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

JOSÉ WEVERTON ALMEIDA BEZERRA

PROSPECÇÃO FITOQUÍMICA E AVALIAÇÃO DO POTENCIAL ANTIMICROBIANO DOS FRUTOS DE *Caryocar coriaceum* Wittm. (CARYOCARACEAE)

Recife
2023

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

Orientador: Prof. Dr. Antonio Fernando Morais de Oliveira

Coorientadora: Profª. Drª. Maria Flaviana Bezerra Morais Braga

Recife

2023

Catalogação na Fonte:
Bibliotecário:Marcos Antonio Soares da Silva
CRB4/1381

Bezerra, José Weverton Almeida.
Prospecção fitoquímica e avaliação do potencial dos frutos de *Caryocar coriaceum Wittm*. (CARYOCARACEAE). / José Weverton Almeida Bezerra – 2024.

153 f. : il., fig.; tab.

Orientador: Antonio Fernando Morais de Oliveira.
Coorientadora: Maria Flaviana Bezerra Morais Braga.

Tese (doutorado) – Programa de Pós-Graduação em Biologia Vegetal da Universidade Federal de Pernambuco, 2024.

Inclui: referências e anexos.

1. Oleagionosa. 2. Extrativismo. 3. Candidíase. 4. Resistência microbiana. 5. Etnobotânica. I. Oliveira, Antonio Fernando Morais de (Orient.). II. Braga, Maria Flaviana Bezerra Morais (Coorient.). III. Título.

580 CDD (22.ed.) UFPE/CB – 2024-029

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Aprovada em: 04/10/2023

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Dedico este trabalho de tese aos meus pais, Valmir Angélico e Maria Vilauba, por acreditarem em minha capacidade e a Sra. Dilma Rousseff, pela valorização ao desenvolvimento da ciência e tecnologia brasileira.

AGRADECIMENTOS

À Universidade Federal de Pernambuco (UFPE), por todas as oportunidades proporcionadas ao longo do doutorado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela bolsa concedida, possibilitando a realização dessa pesquisa.

Ao meu orientador Professor Dr. Antonio Fernando Morais de Oliveira, pela orientação deste trabalho, a qual foi primordial na estruturação de todo o projeto.

À minha coorientadora Professora Dra. Maria Flaviana Bezerra Morais Braga, pela orientação deste trabalho, a qual foi fundamental para o desenvolvimento deste estudo.

Às instituições e laboratórios colaboradores, expresso minha gratidão pelas significativas contribuições ao longo de certas fases desta pesquisa. Dentre eles, o Laboratório de Ecologia Aplicada e Fitoquímica (LEAF-UFPE), Herbário Geraldo Mariz (UFPE), Laboratório de Micologia Aplicada do Cariri (LMAC-URCA), Laboratório de Microbiologia e Biologia Molecular (LMBM-URCA), Laboratório de Pesquisa em Produtos Naturais (LPPN-URCA), Laboratório de Biologia Celular Vegetal (UFC).

À banca examinadora, por todas as contribuições valiosas realizada neste trabalho.

Aos extrativistas da Chapada do Araripe, pelo apoio nas coletas dos frutos.

E a todos que contribuíram com a elaboração deste trabalho.

“Eu vou pro Crato

Comer arroz com pequi

Feijão com rapadura

Farinha do Cariri”

Luiz Gonzaga

RESUMO

Caryocar coriaceum Wittm. é uma espécie arbórea da Família Caryocaraceae, cujo os frutos são utilizados na obtenção de óleo para fins alimentícios e medicinais. Tanto este derivado, quanto os frutos, são empregados na farmacopeia para o tratamento de enfermidades. Nesta perspectiva, o presente trabalho teve como objetivos: 1) Revisar estudos realizados com a espécie, destacando os seus usos farmacológicos, etnomedicinais, importância cultural e socioeconômica. 2) Caracterizar quimicamente o extrato do mesocarpo dos frutos de *C. coriaceum* bem como avaliar sua atividade antimicrobiana e intensificadora de fármacos frente a microrganismos patogênicos. 3) Determinar a composição química e avaliar a atividade antifúngica, antivirulência e toxicidade do óleo fixo dos frutos de *C. coriaceum* frente a três leveduras do gênero *Candida*. Os estudos de revisão foram realizados por meio da análise de dados coletados nas plataformas de pesquisa científica Google ScholarTM, Medline®, ResearchGate, Web of ScienceTM, Scopus®, ScienceDirect® e Pubmed®. Para os estudos de atividade antimicrobiana o extrato metanólico do mesocarpo interno de *C. coriaceum* (EMCC) foi avaliado por meio do teste de microdiluição seriada, frente às bactérias padrões e multirresistentes de *Escherichia coli*, *Pseudomonas aeruginosa* e *Staphylococcus aureus*. Além da atividade intrínseca, foi avaliada a capacidade de modificação de fármacos. Quanto à atividade antifúngica, tanto o extrato quanto o óleo (OFCC) foram avaliados de modo intrínseco e combinados com o fluconazol frente às cepas de *Candida* spp. Além disso, foram investigados os possíveis mecanismos de ação anti-*Candida* bem como a capacidade de inibição de transição morfológica. Quanto à fitoquímica do EMCC, foi realizada a prospecção química qualitativa e determinação do conteúdo de fenóis totais por meio do método de Folin-Ciocalteu. O OFCC transesterificado, por sua vez, foi analisado por cromatografia em fase gasosa acoplada à espectrometria de massas. Quanto à toxicidade do OFCC, este foi avaliado por meio do teste de ingestão, utilizando o organismo-modelo *Drosophila melanogaster*. Os trabalhos demonstram que *C. coriaceum* é uma árvore de grande importância medicinal, econômica e cultural. Medicinalmente, os frutos são utilizados para tratar doenças bronco-pulmonares e tumores, enquanto que o óleo é amplamente empregado principalmente no tratamento de reumatismo. Essas propriedades resultam em alta demanda comercial, impulsionando a economia dos extrativistas. Entretanto, a procura intensa e outros fatores resultaram na classificação da espécie como ameaçada de extinção. Quimicamente O EMCC apresentou flavonas, flavonóis, xantonas, catequinas e flavanonas. Um total de 11,26 mg GAE/g de fenólicos e 5,98 mg QE/g de flavonoides foram encontrados. O OFCC apresenta como compostos majoritários os ácidos oleico (61%) e palmítico (33%). O extrato foi capaz de intensificar a ação da gentamicina e eritromicina frente às cepas multirresistentes. O efeito anti-*Candida* do EMCC e OFCC observado neste estudo ocorre principalmente devido à formação de espécies reativas de oxigênio. Além disso, foram capazes de inibir a transição morfológica de *C. albicans* e *C. tropicalis* em concentrações de relevância clínica (512 µg/mL). Por fim, o OFCC não apresentou toxicidade *in vivo* contra *D. melanogaster*. Dessa forma, os produtos de *C. coriaceum* tornam-se opções para o desenvolvimento de formulações farmacêuticas para tratar infecções.

Palavras-chave: Candidíase; Etnobotânica; Extrativismo; Oleagionosa; Resistência microbiana.

ABSTRACT

Caryocar coriaceum Wittm. is a tree species belonging to the Caryocaraceae family, whose fruits are used to obtain oil for both culinary and medicinal purposes. Both this derivative and the fruits themselves are employed in pharmacopeia for the treatment of ailments. In this perspective, the present study aimed to: 1) Review studies conducted on the species, highlighting its pharmacological and ethnomedicinal uses, as well as its cultural and socioeconomic importance. 2) Chemically characterize the extract from the fruit mesocarp of *C. coriaceum* and assess its antimicrobial activity and drug-potentiating effects against pathogenic microorganisms. 3) Determine the chemical composition and evaluate the antifungal, antivirulence, and toxicity activity of the fixed oil from the fruits of *C. coriaceum* against three yeast strains of the *Candida* genus. The review studies were conducted through the analysis of data collected from scientific research platforms, including Google ScholarTM, Medline®, ResearchGate, Web of ScienceTM, Scopus®, ScienceDirect®, and Pubmed®. For the studies on antimicrobial activity, the methanolic extract of the inner mesocarp of *C. coriaceum* (MECC) was assessed using the serial dilution method against standard and multi-resistant strains of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. In addition to intrinsic activity, the ability to modify drugs was evaluated. Regarding antifungal activity, both the extract and the oil (FOCC) were assessed both intrinsically and in combination with fluconazole against *Candida* spp. strains. Additionally, potential anti-*Candida* mechanisms of action were investigated, along with the ability to inhibit morphological transition. Regarding the phytochemistry of MECC, qualitative chemical prospecting and determination of total phenolic content were performed using the Folin-Ciocalteu method. The transesterified FOCC, in turn, was analyzed by gas chromatography coupled with mass spectrometry. As for the toxicity of FOCC, it was evaluated through ingestion tests using the model organism *Drosophila melanogaster*. The studies demonstrate that *C. coriaceum* is a tree of significant medicinal, economic, and cultural importance. Medicinally, the fruits are used to treat bronchopulmonary diseases and tumors, while the oil is widely employed, particularly in the treatment of rheumatism. These properties have led to high commercial demand, boosting the economy of extractive communities. However, intense demand and other factors have resulted in the classification of the species as threatened with extinction. Chemically, the MECC showed flavones, flavonols, xanthones, catechins, and flavanones. A total of 11.26 mg of gallic acid equivalents per gram (mg GAE/g) of phenolic compounds and 5.98 mg of quercetin equivalents per gram (mg QE/g) of flavonoids were found. The FOCC has oleic acid (61%) and palmitic acid (33%) as its major compounds. The extract was able to enhance the action of gentamicin and erythromycin against multi-resistant strains. The anti-*Candida* effect of MECC and FOCC observed in this study primarily occurs due to the formation of reactive oxygen species. Additionally, they were able to inhibit the morphological transition of *C. albicans* and *C. tropicalis* at clinically relevant concentrations (512 µg/mL). Finally, FOCC showed no *in vivo* toxicity against *D. melanogaster*. Therefore, products from *C. coriaceum* become viable options for the development of pharmaceutical formulations to treat infections.

Keywords: Oilseed; Extractivism; Candidiasis; Microbial resistance; Ethnobotany.

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LISTA DE SIGLAS

ANOVA - Analysis of Variance

APA - Área de Proteção Ambiental

BHI - Brain Heart Infusion

CEME/MS - Central de Medicamentos do Ministério da Saúde

CIM - Concentração Inibitória Mínima

DCFH-DA - 2,7-dichlorofluorescein diacetate

DMSO - Dimetilsulfóxido

DPPH - 2,2-difenil-1-picril-hidrazil

FAME - Fatty Acid Methyl Esters

FID - Flame ionization detector

FOCC - Fixed Oil from *Caryocar coriaceum*

GAE - Gallic Acid Equivalents

GC-MS - Gas chromatography-mass spectrometry

IBAMA - Brazilian Institute of the Environment and Renewable Natural Resources

IC50 - Concentração inibitória média

IUCN - International Union for Conservation of Nature and Natural Resources

MIC - Minimum Inhibitory Concentration

MTT - 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

NTFPs - Non-Timber Forest Products

OMS - Organização Mundial da Saúde

PDA - Potato Dextrose Agar

PNPIC - Política Nacional de Práticas Integrativas e Complementares

QE - Quercetin Equivalents

ROS - Reactive Oxygen Species

SDB - Sabouraud Dextrose Broth

SISBio - Biodiversity Information and Authorization System

SISBio - Sistema de Autorização e Informação em Biodiversidade

SisGen - National System for the Management of Genetic Heritage and Associated Traditional Knowledge

SisGen - Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado

SUS - Sistema Único de Saúde

SUMÁRIO

1 APRESENTAÇÃO

A mortalidade em decorrência de doenças infecciosas e parasitárias teve um aumento expressivo nas últimas décadas. Esse fenômeno é atribuído à resistência microbiana, oriunda do uso indiscriminado de agentes antimicrobianos, o qual leva à seleção de microrganismos resistentes aos medicamentos convencionalmente disponíveis. Agravando a situação, a descoberta de novos antimicrobianos não tem conseguido acompanhar o ritmo evolutivo dos microrganismos. Como resultado, enfrentamos um cenário em que o arsenal terapêutico à disposição não é mais tão eficaz quanto costumava ser, o que torna a luta contra essas infecções mais complexa e desafiadora.

Além disso, um fator adicional que desempenha um papel significativo no aumento das taxas de mortalidade é a dificuldade de acesso a medicamentos nas populações menos favorecidas de países em desenvolvimento e subdesenvolvidos. Como opções terapêuticas, esses indivíduos recorrem ao uso de plantas medicinais para tratar as doenças infecciosas e parasitárias. Essa escolha está diretamente ligada à facilidade de obtenção dessas plantas, ao baixo custo envolvido e à aceitação cultural que elas têm dentro das comunidades.

Essas plantas de usos medicinais desempenham um papel significativo em diversas comunidades ao redor do globo, e o Brasil destaca-se como um dos principais utilizadores desses recursos. Essa tendência está intrinsecamente ligada à riqueza cultural do país e à notável diversidade de espécies vegetais encontradas ao longo dos diversos domínios fitogeográficos. Dentre os diferentes ecossistemas, a Caatinga emerge como um ambiente de destaque, caracterizado por sua vasta diversidade vegetal.

Um número significativo de espécies da flora brasileira desempenha um papel relevante na medicina popular, sendo empregadas no tratamento de diversas enfermidades. No conjunto dessas espécies vegetais, a árvore *Caryocar coriaceum* Wittm. (Caryocaraceae) conhecida como "pequi" destaca-se como produtora de frutos amplamente apreciados na culinária do Nordeste brasileiro. Além disso, essa espécie apresenta uma notável versatilidade em seus usos, com suas propriedades medicinais sendo especialmente notáveis. Entre os diversos usos medicinais, merece destaque a sua aplicação no tratamento de doenças infecciosas e parasitárias.

Do ponto de vista fitoquímico, foram relatados compostos de interesse farmacológico em *C. coriaceum*, como por exemplo ácido gálico, ácido clorogênico, ácido cafeico, rutina e quercetina nas folhas. Além desses compostos nas folhas, a espécie apresenta alcaloides, esteroides, saponinas e taninos. Quanto aos frutos da espécie, estes apresentam flavonoides

tais como quercetina e rutina, sendo também evidenciada a presença de ácidos graxos, dentre eles o ácido palmítico, ácido esteárico, ácido eicosanoico, ácido beênico, ácido lignocérico, ácido linoleico e ácido oleico.

Dessa forma, neste estudo levantou-se a hipótese que o extrato e óleo fixo do mesocarpo interno dos frutos de *C. coriaceum* apresentam atividade biológica contra microrganismos patogênicos. Levando isso em consideração, este trabalho teve como objetivo investigar o potencial biológico de tais produtos contra organismos causadores de infecções, bem como descrever os componentes e identificar os possíveis responsáveis por suas atividades.

2 REFERENCIAL TEÓRICO

2.1 PLANTAS MEDICINAIS

O uso de plantas medicinais pelo ser humano é a forma mais antiga de alternativa de tratamento, sendo utilizada até os dias atuais na medicina tradicional, em diversos países. O desempenho efetivo dessas plantas é fundamentado nas experiências de curandeiros, por meio de tentativas e erros, conhecimento esse que é repassado de pessoa a pessoa ao longo dos séculos (Khan, 2014; Jamshidi-Kia *et al*., 2018).

A importância dos produtos naturais é marcante, pois são fontes de compostos bioativos, na farmacoterapia moderna e antiga são evidenciados que vários medicamentos são derivados da medicina tradicional à base de plantas (Patwardhan *et al*., 2008; Li *et al*., 2020). Tais compostos são produzidos pelas plantas por meio do seu metabolismo secundário, dentre eles estão os terpenos, compostos fenólicos e alcaloides (Li *et al*., 2020).

Apesar desse amplo uso das plantas no tratamento de enfermidade, foi apenas em 1978 que a Organização Mundial da Saúde (OMS) reconheceu o uso de plantas medicinais e fitoterápicos, com finalidade profilática, curativa e paliativa (Boccolini; Boccolini, 2020). Anos mais tarde, em 1982, o governo brasileiro, por meio do Ministério da Saúde criou o Programa de Pesquisa de Plantas Medicinais, pela Central de Medicamentos do Ministério da Saúde (CEME/MS). No ano posterior, esse programa foi reestruturado e passou a ter como objetivo a produção de medicamentos fitoterápicos para o sistema de saúde da época, visto que até então o Sistema Único de Saúde (SUS) não existia. Contudo, no final do século a CEME foi extinta (Simões; Schenkel, 2002; Sant'ana; Assad, 2004).

No início do século XXI a OMS lançou o documento intitulado “Estratégia da OMS sobre Medicina Tradicional” incentivando o uso de fitoterapia no Sistema Nacional de Saúde, bem como a investigação sobre sua eficácia e segurança. Tendo isso em vista, em 2006 o Ministério da Saúde aprovou a Política Nacional de Práticas Integrativas e Complementares (PNPIC) no SUS e incluiu o uso de plantas medicinais e fitoterápicos para a melhoria da qualidade de saúde da população brasileira. Com isso, o governo reconheceu o valor terapêutico das plantas medicinais e incentivou as Unidades Básicas de Saúde a adotarem tais recursos para o tratamento complementar de seus usuários. Ainda no mesmo ano, o governo brasileiro aprovou a Política Nacional de Plantas Medicinais e Fitoterápicos a fim de garantir

acesso seguro, de qualidade, racional e correto de produtos naturais pela população (Figueredo *et al*., 2014; Arada *et al*., 2019).

Esse interesse na implementação de recursos naturais como terapia complementar no Brasil está relacionado à sua diversidade cultural e biológica. Culturalmente falando, o Brasil apresenta uma ampla diversidade étnica e cultural, representados pelas comunidades tradicionais, tais como as indígenas, quilombolas, caiçaras e seringueiros, as quais detém um valioso conhecimento tradicional relacionado ao uso de plantas medicinais para o tratamento de enfermidades. Associado a isso, o território apresenta alta diversidade biológica encontrada ao longo dos diversos domínios fitogeográficos (Magalhães *et al*., 2019).

2.2 PLANTAS MEDICINAIS DA CHAPADA DO ARARIPE

A Chapada do Araripe (Figura 1) está inserida no domínio fitogeográfico da Caatinga, estando localizada no extremo Sul do Ceará, com sua extensão territorial (972.605,18 hectares) abrangendo também os estados do Piauí e Pernambuco. Essa região, devido às condições edafoclimáticas, tais como alta precipitação (1.000 mm/ano), oscilação de altitude (800 - 900 m), temperaturas amenas (24 – 26 ºC) e solos característicos (latossolos vermelho-amarelos), desenvolveu ambientes distintos, denominados de fitofisionomias. Sendo estas: a Caatinga do Sedimentar (Carrasco), Cerrado e Cerradão, Floresta Estacional Sempre-Verde (Mata Úmida do Sedimentar) e a Mata Seca do Sedimentar (Ribeiro-Silva *et al*., 2012; Moro *et al*., 2015; Guerra *et al*., 2020).

Tais fitofisionomias apresentam floras distintas, as quais conferem uma alta diversidade de angiospermas na região. Os estudos mais recentes, indicam que essa região detém em torno de 474 espécies de angiospermas, pertencentes a 79 famílias botânicas (Loiola *et al*., 2015). Devido a essa diversidade vegetal, em conjunto com as comunidades tradicionais que habitam em torno da Chapada do Araripe, há um amplo uso dessas espécies para o tratamento de enfermidades, principalmente infecções (Souza *et al*., 2014; Macêdo *et al*., 2016; Cruz *et al*., 2021).

Essa diversidade etnofarmacológica é tão marcante na região, que é possível observar o relato de diversas espécies medicinais no livro *Travels in the Interior of Brazil* (1846) do médico e botânico escocês George Gardner (1810-1849), fruto de sua expedição pelo interior do Brasil durante a primeira metade do século XIX (Brandão *et al*., 2008; Fagg *et al*., 2015;).

No decurso de sua viagem, ao chegar na região da Chapada do Araripe, mais precisamente na então Vila Real de Crato, o botânico encantarou-se com a região:

Figura 1: Vista panorâmica da Chapada do Araripe – Crato, Ceará.

Fonte: Autor (2020).

> "Impossível descrever o deleite que senti, ao entrar neste distrito, comparativamente rico e risonho, depois de marchar mais de trezentas milhas através de uma região que, naquela estação, era pouco melhor que um deserto. A tarde era das mais belas que me lembra ter visto, com o sol a sumir-se em grande esplendor por trás da Serra do Araripe, longa cadeia de montanhas, a cerca de uma légua para Oeste da Vila, e o frescor da região parece tirar aos seus raios o ardor que pouco antes do poente é tão opressivo ao viajante, nas terras baixas. A beleza da noite, a doçura revigorante da atmosfera, a riqueza da paisagem, tão diferente de quanto, havia pouco, houvera visto, tudo tendia a gerar uma exultação de espírito, que só experimenta o amante da natureza e que, em vão eu desejava fosse duradoura, porque me sentia não só em harmonia comigo mesmo, mas "em paz com tudo em torno". (Gardner, 1849).

O naturalista destacou algumas espécies medicinais utilizadas no Cariri Cearense para tratar uma gama de enfermidades, visto que o sistema de saúde era inexistente na região, fruto de um Brasil colônia que há pouco tempo tornara-se império em 1822. Dentre as espécies medicinais citadas em seus trabalhos destacam-se *Allamanda blanchetii* A.DC. (quatro

patacas; Apocynaceae), *Caryocar coriaceum* Wittm. (pequi; Caryocaraceae), *Passiflora laurifolia* L. (maracujá-peroba; Passifloraceae), *Brunfelsia uniflora* (Pohl) D.Don (manacá; Solanaceae), *Psidium laruotteanum* Cambess.(marangaba; Myrtaceae), dentre outras (Fagg *et al*., 2015).

Ao longo das décadas, mais espécies vegetais foram incorporadas à farmacopeia da região do Araripe, de forma que estudos etnofarmacológicos cresceram exponencialmente (Souza *et al*., 2014; Macêdo *et al.*, 2016; Cruz *et al*., 2021). Dentre as principais espécies que ocorrem na Chapada do Araripe, utilizadas na terapêutica popular, destacam-se *Copaifera langsdorffii* Desf. (pau-d'óleo; Fabaceae), *Lafoensia pacari* A.St.-Hil. (romã braba; Lythraceae); *Hymenaea stigonocarpa* Mart. ex Hayne (jatobá-do-cerrado; Fabaceae), *Astronium urundeuva* (M. Allemão) Engl. (aroeira; Anacardiaceae), *Hancornia speciosa* Gomes (mangaba; Apocynaceae); *Scoparia dulcis* L. (vassourinha; Plantaginaceae), *Himatanthus drasticus* (Mart.) Plumel (janaguba; Apocynaceae), *Ximenia americana* L. (ameixa; Olacaceae); *Libidibia ferrea* (Mart. ex Tul.) L.P.Queiroz (pau-ferro Fabaceae) e *Anacardium occidentale* L. (caju; Anacardiaceae) (Figura 2) (Cruz *et al*., 2021).

As principais indicações terapêuticas relatadas nas comunidades tradicionais dessa região são para doenças do sistema digestório, doenças do sistema geniturinário e doenças do sistema respiratório. São também utilizadas para o tratamento de enfermidades ligadas a outros sistemas corporais, evidenciando que a Chapada do Araripe detém um amplo acervo medicinal para o tratamento de patologias (Souza *et al*., 2014; Macêdo *et al*., 2016).

Dentre os produtos de origem vegetal mais utilizados nessa região, destacam-se o látex, resinas e óleos, estes últimos sendo os de maior valor comercial, visto que demandam tempo e técnicas específicas. Dentre os óleos comercializados na região com fins medicinais e culinários estão o óleo de *Jatropha molissima* (Pohl) Baill. (pinhão-branco; Euphorbiaceae), *Caryocar coriaceum* Wittm. (pequi; Caryocaraceae), *Copaifera langsdorffii* Desf (copaíba; Fabaceae), *Juglans regia* L. (nogueira; Juglandaceae) e *Ricinus communis* L. (mamona; Euphorbiaceae) (Bitu *et al*., 2015; Macedo *et al*., 2016).

Figura 2: Plantas medicinais mais utilizadas na Chapada do Araripe – CE, Brasil.

A = *Copaifera langsdorffii* (pau-d'óleo). B = *Lafoensia pacari* (romã braba). C = *Hymenaea stigonocarpa* (jatobá-do-cerrado). D = *Astronium urundeuva* (aroeira). E = *Hancornia speciosa* (mangaba). F = *Scoparia dulcis* (vassourinha). G = *Himatanthus drasticus* (janaguba). H = *Ximenia americana* (ameixa). I = *Libidibia ferrea* (pau-ferro). J = *Anacardium occidentale* (caju) Fonte: Autor (2020).

2.3 CARYOCARACEAE

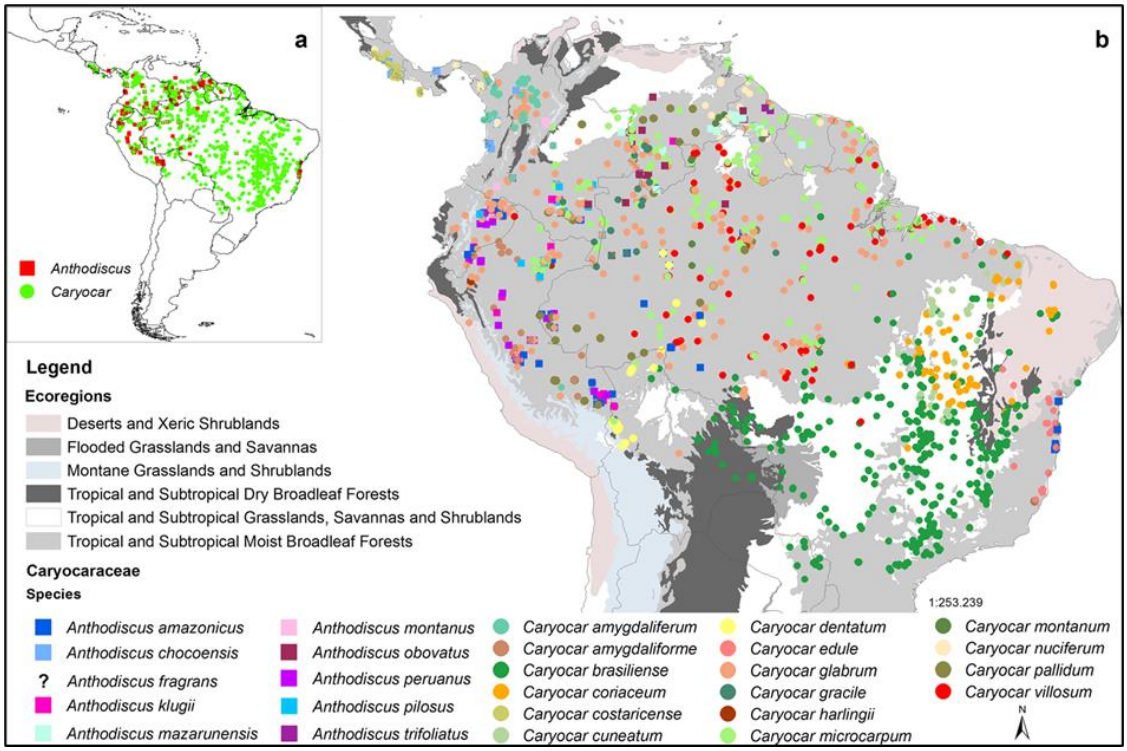
A família botânica Caryocaraceae Voigt (1845) pertence à ordem Malpighiales, classe Magnoliopsida e divisão Magnoliophyta e apresenta atualmente um total de 26 espécies distribuídas em dois gêneros, *Caryocar* L. e *Anthodiscus* G. F. W. Meyer (Tabela 1) (Apg IV, 2016). A distribuição das espécies dessa família é exclusiva da Região Neotropical, podendo ser encontrada ao longo das Américas Central e do Sul (Figura 3) (Ascari *et al*., 2013; Nunes *et al*., 2020).

Tabela 1: Lista de espécies da Família Caryocaraceae.

***Caryocar* L.**	***Anthodiscus* G. F. W. Meyer**
Caryocar amygdaliferum Mutis ex Cav.	*Anthodiscus amazonicus* Gleason & A.C.Sm.
Caryocar amygdaliforme G.Don	*Anthodiscus chocoensis* Prance
Caryocar brasiliense A.St.-Hil.	*Anthodiscus fragrans* Sleumer
Caryocar coriaceum Wittm.	*Anthodiscus klugii* Standl. ex Prance
Caryocar costaricense Donn.Sm.	*Anthodiscus mazarunensis* Gilly
Caryocar cuneatum Wittm.	*Anthodiscus montanus* Gleason
Caryocar dentatum Gleason	*Anthodiscus obovatus* Benth. ex Wittm.
Caryocar edule Casar.	*Anthodiscus peruanus* Baill.
Caryocar glabrum (Aubl.) Pers.	*Anthodiscus pilosus* Ducke
Caryocar gracile Wittm.	*Anthodiscus trifoliatus* G.Mey.
Caryocar harlingii Prance & Encarn.	
Caryocar microcarpum Ducke	
Caryocar montanum Prance	
Caryocar nuciferum L.	
Caryocar pallidum A.C.Sm.	
Caryocar villosum (Aubl.) Pers.	

Fonte: Autor (2021).

Figura 3: Distribuição de espécies da família Caryocaraceae em diferentes ecorregiões da América Central e do Sul.



Fonte: Nunes *et al*. (2020). Com permissão do autor.

Morfologicamente, esse táxon compreende árvores e arbustos com folhas trifolioladas, opostas ou alternadas entre si, apresentando margens serrilhadas, dentadas ou crenadas nas extremidades de seus folíolos. Estípulas, quando presentes, variam de 2 a 4. Suas flores são dispostas em inflorescências do tipo racemos terminais com pedicelos articulados. Tais órgãos reprodutivos são grandes, hermafroditas, actinomorfas, contendo de 5 a 6 sépalas e pétalas, ambas imbricadas. Sendo as pétalas distintas e em alguns poucos casos conectadas na base ou no seu ápice. Os seus estames são numerosos, variando de 55 a 750, os quais são longos e delgados unidos em formato de um anel na sua base. Os estames, que são internos, são curtos, recurvados e geralmente estéreis, nos seus ápices estão fixadas anteras biloculares. Estruturalmente, o ovário é composto e do tipo superior, sendo ligado por um estigma puntiforme distal, e seus carpelos sendo uniloculares, cada um com um único óvulo. Estes são basais, eretos, anátropos ou atroposos, bitegumentados ou unitegumentados. Os ovários quando polinizados, geram frutos do tipo drupas, com 1-4 sementes (*Caryocar* spp.) ou 8-20 sementes (*Anthodiscus* spp.). Apresentam mesocarpo indeiscente, geralmente gorduroso ou carnudo; endocarpo dura e lenhosa, muricatada, tuberculada ou espinulosa na superfície

externa, eventualmente dividindo-se em pirenos ou mericarpos com 1 semente. Estas apresentam endosperma delgado ou até mesmo é ausente, enquanto o embrião apresenta radícula reta, arqueada ou torcida em espiral, com um hipocótilo carnudo e dois pequenos cotilédones (Prance *et al*., 2014).

A família Caryocaraceae é conhecida por apresentar espécies com várias atividades biológicas, dentre elas estão atividade antifúngica (*C. brasiliense* e *C. coriaceum*) (Passos *et al*., 2003; Ascari *et al*., 2010; Araruna *et al*. 2013; Gomes; Ribeiro 2019), antibacteriana (*C. brasiliense*) (Paula Junior *et al*., 2006; Ascari *et al*., 2010), antiparasitária (*C. brasiliense* e *C. coriaceum*) (Herzog-Soares *et al*., 2002; Tomiotto-Pellissier *et al*., 2018), inseticida (*C. brasiliense*) (Morais *et al*., 2020), larvicida (*C. coriaceum*) (Azevedo *et al*., 2021), nematicida (*C. brasiliense*) (Silva *et al*., 2021), moslucicida (*C. brasiliense*) (Bezerra *et al*., 2002) e alelopática (*C. brasiliense*) (Ascari *et al*., 2010; Vargas *et al*., 2021).

Além das atividades biológicas, diversos estudos demonstram que as espécies apresentam alto potencial farmacológico com atividade anticarcinogênica (*C. brasiliense*) (Brito *et al*., 2022), antioxidante (*C. brasiliense*) (Ascari *et al*., 2010; Oliveira *et al*., 2018; Braga *et al*., 2022), anticlastogênica (*C. brasiliense*) (Khouri *et al*., 2007), genoprotetora (*C. brasiliense*) (Miranda-Vilela *et al*., 2008), anti-inflamatória (*C. brasiliense* e *C. villosum*) (Yamaguchi *et al*., 2017; Roll *et al*., 2018), antidiabética (*C. brasiliense*) (Caldeira *et al*., 2021), cicatrizante (*C. brasiliense*) (Bezerra *et al*., 2015; Pires *et al*., 2020), antianêmica (*C. brasiliense*) (Roll *et al*., 2018), neuroprotetora (*C. brasiliense*) (Oliveira *et al*., 2018), gastroprotetora (*C. coriaceum*) (Lacerda-Neto *et al*., 2017) e antinociceptiva (*C. coriaceum*) (Oliveira *et al*., 2015). Tais propriedades estão relacionadas com a composição química das espécies pertences ao táxon em estudo.

Dos dois gêneros da família Caryocaraceae, o mais estudado em todos os aspectos é *Caryocar* L., este gênero apresenta 16 espécies e é representado por árvores conhecidas como "pequizeiros" que dão frutos conhecidos popularmente como "pequi", "piqui", "pequiá", "pekea", "pequi-vinagreiro", "pequiarana" (Ascari *et al*., 2013; Nunes *et al*., 2020).

2.3.1 *Caryocar coriaceum* Wittm.

Caryocar coriaceum é uma espécie pertencente à família Caryocaraceae. A etimologia do gênero é grega, em que *caryon* significa "núcleo" ou "noz", enquanto *kara* significa "cabeça", referindo-se ao fruto globoso da espécie. O epíteto específico *coriaceum* refere-se à "textura de couro", "grosso" e "rígido". A espécie é conhecida popularmente como "pequi",

“piqui” ou “pequizeiro”, tendo essa denominação origem dos povos indígenas de Pindorama (ao território ocupado pelos tupi-guarani) (*py-qui*), onde *py* = pele ou casca e *qui* = espinho, significando “casca espinhenta”, decorrente dos espinhos encontrados no epicarpo (Kerntopf *et al.*, 2013; Silva; Medeiros-Filho, 2006).

Morfologicamente, os indivíduos de *C. coriaceum* são de porte arbóreo (Figura 4), com comprimento do caule variando de 5 a 15 m de altura. As suas folhas são compostas, trifolioladas, com filotaxia oposta. Cada folha possui um pecíolo, este medindo de 1,5 a 4 cm de comprimento. As margens dos folíolos podem ser serreadas ou crenadas. Os folíolos medem de 3,7 a 7 cm de comprimento e 5 a 10 cm de largura e são curtamente peciolados, com limbo oval, de ápice arredondado a ligeiramente retuso. A base dos folíolos é subcuneada. O limbo é glabro, tanto na face abaxial, quanto adaxial, com venação do tipo broquidódroma e uma textura coriácea. Além disso, a espécie apresenta inflorescências do tipo racemo densifloro com 2,5 a 8,5 cm de comprimento. Cada inflorescência apresenta de 10 a 16 flores, as quais são hermafroditas. As flores são compostas por inúmeros estames, chegando a cerca de 300 por flor. O cálice é composto de 5 sépalas de coloração verde-avermelhada e uma corola dialipétala contendo 5 pétalas de cor amarelo-claro. O gineceu possui ovário globoso tri ou tetralocular (Silva; Medeiros-Filho, 2006; Oliveira *et al.*, 2008; Oliveira *et al.*, 2009; Ramos; Souza, 2011; Silva *et al.*, 2013; Ferreira-Júnior *et al.*, 2015; Silva *et al.*, 2016; Nascimento-Silva; Naves 2019; Rodrigues *et al.*, 2019).

Figura 4: Aspecto geral de *Caryocar coriaceum* Wittm. (Caryocaraceae). (A) = Hábito; (B) = Haste; (C) = Folhas; (D) = Flor e botões florais; (E) = Fruta; (F) = Fruto com endocarpo exposto após remoção de parte do epicarpo pelas formigas.

Fonte: Autor (2021).

Após a polinização, que pode ser por autopolinização, por pássaros e por morcegos, um único indivíduo de *C. coriaceum* é capaz de gerar de 500 a 2.000 frutos. Esses diásporos são do tipo drupa ovoide com dimensões variando de 4 a 7 cm de comprimento e 6 a 8 cm de diâmetro, tendo uma massa variando de 100 a 220 g. Os diásporos são formados por um epicarpo coriáceo verde-claro. O mesocarpo externo é esbranquiçado, enquanto o interno é carnoso com uma coloração oscilando do amarelo-creme ao amarelo-intenso, e, algumas vezes, alaranjada. O endocarpo, o qual protege a semente é espinhosa e apresenta uma coloração amarronzada. Geralmente, cada fruto possui de um a quatro caroços, também chamado de putâmen, dependendo de quantos ovários são fecundados e desenvolvidos (Silva; Medeiros-Filho, 2006; Oliveira *et al.*, 2008; Oliveira *et al.*, 2009; Ramos; Souza, 2011; Silva *et al.*, 2013; Cavalcanti *et al.*, 2015; Ferreira-Júnior *et al.*, 2015; Silva *et al.*, 2016; Nascimento-Silva; Naves 2019; Rodrigues *et al.*, 2019).

