatment of infections has grown due to high rates of microbial resistance. Rubiaceae is known for its chemical diversity associated with pharmacological potential, especially in the treatment of infectious diseases; however, some species within this family lack data on their antimicrobial properties. In this regard, the aim of this study was to chemically characterize leaf extracts of *Cordiera myrciifolia* (K. Schum.) C.H. Perss. & Delprete and *Tocoyena formosa* (Cham. & Schltdl.) K. Schum., and evaluate their antimicrobial activity and synergistic effects with drugs. Extracts from both species were analyzed using chromatographic and spectrometric methods. For antibacterial studies, the ethanolic (EECM) and hexane (HEMC) extracts of *C. myrciifolia* and *T. formosa* (EETF and HETF) were evaluated using broth microdilution tests against standard and multi-drug-resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus.* Regarding antifungal activity, extracts were assessed both individually and in combination with Fluconazole against *Candida* spp. Additionally, the inhibitory effect on *Candida* spp. virulence was also evaluated. Chemically, HECM comprised fatty acids, terpenes, alkanes, among others, with lupeol being the major compound. EECM showed the presence of various compounds, particularly flavonoids. Nonacosane and ursolic acid were identified as major compounds in HETF and EETF, respectively. HECM demonstrated bacterial activity against standard strains of *E. coli* and *S. aureus* at concentrations of 8 and 16 µg/mL, respectively. Only the effects of Norfloxacin and Imipenem were potentiated by the extracts against multi-drug-resistant strains of *E. coli* and *S. aureus*, respectively. EECM showed no isolated activity but potentiated Norfloxacin activity against *E. coli*, *P. aeruginosa*, and *S. aureus* when combined. Regarding *T. formosa* extracts, HETF exhibited isolated antibacterial activity against standard strains of *E. coli* and *S. aureus* at concentrations of 128 and 256 µg/mL, respectively. EETF showed activity only against *E. coli* at 256 µg/mL. HETF potentiated *S. aureus* activity when combined with Imipenem. For *P. aeruginosa* and *E. coli* strains, Norfloxacin was potentiated by the combination with EETF, while the only potentiation for *S. aureus* was between Imipenem and EETF. Both *C. myrciifolia* extracts showed significant results against *Candida* spp., with concentrations ranging from 36.6 to 129.1 µg/mL. Antivirulence activity was also observed against *Candida tropicalis*. Concerning *T. formosa*'s anti-*Candida* activity, both extracts demonstrated moderate effects, with concentrations ranging from 57.0 to 378 µg/mL. Antivirulence activity was also observed against *C. tropicalis*. These results support the anticipated potential of Rubiaceae, indicating that these two species may be promising in the development of therapeutic formulations for the treatment of fungal and bacterial infections.

Keywords: Bacterial resistance; Bioactive compounds; Drug potentiation; Candidiasis.

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against *Candida* strains

LISTA DE ABREVIATURAS E SIGLAS

ANOVA	Análise de Variância
ATCC	American Type Culture Collection
BHI	*Brain Heart Infusion*
BSTFA	N,O-bis(trimetilsilil)trifluoroacetamida
CA	*Candida albicans*
CFM	Concentração Fungicida Mínima
MIC	Concentração Inibitória Mínima
CK	*Candida krusei*
CM	Concentração Matriz
CT	*Candida tropicalis*
DHPP	2,2-difenil-1-picril-hidrazil
DMSO	Dimetilsufóxido
DMT	Dimetiltriptamina
EECM	Extrato Etanólico de *Cordiera myrciifolia*
EETF	Extrato Etanólico de *Tocoyena formosa*
FCZ	Fluconazol
FLONA	Floresta Nacional do Araripe
FRAP	Ferric Reducing Antioxidant Power
GC-MS	Gas chromatography coupled to mass spectrometry
HECM	Extrato Hexânico de *Cordiera myrciifolia*
HETF	Extrato Hexânico de *Tocoyena formosa*
IC_{50}	Concentração Inibitória de 50%
INCQS	Instituto Nacional de Controle de Qualidade em Saúde
m/z	Razão massa-carga
mL	Mililitros
nm	Nanômetro
ns	não significativo
º C	graus Celsius
PDA	*Potato Dextrose Agar*
SDA	*Sabourand Dextrose Agar*

SDB	*Sabourand Dextrose Broth*
SISBio	Sistema de Autorização e Informação em Biodiversidade
SISGen	Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado
TMS	Trimetilsilil
UFC	Unidades Formadoras de Colônias
UPLC-MS	Cromatografia Líquida de Ultra Performance acoplada à Espectrometria de Massas
μg/mL	Microgramas por mililitro
μL	Microlitros

SUMÁRIO

1 INTRODUÇÃO

A resistência microbiana é a capacidade que bactérias, fungos e protozoários, têm de crescer e se espalhar na presença de antimicrobianos que normalmente são ativos contra eles (Founou; Founou; Essack, 2017). Essa resistência é parte da adaptação do microrganismo, mas que devido ao uso excessivo e indiscriminado de antibióticos, tanto na saúde humana, quanto na agricultura, tem acelerado esse processo drasticamente (Dadgostar, 2019; Serwecińska, 2020). O crescimento de infecções causadas por microrganismos resistentes se tornou um dos principais problemas de saúde pública no mundo, causando um aumento nas taxas de morbidade e mortalidade (Founou; Founou; Essack, 2017). Desse modo, a indústria farmacêutica não tem conseguido desenvolver fármacos antimicrobianos capazes de reverter/combater ativamente os mecanismos de resistência apresentados pelos microrganismos (Monnet, 2005).

Diante desse cenário, diferentes estratégias têm sido avaliadas para conter a resistência microbiológica, uma delas envolve a busca por metabólitos bioativos encontrados em espécies vegetais (González-Lamothe, 2009). Para isso, pesquisas têm se concentrado no potencial antimicrobiano de diversas plantas, explorando tanto seus extratos brutos, quanto a combinação destes com fármacos comerciais (Chanda; Rakholiya, 2011; Cheesman *et al.*, 2017).

Clardy e Wash (2004) enfatizam que na escolha de uma espécie vegetal para investigação, a análise das suas características botânicas incluindo sua quimiotaxonomia, é de extrema importância, sendo esse processo um componente fundamental para aumentar as chances de descobrir substâncias bioativas, tanto aquelas já conhecidas quanto as novas. Além disso, é importante ressaltar que plantas pouco estudadas, ou ainda não exploradas, possuem uma vasta diversidade de compostos químicos únicos. O que faz da busca por metabólitos ativos em plantas pouco estudadas uma estratégia primordial.

Rubiaceae Juss. é reconhecida por abrigar uma ampla gama de metabólitos secundários com potencial farmacológico, destacando-se especialmente pela produção de alcaloides, muitos dos quais deram origem a medicamentos (Martins; Nunes, 2015). Segundo Martins e Nunez (2015), a diversidade de metabólitos e os produtos originados de Rubiaceae indicam que essa família é uma fonte promissora de substâncias bioativas, que podem resultar em novos produtos farmacológicos.

Contudo, apesar do potencial observado para espécies de Rubiaceae, algumas delas ainda permanecem pouco ou não investigadas, como é o caso da *Cordiera myrciifolia* (K.Schum.) C.H.Perss. & Delprete e *Tocoyena formosa* (Cham. & Schltdl.) K.Schum. Embora alguns estudos tenham identificado uma série de compostos com potencial bioativo nessas espécies (Cesário *et al*., 2018a, 2018b; Militão et al., 2005), pesquisas no âmbito antimicrobiano são escassas.

Cordiera myrciifolia é uma espécie que cresce abundantemente na Bacia do Araripe, Ceará, e nessa região é conhecida popularmente como "café-bravo" ou "marmelinho" (Souza *et al*., 2013). Espécies desse gênero, são utilizadas na medicina popular para o tratamento de afecções de pele e diarreia (Aquino *et al*., 2013; Cruz *et al*., 2021). No entanto, apesar de *C. myrciifolia* não ter relatos de uso etnomedicinal, essa espécie possui compostos químicos que possuem atividades biológicas, como iridoides, flavonoides, triterpenos, entre outros (Luciano *et al*., 2010; Militão *et al*., 2008).

Tocoyena formosa é um arbusto comumente conhecido como "jenipapo-bravo" no semiárido brasileiro, utilizado na medicina popular para o tratamento de distúrbios associados ao trato gastrointestinal (Cesário *et al*., 2019), para inflamações e tratamento de fraturas ósseas (Ribeiro *et al.,* 2014). Essa espécie apresenta grande diversidade química, tendo em sua composição ácidos fenólicos, flavonoides, triterpenos, saponinas e outros (Cesário *et al.*, 2018; Cesário *et al.*, 2019).

Dada a reconhecida riqueza de bioativos em espécies de Rubiaceae, este estudo tem por objetivo investigar as atividades antimicrobianas de *C. myrciifolia* e *T. formosa* contra bactérias e fungos patogênicos humanos, além de contribuir na compreensão e identificação dos metabólitos secundários responsáveis pela bioatividade e assim fornecer subsídios para o desenvolvimento de novos fitoterápicos que auxiliem no combate de microrganismos patogênicos resistentes.

2 FUNDAMENTAÇÃO TEÓRICA

2.1 PLANTAS MEDICINAIS

2.1.1 Breve histórico

Há muito tempo as plantas desempenharam um papel fundamental para o ser humano, fornecendo abrigo, oxigênio, alimentos e remédios. À medida que as sociedades se desenvolveram ao longo do tempo, as pessoas aprenderam a classificar as plantas com base em suas necessidades, incluindo o uso dos poderes curativos das plantas no tratamento de doenças (Mamedov, 2012). Desde os tempos mais antigos, as culturas ao redor do mundo desenvolveram narrativas e histórias que descreviam o uso de plantas como remédios. O registro mais antigo sobre esse uso remonta há 5000 anos atrás. Esse registro foi descoberto em uma laje de argila suméria encontrada em Nagpur e continha receitas que descreviam o uso de aproximadamente 250 plantas na produção de medicamentos. Algumas dessas plantas eram conhecidas por conter alcaloides, como a papoula, o meimendro e a mandrágora (Petrovska, 2012).

Outros registros antigos nos revelam que o uso de plantas com propriedades curativas para tratar doenças foi praticado por várias sociedades em diferentes partes do mundo. Essa prática é mencionada em escritos como a Bíblia (Antigo Testamento), há 1200 anos a.C., e nos livros sagrados indianos, como os Vedas, que descrevem uma ampla variedade de plantas medicinais (Šantić *et al.*, 2017). Outros registros, como o papiro Ebers, que remonta ao período de 1550 a.C., descrevem cerca de 700 espécies de plantas e drogas usadas para terapia, como romã, mamona, babosa, sena, alho, cebola, figo, salgueiro, coentro, zimbro, etc. (Petrovska, 2012). Esses textos antigos contêm informações preciosas sobre o conhecimento e a utilização das propriedades medicinais das plantas ao longo da história. E essa herança cultural diversa nos oferece uma compreensão valiosa sobre o uso terapêutico das plantas e, continua a influenciar a medicina contemporânea.

Nos últimos 100 anos, houve um aumento na produção e uso de medicamentos sintéticos contra diferentes enfermidades. Ainda assim, as plantas continuam sendo reconhecidas como uma fonte segura e alternativa para a prevenção e tratamento de doenças (Calixto, 2019). Estima-se que atualmente, mais de 85 % da população mundial ainda utilize recursos naturais como primeira alternativa ao tratamento de enfermidades,

e, apesar do grande uso, apenas 10 – 15 % das espécies vegetais em todo o mundo foram exploradas quimicamente para aplicações medicinais (Wangchuk, 2018).

O mercado de medicamentos movimenta anualmente cerca 1 trilhão de dólares, sendo que 35 % destes tem origem direta ou indireta de produtos naturais. As plantas, por exemplo, representam 25 % das obtenções em produtos naturais (Calixto, 2019). De acordo com o estudo realizado por Newman e Cragg (2016), no período compreendido entre os anos de 1981 e 2014, constatou-se que, dos 1562 medicamentos aprovados para uso clínico, 64 (4%) eram produtos naturais inalterados, 141 (9,1%) consistiam em medicamentos botânicos (mistura), 320 (21%) eram derivados de substâncias naturais, e 61 (4%) eram drogas sintéticas contendo produtos naturais como farmacóforos.

2.1.2 Uso alternativo de plantas contra a resistência microbiana

Ainda que as pesquisas relacionadas a antimicrobianos de origem vegetais tenham aumentado acentuadamente nos últimos anos, proporcionando alguma esperança de que medicamentos derivados de plantas possam ser efetivamente utilizados no futuro (Chandra *et al*., 2017), até o presente momento, não foi descoberto nenhum medicamento antimicrobiano proveniente do uso de espécies vegetais. No entanto, devido à vasta variedade de compostos químicos de origem vegetal, atrelada às diferentes atividades biológicas e mecanismos de ação, os metabólitos secundários vegetais podem ser promissores nos tratamentos associados à resistência antimicrobiana (Singh, 2017).

Além disso, as plantas permanecem sendo a única fonte possível de alguns compostos, uma vez que não podem ser quimicamente sintetizados, pois são estereoestruturas complexas com muitos centros quirais que podem ser essenciais para a atividade biológica (Ferdes, 2018). Alguns dos princípios ativos de plantas possuem atividade antimicrobiana intrínseca e atividade modificadora de resistência, e embora alguns não apresentem atividades antimicrobianas por si só, quando combinados a antibióticos convencionais ajudam a potencializar os efeitos dos antimicrobianos frente a resistência microbiológica (Vaou *et al*., 2021).

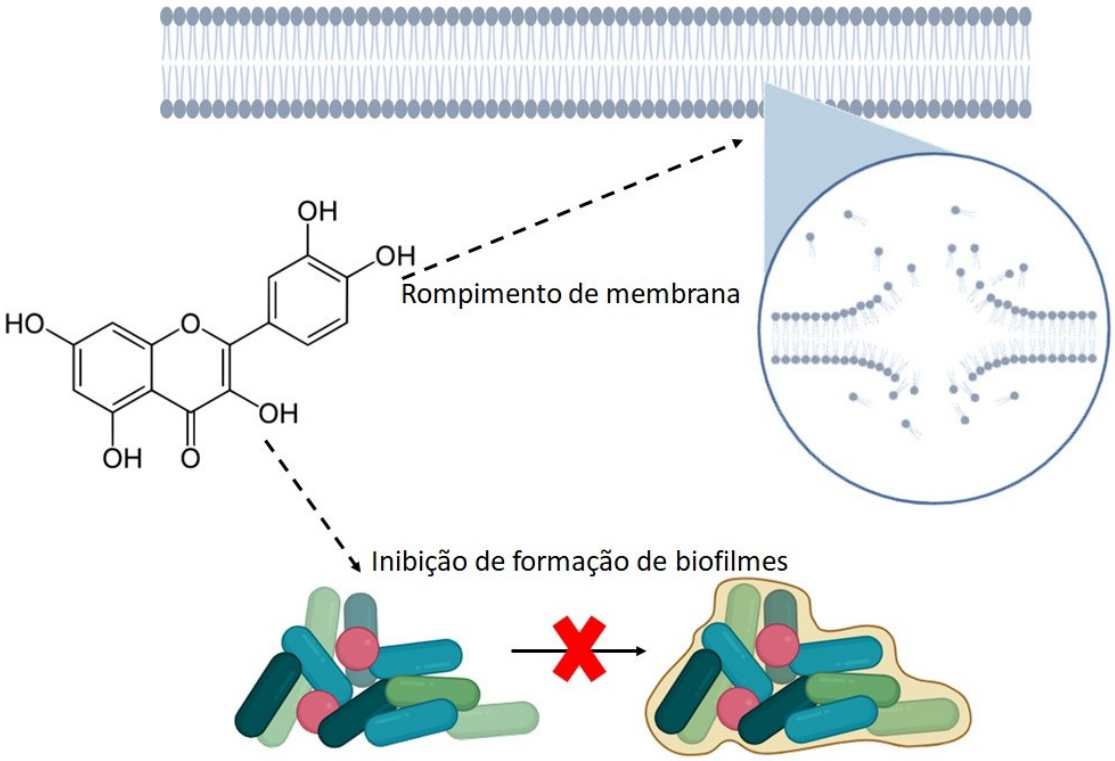
Entre os produtos provenientes da natureza, destacam-se os extratos vegetais como uma escolha amplamente adotada na medicina alternativa, devido à sua acessibilidade, à diversidade de compostos químicos e às interações sinérgicas entre eles.

Esses fatores combinados tornam os extratos uma opção rica e valiosa para aplicações na área da medicina alternativa.

De modo geral, extratos vegetais são compostos por uma mistura complexa de substâncias e sua ação sinérgica pode afetar a viabilidade da célula microbiana de diferentes maneiras (Vaou *et al*., 2021). Geralmente, o primeiro alvo dos compostos bioativos é a membrana plasmática, afetando sua estrutura, integridade e permeabilidade de diferentes formas. Alguns compostos têm como mecanismo de ação a ruptura da membrana plasmática. Como essa membrana é responsável pela osmorregulação, respiração e transporte, biossíntese e reticulação de peptidoglicano (em bactérias), bem como a biossíntese de lipídios, o seu rompimento causa disfunção metabólica levando os microrganismos à morte. Um outro mecanismo de ação que compostos de origem vegetal podem causar, é a inibição da formação de biofilmes microbianos (Górniak *et al*., 2019). A Figura 1 exemplifica esses dois mecanismos de ação que podem ser observados pela ação do flavonol quercetina.

Além desses, outros mecanismos de ação também podem ser observados, como a inibição de bombas de efluxo, inibição da síntese de parece celular, entre outros (Górniak *et al*., 2019).

Figura 1. Mecanismos de ação da quercetina contra microrganismos



Fonte: Autor (2023)

2.2 RUBIACEAE

2.2.1 Características botânicas e taxonomia

Rubiaceae Juss., inicialmente descrita por Antoine Laurent de Jussieu, recebeu este nome a partir do gênero *Rubia* L., termo derivado do latim "*rubium*". Esse termo faz referência à coloração avermelhada obtida a partir das raízes de plantas desse gênero, as quais são tradicionalmente utilizadas na produção de tintas para tingir tecidos (Cronquist, 1981).

Rubiaceae é a quarta maior família em número de espécies dentre as angiospermas, ficando atrás apenas de Orchidaceae, Asteraceae e Fabaceae. Essa família é composta de mais de 13.000 espécies distribuídas mundialmente em 611 gêneros. Possui distribuição cosmopolita, predominantemente Pantropical, com poucas espécies de distribuição extratropical. Quase metade das espécies, e um terço dos gêneros, ocorrem nas regiões neotropicais (Delprete, 2012). No Brasil, ocorrem cerca de 120 gêneros e 1.400 espécies, consistindo de uma das principais famílias da flora brasileira e ocorrendo como um importante elemento em todas as formações naturais (Souza; Lorenzi, 2012).

Rubiaceae está bem representada em todos os tipos de hábitos, ex. ervas, subarbustos, arbustos, árvores e menos frequentemente lianas (Souza; Lorenzi, 2012), com dimensões que variam de 5 mm (*Spermacoce* spp.) a 55 m de altura (*Chimarrhis* spp.) (Delprete, 2012). Morfologicamente, as espécies possuem folhas opostas, raramente verticiladas, simples, quase sempre com estípulas interpeciolares, ocasionalmente transformadas em espinhos, inflorescência geralmente cimosas e frutos do tipo cápsula, esquizocarpo, drupa ou baga (Souza; Lorenzi, 2012).

Segundo resultados de estudos filogenéticos, Rubiaceae é um grupo monofilético dentro da Ordem Gentaniales. No entanto, a classificação taxonômica interna de Rubiaceae é complexa, tendo sofrido diversos rearranjos ao longo das décadas. A última antes do advento dos estudos em filogenia molecular, foi proposta por Robbrecht, que dividiu a família em quatro subfamílias, sendo elas Rubioideae, Cinchonoideae, Antirheoideae e Ixoroideae, e 44 tribos (Robbrecht, 1988). Com o avanço das técnicas moleculares de filogenia, Rubiaceae sofreu mais uma mudança de classificação, sendo melhor representada atualmente por três subfamílias (Rubioideae, Cinchonoideae e Ixoroideae) e 44 tribos (Delprete, 2012).

As espécies estudadas no presente trabalho pertencem a subfamília Ixoroideae. Essa subfamília abriga cerca de 4000 espécies de árvores e arbustos, com distribuição pantropical e subtropical. A estivação da corola varia dentro dessa subfamília, sendo que a maioria das tribos apresenta uma estivação contorcida à esquerda ou valvada (Bremer; Eriksson, 2009). Alguns dos gêneros mais conhecidos da família Rubiaceae, se encontram dentro dessa subfamília, como *Coffea* L.*,* devido ao seu alto valor econômico e *Gardenia* J.Ellis, amplamente utilizada como planta ornamental (Bremer, 2009).

2.2.2 Fitoquímica e atividade antimicrobiana

Espécies de Rubiaceae são conhecidas pela variada produção de compostos bioativos com alto potencial farmacológico. Alguns desses compostos são considerados marcadores taxonômicos para algumas das suas subfamílias. Em Cinchonoideae, por exemplo, são comuns os alcaloides indólicos; em Ixoroideae, prevalecem principalmente os iridoides e terpenos; já em Ruboideae, há uma maior diversidade química, destacando-se a presença predominante de antraquinonas em detrimento de outras classes de compostos químicos (Martins; Nunez, 2015).