Com relação à sua distribuição geográfica, *C. coriaceum* é nativa e endêmica do Brasil, sendo encontrada principalmente na região setentrional do Nordeste brasileiro nos estados da Bahia, Piauí, Pernambuco, Maranhão e Ceará. Neste último, a presença da espécie é mais abundante devido à sua ocorrência em áreas de proteção ambiental (APA), como a APA-Araripe e a Floresta Nacional do Araripe (FLONA). Dentre os municípios do estado do Ceará há registros de coleta em Araripe, Santana do Cariri, Nova Olinda, Crato, Barbalha, Missão Velha, Brejo Santo, Porteiras e Jardim (Costa *et al.*, 2004; Costa; Araújo, 2007; Medeiros *et al.*, 2008; Conceição; Castro, 2009; Medeiros; Walter, 2012; Ribeiro-Silva *et al.*, 2012; Silva *et al.*, 2016; Campos *et al.*, 2018; Bezerra *et al.*, 2020).

Os frutos da espécie são de extrema importância socioeconômica para famílias de comunidades extrativistas da Chapada do Araripe chegando a constituir até 80% da renda total familiar. Para tanto, o trabalho é árduo, visto que o extrativismo dessa fruta inclui um conjunto de atividades como coleta, transporte, processamento e comercialização seja *in natura* ou de seus derivados (óleo), os quais participam todos os membros da família (Augusto; Góes, 2007; Lacerda-Neto *et al.*, 2013; Sousa-Júnior *et al.*, 2013; Feitosa *et al.*, 2014; Cavalcanti *et al.*, 2015; Sobral *et al.*, 2017; Maciel *et al.*, 2018).

Etnofarmacologicamente, quase todas as partes de *C. coriaceum*, exceto as flores e as raízes, são utilizadas para o tratamento de diferentes tipos e enfermidades como as doenças da pele e do tecido subcutâneo, doenças do sistema geniturinário e doenças do sistema respiratório, doenças dos olhos e anexos, doenças do sistema digestivo, doenças do sistema osteomuscular e sistema conjuntivo, doenças endócrinas, nutricionais e metabólicas, doenças infecciosas e parasitárias, lesões, envenenamento e algumas outras consequências de causas externas (Cartaxo *et al.*, 2010; Ferreira-Júnior *et al.*, 2015).

Os seus frutos são os mais utilizados na medicina popular, seja *in natura* (polpa) ou os seus derivados, como o lambedor ou o óleo. O mesocarpo interno é consumido para combater doenças bronco-pulmonares (bronquites, gripes e resfriados) e tumores (Conceição *et al.*, 2011; Ribeiro *et al.*, 2014). O óleo fixo, é amplamente empregado no tratamento de reumatismo, inflamações, dores musculares, dor de garganta, bronquite, tosse com secreções, gripes, eczema, afecções do couro cabeludo, dores nos pulmões, asma, queimaduras, febre, raquitismo, indigestão, sopro no coração, cicatrização de feridas, fadiga e disfunções eréteis (Agra *et al.*, 2007; Matos, 2007; Agra *et al.*, 2008; Batista *et al.*, 2010; Gonçalves, 2010; Lozano *et al.*, 2014; Ribeiro *et al.*, 2014; Lemos *et al.*, 2016; Macêdo *et al.*, 2016; Magalhães *et al.*, 2019).

Apesar das indicações medicinais se concentrarem nos frutos, outros órgãos de *C. coriaceum* também são relatados como agentes terapêuticos. As folhas, por exemplo, são empregadas na regulação do fluxo menstrual, enquanto as cascas são utilizadas no combate à febre e como diurético (Batista *et al.*, 2010; Gonçalves, 2010). Além disso, tanto as folhas, quanto os frutos de *C. coriaceum* são utilizados no tratamento de bronquite, fadiga, nódulo, catarro, para cicatrização, dor de cabeça, dor de dente, dor de garganta, dor nas articulações, ferida na boca, influenza, fraturas, reumatismo e tosse (Silva *et al.*, 2019). Além da utilização para o tratamento de doenças humanas, as folhas de *C. coriaceum* são empregadas na medicina etnoveterinária para eliminar anexos fetais em bovinos e o óleo fixo é aplicado nos cortes e inflamações de vários animais (Gonçalves, 2010; Amorim *et al.*, 2018).

Em adição aos seus usos etnomedicinais, *C. coriaceum* tem sido avaliada cientificamente quanto ao seu potencial medicinal. Dentre os produtos avaliados a partir da espécie, o óleo fixo dos seus frutos é o mais investigado, devido à sua versatilidade e altos índices de indicações terapêuticas. Os estudos são direcionados às atividades antioxidante, antimicrobiana, cicatrizante, anti-inflamatória, gastroprotetora, antinociceptiva, hipolipemiante, anticonvulsionante, antiparasitária, citotóxica, tóxica e alelopática (Leite *et al.*, 2009; Quirino *et al.*, 2009; Costa *et al.*, 2011; Saraiva *et al.*, 2011; Oliveira *et al.*, 2015; Duavy *et al.*, 2019; Almeida-Bezerra *et al*., 2022)

Os frutos e sementes de *C. coriaceum* são uma importante fonte de óleo fixo, constituídos de ácidos graxos saturados e insaturados. Costa *et al.* (2011) e Figueiredo *et al.* (2016) demonstraram que a polpa (mesocarpo interno) do fruto é composta majoritariamente por ácidos graxos insaturados, com um percentual de aproximadamente 64%, seguido de ácidos saturados (≅ 36%). Para o primeiro grupo, o ácido graxo majoritário foi o ácido oleico (C18:1), e para o segundo, o ácido palmítico (C16:0). Similarmente, as sementes apresentaram um perfil lipídico similar, entretanto, foram encontrados lipídios saturados que não estavam presentes na polpa, como o metil-18-metilnonadecanoato (C20:0), ácido docosanóico (C22:0) e ácido lignocérico (C24:0) (Serra *et al.*, 2020). Além disso, ácido araquidônico (C20:0) e ácido linolênico (C18:3) não foram detectados nas sementes, enquanto estavam presentes nos frutos (Pessoa *et al.*, 2015).

Além de compostos do metabolismo primário, *C. coriaceum* apresenta uma variedade de compostos do metabolismo secundário. Dentre estes, fenóis simples, flavonoides, flavonas, flavonóis, xantonas, flavononóis, taninos (pirrogálicos e hidrolisáveis), flavononas, saponinas, leucoantocianidinas, catequinas, esteroides e alcaloides foram identificados nas folhas de *C.*

coriaceum (Duavy *et al.*, 2012; Araruna *et al.*, 2013; Lacerda-Neto *et al.*, 2017; Tomiotto-Pellissier *et al.*, 2018; Amparo *et al.*, 2020).

Os estudos fitoquímicos dos compostos fenólicos de *C. coriaceum* se concentram nos frutos, cascas do caule e folhas, não sendo encontradas pesquisas até o momento que visem a bioprospecção de suas raízes e flores. Dentre os compostos de natureza fenólica identificados na espécie, destacam-se a rutina, quercetina, epicatequina, isoquercitrina, ácido gálico, ácido clorogênico, ácido cafeico e ácido elágico (Alves *et al.*, 2017; Araruna *et al.*, 2013; Araruna *et al.*, 2014; Duavy *et al.*, 2019).

De acordo com a Lista Vermelha da União Internacional para a Conservação da Natureza e dos Recursos Naturais (IUCN), *C. coriaceum* é uma espécie vegetal ameaçada de extinção (Prado, 1998; Bezerra *et al.*, 2020). Tal status de conservação é devido a fatores como extrativismo crescente, germinação lenta, redução de animais dispersores, desmatamento e queimadas (Silva *et al.*, 2006; Sousa-Júnior *et al.*, 2015; Ribeiro *et al.*, 2017; Bezerra *et al.*, 2020).

2.4 RESISTÊNCIA DE MICRORGANISMOS

Em 1928 o médico e cientista Alexander Fleming descobriu acidentalmente o primeiro antibiótico, conhecido por todos como penicilina. Com essa descoberta, foi possível salvar milhões de vidas humanas e aumentar a suas expectativas de vida em 30 anos, principalmente nos países desenvolvidos. Contudo, quase duas décadas depois, o médico, ao ganhar o prêmio Nobel ao lado Ernst Boris Chain e Howard Walter Florey, alertou sobre a possibilidade do surgimento de microrganismos resistentes, caso houvesse uma antibioticoterapia ineficaz, com baixas doses de antibióticos de eficácia duvidosa (Tan; Tatsumura, 2015; Abadi *et al.*, 2019).

Infelizmente Fleming estava certo e, ao longo dos anos, a utilização intensiva e inadequada de antibióticos provocou a seleção de microrganismos resistentes. Estes são microrganismos capazes de se multiplicarem na presença de drogas antibióticas em concentrações que normalmente inibiriam o seu crescimento. Tal resistência é encontrada principalmente em bactérias, mas também se estende ao grupo dos fungos e protozoários, que podem ser classificados como microrganismos nosocomiais o quais causam infecções em

ambiente hospitalar ou clínicas, além de serem transmitidos em equipamentos médicos não esterilizados (Healey; Perlin, 2018; Abadi *et al*., 2019; Fürnkranz; Walochnik, 2021).

A consequência desse uso irracional acarretou na resistência microbiana, um dos maiores problemas de saúde pública enfrentados na atualidade. O problema é tão preocupante que a OMS, divulgou recentemente um relatório expondo a urgência em desenvolvimento de novas estratégias para contornar a resistência microbiana (Fisher; Denning, 2023). A preocupação deve-se ao fato dessa resistência ser a terceira maior causa de mortes, acarretando no óbito de milhões de pessoas em todo o mundo (Abadi *et al*., 2019). Somado a isso, estão os custos aos cofres públicos para o tratamento e hospitalização de pessoas enfermas às infecções, chegando a custos estimados em US$ 30 bilhões anuais no mundo (Fair; Tor, 2014; Patini *et al*., 2020).

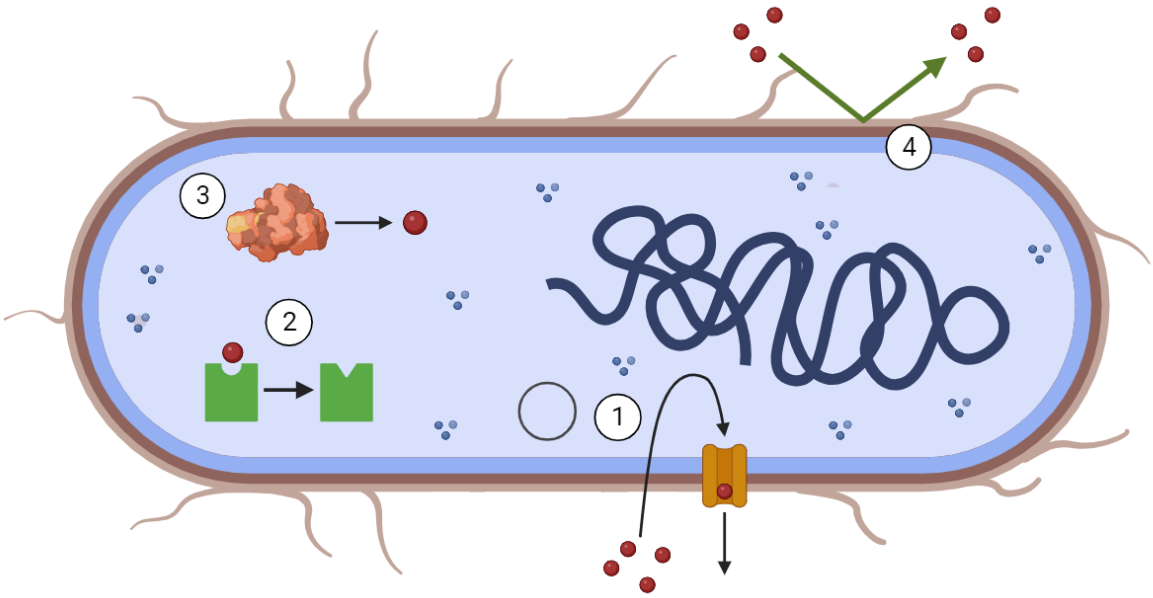
Apesar do desenvolvimento de novas drogas antibióticas após a descoberta da penicilina, como os aminoglicosídeos, tetraciclinas, glicopeptídeos, cefalosporinas, triazólicos, a resistência continua sendo um problema, pelo fato das pessoas utilizarem em excesso tais drogas como medidas profiláticas contra quaisquer possíveis infecções bacterianas (Abadi *et al*., 2019). Com isso a busca dos cientistas por novos agentes terapêuticos cresceu exponencialmente. Como alternativa, estão os produtos naturais, que podem de modo intrínseco ou em combinação com drogas antimicrobianas, apresentarem efeitos biológicos em concentrações de relevância clínica (Gupta; Birdi, 2017; Bezerra *et al*., 2019).

2.4.1 Bactérias

As bactérias são seres unicelulares procariotos que vivem nos mais diversos ecossistemas do planeta (Johnson; Mangel, 2006). Existem bactérias autótrofas, que produzem sua própria energia por meio da quimiossíntese, e as heterotróficas, as quais utilizam compostos orgânicos originados de organismos, vivos ou mortos, para sua nutrição (Kindaichi; Okabe, 2004). Desempenham importantes papeis ecológicos, tais como a ciclagem dos nutrientes e mutualismo. Contudo, algumas dessas bactérias podem ser ou se tornarem patogênicas, como é o caso de bactérias comensais que fazem parte da microbiota do ser humano. Essas convivem normalmente no corpo do seu hospedeiro, contudo em situações específicas, como, um desequilíbrio na homeostase, podem se tornar patogênicas, sendo então denominadas oportunistas (Fast *et al*., 2018; Khan *et al*., 2019).

Como citado anteriormente, as bactérias são capazes de tornarem-se resistentes a antibióticos, expressando diversos mecanismos de resistência como modificações nas moléculas do antibiótico, diminuição da permeabilidade de antibióticos, desenvolvimento de bombas de efluxo, mudanças no sítio alvo do antibiótico, dentre outros mecanismos (Figura 5) (Blair *et al*., 2015). Dentre as principais bactérias resistentes responsáveis por causarem infecções nosocomiais destacam-se *Escherichia coli*, *Pseudomonas aeruginosa* e *Staphylococcus aureus* (Abadi *et al*., 2019).

Figura 5: Principais mecanismos de resistência bacteriana. 1: Efluxo do fármaco antibacteriano; 2: Alteração do sítio-alvo; 3: Inativação enzimática; 4: Bloqueio de entrada.



Fonte: Autor (2023).

O primeiro microrganismo citado, *E. coli* (Enterobacteriaceae), é uma bactéria Gram-negativa que habita de forma comensal o intestino grosso de organismos endotérmicos, dentre eles o ser humano. Tal microrganismo é classificado em três categorias: 1) organismos comensais; 2) cepas que causam doença intestinal diarreica (*E. coli* enteropatogênica, *E. coli* enterotoxigênica, *E. coli* enterohemorrágica, *E. coli* enteroagregativa, *E. coli* enteroinvasiva e *E. coli* difusamente aderente) e 3) cepas que geralmente causam doenças fora do trato intestinal. Patologicamente, *E. coli* é capaz de provocar diarreia aquosa, sanguinolenta, colite hemorrágica, diarreia do viajante, síndrome hemolítica urêmica em aves e humanos (Poolman, 2017; Rodrigues *et al*., 2022).

A bactéria *P. aeruginosa* (Pseudomonadaceae), é Gram-negativas que, por possuírem poucas exigências para o seu crescimento, podem normalmente habitar diversos ambientes, tais como a água, o solo, ambientes hospitalares e a microbiota do ser humano. Neste, os microrganismos quando comensais, habitam a pele, a garganta e o intestino grosso, contudo, caso haja um desequilíbrio na homeostase, podem invadir e infeccionar outros lugares, como por exemplo, o sistema urinário e as vias respiratórias. Em consequência dessas infecções podem causar pneumonias, meningites e até mesmo endocardites (Pang *et al*., 2019; Thi *et al*., 2020). Estudos recentes mostram que essa bactéria é capaz de adquirir resistência à múltiplas classes de antibióticos, incluindo beta-lactâmicos, aminoglicosídeos e fluoroquinolonas. Tal resistência é tão preocupante que a OMS a classificou na categoria "crítica" na lista de patógenos prioritários para o desenvolvimento de novos antibióticos (Botelho *et al*., 2019; Pachori *et al*., 2019).

Por fim, *S. aureus* é uma espécie da família Staphylococcaceae Gram-positiva e dentre as infecções nosocomiais, é a de maior interesse clínico, visto que é causa morbidade e mortalidade em todo o mundo (Cheung *et al*., 2021). Seu hábitat, enquanto comensal no ser humano, é na cavidade oral, na mucosa nasal, na pele e no trato gastrointestinal. Contudo, esta espécie também é oportunista e pode ocasionar no hospedeiro pneumonia e outras infecções do trato respiratório e infecções cardiovasculares. Assim como os demais, este microrganismo apresenta resistência, a qual evolui de modo rápido por diversos meios, dentre eles a transferência horizontal de genes e mutação cromossômica (Craft *et al*., 2019; Okwu *et al*., 2019).

2.4.2 Fungos

Os fungos são microrganismos unicelulares ou multicelulares eucariontes e heterotróficos por absorção e vivem em diferentes ambientes. Diferentemente das bactérias, possuem um sistema de endomembranas, que formam organelas citoplasmáticas com formas e funções variadas, bem como cromossomos lineares (Richards *et al*., 2017; Li *et al*., 2021). Além disso, suas paredes celulares são compostas por polissacarídeos insolúveis em água, como a quitina e os glucanos. O primeiro polímero é formado por longas cadeias de *N*-acetilglucosamina (2-acetamido-2-desoxi-D-glicopiranose) ligadas entre si (Ibe; Munro, 2021), enquanto os glucanos apresentam estruturas altamente variáveis (Ruiz-Herrera; Ortiz-Castellanos, 2019). Além disso, outra característica marcante desse grupo é a presença de certos esteroides em sua membrana plasmática, como por exemplo o ergosterol. Como

destacado anteriormente, eles podem ser formados por uma ou várias células, dessa forma, são classificados em leveduras (unicelulares) ou fungos filamentosos (multicelulares) (Richards *et al*., 2017).

Os fungos são seres extremamente necessários à manutenção da vida na terra, pois estão ligados diretamente à decomposição de matéria orgânica, bem como são capazes de formar associações mutualísticas com outras espécies, como por exemplo a associação com as raízes de árvores originando as micorrizas (Richards *et al*., 2017; Li *et al*., 2021;). Nesse reino, há também associações comensais, como por exemplo algumas leveduras, que habitam a pele e as mucosas dos seres humanos e de outros animais. Contudo, estes são microrganismos oportunistas, ou seja, ocasionam infecções em organismos que tenha sua imunidade comprometida (Pappas, 2010). Dentre os principais fungos causadores de infecções, estão espécies do gênero *Candida*, *Aspergillus*, e *Cryptococcus*, responsáveis por mais de 90% de mortes por doenças micóticas (Lee *et al*., 2020).

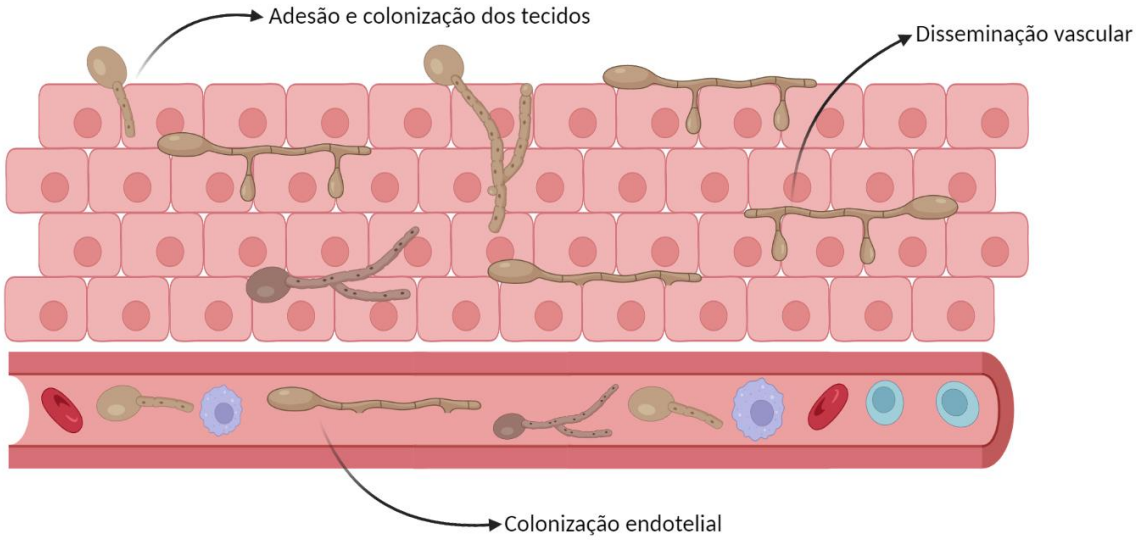
Desses fungos, destacam-se as espécies de levedura do gênero *Candida* (Saccharomycetaceae). Essas leveduras são adquiridas durante o nascimento e tornam-se parte da microbiota humana, sem ocasionar manifestações patológicas em hospedeiros saudáveis. Contudo, quando esse hospedeiro se torna imunocomprometido, esses fungos podem ocasionar infecções conhecidas como candidíase, que em casos mais graves tornam-se invasivas e se disseminam para órgãos internos (Wilson, 2019; Lee *et al*., 2020; Romo, Kumamoto, 2020). Com isso, a candidíase é uma doença fúngica multifacetada oportunista que pode incluir infecções mucocutâneas, viscerais e disseminadas (Ribeiro *et al*., 2020).

Devido à característica oportunista, várias espécies de *Candida* spp. tornou-se um grande problema de saúde pública em todo o mundo, visto que causam altas taxas de mortalidade e altos custos médicos, tanto para governos, quanto para os pacientes hospitalizados (Spampinato; Leonardi, 2013; Wilson, 2019). São vários os fatores de virulência expressos por espécies de *Candida*, sendo os principais a capacidade polimórfica, alteração fenotípica, capacidade de formação de biofilme aderente, produção e secreção de enzimas hidrolíticas, expressão de complexos proteicos de adesina e a invasão de células da epiderme e mucosas (Eix; Nett, 2020; Khan *et al*., 2021; Rosiana *et al*., 2021).

Desses mecanismos de virulência, a capacidade polimórfica ocorre em algumas espécies de *Candida*, isso quer dizer que, as leveduras são capazes de alterar a sua estrutura em circunstâncias específicas. Ao alterar a sua forma, elas passam a ser filamentosas, contando com a presença de pseudo-hifas (cadeias de células de levedura alongadas com constrições) e hifas (cadeias ramificadas de células tubulares sem constrições nos locais de

septação) (Kornitzer, 2019). Patologicamente, essa formação de filamentos tem importância para o sucesso da infecção, pois por meio deles ocorre a invasão tecidual. No caso da candidemia, que é a infecção de *Candida* spp. no sangue, é por meio da formação de pseudo-hifas e hifas que as cepas encontram a corrente sanguínea. Para tanto, inicialmente penetram as células da mucosa, posteriormente alcançam os tecidos subadjacentes e finalmente encontram a corrente sanguínea e espalham-se pelo corpo do hospedeiro (Figura 6) (Kornitzer, 2019; Khan *et al*., 2021). Essa transição morfológica, como citam Khan *et al*. (2021), foi relatada como associada com invasão da camada celular epitelial, ruptura e dano das células endoteliais, evasão de células fagocitárias, tigmotropismo e escape de anticorpos.

Figura 6: Patogênese da invasão e virulência das leveduras de espécies do gênero *Candida*.



Fonte: Autor (2022).

Dentre as espécies do gênero *Candida*, a mais estudada e pesquisada é *Candida albicans*, por ser a mais comum em infecções. No entanto, os estudos não devem ser concentrados apenas nela, visto que as espécies *Candida* não-*albicans* tem causado um quadro grave de infecções ao longo do planeta, dentre elas *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* e *Candida tropicalis* (Montenegro *et al*., 2017; Whaley *et al*., 2017). Assim como as bactérias, essas espécies vêm apresentando uma certa resistência a drogas antifúngicas (Lee *et al*., 2020).

Atualmente são utilizadas três classes de antifúngicos para o tratamento de infecções de tal natureza, sendo eles os pertencentes à classe dos polienos, equinocandinas e azóis, os quais atuam por diferentes mecanismos de ação (Lee *et al*., 2020). Os polienos foram os

primeiros antifúngicos empregados no tratamento de infecções causadas por *Candida* spp., tendo sido introduzido na metade do século XX. Dos polienos, a anfotericina B é a mais conhecida, por atuar contra um amplo espectro de cepas fúngicas de interesse clínico. Tal antifúngico atua por meio da formação de agregados extramembranosos que extraem o ergosterol das membranas celulares das leveduras, atuando como uma "esponja de esterol" fungicida. Contudo, o uso clínico da anfotericina B apresenta certas desvantagens, principalmente pela baixa biodisponibilidade oral e seus efeitos tóxicos dose-dependentes para o paciente, pois estruturalmente o ergosterol assemelha-se ao colesterol encontrado nas membranas das células humanas (Carolus *et al*., 2020; Lee *et al*., 2020).

Os azóis, são os antifúngicos mais utilizados clinicamente, tendo sido empregados pela primeira vez na década de 1980. Estruturalmente são compostos sintéticos heterocíclicos que atuam por meio do bloqueio da síntese do ergosterol, levando à ruptura na estabilidade da membrana plasmática, na sua permeabilidade e na função das enzimas associadas à membrana (Pristov; Ghannoum, 2019). Além disso, antifúngicos azólicos aumentam a concentração de intermediários de esteróis tóxicos às leveduras, incluindo 14-α-metil-3,6-diol. Dessa forma, tais drogas atuam inibindo o crescimento fúngico, ou seja, são fungistáticas, enquanto o organismo do hospedeiro reage a fim de controlar a infecção. O mecanismo de ação utilizado por tal classe é a inibição de enzimas associadas ao citocromo P450 lanosterol 14-α-desmetilase, de forma a bloquear a conversão do lanosterol em ergosterol. Diferentemente das outras duas classes, os azóis apresentam biodisponibilidade oral excepcional e estão disponíveis tanto em formulações orais quanto intravenosas, sendo a mais conhecida o fluconazol. Contudo como o efeito dessas drogas é fungistático, ocorre seleção de microrganismos resistentes, tornando importante a busca por novas drogas antifúngicas (Berman; Krysan, 2020; Lee *et al*., 2020).

Por fim, os antifúngicos da classe equinocandinas foram os últimos a serem descobertos e desenvolvidos. Estes têm origem de produtos naturais e estruturalmente compreendem grandes lipopeptídeos (Healey; Perlin, 2018; Pristov; Ghannoum, 2019). Devido esta forma estrutural atuam rompendo a parede celular fúngica, por meio da ligação à subunidade catalítica da (1,3)-β-D-glucano sintase, o que a tornou uma ótima alternativa terapêutica, visto que tal estrutura não está presente em células eucarióticas de mamíferos. Com esse bloqueio na síntese de (1,3)-β-D-glucana, ocorre a ruptura da parede celular e um desequilíbrio na pressão osmótica, culminando na lise celular. Contudo, assim como alguns polienos, as equinocandinas apresentam baixa absorção oral e, consequentemente, o uso clínico é limitado à administração intravenosa (Healey; Perlin, 2018; Lee *et al*., 2020).

Além dos efeitos colaterais relatados acima, a maioria dos antifúngicos empregados atualmente no combate à candidíase apresentam susceptibilidade à resistência microbiana, principalmente aqueles pertencentes à classe dos azóis (Pristov; Ghannoum, 2019). Infelizmente o desenvolvimento de novos antifúngicos é bastante limitado devido à natureza das células, pois tanto as leveduras quanto as células do seu hospedeiro são eucarióticas (Roemer; Krysan, 2014; Nicola *et al*., 2019). Como alternativa, pesquisas visam combinar produtos naturais e antifúngicos, a fim de avaliar se os produtos naturais são capazes de intensificar a ação dos fármacos (Bezerra *et al*., 2019; Costa *et al*., 2021; Rodrigues *et al*., 2022).

3 ARTIGO 1 – *Caryocar coriaceum* Wittm. (Caryocaraceae): Botany, Ethnomedicinal Uses, Biological Activities, Phytochemistry, Extractivism and Conservation Needs

Artigo Publicado
Revista: *Plants*
ISSN: 2223-7747
Fator de Impacto: 4.5
Qualis – CAPES (2017-2020): A4 em Biodiversidade
Percentil Scopus (2023): 83%

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Abstract: *Caryocar coriaceum* is an endemic tree of Brazil, occurring mainly in the northeast region in the Cerrado environment. The species, popularly known as “pequi”, produces fruits that are used in the manufacture of oil for food and medicinal purposes. This work reviewed studies conducted with the species, highlighting its ethnomedicinal use, its pharmacological potential, including its chemical constituents, and its cultural and socioeconomic importance. Information was obtained through the main scientific research platforms. The keyword “*Caryocar coriaceum*” was used as the main index for searching the following platforms: PubMed®, PubMed Central®, SciElo, Scopus® and Web of ScienceTM. The compiled papers demonstrate that *C. coriaceum* has great medicinal, economic and cultural importance for northeastern Brazil. Popularly, the fruits of *C. coriaceum* are used to treat broncho-pulmonary diseases (bronchitis, colds and flu). The fixed oil is widely used to relieve pain from various causes in the treatment of inflammation, flu, eczema, burns, fever, rickets, indigestion, heart murmurs, fatigue and erectile dysfunction. Some of these uses are corroborated by pharmacological trials, which have demonstrated the antioxidant, healing, anti-inflammatory, gastroprotective, antinociceptive and antiroperties of the species. Chemically, fatty acids and phenolic compounds are the main constituents recorded for the species. Due to its medicinal properties, the fruits and oil of *C. coriaceum* have a high commercial demand and are one of the main forms of subsistence activities for local populations. On the other hand, the extractive practice of the fruits, associated with anthropic factors and its physiological nature, makes the species threatened with extinction. Thus, public management policies are highly necessary in order to avoid its extinction.

Keywords: Oleic acid; Caryocaraceae; extractivism; flavonoids; Chapada do Araripe

1. Introduction

Among the botany families occurring in Brazil, Caryocaraceae (1845) presents a total of 25 species distributed in Central and South America and comprises only two genera, *Anthodiscus* G. Mey. and *Caryocar* L. The species belonging to the genus *Caryocar* are found in varied phytogeographic domains, such as Amazonia, Cerrado or Savannah, Atlantic Forest and Caatinga or Seasonally Dry Tropical Forest [1,2].

Caryocar coriaceum Wittm. is a species that occurs mainly in the Caatinga. In this ecosystem, *C. coriaceum* was described and published in the Flora Brasiliensis in 1886 by the botanist Ludwig Wittmack (1839–1929) [3]. The first reports of the use of this species date from the 19th century [4,5]. The English botanist George Gardner (1810–1849), while passing through the Cariri region in the city of Crato (state of Ceará, northeast region of Brazil), reported that the fruit of "pequi" was used in popular cooking and pharmacopoeia. Its wood, of high quality, was used in the construction of mills [6,7].

The scientific literature shows that *C. coriaceum* is a species widely studied in academia, mainly because it is a well-known species. Thus, a review becomes necessary in order to demonstrate the state of the art for the species, as well as to compile information about the data described in the literature.

Since then, a culture about *C. coriaceum* has been established in the Cariri region, giving this species a high demand due to its versatility. This descriptive work aimed to compile the work done with the species, highlighting its ethnomedicinal uses, pharmacological potential, including phytochemistry, and its cultural and socioeconomic importance in northeastern Brazil.

2. Review

2.1. Botanical Aspects and Geographical Distribution

Caryocar coriaceum is a species belonging to the family Caryocaraceae Voigt, nom. cons. and the order Malpighiales Juss. ex Bercht. & J.Presl [8]. The etymology of the genus is Greek, in which caryon means "core" or "nut", while kara means "head", referring to the globose fruit of the species. The specific epithet *coriaceum* refers to *"*leathery texture*"*, *"*thick*"* and *"*stiff*"*. The species is popularly known as *"*pequi*"*, *"*piqui*"* or *"*pequizeiro*"*, originating from the indigenous people of Pindorama (a territory of present-day Brazil) (pyqui), where py = skin or shell and qui = thorn, meaning *"*spiny shell*"*, arising from the thorns found in the epicarp of the fruit [9–11].

Morphologically, the individuals of *C. coriaceum* are arboreal, with stem lengths varying from 5 to 15 m in height. Its trunk reaches 35 cm in diameter and has a wood density of 0.78 g/cm3 (Figure 1). The trunks have thick bark and thick and angular branches, which can grow to the sides of the plant or close to the ground and which set the species apart from others in Cerrado areas [12–14].

Figure 1. General aspect of *Caryocar coriaceum* Wittm. (Caryocaraceae). (**A**) = Habit; (**B**) = Stem; (**C**) = Leaves; (**D**) = Flower and floral buds; (**E**) = Fruit; (**F**) = Fruit with endocarp exposed after the removal of part of the epicarp by ants.

The leaves of *C. coriaceum* are compound and trifoliolate, with opposite phyllotaxis. Each leaf has a petiole, which measures 1.5 to 4 cm long. The leaf margins can be serrate or crenate. The leaflets measure 3.7 to 7 cm long and 5 to 10 cm wide and are short petiolate, with an oval limb rounded to a slightly rectilinear apex. The base of the leaflets is subcuneate. The limb is glabrous, both on the abaxial and adaxial sides, with venation of the brochidodromous type and a coriaceous texture [15–17].

From the reproductive point of view, *C. coriaceum* presents inflorescences of the densiflorous raceme type that are 2.5 to 8.5 cm long. Each inflorescence presents from 10 to 16 flowers which are hermaphrodite. The flowers are composed of numerous stamens, reaching about 300 per flower. The calyx is composed of five reddish-green sepals and a dialipetal corolla containing five light-yellow petals. The gynoecium has a tri or tetralocular globose ovary [18,19].

After pollination, which can be performed by self-pollination, birds (ornithophily) and bats (chiropterophily), a single individual of *C. coriaceum* is capable of producing 500 to 2000 fruits. These diaspores are of the ovoid drupe type, with dimensions varying from 4 to 7 cm in length and 6 to 8 cm in diameter and a mass varying from 100 to 220 g. The diaspores are formed by a light green coriaceous epicarp. The outer mesocarp is whitish, while the inner one is fleshy, with a color ranging from creamy yellow to intense yellow and sometimes orange. The endocarp, which protects the seed, is spiny and has a brownish color. Generally, each fruit has one to four lumps, also called putamen, depending on how many ovaries are fertilized and developed [15,17,19].

Phenological studies conducted in the Chapada do Araripe (Ceará state, Brazil) showed that the flowering of the species occurs from June to October (dry period), and the maturation of its fruits occurs from October to March (rainy season) [10,13,17,19].

Regarding its geographical distribution, *C. coriaceum* is native and endemic to Brazil, being found mainly in the northern region of the Brazilian Northeast in the states of Bahia, Piauí, Pernambuco, Maranhão and Ceará. In the latter, the presence of the species is more abundant due to its occurrence in areas of environmental protection (APA), such as the APA-Araripe and the Araripe National Forest (FLONA) (Figure 2). Among the municipalities of the Ceará state, there are collection records for Araripe (municipality number 4 on the map), Santana do Cariri (5), Nova Olinda (6), Crato (7), Barbalha (8), Missão Velha (9), Brejo Santo (11), Porteiras (12) and Jardim (13) [15,20–27].

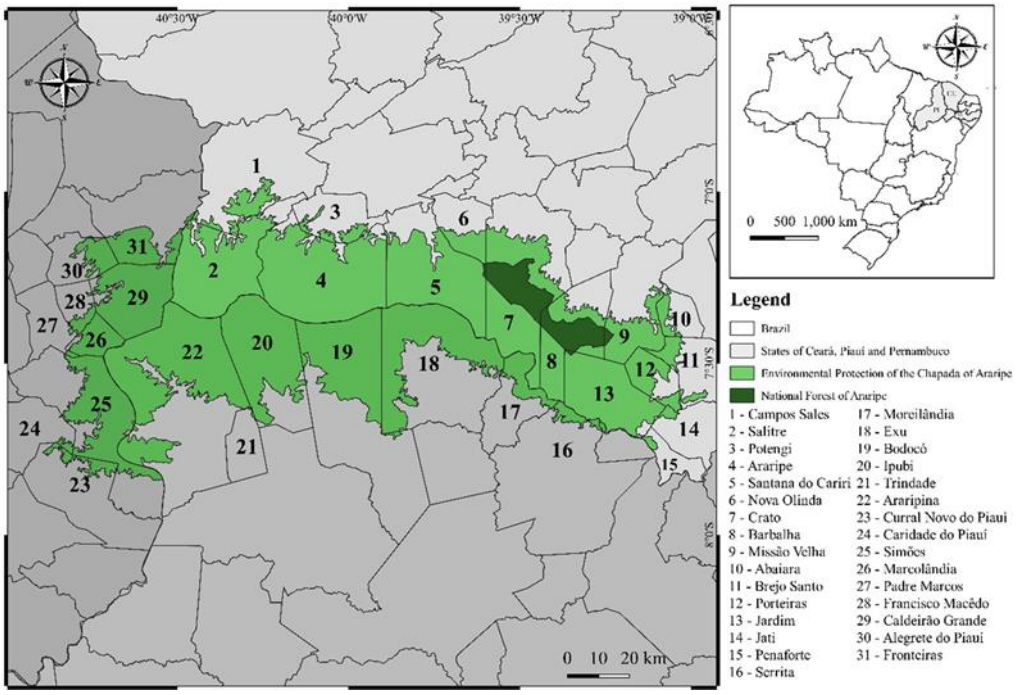


Figure 2. Map of the Araripe Environmental Protection Area (APA—Araripe, Ceará, Brazil) where *Caryocar coriaceum* Wittm. (Caryocaraceae) occurs spontaneously.