Do ponto de vista fitoquímico Rubiaceae apresenta diversos compostos com atividades biológicas conhecidas e de importância médica, como a quinina, extraída do gênero *Cinchona*, a qual foi a primeira cura efetiva para a malária (Gurung; De, 2017). Além dessa, podemos citar como de grande importância a cafeína, um alcaloide obtido de *Coffea arabica* L., que além de atuar como estimulante do sistema nervoso central, é um dos componentes ativos de medicamentos para enxaqueca (Simões *et al*., 2016). Outra substância de Rubiaceae, que demonstra o grande potencial da família, é a dimetiltriptamina (DMT), obtida em grandes quantidades de *Psychotria viridis* Ruiz & Pav., sendo esta utilizada principalmente em rituais religiosos (Callaway; Brito; Neves, 2005).

Diversas variedades de plantas pertencentes à família Rubiaceae desempenharam um papel significativo no tratamento de infecções causadas por microrganismos bem conhecidos, destacando assim o potencial antimicrobiano dessa família. Nesse sentido, um dos gêneros mais relevantes dentro de Rubiaceae no contexto terapêutico é *Uncaria* Schreb. (Abdul *et al.*, 2022). Além disso, *Uncaria* é um dos gêneros mais extensivamente estudados do ponto de vista fitoquímico dentro desta

família (Martins; Nunez, 2015), e seu potencial no combate a bactérias e fungos é bem estabelecido, incluindo eficácia contra as bactérias *Staphylococcus aureus*, *Pseudomonas aeruginosa* e o fungo *Candida albicans* (Abdul *et al.*, 2022).

O óleo essencial de *Ixora coccinea* L., demonstrou ser promissor para o tratamento de doenças do trato respiratório e gastrointestinal provocadas por microrganismos patogênicos, como *Klebsiella pneumonia* e *Escherichia coli*, tendo obtido MIC de 50 e 100 µg/mL, respectivamente (Se *et al*., 2018). Ekon *et al*. (2020), mostraram que o extrato da casca do caule de *Morinda lucida* Benth, tem MIC de 256 µg/mL para a bactéria *Salmonella typhi* e para os fungos *Candida parapsilosis* e *Cryptococcus neoformans.* Já o extrato foliar de *Duroia macrophylla* Huber, testado contra diferentes cepas de *Mycobacterium tuberculosis*, demonstrou MIC extremamente significantes, variando de 6,25 a 25 µg/mL e essa atividade foi atribuída a dois triterpenos pentacíclicos identificados no extrato, sendo eles os ácidos ursólico e oleanólico (Martins *et al.*, 2013). Outras espécies de Rubiaceae com potencial antimicrobiano contra microrganismos patogênicos humanos são mostradas na Tabela 1.

Tabela 1. Espécies de Rubiaceae com atividade antimicrobiana identificada

Espécie	**Parte usada**	**Método**	**Microrganismo**	**Referências**
Gonzalagunia rosea Standl.	Partes aéreas	Diluição em caldo	*C. albicans; Fusarium solani*	Niño *et al*., 2006
Psychotria pubigera Schltdl.; *P. ruelliifolia* (Cham. & Schltdl.) Müll.Arg.; *P. stachyoides* Benth.	Folhas	Ensaio de sal de tetrazol	*Mycobacterium bovis*	Moraes *et al*., 2011
Paederia foetida L.	Planta inteira	Difusão em disco	*Bacillus cereus; Staphylococcus aureus; Escherichia coli; Vibrio mimicus*	Morshed *et al*., 2012
Schumanniophyton magnificum (K.Schum.) Harms	Cascas do caule	Difusão em disco	*Salmonella typhimurium*	Tchouya *et al.,* 2014
Morinda citrifolia L.	Fruto	Diluição em caldo	*S. aureus; E. coli*	Candida *et al.,* 2014
Borreria laevicaulis (Miq.) Ridl.	Planta inteira	Difusão em disco	*S. aureus; Candida albicans*	Niño *et al*., 2006
Simira gardneriana	Sementes	Diluição em caldo	*Enterococcus faecalis; E. coli;Bacillus cereus*	Ferraz *et al.,* 2016
Coffea arabica L.	Polpa do fruto	Difusão em disco	*S. aureus; Staphylococcus epidermidis;*	Duangjai *et al*., 2016

		Diluição em caldo	*Pseudomonas aeruginosa; E. coli*	
Genipa americana L.	Ramos, frutos e folhas	Difusão em disco, diluição em caldo	*E. coli; S. aureus*	Codignoto *et al.*, 2017
Mitracarpusf rigidus (Willd. Ex Roem. & Schult.) K. Schum)	Partes aéreas	Diluição em caldo	*S. aureus; Streptococcus pyogenes*	Lemos *et al.,* 2018
Coffea canephora Pierre ex A. Froehner	Folhas e raízes	Diluição em caldo	*Shigella dysenteria; Candida krusei*	Goulefack *et al*., 2022
Rubia cordifolia L.	Raízes	Difusão em disco	*S. aureus*	Chandrasekhar *et al.*, 2023

2.3 ASPECTOS BOTÂNICOS E BIOATIVOS DE *Cordiera myrciifolia*

Cordiera myrciifolia (K.Schum.) C.H. Perss. & Delprete (Figura 2), é uma espécie arbustiva podendo chegar a 1,2 m de altura e que possui distribuição geográfica em diversos países, sendo eles Panamá, Colômbia, Venezuela, Guiana, Suriname, Guiana Francesa, Brasil e Bolívia (Corrêa, 1984). No Brasil, está presente em todos os estados e ocorrendo nos mais variados domínios fitogeográficos (Flora e Funga do Brasil).

Cordiera myrciifolia apresenta ramos cilíndricos e glabros, com entrenós de 2-5 cm. Suas estípulas são truncadas, medindo cerca de 2,5 mm. As folhas são opostas decussadas, com pecíolo glabro de 0,4-0,6 cm. A lâmina foliar é obovada, variando de 2,5 a 9,5 cm de comprimento por 1,5 a 5 cm de largura, com ápice acuminado de 0,1-1 cm e base aguda. A margem é inteira e revoluta, enquanto as faces são glabras. As nervuras são obliquivênias, com 5-8 pares. A inflorescência estaminada ocorre em cimas fasciculadas terminais, enquanto as flores pistiladas são solitárias. A flor estaminada apresenta botão floral de 7-10 x 1-1,5 mm, cálice truncado de 1,1-1,7 x 1,2-1,5 mm, pubérulo e persistente. Os estames são 4, inclusos, inseridos na base da corola tetrâmera hipocrateriforme, com filamentos filiformes de 0,5-1 cm de comprimento. As anteras são dorsifixas e o tubo externo apresenta velutinosidade, com 6-8 mm de comprimento. O fruto é uma baga, glabra internamente, com lobos ovados a globosos de 9 x 7 mm, pubérulos. As sementes são achatadas, deltóides, medindo 4 x 2 mm, com face dorsal parcialmente velutina e formato oval de 3 x 2 mm (Margalho *et al*., 2009).

Cordiera myrciifolia que tem como sinonímia *Alibertia myrciifolia* Spruce ex K.Schum., foi realocada para o gênero *Cordiera* devido às diferenças no tamanho das flores. No gênero *Cordiera*, as flores são pequenas, com menos de 10 mm, enquanto no gênero *Alibertia*, as flores são maiores, com mais de 10 mm (Delprete; Persson, 2004).

Conhecida popularmente como café-bravo na região da Chapada do Araripe, *C. myrciifolia*, não possui relatos de usos etnomedicinais (Souza *et al*., 2013). No entanto, essa planta integra o grupo de plantas alimentícias não convencionais, sendo seus frutos utilizados para o consumo *in natura* e para a fabricação de doces e geleias (Passos, 2023). Essa espécie apresenta uma ampla diversidade de constituintes químicos identificados. Diferentes autores identificaram cumarinas, flavonoides, triterpenos, iridoides e compostos fenólicos nas partes aéreas de *C. myrciifolia* (Luciano *et al*., 2010; Militão *et al*., 2008).

Para além das análises fitoquímicas, existem poucos relatos na literatura sobre as atividades biológicas de *C. myrciifolia*. Em um estudo realizado por Luciano *et al*. (2010), alguns dos iridoides isolados de *C. myrciifolia* inibiram a formação micelial do fungo *Colletotrichum gloeosporioides*. O ácido geniposídico na concentração de 150 µg/mL pode estar envolvido neste processo. Segundo Militão *et al*. (2008), cinco flavonoides isolados do extrato hexânico de *C. myrciifolia* inibiram o crescimento de células tumorais e o desenvolvimento de ovos de ouriço-do-mar. A atividade dos flavonoides diferiu entre os modelos testados indicando requerimentos estruturais respondem mais a depender do ensaio adotado.

Figura 2. Aspectos gerais de *Cordiera myrciifolia*

A- Ramos de *Cordiera myrciifolia;* B - Fruto maduro em destaque; C e D – Folhas em destaque. Fonte: Autor (2021)

2.4 ASPECTOS BOTÂNICOS E BIOATIVOS DE *Tocoyena formosa*

Tocoyena formosa (Cham. & Schltdl.) K.Schum. (Figura 3), é uma espécie arbustiva nativa da América do Sul, encontrada em território brasileiro, paraguaio e boliviano. Ela é comumente encontrada em formações xeromórficas, tais como o Cerrado, a Caatinga e a Restinga. No Brasil, essa espécie apresenta uma ampla

distribuição, ocorrendo nas regiões Norte, Nordeste, Centro-Oeste, Sudeste e Sul, estendendo-se até o estado do Paraná (Delprete, 2008).

Tocoyena formosa é um arbusto monoico de 2 a 2,5 metros de altura, com caule cilíndrico, entrenós de 20 a 80 mm e estípulas persistentes. Suas folhas opostas têm pecíolo velutino, lâmina de 90 a 200 mm de comprimento, base cuneada e ápice agudo, com pubescência na face adaxial e tomento na face abaxial, apresentando venação broquidódroma com 7 a 11 pares de nervuras. As inflorescências são corimbosas, terminais, com 4 a 7 flores, e as brácteas foliáceas. As flores são amarelas, hipocrateriformes, com cálice tomentoso, corola de tubo tomentoso externamente, estames exsertos e inseridos na fauce, anteras elípticas de 7 a 9 mm e estigma bífido, estilete de 90 a 130 mm. Os frutos são bagas globosas de 18 a 25 mm por 15 a 35 mm, tornando-se negros na maturação, com superfície pubérula a híspida. As sementes são orbiculares, comprimidas, de 4 a 5 mm por 3,8 a 5 mm, com exotesta lisa (Sousa et al., 2013).

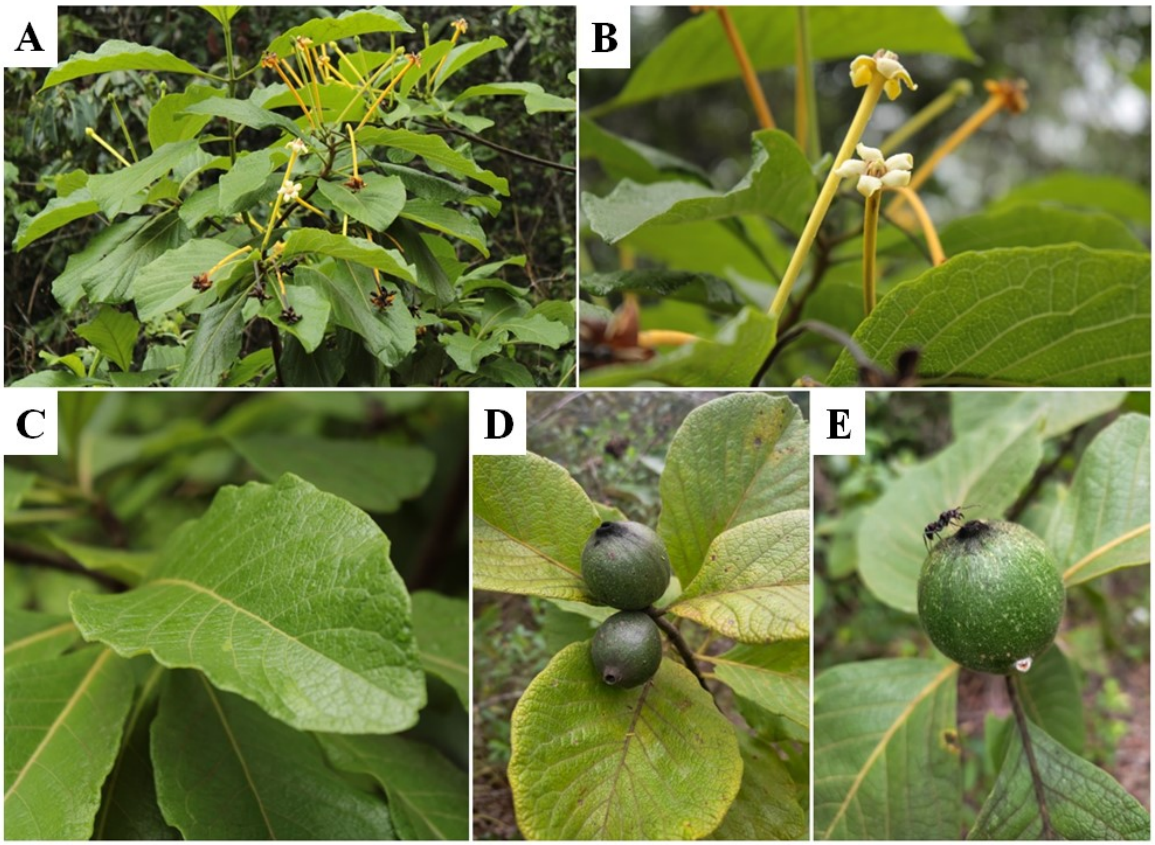
Popularmente, *T. formosa*, é conhecida como jenipapo-bravo ou jenipapo do campo, no semiárido do Brasil, sendo também conhecida em outras regiões brasileira como cafezinho, genipapinho, pau de cera e trombeta, suas folhas são usadas tradicionalmente para o tratamento de reumatismos (Agra *et al*., 2017), como anti-inflamatória e para dor lombar, mialgias e sintomas gastrointestinais (Cesário *et al*., 2018). As cascas do caule são usadas para tratar fraturas, lesão nos pés e mãos, reumatismo, contusão, torção, pancadas, inchaço, cicatrização e luxação (Macedo *et al*., 2018; Ribeiro *et al*., 2014). Quimicamente, *T. formosa*, apresenta em sua composição uma grande diversidade de metabólitos, incluindo ácidos fenólicos, flavonoides, flavonóis, flavonas, triterpenos, alcaloides, saponinas, dentre outras (Cesário *et al*., 2019).

Biologicamente, *T. formosa*, tem alguns dos seus usos etnomedicinais comprovados. No estudo de Cesário *et al*. (2018b), por exemplo, foi observado que o extrato hidroalcóolico foliar de *T. formosa,* nas doses de 200 e 400 mg/kg, diminuiu significativamente as contorções abdominais em camundongos, além de reduzir a lambedura das patas no teste de nocicepção induzido por formalina tanto na fase neurológica como na fase inflamatória. Nas doses de 100, 200 e 400 mg/kg o extrato aumentou o tempo de latência dos animais à estimulação térmica após 30 min de administração do tratamento. Concluiu-se que *T. formosa* tem ação nociceptiva, ao qual está ligada a diversas mecanismos de ação.

Os testes de FRAP (Ferric Reducing Antioxidant Power) e DPPH (2,2-diphenyl-1-picrylhydrazyl) confirmaram a atividade antioxidante do extrato hidroalcóolico de *T. formosa*. Além disso, no teste de gastroproteção conduzido por Cesário *et al*. (2018a), foi verificada a formação de uma camada protetora associada ao muco do estômago.

Quanto às propriedades antimicrobianas, o extrato etanólico de *T. formosa* na concentração de 1000 µg/mL, inibiu totalmente o crescimento bacteriano de *Mycobacterium abscessus* subsp. *massiliense*, agente causador da tuberculose (Neves *et al*., 2019).

Figura 3: Aspectos gerais de *Tocoyena formosa* (Cham. & Schltdl.) K.Schum



A – Ramos de *Tocoyena formosa*; B – Flores em destaque; C – Folhas em destaque; D e E; Frutos em destaque. Fonte: Autor (2021)

2.5 ASPECTOS HISTÓRICOS SOBRE OS ANTIMICROBIANOS NATURAIS

É conhecido que civilizações antigas já faziam uso de diferentes produtos naturais, tais como, ervas, mel e até fezes de animais para tratar diferentes tipos de infecções (Dhingra *et al.,* 2020). Um dos primeiros relatos, por exemplo, remonta há

milênios com a utilização do pão mofado para o tratamento de infecções de pele decorrentes de feridas abertas no Egito, Sérvia, Grécia e China (Hutchings *et al*., 2019).

No entanto, foi apenas em 1928 que Alexander Fleming, após analisar uma placa de Petri contendo uma cultura de *Staphylococcus* spp., contaminada por fungos, constatou que o crescimento fúngico reduzia a cultura bacteriana. Posteriormente constatou que o fungo contaminante pertencia ao gênero *Penicillium*. A substância ativa do fungo foi dada o nome de “Penicilina” o que viria a revolucionar a medicina (Dhingra *et al*. 2020; Lobanovska; Pilla, 2017). A descoberta da Penicilina marcou o início de uma época conhecida como a “Era de Ouro dos Antibióticos”, durante a qual foi encontrada uma grande variedade de antibióticos de origem natural, e que teve seu ápice entre as décadas de 1940 e 1960 (Lobanovska; Pilla, 2017).

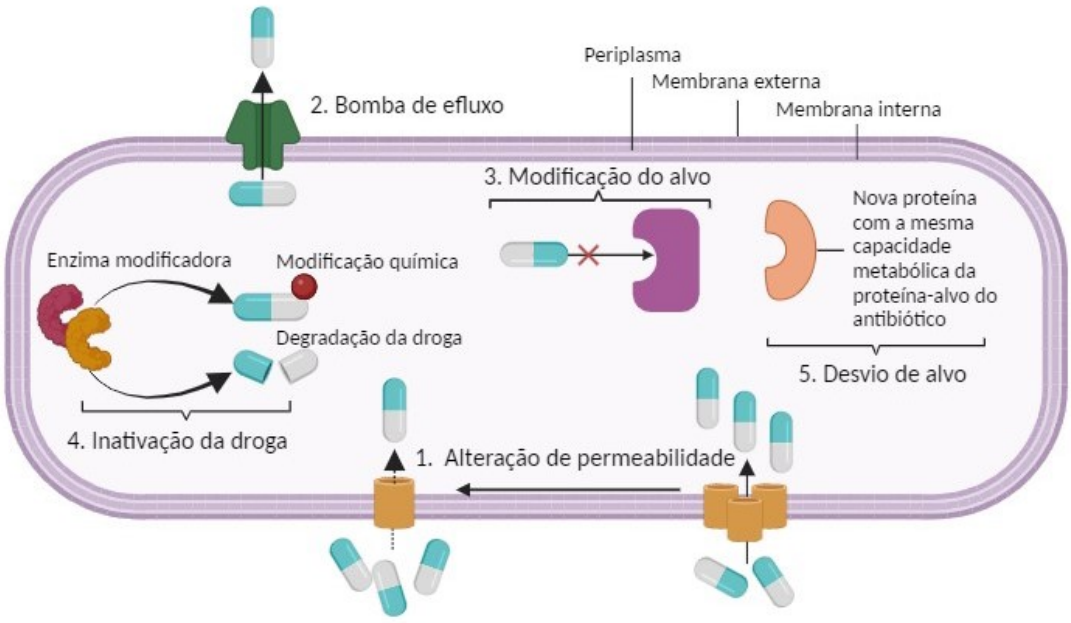
A palavra "antibiótico", pode ser rastreada da palavra "antibiose", que era usada como um antônimo de "simbiose", com o propósito de descrever a ação antagônica entre dois microrganismos. Posteriormente, o termo antibiótico passou a ser usado para descrever metabólitos produzidos por microrganismos que possuem atividades inibitórias de crescimento ou capazes de matar bactérias ou fungos. Atualmente, é empregado para descrever compostos com a capacidade de inibir ou erradicar microrganismos, inclusive aqueles fabricados sinteticamente. Para distinguir medicamentos destinados, exclusivamente, a bactérias ou fungos, são utilizados os termos "antibacteriano" e "antifúngico", respectivamente (Nicolaou; Rigol, 2018).

2.6 RESISTÊNCIA MICROBIANA

No ambiente natural, antibióticos são produzidos por populações microbianas como uma ferramenta de competição por recursos nutricionais e por espaço dentro do micro-habitat que ocupam (Costa; Silva Junior, 2017). Dessa forma, a resistência microbiana surgiu como mecanismo de defesa em resposta a ação dos antibióticos, tornando-a um aspecto natural do processo evolutivo dos microrganismos (Durand; Raoult; Dubourg, 2019). Esse processo, apesar de natural, tem evoluído de maneira expressiva devido ao uso abusivo de antibióticos e aos tratamentos incompletos tanto na saúde humana quanto na agropecuária, que contribuem ativamente para o aumento da resistência, tornando-a um dos principais problemas de saúde pública global e de grande impacto ambiental e socioeconômico (Dyary *et al.*, 2023; Ohore *et al*., 2022).

A resistência bacteriana é caracterizada como a habilidade inerente das bactérias de neutralizar os efeitos dos agentes antibióticos, sejam eles de natureza bacteriostática (inibindo o crescimento) ou bactericida (causando a morte das bactérias). A Figura 4 ilustra alguns dos principais mecanismos utilizados por bactérias no desenvolvimento de resistência, a saber: 1. Redução da permeabilidade da membrana, mecanismo intrínseco em bactérias Gram-negativas, que as torna menos suscetíveis aos antibióticos devido à presença de uma dupla membrana. Além disso, bactérias têm a capacidade de elaborar adaptações no envoltório celular, tais como a redução de porinas ou modificações nos componentes de fosfolipídeos e ácidos graxos; 2. Bombas de efluxo, que consistem na atividade de proteínas transmembranas de extrair do interior da célula compostos tóxicos; 3. Modificações do alvo, envolvendo alterações genéticas na proteína-alvo do antibiótico; 4. Inativação de antibióticos por enzimas que degradam a droga ou a modificam quimicamente; 5. Desvio de alvo, criando uma via alternativa que torna a rota principal redundante e menos suscetível aos antibióticos (Darby *et al*., 2023; Hassan; Al-Harmoosh, 2020; Peterson; Kaur, 2018; Uddin *et al*., 2021).

Figura 4. Mecanismos de resistência bacteriana



Fonte: Autor (2023)

No que tange aos fatores de resistência, as bactérias despontam como sendo os microrganismos mais amplamente investigados. Contudo, a resistência de outros grupos

de organismos, como o dos fungos, também tem se destacado por se apresentarem como um importante problema clínico (Osset-Trénor; Pascual-Ahuir; Prof, 2023).

Além da resistência bacteriana, a ciência tem testemunhado uma contínua ascensão das infecções fúngicas, onde parte significativa dos casos tem sido causada por fungos oportunistas. Essas infecções são oriundas de procedimentos cirúrgicos complexos, estados de supressão imunológica ou redução da resposta imune do paciente (Berman; Krysan, 2020). De forma equivalente ao que acontece com as bactérias, a crescente utilização de antifúngicos de formas indiscriminada e incorreta, tem acarretado um cenário ascendente de fungos resistentes às drogas.

Em um contexto de infecção fúngica, outras variáveis também influenciam na resistência às drogas. São os casos de (1) restrição quantitativa das classes antifúngicas disponíveis e (2) sobreposição entre antifúngicos empregados seja em doenças humanas seja em fitopatologia, uma vez que utilizam de mecanismos de ação similares que possibilitam que patógenos humanos adquiram resistência aos azóis (uma classe de antifúngicos) ao serem expostos a produtos agrícolas. Adicionalmente a essas, existe a resistência intrínseca que alguns fungos demonstram ter a determinadas categorias de antifúngicos (Chang *et al*., 2019).

Os mecanismos de resistência em fungos são muitos e variam conforme a classe de antifúngicos. Entre as categorias de antifúngicos disponíveis, destacam-se três como as principais: polienos, azóis e equinocandinas. As duas primeiras atuam diretamente sobre um componente crucial da membrana celular dos fungos, o esteroide ergosterol; enquanto a terceira incide na parede celular fúngica.

Os azóis despontam como uma importante classe de antifúngicos, sendo geralmente a classe de primeira escolha para o tratamento de infecções fúngicas (Martínez-Matías; Rodríguez-Medina, 2018.). Os azóis são divididos em imidazóis (caracterizados pela presença de um anel azol com dois nitrogênios) e triazóis (caracterizados pela presença de um anel azol com três nitrogênios) e que possuem o Fluconazol ─ um triazól de primeira classe ─ como um de seus principais representantes (Zonios; Bennett, 2008). Além de ter um amplo espectro de tratamento contra *Candida* spp., *Cryptococcus neoformans* e fungos dimórficos, o Fluconazol desponta como uma alternativa mais efetiva para o tratamento de infecções fúngicas invasivas quando comparado a outros azóis, por apresentar uma alta taxa de absorção e, ao mesmo tempo, menores efeitos colaterais (Allen *et al*., 2015).

Os azóis, de maneira geral, agem inibindo a enzima lanosterol-14-*α*-desmetilase, enzima dependente do citocromo P450 (conhecido como *ERG11* em leveduras) e responsável por converter o lanosterol em ergosterol para a membrana fúngica (Cowen *et al*., 2023). A interrupção da via biossintética do ergosterol, promove o acúmulo de um esterol tóxico para a célula fúngica, o 14-*α*-metilesterol, que altera a estabilidade e permeabilidade da membrana e a ação de enzimas ligadas a ela (Cowen *et al*., 2023; Houšť; Spížek; Havlíček, 2020).

Devido à sua natureza fungistática, os azóis operam em cooperação com o sistema imunológico do hospedeiro para combater as infecções fúngicas. Entretanto, o seu uso cada vez mais frequente e prolongado, tem apresentado como consequência o aumento nos casos de resistências à essa classe. A resistência aos azóis pode manifestar-se através de diversas formas, das quais destacam-se (1) mutações no gene *ERG11*, que resultam tanto na redução da afinidade da enzima lanosterol-14-*α*-desmetilase pelos compostos azólicos, como a superexpressão dessa enzima, que implica em sua maior presença nas células e, por conseguinte, na necessidade de uma concentração mais elevada do fármaco para que este apresente eficácia (Zavrel; White, 2015) e (2) a resultante de amplificação da atividade das bombas de efluxo de drogas ─ mecanismo predominante de resistência aos azóis (Berman; Krysan, 2020).

2.7 MICRORGANISMOS DE INTERESSE CLÍNICO

2.7.1 Bactérias

Bactérias são organismos unicelulares, dotados de um material genético não envolto por membrana nuclear, que exibem uma variada morfologia e se reproduzem por meio de fissão binária. Algumas têm a capacidade de sintetizar seu próprio alimento por meio de quimiossíntese, mas a grande maioria depende de compostos orgânicos, provenientes de organismos vivos ou inativos. Algumas bactérias constituem um importante parte da microbiota humana e, ocasionalmente, podem sofrer desregulação, adquirindo características patogênicas (Tortora; Case; Funke, 2016).

Algumas das bactérias se destacam por sua capacidade oportunista, e por apresentarem mais dificuldade de tratamento devido a sua alta taxa de resistência a antibióticos, como *Pseudomonas aeruginosa*, *Staphylococcus aureus* e *Escherichia coli* (Mancuso *et al*., 2021). *Escherichia coli*, por exemplo, é uma bactéria Gram-negativa da família Enterobiaceae, sendo o microrganismo facultativo mais abundante no sistema

gastrointestinal de humanos e animais. Normalmente, *E. coli*, coloniza de forma comensal o intestino grosso de mamíferos, raramente causando doenças em indivíduos saudáveis, exceto em indivíduos imunocomprometidos ou onde as barreiras gastrointestinais normais são quebradas (Kaper; Nataro; Mobley, 2004). *E. coli* é um microrganismo extremante versátil, tendo além das cepas comensais, aquelas patogênicas capazes de causar diversos tipos de doenças, normalmente caracterizadas em doenças intestinais ou extra intestinais, e classificadas de acordo com sua virulência (Paitan, 2018; Poirel *et al*., 2018). Diversos fatores de resistência estão associados a *E. coli*, como a inativação de antibióticos, bombas de efluxo, mutações no alvo do antibiótico, "by-pass" (desvio de alvo), dentre outros (Paitan, 2018).

Staphylococcus aureus é uma bactéria Gram-positiva, pertencente à família Staphylococcaceae. Dentre os diversos tipos de microrganismos, *S. aureus* é o mais relevante do ponto de vista clínico. Esse microrganismo está presente na microbiota comensal da mucosa nasal em aproximadamente 20-40 % da população em geral. Quando ocorre a ruptura das barreiras cutâneas e mucosas, como resultado de condições crônicas da pele, feridas ou procedimentos cirúrgicos, *S. aureus* tem a oportunidade de invadir os tecidos subjacentes ou entrar na corrente sanguínea, podendo desencadear uma infecção (Lee *et al*., 2018). Um dos modos pelos quais o *S. aureus* desenvolve resistência é gerando moléculas alvo alternativas que não são afetadas pelo antibiótico. Ao mesmo tempo, persiste na produção das moléculas alvo originais, evitando assim a inibição causada pelo antibiótico, como é o caso da resistência à Meticilina (Loureiro et al., 2016).

A bactéria *P. aeruginosa* é classificada como Gram-negativa e tem uma morfologia de bastonete, apresentando-se como um organismo asporogênico e com um único flagelo. *P. aeruginosa* é amplamente distribuída no ambiente e possui a habilidade de sobreviver em diversas condições ambientais. Além de provocar enfermidades em plantas e animais, essa bactéria também é patogênica para seres humanos, ocasionando infecções severas em indivíduos com sistema imunológico comprometido, como pacientes oncológicos, bem como em pacientes com queimaduras graves e portadores de fibrose cística (Wu *et al*., 2015). Quando comparada às outras espécies de bactérias, *P. aeruginosa*, é especialmente considerada difícil de erradicar, uma vez que apresenta uma alta taxa de resistência intrínseca a diversos grupos de antibióticos (Breidenstein; Fuente-Núñez; Hancock, 2011). Os principais mecanismos de resistência incluem a superexpressão de bombas de efluxo, a redução da

permeabilidade da membrana externa e a aquisição ou mutação de genes de resistência (Mancuso *et al*., 2021).

2.7.2 Fungos

Os fungos abrigam uma variedade de organismos, incluindo bolores, cogumelos, liquens, ferrugens e leveduras. Esses eucariotos possuem histórias de vida muito distintas e desempenham papéis cruciais na biosfera, bem como na indústria, na medicina e na pesquisa científica (Stajich *et al*., 2009). Os fungos podem apresentar diferentes tamanhos e formas, mas, de modo geral, podem ser divididos em três grupos: fungos multicelulares filamentosos, fungos unicelulares e fungos dimórficos (aqueles capazes de mudar para a forma multicelular ou unicelular dependendo das condições do ambiente) (Campbell; Johnson, 2013). Os fungos desempenhando um papel fundamental na decomposição de matéria orgânica, no entanto, embora muitos sejam importantes para a ciclagem de elementos, outros podem ser responsáveis por doenças em plantas, animais e seres humanos, resultando em impactos adversos na vida humana (Hyde *et al*., 2019).

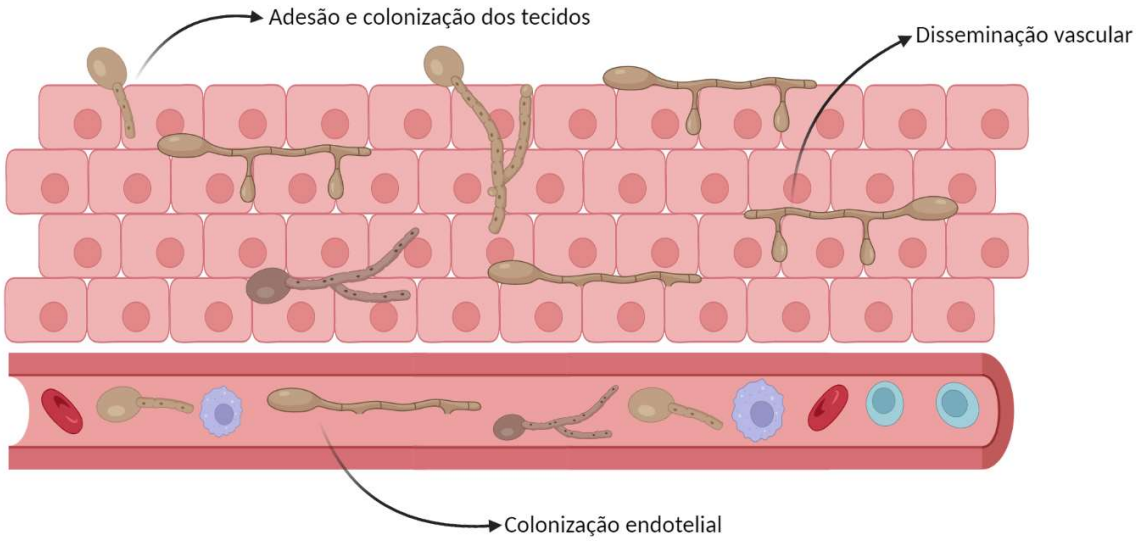
Existem cerca de 600 espécies de fungos que são conhecidas por causarem doenças humanas, que podem variar de infecções comuns a fatais (Brown; Denning; Levitz, 2012). Dentre os principais fungos que atuam como agentes de infecções, estão *Candida* spp., *Aspergillus* spp., e *Cryptococcus* spp. Estes, são responsáveis por mais de 90 % das mortes relacionadas às infecções micóticas (Lee *et al*., 2020).

Dentre os fungos patogênicos que mais acometem os seres humanos, estão os pertencentes ao gênero *Candida*. Os fungos pertencentes a esse gênero são unicelulares, leveduras e com um processo de reprodução por brotamento (Sidrim; Rocha, 2010). Esses fungos são adquiridos por transmissão vertical da mãe para o filho durante o nascimento, e formam uma associação vitalícia com o hospedeiro (Neville; d'Enfert; Bougnoux, 2015). Fungos desse gênero colonizam a pele, mucosas e tratos gastrointestinal e reprodutivo, podendo causar infecções superficiais (pele e mucosas) e invasivas (órgãos internos) (Romo; Kumamoto, 2020). Apesar da alta frequência, infecções superficiais por *Candida* spp. não são letais, ao contrário das infecções sistêmicas que estão associadas a uma alta taxa de mortalidade (Mayer; Wilson; Hube, 2013).

Dentre as espécies do gênero *Candida*, *C. albicans* se destaca como sendo a espécie de maior prevalência em infecções fúngicas. O sucesso das infecções por *C. albicans*, está diretamente associado aos seus mecanismos de virulência, os quais incluem tigmotropismo (capacidade de detectar e responder a estímulos mecânicos), a expressão de adesinas e invasinas (produção de proteínas que desempenham papéis-chave na aderência e invasão das células hospedeiras), a formação de biofilmes (comunidades de fungos que aderem a superfícies e se envolvem em uma matriz protetora) e a transição morfológica de levedura para hifas (Mayer; Wilson; Hube, 2013; Nicholls et al., 2011).

A mudança na morfologia de levedura para hifas é um mecanismo de extrema importância durante o processo infeccioso, uma vez que é através dessa transformação de células de levedura para formas filamentosas que ocorre a invasão dos tecidos. Inicialmente, essa invasão envolve a penetração das mucosas, seguida pela invasão dos tecidos subjacentes e, posteriormente, a entrada na corrente sanguínea, no caso de candidemia (Figura 5) (Kadosh; Mundodi, 2020).

Figura 5. Patogênese da invasão e virulência das leveduras de espécies do gênero



Candida.

Fonte: Bezerra, 2022

Apesar de *C. albicans* ser a espécie mais conhecida do gênero, outras espécies *Candida* não-*albicans*, têm ganhado destaque devido as suas altas taxas de incidência e

resistência, como *C. tropicalis* e *C. krusei*. Durante muitos anos, *C. tropicalis* foi considerada a segunda espécie mais relevante em termos de virulência e importância clínica (Chai; Denning; Warn, 2010). Atualmente, as espécies de *Candida* não-*albicans* com maior prevalência são *C. glabrata* (18,7 %), *C. parapsilosis* (15,9 %), *C. tropicalis* (9,3 %), e *C. krusei* (2,8 %) (Pfaller *et al*., 2019).

No que diz respeito aos fatores de virulência, *C. tropicalis* e *C. krusei* compartilham semelhanças com *C. albicans*, uma vez que empregam mecanismos similares (Jamiu *et al*., 2020; Zuza-Alves; Silva-Rocha; Chaves, 2017). Contudo, é importante observar que *C. krusei* apresenta uma transição morfológica da forma de levedura para pseudohifas, ao contrário das outras espécies, que desenvolvem hifas verdadeiras. No contexto da virulência, a formação de pseudohifas em *C. krusei* é um fator significativo. No entanto, é válido ressaltar que as hifas de *C. albicans* demonstram maior eficiência na colonização e penetração de tecidos em comparação com as pseudohifas de *C. krusei* (Jamiu *et al*., 2020).

3 ARTIGO 1 – CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF *Cordiera myrciifolia* LEAVES AGAINST PATHOGENIC BACTERIA AND FUNGI: DRUG POTENTIATION ABILITY AND INHIBITION OF VIRULENCE

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Abstract: *Cordiera myrciifolia* is an abundant species in Northeast Brazil that presents metabolites of biological/therapeutic interest. From this perspective, the present study aimed to investigate the chemical constituents and evaluate the *in vitro* antimicrobial activity of hexane (HECM) and ethanolic (EECM) extracts of *C. myrciifolia* leaves. The extracts were analyzed by chromatographic techniques (GC and UPLC) coupled with mass spectrometry. The antimicrobial activity of the extracts and the extracts combined with conventional drugs was evaluated by microdilution. The *in vitro* effect of the treatments on *Candida*'s morphological transition was verified through

cultivation in humid chambers. In HECM, 11 constituents including fatty acids, and triterpenes, including phytosterols, alkanes, tocols, and primary alcohols were identified. Triterpenes represented more than 40% of the identified constituents, with Lupeol being the most representative. In EECM, 13 constituents were identified, of which eight belonged to the class of flavonoids. High antibacterial activity of HECM was detected against *Escherichia coli* and *Staphylococcus aureus*, with Minimum Inhibitory Concentrations of 8 and 16 µg/mL, respectively. The combined activity was more effective when combined with Norfloxacin and Imipenem. In anti-*Candida* activity, the IC_{50} of the extracts ranged from 36.6 to 129.1 µg/mL. There was potentiating effect when associated with Fluconazole. Both extracts inhibited the filamentous growth of *C. tropicalis* at a concentration of 512 µg/mL. *C. myrciifolia* extracts prove to be candidates for the development of new therapeutic formulations to treat bacterial and fungal infections.

Keywords: café-bravo; *Alibertia myrciifolia*; multidrug resistance bacteria; fungal virulence

1. Introduction

Infections caused by drug-resistant microorganisms, such as bacteria and fungi, are a major global public health problem, being the leading cause of death worldwide [1]. Certain microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, stand out as opportunistic and contribute to deaths associated with antibiotic resistance [2]. Among fungi, *Candida albicans* is the pathogen that most commonly causes infections. However, other species of *Candida* have been progressively emerging as opportunistic pathogens [3].

Many efforts have been made to find new substances of natural origin to reverse microbial resistance, either through their intrinsic action or through their combined action with antibiotics [4,5]. Plants are sources of various substances that confer medicinal properties to them. Therefore, for thousands of years, medicinal plants have been part of human therapeutic practices. Despite recent advances in synthetic drug production, plants remain a valuable source for drug development [6].

The therapeutic potential of plant species depends on the presence of biologically active metabolites and the beneficial effects they cause in the human body [7]. In this regard, the Rubiaceae family stands out due to the presence of biologically

active secondary metabolites [8]. Various classes of substances and their bioactivities have already been documented for species of Rubiaceae [9]. The antimicrobial activity of some species within this family has promising results [10–13].

The genus *Cordiera* A.Rich. ex DC. comprises approximately 23 species distributed from Costa Rica to southern Brazil and Argentina [14]. Some of its representatives, such as *Cordiera edulis* (Rich.) Kuntze [15], *Cordiera macrophylla* (K. Schum.) Kuntze [15], and *Cordiera sessilis* (Vell.) Kuntze [15–17], are widely used by traditional Brazilian communities for the treatment of helminthiasis, flu, diarrhea, and toothache. These ethnomedicinal uses drive the development of experimental research to evaluate the pharmacological potential of products obtained from plants of this genus.

Cordiera myrciifolia (K. Schum.) C. Persson & Delprete, synonym *Alibertia myrciifolia* Sprunge ex. Schum. is commonly known as "café-bravo" that grows abundantly in Northeast Brazil, especially in the Araripe Basin (state of Ceará) [18]. Phytochemically, the species contains flavonoids, iridoids, and triterpenes as some of its main constituents. [18–20]. Despite presenting compounds of pharmacological interest, few studies have evaluated the biological activities of *C. myrciifolia* [18,20,21]. According to Luciano et al. [18], the isolated geniposidic acid from this plant inhibited the development of the mycelial mass of *Colletotrichum gloeosporioides*, a phytopathogenic fungus known to affect the productivity of various agricultural crops [22,23]. However, to date, the antimicrobial effects of *C. myrciifolia* against microorganisms that affect human and animal health have not yet been reported in the literature.

From this perspective, the present study aimed to investigate, for the first time, the chemical constituents and the *in vitro* biological potential of hexane (HECM) and ethanolic (EECM) extracts of *C. myrciifolia* leaves against pathogenic bacteria and fungi that cause diseases in humans and animals. The modulatory effect of these extracts in combination with conventional antimicrobials was also evaluated in this study.

2. Material and Methods

2.1 Plant material

Leaves of *Cordiera myrciifolia* were collected in the Araripe National Forest - Flona, in the state of Ceará, municipality of Barbalha, under the coordinates 7°23'1.39"S

and 39°21'7.36" W at an altitude of 924 m. The species was identified, and a voucher specimen was deposited in the Dárdano de Andrade-Lima Herbarium at the Regional University of Cariri - URCA, with the identification number 15.005. Previously, this study was registered in the Biodiversity Authorization and Information System (SISBio - Brazil) and the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen - Brazil) under registration numbers 77744 and A236850, respectively.

2.2 Plant extraction

The leaves of *C. myrciifolia* (200 g) were crushed and subjected to maceration with exhaustive extraction with *n*-hexane for 72 hours to obtain an extract predominantly composed of low-polar compounds. After filtration, the residue underwent a new extraction with ethanol for 72 hours. After solvent removal in a rotary evaporator, the hexane (HECM) and ethanolic (EECM) extracts were stored for phytochemical analysis and *in vitro* antimicrobial tests. The yields of HECM and EECM extracts were 3.14 g and 9.53 g, respectively.