In Chapada do Araripe, *C. coriaceum* occurs in Cerrado *sensu stricto* enclaves. This vegetation occurs at the top of the Chapada and is characterized by being a semideciduous savannah vegetation. It covers aluminized leached soils, which are responsible for the twisted branches and trunks of the plant species that occur in this environment, showing sinuses of hemicryptophytes, cryptophytes-geophytes, chamaephytes and phanerophytes, twisted with irregular branching, perennial or deciduous, sometimes with a well- developed cortex [28].

2.2 Ethnomedicinal Uses

Among the species with medicinal potential belonging to the genus *Caryocar* L., *C. brasiliense* Cambess., *C. villosum* (Aubl.) Pers. and *C. coriaceum* Wittm. stand out. These three species are popularly used in Brazil for the treatment of swellings, respiratory diseases, wound injuries, gastric and inflammatory diseases, muscle pain and chronic arthritis [14,29].

Historically, the Kariri Indians of the Chapada do Araripe called individuals of *C. coriaceum "Pyrantecaira"* (i.e., which gives vigor and strength). Unfortunately, due to the colonization of the region by the Portuguese (1683–1713), the genocide of these peoples occurred, and along with them, some of the traditional knowledge associated with the species was lost [30,31].

Among the 67 plant species occurring in the Chapada do Araripe with medicinal potential, *C. coriaceum* is one of the most cited by rural communities, presenting 47 therapeutic indications. Ethnobotanically, almost all parts of *C. coriaceum*, except the flowers and roots, are used for the treatment of different types and diseases such as diseases of the skin and subcutaneous tissue, diseases of the eyes and annexes, diseases of the digestive system, diseases of the osteomuscular system and connective system, endocrine, nutritional and metabolic diseases, infectious and parasitic diseases, injuries, poisoning and some other consequences of external causes, diseases of the genitourinary system and diseases of the respiratory system [16,32].

The fruits of *C. coriaceum* are the most used organ in folk medicine, either in natura (pulp) or its derivatives, such as syrup or oil [33,34]. The internal mesocarp is consumed to combat broncho-pulmonary diseases (bronchitis, colds and flu) and tumors [35–37]. The fixed oil is widely used in the treatment of rheumatism, inflammation, muscle pain, sore throat, bronchitis, cough with secretions, flu, eczema, scalp disorders, lung pain, asthma, burns, fever, rickets, indigestion, heart murmur, wound healing, fatigue and erectile dysfunction [38–42].

Although medicinal indications focus on the fruits, other organs of *C. coriaceum* are also reported as therapeutic agents. The leaves, for example, are employed in the regulation of catamenial flow, while the bark is used to combat fever and as a diuretic [38,40]. According to Silva et al. [43], the leaves and fruits of *C. coriaceum* are used in the treatment of bronchitis, fatigue, nodule, catarrh, cicatrization, headache, toothache, sore throat, joint pain, mouth sore, influenza, broken bone, rheumatism and cough. In addition to the use for the treatment of human diseases, *C. coriaceum* leaves are employed in ethnoveterinary medicine to eliminate fetal attachments in cattle, and the fixed oil is applied to cuts and inflammations in various animals [31,44].

2.3 Biological and Pharmacological Activities

In addition to its ethnomedicinal uses, *C. coriaceum* has been scientifically evaluated for its medicinal potential. Due to its versatility and high rates of therapeutic indications, the fixed oils obtained from the fruits are the most investigated products by different authors [45–51]. Studies that have demonstrated the antimicrobial, healing, anti-inflammatory and gastroprotective antioxidant potential, in addition to other activities of *C. coriaceum*, are presented below.

2.3.1 Antioxidant Activity

Among the various published studies on *C. coriaceum*, the antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical reduction method is the most investigated (Table 1). In this method, a total of 2.5 mL of *C. coriaceum* samples are added to a 1 mL solution of DPPH (60 µM), obtaining varying concentrations depending on the study, reaching a maximum of 1000 µg/mL. In addition, blank tests are used, which aim to measure the absorbance of the extraction solvents (ethanol, methanol or water). As a positive control, in this assay, known antioxidant agents are used, such as vitamin C (ascorbic acid) or BHT (butylhydroxytoluene). As a negative control, only the DPPH solution is used for comparative purposes. Finally, with the aid of a spectrophotometer, the absorbance of the solutions is measured after 30 min of reaction in an environment devoid of light. When the product shows antioxidant activity, the sample that has an original purple color tends to discolor to a yellowish color. So, the more yellow it is, the greater the antioxidant potential.

The aqueous extract of the leaves of the species, for example, showed the highest capacity in reducing DPPH, with an IC_{50} value 15 times lower than ascorbic acid, a positive control [52]. Duavy et al. [53] reported that, at concentrations of 100 and 250 µg/mL, the DPPH radical scavenging exhibited by *C. coriaceum* leaf and fruit peel extracts was similar to that found for ascorbic acid. Alves et al. [54] also demonstrated that the peels and pulp of *C. coriaceum* fruits showed antioxidant potential, with the pulp, with an IC_{50} value of 49.4 µg/mL, being the most active part.

In vivo antioxidative studies were conducted by Duavy et al. [50]. These authors found that the leaf extracts and oil from the pulp of *C. coriaceum* fruits conferred protection to *Drosophila melanogaster* (fruit fly) against the oxidant agent paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride). Methodologically, the researchers fed the flies with a sucrose solution (4%) containing paraquat (1 mM) and a group which was added to this solution: the aqueous extract of *C. coriaceum* leaves in varying concentrations (1–5 mg/mL), accompanied by a control (without paraquat and without extract). After a period of one week, the live flies were anesthetized on ice and manually homogenized in a phosphate buffer solution (20 mM), pH 7.4 (50 flies/mL), and the homogenate was centrifuged at 3500×g for 10 min at 4 °C. After centrifugation, the supernatant was collected and kept on ice until testing. Such procedures were carried out to determine the levels of reactive species in order to assess whether the product had an antioxidant effect. For this, the levels of reactive species were measured using the DCFDA assay, which is based on the deacetylation of 2’,7’-dichlorofluorescein diacetate (DCF-DA). The medium contained pH 7.4 potassium phosphate

buffer (75 mM), DCFH-DA (5 µM final) and the fly supernatant (10 µL). Fluorescence was determined at 488 nm excitation and 525 nm emission, respectively, for 20 min in a Shimadzu spectrophotometer. The results were expressed as arbitrary fluorescence units (AUF), using a standard curve with DCF. In addition, lipid peroxidation was evaluated by measuring the levels of thiobarbituric acid reactive substances (TBARS) in the samples. In such an assay, the authors incubated the supernatant solution in an acidic medium (8.1% SDS (100 µL), 0.8% TBA (500 µL) and 20% acetic acid pH 3.5 (500 µL)) for an hour at 100 °C. TBARS were determined spectrophotometrically at 532 nm using malondialdehyde (MDA). The natural products were able to reduce the levels of reactive oxygen species (ROS) and lipidic peroxidation, as well as decrease the activity of the antioxidant enzymes catalase and glutathione-S-transferase. In addition, leaf and pulp extracts were able to down-regulate the mRNA expression of stress-related genes for catalase, superoxide dismutase, thioredoxin reductase and Keap-1 (Kelch-like ECH-associated protein 1).

Table 1. *In vitro* antioxidant activity of *Cariocar coriaceum* Wittm. (Caryocaraceae) using the free radical DPPH (2,2-difenil- 1-picril-hidrazil).

Plant organ	**Preparation**	**IC_{50} µg/mL (extract/oil)**	**IC_{50} µg/mL (positive control)**	**Reference**
Leaves	Aqueous extract	2.70	42.0 (Ascorbic acid)	[52]
Leaves	Ethanolic extract	3.24	42.0 (Ascorbic acid)	[52]
Leaves	Ethanolic extract	26.37	6.5 (Ascorbic acid)	[53]
Leaves	Aqueous extract	27.20	6.5 (Ascorbic acid)	[53]
Fruit peels	Ethanolic extract	38.66	6.5 (Ascorbic acid)	[53]
Leaves	Hydroethanolic extract	6.06	77.76 (Ascorbic acid)	[55]
Leaves	Methanolic extract	5.02	77.76 (Ascorbic acid)	[65]
Leaves	Hydroethanolic extract	9.70	6.20 (Butylated hydroxytoluene)	[56]
Fruit peels	Ethanolic extract	49.40	13.7 (Rutin)	[54]
Pulp	Ethanolic extract	25.50	13.7 (Rutin)	[54]
Pulp	Fixed oil	10.21	13 (Ascorbic acid)	[57]

2.3.2 Antimicrobial Activity

The antimicrobial activity of products from *C. coriaceum*, especially its fixed oil, against bacteria of clinical interest has been determined by different authors. For the assays to determine the Minimum Inhibitory Concentration (MIC) of the antibacterial assays using the microdilution method, a serial dilution of the natural product (1024–1 µg/mL) in BHI broth (Brain Heart Infusion) was used, which contained 10% of the bacterial inoculum in suspensions of 105 CFU/mL. Subsequently, the culture plates were placed in bacteriological incubators for incubation at 37 °C for 24 h. After this period, solutions (20 uL) of liquid resazurin were added to the plates in order to carry out the redox reactions. The MIC was defined in such studies as the lowest concentration at which there was no bacterial growth. Furthermore, in some studies, the researchers evaluated the ability of *C. coriaceum* to enhance the action of antibiotics against multidrug-resistant bacteria using the same methodology; however, the product concentrations were sub-inhibitory (MIC/8).

Saraiva et al. [48] demonstrated through microdilution assays that the oil presents a minimum inhibitory concentration (MIC) of 512 µg/mL against *Escherichia coli* ATCC 25922, *E. coli* EC 27, *Staphylococcus aureus* ATCC 12692 and *S. aureus* SA 358; however, it proved ineffective against *Pseudomonas aeruginosa* ATCC15442 and *Proteus vulgaris* ATCC13315. These authors also demonstrated that the fixed oil of *C. coriaceum* was able to reduce the MIC of aminoglycoside antimicrobials (gentamicin, kanamycin, amikacin and neomycin) against *E. coli* and *S. aureus*.

Costa et al. [47] found through the disc diffusion method that the fixed oil of *C. coriaceum* at a concentration of 10 µg/disc showed an antibacterial effect on *Salmonella choleraesuis* ATCC 13314 (15 mm halo), *S. aureus* ATCC 12692 (13.7 mm), *P. aeruginosa* ATCC 15442 (10.3 mm) and *Streptococcus pneumoniae* ATCC 6314 (7.7 mm). In the case of the agar diffusion method, the bacteria are replicated in Petri dishes containing MüllerHilton agar, using a sterile swab. After sowing, paper discs (6 mm in diameter) are impregnated with a solution (20 µL) of the natural product of *C. coriaceum* in different concentrations (1.25–10%), which are placed in the center of the plate agar. Ampicillin (AMP, 100 µg/disk) and chloramphenicol (CLO, 100 µg/disc) are used as positive controls, and Tween 80 and distilled water are used as negative controls. Subsequently, these plates were incubated at 37 °C for 24 h. Finally, the inhibition halos of each concentration were measured.

Despite the bactericidal effects of the fixed oil reported above, Pereira et al. [58] found no *in vitro* antibacterial action of the oil at concentrations of clinical relevance (1024 µg/mL) against the strains of *Proteus vulgaris* ATCC 13315, *Klebsiella pneumoniae* ATCC 10031,

Shigella flexneri ATCC 12022, *P. aeruginosa* ATCC 9027, *E. coli* 06, *Bacillus cereus* ATCC 33018, *S. aureus* ATCC 6538 and *S. aureus* 10.

According to Araruna et al. [59], the leaf extracts of *C. coriaceum* have an antibiotic modifying action. In this study, the authors found that the hydroethanolic extracts and the methanolic fraction were able to enhance the activity of different aminoglycoside antibiotics against *E. coli* 27 and *S. aureus* 358. Lacerda-Neto et al. [56] also observed that the hydroalcoholic leaf extracts of *C. coriaceum* enhance the effect of penicillins, such as benzylpenicillin, against *E. coli*.

The antimicrobial effect of *C. coriaceum* also extends to fungal strains of veterinary interest, such as *Microsporum canis* and *Malassezia* spp. [54]. In such a study, spore suspension solutions were used for growth on potato dextrose agar (PDA) and placed in microbial growth ovens at 28 °C for a period of 7 days. After growth, the spores were quantified in a Neubauer chamber to reach a concentration of 105 to 106 cells. In 96-well microplates, a solution of 100 μL of RPMI medium was added, which was microdiluted with *C. coriaceum* extracts starting from a concentration of 2500 until reaching 2.44 µg/mL. Finally, 50.0 µL of the fungal suspension was added to all wells of the plates, except for the lines intended for the control of the sterile medium. The readings were made by verifying the MIC, with the aid of stereoscopic verification of the lowest concentration of the samples capable of inhibiting 100% of the growth of the microorganism, after 5 days of incubation. In this study, the authors demonstrated that the ethanolic extracts of the fruit peel and pulp are excellent antifungal agents, since both extracts showed an MIC of 4.88 µg/mL against *M. canis*.

In contrast, the antifungal effect was not observed against species of the *Candida* genus of clinical interest, such as *Candida albicans*, *Candida glabrata*, *Candida krusei* and *Candida tropicalis* [59,60].

2.3.3 Healing and Anti-Inflammatory Activity

Quirino et al. [46] showed that the oil from the pulp of the fruit of *C. coriaceum* had a significant healing effect in rats on the sixteenth day after administration. Therefore, acute gastric lesions were induced in mice (n = 8/group) by the oral administration of absolute ethanol (96%) in a volume of 0.2 mL (using an orogastric metal tube), and *C. coriaceum* oil dissolved in Tween 80 (2% in distilled water as a vehicle) was administered in oral doses of 200 and 400 mg/kg, 60 min before ethanol application. The vehicle treated group (2% Tween

80) was included as a negative control. After a period of 30 min after the administration of the solvent, the animals were killed by cervical dislocation; following ethical procedures, the stomachs were removed and opened along the greater curvature, and the area of the gastric lesions was measured by planimetry using a transparent grid.

Oliveira et al. [61] also evaluated such effect on excisional skin lesions in mice. Methodologically, mice were treated with ointment containing the fixed oil of *C. coriaceum* at 6% (*v*/*w*) and 12% (*v*/*w*), while the control group consisted of mice treated with solid ointment (vaseline and lanolin—1: two). As a positive control, 5% *w*/*w* clostebol acetate and neomycin sulfate cream were used, and T5 received 0.9% saline as a negative control. After treatment, the mice were anesthetized by the open mask method, and their dorsal surface was scraped with a sterile blade. In this area, the holidays were left naked in the open environment, and daily observations were made. Treatments were applied topically once a day from wound induction to complete healing in sufficient amounts to cover all wounds. These authors showed that, on the seventh day of treatment, the 12% oil caused a contraction of up to 96.5% of the wounds compared to 88% of the control drug.

Batista et al. [40] added *C. coriaceum* oil to a base cream. To achieve the results, the authors divided mice into two groups. One consisted of 20 mice with skin wounds treated with the topical application of a cream base with 10% *C. coriaceum* oil. The second group included the same number of animals that received the topical application of the base cream without the natural product. After antisepsis and local anesthesia, a circular wound of 1 cm in diameter was surgically produced in the dorsum lumbar region. The skin lesions were evaluated under the clinical, morphometric and histological aspects on the 3rd, 7th, 14th and 21st postoperative days. At the end of the experiment, the skin wounds of the rats were fully healed, with complete closure of the edges, while the wounds of the animals in the control group still required more time for healing.

Regarding the anti-inflammatory potential, Oliveira et al. [61] showed that the fixed oil of *C. coriaceum* attenuated xylene-induced inflammatory edema in albino Swiss mice (*Mus musculus*) in a dose-dependent manner. The methods consisted of randomly allocating animals into nine groups. The first received only xylene (positive control), while the second received the fixed oil of *C. coriaceum in natura*. Another four groups received the oil in varying concentrations (6%, 12%, 25% and 50%), while the other two groups received dexamethasone (2.5 mg/kg) and indomethacin (5 mg/kg) orally for 3 days. One hour after the last oral treatment of mice, xylene was applied. Edema was induced by the topical application of xylene to the inner and outer surfaces of the right ear lobe. The left ear was considered as a

control. Fifteen minutes or one hour after the induction of inflammation, the mice were sacrificed by an overdose of ether anesthesia, and both ears were removed. Circular sections were made using a cork drill with a diameter of 5 mm and weighed. The edematous response was measured as the difference in weight between the right and left ears. In this study, the crude oil reduced inflammation by 38.01% in just 15 min. According to these authors, the fixed oil of *C. coriaceum* accelerates the repair of cutaneous wounds, validating its popular use.

Similar results were also found by Saraiva et al. [62]. *C. coriaceum* oil at a concentration of 8 mg/ear was responsible for inhibiting 28.5% of *Croton* oil-induced inflammation in mice. After 48 h of application, a significant reduction in ear thickness compared to the group treated with the saline solution was also found.

Using the method of inflammation induced in the paw of mice by carrageenan, Figueiredo et al. [63] demonstrated that doses of 500 and 1000 mg/Kg of the fixed oil of *C. coriaceum* reduced the induced edema by 21% and 31%, respectively, after seven days of treatment. Methodologically, different groups of animals were treated with *C. coriaceum* oil at different doses (500, 1000 and 2000 mg/kg and 0.9% saline solution) for 7, 15 and 30 days, and at the end of each period, the initial volume (Vi) of the right paw was recorded using a plethysmograph. After 1 h, each animal received an intraplantar injection of 2% (*w*/*v*) carrageenan in the right hind paw (0.2 mL/paw). The volume of the right hind paw of each animal was evaluated again by the plethysmograph at 1, 2, 3 and 4 h after the injection of the phlogistic agent.

Silva et al. [15] verified, in rats, an anti-inflammatory action of the fixed oil after tendonitis induced with the intratendinous injection of collagenase in the calcaneal tendon. In this research, a total of 36 male rats were divided into groups: control, ultrasound associated with *C. coriaceum* oil and pure. In order to induce tendinitis, the intratendinous injection of collagenase was used in the right Achilles tendon. The treatment consisted of the daily application of ultrasound + oil or oil alone to the tendon. Macroscopic analysis was performed with a caliper on the 1st, 7th and 14th days. Subsequently, the rats were sacrificed, and then the tendon was dissected and removed to allow for histological analysis with Hematoxylin & Eosin (HE). Such action was evidenced in the reduction of neutrophils (inflammatory cells) after seven days of topical treatment with the oil. The researchers showed that when the tendinitis was submitted to ultrasonic waves, the process of tissue repair was more effective, because there was an induction of fibroblasts increase.

Oliveira et al. [49] induced knee arthritis in rodents using zymosan, a polysaccharide from the cell wall of *Saccharomyces cerevisiae* that produces acute and severe inflammation. In this research, ethyl acetate extract from the pulp of was used, which was diluted in 2% of Tween 80 concentrations and administered by oral gavage at doses of 100, 200 and 400 mg/kg 45 min before zymosan-induced arthritis or for 7 consecutive days at the same time each day. The zymosan injection was performed 24 h after the last administration of the extract. After the entire experimental protocol, the animals were then euthanized following ethical protocols for collecting fluid and synovial tissue to evaluate leukocyte recruitment, myeloperoxidase (MPO) activity and cytokine release and immunohistochemistry. Changes in vascular permeability were assessed by the extravasation of Evans Blue dye into joint tissue 6 h after zymosan injection. Thus, they demonstrated that the fixed oil of *C. coriaceum*, at doses of 100 mg/Kg, showed anti-inflammatory potential by reducing the influx of leukocytes and neutrophils into the joint cavity. In addition to the fruit, the leaves of *C. coriaceum* also show anti-inflammatory potential in mice. According to Araruna et al. [55], the hydroethanolic and methanolic leaf extracts of *C. coriaceum* reduced edema in Swiss mice (*Mus musculus*) caused by different sensitizing agents such as arachidonic acid, *Croton* oil, phenol and histamine.

2.3.4 Gastroprotective Activity

The gastroprotective effect of *C. coriaceum* has been demonstrated by different authors. Leite et al. [45] found that, at a dose of 200 mg/Kg, the oil from the *C. coriaceum* fruit pulp was able to inhibit 60.5% of the gastric mucosal lesions induced by ethanol in Swiss mice (*M. musculus*). To achieve these objectives, the animals were deprived of their food and were induced to ingest, 12 h after this period, *C. coriaceum* oil at the concentration (200 and 400 mg/kg) or vehicle (tap water 10 mL/kg, control). One hour later, each animal was orally given 0.2 mL of ethanol (96%), and the animals were killed 30 min later. Their stomachs were excised and opened along the greater curvature, and the mucosal lesion area was measured by planimetry with a transparent grid placed on the surface of the glandular mucosa. Quirino et al. [46] also evidenced the pharmacological effect of *C. coriaceum* pulp oil on ethanol-induced ulcers and demonstrated that the activity involves α2-receptor mechanisms, endogenous prostaglandins, nitric oxide and K+ATP.

The gastroprotective effect is not restricted to the pulp oil but extends to the leaves of *C. coriaceum*, as demonstrated by Lacerda-Neto et al. [56]. According to these authors, the

oral administration of the hydroethanolic extract of *C. coriaceum* leaves, at a dose of 100 mg/Kg, reduced gastric lesions by up to 86%. This study also demonstrated that opioid receptors, α2-adrenergic receptors and capsaicin-sensitive primary afferent neurons were involved in the gastric protection mechanism used by the extract of pequi leaves.

2.3.5 Other Activities

Oliveira et al. [49] found that the oil of *C. coriaceum* at a dose of 400 mg/kg showed an antinociceptive effect in rats when compared to the control group. The effect found was similar to that of dexamethasone, a corticosteroid widely used to treat symptoms associated with various diseases. Experimentally, *C. coriaceum* oil was diluted in 2% Tween 80 concentrations and administered 45 min before induced arthritis or for 7 consecutive days by oral gavage at doses of 100, 200 and 400 mg/kg. Induced arthritis was performed using zymosan, which was administered as an injection 24 h after the last administration of the oil. In addition, there was a negative control, which received only saline and Tween 80 (2%). Another group was treated with the standard drug (dexamethasone) in each single-dose experimental trial 2 h before arthritis induction. The experimental groups were divided into subgroups to assess joint disability and joint swelling. Finally, the fluid and synovial tissue were collected for the evaluation of leukocyte recruitment, myeloperoxidase activity and cytokine release and immunohistochemistry. Changes in vascular permeability were assessed by the extravasation of Evans Blue dye into joint tissue 6 h after zymosan injection.

Another pharmacological effect for the oil from the fruits of *C. coriaceum* is its hypolipidemic action. Figueiredo et al. [63] treated Wistar rats (*Rattus norvegicus*) for 15 days with the fixed oil of “pequi” and subsequently induced dyslipidemia (elevation of cholesterol and triglycerides in plasma) through the administration of Triton WR-1339 (Tyloxapol). As a result, they showed that, at a dose of 2 g/kg, the oil was capable of reducing serum cholesterol levels by 16% and serum triglyceride levels by 23%. Another effect observed was a significant increase in the levels of HDL-C in the rats. The fixed oil of *C. coriaceum* was also able to prevent lung injury in rats subjected to short-term exposure [64].

Oliveira et al. [57] evaluated the effect of *C. coriaceum* oil in the treatment of seizures. In general, the authors first administered the fixed oil from the pulp of *C. coriaceum* to rats in increasing doses (25, 50 or 100 mg/kg). Subsequently, the animals were induced to seizure by PTZ and were immediately transferred to a cubic glass arena and video monitored for 15 min for the appearance of seizures. Unfortunately, no anticonvulsant action was found, but the administration at a dose of 100 mg/Kg was able to increase the latency to the first

myoclonic spasm and the first generalized tonic-clonic seizures induced by pentylenetetrazol. These authors also demonstrated that the oil did not cause any significant adverse behavioral effect by means of the open field, rotarod, forced swimming and object recognition tests.

Regarding the antiparasitic effect, Alves et al. conducted the following experiment [54]. Antiparasitic activity was performed in 24-well microtiter plates, each well containing 1000 μL of 199 culture media supplemented with 1 × 106 stationary phase promastigote forms with or without the extracts of interest at final concentrations of 0.1, 0, 05 and 0.025 mg/mL. After growth in contact with *C. coriaceum* extracts, viable cells were counted in a Neubauer chamber after 24, 48 and 72 h of treatment. They found that the ethanolic extracts of the bark and fruit pulp of *C. coriaceum* were effective against promastigote forms of *Leishmania* (*Leishmania*) *amazonensis* (MHOM/BR/1989/166MJO), with IC_{50} values of 38 and 30 µg/mL, respectively. These results were similar to those verified for the positive controls (pentamidine and meglumine antimoniate). These authors also concluded that the extracts showed anti-acetylcholinesterase activity compared to physiostigmine.

Tomiotto-Pellissier et al. [65] verified the leishmanicidal activity of *C. coriaceum* leaf extracts against *Leishmania* (L.) *amazonensis*. These authors used promastigote forms of L. amazonensis (106 cells/mL), which were subjected to different concentrations (25, 50 and 100 µg/mL) of extracts from the extracts of *C. coriaceum*. After the growth of 24, 48 and 72 h of treatment, the parasites were counted in a Neubauer chamber. From 24 h, all of the concentrations tested showed a significant reduction in the proliferation of *L. amazonensis* compared to the control (amphotericin B) or vehicle. The extracts also induced the loss of mitochondrial membrane potential, the production of reactive oxygen species (ROS), plasma membrane damage and phosphatidylserine exposure in promastigotes. Most parasites entered a process of late apoptosis.

Tomiotto-Pellissier et al. [66] first reported that the extracts of *C. coriaceum* fruit pulp and peel could induce the death of promastigote forms of *L. amazonensis* through apoptosis-like mechanisms and were also active against amastigote forms by activating the Nrf2/HO-1/ferritin pathway, reducing iron availability for parasite survival. In general, to identify such mechanisms, the authors treated the protozoa with extracts from the pulp and peel of the fruits of *C. coriaceum* 50 µg/mL during a period of 24 h, which were later processed and analyzed in scanning electron microscopy in order to assess whether the products caused any changes in the cells.

Despite the widespread popular use of plant products as therapeutic alternatives, there is evidence that they can be potentially toxic. In this sense, some authors have evaluated the *in*

vitro and *in vivo* toxic effects of products obtained from *C. coriaceum*. Alves et al. [54], for example, evaluated *in vitro* the cytotoxic effect of the extracts of the bark and fruit pulp of *C. coriaceum* against macrophages. Experimentally, the viability of peritoneal macrophages treated with *C. coriaceum* extracts was evaluated using the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. BALB/c peritoneal macrophages (5 × 105 U/mL) were cultured on media plates for 2 h for adherence at 37 °C and 5% CO_2. The cells were washed with PBS, and then adherent cells were incubated with different concentrations of extracts (2.5–0.025 mg/mL) or with the vehicle (0.1% DMSO) and kept in the culture for 24 h under the previous conditions. After incubation with the extracts, the macrophages were washed with PBS, and MTT was added to a final concentration of 5 µg/mL in each well, followed by incubation for 4 h and the conditions mentioned above. The MTT formazan product was solubilized with 300 μL of DMSO, and the plates were read at 570 nm in a spectrophotometer. They verified moderate cytotoxicity, with IC_{50} values of 454 and 253 µg/mL, respectively. In contrast, against human erythrocytes, these products show low hemolytic activity at a concentration of 500 µg/mL.

In order to assess toxicity, Duavy et al. [52] tested the effect of the ethanolic and aqueous extract of the leaves of the species under study against the microcrustacean *Artemia salina* Leach. For that, the nauplii were exposed to different concentrations of the respective extracts (1–1000 µg/mL) during a period of 24 h. After this period, the surviving larvae were read. Concomitantly, a positive control group was prepared with aquamarine and potassium dichromate ($K_2Cr_2O_7$), and a negative control was prepared with aquamarine and Tween 80. The ethanolic and aqueous extracts of *C. coriaceum* leaves presented, *in vivo*, a high toxicity on *A. salina* nauplii, with LC_{50} values of 14.9 and 18.5 µg/mL, respectively—more toxic than the positive control (potassium dichromate), whose LC_{50} was 55.9 µg/mL [52]. However, when using *Drosophila melanogaster* as a model, these authors found no toxicity of the aqueous extract at the concentration of 5 mg/mL during 5 days of the experiment [50]. In this trial, flies were fed a corn diet supplemented or not supplemented with *C. coriaceum* fixed oil in varying concentrations (1, 5 or 10 mg/g) for 7 days.

The allelopathic and larvicidal potential of *C. coriaceum* have also been reported [67,68]. Silva et al. [67] demonstrated that aqueous extracts of the leaves, fruits and stem of *C. coriaceum* possess allelochemicals capable of interfering with the germination of the seeds of *Lactuca sativa* L. (lettuce). These researchers evaluated the action of aqueous extracts at concentrations of 25, 50, 75 and 100% of the stem, leaf and fruit of *C. coriaceum* on the germination and growth of *L. sativa*. For that, they distributed the seeds of the donor species.

The seeds were distributed in Petri dishes and spaced in order to facilitate the individual evaluation. Subsequently, they were placed in a B.O.D.-type chamber and were evaluated daily. Finally, 20 seedlings were randomly selected to obtain the averages referring to the length of the aerial and underground parts. Moreover, the extracts were able to interfere in the growth of the roots and stem of the recipient species. Regarding the larvicidal effect, Azevedo et al. [68] reported that *C. coriaceum* oil caused 100% mortality of *Aedes aegypti* larvae in a period of 120 h. In this study, methodologically, the researchers used the fixed oil from the fruits of *C. coriaceum*. They evaluated the larvicidal effect at different concentrations of the product (500, 1000, 1500, 2000 and 2500 ppm), using Tween® 20 as a surfactant to aid in the dilution of oils in water. Ten *A. aegypti* larvae were used in instar L3 for each repetition. The larvae were submitted to treatments for 24, 48, 72, 96 and 120 h of exposure. Mortality was assessed when the dead larvae did not react to the mechanical stimulation of fine-tipped forceps.

2.4 Phytochemistry

The fruit and seeds of *C. coriaceum* are an important source of fixed oil (Table 2). Costa et al. [47], Figueiredo et al. [63] and Borges et al. [69] found that the pulp of the fruit of *C. coriaceum* is composed mostly of unsaturated fatty acids (> 60%). Among the predominant unsaturated fatty acids, oleic acid (C18:1) stands out, while palmitic acid (C16:0) predominates among the saturated acids. In the lipid profile of the seeds, saturated fatty acids were found that were not present in the pulp, such as methyl-18-methylnon-adecanoate (C20:0), docosanoic acid (C22:0) and lignoceric acid (C24:0) (Serra et al., 2020). On the other hand, arachidic acid (C20:0) and linolenic acid (C18:3), present in the fruits, were not detected in the seeds [70].

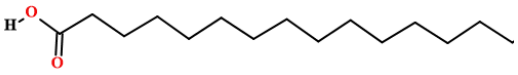
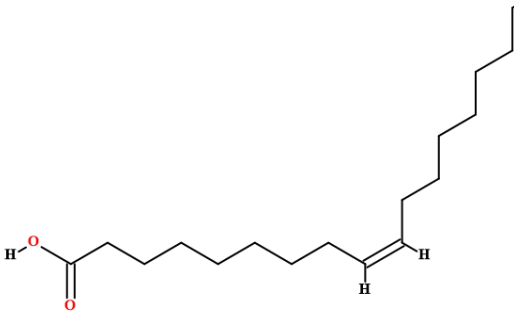
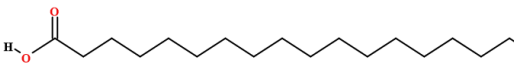
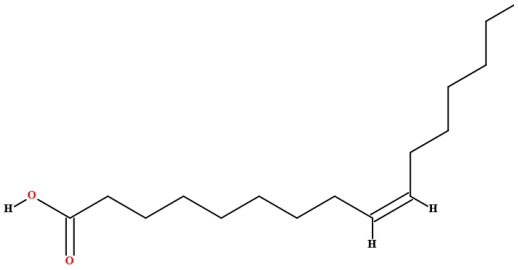
Besides compounds of the primary metabolism, *C. coriaceum* presents a wide variety of secondary compounds. Simple phenolics, flavones, flavonols, flavonones, flavononols, xanthones, tannins (pyrrobalic and hydrolysable), saponins, leucoanthocyanidins, catechins, steroids and alkaloids have been identified in *C. coriaceum* leaves [52,56,59,65,71].

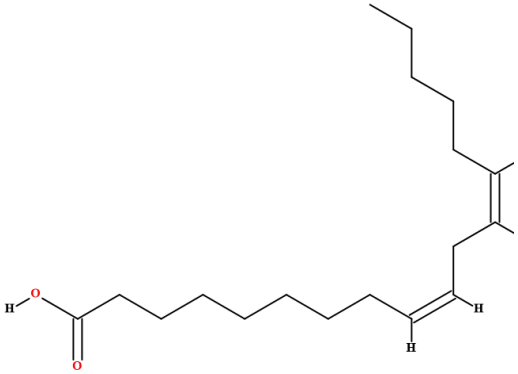
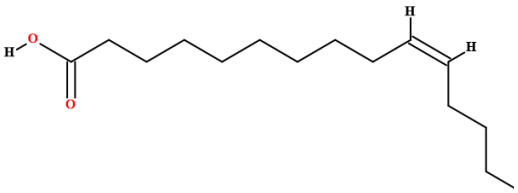
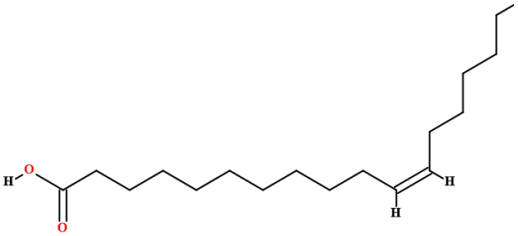
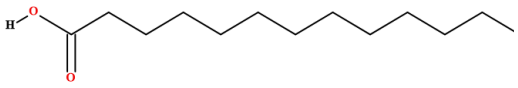
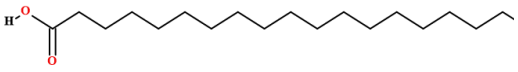
The phytochemical studies of the phenolic compounds of *C. coriaceum* have concentrated on the fruits, bark and leaves (Table 2), with no research to date aimed at the bioprospecting of its roots and flowers. Among the compounds of a phenolic nature identified in the species, rutin, quercetin, epicatechin, isoquercitrin, gallic acid, chlorogenic acid, caffeic

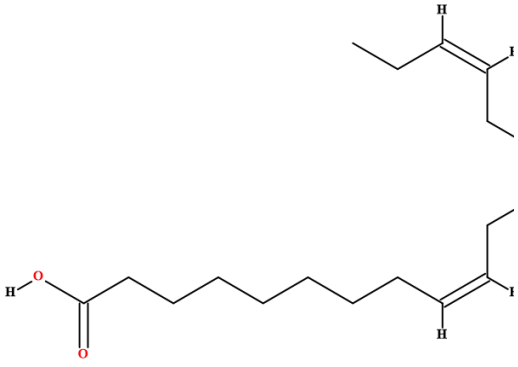
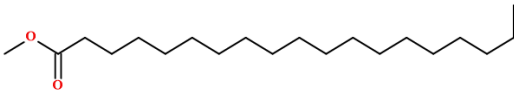
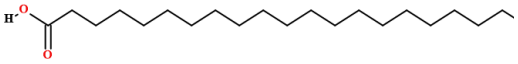
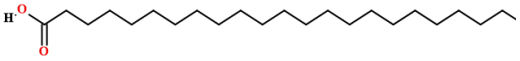
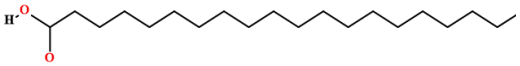
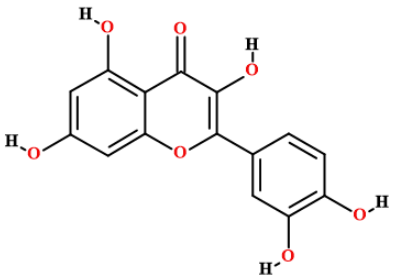
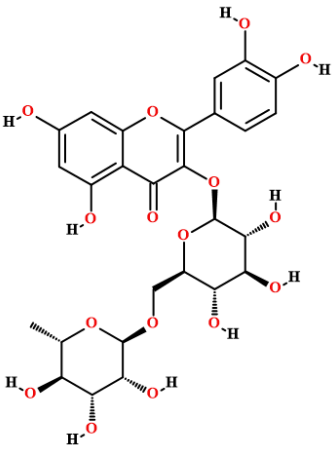
acid and ellagic acid stand out [50,54,55,59]. Such chemical heterogeneity may be responsible for the different activities verified for *C. coriaceum*.