2.3 Gas Chromatography coupled to Mass Spectrometry (GC-MS)

A defined amount of HECM was solubilized in *n*-hexane (1:1 v/v), and the extract was filtered over anhydrous sodium sulfate. An aliquot of 1 mL of HECM was further filtered through a 0.22 nm PTFE filter into a vial with a capacity of 2 mL. The solvent was completely evaporated under nitrogen gas, and the extract was prepared for analysis through a derivatization process, employing a silylation reaction. In the vial, 100 µL of N, O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Sigma-Aldrich), and 70 µL of pyridine were introduced [24]. The mixture was heated in a water bath at 80 °C for a period of 1 h. HECM was analyzed by gas chromatography coupled with mass spectrometry (GCMS–TQ8040 NX, Shimadzu, Kyoto, Japan) using a Shimadzu SH–5MS column (30 m x 0.25 mm x 0.25 µm). The following operational conditions were established: initial oven temperature of 150 °C (maintained for 5 min), increase of 10°C/min up to 300 °C (maintained for 20 min). The total analysis time was 40 min. The injector, ion source, and interface temperatures were set at 250, 250, and 300 °C, respectively. Electron impact ionization was used at 70 eV in full-scan acquisition mode, ranging between 45–750 m/z. Identification of constituents was performed by

comparing the mass spectra of each detected compound with the NIST 2020 library (National Institute of Standards and Technology, Gaithersburg, MD, USA).

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

The EECM was analyzed using an Acquity UPLC (Waters, USA) system coupled to a mass system (Q–TOF, Waters). A Waters Acquity BEH C18 column for separation condition (150 mm × 2.1 mm, 1.7 μm) was set at 40 °C. An injection volume of 5 μL aliquot of EECM 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): 2–100% B (22.0 min), 100% B (22.1-25.0 min), 2% B (26.0-30 min) with a flow rate of 0.3 mL/min [25].

Ionization was performed using an electrospray ionization source in negative mode (ESI), acquired in the range of 110–1180 Da and the optimized instrumental parameters were as follows: capillary voltage at 3.2kV, cone voltage at 15 V, source temperature at 120 ºC, desolvation temperature at 350 ºC, desolvation gas flow at 500 L/ h. The MassLynx 4.1 software (Waters MS Technologies, Manchester, United Kingdom) software was used to control the system and assign the exact masses, as well as the molecular formula of the compounds. Data were analyzed via comparison with those described in the literature and peak identification was determined by the *m/z* values.

2.5 Antibacterial activity

For the *in vitro* antibacterial assay, 10 mg of HECM and EECM were used and diluted in 500 μL of dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany). These solutions were placed in Falcon tubes and distilled water was added to reach an initial concentration of 1024 μg/mL. The reference antibiotics used were Gentamicin, Norfloxacin, and Imipenem (Sigma-Aldrich), all at a concentration of 1024 μg/mL diluted in distilled water. All analysis were performed in triplicate.

The bacterial cultures (*Escherichia coli* ATCC 25922, *Escherichia coli* 06, *Staphylococcus aureus* ATCC 22923, *Staphylococcus aureus* 10, *Pseudomonas aeruginosa* ATCC 25924, and *Pseudomonas aeruginosa* 24) were grown on Heart

Infusion Agar (HIA) medium and placed in an incubator at 37 °C for 24 h. After this period, using a nickel-chromium inoculation loop, the bacterial strains were suspended in test tubes containing 3 mL of sterile NaCl (0.9%). Following this procedure, the turbidity of the solution was adjusted with the McFarland standard solution to 0.5 McFarland, equivalent to 10^5 CFU/mL. Eppendorf tubes were prepared in triplicate for each bacterium and antibiotics (Gentamicin, Norfloxacin, and Imipenem), each containing 1350 µL of 10% Brain Heart Infusion (BHI) + 150 µL of the inoculum for MIC testing.

2.5.1 Minimum Inhibitory Concentration - MIC

To determine the Minimum Inhibitory Concentration (MIC), standard strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 22923, and *Pseudomonas aeruginosa* ATCC 25924) and multidrug-resistant strains (*Escherichia coli* 06, *Staphylococcus aureus* 10, and *Pseudomonas aeruginosa* 24) were used, whose resistance profile can be checked in Rodrigues et al. (2022). For drug potentiation assays, only the multidrug-resistant strains were utilized.

Brain Heart Infusion (BHI) culture medium and inoculum (10%) were prepared for distribution in a 96-well microdilution plate. Aliquots of 100 µL of this solution were added to each well, followed by serial dilution of 100 µL of HECM and EECM with concentrations ranging from 512 to 4 µg/mL. Commercial antibiotics (Gentamicin, Norfloxacin and Imipenem) were used as positive controls at the same concentrations as the extracts and applied only to multidrug-resistant strains, since these pathogens were used for drug potentiation assays. Subsequently, the plates were transferred to a bacteriological oven for a period of 24 hours at 37 ºC [26]. Antibacterial activity was detected by adding 20 µL of sodium resazurin solution (Sigma-Aldrich) to each well at the end of the incubation period. Bacterial growth was monitored by the irreversible reduction of resazurin, characterized by a color change from blue to pink. The MIC was determined based on the lowest concentration that inhibited microbial growth [27].

2.5.2 Antibiotic Potentiation Effect

In this assay, the HECM and EECM were tested at sub-inhibitory concentrations (MIC/8) [28]. Eppendorf tubes containing culture medium, 10% inoculum and both extracts at subinhibitory concentrations were prepared. In each well of the plates, 100 µL aliquots of this solution were added. Subsequently, 100 µL of conventional

antibiotics were mixed and serial dilutions were performed from the first to the penultimate well of the plates (1 to 512 µg/mL). The determination of the modulating effect was carried out by adding 20 µL of resazurin aqueous solution [29].

2.6 Antifungal activity

For the *in vitro* antifungal assay, 10 mg of HECM and EECM were dissolved in 1 mL of DMSO and subsequently in *Sabouraud Dextrose Broth* (SDB) culture medium to obtain a stock concentration of 1024 µg/mL. The reference antifungal used was Fluconazole (capsule-FLUCOMED), diluted under the same conditions as the extracts. All analysis were performed in quadruplicate.

For the *in vitro* antifungal assay, standard strains of *Candida albicans* (INCQS 40006), *Candida tropicalis* (INCQS 40042), and *Candida krusei* (INCQS 40095) were used. The culture media used were SDB, *Sabouraud Dextrose Agar* (SDA), and *Potato Dextrose Agar* (PDA) (Difco, BD Diagnostic Systems, Sparks, MD, USA).

2.6.1 Determination of Fungal Growth Curve and 50% Inhibitory Concentration - IC_{50}

The 50% inhibitory concentration (IC_{50}) was determined according to the method described by the Clinical and Laboratory Standards Institute [30]. The yeasts (*Candida albicans* INCQS 40006, *Candida tropicalis* INCQS 40042, and *Candida krusei* INCQS 40095) were inoculated in SDA and incubated for 24 h at 35 ºC. Aliquots of the yeast were transferred to test tubes containing sterile saline solution (NaCl 0.9%) and compared with the McFarland scale [31]. Subsequently, they were dissolved in SDB resulting in a concentration of $1–5\times10^3$ colony-forming units (CFU)/mL.

Solutions containing SDB and extracts of *C. myrciifolia* at each concentration tested (1 to 512 µg/mL) were added to 96-well plates. To compare the different treatments, growth control, sterility control, and positive control with Fluconazole were included. The plates were incubated at 35 °C for 24 h in a bacteriological oven and, subsequently, read in an ELISA spectrophotometer (Thermoplate®) at 630 nm [31]. The results obtained were expressed in a fungal growth curve and IC_{50}, which determines the concentration necessary to inhibit fungal growth by 50% [32].

2.6.2 Determination of Minimum Fungicidal Concentration (MFC)

The MFC was performed to determine if the HECM, EECM, and fluconazole affected the viability of *Candida*. An aliquot of 5 µL of the solution from each well of the IC_{50} test plate was removed and then transferred to Petri dishes containing solid SDA medium After 24 and 48 hours in a bacteriological oven, the plates were analyzed, and the concentration at which no growth of fungal colonies was observed was considered the MFC [33].

2.6.3 Antifungal Potentiation Effect

To evaluate the possible potentiating effects of Fluconazole, HECM and EECM were evaluated at subinhibitory concentrations based on the Matrix Concentration (MC/8), according to the method proposed by Coutinho et al. (2008), with modifications. Initially, aliquots of 100 µL of SDB and 100 µL of HECM and EECM were mixed and serially diluted in a 1:1 ratio up to the tenth well of the plates. In the last wells, the growth control and the culture medium sterility control were established, respectively [32]. The reference antifungal, Fluconazole, was added in a volume of 100 µL to each well of the plate. Finally, 20 µL of the fungal inoculum prepared according to CLSI was added [30].

2.6.4 Evaluation of fungal virulence inhibition

The micromorphology assay was used to determine whether the HECM and EECM cause changes in fungal morphology by inhibiting the emission of filamentous structures. The analysis was conducted following the method proposed by Morais-Braga et al. (2016), where microculture chambers were prepared for yeast observation. On the chamber slide, 3 mL of enriched PDA medium supplemented with bacterial agar-containing extracts at Matrix Concentrations (MC) of MC/2 = 512 µg/mL and MC/8 = 128 µg/mL, were dispensed [34].

Aliquots of the fungal inoculum were transferred from Petri dishes containing SDA to solidified PDA medium and incubated at 35 °C for 24 h. After incubation, morphological analysis was performed by optical microscopy to capture images using 4x and 10x objectives. A control for hyphal emission stimulated by the impoverished medium, as well as a control with Fluconazole, was used for comparative purposes [35].

2.7 Statistical analysis

All statistical analyses were performed using GraphPad Prism 6.0 Software (GraphPad Software, Inc., San Diego, CA). Data were analyzed using one-way ANOVA with the Tukey test. *p* values were set at < 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 > 0.05 (ns = not significant), according to the GraphPad Prism guide. IC_{50} values were obtained by nonlinear regression.

3. Results

3.1. GC-MS identification

The chromatographic analysis of the HECM (Table 1) identified 11 compounds, corresponding to 89.01% of the total identified composition. Triterpenes predominated in the extract, mainly lupeol (28.97). Fatty acids, terpenes, and alkanes were also identified. The chemical structures of the identified compounds can be seen in Fig. 1.

Table 1. Identification of phytoconstituents in the hexane leaf extract of *Cordiera myrciifolia* by gas chromatography and mass spectrometry (GC–MS).

Compounds	R_t (min)	Chemical Class	Abundance (%)
Hexadecanoic acid*	14.10	Fatty acid	2.01
Octadecanoic acid*	16.07	Fatty acid	0.17
Squalene	21.09	Triterpene	10.25
Nonacosane	21.57	Alkane	14.38
Hentriacontane	23.67	Alkane	8.90
α–Tocopherol*	24.68	Tocol	8.30
Triacontanol*	27.22	Fatty alcohol	3.41
β–Sitosterol*	28.37	Phytosteroid	3.55
β–Amyrin*	28.56	Triterpene	3.60
Lupeol*	29.51	Triterpene	28.97
Docotriacontanol*	31.61	Fatty alcohol	5.47
Identified			89.01
Not identified			10.99

* Compounds identified as TMS derivatives.

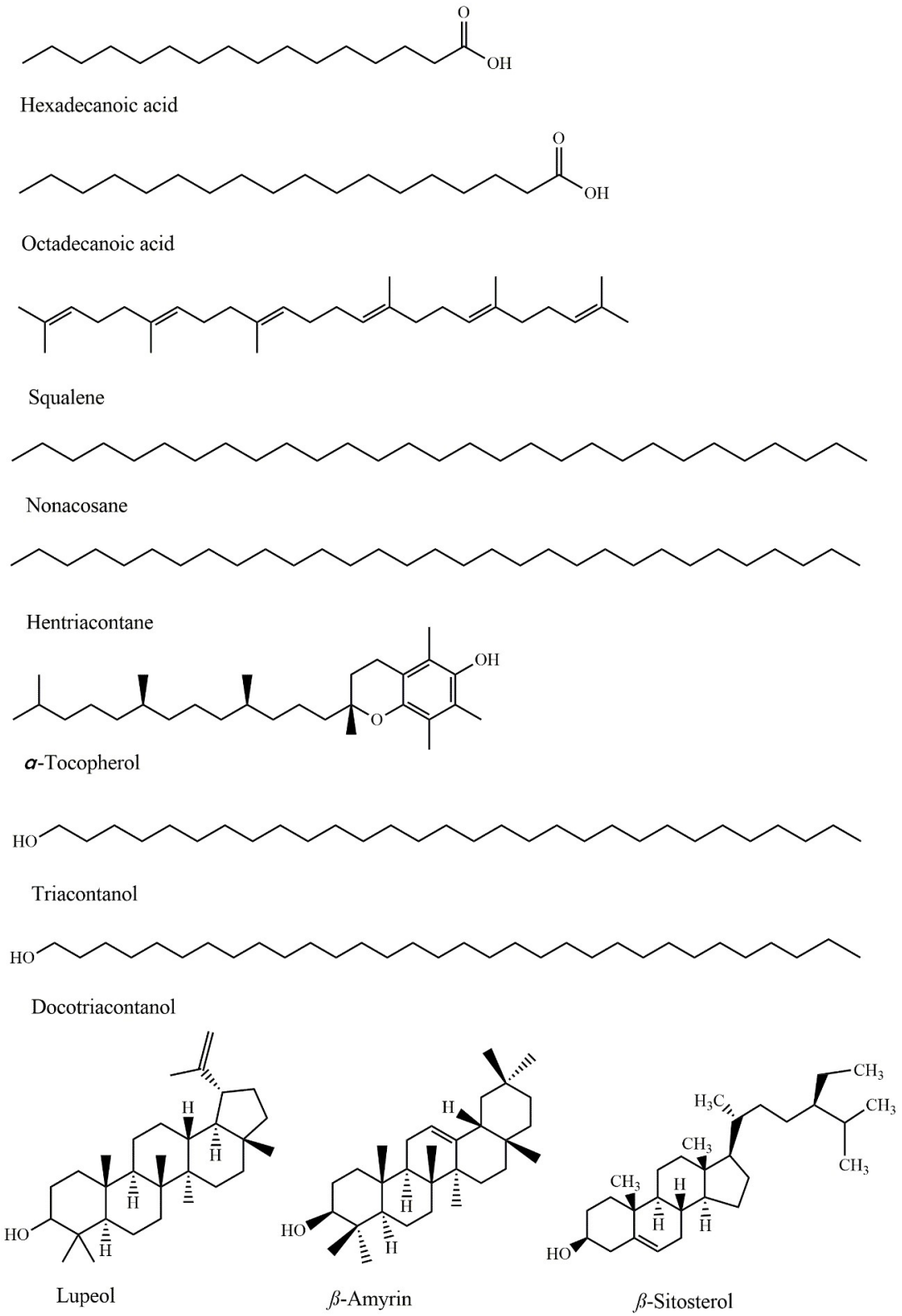

Fig. 1. Chemical structures of the compounds identified in the hexane extract of *Cordiera myrciifolia* by GC-MS.

3.2. UPLC–MS^E identification

Table 2 presents the chemical composition of the EECM. A total of 13 chemical constituents were identified. According to the obtained mass spectra, the EECM contains a polyol (quinic acid, 1), a glycosylated iridoid (geniposidic acid, 2), a phenylpropanoid (chlorogenic acid, 3), and eight flavonoids: myricetin-3-*O*-rhamnosylglucoside (4), rutin (5), quercetin derivative (6), kaempferol-*O*-hexose-deoxyhexose (7), isorhamnetin-*O*-rutinoside (8), kaempferol-*O*-rhamnoside (9), dihydroxy-trimethoxyflavone (12), and dihydroxy-methoxyisoflavone (13). Additionally, two compounds were not identified (10 and 11). Figure 2 shows the chemical structures of the identified compounds.

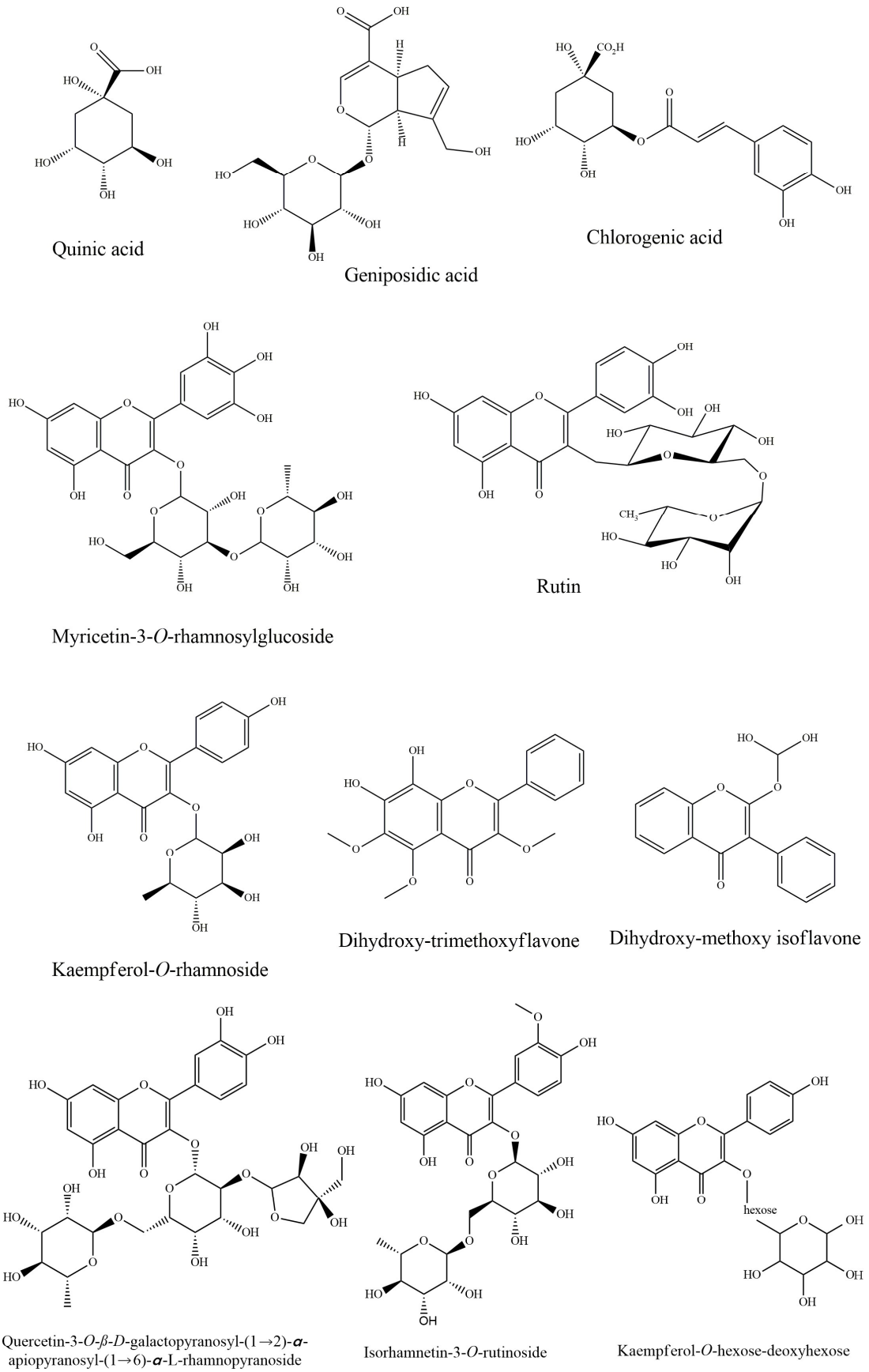

Fig. 2. Chemical structures of the compounds identified in the ethanolic extract of *Cordiera myrciifolia* by UPLC-ESI-QTOF-MS^{E}.

Table 2. Identification of compounds by UPLC-ESI-QTOF-MS^E in negative ionic mode from ethanolic extract from *Cordiera myrciifolia*

Peak	Rt min	[M-H]$^-$ Observed	[M-H]$^-$ Calculated	Product Ions (MS/MS)	Empirical Formula	Ppm (error)	Putative Name	References
1	1.20	191.0554	191.0556	-	$C_7H_{11}O_6$	-1.0	Quinic acid	[36,37]
2	3.12	373.1121	373.1167	211, 149, 123	$C_{16}H_{21}O_{20}$	-3.8	Geniposidic acid	[38,39]
3	4.21	353.0872	353.0873	191	$C_{16}H_{17}O_9$	-0.3	Chlorogenic acid	[37,40]
4	5.20	625.1395	625.1405	317, 316	$C_{27}H_{29}O_{17}$	-1.6	Myricetin-3-*O*-rhamnosylglucoside	[41,42]
5	5.33	741.1860	741.1878	301	$C_{32}H_{37}O_{20}$	-2.4	Quercetin-3-*O*-*β*-*D*-galactopyranosyl-(1→2)-*α*-apiopyranoside-(1→6)-*α*-L-rhamnopyranoside	[43]
6	5.76	609.1433	609.1456	301, 300	$C_{27}H_{29}O_{16}$	-3.8	Rutin	[39,44,45]
7	6.29	593.1501	593.1506	285	$C_{27}H_{29}O_{16}$	-0,8	Kaempferol-*O*-hexose-deoxyhexose	[46]
8	6.43	623.1636	623.1612	315	$C_{28}H_{31}O_{16}$	3.9	Isorhamnetin-*O*-rutinoside	[47]
9	7.29	431.0981	431.0978	285	$C_{21}H_{19}O_{10}$	0.7	Kaempferol-*O*-rhamnoside	[48]
10	9.68	1087.5687	1087.5689	763	$C_{54}H_{87}O_{22}$	3.1	Unknown	-
11	10.84	925.5160	925.5161	763, 455	$C_{48}H_{77}O_{17}$	-0.1	Unknown	-
12	11.67	343.0830	343.0818	328, 313	$C_{18}H_{15}O_7$	3.5	Dihydroxy-trimethoxyflavone	[49]
13	12.10	283.0611	283.0606	268	$C_{16}H_{11}O_5$	1.8	Dihydroxy-methoxy isoflavone	[50]

3.3. Antibacterial activity

3.3.1. Minimum Inhibitory Concentration – MIC

The antibacterial activity of the extracts was observed exclusively against two standard strains (Table 2). The HECM showed antimicrobial activity against *E. coli* and *S. aureus* strains, with MIC values of 8 µg/mL and 16 µg/mL, respectively. On the other hand, the ethanolic extract did not exhibit antibacterial activity at any of the tested concentrations.

Table 3. Minimum Inhibitory Concentration (MIC) in µg/mL of *Cordiera myrciifolia* extracts.

Strains	**Extracts (µg/mL)**	
	HECM	**EECM**
E. coli ATCC 25922	8	> 512
S. aureus ATCC 22923	16	> 512
P. aeruginosa ATCC 25924	> 512	> 512
E. coli 06	> 512	> 512
S. aureus 10	> 512	> 512
P. aeruginosa 24	> 512	> 512

HECM: Hexane extract of *Cordiera myrciifolia*; EECM: Ethanolic extract of *Cordiera myrciifolia*.

3.3.2. Potentiation of Antibiotic Effect

Fig. 3 illustrates the interaction between HECM and different antibiotics against multidrug-resistant bacterial strains. The HECM did not show significant antibacterial activity when combined with the antibiotics Gentamicin and Imipenem against *P. aeruginosa*. Moreover, it reduced the effectiveness of Norfloxacin. For *E. coli*, only the combination with Norfloxacin showed significant results. The combination of the HECM with Imipenem significantly increased the antimicrobial potential of this antibiotic against *S. aureus*. However, when combined with Gentamicin and Norfloxacin, the hexane extract reduced the effectiveness of these antibiotics against *S. aureus.*

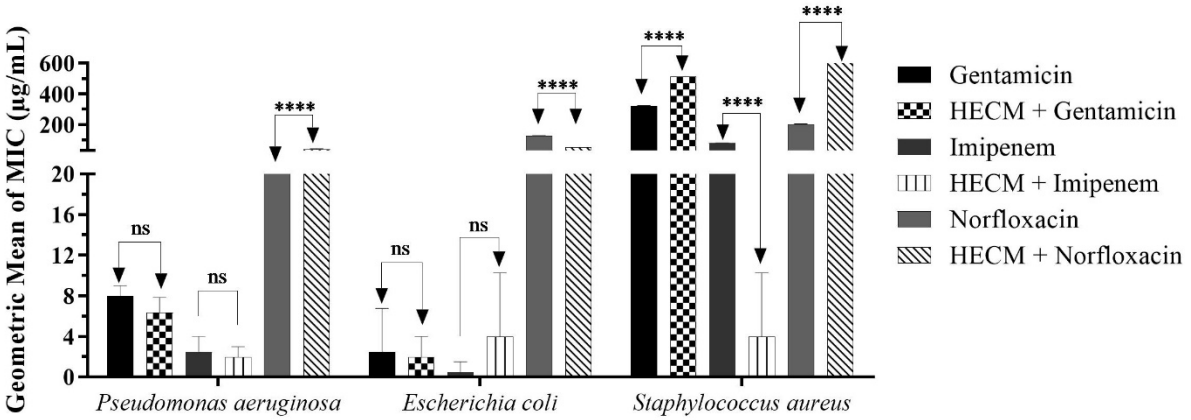

Fig 3. Minimum inhibitory concentration (MIC) of isolated antibiotics and in combination with the leaf hexane extract of *Cordiera myrciifolia* (HECM) against multidrug-resistant bacterial strains. ns = not significant ($P > 0.05$), **** = $P < 0.0001$. Bars represent the standard deviation (n = 3).

In Fig. 4, the results of the combination between EECM and different antibiotics against multidrug-resistant bacterial strains are presented. It was observed that Norfloxacin combined with EECM was the only tested antibiotic that showed significant results against all three resistant bacterial strains.

On the other hand, both Gentamicin and Imipenem in combination did not show significant results against *P. aeruginosa* and *E. coli*. Regarding *S. aureus*, distinct interactions occurred. The combination of the ethanolic extract with Gentamicin increased MIC, indicating an antagonistic effect. On the other hand, the combination with Imipenem reduced the MIC values, indicating a potentiating effect.

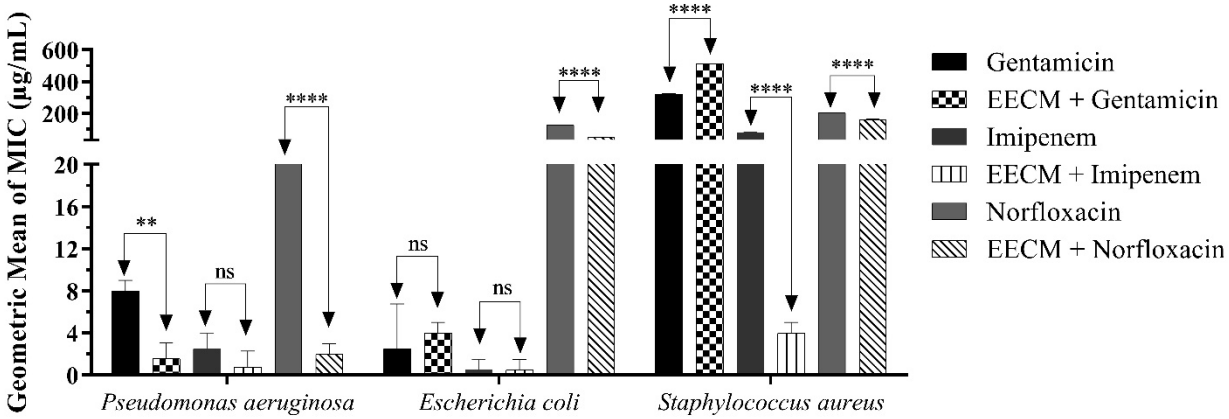

Fig. 4. Minimum Inhibitory Concentration (MIC) of antibiotics alone and in combination with the ethanolic leaf extract of *Cordiera myrciifolia* (EECM) against multidrug-resistant

bacterial strains. ns = not significant ($P > 0.05$), **** = $P < 0.0001$. Bars represent the standard deviation (n = 3).

3.4. Antifungal activity

3.4.1. $IC_{50,}$ MFC, and Fungal Growth Curve

The IC_{50} values of HECM and EECM were higher when compared to Fluconazole (Table 4). Nevertheless, the extracts exhibited antifungal activity at concentrations ranging from 36.6 to 129.1 µg/mL. When combined with Fluconazole, both extracts did not reduce the IC_{50} of the conventional antifungal against strains of *C. albicans* and *C. krusei*. Against *C. tropicalis*, the IC_{50} values of the combined drugs were slightly lower than those obtained with Fluconazole. Due to the absence of inhibition at the evaluated concentrations, the MFC of the tested extracts, as well as Fluconazole, is above 512 µg/mL.

Table 4. The concentration that inhibits 50% of the fungal growth (IC_{50}) of *Cordiera myrciifolia* extracts and Fluconazole against *Candida* strains

Treatments	Strains		
	CA INCQS 40006	CT INCQS 40042	CK INCQS 40095
FCZ	2.8	1.6	36.5
HECM	129.1	69.2	75.0
FCZ + HECM	3.7	1.1	76.6
EECM	40.3	94.9	36.6
FCZ + EECM	3.3	1.2	65.6

CA – *C. albicans*; CT – *C. tropicalis*; CK – *C. krusei*; FCZ – Fluconazole; HECM - Hexane extract of *Cordiera myrciifolia*; EECM – Ethanolic extract of *Cordiera myrciifolia*

Regarding the fungal growth curve as a function of concentration, it is possible to observe that *C. tropicalis* and *C. krusei* exhibited greater susceptibility to the HECM, showing a notable reduction in growth from the concentration of 64 µg/mL (Figs. 5B and 5C). In contrast, *C. albicans* demonstrated this reduction in growth only when exposed to the concentration of 128 µg/mL (Fig. 5A). On the other hand, *C. albicans* and *C. tropicalis* strains showed greater sensitivity to the combination with Fluconazole, leading to a decrease in fungal growth at concentrations of 4 and 2 µg/mL, respectively (Figs. 5A and 3B).

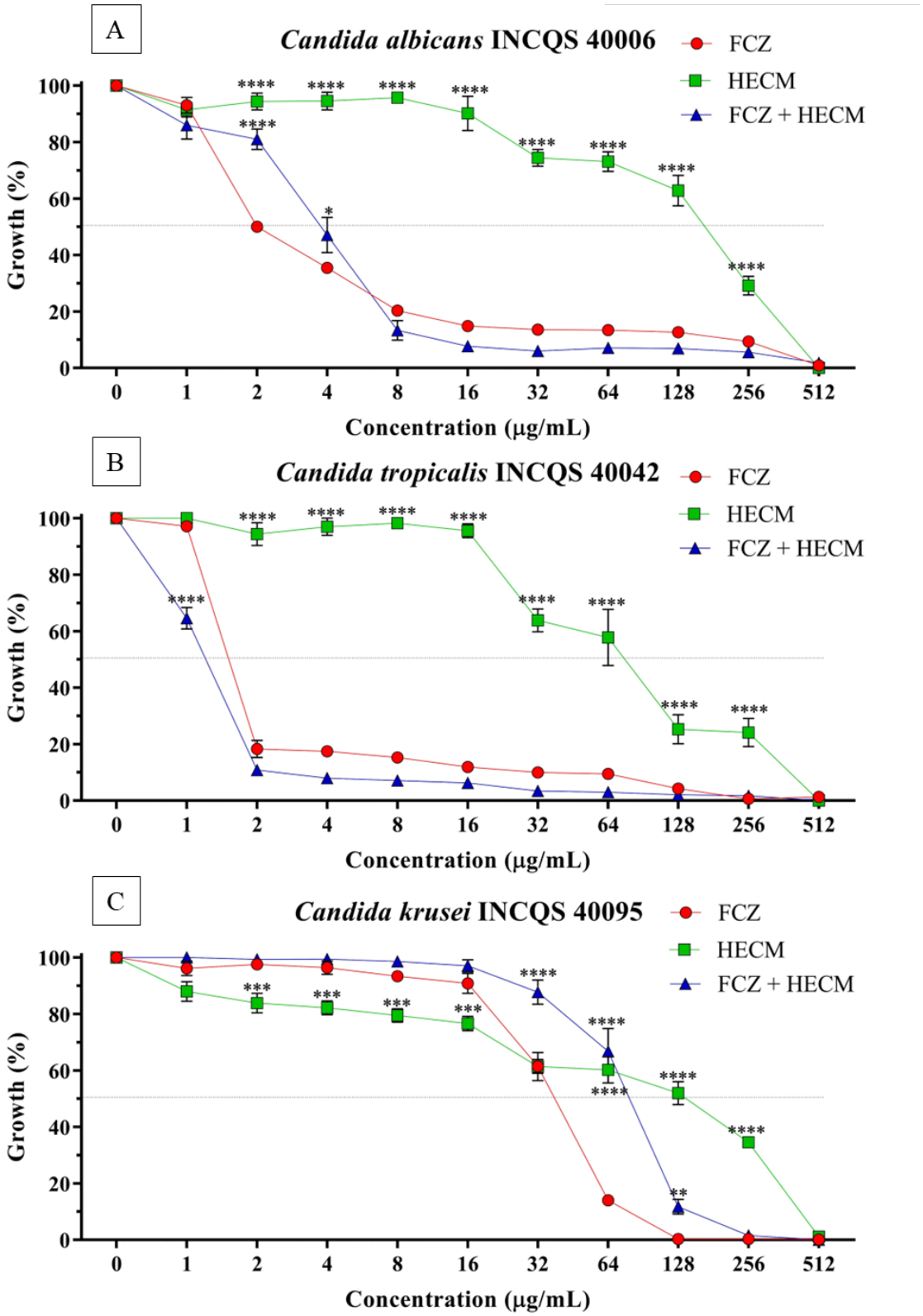

Fig. 5. Fungal growth curve and IC_{50} value (dotted line) of different concentrations of hexane extract of the leaves of *Cordiera myrciifolia* (HECM), Fluconazole (FCZ), and their combination against *Candida* strains. The bars represent the standard error of the mean (n = 4). ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$ compared to Fluconazole.

Regarding the EECM, a decrease in the fungal growth curve was observed from the concentration of 32 µg/mL for *C. albicans* (Fig. 6A). In contrast, for *C. tropicalis*, the concentration at which this reduction was observed was only from 128 µg/mL, suggesting lower sensitivity of *C. tropicalis* to the ethanolic extract (Fig. 6B). Regarding *C. krusei*, its greater susceptibility to the ethanolic extract is highlighted, with growth inhibited from 16 µg/mL (Fig. 6C). However, when in association, it was observed that the growth of *C. krusei* was less impacted, with a reduction in growth only at the concentration of 128 µg/mL (Fig. 6C). The growth curve of *C. albicans* (Fig. 6A) and *C. tropicalis* (Fig. 6B) was reduced from concentrations of 4 and 2 µg/mL, respectively.

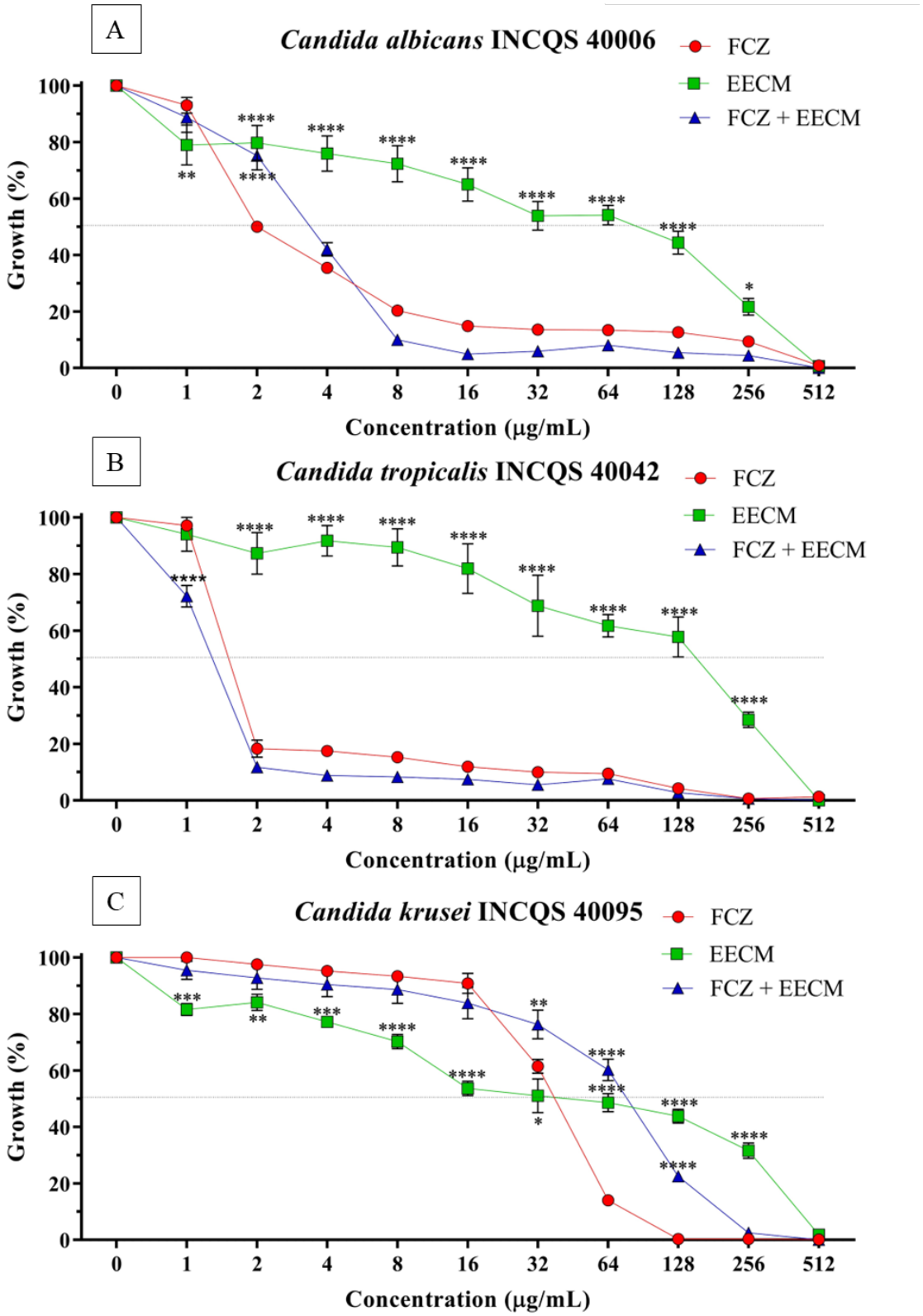

Fig. 6. Fungal growth curve and IC_{50} value (dotted line) of different concentrations of ethanolic extract of the leaves of *Cordiera myrciifolia* (EECM), Fluconazole (FCZ), and their combination against *Candida* strains. The bars represent the standard error of the mean (n = 4). * = $P < 0.05$, ** = $P < 0.01$, **** = $P < 0.0001$ compared to Fluconazole.

3.4.2. Inhibition of fungal virulence

Both extracts (HECM and EECM) at a concentration of 512 µg/mL were only effective against *C. tropicalis*, completely preventing the development of hyphae (Fig. 7). The conventional drug fluconazole was able to inhibit the development of hyphae, preventing the morphological transition of *C. albicans* and *C. tropicalis*. However, at the lowest concentration tested, this antifungal did not inhibit the development of hyphae in *C. krusei*.

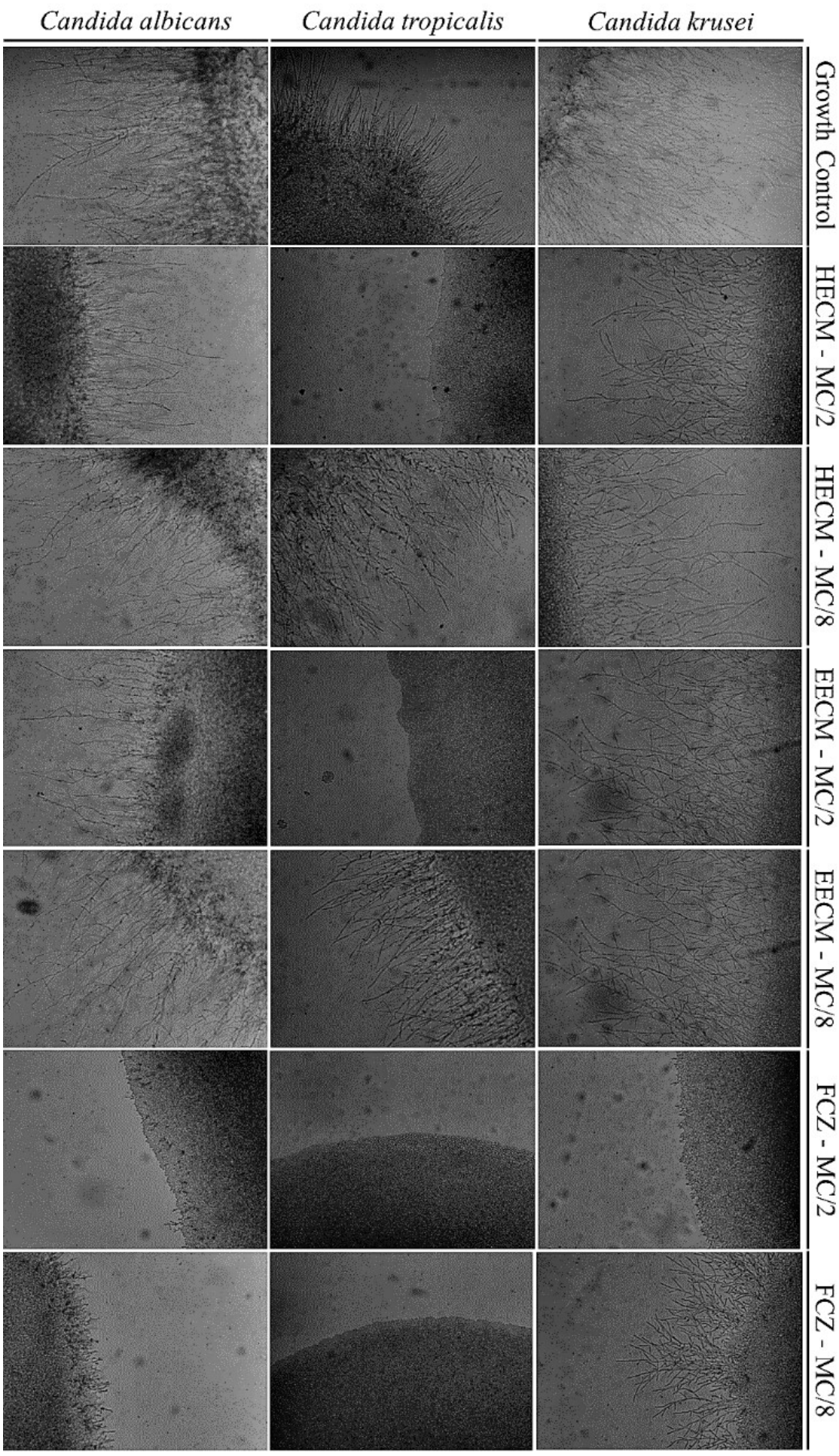
Candida albicans
Candida tropicalis
Candida krusei
Growth Control
HECM - MC/2
HECM - MC/8
EECM - MC/2
EECM - MC/8
FCZ - MC/2
FCZ - MC/8

Fig. 7. Effects of the hexanoic (HECM) and ethanolic (EECM) extracts of *Cordiera myrciifolia* on the dimorphism of *Candida albicans* INCQS 40006, *Candida tropicalis* INCQS 40042 and *Candida krusei* INCQS 40095 at 100× magnification.