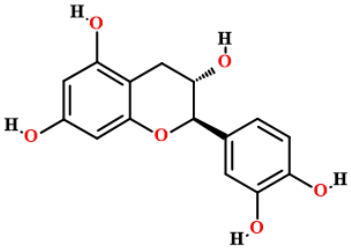
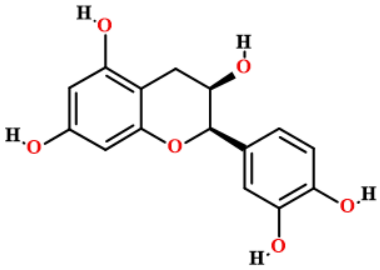
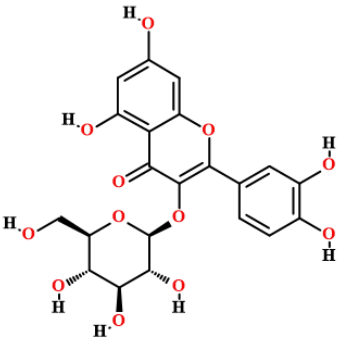
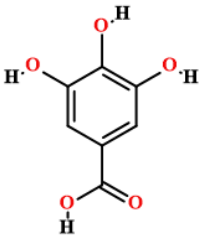
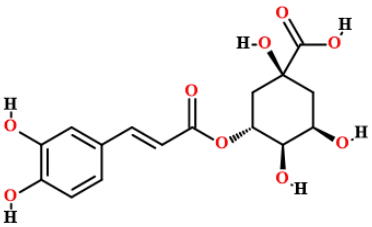
Although numerous volatiles have been identified in *C. brasiliensis* [72–74], to our knowledge, there is no research targeting these constituents in *C. coriaceum.*

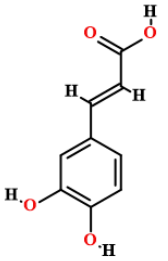
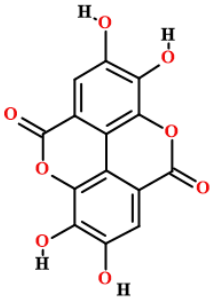
Table 2. Substances identified in *Caryocar coriaceum*.

Compounds	**Structure**	**Product**	**Plant source**
Fatty acids			
Palmitic acid [46,47,58,61,63,64,70,75,76-79]		Fixed oil	Fruits (internal mesocarp and endocarp) and seeds
Oleic acid [46,47,58,61,63,70,75,76–79]		Fixed oil	Fruits (internal mesocarp and endocarp) and seeds
Stearic acid [39,40,54,56,64,70,75–78]		Fixed oil	Fruits (internal mesocarp and endocarp) and seeds
Palmitoleic acid [45,47,63,70,77,79]		Fixed oil	Fruits (internal mesocarp and endocarp) and seeds

Linoleic acid [46,47,61,63,64,70,76–79]		Fixed oil	Fruits (internal mesocarp and endocarp) and seeds
Heptadecenoic acid [46,47,61,63,79]		Fixed oil	Fruits (internal mesocarp and endocarp) and seeds
Eicosenoic acid [46,47,63,64,79]		Fixed oil	Fruits (internal mesocarp and endocarp) and seeds
Myristic acid [70,76,78]		Fixed oil	Fruits (internal mesocarp) ; Seeds
Arachidic acid [70]		Fixed oil	Fruits (internal mesocarp)

Linolenic acid [58,70]		Fixed oil	Fruits (internal mesocarp)
Methyl-18-methylnonadecanoate [64]		Fixed oil	Seeds
Docosanoic acid [64]		Fixed oil	Seeds
Lignoceric acid [64]		Fixed oil	Seeds
Heneicosanoic acid [58]		Fixed oil	Seeds
Phenolic compounds			
Quercetin [50,54,55,59]		Ethanol, hydroethanolic, methanolic and aqueous extract	Fruits (internal mesocarp); Leaves
Rutin [50,54,55,59]		Ethanol, hydroethanolic, methanolic and aqueous extract	Fruits (internal mesocarp); barks; Leaves

Catechin [50]		Aqueous extract	Leaves
Epicatechin [50]		Aqueous extract	Leaves
Isoquercitrin [50]		Aqueous extract	Leaves
Gallic acid [50,55,59]		Ethanol, hydroethanolic, methanolic and aqueous extract	Leaves
Chlorogenic acid [50,55,59]		Ethanol, hydroethanolic, methanolic and aqueous extract	Leaves

Caffeic acid [50,55,59]		Ethanol, hydroethanolic, methanolic and aqueous extract	Leaves
Ellagic acid [50]		Aqueous extract	Leaves

2.5 Extractivism and Social, Economic and Cultural Importance

The fruits of *C. coriaceum* are of extreme socioeconomic importance for families of extractivist communities in the Chapada do Araripe, constituting up to 80% of the total family income. For this, the work is hard, since the extractivism of this fruit includes a set of activities such as collection, transport, processing and marketing either “in natura” or for its derivatives (oil), in which all family members participate [19,80–85].

For the extractive activity, initially, the families set up camps at the beginning of December around the forest to facilitate collection. Other camps are set up near the highways to sell the fruit to drivers passing through. The best known camps are located in the municipality of Jardim, receiving families from different districts of the Araripe region [19,86,87]. The camps are simple constructions of wood and clay and, in some cases, are constructed only with straw, with rare exceptions of brick. Most camps bring risks to the health of the inhabitants, such as the contact with insect vectors of *Trypanosoma cruzi*, the cause of Chagas disease. Due to the distance of the camps from the headquarters of the municipality, there is no electricity and drinking water in these places, and the only source of drinking water is the tanker trucks that pass by, supplying them every other fifteen days [19].

After settling in the camps in forest areas, the process of fruit gathering begins. This activity is carried out by all family members, with the women and children collecting in the areas closest to the camp and the men, due to their physical resistance, burrowing into the closed forests to harvest the fruit [19,81]. The peak of fruitfall occurs at 03:00 p.m., probably

due to a higher production of ethylene gas that is strictly related to the high temperature in the environment (personal observation). Thus, the collection occurs the next day, around 4:00 a.m., due to a maximization of the fallen fruits and because, at this time, the climate is milder. It is worth mentioning that the fruit cannot be picked directly from the tree, since the product is still immature and of inferior quality to fruit picked from the ground. Thus, the community establishes certain rules for fruit collection, such as collecting only the fallen fruit and not shaking the trees [19,82,86]. However, there are extractivists who occasionally do not follow the rules, collecting immature fruits directly on the plant when they do not find them on the ground [82,86].

Figure 3. Car commonly used to transport *Caryocar coriaceum* Wittm. during the harvest.

Processing the fruit for oil production is done by means of a technique called "rolling". In this technique, a sharp-surfaced object, usually a knife, rotates around the fruit so that its skin is cut in two. Care must be taken not to cut the seeds, which are removed from the fruit with light pressure. This activity is performed by all family members, except children, due to the risk of accidents, but they can observe the procedure in order to learn the technique [81,82]. After "rolling", the fruit peels can be used as fodder for animals (pigs and cattle) or as fertilizers. Occasionally, some extractivists discard the peels in the vicinity of the camps, causing the propagation of insects and other pests. The fruit "stones", necessary for oil production, are stored in straw baskets until the moment of the oil extraction [81,89].

To obtain oil, forest wood resources are needed to act as fuel. The men are responsible for obtaining the wood and obtaining the oil, due to their physical resistance, and they enter the forest in search of dry wood. Some families extract the oil using liquefied gas, but due to its high price on the commercial market and the yield being significantly lower, this practice is not so common. It is worth noting that green wood, besides being forbidden to be obtained in the forest, is not very combustible [19,81,90].

The oil production process is arduous, as the first stage can take up to 5 h of cooking until a brownish mixture is obtained and the inner mesocarp becomes soft. After this process, the stones are rubbed with the help of a grater, which is made up of a wooden handle and a metal cylinder full of sharp points, in order to separate the mesocarp from the spiny epicarp (Figure 4). The work consists of repetitive movements inside the boilers, providing at the end of the process a darker brown oil with a pasty consistency [81].

Figure 4. Process of cooking the fruits of *Caryocar coriaceum* Wittm. in boilers for the production of oil at the Barreiro Novo camp, Jardim—CE, Brazil.

After this process, the stones that are not pulped are removed from the boilers using a handmade skimmer. Before being discarded, they are washed with drinking water, and the solution returns to the boiler. This, in turn, continues the cooking process in order to agglutinate the oil. To do so, the extractors spend five hours continuously stirring the solution in the boiler with the help of a piece of wood, usually from the *C. coriaceum* trunk itself. This

phase is very important for a better quality of the oil, because it can end up being cooked for a longer time than that which is ideal [81,90].

Finally, through agglutination, dark yellow spots appear, creating a superficial layer of oil in the boiler solution. With due care, the oily product is separated from the aqueous solution, brought back to the fire for two hours and subsequently filtered and bottled so that it can be marketed or used by the families themselves [81,90]. It is worth noting that the oil can be extracted from the stone (internal mesocarp + endocarp + almond) or from the kernel. The final difference is in the quality of the product, because the oil from the kernel, according to producers, is purer because it has a lighter color. However, this extraction is more arduous because the epicarp is of the thorny type, which can cause accidents during cutting and handling. Due to this set of factors, the oil from the almonds has a higher cost in the commercial market [81].

The marketing of oil is driven mainly by the pilgrimages, which are pilgrimages to religious sites or places of devotion and occur annually in the city of Juazeiro do Norte in devotion to Father Cícero (1844–1934), a great religious and political leader of the region [90–92]. Among the pilgrimages that are more profitable are those that occur in the off-season period, such as the Anniversary of the Death of Father Cícero (July 20), the Pilgrimage of Our Lady of Sorrows (September 15), the Pilgrimage of Saint Francis of the Wounds (October 4) and the Pilgrimage of The Day of The Dead (October 30 to November 1). These pilgrimages attract thousands of faithful from all over Brazil, who buy *C. coriaceum* products for medicinal, food and commercial purposes [87,90,93–96].

At the end of every *C. coriaceum* harvest, important cultural events take place in the region, such as the “Pequis Festival”. This festival takes place over two days, usually in March. During this period, there is a Holy Mass of Thanksgiving, cattle-catching competitions and regional musical attractions (“forró”). Such events are used to commemorate and give thanks for another year of harvesting, as well as to celebrate and bring together friends and family [30,81,90]. Traditionally, recipes are prepared using the fruits of *C. coriaceum*, such as baião de dois (a combination of rice and beans), munguzá (a food that uses corn, beans, pork and vegetables) and the famous pequizada, which is prepared with milk, spices and *C. coriaceum* [30,90].

2.6 Conservation of Caryocar coriaceum

According to the International Union for Conservation of Nature and Natural Resources (IUCN) Red List, *C. coriaceum* is an endangered plant species [31]. Such

conservation status is due to a number of factors, such as increasing extractivism, slow germination, the reduction of dispersing animals, deforestation and fires in the Cerrado [10,24,88].

The fruits of *C. coriaceum* suffer great anthropic pressure because they are highly appreciated in cuisine and popular medicine. To meet the demand, thousands of fruits are used to produce oil and to be sold in natura. Thus, if there is no proper control of extractivism in accordance with the replacement standards, this overexploitation may lead to the collapse of these natural resources, because, with the extractive pressure and the reduced amount of diaspores at the end of the harvest, there is a low recruitment of seedlings, which already present a low frequency and restricted distribution in the ecosystem [24,97]. In addition to these factors, the seeds of *C. coriaceum* present a slow germination. Such phenomenon is due to the endogenous dormancy that the seeds present, which can take a year to germinate. This slow and delayed germination further aggravates the conservation of the species [10,85].

The reduction of dispersing animals of *C. coriaceum* diaspores is a rarely reported factor, but it is one that deserves attention. The process of the fruit dispersal of this species is an essential step in the regeneration of individuals, as well as in the biological maintenance of natural environments [98]. Among the natural dispersers are the beetles of the Scarabaeidae family, which are known as “dung beetles”. These insects use the decomposing pulp of *C. coriaceum* fruits as a food resource, thus reducing the thorny endocarp, besides burying the fruits, favoring seed germination [88,98,99]. Unfortunately, these arthropods, on account of having a sedentary life, are more vulnerable to climate change, and their local distribution is strongly influenced by vegetation cover. As the Chapada do Araripe has been constantly targeted by anthropic actions such as deforestation and burning, the populations of these beetles tend to decrease [99–101].

Besides insects, there are vertebrates that participate in the dispersal of *C. coriaceum*, such as *Dasyprocta prymnolopha* Wagler, 1831 (Dasyproctidae), popularly called “cutia”. This rodent helps in the dissemination of seeds, bringing benefits such as reducing attacks by natural predators, assisting in the colonization of new environments and increasing genetic variability over space [98]. Unfortunately, these mammals are targets of hunters in the region, which are used for food purposes [102]. With the above, it is evident that forest fragmentation, associated with hunting, has reduced the local fauna and, consequently, the dispersers of *C. coriaceum* fruits [98,103].

Finally, deforestation and burning are the main factors that accelerate this process of extinction of the species. Such criminal practices are used with the intent of obtaining pasture

areas for livestock. One of the largest fires in Chapada do Araripe occurred in early 2020, which affected about two thousand hectares of forest. This destruction had a direct impact on the production of the fruit, with 80% of the production being affected [83,104].

It is clear that *C. coriaceum* receives great anthropic pressure, and measures are necessary to ensure that the extinction of the species does not occur. As a strategy, there are numerous measures that can be taken—for example, reducing the exploitation of the fruits. The excessive collection of fruits prevents the propagation of new individuals and the regeneration of populations in deforested areas. The creation of public policies that focus on the protection of *C. coriaceum* dispersing animals, as well as on greater forest surveillance and the planting of seedlings of the species in several areas of the fragmented forest, should also be considered [87,88].

One of the advances in conservation was the establishment of law 9.985 in 2000 by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA), which prohibited practices or any activities that impede the natural regeneration of ecosystems. Among the prohibited activities is the entry of cattle into the forest, an activity widely used by extractivists, because the animals, by opening paths in the forest, facilitate the collection of the fruits of *C. coriaceum* [81].

3. Materials and Methods

3.1 Search in Databases and Inclusion and Exclusion Criteria

The keyword "*Caryocar coriaceum*" was used as the main index for searching the following platforms: PubMed®, PubMed Central®, SciElo, Scopus® and Web of ScienceTM. Articles published in English and Portuguese in the last 39 years (1983–2022) were considered. Abstracts published in conference proceedings, course completion papers, dissertations and theses and those without mention of the ethnomedicinal uses, biological activities, phytochemistry, extraction and conservation of *C. coriaceum* were discarded.

3.2 Data Screening and Categorization of Information

A total of 216 scientific documents were found in the databases searched. After an initial analysis, 75 documents were excluded for duplicity. A total of 141 documents were analyzed in full (title, abstract, keywords and full text), 33 being excluded for not meeting the scope of the review. A total of 107 documents were used in the qualitative analysis, and another 12 were used to compose the present review (Figure 5). Information management was

performed with the help of Mendeley Reference Manager© software version 2.67.0 (Mendeley Ltd., London, UK).

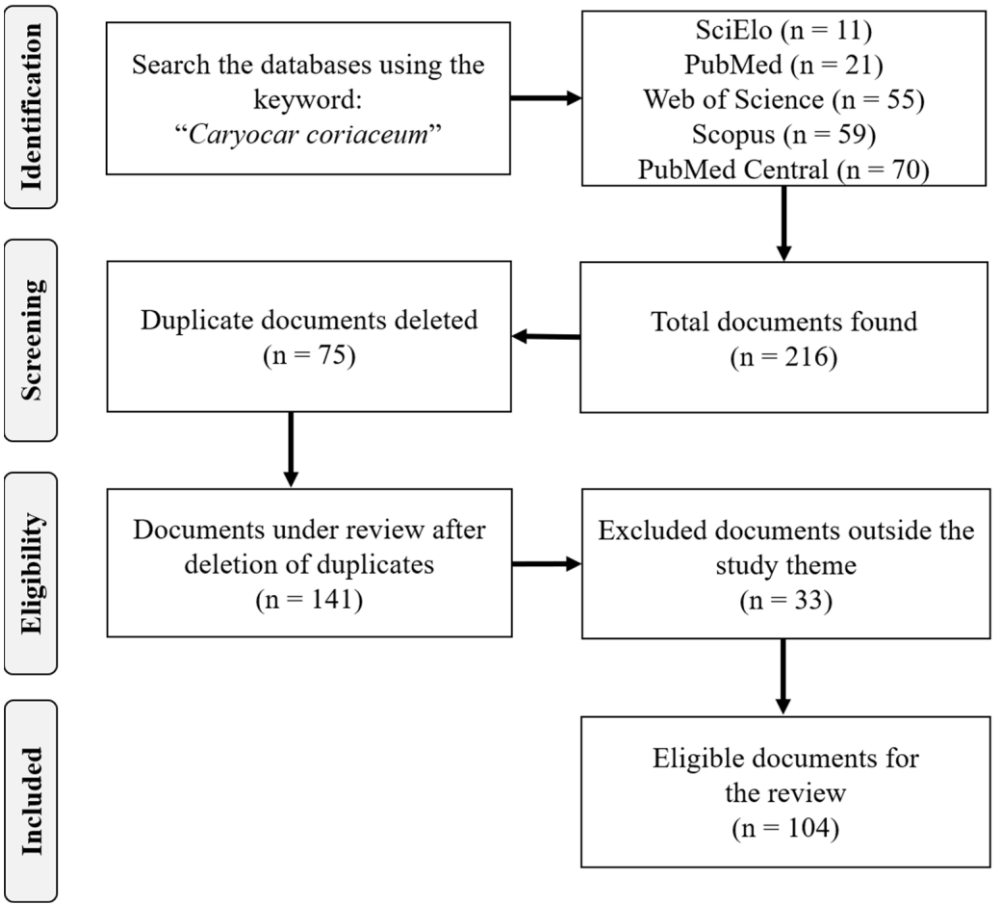


Figure 5. Flowchart describing the search and selection strategies.

The information found was categorized into: (1) botanical aspects and geographical distribution; (2) ethnobotanical uses; (3) biological and pharmacological activities; (4) phytochemistry; (5) extraction and social, economic and cultural importance; and (6) conservation of *Caryocar coriaceum*.

4. Conclusions and Future Prospects

Caryocar coriaceum is a tree of great economic, cultural and medicinal importance for the inhabitants of the Chapada do Araripe and neighboring regions. Such importance is noted for the use of the fruits and its by-products such as oil. In this review, we demonstrate that the fixed oil of *C. coriaceum* is widely used in folk medicine in communities in the Chapada do Araripe. Such use is supported by pharmacological tests, which show that the fatty acids present in the natural product are possibly responsible for the pharmacological property, one

of the reasons for having patents with such a product. However, even though it is a very relevant species and is known for its therapeutic effects, it is still rarely studied by the scientific community when compared to another species of the same genus, *C. brasiliense*— also known as “pequi”.

Author Contributions: Conceptualization, J.W.A.-B.; methodology, J.J.L.B.; investigation, J.W.A.-B. and V.B.d.S.; resources, H.D.M.C. and N.C.-M.; data curation, A.F.M.d.O. and J.G.M.d.C.; writing— original draft preparation, J.W.A.-B. and S.A.d.M.; writing—review and editing, M.F.B.M.-B., N.C.- M. and C.H.; visualization, A.F.M.d.O.; supervision, A.F.M.d.O.; project administration, A.F.M.d.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: We thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. In addition, we are especially grateful to the extractivists from Chapada do Araripe, especially José da Cruz da Serra Gravatá (Ceará, Brazil), who was instrumental in conducting this work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nunes, C.S.; Gil, A.S.B. Flora das cangas da Serra dos Carajás, Pará, Brasil: Caryocaraceae. *Rodriguésia* **2016**, *67*, 1281–1283.
2. Guedes, A. Pequi: A brazilian fruit with potential uses for the fat industry. *OCL* **2017**, *24*, D507.
3. Wittmack, L. Rhizoboleae. *Fl. Bras.* **1886**, *12*, 335–362.
4. Gardner, G. *Travels in the Interior of Brazil: Principally through the Northern Provinces, and the Gold and Diamond Districts, during the Years 1836–1841*; Reeve, Benham & Reeve: London, UK, 1849.
5. Santana, R.N.R. História e natureza. *Rev. Espac.* **2014**, *7*, 196–216.

6. Fagg, C.W.; Lughadha, E.N.; Milliken, W.; Hind, D.N.; Brandão, M.G. Useful brazilian plants listed in the manuscripts and publications of the scottish medic and naturalist George Gardner (1812–1849). *J. Ethnopharmacol.* **2015**, *161*, 18–29.
7. Vieira, J.F. *Dormindo à Borda do Abismo: A Medicina No Cariri cearense 1800–1900*; Expressão Gráfica e Editora: Fortaleza, Brazil, 2018.
8. Group, A.P.; Chase, M.W.; Christenhusz, M.J.; Fay, M.F.; Byng, J.W.; Judd, W.S.; Soltis, D.E.; Mabberley, D.J.; Sennikov, A.N.; Soltis, P.S. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* **2016**, *181*, 1–20.
9. Kerntopf, M.R. Óleo de pequi (*Caryocar coriaceum* W.) e a potencial atividade cardioprotetora. *Ens. Ciência Ciências Biológicas Agrárias Saúde* **2013**, *17*, 117–125.
10. Silva, M.A.P.; Medeiros Filho, S. Emergência de plântulas de pequi (*Caryocar coriaceum* Wittm). *Rev. Ciênc. Agron.* **2006**, *37*, 381– 385.
11. Oliveira, M.E.B.; Guerra, N.B.; Maia, A.H.N.; Alves, R.E.; Xavier, D.S.; Matos, N.M.S. Caracterização física de frutos do pequizeiro nativos da chapada do Araripe-CE. *Rev. Bras. Frutic.* **2009**, *31*, 1196–1201.
12. Oliveira, M.E.B.; Guerra, N.B.; Barros, L.M.; Alves, R.E. *Aspectos Agronômicos e de Qualidade do Pequi*; Embrapa Agroindústria Tropical: Fortaleza, Brazil, 2008.
13. Ramos, K.M.C.; Souza, V.A.B. Características físicas e químico-nutricionais de frutos de pequizeiro (*Caryocar coriaceum* Wittm.) em populações naturais da região meio-norte do Brasil. *Rev. Bras. Frutic.* **2011**, *33*, 500–508.
14. Nascimento-Silva, N.R.R.; Naves, M.M.V. Potential of whole Pequi (*Caryocar* Spp.) fruit-pulp, almond, oil, and shell-as a me- dicinal food. *J. Med. Food* **2019**, *22*, 952–962.
15. Silva, L.F.B.P.; Poty, J.A.C.; Martins, M.; Coelho, N.P.M.F.; Maia Filho, A.L.M.; Costa, C.L.S. Anti-inflammatory action of pequi oil associated to ultrasound in tendinitis in rats: Macroscopic and histological analysis. *Man. Ther. Posturology Rehabil. J.* **2016**, *14*, 1–6.
16. Ferreira-Júnior, W.S.; Santoro, F.R.; Nascimento, A.L.B.; Avilez, W.M.T.; Zank, S.; Silva, N.F.; Albuquerque, U.P.; Araújo, E.L. Check-List das plantas medicinais na Chapada do Araripe. In: *Sociobiodiversidade na Chapada do Araripe*; NUPEEA: Recife, Brazil, **2015**.
17. Rodrigues, B.S.; Ferreira, M.A.; Oliveira, T.C.S.; Oliveira, M.C.P. Morphobiometry and ecophysiology of *Caryocar coriaceum* Wittm. (Pequi) in cerrado areas of Northeast Brazil. *J. Exp. Agric. Int.* **2019**, *41*, 1–7.

18. Silva, M.A.P.; Medeiros Filho, S.; Duarte, A.E.; Mendonça, A.C.A.M.; Santos, A.C.B.; Souza, M.M.A. Fenologia de *Caryocar coriaceum* Wittm. *Caryocaraceae*, ocorrentes na Chapada do Araripe–Crato-CE-Brasil. *Cad. Cult. Ciênc.* **2013**, *12*, 21–31.
19. Cavalcanti, M.C.B.T.; Campos, L.Z.O.; Sousa, R.S.; Albuquerque, U.P. Pequi (*Caryocar coriaceum* Wittm., *Caryocaraceae*) oil pro- duction: A strong economically influenced tradition in the Araripe region, Northeastern Brazil. *Ethnobot. Res. Appl.* **2015**, *14*, 437–452.
20. Medeiros, M.B.; Walter, B.M.T. Composição e estrutura de comunidades arbóreas de cerrado stricto sensu no norte do Tocantins e sul do Maranhão. *Rev. Árvore* **2012**, *36*, 673–683.
21. Medeiros, M.B.; Walter, B.M.T.; Silva, G.P. Fitossociologia do cerrado stricto sensu no município de Carolina, MA, Brasil. *Cerne* **2008**, *14*, 285–294.
22. Ribeiro-Silva, S.; Medeiros, M.; Gomes, B.; Silva, M. Angiosperms from the Araripe National Forest, Ceará, Brazil. *Check List* **2012**, *8*, 744–751.
23. Campos, L.Z.; Nascimento, A.L.B.; Albuquerque, U.P.; Araújo, E.L. Use of local ecological knowledge as phenology indicator in native food species in the Semiarid region of Northeast Brazil. *Ecol. Indic.* **2018**, *95*, 75–84.
24. Bezerra, J.S.; Linhares, K.V.; Júnior, J.T.C.; Duarte, A.E.; Mendonça, A.C.A.M.; Pereira, A.E.P.; Batista, M.E.P.; Bezerra, J.W.A.; Campos, N.B.; Pereira, K.S. Floristic and dispersion syndromes of cerrado species in the Chapada do Araripe, Northeast of Brazil. *Res. Soc. Dev.* **2020**, *9*, e864997934.
25. Costa, I.R.; Araújo, F.S.; Lima-Verde, L.W. Flora e aspectos auto-ecológicos de um encrave de Cerrado na Chapada do Araripe, Nordeste do Brasil. *Acta Bot. Bras.* **2004**, *18*, 759–770.
26. Costa, I.R.; Araújo, F.S. Organização comunitária de um encrave de cerrado sensu stricto no bioma Caatinga, Chapada do Araripe, Barbalha, Ceará. *Acta Bot. Bras.* **2007**, *21*, 281–291.
27. Conceicao, G.M.; Castro, A. Fitossociologia de uma área de cerrado marginal, Parque Estadual do Mirador, Mirador, Maranhão. *Sci. Plena* **2009**, *5*, 105401.
28. Loiola, M.I.B.; Araújo, F.S.; Lima-Verde, L.W.; Souza, S.S.G.; Matias, L.Q.; Menezes, M.O.T.; Silva, M.A.P.; Souza, M.A.; Mendonça, A.; Macedo, M.S. Flora da Chapada do Araripe. In *Sociobiodiversidade na Chapada do Araripe*; NUPEEA: Recife, Brazil, 2015; Volume 1, pp. 103–148.

29. Torres, L.R.O.; Santana, F.C.; Shinagawa, F.B.; Mancini-Filho, J. Bioactive compounds and functional potential of pequi (*Caryocar* spp.), a native Brazilian fruit: A review. *Grasas Aceites* **2018**, *69*, e257–e257.
30. Gonçalves, C.U. A organização dos piquizeiros na Chapada do Araripe. **2007**, *4*, 4.
31. Gonçalves, C.U. Os piquizeiros da Chapada do Araripe. *Geografia* **2010**, *21*, 88–103.
32. Cartaxo, S.L.; Souza, M.M.A.; Albuquerque, U.P. Medicinal plants with bioprospecting potential used in semi-arid northeastern Brazil. *J. Ethnopharmacol.* **2010**, *131*, 326–342.
33. Conceição, G.M.; Ruggieri, A.C.; Araújo, M.F.V.; Conceição, T.T.M.M.; Conceição, M.A.M.M. Plantas do cerrado: comercialização, uso e indicação terapêutica fornecida pelos raizeiros e vendedores, Teresina, Piauí. *Sci. Plena* **2011**, *7*, 129902.
34. Ribeiro, D.; Oliveira, L.; Macêdo, D.; Menezes, I.R.; Costa, J.G.; Silva, M.; Lacerda, S.; Souza, M. Promising Medicinal plants for bioprospection in a cerrado area of Chapada Do Araripe, Northeastern Brazil. *J. Ethnopharmacol.* **2014**, *155*, 1522–1533.
35. Agra, M.F.; Freitas, P.F.; Barbosa-Filho, J.M. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. *Rev. Bras. Farmacogn.* **2007**, *17*, 114–140.
36. Agra, M. de F.; Silva, K.N.; Basílio, I.J.L.D.; de Freitas, P.F.; Barbosa-Filho, J.M. Survey of medicinal plants used in the region Northeast of Brazil. *Rev. Bras. Farmacogn.* **2008**, *18*, 472–508.
37. Lemos, I.; Delmondes, G.; Santos, A.; Caprara, A.; Barbosa, R.; Menezes, I.R.; Coutinho, H.; Kerntopf, R.; Fernandes, G. Ethno- biological survey of plants and animals used for the treatment of acute respiratory infections in children of a traditional com- munity in the municipality of Barbalha, Ceará, Brazil. *Afr. J. Tradit. Complement. Altern. Med.* **2016**, *13*, 166–175.
38. Magalhães, K.; Sagástegui Guarniz, W.; Miranda Sá, K.; Freire, A.; Monteiro, M.; Nojosa, R.; Bieski, I.; Custódio, J.; Balogun, S.; Bandeira, M. Medicinal plants of the Caatinga, Northeastern Brazil: Ethnopharmacopeia (1980–1990) of the late professor Francisco José de Abreu Matos. *J. Ethnopharmacol.* **2019**, *237*, 314–353.
39. Matos, F.J.A. *Plantas Medicinais: Guia de Seleção e Emprego de Plantas Usadas em Fitoterapia no Nordeste do Brasil*, 3rd ed.; Imprensa Universitária: Fortaleza, Brazil, 2007; ISBN 9788574850085.
40. Batista, J.S.; Silva, A.E.; Rodrigues, C.M.F.; Costa, K.; Oliveira, A.F.; Paiva, E.S.; Nunes, F.V.A.; Olinda, R.G. Avaliação da atividade cicatrizante do óleo de pequi (*Caryocar coriaceum* Wittm) em feridas cutâneas produzidas experimentalmente em ratos. *Arq. Inst. Biol.* **2020**, *77*, 441–447.

41. Lozano, A.; Araújo, E.; Franco, M.; Albuquerque, U. The apparency hypothesis applied to a local *Pharmacopoeia* in the Brazilian northeast. *J. Ethnobiol. Ethnomed.* **2014**, *10*, 2.
42. Macêdo, D.; Menezes, I.R.; Lacerda, S.; Silva, M.; Ribeiro, D.; Macêdo, M.; Oliveira, L.; Saraiva, M.; Alencar, S.; Oliveira, S.; et al. Versatility and consensus of the use of medicinal plants in an area of cerrado in the Chapada do Araripe, Barbalha-CE-Brazil. *J. Med. Plant Res.* **2016**, *10*, 505–514.
43. Silva, N.F.; Hanazaki, N.; Albuquerque, U.P.; Campos, J.L.A.; Feitosa, I.S.; Araujo, E.L. Local knowledge and conservation pri- orities of medicinal plants near a protected area in Brazil. *Evid. Based Complement. Altern.* **2019**, *2019*, 8275084.
44. Amorim, W.R.; Sousa, C.P.; Martins, G.N.; Melo, E.S.; Silva, I.C.R.; Corrêa, P.G.N.; Santos, A.R.S.S.; Carvalho, S.M.R.; Pinheiro, R.E.E.; Oliveira, J.M.G. Estudo etnoveterinário de plantas medicinais utilizadas em animais da microrregião do Alto Médio Gurguéia–Piauí. *PUBVET* **2018**, *12*, 131.
45. Leite, G.; Penha, A.; Quirino, G.; Colares, A.; Rodrigues, F.; Costa, J.G.; Herzog, A.; Campos, A. Gastroprotective effect of medicinal plants from Chapada do Araripe, Brazil. *J. Young Pharm.* **2009**, *1*, 54–56.
46. Quirino, G.; Leite, G.; Rebelo, L.; Tomé, A.; Costa, J.G.; Herzog, A.; Campos, A. Healing potential of pequi (*Caryocar coriaceum* Wittm.) fruit pulp oil. *Phytochem. Lett.* **2009**, *2*, 179–183.
47. Costa, J.G.; Brito, S.A.; Nascimento, E.M.; Botelho, M.A.; Rodrigues, F.F.; Coutinho, H.D. Antibacterial properties of pequi pulp oil (*Caryocar coriaceum*–Wittm.). *Int. J. Food Prop.* **2011**, *14*, 411–416.
48. Saraiva, R.A.; Matias, E.F.F.; Coutinho, H.D.M.; Costa, J.G.M.; Souza, H.H.F.; Fernandes, C.N.; Rocha, J.B.T.; Menezes, I.R.A. Synergistic action between *Caryocar coriaceum* Wittm. fixed oil with aminoglycosides *in vitro*. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 967–972. https://doi.org/10.1002/ejlt.201000555.
49. Oliveira, F.F.B.; Araújo, J.C.B.; Pereira, A.F.; Brito, G.A.C.; Gondim, D.V.; Ribeiro, R.A.; Menezes, I.R.A.; Vale, M.L. Antinocicep- tive and anti-inflammatory effects of *Caryocar coriaceum* Wittm fruit pulp fixed ethyl acetate extract on zymosan-induced arthri- tis in rats. *J. Ethnopharmacol.* **2015**, *174*, 452–463.
50. Duavy, S.; Ecker, A.; Salazar, G.; Loreto, J.; Costa, J.G.; Barbosa, N. Pequi enriched diets protect *Drosophila melanogaster* against paraquat-induced locomotor deficits and oxidative stress. *J. Toxicol. Environ. Health Part A* **2019**, *82*, 664–667.

51. Ribeiro Neto, J.A.; Tarôco, B.R.P.; Santos, H.B.; Thomé, R.G.; Wolfram, E.; Ribeiro, R.I.M.A. Using the plants of brazilian cerrado for wound healing: From traditional use to scientific approach. *J. Ethnopharmacol*. **2020**, *260*, 112547.
52. Duavy, S.M.P.; Silva, L.J.; Costa, J.G.M.; Rodrigues, F.F.G. Atividade biológica de extratos de folhas de *Caryocar coriaceum* Wittm.: Estudo *in vitro*. *Cad. Cult. Ciência* **2012**, *11*, 13–19.
53. Duavy, S.; Ramos, A.; Torres, G.; Ecker, A.; Seeger, R.; Rocha, J.B.; Costa, J.G.; Barbosa, N. Preliminary *in vitro* evaluation of *Caryocar coriaceum* Wittm. leaf and bark extracts as antioxidants. *Int. J. Appl. Res. Nat. Prod.* **2016**, *9*, 18–25.
54. Alves, D.R.; Morais, S.M.; Tomiotto-Pellissier, F.; Miranda-Sapla, M.M.; Vasconcelos, F.R.; Silva, I.N.G.; Sousa, H.A.; Assolini, J.P.; Conchon-Costa, I.; Pavanelli, W.R. Flavonoid composition and biological activities of ethanol extracts of *Caryocar coriaceum* Wittm., a native plant from caatinga biome. *Evid. Based Complement. Altern.* **2017**, *2017*, 6834218.
55. Araruna, M.K.; Nogara, P.A.; Boligon, A.A.; Athayde, M.L.; Rodrigues, L.B.; Costa, R.H.S.; Santana, F.R.A.; Coutinho, H.D.M.; Menezes, I.R. Effect of pequi tree *Caryocar coriaceum* Wittm. leaf extracts on different mouse skin inflammation models: Inference with their phenolic compound content. *Afr. J. Pharm. Pharmacol.* **2014**, *8*, 629–637.
56. Lacerda Neto, L.J.; Ramos, A.G.B.; Kerntopf, M.R.; Coutinho, H.D.M.; Quintans-Junior, L.J.; Almeida, J.; Ribeiro-Filho, J.; Menezes, I.R.A. Modulation of antibiotic activity by the hydroalcoholic extract from leaves of *Caryocar coriaceum* Wittm. *Nat. Prod. Res.* **2018**, *32*, 477–480.
57. Oliveira, C.C.; Oliveira, C.V.; Grigoletto, J.; Ribeiro, L.R.; Funck, V.R.; Meier, L.; Fighera, M.R.; Royes, L.F.F.; Furian, A.F.; Menezes, I.R.A.; et al. Anticonvulsant activity of *Caryocar coriaceum* Wittm. fixed pulp oil against pentylenetetrazol-induced seizures. *Neurol. Res.* **2017**, *39*, 667–674.
58. Pereira, F.; Feitosa, M.; Costa, M.; Tintino, S.; Rodrigues, F.; Menezes, I.R.; Coutinho, H.; Costa, J.G.; Sousa, E. Characterization, Antibacterial Activity and Antibiotic modifying action of the *Caryocar coriaceum* Wittm. pulp and almond fixed oil. *Nat. Prod. Res.* **2019**, *34*, 3239–3243.
59. Araruna, M.K.; Santos, K.K.; da Costa, J.G.; Coutinho, H.D.; Boligon, A.A.; Stefanello, S.T.; Athayde, M.L.; Saraiva, R.A.; Rocha, J.B.T.; Kerntopf, M.R. Phenolic composition and *in vitro* activity of the brazilian fruit tree *Caryocar coriaceum* Wittm. *Eur. J. Integr. Med.* **2013**, *5*, 178–183.