4. Discussion

Chemically, Rubiaceae species are known for their secondary metabolites of significant biological/therapeutic value [8]. In this study, the selection of *C. myrciifolia* was made due to it being a representative of the family with significant phytochemical diversity [18,20], demonstrating significant potential. However, few biological activities have been explored with the species, particularly regarding its antimicrobial potential.

The chemical analysis of the HECM resulted in the identification of 11 constituents, none of which have been reported previously for the species. Among the identified constituents, only two, octadecanoic acid and hexadecanoic acid, have been previously identified in another species of the genus, *Cordiera sessilis* (Vell.) K. Schum [51]. As for the ethanolic extract of *C. myrciifolia*, a significant presence of flavonoids was observed. Among the identified compounds, only rutin and geniposidic acid had been previously identified in the species [18,21]. Previous studies have shown that extracts of *C. myrciifolia* are rich in flavonoids, notably flavones [19,20]. However, the flavones found in our study are being reported for the first time for the species.

For the genus *Cordiera*, only one study evaluated the antibacterial potential against aerobic and anaerobic bacteria. The essential oil and partitions of the ethanolic extract of *C. sessilis* demonstrated antimicrobial activity with MIC ranging from 50 to 400 µg/mL [51]. In our study, we observed high antibacterial activity of the HECM against two of the tested strains, *E. coli* and *S. aureus*, with MIC of 8 and 16 µg/mL, respectively. Our findings support studies indicating that non-polar extracts exhibit more pronounced antimicrobial properties compared to polar extracts [52–54]. Non-polar extracts, with a predominance of lipophilic compounds, may interact more intensely with the bacterial cell membrane, resulting in more significant cellular damage and compromising the integrity and cellular function of the pathogen. [55].

On the other hand, the EECM combined with antibiotics exerted greater antimicrobial activity, which may be related to the quantity of phenolic compounds in the extract. According to some studies, the combination of flavonoids and antibiotics may be a promising source for the development of new broad-spectrum therapies to help treat infections caused by resistant strains [56,57]. In the study conducted by Amim et al. [58], for example, isolated

flavonoids showed good activity against bacteria, but when combined with antibiotics, there was an enhancement of antimicrobial effects.

Regarding the antifungal potential, HECM and EECM, regardless of concentration, do not enhance the activity of Fluconazole. However, when evaluated individually, the extracts showed significant inhibitions with concentrations ranging from 36.6 to 129.1 µg/mL. According to Alves et al. [59], good activity against *Candida* is defined with values ranging from 26 to 125 µg/mL, and between 126 and 500 µg/mL, the activity is considered moderate. Although the extract did not achieve IC_{50} values better than Fluconazole, it may be an alternative for the treatment of *Candida* infections, as Fluconazole, being fungistatic, may favor the resistance of certain pathogens [5], in addition to presenting side effects[60].

Some of the compounds identified in *C. myrciifolia* show high antifungal activity in previous studies. Lupeol, the major compound of HECM, proved to be active against *C. albicans* [61], and the combination of lupeol and *β*-Amyrin, led to an MIC value of 16 µg/mL against the same yeast [62]. *β*-Amyrin, in isolation, exhibited antifungal activity against *C. albicans*, and various mechanisms of action have been proposed, such as excessive intracellular ROS generation and mitochondrial dysfunction [63]. Another compound that acts similarly in inhibiting *Candida* sp. is hexadecanoic acid, which inhibits virulence and biofilm formation in *C. tropicalis* through ROS-mediated mitochondrial dysfunction [64].

The geniposidic acid, present in the EECM, can inhibit the mycelial formation of the fungus *Colletotrichum gloeosporioides* at a concentration of 150 µg/mL [18]. In the study by Muthamil et al. [65], quinic acid had a synergistic effect when combined with undecanoic acid against *Candida* spp., inhibiting virulence characteristics such as the yeast-to-hypha transition, production of extracellular polymeric substances, secretion of hydrolytic enzymes, and ergosterol biosynthesis. Although it is not possible to attribute this activity to a specific chemical compound, the antifungal compounds mentioned earlier occurring in *C. myrciifolia* provide strong evidence of a close correlation between its phytochemical profile and the found antimicrobial activity.

The morphological transition from yeast to a filamentous form in *Candida* is one of the main factors of its virulence, and inhibiting hyphal development is a viable strategy against fungal infections [66]. In this study, we demonstrated that although there was inhibition of fungal growth in all three species tested at the concentration of 512 µg/mL, as observed in the growth curve (Figs. 5 and 6), at this same concentration, the extracts inhibited only the yeast-to-filamentous transition in *C. tropicalis* (Fig. 7).

As mentioned previously, some of the compounds identified in *C. myrciifolia* extracts exhibit potential in combating fungal virulence [19,65], such as hexadecanoic acid present in the hexane extract of *C. myrciifolia* [64]. Some studies suggest that flavones and flavonols act in synergy by inhibiting filament emission and affecting the virulence of *Candida*. The mechanisms behind the activity of these flavonoids are related to the excessive generation of ROS during the germ tube formation phase, a phase that precedes hyphal formation [67], and the inhibition of genes encoding this morphological conversion [68].

In this study, we identified some antagonistic interactions, emphasizing the importance of considering the variables involved. The results indicate that different microbial strains when exposed to different antimicrobials and extracts, show varied responses. This diversity of results emphasizes the need for a detailed analysis of interactions between extracts and antibiotics.

5. Conclusions

The antimicrobial activity present in *Cordiera myrciifolia*, along with its phytochemical composition, reinforces the therapeutic potential of the Rubiaceae family. Our research revealed that *C. myrciifolia* stands out as a promising species for the treatment of microbial infections, especially its ethanolic extract due to the presence of flavonoids, compounds with remarkable antimicrobial activity. Against bacteria, the hexane extract proved to be more effective, especially against *E. coli* and *S. aureus* strains. Regarding the antifungal assay, a more significant activity of the combination between extracts and Fluconazole was evident, with particular susceptibility of *C. albicans* and *C. tropicalis* strains. These findings point to a promising perspective in the search for effective therapeutic solutions and pave the way for more detailed investigations into the medicinal potential of *C. myrciifolia.*

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4 ARTIGO 2 – EVALUATION OF THE ANTIMICROBIAL ACTIVITY AND GC-MS ANALYSIS OF HEXANE AND ETHANOLIC EXTRACTS OF *Tocoyena formosa* LEAVES (RUBIACEAE)

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Abstract

Tocoyena formosa is a medicinal species used to treat gastrointestinal disorders, which may include infections. In this study, we chemically analyzed its hexane (HETF) and ethanolic (EETF) extracts and investigated their potential to combat infectious microorganisms. The extracts were identified by gas chromatography-mass spectrometry, and their antimicrobial and drug-potentiating activities against multidrug-resistant bacteria and fungi of the *Candida* genus were evaluated by the broth microdilution method. HETF was mainly composed of *n*-alkanes (e.g., *n*-nonacosane), and EETF was predominantly composed of pentacyclic triterpenes such as ursolic and oleanolic acids. Both extracts revealed effective antibacterial activity, with HETF being active against *E. coli* and *S. aureus*, with MIC values of 128 and 256 μg/mL, respectively, while EETF had a MIC of 256 against *E. coli*. The combination with the antibiotic Imipenem was significant for both extracts against *S. aureus*. Regarding antifungal activity, a high activity of the EETF against *Candida albicans* was observed, with an IC_{50} of 57.0 μg/mL. Reduction in fungal growth was also observed for strains of *C. albicans* and *C. tropicalis*. The extracts were also able to inhibit the morphogenesis of *C. tropicalis* at a concentration of 512 μg/mL. Thus, *T. formosa* extracts proved to be an

important source of bioactive compounds, especially n-alkanes and triterpenes, acting either alone or potentiating the action of conventional drugs against infectious microorganisms, making them a promising source for the development of pharmaceutical products targeting bacteria and fungi.

Key words: Jenipapo-bravo; multidrug resistance; ursolic acid; nonacosane; Rubiaceae; *Candida* morphogenesis

1. INTRODUCTION

The discovery of antibiotics at the beginning of the 20th century by Alexander Fleming was one of the greatest achievements in medicine (Dhingra et al., 2020). However, the indiscriminate use of these agents and the ability of microorganisms to develop resistance to them, among other factors, have represented a challenge in the fight against microbial infections (Chiș et al., 2022; Ohore et al., 2022).

Although there is a significant demand for new antimicrobials, the high costs of their research and development, the long period for approval, the risk of ineffectiveness over time, stringent regulations for approval, and strict price controls pose obstacles to production (Norrby et al., 2005). Therefore, other treatment approaches have been encouraged, such as the combined use of antibiotics and/or their combination with plant-derived products (Coates et al., 2020; Ju et al., 2022; Silva et al., 2019b).

Combining drugs with natural products implies a decrease in the minimum inhibitory concentration of the synthetic drug and greater effectiveness in the final antimicrobial activity, demonstrating positive results in combating resistant infectious pathogens (Silva et al., 2019a). Thus, the combination of plant extracts with antimicrobial drugs has proven to be the most effective alternative in combating antibiotic resistance (Cheesman et al., 2017).

Although the antimicrobial potential of plant species has been constantly investigated, many species have not yet been evaluated, especially those that occur in Brazil – a country that holds the world's greatest biodiversity. This includes some species of Rubiaceae, for example, the fourth-largest family of angiosperms and one of the main families in the Brazilian flora (Souza and Lorenzi, 2005).

Rubiaceae is characterized by presenting secondary compounds of significant biopharmacological value, such as alkaloids and iridoids (Martins and Nunez, 2015). Representatives of this family have a long history of activity against microorganisms and

have great therapeutic success, being used in traditional medicine as an anti-inflammatory, analgesic, antibacterial, antiviral, and antioxidant, among others (Abdul et al., 2022; Ehiabhi Okhale, 2018; Martins and Nunez, 2015; Suksungworn and Duangsrisai, 2021).

Additionally, Rubiaceae includes species whose metabolites have led to the development of important clinical medications, such as quinine (Jovanović and Krajnović, 2023). According to Martins and Nunez (2015), the Rubiaceae family is promising for developing new bioactive molecules or drug prototypes due to its great phytochemical diversity and reported bio-pharmacological potential.

Among the species of Rubiaceae, *Tocoyena formosa* (Cham. & Schltdl.) K.Schum, known in the Brazilian semi-arid region as "jenipapo-bravo", stands out in traditional medicine for treating disorders associated with the gastrointestinal tract (Cesário et al., 2018). Such disorders may include symptoms caused by pathogenic microorganisms such as bacteria and fungi (Erdogan and Rao, 2015; Negrut et al., 2020; Sotelo-Coronado et al., 2016).

The antimicrobial potential of *T. formosa* has not been fully established; however, considering its use in the treatment of gastrointestinal problems, which may include infections by microorganisms, we hypothesize that this species may have activity against different pathogens that cause infections.

Thus, based on the mentioned ethnomedicinal use of *T. formosa*, this study aimed to (1) determine the chemical composition of leaf extracts of this species, (2) evaluate its isolated antimicrobial effect against pathogenic bacteria and fungi, (3) evaluate its drug-modifying action, and (4) determine the effectiveness of the extracts in inhibiting the morphological transition of *Candida*.

2. MATERIAL AND METHODS

2.1 Plant material and extraction

The collection of *T. formosa* plant material was previously recorded and authorized by the Biodiversity Authorization and Information System (SISBio) under registration number 77744 and registered in the National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration A236850. Healthy leaves of *T. formosa* were collected in the Araripe National Forest - Flona, in the municipality of Barbalha, Ceará, at an altitude of 924 meters, at coordinates 7°23'1.39"S and 39°21'7.36" W. Branches and flowers were also collected for identification and subsequent deposit in the Dárdano de Andrade-Lima Herbarium of the Regional University of Cariri under the number 15.004.

The leaves of *T. formosa* (108 g) were dried in the shade, crushed, and subjected to exhaustive extraction with *n*-hexane for 72 hours to remove low-polarity compounds. After filtration, the residue underwent a subsequent extraction with absolute ethanol for 72 hours. After the solvents were removed using a rotary evaporator, the hexane extract (HETF) and the ethanolic extract (EETF) were stored at room temperature until chemical analysis and antimicrobial tests. The yields of the HETF and EETF extracts were 0.96 g and 6.0 g, respectively.

2.2 Phytochemical analysis

The hexane extract (HETF) and ethanolic extract (EETF) of *T. formosa* were analyzed by gas chromatography coupled to mass spectrometry (GC-MS-TQ8040 NX, Shimadzu, Kyoto, Japan). To prepare the samples for analysis, both extracts underwent a derivatization process, wherein 100 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, Sigma-Aldrich) and 70 µL of pyridine were added to the extract, and the mixture was heated to 80 °C for 1 hour.

For the analysis, an SH-5M column (30 m x 0.25 mm x 0.25 µm) was used, and the following operational conditions were established: initial oven temperature of 150 °C (maintained for 5 minutes), followed by an increase of 10°C/min to 300 °C (maintained for 20 minutes). The total analysis time was 40 minutes. The injector, ion source, and interface temperatures were set at 250, 250, and 300 °C, respectively. Electron impact ionization mode was 70 eV. The sample injection volume was 1 µL with helium as the carrier gas with a constant flow of 0.81 mL/min and a split ratio 1/50. Mass spectra were obtained in full-scan acquisition mode, ranging from 45 to 750 m/z. Peaks were identified by comparison with the mass spectra obtained from NIST20 (National Institute of Standards and Technology, Gaithersburg, MD, USA).

2.3 Antibacterial activity

2.3.1 Drugs, reagents, solutions, bacterial strains, and culture media

To prepare an initial concentration of 1024 µg/mL for the antibacterial tests, 10 mg of each extract were diluted in 500 µL of dimethyl sulfoxide (DMSO, Merck, Darmstadt, Germany), and subsequently placed in Falcon tubes and topped up with distilled H_2O. Three reference antibiotics were used: Gentamicin (aminoglycoside), Norfloxacin

(fluoroquinolones), and Imipenem (*β*-lactam) from Sigma Aldrich, St. Louis, Missouri, USA, and diluted in distilled water to reach a concentration of 1024 µg/mL.

Minimum Inhibitory Concentration (MIC) was determined with standard strains (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 22923 e *Pseudomonas aeruginosa* ATCC 25924) and multi-resistant strains (*Escherichia coli* 06, *Staphylococcus aureus* 10 e *Pseudomonas aeruginosa* 24), with their resistance profile available in Rodrigues et al. (2022). Antibiotic potentiation assays were conducted only with multi-resistant strains.

The bacterial cultures were cultured on *Heart Infusion Agar* (HIA) and incubated in a bacteriological oven at 37 °C for 24 hours. After this period, an aliquot of each microbial culture was diluted in labeled test tubes, in triplicate, and compared to the McFarland standard solution at 10^5 CFU/mL. Eppendorf tubes were prepared, in triplicate, for each bacterium and antibiotic (Gentamicin, Norfloxacin, and Imipenem), each containing 1350 µL of 10% Brain Heart Infusion (BHI) + 150 µL of the inoculum (equivalent to 10% of the total solution) for MIC.

2.3.2 Minimum Inhibitory Concentration

Brain Heart Infusion (BHI) culture medium and inoculum (10%) were distributed in a 96-well microdilution plate. 100 µL of this solution was placed in each well of the plate, and then serial dilution was performed with 100 µL of the extracts, with concentrations ranging from 4 to 512 µg/mL. After a 24-hour incubation period at 37 ºC, antibacterial activity was detected by adding 20 µL of a 0.01% aqueous solution of resazurin (Sigma Aldrich, St. Louis, Missouri, USA). The reduction of resazurin caused a color change from blue to pink, indicating bacterial growth. The lowest concentration at which no bacterial growth was observed was determined as the MIC (Santos et al., 2019).

2.3.3 Potentiating effect of antibiotics

For the potentiation assays, the extracts were tested at sub-inhibitory concentrations (CIM/8). A solution containing culture medium, 10% inoculum, and extracts at sub-inhibitory concentration was prepared. 100 µL of this solution was added to each well of the microdilution plate. Then, 100 µL of the antibiotics were mixed in the first well of the plate, in alphabetical order, proceeding with serial dilution to the penultimate well (512 to 1 µg/mL).

The potentiation ability of the extracts combined with antibiotics was observed by adding 20 µL of an aqueous solution of resazurin (Coutinho et al., 2008).

2.4 Anti-*Candida* activity

2.4.1 Drugs, Reagents, Solutions and Fungal Strains

To obtain a matrix concentration of 1024 µg/mL, 10 mg of *T. formosa* extracts were first dissolved in 1 mL of dimethyl sulfoxide (DMSO) and then in *Sabouraud Dextrose Broth* (SDB) culture medium. The antifungal Fluconazole (FLUCOMED capsule) was used as a positive control and was diluted under the same conditions as the extracts. The culture media used were *Sabouraud Dextrose Broth* (SDB), *Sabouraud Dextrose Agar* (SDA), and *Potato Dextrose Agar* (PDA) prepared according to supplier guidelines (Difco, BD Diagnostic Systems, Sparks, MD, USA) and autoclaved at 120 °C for 15 min. Antifungal assays were performed using standard strains of *Candida* spp. obtained from the National Institute of Quality Control in Health (Rio de Janeiro, Brazil), namely: *C. albicans* INCQS 40006, *C. tropicalis* INCQS 40042, and *C. krusei* INCQS 40095.

2.4.2 Fungal Growth Curve and 50% Inhibitory Concentration - IC_{50}

The growth curve and determination of IC_{50} followed the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI, 2017). The yeasts were inoculated in ASD and incubated for 24 h at 35 ºC. Small aliquots of yeasts were transferred to test tubes containing sterile saline solution and compared to the McFarland scale. Subsequently, they were dissolved in SDB resulting in a concentration of $1–5\times10^3$ cells with colony-forming units (CFU)/mL.

The solutions containing SDB and inoculum at the concentration mentioned and the extracts of *T. formosa* at each concentration to be tested (1-512 µg/mL) were dispensed in a volume of 100 µL in 96-well flat-bottom microdilution plates. For each plate, a growth control and a sterility control of the culture medium were performed. The same procedure was carried out for Fluconazole. The plates were incubated at 35 °C for 24 h and subsequently read on an ELISA spectrophotometer at a wavelength of 630 nm (Thermoplate®). The results obtained were used to construct the fungal growth curve and the IC_{50} of the extracts and Fluconazole (Morais-Braga et al., 2016). IC_{50} was defined as the concentration capable of reducing the fungal growth curve by 50%.

2.4.3 Minimum Fungicidal Concentration (MFC)

The Minimum Fungicidal Concentration (MFC) assay was used to determine if the extracts and Fluconazole affected the viability of *Candida* spp. The assay helped to determine if these treatments had a fungistatic effect (inhibiting growth) or fungicidal effect (leading to fungal cell death). Using an automatic pipette, 5 µL of the solution from each well of the IC_{50} test plate was aspirated and transferred to Petri dishes containing solid SDA medium, distributed according to an orientation card fixed just below the plate. These plates were used for the subculture of yeasts, followed by the evaluation of colony formation. After 24 and 48 hours of incubation, the plates were analyzed, and the concentration at which no fungal colony growth was observed was considered the MFC (Fonseca et al., 2022).

2.4.4 Potentiating Effect of the Fluconazole

The extracts were tested at sub-inhibitory concentrations starting from the Matrix Concentration (MC/8), following the method proposed by Coutinho et al. (2008), with modifications. SDB culture medium was added to each well of the microdilution plate, and then 100 µL of each extract was mixed in the first well and serially diluted at a ratio of 1:1 up to the tenth well of the plate. The last two wells of the plate were reserved for the growth control and the sterility control of the culture medium, respectively (Morais-Braga et al., 2016). The reference antifungal, Fluconazole, was added in a volume of 100 µL to each well of the plate. Finally, 20 µL of the fungal inoculum prepared according to the Clinical and Laboratory Standards Institute was added (CLSI, 2017).

2.4.5 Effect of the extracts on fungal morphology

This assay was used to determine if the hexane and ethanolic extracts of *T. formosa* cause alterations in the morphology of *Candida* by inhibiting the emission of filamentous structures (hyphae and pseudohyphae). The analysis was conducted following the method proposed by Morais-Braga et al. (2016), where microculture chambers were prepared for yeast observation. A nutrient-poor medium with bacterial agar and the extracts at two concentrations, MC/2 = 512 µg/mL and MC/8 = 128 µg/mL (where MC is the matrix concentration) was dispensed on a chamber slide, with 3 mL of the mixture (Silva et al., 2024).

Two parallel streaks of the fungal inoculum were taken from Petri dishes containing SDA and placed on a depleted PDA medium. Subsequently, they were covered with a sterile coverslip and incubated at 37 ºC for 24 hours. Optical microscopy was used to capture images at 40x and 100x magnification. A control for hyphae stimulation by the depleted medium, as well as a pharmacological control with Fluconazole, were used for comparative purposes.

3. RESULTS

3.1 GC-MS analysis

Chemical analyses were conducted on the extracts and the total ion chromatogram of the hexane (HETF) and ethanolic (EETF) extracts of *Tocoyena formosa* are shown in Fig. 1 (A and B). The analysis identified 12 and 9 compounds in HETF and EETF and the results are presented in Tables 1 and 2, respectively. In HETF, it was possible to identify 94.39% of its composition, with a predominance of n-alkanes, and *n*-nonacosane being the major compound in the extract (70.02%).

As for EETF, a total of 97.72% of the extract constituents were identified. The main components of this extract were pentacyclic triterpenes, corresponding to 88.38% of the total composition, with ursolic acid as the major compound (72.08%), followed by oleanolic acid (16.30%).