60. Gomes, A.B.; Ribeiro, I.A. Evaluation of antifungal potential of oil of the species *Caryocar coriaceum* front of *Candida* species isolated in the oral cavity of oncological pediatric patients in antineoplastic treatment. *Int. J. Pediatr. Res. Rev.* **2019**, *2*, 11.
61. Oliveira, M.L.M.; Nunes-Pinheiro, D.C.S.; Tomé, A.R.; Mota, E.F.; Lima-Verde, I.A.; Pinheiro, F.G.M.; Campello, C.C.; Morais, S.M. *In vivo* topical anti-inflammatory and wound healing activities of the fixed oil of *Caryocar coriaceum* Wittm. seeds. *J. Ethnopharmacol*. **2010**, *129*, 214–219.
62. Saraiva, R.A.; Araruna, M.K.A.; Oliveira, R.C.; Menezes, K.D.P.; Leite, G.O.; Kerntopf, M.R.; Costa, J.G.M.; Rocha, J.B.T.; Tomé, A.R.; Campos, A.R.; et al. Topical anti-inflammatory effect of *Caryocar coriaceum* Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. *J. Ethnopharmacol.* **2011**, *136*, 504–510.
63. Figueiredo, P.R.L.; Oliveira, I.B.; Neto, J.B.S.; Oliveira, J.A.; Ribeiro, L.B.; Viana, G.S.B.; Rocha, T.M.; Leal, L.K.A.M.; Kerntopf, M.R.; Felipe, C.F.B. *Caryocar coriaceum* Wittm. (Pequi) fixed oil presents hypolipemic and anti-inflammatory effects *in vivo* and *in vitro*. *J. Ethnopharmacol.* **2016**, *191*, 87–94.
64. Serra, D.S.; Sousa, A.M.; Silva Andrade, L.C.; Gondim, F.L.; Santos, J.E.Á.; Oliveira, M.L.M.; Pimenta, A.T.Á. Effects of fixed oil of *Caryocar coriaceum* Wittm. seeds on the respiratory system of rats in a short-term secondhand-smoke exposure model. *J. Ethnopharmacol.* **2020**, *252*, 112633.
65. Tomiotto-Pellissier, F.; Alves, D.R.; Miranda-Sapla, M.M.; Morais, S.M.; Assolini, J.P.; Bortoleti, B.T.S.; Goncalves, M.D.; Cata- neo, A.H.D.; Kian, D.; Madeira, T.B. *Caryocar coriaceum* extracts exert leishmanicidal effect acting in promastigote forms by apoptosis-like mechanism and intracellular amastigotes by Nrf2/HO-1/Ferritin dependent response and iron depletion: Leish- manicidal effect of *Caryocar coriaceum* leaf exracts. *Biomed. Pharmacother.* **2018**, *98*, 662–672.
66. Tomiotto-Pellissier, F.; Alves, D.R.; Morais, S.M.; Bortoleti, B.T.S.; Gonçalves, M.D.; Silva, T.F.; Tavares, E.R.; Yamauchi, L.M.; Costa, I.N.; Marinho, E.S. *Caryocar coriaceum* Wittm. fruit extracts as *Leishmania* inhibitors: *In-vitro* and *in-silico* approaches. *J. Biomol. Struct. Dyn.* **2021**, 1–16.
67. Silva, M.A.P.; Medeiros Filho, S.; Duarte, A.E.; Moreira, F.J.C. Potencial alelopático de *Caryocar coriaceum* Wittm na germinação e crescimento inicial de plântulas de alface. *Cad. Cult. Ciência* **2014**, *13*, 16–24.

68. Azevedo, F.R.; Bezerra, L.L.A.; Silva, T.I.; Silva, R.A.; Feitosa, J.V. Larvicidal activity of vegetable oils against aedes *Aegypti larvae*. *Rev. Fac. Nac. Agron. Medellin* **2021**, *74*, 9563–9570.
69. Borges, O.M.A.; Araújo, Í.M.S.; Canuto, K.M.; Carvalho, J.D.G.; Magalhães, H.C.R.; Rodrigues, T.H.S.; Carioca, J.O.B.; Gaban, S.V.F. Pequi pulp oil: Effect on the physicochemical, nutritional, and textural properties of cottage cheese. *Food Sci. Technol.* **2022**, *42*, e37221.
70. Pessoa, A.; Podestá, R.; Block, J.; Franceschi, E.; Dariva, C.; Lanza, M. Extraction of Pequi (*Caryocar coriaceum*) pulp oil using subcritical propane: Determination of process yield and fatty acid profile. *J. Supercrit. Fluids* **2015**, *101*, 95–103.
71. Amparo, T.R.; Seibert, J.B.; Vieira, P.M.A.; Teixeira, L.F.M.; Santos, O.D.H.; Souza, G.H.B. Herbal medicines to the treatment of skin and soft tissue infections: Advantages of the multi-targets action. *Phytother. Res.* **2020**, *34*, 94–103.
72. Passos, X.; Castro, A.; Pires, J.; Garcia, A.; Campos, F.; Fernandes, O.; Paula, J.; Ferreira, H.; Santos, S.; Ferri, P.; et al. Composition and antifungal activity of the essential oils of *Caryocar brasiliensis*. *Pharm. Biol.* **2003**, *41*, 319–324.
73. Geőcze, K.C.; Barbosa, L.C.A.; Fidêncio, P.H.; Silvério, F.O.; Lima, C.F.; Barbosa, M.C.A.; Ismail, F.M.D. Essential oils from pequi fruits from the brazilian cerrado ecosystem. *Food Res. Int.* **2013**, *54*, 1–8.
74. Cordeiro, M.W.S.; Cavallieri, Â.L.F.; Ferri, P.H.; Naves, M.M.V. Características físicas, composição químico-nutricional e dos óleos essenciais da polpa de *Caryocar brasiliense* nativo do estado de Mato Grosso. *Rev. Bras. Frutic.* **2013**, *35*, 1127–1139.
75. Alencar, J.; Alves, P.B.; Craveiro, A.A. Pyrolysis of tropical vegetable oils. *J. Agric. Food Chem.* **1983**, *31*, 1268–1270.
76. Figueiredo, R.W.; Maia, G.A.; Figueiredo, E.A.T. Propriedades físico-químicas e composição dos ácidos graxos da fração lipídica da polpa e amêndoa do Pequi (*Caryocar coriaceum* Wittn.). *Ciênc. Agron.* **1989**, *20*, 135–139.
77. Dresen, H.; Prasad, R.B.N.; Gülz, P.-G. Composition of lipids of piqui (*Caryocar coriaceum* Wittm.) seed and pulp oil. *Z. Naturforsch. C* **1989**, *44*, 739–742.
78. Lima, J.; Souza, A.; Magalhães, H.; Pinto, C. Pequi kernel oil extraction by hydraulic pressing and its characterization. *Rev. Bras. Frutic.* **2020**, *42*, e-456.
79. Sena, D.M.; Rodrigues, F.F.G.; Freire, P.T.C.; Lima, S.G.; Coutinho, H.D.M.; Carvajal, J.C.L.; Costa, J.G.M. Physicochemical and spectroscopical investigation of pequi (*Caryocar coriaceum* Wittm.) pulp oil. *Grasas Aceites* **2010**, *61*, 191–196.

80. Feitosa, I.S.; Albuquerque, U.P.; Monteiro, J.M. Knowledge and extractivism of *Stryphnodendron rotundifolium* Mart. in a local community of the Brazilian savanna, northeastern Brazil. *J. Ethnobiol. Ethnomed.* **2014**, *10*, 64.
81. Maciel, T.C.M.; Marco, C.A.; Silva, E.E.; da Silva, T.I.; dos Santos, H.R.; de Oliveira Alcantara, F.D.; Chaves, M.M. Pequi (*Caryocar coriaceum* Wittm.) extrativism: Situation and perspectives for its sustainability in *Cariri cearense*, Brazil. *Acta Agron.* **2018**, *67*, 238– 245.
82. Sousa Júnior, J.R.; Albuquerque, U.P.; Peroni, N. Traditional knowledge and management of *Caryocar coriaceum* Wittm. (Pequi) in the Brazilian savanna, Northeastern Brazil. *Econ. Bot.* **2013**, *67*, 225–233.
83. Augusto, L.G.S.; Góes, L. Compreensões integradas para a vigilância da saúde em ambiente de floresta: o caso da Chapada do Araripe, Ceará, Brasil. *Cad. Saúde Pública* **2007**, *23*, S549–S558.
84. Lacerda-Neto, L.J.; Ramos, A.G.B.; Vidal, C.S. Serviços Ecossistêmicos: O caso do *Caryocar coriaceum* Wittm. (Pequi) na Chapada do Araripe. *Rev. Biol. Farm.* **2013**, *9*, 34–40.
85. Sobral, A.; La Torre, M.Á.; Alves, R.R.N.; Albuquerque, U.P. Conservation efforts based on local ecological knowledge: The role of social variables in identifying environmental indicators. *Ecol. Indic.* **2017**, *81*, 171–181.
86. Silva, R.R.; Gomes, L.J.; Albuquerque, U.P. Plant extractivism in light of game theory: A case study in Northeastern Brazil. *J. Ethnobiol. Ethnomed.* **2015**, *11*, 6.
87. Silva, R.R.V.; Gomes, L.J.; Albuquerque, U.P. What are the socioeconomic implications of the value chain of biodiversity prod- ucts? A case study in Northeastern Brazil. *Environ. Monit. Assess.* **2017**, *189*, 64.
88. Sousa-Júnior, J.R.; Santos, G.C.; Campos, L.Z.O.; Sousa, R.S.; Cordeiro, P.S.; Almeida, A.L.S.; Cavalcanti, M.C.B.; Albuquerque, U.P. O Pequi (*Caryocar coriaceum* Wittm.-*Caryocaraceae*) na Chapada do Araripe. In *Sociobiodiversidade na Chapada do Araripe*; NUPEEA: Recife, Brazil, 2015.
89. Maciel, T.; Silva, T.; Alcantara, F.; Marco, C.; Ness, R. Substrato à base de pequi (*Caryocar coriaceum*) na produção de mudas de tomate e pimentão. *Rev. Agric. Neotrop.* **2017**, *4*, 9–16.
90. Cavalcanti, M.C.B.; Ramos, M.A.; Araújo, E.L.; Albuquerque, U.P. Implications from the use of non-timber forest products on the consumption of wood as a fuel source in human-dominated semiarid landscapes. *Environ. Manag.* **2015**, *56*, 389–401.

91. Braga, A. Devoção, lazer e turismo nas romarias de Juazeiro Do Norte, CE: Reconfigurações romeiras dos significados das romarias a partir de tensões entre as categorias turismo e devoção. *Rev. Estud. Relig.* **2010**, *1*, 149–161.
92. Silva, A.T. Almoçando entre os romeiros de padre Cícero: Memórias do escultor Agostinho Balmes Odísio sobre práticas alimentares no interior do Ceará (1934–1935). *História Cult.* **2020**, *9*, 227–243.
93. Lira Neto. *Padre Cícero—Poder, Fé e Guerra No Sertão*; Editora Companhia das Letras: Sao Paulo, Brazil, 2009.
94. Comblin, J. *Padre Cícero de Juazeiro*; Pia Sociedade de São Paulo-Editora Paulus: Sao Paulo, Brazil, 2014.
95. Duarte, C.M.; Pereira, A.M.B.; Pereira, P.S.; Barros, L.M.; Duarte, A.E. A religiosidade e o turismo em uma cidade do interior do Ceará. *InterSciencePlace* **2016**, *11*, 136-150.
96. Prado, D. *Caryocar coriaceum*. In *The IUCN Red List of Threatened Species*; International Union for Conservation of Nature: Gland, Switzerland, 1998.
97. Peres, C.; Baider, C.; Zuidema, P.; Wadt, L.; Kainer, K.; Gomes-Silva, D.; Salomão, R.; Simões, L.; Franciosi, E.; Valverde, F.; et al. Demographic threats to the sustainability of Brazil nut exploitation. *Science* **2003**, *302*, 2112–2114. Santos, G.C.; Schiel, N.;
98. Araújo, E. de L.; Albuquerque, U.P. *Caryocar coriaceum* (Caryocaraceae) diaspore removal and dispersal distance on the margin and in the interior of a cerrado area in Northeastern Brazil. *Rev. Biol. Trop.* **2016**, *64*, 1117–1127.
99. Azevedo, F.R.; Moura, M.A.R.; Arrais, M.S.B.; Nere, D.R. Composição da entomofauna da floresta nacional do Araripe em diferentes vegetações e estações do ano. *Rev. Ceres* **2011**, *58*, 740–748.
100. Halffter, G.; Arellano, L. Response of dung beetle diversity to human–induced changes in a tropical landscape. *Biotropica* **2002**, *34*, 144–154.
101. Kimberling, D.; Karr, J.; Fore, L. Measuring human disturbance using terrestrial invertebrates in the shrub-Steppe of eastern Washington (USA). *Ecol. Indic.* **2001**, *1*, 63–81.
102. Melo, R.S.; Silva, O.C.; Souto, A.; Alves, R.R.N.; Schiel, N. The role of mammals in local communities living in conservation areas in the Northeast of Brazil: An ethnozoological approach. *Trop. Conserv. Sci.* **2014**, *7*, 423–439.
103. Albuquerque, U.P.; Gonçalves, P.H.S.; Júnior, W.S.F.; Chaves, L.S.; Silva Oliveira, R.C.; Silva, T.L.L.; Santos, G.C.; Araújo, E.L. Humans as niche constructors: Revisiting

the concept of chronic anthropogenic disturbances in ecology. *Perspect. Ecol. Conserv.* **2018**, *16*, 1–11.

104. Santiago, D.B.; Correia Filho, W.L.F.; Oliveira-Júnior, J.F.; Silva Junior, C.A. Mathematical modeling and use of orbital products in the environmental degradation of the araripe forest in the brazilian northeast. *Model. Earth Syst. Environ.* **2019**, *5*, 1429–1441.

3 ARTIGO 2 – *Caryocar coriaceum* Fruits as a Potential Alternative to Combat Fungal and Bacterial Infections: *In Vitro* Evaluation of Methanolic Extracts

Artigo Publicado

Revista: *Microbial Pathogenesis*

ISSN: 0882-4010

Fator de Impacto: 3.8

Qualis – CAPES (2017-2020): A3 em Biodiversidade

Percentil Scopus (2023): 75%

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Abstract: *Caryocar coriaceum*, commonly known as ‘pequi’, is a medicinal species used traditionally for the herbal treatment of infectious and parasitic diseases in the Brazilian Northeast region. In this study, we investigated whether the fruits of *C. coriaceum* have bioactive chemical constituents against etiological agents of infectious diseases. The methanolic extract of the internal mesocarp of the fruits of *C. coriaceum* (MECC) was chemically analyzed and evaluated for its antimicrobial and drug-enhancing activity against multidrug-resistant pathogenic bacteria (*Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus*), and *Candida* spp. strains. The extract had flavones, flavonols, xanthones, catechins, and flavanones as major classes. A total of 11.26 mg GAE/g of phenolics, and 5.98 mg QE/g of flavonoids were found. No intrinsic antibacterial activity was observed; however, the extract was able to intensify the action of gentamicin and erythromycin against multi-resistant strains. The anti-*Candida* effect observed in this study was mainly due to the formation of reactive oxygen species. The extract was capable of causing damage to the plasmatic membrane of *Candida tropicalis* through pores formation. Our findings partially support the ethnopharmacological uses of the fruit pulp of *C. coriaceum* against infectious diseases.

Keywords: Candidiasis, Chapada do Araripe, Infectious and Parasitic Diseases.

1 Introduction

The mortality as a result of infectious and parasitic diseases (IPDs) has grown in recent decades, mainly due to microbial resistance originating from the indiscriminate use of antibiotics. Pathogenic bacteria, fungi, and protozoa have been shown to overcome the action of commercially available drugs, and the discovery of new drugs has not kept pace with the adaptation speed of these microorganisms [1-3].

Another factor that significantly contributes to the increase in mortality due to infectious and parasitic diseases, is the lack of access to appropriate medicines by the population, especially in underdeveloped countries. On the other hand, medicinal plants have stood out as the therapeutic alternative for the treatment of IPDs, due to their easy access, low cost, and traditional and cultural usage by many communities [4,5]. Therefore, the investigation of the bioactive potential of plant species becomes important to formulate new medicines or drug enhancers [6,7].

In Brazil, medicinal plants are widely used by different communities, the country is one of the top ten consumers of *in natura* plant therapeutic resources [8-14]. Brazil has a huge diversity in terms of traditional communities which make use of herbal medicine (e.g., indigenous peoples, afro-descendant quilombos, rubber tappers, and traditional inhabitants of the coastal regions, among others). However, the use of medicinal plants is not only related to these cultural aspects but also to the plant diversity found in the different Brazilian phytogeographic domains [15].

Among Brazilian ecosystems, the Caatinga, a type of seasonally dry tropical forest, located in the Northeast region of Brazil, accounts for 17.2% of the total botanical taxa found in the country [15]. More than 700 spermatophytes are endemic to this region. Many of these species are used in traditional medicine to treat various diseases [6,15]. In the northern region of the Northeast, in the states of Ceará, Pernambuco and Piauí, more precisely in the area of Chapada do Araripe, a tree that stands out is *Caryocar coriaceum* Wittm. (Caryocaraceae), popularly known as “pequi” and “pequizeiro” and by the native indigenous Kariri as “*pyrantecaira*”. This species produces fruits that are used in oil production, which is highly appreciated in regional cuisine. In addition to food use, this oil is highly versatile, displaying a notorious medicinal use [15-19].

In ethnopharmacology, the fruits of *C. coriaceum* and its by-products are used against infectious and parasitic diseases, also a series of digestive, genitourinary, skin, and subcutaneous diseases [19]. These medicinal properties can be attributed to the chemical

composition of the fruits since they have flavonoids in their constitution [20]. In this context, considering the increasing resistance of microorganisms to the standard drugs, and the rising search for natural products with biological properties, it is pertinent to investigate the biological potential of *C. coriaceum* against microorganisms that cause infections, as well as to describe its chemical composition.

This study hypothesized that the extract of the fruit pulp of *C. coriaceum* has biological activity against microorganisms that cause infections. The objective of this research was to investigate whether the fruits of *C. coriaceum* have bioactive chemical constituents against etiological agents of infectious diseases, and also its antimicrobial and drug-enhancing activity against multidrug-resistant pathogenic fungi (*Candida* spp.) and strains bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*).

2 Methodology

2.1 Collection of Botanical Material and Environmental Licenses

Ripe and undamaged fruits of *C. coriaceum* were collected in February 2021 in the Environmental Protection Area (APA) of Chapada do Araripe, belonging to the municipality of Jardim - CE, Brazil (Figure 1), under coordinates 07°29'269"S and 39°18'050"W, and at an altitude of 925 m. Concurrently, branches with fertile parts were collected, and herborization techniques were applied to make an exsiccate. Following, it was deposited in the Herbarium UFP - Geraldo Mariz under registration number 88,948. This study was registered in the Biodiversity Information and Authorization System (SISBio) under registration number 77450-1; and at the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number A4848B1.

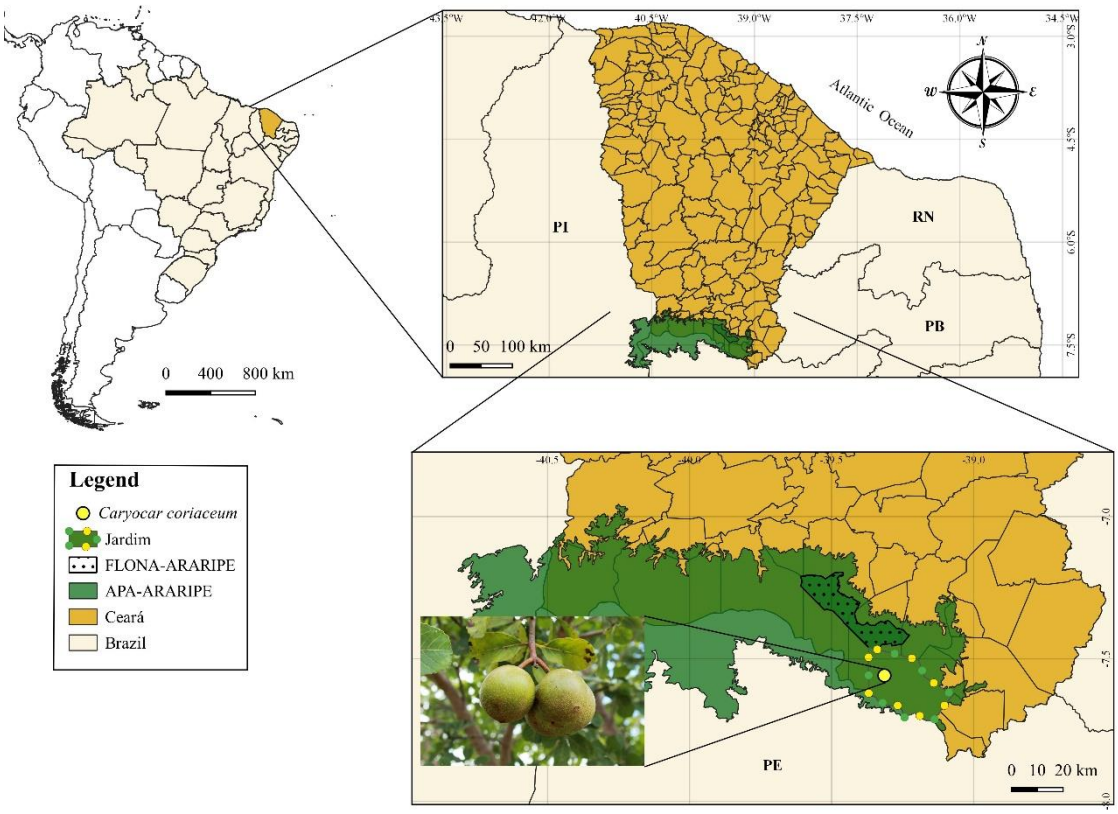

Figure 1: *Caryocar coriaceum* Wittm. fruit collection location map in the municipality of Jardim, in the Environmental Protection Area of Chapada do Araripe, Brazil.

2.2 Extract Obtaining

The internal mesocarp of *C. coriaceum* harvested from 300 fruits was subjected to dehydration at 40 ºC for seven days, obtaining 760 g of dehydrated pulp. The pulp was subjected to exhaustive extraction with *n*-hexane for 72 h to remove compounds with low polarity. The residue was subjected to a new extraction with methanol for the same period. After filtration, the extract was concentrated on a rotary evaporator to obtain the crude extract (yield = 4.84%). The methanolic extract of *C. coriaceum* (MECC) was stored in an amber bottle at room temperature until chemical analysis and biological tests [21,22].

2.3 Phytochemical Analysis

2.3.1 Qualitative Chemical Prospection

Were evaluated the presence of secondary metabolites in the extract via colorimetric alteration and formation of precipitates [23]. Tests were carried out for the detection of

pyrogallic tannins, condensed tannins, anthocyanins, anthocyanidins, flavones, flavonols, xanthones, chalcones, aurones, leucoanthocyanidins, catechins, flavanones, and alkaloids.

2.3.2 Total phenols and flavonoids

To determine the total phenol content, the Folin-Ciocalteu method was used, according to Singleton et al. [24], with some adaptations. An ethanolic solution (1 mg/mL) of *C. coriaceum* extract was added to a volumetric flask, plus 250 µL of Folin-Ciocalteu reagent, and 3 mL of distilled water. This solution was stirred for 0.5 min, and 1 mL of 15% sodium carbonate (Na_2CO_3) was added. After two hours, the absorbance was read in a spectrophotometric device at 760 nm (SmartSpec Plus, Bio Rad, USA). The total phenolic content was expressed in µg of gallic acid equivalents per mg of *C. coriaceum* extract (GAE/mg).

For the quantification of total flavonoids, the method described by Woisky and Salatino [25] was used with some alterations. In an aqueous solution of the extract (1 mg/mL), were added 3 mL of methanol and 1 mL of 5% aluminum chloride, left resting for 30 min. After this period, the absorbance of the solution was measured at 425 nm in a spectrophotometer device (SmartSpec Plus, Bio Rad, USA). Total flavonoid content was determined using a quercetin standard curve (Sigma-Aldrich®). The total flavonoid content was expressed in µg of quercetin equivalents per mg of *C. coriaceum* extract (QE/mg).

2.4 Antifungal Activity

2.4.1 Strains, Culture Medium, Drugs, Reagents, and Preparation of Solutions

The standard strains of *Candida albicans* CA INCQS 90028, *Candida krusei* – CK INCQS 40095, and *Candida tropicalis* CT INCQS 40042 obtained from the Microbial Collection and Reference in Health Surveillance – CMRVS of the Instituto Nacional de Controle de Qualidade em Saúde (FIOCRUZ-INCQS) were used.

The fungal strains were inoculated in Petri dishes containing *Sabouraud Dextrose Agar* (SDA, Kasvi), and then incubated in a microbial incubator at 37 °C for 24 h. After growth, aliquots were collected and transferred to test tubes containing 3 mL of 0.9% saline solution, and adjusted to 0.5 McFarland turbidity (1×10^8 CFU/mL) [26]. For the microdilution assays and determination of the average inhibitory concentration (IC_{50}), *eppendorfs* containing *Sabourand Dextrose Broth* (CSD, Himedia) were prepared using a two-fold concentration. For the virulence inhibition assays, a *Potato Dextrose Agar* (PDA, Kasvi)

medium depleted of nutrients was prepared to stimulate the formation of hyphae and pseudohyphae [27].

The methanolic extract of *C. coriaceum* (20 mg) was initially diluted in dimethylsulfoxide (0.5% DMSO, Merck, Darmstadt, Germany), and later in sterile distilled water, until reaching the concentration of the stock solution (2,048 µg/mL). As a positive control, fluconazole (Capsule – FLUCOMED, São Paulo, Brazil) was used, this compound acts in the synthesis of ergosterol, which was diluted in sterile water [28].

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

To determine the anti-*Candida* action of the *C. coriaceum* extract, the broth microdilution technique was used, using a 96-well plate (Kasvi). During the procedure, each well was initially filled with 90 µL of *Sabouraud Dextrose Broth* (SDB), which preceded a serial microdilution (1:1 v/v) with the natural product (MECC) until the penultimate well of the plate, in numerical order. The concentrations obtained ranged from 1 to 1,024 µg/mL. After this process, 10 µL of inoculum was added. No tested products were added in the last well, it was kept for fungal growth control. Using the same method for the positive control (fluconazole). Following, the plates were kept in a microbial incubator for 24 h at 37 °C. The samples were read using an ELISA spectrophotometer model DR.-200BS-NM-BI (Kazuaki, Wuxi, China) at a wavelength of 630 nm. Dilution controls for the natural product and drug (with 0.9% sodium chloride solution instead of the inoculum), and sterility controls, were also performed [29,30].

The absorbance results were used to determine the Minimum Inhibitory Concentration (MIC) and design the cell survival curve. Based on the average cell survival curve, the IC_{50} values of the *C. coriaceum* extract and fluconazole were calculated. The MIC was considered to be the concentration responsible for completely inhibiting fungal growth. When there was no MIC, the matrix concentration (2048 µg/mL) was considered as the starting point for the sub-inhibitory concentrations.

2.4.3 Assessment of Fluconazole Modifier Activity

After determining the MIC, the assessment of the Fluconazole modifier activity was carried out using sub-inhibitory concentrations (MIC/8) according to Morais-Braga et al. [2]. During the testes, 96-well flat-bottomed plates (Kasvi) were used, which were added with *Sabouraud Dextrose Broth* (SDB) medium (SDB, Himedia) containing the methanolic extract

of *C. coriaceum* in its sub-inhibitory concentrations. Subsequently, this solution was microdiluted with Fluconazole (1:1 v/v) up to the penultimate row of the wells. The concentrations ranged from 1,024 to 1 µg/mL. Finally, the inoculum of *Candida* spp. was added until obtaining a concentration of 10% in each well of the plate. This procedure followed the same methodology as described in section 2.4.2.

2.4.4 Evaluation of Fungal Virulence Inhibition

On a sterile microscope slide, 3 mL of Potato Dextrose Agar (PDA) depleted of nutrients containing the methanolic extract of *C. coriaceum* at sub-inhibitory concentrations (MIC/2 and MIC/4) was added. After solidification and stabilization of the medium on the slide, two parallel streaks of *Candida* spp. inoculum were made, which were placed in a humid chamber (Figure 2), and kept in a microbial incubator under the same conditions previously mentioned. After a period of 24 h, the slides were observed under an optical microscope at a magnification of 400×. It captured images where there was emission or inhibition of filamentous structures. As a positive control, fluconazole was used at the same sub-inhibitory concentrations mentioned previously. While the growth control consisted of the presence of PDA and fungal strains [27].

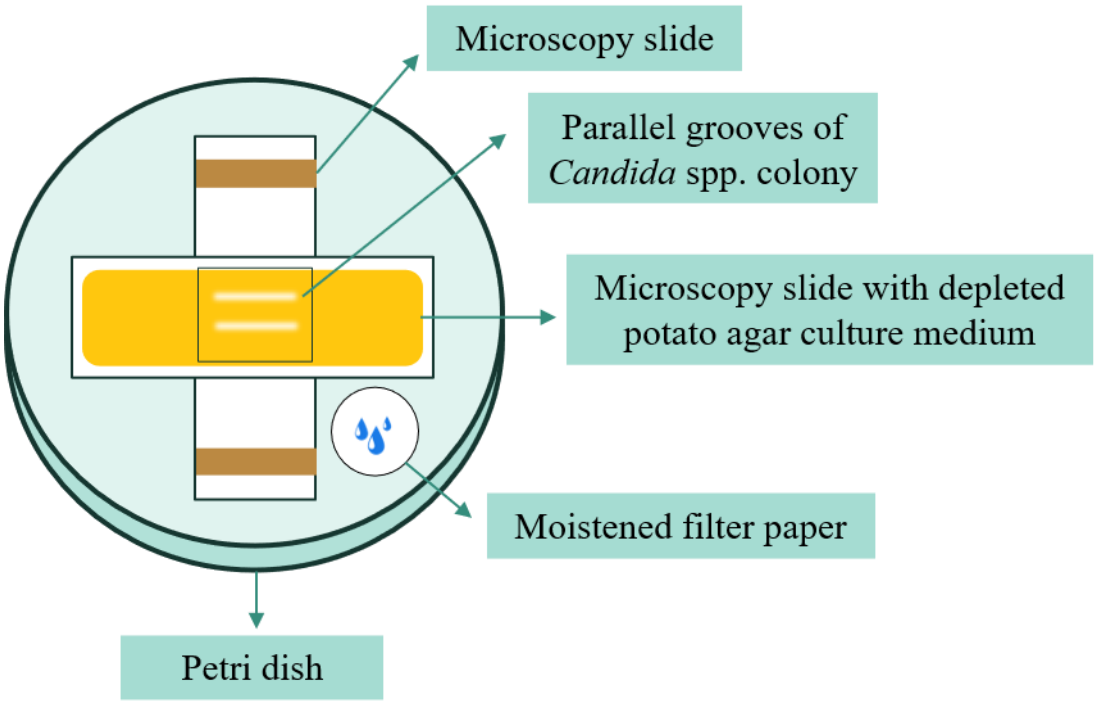

Figure 2: Humid chamber scheme used for the induction of morphological transition in *Candida* yeasts.

2.4.5 Induction of Reactive Oxygen Species (ROS)

Candida spp. were initially cultivated in SDB medium, for a period of 18 h at 37 °C. Subsequently, 100 µL of the fungal inoculum (10^6 cells/mL) were incubated with 100 µL of the methanolic extract of *C. coriaceum* at the concentrations of their IC_{50} (µg/mL) against their respective strains of *Candida* spp., at 37 °C for 24 h in an environment completely absent of light. Following, the yeasts from each treatment were incubated with 2,7-dichlorofluorescein diacetate (DCFH-DA) at 37 °C for 30 min in the dark. The yeasts were then centrifuged (Mikro 200R, Hettich, Germany) at 3000 × g for 5 min at 22 °C, and washed three times using NaCl (0.15M). Finally, they were properly prepared for observation under a fluorescence microscope (Olympus System BX 60; excitation and emission wavelengths of 488 and 525 nm, respectively). The positive control (Fluconazole), and the negative control (NaCl 150 mM) were preceded by the same method [31].

2.4.6 Determination of Cell Membrane Integrity

Yeast cells were incubated in SDB for 24 hours at 37 °C, plus methanolic extract of *C. coriaceum* in its IC_{50} concentration, and with 150 mM NaCl (negative control), or with fluconazole (positive control). After incubation, aliquots (100 µL) of treated cells were incubated with 1 mM propidium iodide for 30 min, at 37 ºC, under moderate agitation (75 rpm). Cell visualization was performed using a fluorescence microscope (Olympus BX 60 System, excitation wavelength – 490 nm; emission wavelength – 520 nm) [32].

2.5 Antibacterial Activity

2.5.1 Strains, Culture Medium, Drugs, Reagents, and Preparation of Solutions

For the evaluation of the antibacterial activity, the standard bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853, and *Staphylococcus aureus* ATCC 22923), and the multi-resistant strains *Escherichia coli* 06, *Pseudomonas aeruginosa* 24, both urine culture derived, and *Staphylococcus aureus* 10, from rectal tissue culture (Table 1) were used. These bacteria were grown in Petri dishes containing Heart Infusion Agar (HIA), and placed in a microbial incubator at 37 °C for 24 h. After growth, samples of each bacterial culture were collected, and diluted in saline solution (0.9%), reaching a turbidity of 0.5 on the McFarland scale (1×10^8 CFU/mL). From these inoculums, 150 µL were taken and added to a 10% Brain Heart Infusion (BHI) solution, to be used in antibacterial assays.

Table 1. Antibacterial resistance profile of clinical bacterial isolates. Source: Laboratory of Microbiology and Molecular Biology – LMBM, Regional University of Cariri - URCA.

Bacteria	Resistance profile
Escherichia coli 06	Cephalotin, cephalexin, cefadroxil, ceftriaxone, cefepime, ampicillin-sulbactam, amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, cefepime
Pseudomonas aeruginosa 24	Amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, merpenem, cefepime
Staphylococcus aureus 10	Cefadroxil, cephalexin, cephalothin, oxacillin, penicillin, ampicillin, amoxicillin, moxifloxacin, ciprofloxacin, levofloxacin, ampicillin-sulbactam, amoxicillin / ac. clavulanic, erythromycin, clarithromycin, azithromycin, clindamycin

For the determination of the minimum inhibitory concentration (MIC), the methanolic extract of *C. coriaceum* was diluted in dimethylsulfoxide (DMSO, Merck, Darmstadt, Germany) reaching a concentration of 20,000 µg/mL, later this solution underwent a new dilution with sterile distilled water until reaching a concentration of 1,024 µg/mL. The antibacterial drugs used in the intensifier activity assays were norfloxacin (fluoroquinolone class), gentamicin (aminoglycosides), and erythromycin (macrolide group).

2.5.2 Minimum Inhibitory Concentration - MIC

For MIC determination, 96-well flat bottom plates (Kasvi) were used, which were filled with 100 µL of BHI solution + inoculum. Following, a serial microdilution (1:1 v/v) was performed with the *C. coriaceum* extract, obtaining different concentrations (0.5 – 512 µg/mL). These plates were placed in a microbial incubator for 24 h at 37 ºC. After this period, a solution of 20 µL resazurin (Sigma Aldrich, St. Louis, Missouri, USA) at 0.01% was added to each well of bacterial growth, targeting the occurrence of redox reactions. It was considered the highest MIC which inhibited bacterial growth [27].

2.5.3 Drug-Enhancing Activity

After determining the MIC, the drug-enhancing activity test was performed, in which the product was evaluated at sub-inhibitory concentrations (MIC/8) [7]. A BHI solution was

prepared in association with the inoculum (10%), and the natural product at a sub-inhibitory concentration, which was distributed in the wells of the plates. Then, serial microdilution (1:1 v/v) was performed using the aforementioned antibacterial drugs in varied concentrations (0.5-512 μg/mL). After bacterial growth (24 h at 37 °C), the reading was performed with the addition of 20 μL of aqueous resazurin solution.

2.6 Statistical analysis

All assays were performed in triplicate. Data were expressed as means, and their respective standard errors (± SEM). Subsequently, they were submitted to a one-way analysis of variance (ANOVA One-way) using Tukey's test at 95% reliability. *P* values were defined as < 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). The average inhibitory concentrations (IC_{50}) values were calculated using non-linear regression. All analyzes were performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, United States). The photomicrographs of the mechanisms of action were analyzed using the point picker tool in the ImageJ software (National Institutes of Health, Bethesda, Maryland, USA) version 1.53.

3. Results

3.1 Chemical Composition

The MECC phytochemical tests showed the presence of flavones, flavonols, xanthones, catechins, and flavanones, and negative results for the other classes investigated (Table 2). Quantitatively, the total phenols and flavonoids represented 11.26 mg GAE/g and 5.98 mg QE/g of extract, respectively.

Table 2: Total phenolic and flavonoid content of the methanolic extract of *Caryocar coriaceum* (MECC).

Sample	Total Phenolics (mg gallic acid/g)	Total Flavonoids (mg quercetin/g)
MECC	11.26 ± 1.01	5.98 ± 1.16

3.2 Antifungal activity

3.2.1 Cell survival curve and average inhibitory concentration

According to Figure 3, the MECC was not effective in reducing the fungal growth of the three tested strains. Only the highest extract concentration (1024 µg/mL) reduced the cell survival curve of C. *albicans* (96.61%), and *C. tropicalis* (85.11%). For *C. krusei*, the reduction was only 22%.