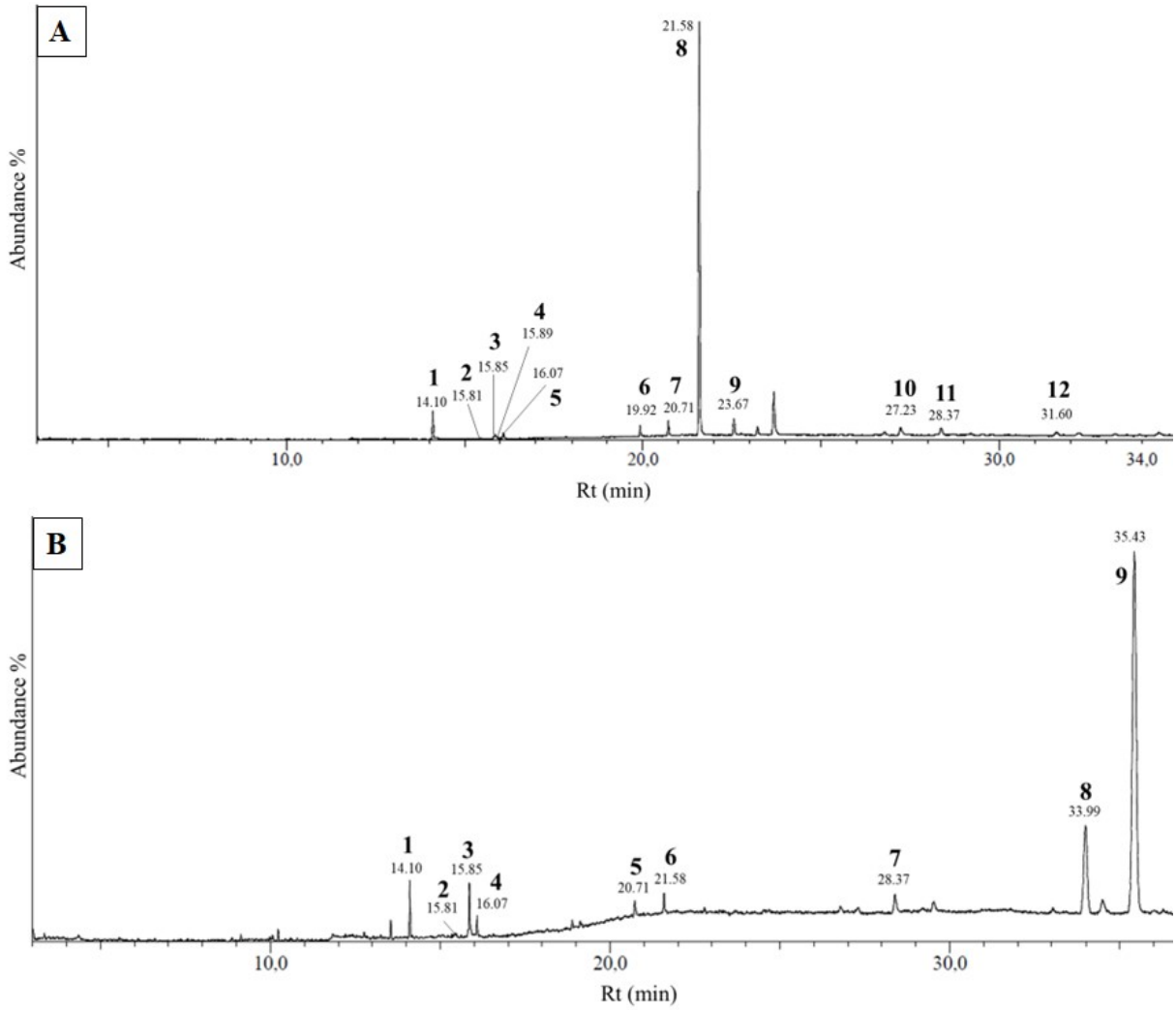

Fig. 1. Total ion chromatogram of the hexane (A) and ethanolic (B) extracts from *Tocoyena formosa*

Table 1. Identification of phytoconstituents in the hexane leaf extract of *Tocoyena formosa* by gas chromatography - mass spectrometry (GC–MS).

Compounds	R_t (min)	Classes	Abundance (%)
Palmitic acid*	14.10	Fatty acid	3.45
Linoleic acid*	15.81	Fatty acid	0.12
Oleic acid*	15.85	Fatty acid	0.29
α-Linolenic acid*	15.89	Fatty acid	0.11
Stearic acid*	16.07	Fatty acid	0.68
n-Heptaconsane	19.92	Alkane	1.40
n-Octacosane	20.71	Alkane	2.20
n-Nonacosane	21.58	Alkane	70.02

n-Hentriacontane	23.67	Alkane	9.93
Triacontanol*	27.23	Fatty alcohol	2.31
β-sitosterol*	28.37	Phytosteroid	2.28
Dotriacontanol*	31.60	Fatty alcohol	1.60
Total identified			94.39
Not identified			5.61

* Compounds identified as TMS derivatives.

Table 2. Identification of phytoconstituents in the ethanolic leaf extract of *Tocoyena formosa* by gas chromatography - mass spectrometry (GC–MS).

Compounds	R_t (min)	Classes	Abundance (%)
Palmitic acid*	14.10	Fatty acid	2.58
Linoleic acid*	15.81	Fatty acid	0.10
Oleic acid*	15.85	Fatty acid	1.82
Stearic acid*	16.07	Fatty acid	0.72
n-Octacosane	20.71	Alkane	0.94
n-Nonacosane	21.58	Alkane	1.11
β-sitosterol*	28.37	Phytosteroid	2.07
Oleanolic acid*	33.99	Triterpene	16.30
Ursolic acid*	35.43	Triterpene	72.08
Total identified			97.72
Not identified			2.28

* Compounds identified as TMS derivatives.

3.2 Antibacterial and antibiotic-potentiating activities

According to Table 3, *T. formosa* extracts exhibit antibacterial activity against standard strains of *E. coli* and *S. aureus*. The HETF and EETF extracts showed MIC values of 128 and 256 μg/mL against *E. coli*, respectively. For the *S. aureus* strain, only EHTF showed activity, with an MIC value of 256 μg/mL. Furthermore, it was found that the *P. aeruginosa* strain was not susceptible to the extracts at the evaluated concentrations.

Table 3. Minimum Inhibitory Concentration (MIC) in μg/mL of *Tocoyena formosa* extracts

Extracts	Bacteria					
	E. coli ATCC	*E. coli* *06*	*S. aureus* ATCC	*S. aureus* *10*	*P. aeruginosa* ATCC	*P. aeruginosa* *24*
EHTF	128	> 512	256	> 512	> 512	> 512
EETF	256	> 512	> 512	> 512	> 512	> 512

HETF – Hexane extract of *Tocoyena formosa*; EETF – Ethanolic extract of *Tocoyena formosa*

Fig. 2 shows the combination of the hexane extract from *T. formosa* and antibiotics. In it, we can observe that HETF was able to significantly reduce the MIC of Imipenem from 80 µg/mL to 32 µg/mL against the multidrug-resistant strain of S. aureus. However, when combined with Norfloxacin, HETF interfered with the antibiotic action against all three strains, substantially increasing their respective MICs.

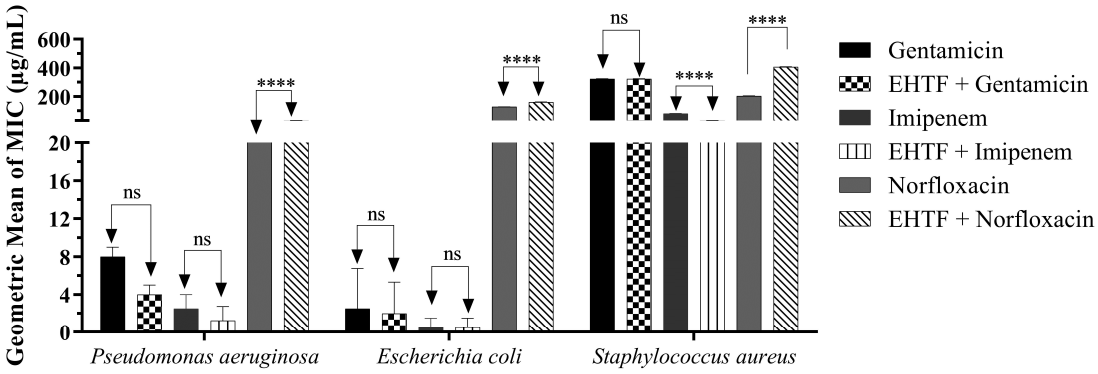

Fig. 2. Minimum Inhibitory Concentration (MIC) of isolated antibiotics and in combination with the hexane leaf extract of *Tocoyena formosa* (HETF) against multidrug-resistant bacterial strains. ns = not significant ($P > 0.05$), **** = $P < 0.0001$. Bars represent the standard deviation (n = 3).

The EETF, on the other hand, did not enhance the action of drugs against *P. aeruginosa* (Fig. 3). However, when associated with Norfloxacin, the extract was able to intensify its action, reducing its MIC against the *E. coli* strain. Interestingly, against the Gram-positive bacterium *S. aureus*, while the extract reduced the activity of Norfloxacin; it potentiated the action of the antibiotic Imipinem, reducing its MIC from 80 µg/mL to 20 µg/mL.

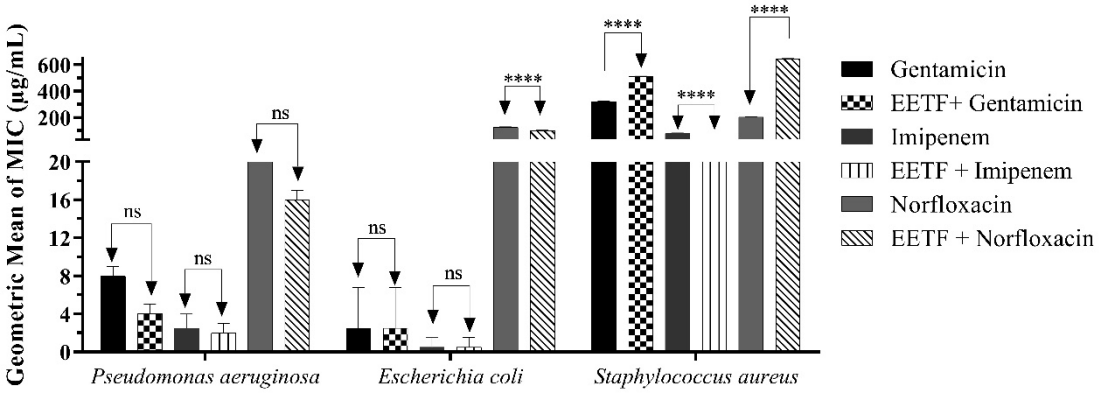

Fig. 3. Minimum Inhibitory Concentration (MIC) of isolated antibiotics and in combination with the ethanolic leaf extract of *Tocoyena formosa* (EETF) against multidrug-resistant bacterial strains. ns = not significant ($P > 0.05$), **** = $P < 0.0001$. Bars represent the standard deviation (n = 3).

3.3 Anti-*Candida* activity

3.3.1 IC_{50}, MFC, and fungal growth curve

Table 4 presents the IC_{50} values related to the antifungal activity of Fluconazole, *T. formosa* extracts, and their combination. It can be observed that the isolated extracts demonstrated moderate activity, with IC_{50} values ranging from 271.5 to 378 µg/mL. However, it is noteworthy that EETF exhibited a relevant result against the *C. albicans* strain, with an IC_{50} of 57 µg/mL. Regarding the potentiation of the antifungal, an extremely significant result was observed for *C. tropicalis*, where the IC_{50} values dropped to 0.2 and 0.3 µg/mL for EETF and HETF, respectively. At the same time, Fluconazole showed 1.6 µg/mL alone. When combined with the standard drug and evaluated against *C. krusei*, the *T. formosa* extracts led to a reduction in the anti-*Candida* activity of Fluconazole. Due to the absence of inhibition at the tested concentrations, the Minimum Fungicidal Concentration (MFC) of the extracts as well as Fluconazole is above 512 µg/mL.

Table 4. The concentration that inhibits 50% of fungal growth (IC_{50}) of extracts from *Tocoyena formosa*, Fluconazole, and the combination of both against *Candida* strains

Treatments	Strains/IC_{50} µg/mL		
	CA INCQS 40006	CT INCQS 40042	CK INCQS 40095
FCZ	2.8	1.6	36.5

HETF	307.2	328.2	378.0
FCZ + HETF	3.0	0.3	56.4
EETF	57.0	271.5	327.0
FCZ + EETF	2.5	0.2	56.0

CA – *C. albicans*; CT – *C. tropicalis*; CK – *C. kruzei*; INCQS – National Institute of Health Quality Control; FCZ - Fluconazol; HETF - Hexane Extract of *Tocoyena formosa*; EETF - Ethanolic Extract of *Tocoyena formosa*

Fig. 4 depicts the effects of HETF and its combination with Fluconazole on fungal growth. It is possible to observe that for *C. albicans* (Fig. 4A), HETF shows anti-*Candida* activity notably at concentrations of 256 µg/mL and 512 µg/mL, considering that it reduced growth by 28% and 94%. For the combination of the extract with Fluconazole, it can be noted that there was no change in the activity. Similar data were observed for *C. tropicalis* (Fig. 4B). In this case, the combination of HETF and Fluconazole was more significant when compared to the antifungal tested alone, inhibiting 60% of fungal growth from a concentration of 1 µg/mL. Fig. 4C, when compared to the others, shows that *C. krusei* was the least susceptible strain to pure HETF and its combination with Fluconazole, requiring higher concentrations for the inhibition of fungal growth to be observed.

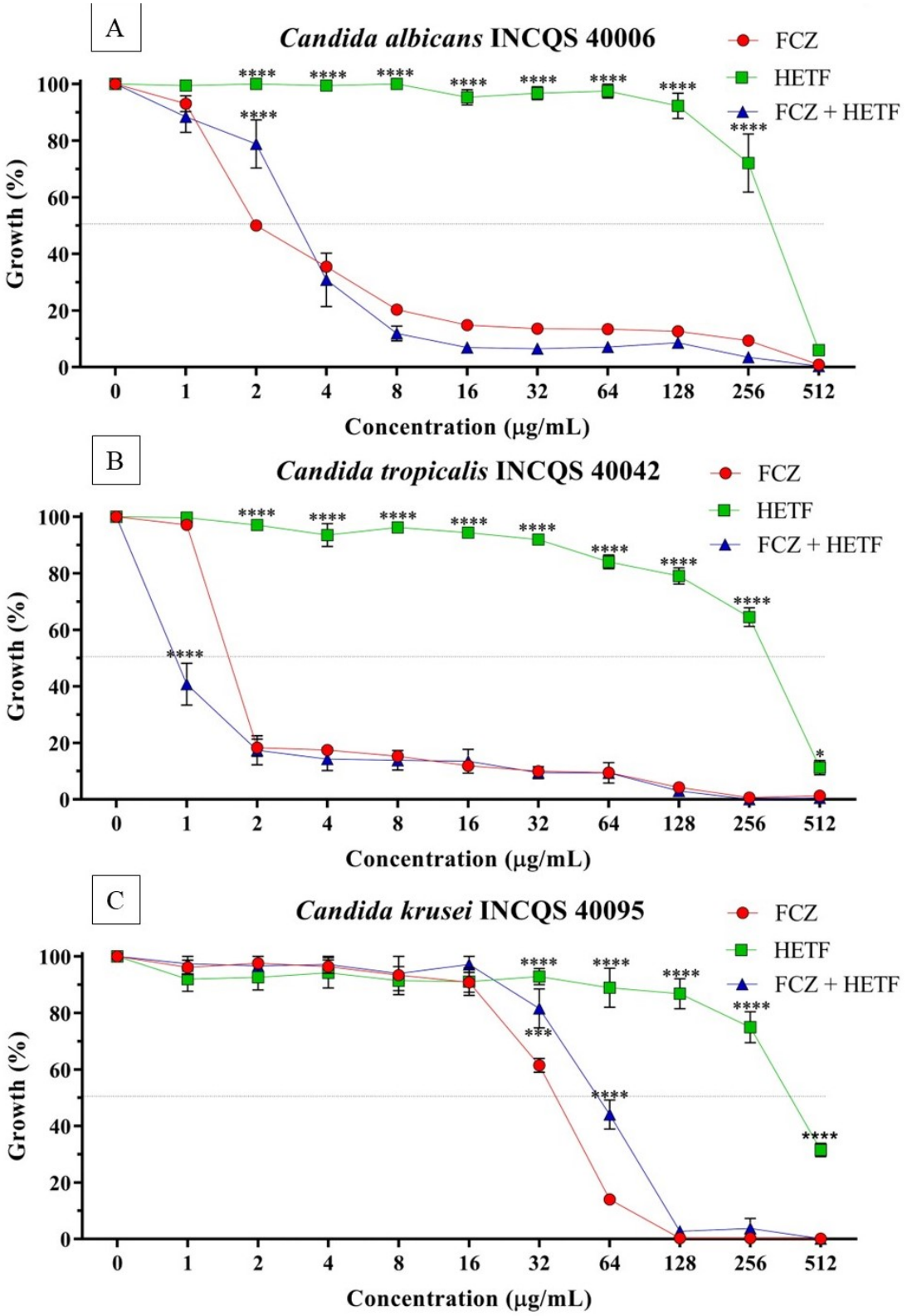

Fig. 4. Growth curve and IC_{50} (dotted line) of different concentrations of hexane leaf extract of *Tocoyena formosa* (HETF), Fluconazole (FCZ), and their combination (FCZ + HETF) against *Candida* strains. Bars represent standard error (n = 4). ** = P < 0.01, **** = P < 0.0001 compared to Fluconazole.

The effects of EETF and its combination with Fluconazole are observed in Fig. 5. Fig. 5A shows this effect for *C. albicans*, where it can be observed that the extract exhibited anti-*Candida* action at clinically relevant concentrations, as there was a reduction in yeast growth from concentrations of 2 µg/mL. Furthermore, the highest concentration evaluated (512 µg/mL) was able to completely inhibit the growth of the fungal strain. When evaluating the potentiating activity of EETF in relation to the drug, no relevant impact was evidenced.

Similarly, to the hexane extract, *C. tropicalis* (Fig. 5B) was the most susceptible strain to the combination of EETF and Fluconazole, with its growth being reduced by 60% from a concentration of 1 µg/mL. *Candida krusei* (Fig. 5C), on the other hand, was the least susceptible to the effects of the extract and the combination, showing no significant differences when compared to Fluconazole alone.

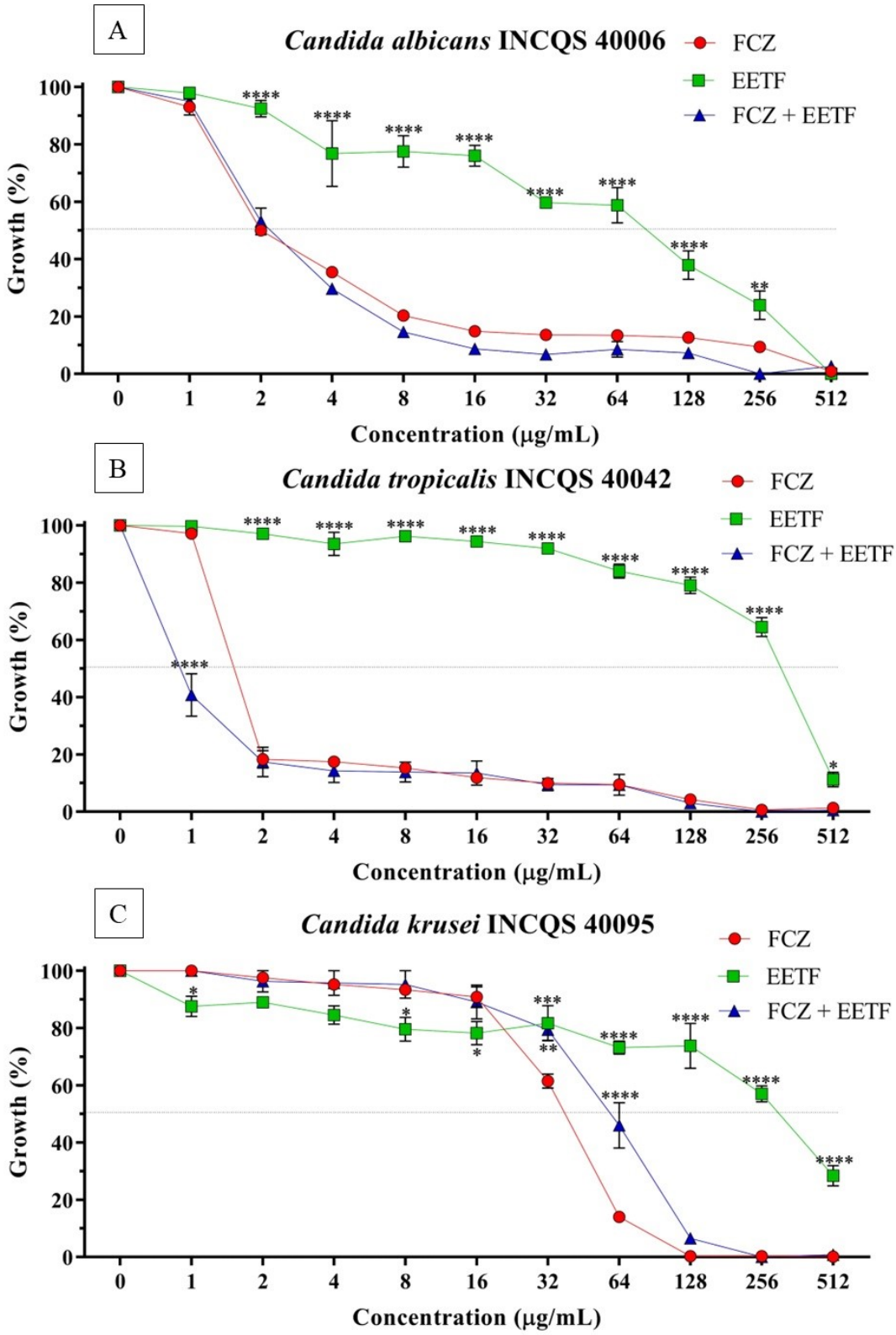

Fig. 5. Growth curve and IC_{50} (dotted line) of different concentrations of ethanolic leaf extract of *Tocoyena formosa* (EETF), Fluconazole (FCZ), and their combination (FCZ + EETF) against *Candida* strains. Bars represent standard error (n = 4). ** = P < 0.01, **** = P < 0.0001 compared to Fluconazole.