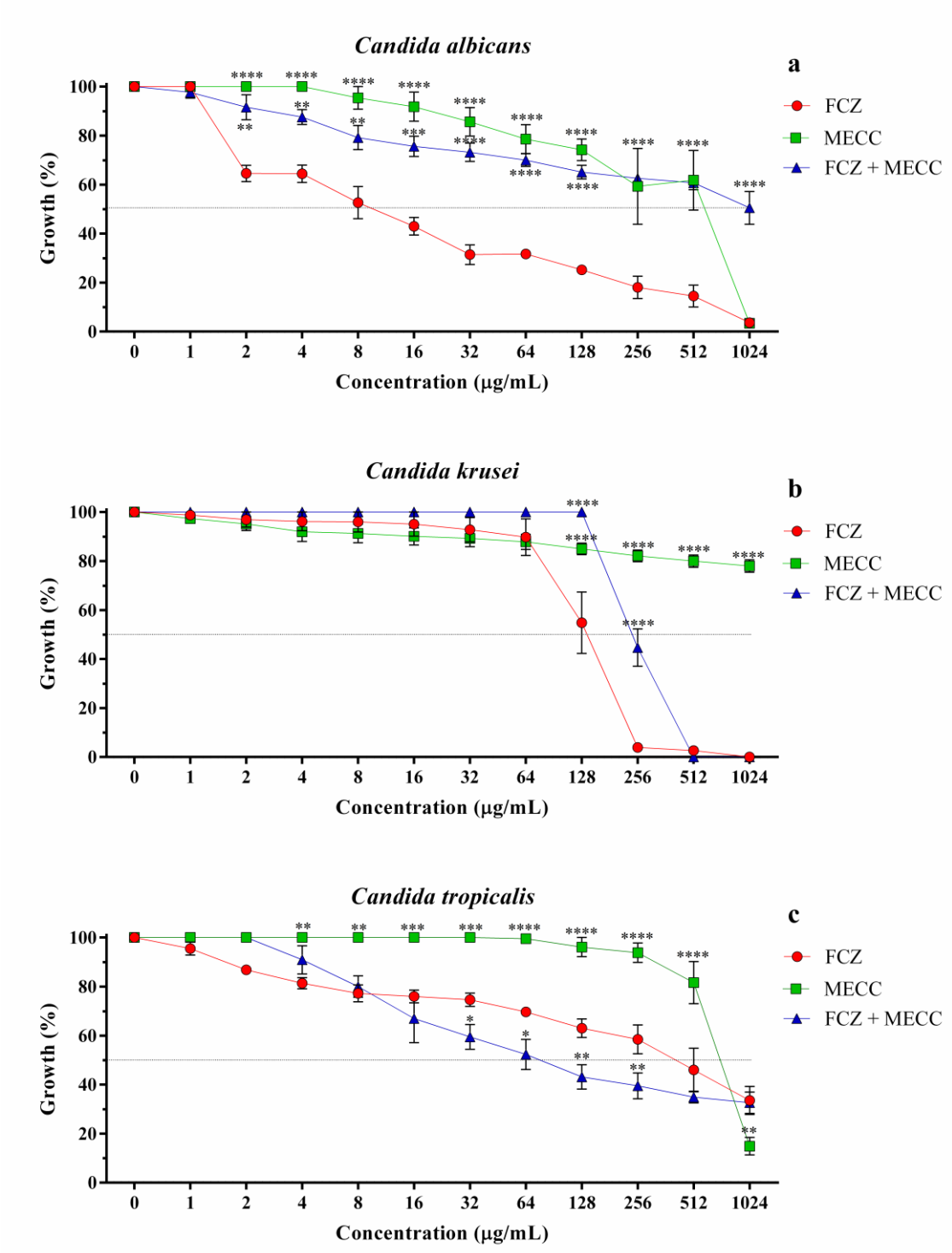

Figure 3: Cell viability curve and IC_{50} value (dotted line) of different concentrations of methanolic extract of *Caryocar coriaceum* (MECC), fluconazole (FCZ) and its combination

(FCZ + MECC) against *Candida albicans* (INCQS 90028) (3a), *Candida krusei* (INCQS 40095) (3b) and *Candida tropicalis* (INCQS 40042) (3c). * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$. The bars represent the standard error of the mean (n = 3).

Regarding the intensifier activity of fluconazole, it was possible to notice that MECC was not able to increase the antifungal effect against *C. albicans* and *C. krusei* strains (Figure 3). However, against *C. tropicalis*, the intensifying action of MECC was significant at concentrations from 32 to 256 µg/mL (* = $p < 0.05$, ** = $p < 0.01$, respectively).

The IC_{50} values (µg/mL) for the anti-*Candida* activity of fluconazole, MECC, and MECC + fluconazole, indicate that the IC_{50} values for the MECC were higher than those for fluconazole, demonstrating the ineffectiveness of the extracts (Table 3). Nevertheless, the IC_{50} of the MECC against *C. albicans* could be considered relevant, since the concentration of 351 µg/mL was able to inhibit the yeast growth by 50%. This value is similar to that of fluconazole (362 µg/mL) against *C. tropicalis*.

Table 3: Median inhibitory concentration (IC_{50}) values in µg/mL of methanolic extract of *Caryocar coriaceum* (MECC), fluconazole (FCZ), as well as in combination (FCZ + MECC), against strains of *Candida albicans* (INCQS 90028), *Candida krusei* (INCQS 40095) and *Candida tropicalis* (INCQS 40042).

Treatment	IC_{50} (µg/mL)		
	Candida albicans	***Candida krusei***	***Candida tropicalis***
MECC	351	>1024	703
FCZ	12.33	131.6	362
FCZ + MECC	1144	254.3	109.9

Regarding the synergistic effect, the antifungal effect of fluconazole was intensified when associated with MECC against *C. tropicalis* strains. The IC_{50} value was reduced by almost 70% after association with the extract, which is a promising result. However, MECC reduces the antifungal activity of fluconazole against *C. albicans* and *C. krusei* (Table 3).

3.2.2 Inhibition of Fungal Virulence

In addition to the direct anti-*Candida* activity of the extracts, their ability to inhibit fungal virulence through hyphal suppress the development was also evaluated. In Figures 4

and 6, it is possible to observe the natural formation of hyphae of *C. albicans* and *C. tropicalis* (control group), which were inhibited by MECC at a concentration of 512 µg/mL. Although MECC at a concentration of 256 µg/mL was less effective in inhibiting hyphal formation compared to fluconazole, the inhibition was noticeably greater compared to the control group. Thus, MECC can inhibit one of the virulence mechanisms present in these pathogens.

On the other hand, MECC was not able to inhibit the formation of hyphae of *C. krusei* at the tested concentrations. Interestingly, the lowest concentration (512 µg/mL) considerably increased the formation of hyphae and pseudohyphae in *C. krusei*. While fluconazole (256 and 512 µg/mL) completely inhibited the formation of these filamentous structures (Figure 5).

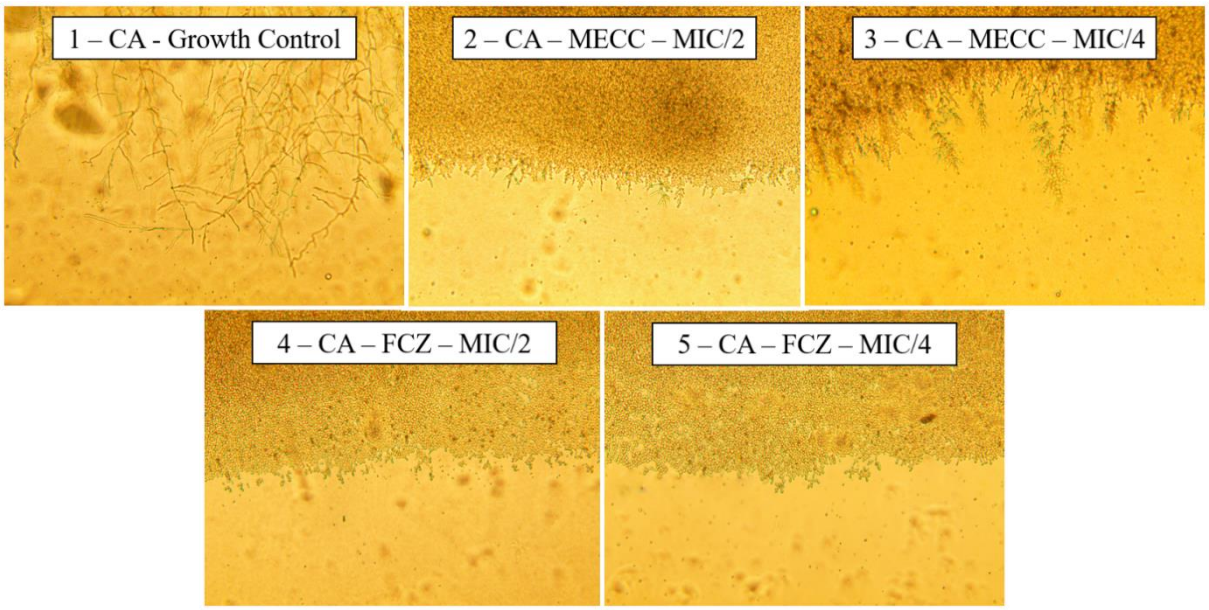

Figure 4: Effects of methanolic extract of *Caryocar coriaceum* (MECC) on the dimorphism of *Candida albicans* INCQS 90028. Slide (S1): Growth control; S2-3: Effect of MECC on the concentration of 512 µg/mL (S2) and 256 µg/mL (S3). S4-5: Effect of fluconazole at the concentration of 512 µg/mL (S4) and 256 µg/mL (S5). 400× magnification.

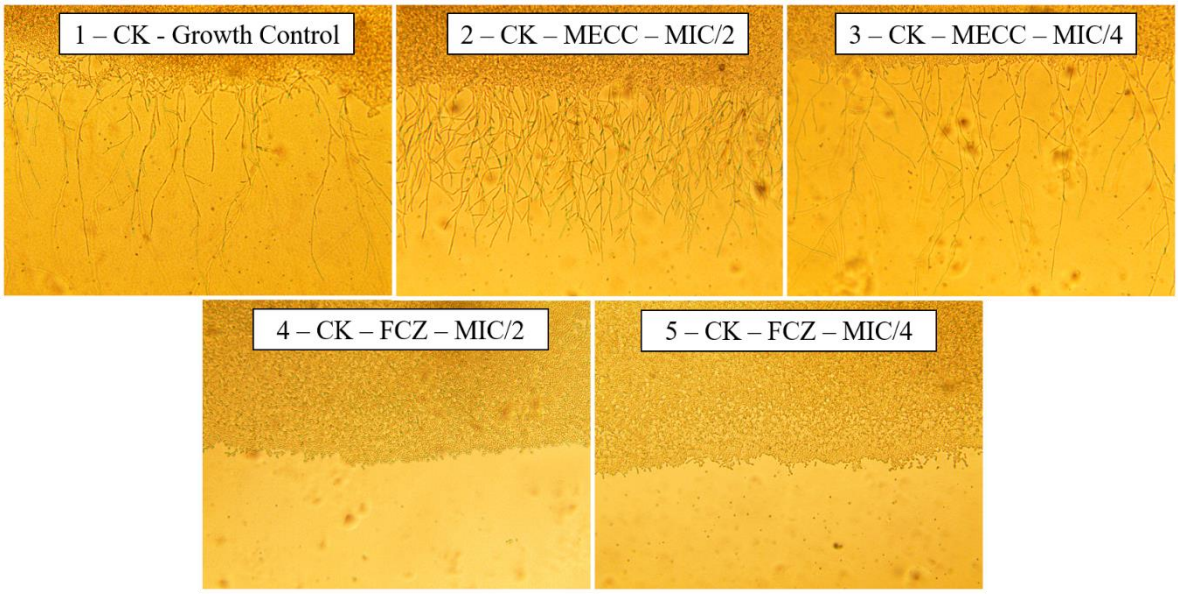

Figure 5: Effects of methanolic extract of *Caryocar coriaceum* (MECC) on the dimorphism of *Candida krusei* INCQS 40095. Slide (S1): Growth control; S2-3: Effect of MECC on the concentration of 512 µg/mL (S2) and 256 µg/mL (S3). S4-5: Effect of fluconazole at the concentration of 512 µg/mL (S4) and 256 µg/mL (S5). 400× magnification.

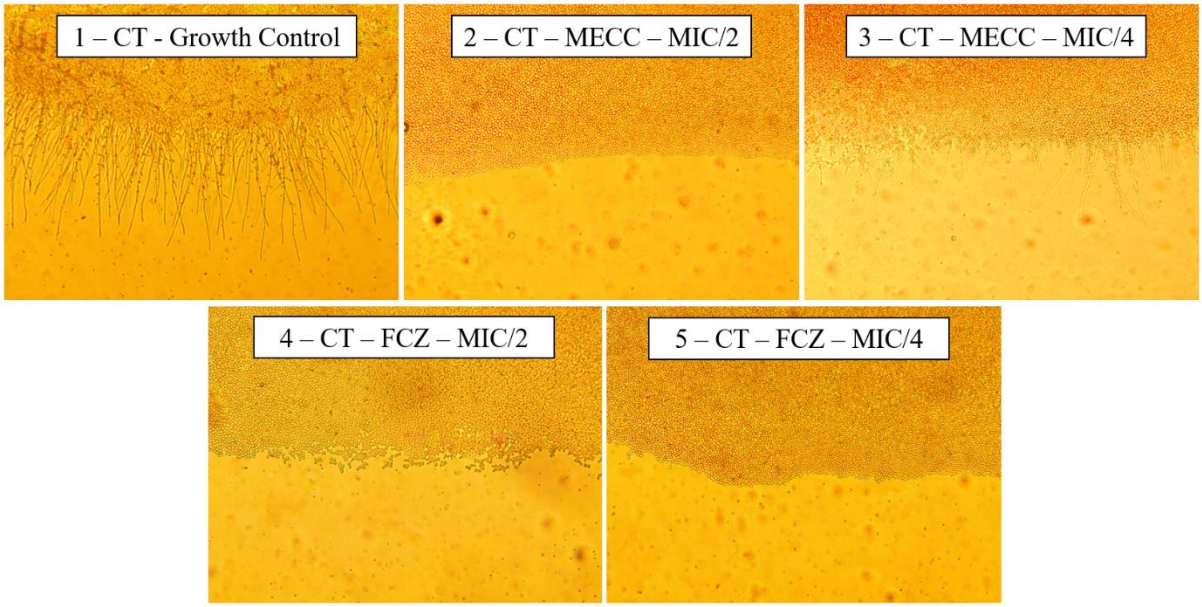

Figure 6: Effects of methanolic extract of *Caryocar coriaceum* (MECC) on the dimorphism of *Candida tropicalis* INCQS 40042. Slide (S1): Growth control; S2-3: Effect of MECC on the concentration of 512 µg/mL (S2) and 256 µg/mL (S3). S4-5: Effect of fluconazole at the concentration of 512 µg/mL (S4) and 256 µg/mL (S5). 400× magnification.

3.2.3 Mechanisms of Action

As observed in Figures 7, 8, and 9, the main mechanism to reduce yeast growth was the formation of reactive oxygen species (ROS). In all *Candida* species, the verification of fluorescent fungal cells labeled with 2 ',7'-dichlorofluorescein was higher than the negative control (150 mM NaCl). Among the three strains evaluated, *C. krusei* was the most susceptible to the effect of MECC, which was able to produce a significantly higher amount of ROS compared to fluconazole (Figure 8). In addition to ROS formation, it was observed during fluorescence microscopy that MECC can act by permeabilizing the cell membrane. *C. tropicalis* strains showed to be susceptible to this mechanism of action (Figure 9). On the other hand, there was no change in cell membrane permeabilization in *C. albicans* (Figure 7), and *C. krusei* strains (Figure 8).

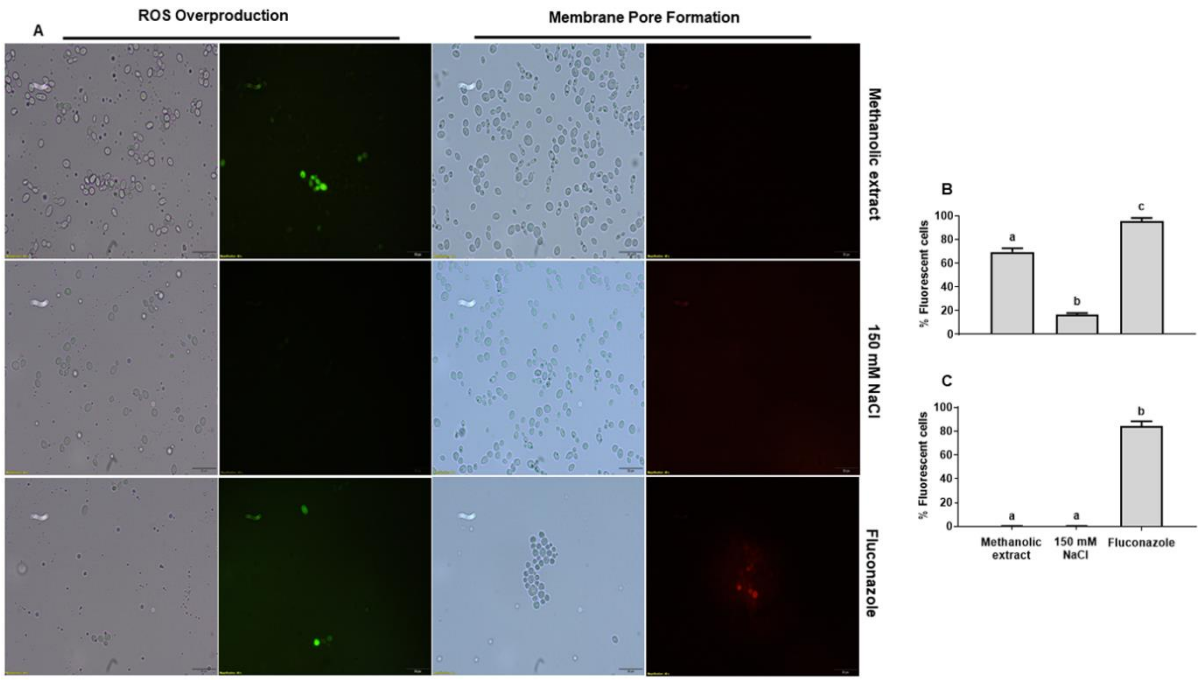

Figure 7: Effect of methanolic extract of *Caryocar coriaceum* on *Candida albicans* (INCQS 90028). (A - Left) Reactive Oxygen Species. (A - Right) Cell membrane permeabilization. (B) Percentage of fungal cells labeled with 2',7'-dichlorofluorescein. (C) Percentage of fungal cells stained with propidium iodide. Different letters represent statistical difference between means ($p < 0.05$). Results are presented as mean ± standard deviation.

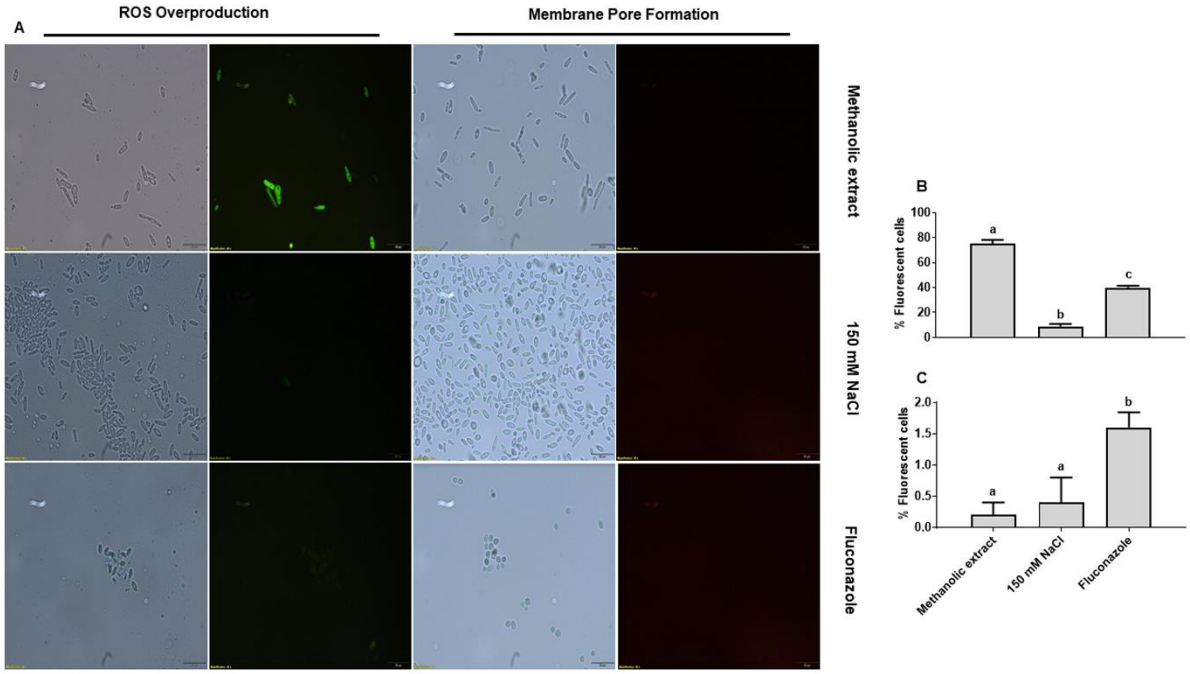

Figure 8: Effect of methanolic extract of *Caryocar coriaceum* on *Candida krusei* (INCQS 40095) (A - Left) Reactive Oxygen Species. (A - Right) Cell membrane permeabilization. (B) Percentage of fungal cells labeled with 2',7'-dichlorofluorescein. (C) Percentage of fungal cells stained with propidium iodide. Different letters represent statistical difference between means ($p < 0.05$). Results are presented as mean ± standard deviation.

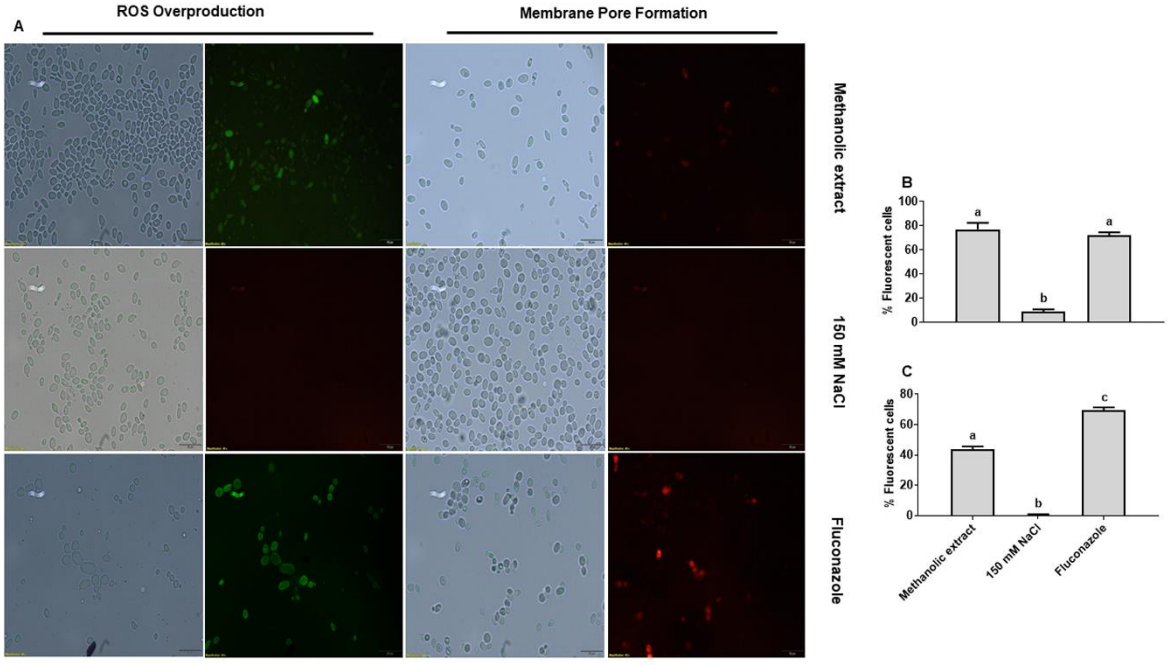

Figure 9: Effect of methanolic extract of *Caryocar coriaceum* on *Candida tropicalis* (INCQS 40042) (A - Left) Reactive Oxygen Species. (A - Right) Cell membrane permeabilization. (B) Percentage of fungal cells labeled with 2',7'-dichlorofluorescein. (C) Percentage of fungal

cells stained with propidium iodide. Different letters represent statistical difference between means ($p < 0.05$). Results are presented as mean ± standard deviation.

3.3 Antibacterial activity

The MECC did not show direct activity against the standard multi-resistant strains of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*. The MIC found was > 512 µg/mL, indicating that MECC was not able to inhibit the growth of these multi-resistant bacteria at concentrations of clinical interest. However, when combining the MECC with standard drugs it was possible to verify that there was a modifying action (Figure 10). MECC combined with gentamicin significantly increased the antibacterial activity against the three multidrug-resistant strains evaluated (** = $p < 0.01$, **** = $p < 0.0001$). In addition, the extract was able to significantly reduce (**** = $p < 0.0001$) the MIC of erythromycin against strains of *P. aeruginosa* and *E. coli*, potentializing drug activity. Interestingly, the extract of *C. coriaceum* reduced the antibacterial activity of norfloxacin, as it significantly increased (**** = $p < 0.0001$) its MIC in all multi-resistant bacteria evaluated.

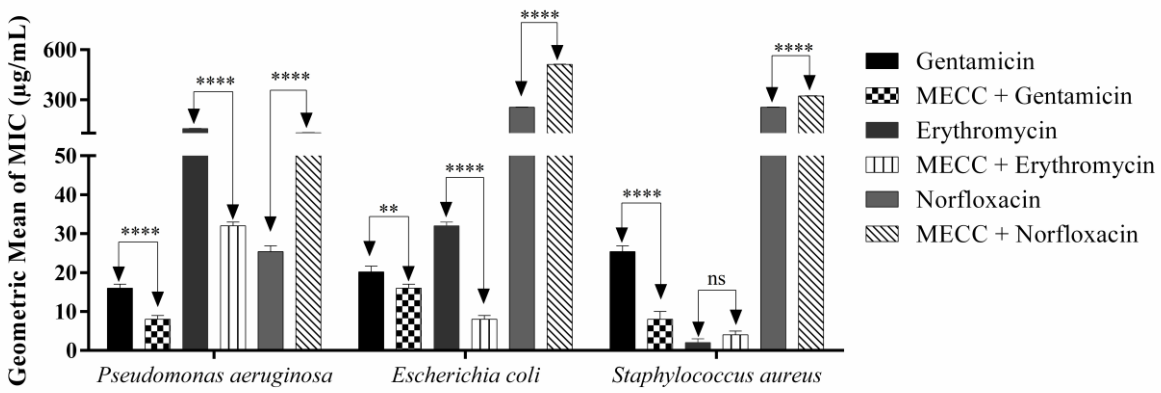

Figure 10: Geometric mean minimum inhibitory concentration (MIC) in µg/mL of methanolic extract of *Caryocar coriaceum* (MECC) in association with different antibiotics against different multidrug-resistant bacterial strains. ns = $p > 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$. The bars represent the standard error of the mean (n = 3).

4 Discussion

Located in Northeast Brazil, Chapada do Araripe has a diversity of flora throughout its territory [33]. This botanical richness associated with the cultural plurality of the region, resulted in a diverse and important ethnopharmacological knowledge of many plant species, in which *C. coriaceum* has a prominent place [34,35]. Among which stands out *C. coriaceum*,

a species used in the popular pharmacopoeia for centuries in the aforementioned region [19]. Among the ethnopharmacological indications, the use of fruits for the treatment of infectious and parasitic diseases stands out [17-19].

Such medicinal indications may be directly linked to the chemical composition of the fruits of *C. coriaceum*. Previous studies identified the flavonoids rutin, isoquercitrin, and quercetin in extracts from the peel and pulp of *C. coriaceum* fruits [22]. The occurrence of flavonoids in other species of the genus *Caryocar* [36], demonstrates that in addition to lipids, fruits are also sources of compounds with a phenolic nature. Our findings showed that MECC contains flavones, flavonols, xanthones, catechins, and flavanones, reinforcing the occurrence of phenolic compounds in *C. coriaceum* fruits.

In our study, it was evidenced the absence of intrinsic antibacterial activity of the fruit extract of *C. coriaceum* at clinically relevant concentrations [37]. This lack of activity may be related to the low content of total flavonoids present in the internal mesocarp. It is known that extracts with high levels of flavonoids tend to have antibacterial activity, and several flavonoid mechanisms of action have already been described [38-40]. However, Lacerda Neto et al. [41] demonstrated that the ethanolic extract of the leaves of *C. coriaceum*, which is rich in flavonoids, was able to inhibit bacterial growth at low concentrations against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 12692, and *Pseudomonas aeruginosa* ATCC 15442. Thus, the antimicrobial activity also depends on the qualitative composition of the extract.

Due to its ethnopharmacological use, the hypothesis was raised that the inner mesocarp of C. coriaceum fruits would present antibacterial activity, which was not verified. However, according to Costa et al. [42], the fixed oil of the internal mesocarp of *C. coriaceum* showed to reduce the growth of *Salmonella choleraesuis* ATCC 13314, *Staphylococcus aureus* ATCC 12692, *Pseudomonas aeruginosa* ATCC 15442, and *Streptococcus pneumoniae* (ATCC6314). Thus, the antimicrobial potential of the mesocarp of *C. coriaceum* is more directly related to its lipidic composition, and not to the phenolic constituents of the internal mesocarp.

Despite the absence of intrinsic antibacterial activity, the combination of the *C. coriaceum* extract with gentamicin and erythromycin showed an intensifying action of these antibiotics against the strains, especially when associated with gentamicin, which belongs to the class of aminoglycosides. Drug potentiating activity in microbiological assays refers to the assessment of the ability of a substance or compound to enhance or enhance the effects of a given drug. This type of assay is important in pharmaceutical research and drug development,

as it allows identifying substances that can increase the therapeutic efficacy of a drug or reduce the dose required to obtain a certain effect [2,7]. The fundamentals of this assay involve performing experiments in biological models or *in vitro* systems to assess the synergistic or additive effects of two or more substances. The substance or compound being tested is added to the parent drug in varying concentrations, and the resulting effect is compared with the effect produced by the parent drug alone [27,28].

Among the mechanisms of bacterial resistance to antibiotics, the most common against the class of aminoglycosides is the enzymatic destruction or inactivation of the drug. This can occur through the production of acetyltransferases, nucleotidyltransferases., and phosphotransferases [43]. Górniak, Bartoszewski, and Króliczewski [38] reported that some flavonoids, even at low concentrations, can act as inhibitors of these enzymes. Thus, the intensifying action found in the present study may be due to the activity of the extract in combination with gentamicin which could cause the inactivation of these bacterial enzymes.

As mentioned, the fruits of *C. coriaceum* have been used in traditional medicine to treat infections associated with the genitourinary tract [19], which are often caused by yeasts of the genus *Candida* [44,45]. Thus, the symptoms related to candidiasis are popularly treated with derivatives of *C. coriaceum* fruits, such as fixed oil [19]. Our findings corroborate the ethnopharmacological use of *C. coriaceum* since the MECC was able to reduce the growth of *Candida* spp. yeasts, as well as inhibit one of its virulence factors.

The anti-*Candida* effect of the MECC may be related to the presence of flavonoids in the chemical composition of the mesocarp of *C. coriaceum* fruits. Flavonoids are known to have significant antifungal effects [46,47]. These displayed effects against *Candida* spp. by various mechanisms, including induced disruption of the plasma membrane, inhibition of cell wall formation, induced mitochondrial dysfunction, inhibition of cell division, inhibition of efflux pumps, inhibition of the RNA/DNA, and inhibition of protein synthesis [48-50]. Alves et al. [20] reported that the internal mesocarp of *C. coriaceum* fruits showed an antifungal effect against *Malassezia* spp. (MIC: 19.53 µg/ml), and *Microsporum canis* (MIC: 4.88 µg/ml). These authors associated the biological effect with the presence of flavonoids in *C. coriaceum*, such as quercetin, rutin, and isoquercetin.

The antifungal effect of MECC was not only restricted to reducing yeast growth. Also, was observed inhibition in the morphological transition of the yeast, one of the virulence factors of *Candida* spp. [50]. This mechanism inherent to the pathogen, consists of the transition from yeast forms to filamentous forms, characterizing them as polymorphic. Such change is associated with tissue penetration and invasion, as well as escape macrophage

killing. If a natural or synthetic product inhibits the filamentous form of the fungi, a certain infected body via the immune system may be able to fight the infection itself [49]. Among the mechanisms of action caused by natural products, the inhibition of the expression of proteins responsible for phenotypical transformation stands out [51].

In our findings, the MECC probably acted in the formation of reactive oxygen species (ROS). The extract tested may have induced the overproduction of ROS in all evaluated strains (Figure 11). These ROS act in the intracellular environment, causing damage to important molecules, such as proteins, nucleic proteins (DNA and RNA), and membrane lipids, therefore, threatening cell integrity (Figure 11). The increased production of ROS can lead to oxidative stress in *Candida* spp. yeasts, leading them to apoptosis [52,53]. It was also observed the formation of pores in the membrane when tested against the strains of *C. tropicalis*. Pore formation is due to electrolyte imbalance, causing damage to the plasmatic membrane. Thus, the yeast will lose its cytoplasmic content, which may lead to cell death [54].

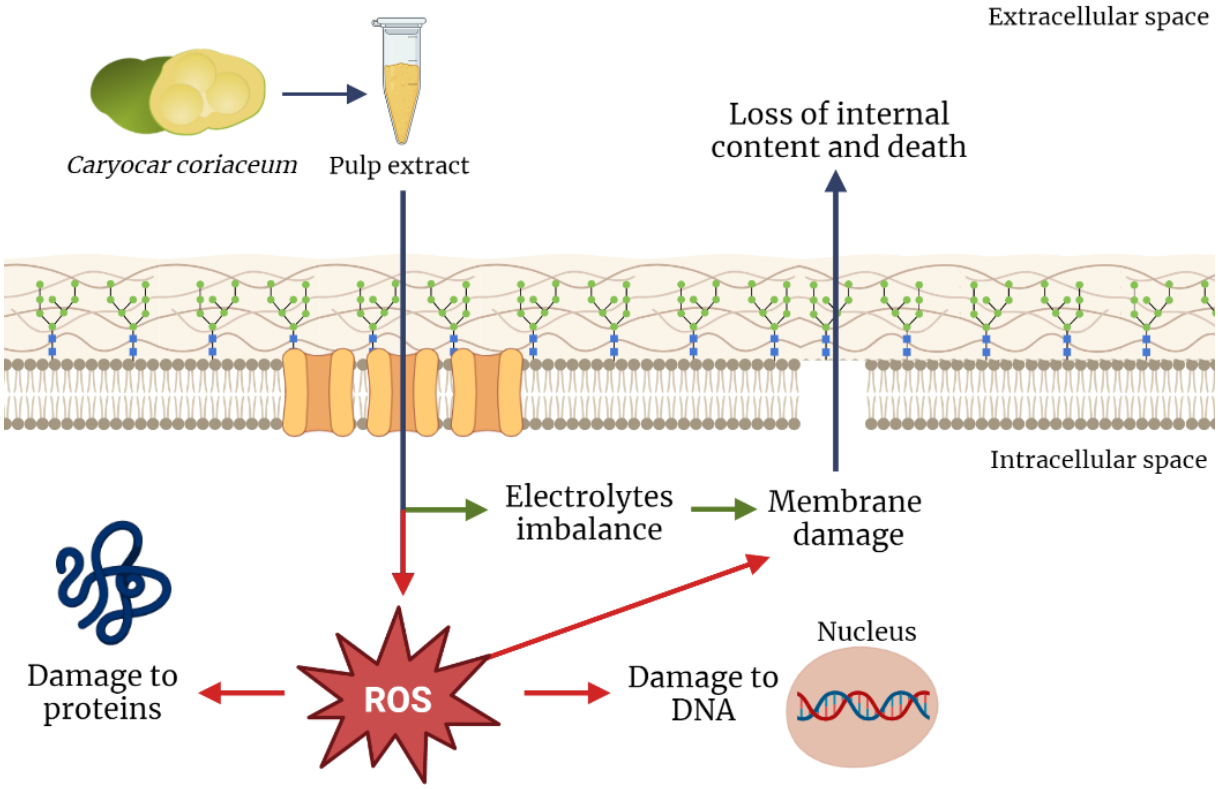

Figure 11: Diagram indicating the possible mechanisms of action for the anti-*Candida* activity of the methanolic extract of *Caryocar coriaceum*.

In addition to the intrinsic antifungal activity, an intensifying effect of fluconazole activity against *C. tropicalis* strains was verified. One of the possibilities of this synergism, is the change in membrane permeability of the yeast, favoring the entry of fluconazole, and consequently increasing its intracellular concentration. Fluconazole is known to inhibit the biosynthesis of ergosterol, one of the main constituents of the fungal membrane [48,55]. This association of products of natural origin with standard drugs is promising, as it becomes an alternative in antibiotic therapy, as the synergistic effect reduces the dosage needed during the use of commercial drugs [56-58].