3.3.2 Effect of extracts on fungal morphology

Fig. 6 presents the effects of HETF and EETF extracts from *Tocoyena formosa* on filament emission in *Candida* spp. It can be observed that both, at the concentration of 512 µg/mL (MC/2), were able to inhibit filament emission in *C. tropicalis*. For *C. albicans*, it was observed that only HETF had an impact on filamentous structures, considerably reducing their formation, but not preventing their development. It was also noted that the extracts did not prevent the morphological transition of *C. krusei* at any of the concentrations when compared to the growth control.

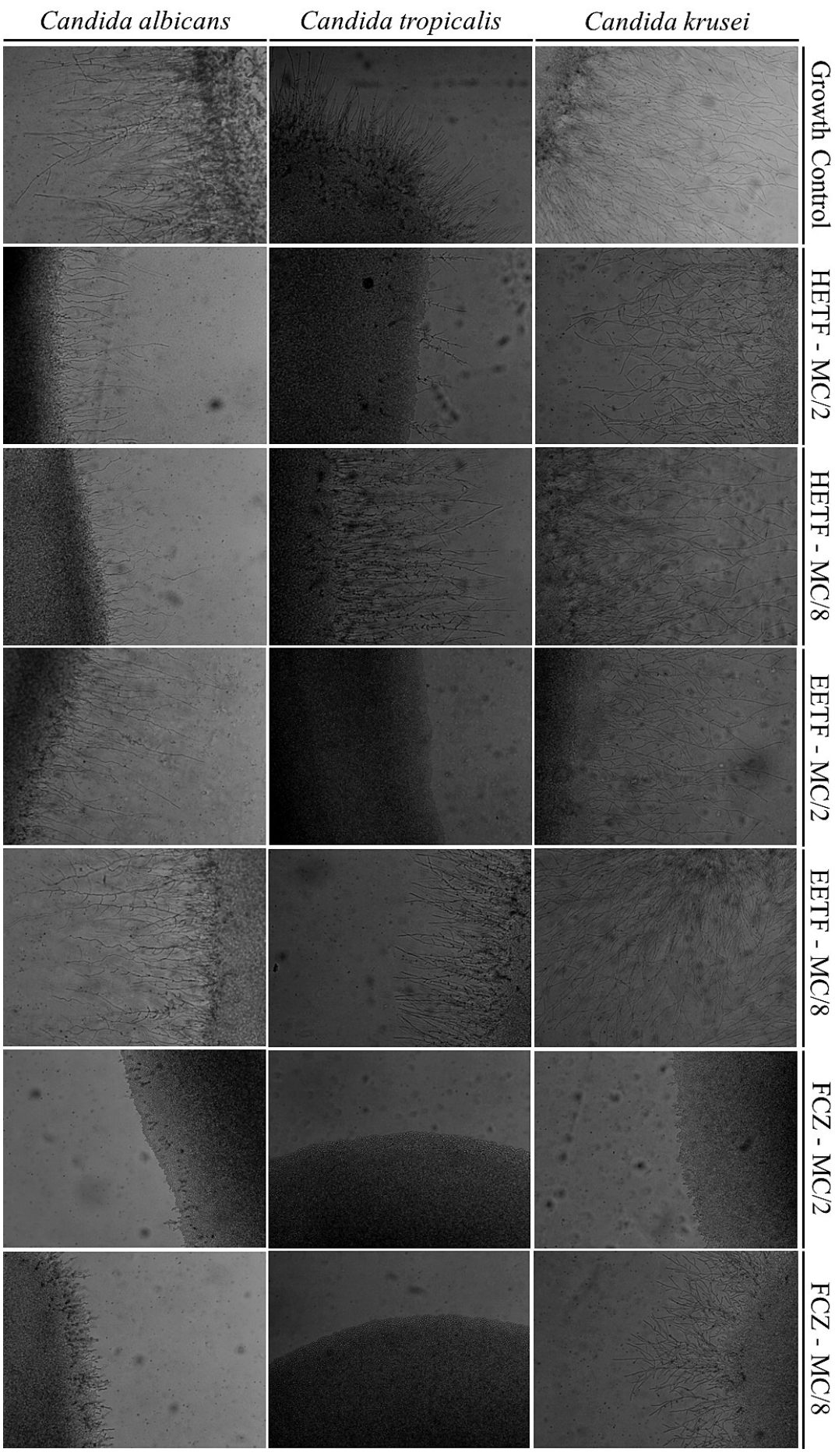
Candida albicans
Candida tropicalis
Candida krusei
Growth Control
HETF - MC/2
HETF - MC/8
EETF - MC/2
EETF - MC/8
FCZ - MC/2
FCZ - MC/8

Fig. 6. Effects of the hexane (HETF) and ethanolic (EETF) extracts of *Tocoyena formosa* on the dimorphism of *Candida albicans* INCQS 40006, *Candida tropicalis* INCQS 40042 and *Candida krusei* INCQS 40095 at 100× magnification.

4. DISCUSSION

The Rubiaceae family presents a high diversity of species used in ethnopharmacology, including *T. formosa*, which stands out for the treatment of symptoms associated with the gastrointestinal tract, such as infections (Cesário et al., 2018). Considering this reality, the hypothesis was raised that its leaves contain biologically active chemical constituents against etiological agents that cause infections - a hypothesis later supported by our findings.

The phytochemical constituents identified in the mentioned species (Tables 1 and 2) had not been reported before and were reported for the first time in our study. The analysis of the hexane extract of *T. formosa* identified a total of twelve chemical constituents, with n-nonacosane being the major constituent, a long-chain alkane. The ethanolic extract, in turn, presented ursolic acid as the major constituent, characterized as a pentacyclic triterpene. Some studies have identified that the composition of *T. formosa* includes the presence of polyphenols, flavonoids, triterpenes, and iridoids, among other phytochemical groups (Bolzani et al., 1997; Cesário et al., 2019). The *n*-Alkanes class, described as the majority of constituents in our hexane extract, was previously reported for *T. formosa* only in its cuticular composition (Oliveira and Salatino, 2000).

In a study conducted by Neves et al. (2019), the ethanolic extract of *T. formosa* showed activity against the bacteria *Mycobacterium abscessus* subsp. *massiliense*, the etiological agent of tuberculosis. The results showed that at a concentration of 1000 µg/mL, this extract presented 100% inhibition of bacterial growth. However, it is important to note that concentrations above 1000 µg/mL are considered ineffective for clinical application (Houghton et al., 2007). In this regard, our work demonstrated that *T. formosa* has antibacterial activity at concentrations considered moderate, as observed for *E. coli*, with MICs of 128 and 256 µg/mL, respectively, for the hexane and ethanolic extracts; and for *S. aureus*, with an MIC of 256 µg/mL for the hexane extract (Table 1). It is noteworthy that the results of our work and the study conducted by Neves et al. (2019) were different, but it is important to emphasize that different bacterial strains may have different susceptibility patterns to the same treatments.

Another determining factor for the performance of a good biological activity is the chemical composition of the extract. In our study, *n*-nonacosane, the main metabolite of the hexane extract of *T. formosa*, may have been responsible for the observed antibacterial activity against the strains of *E. coli* and *S. aureus*. A previous study identified that nonacosane has MICs that ranged from 125 to 250 µg/mL when tested against phytopathogenic bacteria (*Agrobacterium tumefaciens*, *Erwinia carotovora*, *Rastonia solanacearum*) (Wei et al., 2023). From a bioactive perspective, *n*-alkanes are relatively unexplored compounds, and for this reason, the mechanisms of action of these metabolites are still unknown.

The ethanolic extract demonstrated antibacterial activity in isolation against the *E. coli* strain, justified at least partially by the presence of ursolic acid, the major compound in this extract (70.40%). The aforementioned acid is widely known in the literature for its bioactive properties, including activity against pathogenic bacteria (Jesus et al., 2015); and for its ability to inhibit the formation of *E. coli* biofilms, one of its virulence factors, by 6 to 20 times, as observed in the study by Ren et al. (2005).

Our study also highlighted the potentiating effect of *T. formosa* when combined with the antibiotics Norfloxacin for *E. coli* and Imipenem for *S. aureus* (Fig. 2 and 3). Many studies have corroborated the effectiveness of combining plant extracts, especially those of a more polar nature, with standard antibiotics in combating pathogen resistance (Demgne et al., 2022; Ngongang et al., 2020). The study carried out by Barbosa-Filho et al. (2015), as evidenced in our research, highlighted the increased action potentials of fluoroquinolones and aminoglycosides against *E. coli* and *S. aureus* strains when combined with extracts. The results of this study revealed a relevant reduction in the MIC value of the antibiotic, demonstrating the effectiveness of these combinations.

Some studies suggest that pentacyclic triterpenes such as ursolic and oleanolic acids, in combination with antimicrobial drugs, are capable of enhancing the effects of these drugs. In the study by Dwivedi et al. (2015), ursolic acid and its derivatives, tested in combination with antibiotics against multidrug-resistant strains of *E. coli*, proved effective in reversing the resistance of this pathogen by inhibiting efflux pumps that reduce its cell viability.

In our study, we observed that the isolated ethanolic extract of *T. formosa* exhibits satisfactory anti-*Candida albicans* activity, with an IC_{50} value of 57.0 µg/mL (Table 2). Although the ethanolic extract did not outperform Fluconazole when tested alone on *Candida albicans*, it is important to highlight the relevance of the value discovered, as it suggests that

the *T. formosa* extract can be considered as an alternative treatment option, since the Fluconazole may cause unwanted side effects.

Some studies on the antifungal potential of the genus *Tocoyena* have been conducted, revealing that it harbors a variety of chemical compounds with the potential to combat various fungal strains. One study evaluated the potential of triterpenic saponins isolated from *Tocoyena brasiliensis* against filamentous fungi and indicated antifungal activity primarily attributed to quinovic acid 28-O-β-D-glucopyranoside (Hamerski et al., 2005). Other assays were conducted to evaluate the activities of the iridoids α and β-gardiol, isolated from *T. formosa*, in mutant strains of *Saccharomyces cerevisiae*. The results indicated MIC values ranging between 90 and 126 μg/mL, indicating the fungitoxic activity of these compounds (Bolzani et al., 1997). Previously, Bolzani et al. (1996), had already identified a new iridoid in *T. formosa* – 11-O-trans-teucrein ferulate – which also has antifungal activity.

The aforementioned studies confirmed that *Tocoyena* is a genus rich in isolated compounds with antifungal activity, however, no study had evaluated the crude extracts of *T. formosa* either in isolation or in combination with antibiotics. Our research further corroborates this premise, since our results indicate that the antifungal activity observed for the hexane and ethanolic extracts is attributed to their major compounds, such as the *n*-alkane nonacosane and the pentacyclic triterpene ursolic acid, respectively.

In our study, we used Fluconazole as a standard - a first-choice medication for the treatment of fungal infections, especially those caused by the genus *Candida* (Lu et al., 2021). Since this medication is fungistatic, meaning it can inhibit the growth of fungal strains rather than eliminating them; increases the possibility of strains developing resistance to it (Carneiro et al., 2020). In this context, extensive research on the combination of Fluconazole with plant extracts has been carried out; and several have corroborated the effectiveness of this association in controlling infections caused by *Candida*. In this context, extensive research on the combination of Fluconazole with plant extracts has been conducted, and several studies have supported the effectiveness of this association in controlling infections caused by *Candida*. For example, a synergistic interaction between the antifungal and the extract from the bark of *Uncaria tomentosa* D.C. – a species of the Rubiaceae family – was observed against strains of *Candida krusei* and *C. glabrata*, resulting in a probable mechanism of action directly on the cell walls of the microorganisms. (Moraes et al., 2017).

Candida species possess the ability to modify their yeast morphology into a filamentous form, a phenomenon known as morphogenesis. This phenomenon is associated with their virulence and pathogenesis and is a response to the diverse environmental

conditions within the host (Kadosh, 2019). This contributes to adhesion and tissue invasion and leads to the establishment of the infection (Menezes et al., 2013; Vediyappan et al., 2013). Therefore, the search for bioactive that inhibit the morphological transition of *Candida* has been a viable strategy against fungal infections (Khan et al., 2021). Our study showed that HETF and EETF inhibited the morphological transition of *C. tropicalis*. Although they were not able to completely inhibit the development of hyphae in *C. albicans*, they reduced their formations (Fig. 6).

The results of this study reveal the promising antimicrobial activity of leaf extracts from *T. formosa*, as it manifests at clinically relevant concentrations. Furthermore, Cesário et al. (2019) demonstrated through acute toxicity tests in mice that *T. formosa* extracts do not present toxicity at doses of clinical relevance. Therefore, research indicates that the species, in addition to being promising, is safe for treating infections.

However, it is important to note that these findings cannot be generalized, as we observed a reduction in the activity of some of the standard drugs tested when combined with the extracts. This discovery highlights the complexity of interactions and emphasizes that the variation in activity depends on specific combinations of products used and the characteristics of the microorganisms involved. Furthermore, this reduction in drug potential may be partially due to the interaction/mutual chelation of the antibiotic with the chemical components of plant extracts (Barbosa-Filho et al., 2015). Therefore, more in-depth studies are necessary for a more comprehensive understanding of the mechanisms underlying these synergistic/antagonistic interactions.

5. CONCLUSION

Our results indicate that *Tocoyena formosa* is a species with the potential to be used as an antimicrobial agent, reinforcing the initial hypothesis and highlighting the therapeutic potential of the Rubiaceae family. *T. formosa* proved to be a very diverse species from a chemical point of view, with numerous aliphatic constituents (n-alkanes) and some cyclic constituents identified (e.g. triterpenes). This study found that the hexane and ethanolic extracts of *T. formosa* combined with drugs can reverse the resistance of *E. coli* and *S. aureus*. Additionally, antifungal activity was especially significant against strains of *C. albicans* and *C. tropicalis*, inhibiting the morphogenesis of the latter. Based on the observed results, we can conclude that *T. formosa* is promising in the development of therapeutic products for the treatment of infections.

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5 CONSIDERAÇÕES FINAIS

De forma geral, espécies da família Rubiaceae são caracterizadas pela sua diversidade química associada ao seu potencial biofarmacológico. Nesta tese, foram obtidos resultados significativos que destacam a relevância das propriedades bioativas de duas espécies pertencentes à essa família.

Ao longo desta pesquisa, conseguimos aprofundar nosso conhecimento sobre a fitoquímica da espécie *Cordiera myrciifolia*, permitindo-nos identificar uma ampla variedade de constituintes, incluindo triterpenos no extrato hexânico e flavonoides, especialmente flavonas, no extrato etanólico, como alguns de seus principais compostos. Além disso, a espécie *Tocoyena formosa* também demonstrou diversidade química, com alguns de seus metabólitos presentes nos dois extratos, como ácidos graxos e fitoesteróis, enquanto que triterpenos pentacíclicos e *n*-alcanos, foram observados apenas nos extratos etanólico e hexânico, respectivamente.

As atividades antimicrobianas foram confirmadas para cepas bacterianas e fúngicas, tanto nos extratos brutos, quanto em combinação com fármacos padrões. Quanto a capacidade antibacteriana dos extratos, demonstramos que estes possuem atividade isolada para as bactérias *E. coli* e *S. aureus*, além de potencializar os efeitos de antibióticos. A atividade contra *Candida* spp. determinou que as cepas de *C. albicans* e *C. tropicalis* são as cepas mais sensíveis a ação dos extratos isolados e em combinação com o fármaco Fluconazol.

Além disso, os extratos de ambas as espécies apresentaram a habilidade de inibir a transição morfológica em *C. tropicalis*, destacando-se como um elemento crucial na redução da virulência e, consequentemente, na limitação da capacidade de invasão tecidual dessa cepa. Ambas as espécies foram determinadas como promissoras para o desenvolvimento de produtos com aplicações farmacológicas, tanto para fungos quanto bactérias.

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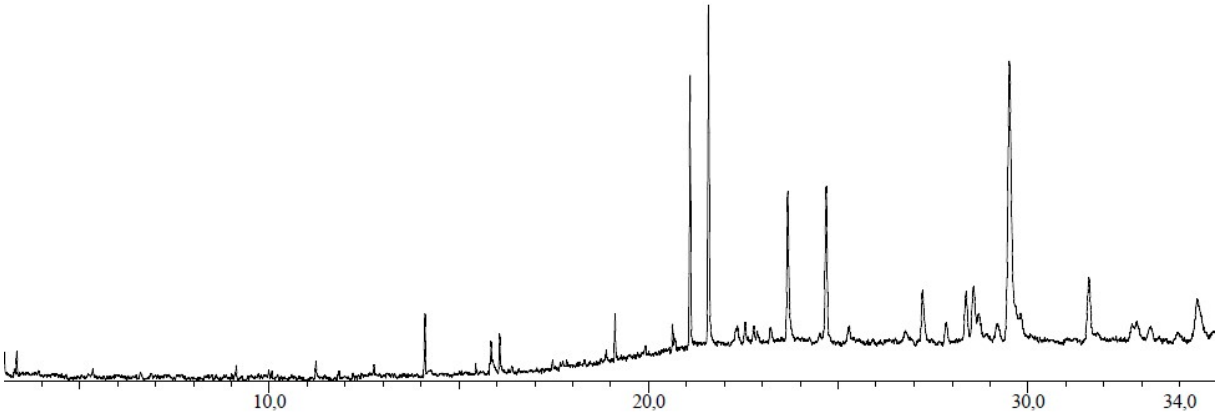
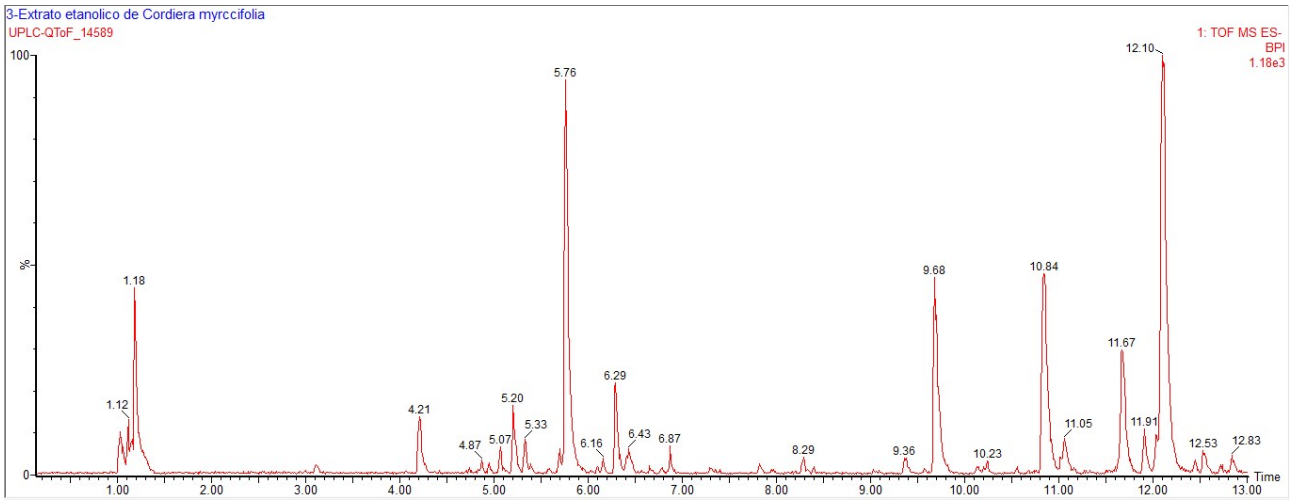
ANEXO A – CROMATOGRAMA DO EXTRATO HEXÂNICO DE *Cordiera myrciifolia*



ANEXO B – CROMATOGRAMA DO EXTRATO ETANÓLICO DE *Cordiera myrciifolia*



ANEXO C – COMPROVANTE DE AUTORIZAÇÃO DE COLETA

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: 77744-1	Data da Emissão: 24/02/2021 07:08:29	Data da Revalidação*: 24/02/2022
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: FELICIDADE CAROLINE RODRIGUES	CPF: 605.939.063-30
Título do Projeto: Autorização para coleta de material botânico na FLONA Apodi	
Nome da Instituição: Universidade Federal de Pernambuco - UFPE	CNPJ: 24.134.488/0001-08

ANEXO D - CERTIDÃO DE CADASTRO NO 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 A236850

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:	A236850
Usuário:	Felicidade Caroline Rodrigues
CPF/CNPJ:	605.939.063-30
Objeto do Acesso:	Patrimônio Genético
Finalidade do Acesso:	Pesquisa

Espécie

Cordiera myrciifolia

Tocoyena formosa

Título da Atividade:	COMPOSIÇÃO QUÍMICA, CUTICULAR E BIOATIVIDADE DE EXTRATOS FOLIARES DE DUAS ESPÉCIES DE RUBIACEAE

Equipe

Felicidade Caroline Rodrigues	Universidade Federal de Pernambuco
Antonio Fernando Morais de Oliveira	Universidade Federal de Pernambuco

ANEXO E – COMPROVANTE DE PUBLICAÇÃO DO ARTIGO 1

Fitoterapia 176 (2024) 106027

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Chemical composition and antimicrobial activity of *Cordiera myrciifolia* leaves against pathogenic bacteria and fungi: Drug potentiation ability and inhibition of virulence

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