The MECC anti-*Candida* results demonstrated in this study are promising for pharmaceutical industries to develop new products. Because the antifungal activities occurred in concentrations of clinical relevance, making it a promising candidate for therapeutic applications [37]. However, additional tests must be carried out to evaluate the pharmacological profile of extracts from *C. coriaceum* fruits, such as the evaluation of acute, subacute, and chronic toxicity and the evaluation of pharmacokinetics and pharmacodynamics, to guarantee the safety of using these extracts in medicines. In addition, it is essential to carry out chemical stability tests on the extracts to assess the degradation of the active principles, in order to guarantee the effectiveness of possible drugs. These tests are essential to prove the efficacy and safety of the drugs that will be produced using the extracts [59,60].

5 Conclusion

Our study demonstrated that the ethnopharmacological use of fruit pulp of *C. coriaceum* by communities in Chapada do Araripe (Brazil) for the treatment of infectious and parasitic diseases was partially supported by our findings. The *C. coriaceum* extract did not show a direct effect against pathogenic bacteria, but it was able to intensify the action of antibiotics against multi-resistant microorganisms. Regarding the antifungal activity, the extract reduced the growth of *Candida* spp., acting through the formation of reactive oxygen species. Furthermore, it was able to inhibit the morphological transition of the yeasts, one of their virulence mechanisms. *C. coriaceum* extract even intensified the activity of fluconazole.

References

[1] C.D.M. Oliveira-Tintino, S.R. Tintino, P.W. Limaverde, F.G. Figueredo, F.F. Campina, F.A.B. Cunha, R.H.S. Costa, P.S. Pereira, L.F. Lima, Y.M.L.S. Matos, H.D.M.

Coutinho, J.P. Siqueira-Júnior, V.Q. Balbino, T.G. Silva, Inhibition of the essential oil from *Chenopodium ambrosioides* L . and α - terpinene on the NorA e ffl ux-pump of *Staphylococcus aureus*, Food Chem. 262 (2018) 72–77. https://doi.org/10.1016/j.foodchem.2018.04.040.

[2] M.F.B. Morais-Braga, J.N.P. Carneiro, A.J.T. Machado, D.L. Sales, A.T.L. Santos, A.A. Boligon, M.L. Athayde, I.R.A. Menezes, D.S.L. Souza, J.G.M. Costa, H.D.M. Coutinho, Phenolic composition and medicinal usage of *Psidium guajava* Linn .: Antifungal activity or inhibition of virulence ?, Saudi J. Biol. Sci. 24 (2017) 302–313. https://doi.org/10.1016/j.sjbs.2015.09.028.

[3] T.B. Abadi, A.A. Rizvanov, T. Haertlé, N.L. Blatt. World Health Organization report: current crisis of antibiotic resistance. BioNanoScience. 9 (2019) 778-788. https://doi.org/10.1007/s12668-019-00658-4

[4] F. Jamshidi-Kia, Z. Lorigooini, H. Amini-Khoei, Medicinal plants: Past history and future perspective, J. HerbMed Pharmacol. 7 (2018) 1–7. https://doi.org/10.15171/jhp.2018.01.

[5] L.C. Saalu, Nigerian Folklore Medicinal Plants with Potential Antifertility Activity in Males : A Scientific Appraisal, Res. J. Med. Plants. 10 (2016) 201–227. https://doi.org/10.3923/rjmp.2016.201.227.

[6] U.P. Albuquerque, P.M. Medeiros, A.L.S. Almeida, J.M. Monteiro, E.M.F. Lins Neto, J.G. Melo, J.P. Santos, Medicinal plants of the caatinga (semi-arid) vegetation of NE Brazil: A quantitative approach, J. Ethnopharmacol. 114 (2007) 325–354. https://doi.org/10.1016/j.jep.2007.08.017.

[7] H.D.M. Coutinho, G.M. Costa, E.O. Lima, V.S. Falcão-Silva, J.P. Siqueira-Júnior, Enhancement of the Antibiotic Activity against a Multiresistant *Escherichia coli* by *Mentha arvensis* L . and Chlorpromazine, Chemotherapy. 54 (2008) 328–330. https://doi.org/10.1159/000151267.

[8] S.G.D. Oliveira, F.R.R. Moura, F.F. Demarco, P.S. Nascente, F.A.B.D. Pino, R.G. Lund, An ethnomedicinal survey on phytotherapy with professionals and patients from Basic Care Units in the Brazilian Unified Health System, J. Ethnopharmacol. 140 (2012) 428–437. https://doi.org/10.1016/j.jep.2012.01.054.

[9] M. Lenzi, V. Cocchi, A. Novaković, M. Karaman, M. Sakač, A. Mandić, M. Pojić, M.C. Barbalace, C. Angeloni, P. Hrelia, M. Malaguti, S. Hrelia. *Meripilus giganteus* ethanolic extract exhibits pro-apoptotic and anti-proliferative effects in leukemic cell

lines, BMC Complement Altern. Med., 18 (2018) 1-14. https://doi.org/10.1186/s12906-018-2366-7.

[10] C. Lu, Z. Wei, N. Jiang, Y. Chen, Y. Wang, S. Li, Q. Wang, B. Fan, X. Liu, F. Wang. Soy isoflavones protects against cognitive deficits induced by chronic sleep deprivation via alleviating oxidative stress and suppressing neuroinflammation, Phytother Res. 36 (2022) 2072-2080. https://doi.org/10.1002/ptr.7354.

[11] S. Mitra, R. Das, T.B. Emran, R.K. Labib, Noor-E-Tabassum, F. Islam, R. Sharma, I. Ahmad, F. Nainu, K. Chidambaram, F.A. Alhumaydhi, D. Chandran, R. Capasso, P. Wilairatana. Diallyl Disulfide: A Bioactive Garlic Compound with Anticancer Potential, Front Pharmacol. 13 (2022) 1-21. https://doi.org/10.3389/fphar.2022.943967.

[12] E.G. Ferrarini, R.S. Paes, G.M. Baldasso, P.M. Assis, M.C. Gouvêa, P. Cicco, N.R.B. Raposo, R. Capasso, E.L.G. Moreira, R.C. Dutra. Broad-spectrum cannabis oil ameliorates reserpine-induced fibromyalgia model in mice, Biomed Pharmacother. 154 (2022) 1-9. https://doi.org/10.1016/j.biopha.2022.113552.

[13] D. Ağagündüz,T.Ö. Şahin, B. Yılmaz, K.D. Ekenci, D.Ö. Şehriban, R. Capasso. Cruciferous Vegetables and Their Bioactive Metabolites: from Prevention to Novel Therapies of Colorectal Cancer. BMC Complement. Altern. Med., 2022 (2022) 1-20. https://doi.org/10.1155/2022/1534083.

[14] J. Fernández, B. Silván, R. Entrialgo-Cadierno, C.J. Villar, R. Capasso, J.A., Uranga, F. Lombó, R. Abalo, Antiproliferative and palliative activity of flavonoids in colorectal cancer, Biomed. Pharmacother. 143 (2021) 1-11. https://doi.org/10.1016/j.biopha.2021.112241

[15] K.N. Magalhães, W.A.S. Guarniz, K.M. Sá, A.B. Freire, M.P. Monteiro, R.T. Nojosa, I.G.C. Bieski, J.B. Custódio, S.O. Balogun, M.A.M. Bandeira, Medicinal plants of the Caatinga, northeastern Brazil: Ethnopharmacopeia (1980–1990) of the late professor Francisco José de Abreu Matos, J. Ethnopharmacol. 237 (2019) 314–353. https://doi.org/10.1016/j.jep.2019.03.032.

[16] V.C.N. Bitu, V.C.N. Bitu, E.F.F. Matias, W.P. Lima, A.C. Portelo, H.D.M. Coutinho, I.R.A. Menezes, Ethnopharmacological study of plants sold for therapeutic purposes in public markets in Northeast Brazil, J. Ethnopharmacol. 172 (2015) 265–272. https://doi.org/10.1016/j.jep.2015.06.022.

[17] M.D.F. Agra, K.N. Silva, I.J.L.D. Basílio, P.F. Freitas, J.M. Barbosa-Filho, Survey of medicinal plants used in the region Northeast of Brazil, Brazilian J. Pharmacogn. 18 (2008) 472–508. https://doi.org/10.1590/S0102-695X2008000300023.

[18] I.C.S. Lemos, G.A. Delmondes, E.S. Santos, D.R. Oliveira, P.R.L. Figueiredo, D.A. Alves, R. Barbosa, I.R.A. Menezes, H.D.M. Coutinho, M.R. Kerntopf, G.P. Fernandes, Ethnobiological survey of plants and animals used for the treatment of acute respiratory infections in children of a traditional community in the municipality of Barbalha, Ceará, Brazil, African J. Tradit. Complement. Altern. Med. 13 (2016) 166–175. https://doi.org/10.21010/ajtcam.v13i4.22.

[19] J.W. Almeida-Bezerra, J.J.L. Bezerra, V.B. da Silva, H.D.M. Coutinho, J.G.M. da Costa, N. Cruz-Martins, C. Hano, S.A. de Menezes, M.F.B. Morais-Braga, A.F.M. de Oliveira, *Caryocar coriaceum* Wittm. (Caryocaraceae): Botany, Ethnomedicinal Uses, Biological Activities, Phytochemistry, Extractivism and Conservation Needs, Plants. 11 (2022) 1685. https://doi.org/10.3390/plants11131685.

[20] D.R. Alves, S.M. De Morais, F. Tomiotto-pellissier, M.M. Miranda-sapla, F.R. Vasconcelos, I.N.G. Silva, H.A. Sousa, J.P. Assolini, I. Conchon-costa, W.R. Pavanelli, F.C.O. Freire, Flavonoid Composition and Biological Activities of Ethanol Extracts of *Caryocar coriaceum* Wittm., a Native Plant from Caatinga Biome, Evidence-Based Complement. Altern. Med. 2017 (2017) 1–7.

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

[22] F. Tomiotto-Pellissier, D.R. Alves, S.M. de Morais, B.T. da S. Bortoleti, M.D. Gonçalves, T.F. Silva, E.R. Tavares, L.M. Yamauchi, I.N. Costa, E.S. Marinho, M.M. Marinho, I. Conchon-Costa, M.M. Miranda-Sapla, W.R. Pavanelli, *Caryocar coriaceum* Wittm. fruit extracts as *Leishmania* inhibitors: *in-vitro* and *in-silico* approaches, J. Biomol. Struct. Dyn. 2021 (2021) 1–16. https://doi.org/10.1080/07391102.2021.1905557.

[23] F.J.A. Matos, Introdução a fitoquímica experimental, 3rd ed., UFC, Fortaleza, 2009.

[24] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, in: 1999: pp. 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1.

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

[26] M.A. Wikler, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard., in: CLSI, 2006.

[27] J.N.P. Carneiro, R.P. Cruz, J.C.P. Silva, J.E. Rocha, T.S. Freitas, D.L. Sales, C.F. Bezerra, W.O. Almeida, J.G.M. Costa, L.E. Silva, W. do Amaral, R.A. Rebelo, I.M. Begnini, H.D.M. Coutinho, M.F.B. Morais-Braga, *Piper diospyrifolium* Kunth.: Chemical analysis and antimicrobial (intrinsic and combined) activities, Microb. Pathog. 136 (2019) 1–9. https://doi.org/10.1016/j.micpath.2019.103700.

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

[29] J.N.P. Carneiro, R.P. Cruz, F.F. Campina, M. do S. Costa, A.T.L. Santos, D.L. Sales, C.F. Bezerra, L.E. Silva, J.P. Araujo, W. Amaral, R.A. Rebelo, I.M. Begnini, L.F. Lima, H.D.M. Coutinho, M.F.B. Morais-Braga, GC/MS analysis and antimicrobial activity of the *Piper mikanianum* (Kunth) Steud. essential oil, Food Chem. Toxicol. 135 (2020) 1–8. https://doi.org/10.1016/j.fct.2019.110987.

[30] M.M. Javadpour, M.M. Juban, W.C.J. Lo, S.M. Bishop, J.B. Alberty, S.M. Cowell, C.L. Becker, M.L. McLaughlin, De novo antimicrobial peptides with low mammalian cell toxicity, J. Med. Chem. 39 (1996) 3107–3113. https://doi.org/10.1021/jm9509410.

[31] I.K. Maurya, S. Pathak, M. Sharma, H. Sanwal, P. Chaudhary, S. Tupe, M. Deshpande, V.S. Chauhan, R. Prasad, Antifungal activity of novel synthetic peptides by accumulation of reactive oxygen species (ROS) and disruption of cell wall against *Candida* albicans, Peptides. 32 (2011) 1732–1740. https://doi.org/10.1016/j.peptides.2011.06.003.

[32] M. Regente, G.B. Taveira, M. Pinedo, M.M. Elizalde, A.J. Ticchi, M.S.S. Diz, A.O. Carvalho, L. de la Canal, V.M. Gomes, A Sunflower Lectin with Antifungal Properties and Putative Medical Mycology Applications, Curr. Microbiol. 69 (2014) 88–95. https://doi.org/10.1007/s00284-014-0558-z.

[33] S. Ribeiro-Silva, M.B. De Medeiros, B.M. Gomes, E.C.S. Naiana, M.A.P. Silva, Angiosperms from the Araripe National Forest, Ceará, Brazil, Check List. 8 (2012) 744–751. www.checklist.org.br.

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

[35] R.K.D. Souza, M.A.P. da Silva, I.R. de M. Alencar, D.A. Ribeiro, L.R. Bezerra, M.M. de A. Souza, Ethnopharmacology of medicinal plants of carrasco, northeastern Brazil, J. Ethnopharmacol. 157 (2014) 99–104.

[36] K.K. de L. Yamaguchi, C.V. Lamarão, E. Aranha, R.O.S. Souza, P.D. Oliveira, M. Vasconcellos, E.S. Lima, V.F. Veiga-Junior, HPLC-DAD profile of phenolic compounds, cytotoxicity, antioxidant and anti-inflammatory activities of the amazon fruit *Caryocar villosum*, Quim. Nova. (2017). https://doi.org/10.21577/0100-4042.20170028.

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

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

[39] D.H.A. Baker, An ethnopharmacological review on the therapeutical properties of flavonoids and their mechanisms of actions: A comprehensive review based on up to date knowledge, Toxicol. Reports. 9 (2022) 445–469. https://doi.org/10.1016/j.toxrep.2022.03.011.

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

[41] L.J. Lacerda Neto, A.G.B. Ramos, M.R. Kerntopf, H.D.M. Coutinho, L.J. Quintans-Júnior, J.R.G.S. Almeida, J. Ribeiro-Filho, I.R.A. Menezes, Modulation of antibiotic activity by the hydroalcoholic extract from leaves of *Caryocar coriaceum* WITTM, Nat. Prod. Res. 32 (2018) 477–480. https://doi.org/10.1080/14786419.2017.1312396.

[42] J.G.M. Costa, S.A. Brito, E.M.M. Nascimento, M.A. Botelho, F.F.G. Rodrigues, H.D.M. Coutinho, Antibacterial Properties of Pequi Pulp Oil (*Caryocar coriaceum* – WITTM.), Int. J. Food Prop. 14 (2011) 411–416. https://doi.org/10.1080/10942910903207744.

[43] M.S. Ramirez, M.E. Tolmasky, Aminoglycoside modifying enzymes, Drug Resist. Updat. 13 (2010) 151–171. https://doi.org/10.1016/j.drup.2010.08.003.

[44] H.M.E. Willems, S.S. Ahmed, J. Liu, Z. Xu, B.M. Peters, Vulvovaginal Candidiasis: A Current Understanding and Burning Questions, J. Fungi. 6 (2020) 27. https://doi.org/10.3390/jof6010027.

[45] S. Bhattacharya, S. Sae-Tia, B.C. Fries, Candidiasis and Mechanisms of Antifungal Resistance, Antibiotics. 9 (2020) 312. https://doi.org/10.3390/antibiotics9060312.

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

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

[48] M.A.S. Aboody, S. Mickymaray, Anti-Fungal Efficacy and Mechanisms of Flavonoids, Antibiotics. 9 (2020) 45–87. https://doi.org/10.3390/antibiotics9020045.

[49] S. Soliman, D. Alnajdy, A. El-Keblawy, K. Mosa, G. Khoder, A. Noreddin, Plants' natural products as alternative promising anti-*Candida* drugs, Pharmacogn. Rev. 11 (2017) 104. https://doi.org/10.4103/phrev.phrev_8_17.

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

[51] Q.-R. Bu, M.-Y. Bao, Y. Yang, T.-M. Wang, C.-Z. Wang, Targeting Virulence Factors of *Candida albicans* with Natural Products, Foods. 11 (2022) 2951. https://doi.org/10.3390/foods11192951.

[52] N. Delattin, B.P. Cammue, K. Thevissen, Reactive oxygen species-inducing antifungal agents and their activity against fungal biofilms, Future Med. Chem. 6 (2014) 77–90. https://doi.org/10.4155/fmc.13.189.

[53] Q. Yu, B. Zhang, J. Li, B. Zhang, H. Wang, M. Li, Endoplasmic reticulum-derived reactive oxygen species (ROS) is involved in toxicity of cell wall stress to *Candida albicans*, Free Radic. Biol. Med. 99 (2016) 572–583. https://doi.org/10.1016/j.freeradbiomed.2016.09.014.

[54] P.G. Lima, P.F.N. Souza, C.D.T. Freitas, J.T.A. Oliveira, L.P. Dias, J.X.S. Neto, I.M. Vasconcelos, J.L.S. Lopes, D.O.B. Sousa, Anticandidal activity of synthetic peptides:

Mechanism of action revealed by scanning electron and fluorescence microscopies and synergism effect with nystatin, J. Pept. Sci. 26 (2020). https://doi.org/10.1002/psc.3249.

[55] R.C. Moraes, A.R. Carvalho, A.J.D. Lana, S. Kaiser, B. Pippi, A.M. Fuentefria, G.G. Ortega, *In vitro* synergism of a water insoluble fraction of Uncaria tomentosa combined with fluconazole and terbinafine against resistant non- *Candida albicans* isolates, Pharm. Biol. 55 (2017) 406–415. https://doi.org/10.1080/13880209.2016.1242631.

[56] O.A. Aiyegoro, A.I. Okoh, Use of bioactive plant products in combination with standard antibiotics: implications in antimicrobial chemotherapy, J. Med. Plants Res. 3 (2009) 1147–1152.

[57] D.M. Silva, P.A. Costa, A.O.B. Ribon, G.A. Purgato, D.-M. Gaspar, M.A.N. Diaz, Plant Extracts Display Synergism with Different Classes of Antibiotics, An. Acad. Bras. Cienc. 91 (2019) 1–8. https://doi.org/10.1590/0001-3765201920180117.

[58] D. Ağagündüz,E. Cocozza, Ö. Cemali, A.D. Bayazıt, M.F. Nanì, I. Cerqua, F. Morgillo, S.K. Saygili, C.R. Berni, Canani, P. Amero, R. Capasso. Understanding the role of the gut microbiome in gastrointestinal cancer: A review, Front. Pharmacol. 14 (2023) 1-17. https://doi.org/10.3389/fphar.2023.1130562.

[59] S. Bajaj, D. Singla, N. Sakhuja. Stability testing of pharmaceutical products, J. Appl. Pharm. Sci. 2 (2012), 129-138.

[60] K.H. Denny, C.W. Stewart. Acute, subacute, subchronic, and chronic general toxicity testing for preclinical drug development. In A comprehensive guide to toxicology in nonclinical drug development. Academic Press. (2017) 109-127. https://doi.org/10.1016/B978-0-12-803620-4.00005-0.

4 ARTIGO 3 – Chemical composition, toxicity and mechanisms of action involved in the anti-*Candida* activity of the fixed oil from *Caryocar coriaceum* Wittm. (Caryocaraceae)

Manuscrito a ser submetido ao periódico *Journal of Ethnopharmacology*

ISSN: 0378-8741

Fator de Impacto: 5.4

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

Percentil Scopus (2023): 82%

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Abstract: The fixed oil from the inner mesocarp of *Caryocar coriaceum* Wittm. is used in the Chapada do Araripe region of Brazil for the treatment of genitourinary system diseases, such as candidiasis. This study aimed to evaluate the chemical composition, antifungal activity, reduction of fungal virulence, and toxicity of the fixed oil from the inner mesocarp of *C. coriaceum* tested against three *Candida* yeasts. The oil was characterized by gas chromatography (GC-MS and GC-FID). Antifungal activity was assessed using the serial microdilution method. Additionally, the potential of the oil as an enhancer of fluconazole action was tested at sub-inhibitory concentrations (MIC/8). The mechanisms of action of the fixed oil of *C. coriaceum* on the morphological transition of *Candida* spp. strains and their virulence factors. The chemical composition of the fixed oil of *C. coriaceum* comprised both unsaturated and saturated fatty acids. Oleic (61%) and palmitic (33%) acids were the major constituents. In terms of its anti-*Candida* activity, the oil reduced the growth of *C. albicans* (IC_{50}: 371 µg/mL) and *C. tropicalis* (IC_{50}: 830 µg/mL). Furthermore, the oil reversed the antibiotic resistance of *C. albicans* and *C. tropicalis*, redering them sensitive to fluconazole and reducing their IC_{50} from 12.33 µg/mL and 362 µg/mL to 0.22 µg/mL and 13.93 µg/mL, respectively. The observed antifungal activity may be attributed to the overproduction of reactive oxygen species. The fixed oil of *C. coriaceum* completely inhibited the morphological transition of *C. albicans* and *C. tropicalis* at a concentration of 512 µg/mL, but exhibited limited low antifungal potential against *C. krusei*. Additionally, the oil did not display *in vivo* toxicity against *Drosophila melanogaster*. The fixed oil from the inner mesocarp of *C. coriaceum* emerge as a strong candidate for the development of new pharmaceutical formulations to treat infections caused by *Candida* spp.

Keywords: Candidiasis, Dimorphism, oxidative stress, oleaginous, pequi

1. Introduction

The genus *Candida* comprises approximately 150 yeast species. Several species of this genus are commensal in humans and can be found on the skin in the gastrointestinal and genital tracts (Romo; Kumamoto, 2020; Willems et al., 2020). Eventually, some *Candida* species can cause infections in susceptible hosts, such as the elderly, hospitalized individuals, or those with weakened immune systems. While most infections are caused by *Candida albicans*, ofthere has been an increase in non-*albicans Candida* infections, including as *Candida krusei* and *Candida tropicalis*, in recent years (Bhattacharya et al., 2020; Ciurea et al., 2020; Vila et al., 2020).

Due to its opportunistic pathogenic nature, *Candida* spp. has become a significant global public health concern, result in high mortality rates and costly treatments for both hospitalized patients and governments (Spampinato, Leonardi, 2013; Pfaller; Castanheira, 2016; Wilson, 2019). Several factors contribute to the virulence of *Candida* spp., with the primary ones being polymorphic capacity, phenotypic alteration, ability to form adherent biofilms, production and secretion of hydrolytic enzymes, expression of adhesin protein complexes, invasion of epidermal cells and mucous membranes (Eix; Nett, 2020; Khan et al., 2021; Rosiana et al., 2021).

Among these virulence mechanisms, some *Candida* species have the ability to alter their structure under specific conditions. Through this polymorphism, yeasts transition into filamentous forms, displaying pseudohyphae (chains of elongated yeast cells) and hyphae (branched chains of tubular cells without constrictions at septation sites). The formation of these filamentous structures is crucial for the success of the infection because tissue invasion occurs through them (Kornitzer, 2019). Another form of infection is candidemia, which occurs when yeasts gain access to the bloodstream host’s. In this case, the yeasts penetrate mucosal cells, subsequently reaching underlying tissues, eventually entering the bloodstream and spreading throughout the body (Fig. 1) (Kornitzer, 2019; Khan et al., 2021).

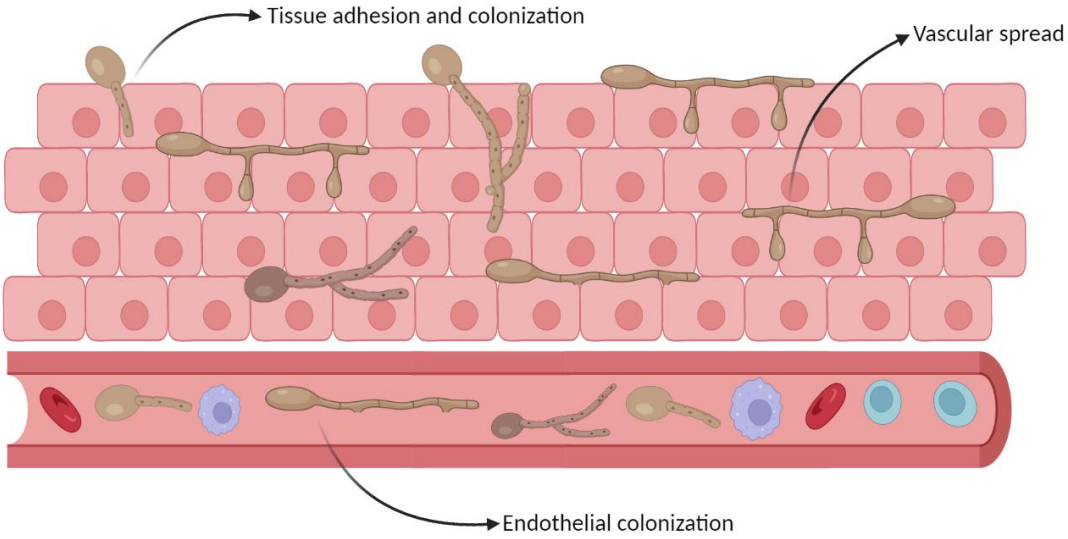

Fig. 1. Pathogenesis of invasion and virulence of yeast species of the genus *Candida*.

Currently, treatments for *Candida* spp. infections rely on antifungal drugs (e.g., polyenes, echinocandins, and azoles). These drugs act through various mechanisms (Lee et al., 2020). Azoles are the most commonly used antifungals in clinical practice, having been introduced in the 1980s, with fluconazole being the most recognized among them. Regarding their structure, they are heterocyclic synthetic compounds that block the synthesis of ergosterol, leading to destabilization of the plasma membrane, increased permeability, and disruption of associated enzymes (Pristov; Ghannoum, 2019). Azoles work by inhibiting fungal growth they are fungistatic, allowing the host organism to control the infection. However, their use can lead to the selection of resistant microorganisms, underscoring the importance of finding new, more effective, and less toxic antifungal drugs (Berman; Krysan, 2020; Lee et al., 2020).

Development of new antifungals is challenging due to the similarity between *Candida* spp. cells and host cells, being eukaryotic. This cellular resemblance makes the search for specific targets an even greater challenge (Roemer; Krysan, 2014; Nicola et al., 2019). As an alternative to synthetic antifungals, there are products derived from medicinal plants that can exert biological effects against fungal infections, at clinically relevant concentrations. These antifungal effects may arise from the intrinsic properties of plant products, or can be enhanced when combined with other antifungal drugs. Prominent among these products are latex, exudates, resin, extracts, essential oils, and fixed oils (Gupta; Birdi, 2017; Bezerra et al., 2019). For instance, plant oils, have demonstrated significant activity against various fungal species (Al Ashaal et al., 2010; Viana et al., 2022; Sampaio et al., 2023).

Caryocar coriaceum Wittm. (Caryocaraceae), commonly known as “pequi”, is a species extensively harvested widely traded by extractive workers in the region of Chapada do Araripe (state of Ceará, Brazil). The fruits of this species find broad usage in Brazilian herbal medicine for treating bronchopulmonary diseases (bronchitis, colds, and flu), as well as in combatting tumors (Agra et al., 2007; Agra et al., 2008; Lemos et al., 2016). The fixed oil derived from the fruits of *C. coriaceum* employed in the genitourinary system (GUS), such as candidiasis (Magalhães et al., 2019). This use may be linked to its chemical composition, particularly its fatty acid content (Oliveira et al., 2010; Almeida-Bezerra et al., 2020).

Given these considerations, this study hypothesized that the oil derived from the fruits of *C. coriaceum* exhibits antifungal activity as well as being able to inhibit the yeast-like morphological transition to the filamentous form against *Candida* spp. This research investigated the chemical composition, toxicity, antifungal properties and fluconazole-modifying effects of the fixed oil from *C. coriaceum* fruits. Additionally, it explored the role of *C. coriaceum* oil in inhibiting the morphological transition in *Candida* spp. and the underlying mechanisms involved in its anti-*Candida* activity.

2. Material and methods

2.1 Collection of Botanical Material and Environmental Licenses

In February 2021, a total of 300 ripe and undamaged fruits of *C. coriaceum* were collected in Serra do Pequi, an Environmental Protection Area (APA) located in Chapada do Araripe, Jardim city, state of Ceará (CE), Brazil (07°29'269"S and 39°18'050"W). Exsicates were deposited at the Herbarium UFP - Geraldo Mariz under registration number 88.948. This study was registered in the Sistema de Autorização e Informação em Biodiversidade (SISBio) under registration number 77450-1, and in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen), under registration code A4848B1.

2.2 Extraction of the Fixed Oil

Initially, the husks (epicarp and outer mesocarp) were removed and disposed of. The inner mesocarp was manually extracted, then dehydrated in an oven at 40 ºC for seven days, resulting in 760 g of dehydrated inner mesocarp. The inner mesocarps were crushed and subjected to exhaustive extraction with *n*-hexane at room temperature for 72 hours. After the solvent was evaporated using a rotary evaporator, the fixed oil of *C. coriaceum* (FOCC)

yielded 291.06 g (38.29% yield). This oil was stored in an amber bottle until the chemical analysis, antifungal, and toxicological tests (Costa et al., 2011).

2.3 Oil hydrolysis and identification of fatty acids

The oil (0.2 g) was saponified for 10 minutes under reflux at 45 ºC, using a solution of potassium hydroxide in methanol (1.5 g of KOH in 35 mL of CH_3OH) following the method by Geris et al. (2007). Fatty acid methyl esters (FAME) were then extracted from the reaction medium using dichloromethane. The organic phase was subsequently washed using distilled water, dried with a desiccant agent, and filtered. The identification of the chemical constituents of FOCC was performed by gas chromatography-mass spectrometry (GC-MS). The trans-esterified FOCC was diluted in 1% dichloromethane, and 1.0 µL of the solution was injected, with a 1:20 flow split, into the Agilent 6890 chromatograph (Palo Alto, CA, USA), coupled to a selective mass detector Agilent 5973N. The injector temperature was maintained at 250 °C. The constituents were separated using an HP-5MS capillary column (5%-phenyl-95%-dimethylpolysiloxane, 30 m x 0.25 mm x 0.25 µm), and helium was employed as the carrier gas (1.0 mL/min). The oven temperature was programmed to begin at 60 °C, gradually increasing at a rate of 3 °C/min until reaching 240 °C. The mass detector operated in electronic ionization mode (70 eV), with a scan rate of 3.15 sweeps s^{-1}, and a mass range of 40 to 450 u. The transfer line was kept at 260°C, the ion source at 230°C, and the analyzer (quadrupole) at 150°C. The identification of the chemical constituents was performed by comparing their mass spectra with those at the NIST library (2.2. Mass Spectral Library (NIST/EPA/NIH) (2016) National Institute of Standards and Technology, Gaithersburg, MD, USA).

For quantification, the diluted samples were injected into an Agilent 7890A chromatograph equipped with a flame ionization detector (FID), operating at 280 ºC. The same analytical conditions described above were used, except for the hydrogen carrier gas, at a flow rate of 1.5 mL/min. The percentage composition was calculated by electronically integrating integration of the FID signal, dividing the peak area of each component by the total area (area %).

2.4 Antifungal activity

2.4.1. Strains, Culture Medium, Drugs, Reagents, and Preparation of Solutions

For the antifungal assays, were used the standard strains *Candida albicans* CA INCQS 90028, *Candida krusei* – CK INCQS 40095, and *Candida tropicalis* CT INCQS 40042 from the Collection of Microorganisms and Reference in Health Surveillance – CMRVS of the Instituto Nacional de Controle de Qualidade em Saúde (FIOCRUZ-INCQS, Brazil).

The strains were spread on Petri dishes (Kasvi, São José dos Pinhais, PR, Brazil) containing Sabouraud Dextrose Agar (SDA). The plates were the incubated in a microbiological incubator at 37 °C for 24 hours. After growth, aliquots were collected and transferred to test tubes with 3 mL of 0.9% saline solution, adjusted to turbidity of 0.5 on the McFarland scale (1×10^8 CFU/mL) (Wikler, 2006). For the microdilution assays and determination of the half-maximal inhibitory concentration (IC_{50}), Eppendorf's were prepared with Sabouraud Dextrose Broth (SDB, Himedia, Paraná, Brazil), twice concentrated. For the virulence inhibition assays, Potato Dextrose Agar (PDA, Kasvi) depleted of nutrients was used to stimulate the formation of hyphae and pseudohyphae (Carneiro et al., 2019).

A total of 20 mg of FOCC was diluted in Dimethylsulfoxide (DMSO 0.5%, Merck, Darmstadt, Germany). Following, the solution was further diluted in sterile distilled water, until reaching the concentration of the stock solution (2,048 µg/mL). The azole antifungal fluconazole (Capsule – FLUCOMED, São Paulo, Brazil) was used as a positive control, it was diluted in sterile distilled water (Morais-Braga et al., 2016).

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

The broth microdilution technique was performed to evaluate the antifungal activity of FOCC, using a 96-well plate (Kasvi). To each well, 90 µL of SDB was added, proceeding with a serial microdilution (1:1 v/v) using FOCC solution or fluconazole, until the penultimate well in numerical order. The concentrations ranged from 1 to 1,024 µg/mL. At the end of the serial microdilution, 10 µL of *Candida* spp. were added to each well. Subsequently, the plates were placed in a microbiological incubator for 24 hours at 37 °C. The readings were performed using the ELISA spectrophotometer model DR-200BS-NM-BI (Kazuaki, Wuxi, China), at a wavelength of 630 nm. In parallel, FOCC and fluconazole dilution controls (0.9% sodium chloride solution instead of the inoculum), and sterility controls were also prepared (Javadpour et al., 1996; Carneiro et al., 2020).

After absorbance readings, the data were used to determine the Minimum Inhibitory Concentration (MIC) and generate the cell survival curve. From this, IC_{50} values of FOCC and fluconazole were calculated. The MIC was established as the concentration that

completely inhibited fungal growth. When there was no MIC, the matrix concentration (2,048 µg/mL) was considered as the starting point for the sub-inhibitory concentrations.

2.4.3. Evaluation of the Fluconazole Modifying Activity

After determining the MIC, the fluconazole modifying activity tests were performed according to Morais-Braga et al. (2017). In 96-well flat-bottomed plates (Kasvi), 90 µL of Sabouraud Dextrose Broth solutions (SDB, Himedia) were added to all wells, containing the FOCC in its sub-inhibitory concentrations (MIC/8), except in the last line that was kept as control of the fungal growth. Subsequently, this solution was microdiluted with fluconazole (1:1 v/v) up to the penultimate row of wells, in concentrations ranging from 1 to 1,024 µg/mL. Finally, *Candida* spp. was added until reaching a concentration of 10% in each well of the plate, using the same method previously mentioned.

2.4.4. Evaluation of the morphological transition inhibition in *Candida* spp.

A volume of 3 mL of Potato Dextrose Agar (PDA) depleted of nutrients solution, containing FOCC at sub-inhibitory concentrations (MIC/2 and MIC/4), was placed onto a sterile microscope slide. After solidification and stabilization of the growth medium on the slide, two parallel streaks of the inoculum (*Candida* spp.) were made. The slides were placed in a humid chamber and kept in a microbiological incubator at 37º C for 24 h. After this period, the slides were observed under an optical microscope (Model L-2000i-BINO, Bioval) at a magnification of 400×. The images captured showed emission or inhibition of filamentous structures in *Candida* spp. strains. Fluconazole was used as a positive control at the same sub-inhibitory concentrations previously mentioned, while the growth control consisted solely of PDA and the fungal strains (Carneiro et al., 2019).

2.4.5. Induction of Reactive Oxygen Species (ROS) Production

To evaluate the mechanisms of action involved in the anti-*Candida* activity of FOCC, was followed the methodology by Maurya et al. (2011). The yeasts were cultured in SDB for 18 hours at 37° C. Subsequently, a volume of 100 µL of *Candida* spp. (10^6 cells/mL) were incubated with 100 µL of FOCC at their IC_{50} concentrations (µg/mL) at 37 °C for 24 hours in the dark. Subsequently, the yeasts from each treatment were incubated with 2,7-dichlorofluorescein diacetate (DCFH-DA) at 37 °C for 30 min in the dark. The yeasts were then centrifuged (Mikro 200R, Hettich, Germany) at 3000 × g for 5 min at 22 °C, and washed

three times with NaCl (0.15M). The samples were properly prepared for observation via Olympus System BX 60 fluorescence microscope (Tokyo, Japan), under excitation and emission wavelengths of 488 and 525 nm, respectively. The same procedure was performed for the negative (150 mM NaCl) and positive (fluconazole) controls.

2.4.6. Evaluation of the Cell Membrane Integrity

Following the methodology by Regente et al. (2014), *Candida* spp. was incubated in SDB at 37 °C for 24 hours in the presence of FOCC at the IC_{50}. Fluconazole was used as the positive control, while 150 mM NaCl was used as the negative control. After the growth, aliquots of 100 µL of the yeast solution treated with FOCC, fluconazole, or NaCl were incubated with 1 mM propidium iodide for 30 min at 37 ºC, under moderate agitation (75 rpm). Finally, they were visualized in a fluorescence microscope (Olympus BX 60 System), under excitation and emission wavelengths of 490 and 520 nm, respectively.

2.5. Toxicological Activity

For the toxicity tests, it was performed the ingestion test using flies of the species *Drosophila melanogaster*. Twenty adult flies (male and female) at 4 days old were used. These flies had been deprived of feed for 6 hours, they were kept under diapause and transferred to 250 mL flasks. These flasks had at the bottom, FOCC in varied concentrations (0.5 – 100 mg/g) mixed with the basal diet of the flies (Bezerra et al., 2017). The flasks were kept in a controlled room under a 12-hour light/dark cycle, at 25 °C and 60% relative humidity. Negative controls and the control of the dilution vehicle of the extract (DMSO) were prepared at a concentration of 10%. After 7 days, mortality readings were performed daily until the end of the experimental period (Bezerra et al., 2017, Costa et al., 2020).

2.6. Statistical Analysis

The assay values are displayed as means (n = 3), with their respective standard errors (± SE). Data were submitted to one-way analysis of variance (ANOVA One-way), using the posthoc Tukey test, at 95% reliability. *P* values were established as < 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). Additionally, the IC_{50} were calculated using non-linear regression analysis. These analyzes were performed using the statistical software GraphPad Prism 6.0 (GraphPad Software, San Diego, CA,

United States). The photomicrographs of the mechanisms of action were analyzed using the point picker tool in ImageJ software version 1.53t (U. S. National Institutes of Health, Bethesda, Maryland, USA).

3 Results

3.1 Chemical composition of the fixed oil from *Caryocar coriaceum*

The fixed oil extracted from the inner mesocarp of *C. coriaceum* exhibited a light-yellow hue, and the fruits emitted a distinctive aroma during the cooking process. In terms of chemical composition, unsaturated fatty acids predominated, comprising 63.3% of in the fixed oil composition, and oleic acid was the main compound constituting 61.0%. Palmitic acid, a saturated fatty acid, was the second most abundant at 33.0%. Together, these two fatty acids accounted for 94.0% of the total composition of the *C. coriaceum* oil. Additionally other fatty acids were identified, but in lower concentrations such as palmitoleic acid, linoleic acid, stearic acid, elaidic acid, and arachidonic acid (Table 1).

Table 1. Composition of the fatty acid methyl esters (FAME) of the fixed oil from the inner mesocarp of *Caryocar coriaceum*.

Fatty acids	**Chemical structure**	**[%]**
Palmitoleic acid (C16:1)	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	*tr*
Palmitic acid (C16:0)	$CH_3(CH_2)_{14}COOH$	33.0
Linoleic acid (C18:2)	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	1.3
Oleic acid (C18:1*cis*)	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	61.0
Stearic acid (C18:0)	$CH_3(CH_2)_{16}COOH$	1.8
Elaidic acid (C18:1*trans*)	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	1.0
Arachidonic acid (C20:0)	$CH_3(CH_2)_{18}COOH$	*tr*
Total saturated		34.8
Total unsaturated		63.3

tr = trace (content less than 1%).

3.2 Antifungal activity

3.2.1 Cell survival curve and median inhibitory concentration (IC_{50})

As observed in Fig. 2, FOCC demonstrated antifungal activity against *C. albicans* and *C. tropicalis* strains at clinically relevant concentrations. The antifungal activity against *C. albicans*, was evident a concentration of 512 µg/mL (Fig. 2a). *C. tropicalis* exhibited sensitivity to FOCC only at the highest concentration evaluated (1024 µg/mL) (Fig. 2c). At this concentration (1024 µg/mL), fungal growth inhibition was 86.3% for both strains. FOCC did not exhibit antifungal activity against *C. krusei* in any of the tested concentrations (Fig. 2b).

Regarding the modifying activity of the antifungal fluconazole, FOCC at sub-inhibitory concentrations, was able to significantly intensify the antifungal action of the fluconazole against *C. albicans* in concentrations of 1 to 64 µg/mL of fluconazole (** = $p < 0.01$, **** = $p < 0.0001$) (Fig. 2a). Against *C. tropicalis* (Fig. 2c), FOCC at sub-inhibitory concentrations enhanced the activity of the fluconazole in concentrations of 8 to 512 of fluconazole (**** = $p < 0.0001$). However, the combination of FOCC with fluconazole was not effective against *C. krusei* (Fig. 2b).

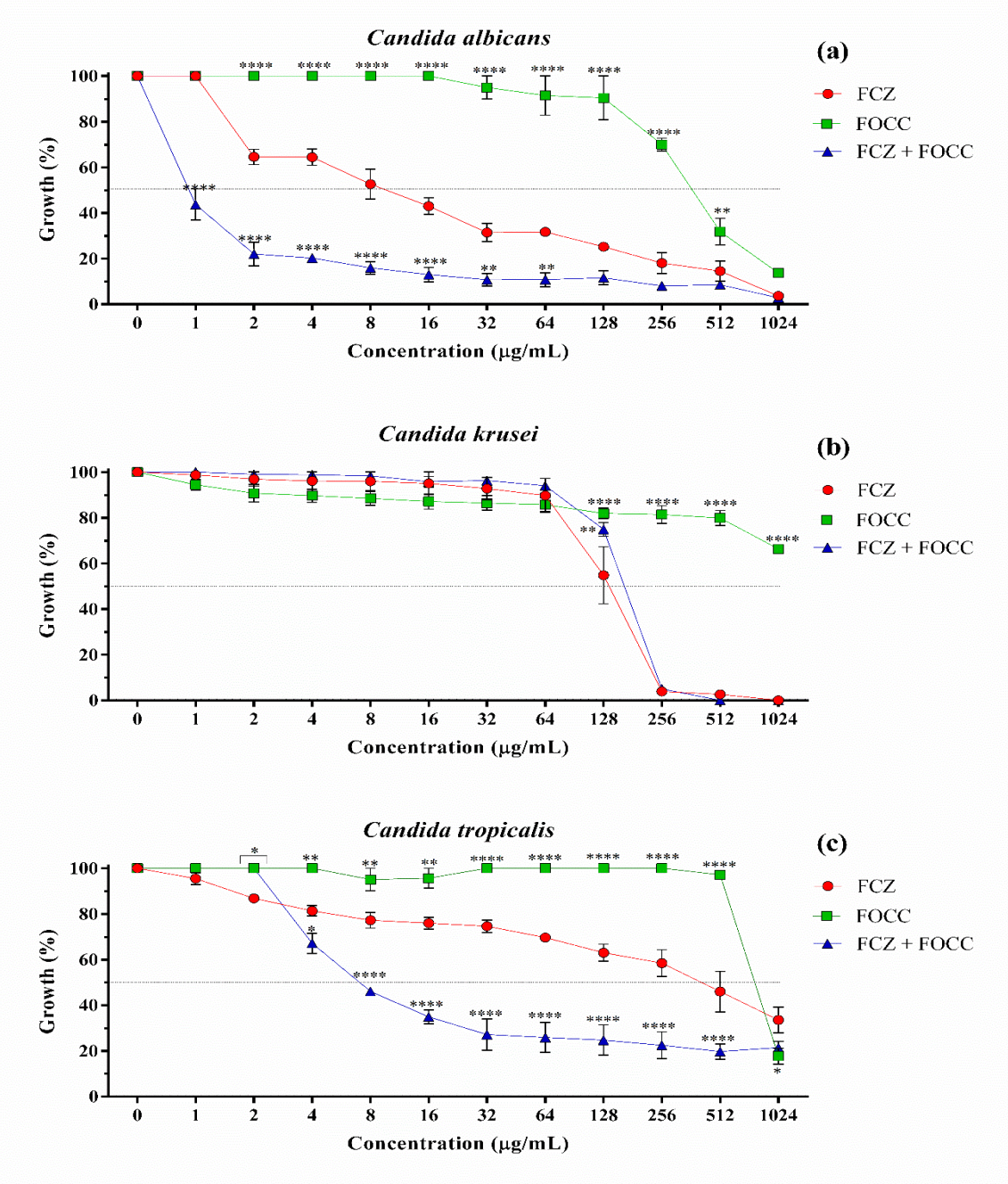

Fig. 2. Cell survival curve and half-maximal inhibitory concentration (IC_{50}) (dotted line) of different concentrations of the fixed oil from the inner mesocarp of *Caryocar coriaceum* (FOCC), fluconazole (FCZ), and their combination (FCZ + FOCC) at sub-inhibitory concentration (MIC/8) against *Candida albicans* (INCQS 90028) (a), *Candida krusei* (INCQS 40095) (b) and *Candida tropicalis* (INCQS 40042) (c). * = $p < 0.05$, ** = $p < 0.01$, **** = $p < 0.0001$. The bars represent the standard error of the mean (n = 3).

The IC_{50} values of FOCC and its combination with fluconazole are displayed in Table 2. FOCC exhibited IC_{50} values of clinical interest solely against *C. albicans* (IC_{50} of 371

µg/mL). Notably, it demonstrated an augmenting effect on the activity of fluconazole against both *C. albicans* and *C. tropicalis*, with IC_{50} values of 0.72 and 7.93 µg/mL, respectively. This represented a remarkable reduction of 98.2% in the IC_{50} of fluconazole when combined with FOCC against *C. albicans*. Additionally, in the case of *C. tropicalis*, the fixed oil intensified the activity of fluconazole by 96.24%.

Table 2. Values of the half-maximal inhibitory concentration (IC_{50}) (µg/mL) of the fixed oil from the inner mesocarp of *Caryocar coriaceum* (FOCC), fluconazole (FCZ), and the combination (FCZ + FOCC), against strains of *Candida albicans* (INCQS 90028), *Candida krusei* (INCQS 40095) and *Candida tropicalis* (INCQS 40042).

Treatment	***Candida albicans***	***Candida krusei***	***Candida tropicalis***
FOCC	371	>1024	830
FCZ	12.33	131.6	362
FCZ + FOCC	0.72	155	7.93

3.2.2 Inhibition of Fungal Virulence

In addition to its antifungal activity against *C. albicans* and *C. tropicalis* strains, FOCC at a concentration of 512 µg/mL demonstrated the ability to inhibit the morphological transition of these yeasts into their filamentous forms (Fig. 3). Regarding *C. krusei*, both FOCC and ineffective in reducing the virulence of this strain. Although FOCC at a concentration of 256 µg/mL did not completely inhibit virulence, it did lead to a reduction in the quantity and size of hyphae and pseudohyphae compared to the control group.

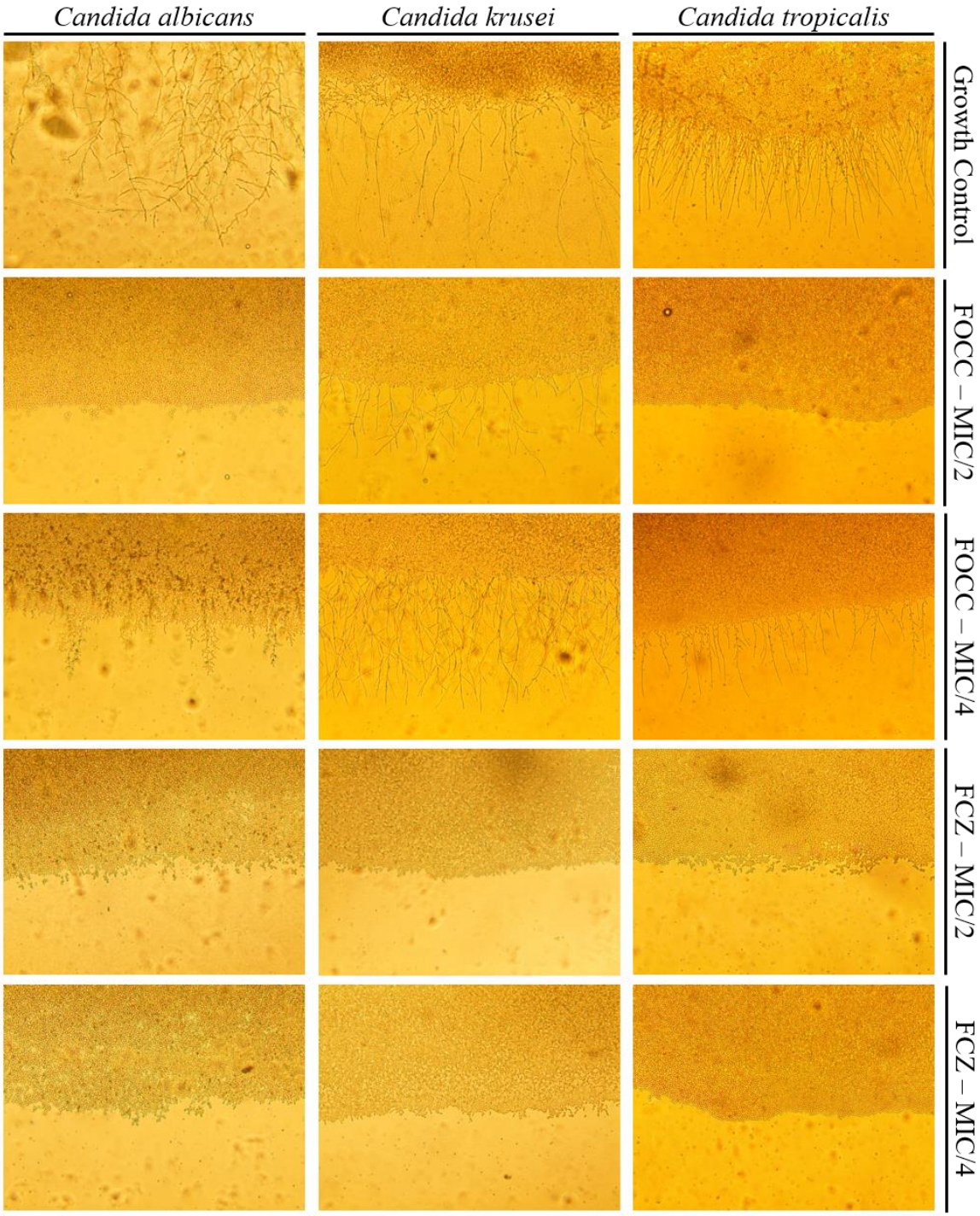

Fig. 3: Effects of the fixed oil from the inner mesocarp of *Caryocar coriaceum* (FOCC) on the dimorphism of *Candida albicans* INCQS 90028, *Candida krusei* INCQS 90095 and *Candida tropicalis* INCQS 90042. 400× magnification. MIC/2: 512 µg/mL and MIC/4: 256 µg/mL.

3.2.3 Mechanisms of Action

According to Fig. 4, the antifungal activity of FOCC against *C. albicans* strains is attributed to the formation of Reactive Oxygen Species (ROS), as evidenced by the intense fluorescence observed in yeast micrographs (Fig. 4A – left and 4B). Interestingly, when analyzing the formation of pores in the membranes of *C. albicans* yeasts, FOCC did not show significant differences compared to the negative control (NaCl 150 mM) (Fig. 4A - right and 4C). In line with the cell survival assays, FOCC did not demonstrate activity against *C. krusei* strains, as it did not induce the formation of ROS and pores in the membrane at a concentration of 1024 µg/mL (Fig. 5).

The strains of *C. tropicalis* displayed the highest susceptibility to FOCC, as demonstrated in Fig. 6, based on the observed formation of ROS and pores in the yeast membrane. In the initial mechanism, FOCC exhibited the same percentage of fluorescence as the standard drug.

Notably, there was a remarkable susceptibility of *C. tropicalis* strains to FOCC, as evident from the significant formation of Reactive Oxygen Species (ROS) in the micrographs (Fig. 6A (Left) and 6B). FOOC showed a fluorescence percentage comparable to the standard drug that was tested. This indicates that FOCC may share similar pathways of action with the standard drug, at least in terms of ROS production and its association with antifungal activity. Addition to these mechanisms of action, FOCC induced the formation of pores in the membrane of *C. tropicalis* yeasts (Fig. 6A – Left and 6C).

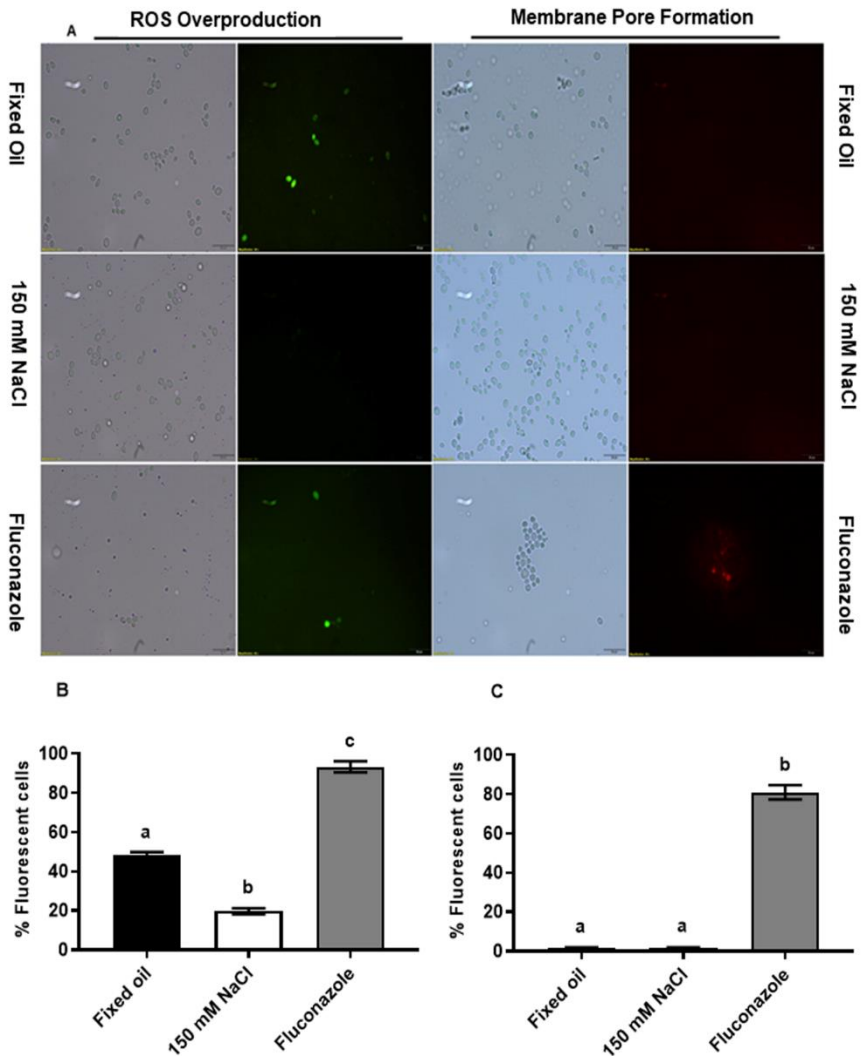

Fig. 4. Effect of the fixed oil from the inner mesocarp of *Caryocar coriaceum* against *Candida albicans* (INCQS 90028). (A - Left) Reactive Oxygen Species. (A - Right) Cell membrane permeabilization. (B) Percentage of fungal cells labeled with 2',7'-dichlorofluorescein. (C) Percentage of fungal cells stained with propidium iodide. Different letters represent statistical differences between means ($p < 0.05$). Results are displayed as mean ± standard deviation.

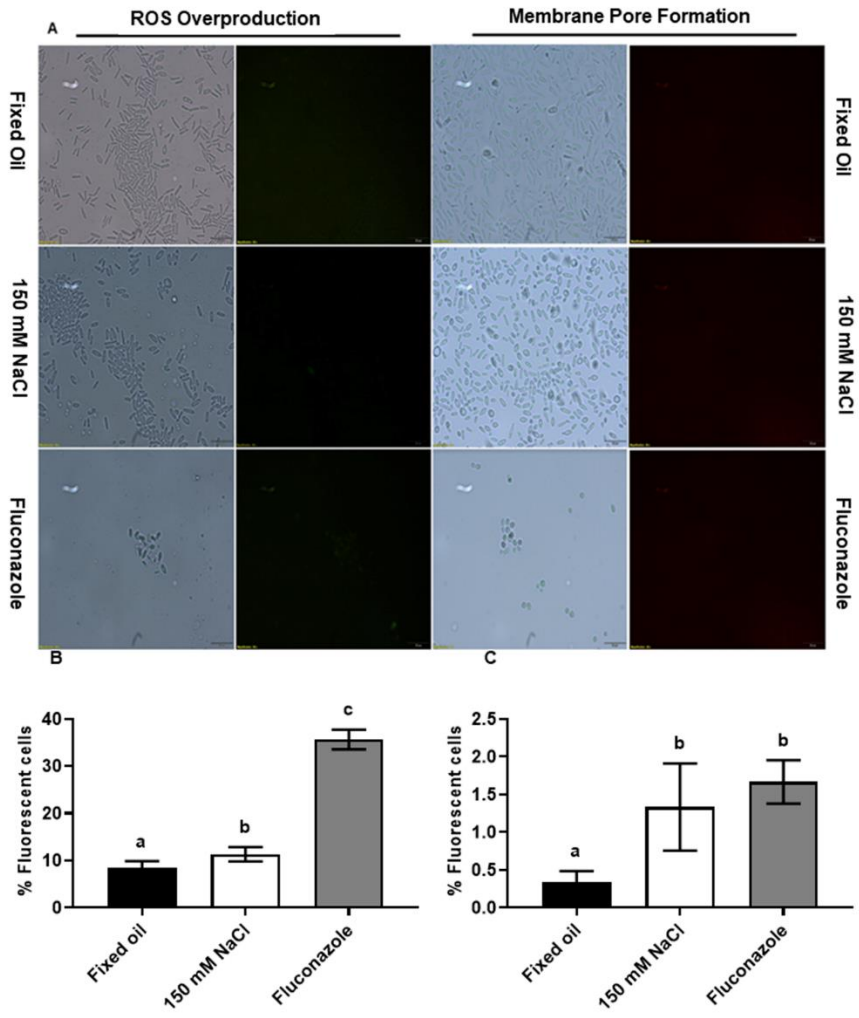

Fig. 5. Effect of the fixed oil from the inner mesocarp of *Caryocar coriaceum* against *Candida krusei* (INCQS 90028). (A - Left) Reactive Oxygen Species. (A - Right) Cell membrane permeabilization. (B) Percentage of fungal cells labeled with 2',7'-dichlorofluorescein. (C) Percentage of fungal cells stained with propidium iodide. Different letters represent statistical differences between means ($p < 0.05$). Results are displayed as mean ± standard deviation.

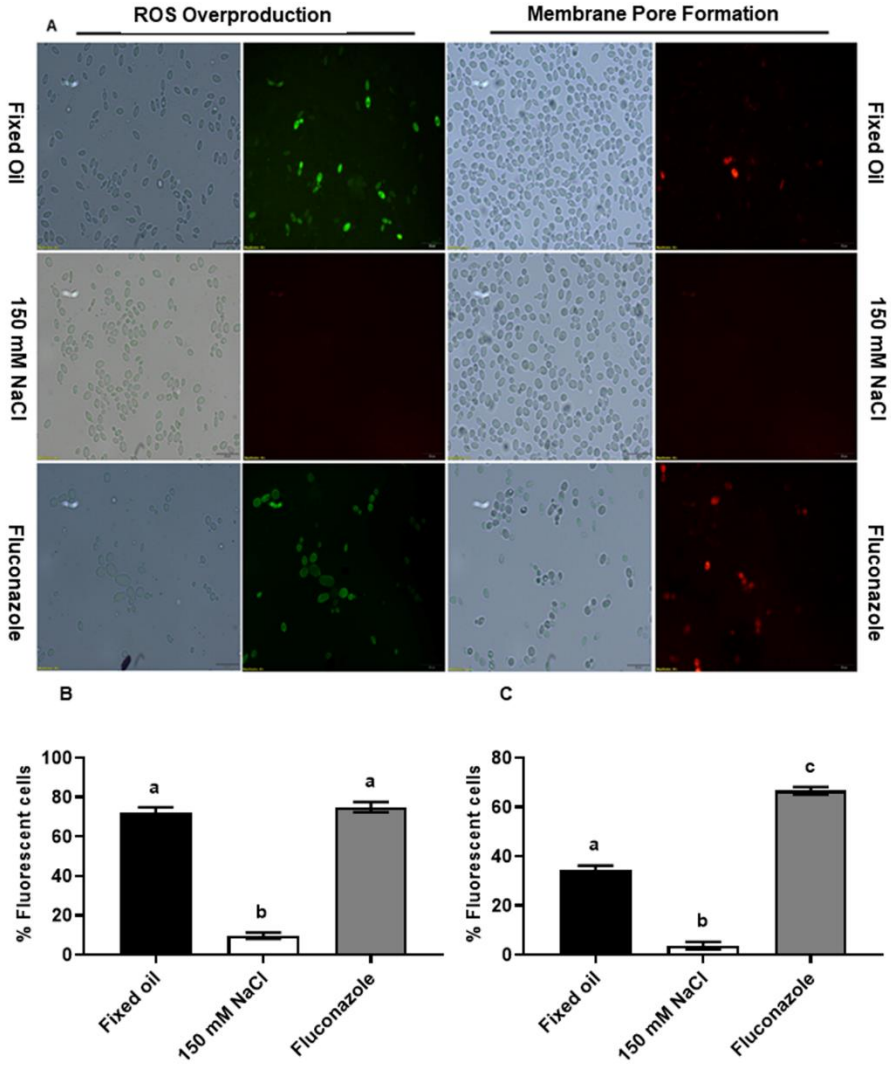

Fig. 6. Effect of the fixed oil from the inner mesocarp of *Caryocar coriaceum* against *Candida tropicalis* (INCQS 90028). (A - Left) Reactive Oxygen Species. (A - Right) Cell membrane permeabilization. (B) Percentage of fungal cells labeled with 2',7'-dichlorofluorescein. (C) Percentage of fungal cells stained with propidium iodide. Different letters represent statistical differences between means ($p < 0.05$). Results are displayed as mean ± standard deviation.

3.3 Toxicity

In the toxicity assay using *D. melanogaster*, FOCC did not demonstrate toxicity through ingestion, even at clinically relevant concentrations (0.5 mg/g), indicating its safety at low concentrations (Fig. 7). However, it exhibited dose-dependent toxicity. At 1 mg/g, FOCC result in a 35% mortality rate on the fifth day of the experiment. The concentration of 10 mg/g showed toxicity from the 3rd day of exposure to the natural product. The highest

concentration tested (100 mg/g) showed toxic effects on the first day of exposure, with a 25% mortality rate, which increased to 78.3% by the end of the test.

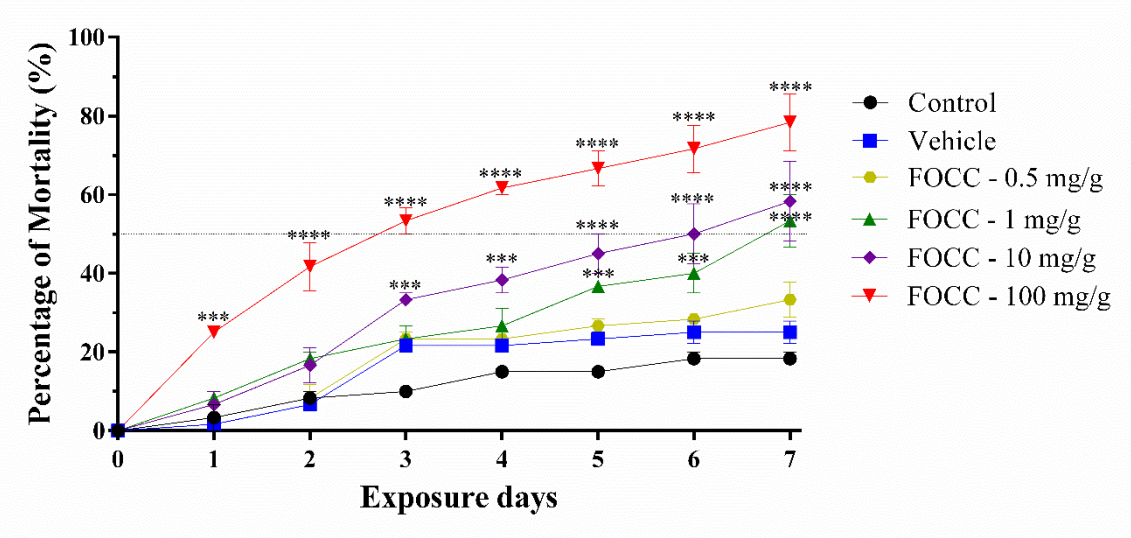

Fig. 7. Toxicity of the fixed oil from the inner mesocarp of *Caryocar coriaceum* (FOCC) at different concentrations ingested by *Drosophila melanogaster*. *** = $p < 0.001$ **** = $p < 0.0001$. The bars represent the standard error of the mean (n = 3).

4. Discussion

The versatile applications of the fixed oil derived from the fruits of *C. coriaceum* have prompted numerous researchers to explore its chemical composition and therapeutic potential (Oliveira et al., 2010; Costa et al., 2011; Figueiredo et al., 2016; Almeida-Bezerra et al., 2022). The phytochemical profile of the fruit oil, specifically from the inner mesocarp of *C. coriaceum*, has been extensively documented, as outlined in the review by Almeida-Bezerra (2022). Notably, the inner mesocarp, predominantly contains unsaturated fatty acids (> 60%), particularly C18:1, followed by C16:0, a saturated fatty acid (Costa et al., 2011; Figueiredo et al., 2016; Borges et al., 2022). Our findings align with therefore, corroborate these studies. The prevalence of C18:1 and C16:0 has also been reported in for the inner mesocarp and pulp of other *Caryocar* species like *C. brasiliense* Cambess. (Araujo, 1995), and *C. villosum* (Aubl.) Pers. (Ascari et al., 2013).

Fixed oils present a promising alternative in combating fungal infections, including candidiasis (Sampaio et al., 2023; Kumar et al., 2020). Our results illustrate that the fixed oil extracted from the internal mesocarp of *C. coriaceum* exhibits anti-*Candida* activity, along with the ability to inhibit the formation of filamentous structures. This activity is likely attributable to the synergism effects of its major chemical constituents (oleic acid and palmitic

acid). These isolated compounds have been reported to possess antifungal properties against *C. albicans* and *C. tropicalis* (Muthamil et al., 2020; Prasath et al., 2020).

In this study, the observed effects of FOCC on the reduction of *C. albicans* and *C. tropicalis* growth may be attributed to the high content of oleic and palmitic acids in the oil. According to Muthamil et al. (2020), oleic acid deactivates proteins involved in glucose metabolism, ergosterol biosynthesis, lipase production, iron homeostasis, and amino acid biosynthesis, thus inhibiting biofilm formation and *Candida* spp. virulence. Additionally, oleic acid can induce oxidative stress in various strains of *Candida* spp., a finding supported by our results, which demonstrated that FOCC led to the formation of ROS in *C. albicans* and *C. tropicalis* strains, in line with the studies by Muthamil et al. (2020). Prasath et al. (2020), suggest that palmitic acid (C16:0), the second major fatty acid in *C. coriaceum* oil, effectively inhibits biofilm formation in *C. tropicalis*. Moreover, it triggers apoptosis through ROS-mediated mitochondrial dysfunction. The acid palmitic also regulates other virulence factors, such as cell surface hydrophobicity, ergosterol biosynthesis, protease, and lipase. While we did not investigate all the different mechanisms of action of FOCC, our results align with the findings regarding the antifungal activity of the major fatty acids found in *C. coriaceum* oil.

Other oleaginous medicinal species native to Chapada do Araripe, such as *Acrocomia aculeata* (Jacq.) Lodd stands out. ex Mart. (Arecaceae) and *Syagrus cearensis* Noblick (Arecaceae), have demonstrated biological effects against *Candida* species. Theses species, with lauric acid (C12:0) as the major compound, showed the ability to reduce the growth of *C. albicans*, *C. krusei* and *C. tropicalis* (Sampaio et al., 2023).

The anti-*Candida* activity of FOCC observed in this study was likely due to the overproduction of ROS, as demonstrated against *C. albicans* and *C. tropicalis* strains. These reactive species act within the cells of *Candida* spp. causing molecular level alterations. Consequently, they exert detrimental effects on proteins, nucleic acids (DNA and RNA), and lipids, compromising the yeast's integrity. Moreover, when ROS levels significantly increase, yeast may undergo oxidative stress leading to apoptosis (Yu et al., 2016). FOCC also induced the formation of pores in the membrane of *C. tropicalis*, resulting in an electrolyte imbalance in the intracellular environment. This process causes the yeast to lose its cytoplasmic content, ultimately leading to its demise (Lima et al., 2020).

Our results also demonstrated that FOCC intensified the action of fluconazole against *C. albicans* and *C. tropicalis*, effectively reversing drug resistance in these strains. The combination with FOCC reduced the required dose for antifungal action of fluconazole. This synergism effect may be attributed to the lipophilic characteristics of the fatty acids present in

the oil. Hydrophobic compounds can interact with the fungal membrane, facilitating drug entry, causing membrane damage, and interrupting the activity of the efflux pumps (Jamiu et al., 2021). Consequently, the antifungal results of the combination between FOCC and fluconazole are promising, especially given the need for therapeutics against resistant pathogens (Lu et al., 2017). According to Pierce & Lopez-Ribot (2013), the pathogenicity of *C. albicans* is multifactorial. Our results demonstrated that the combination of FOCC and fluconazole was effective against this yeast, and also against *C. tropicalis*.

Additionally, FOCC, at clinically relevant concentrations, was observed to inhibit and reduce the morphological transition of *C. albicans* and *C. tropicalis*. Inhibiting these virulence factors is crucial, as filamentous forms of *Candida* spp. can invade host tissues and evade macrophage action. According to Bu et al. (2022), the isolated or synergistic action of chemical constituents, inhibits the expression of genes responsible for morphological transition. For instance, oleic acid formation in *C. albicans* by inhibiting the expression of hyphal elongation and filamentation genes (Muthamil et al., 2020). The FOCC strongly inhibited or reduce the morphological transition of *C. albicans* and *C. tropicalis*, underscoring the potent antifungal properties of fatty acids. Practically, inhibiting filamentous forms enables the host's immune system to combat fungal infection (Soliman et al., 2017).

The discovery of products with antifungal effects poses a significant challenge in new drug development given that fungi are eukaryotic organisms, similar to human cells (Pierce and Lopez-Ribot, 2013; Simon and Bedalov, 2004). In our study, we demonstrated that FOCC at clinically relevant concentrations (below 1000 µg/mL), did not induce *in vivo* toxicity in *D. melanogaster*. The lack of toxicity of *C. coriaceum* oil at clinically relevant concentrations was also corroborated by Silva et al. (2022), using macrophages (lineage J774G8). These authors reported that 150 mg of the nano-encapsulated oil did not cause skin irritation, highlighting the product's safety for topical use. Considering that the anti-*Candida* activity and antivirulence effects of FOCC occurred at clinically relevant concentrations (Houghton et al., 2007), and without showing any toxicity, the potential use of FOCC as an antifungal is promising, particularly the context of new pharmaceutical drug development. Nevertheless, additional preclinical studies must be conducted to evaluate the pharmacokinetics and pharmacodynamics of FOCC. These are some of the requirements before launching a new pharmaceutical product (Bajaj et al., 2012).

Conclusion

The fixed oil of *Caryocar coriaceum* is rich in oleic acid and palmitic. This oil is commonly used by the population of the Chapada do Araripe to treat diseases of the genitourinary system, for example, candidiasis. The findings of this study support the anti-*Candida* potential of the oil. It was evidenced that one of the factors involved in the antifungal activity of this oil is the overproduction of reactive oxygen species, which can induce apoptosis in yeast cells. The fixed oil of *C. coriaceum* was able to inhibit or reduce the morphological transition of *C. albicans* and *C. tropicalis*, an important virulence factor, consequently affecting the potential for tissue invasion by these strains. The oil also intensified the action of fluconazole, reversing the antibiotic resistance by *C. albicans* and *C. tropicalis* strains, turning them more sensitive to the drug. Finally, it was demonstrated that the fixed oil had no toxic effect at clinically relevant concentrations against *D. melanogaster*, which was used as the biological model in the study. Therefore, *C. coriaceum* oil needs to be further investigated regarding its pharmacokinetics and pharmacodynamics. This can help in the development of new pharmaceutical products against the fungal strains evaluated in the present study.

References

Adams, R.P., 2017. Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream, IL, USA: Allured Publishing Corporation.

Agra, M. F., Silva, K.N., Basílio, I.J.L.D., Freitas, P.F., Barbosa-Filho, J.M., 2008. Survey of medicinal plants used in the region Northeast of Brazil. Rev. Bras. Farmacogn.18, 472–508. https://doi.org/10.1590/S0102-695X2008000300023

Agra, M.F., Freitas, P.F., Barbosa-Filho, J.M., 2017. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. Rev. Bras. Farmacogn. 17, 114–140. https://doi.org/10.1590/S0102-695X2007000100021

Ahmad, A., Khan, A., Manzoor, N., Khan, L.A., 2010. Evolution of ergosterol biosynthesis inhibitors as fungicidal against *Candida*. Microb. Pathog. 48(1), 35-41. https://doi.org/10.1016/j.micpath.2009.10.001

Al Ashaal, H. A., Farghaly, A.A., Abd El Aziz, M.M., Ali, M.A., 2010. Phytochemical investigation and medicinal evaluation of fixed oil of *Balanites aegyptiaca* fruits (Balantiaceae). J. Ethnopharmacol. 127(2), 495-501. https://doi.org/10.1016/j.jep.2009.10.007

Almeida-Bezerra, J. W., Bezerra, J. J. L., Silva, V. B. D., Coutinho, H. D. M., Costa, J. G. M. D., Cruz-Martins, N., Hano, C., Menezes, S. A., Morais-Braga, M.F.B., Oliveira, A. F. M., 2022. *Caryocar coriaceum* Wittm.(Caryocaraceae): Botany, Ethnomedicinal Uses, Biological Activities, Phytochemistry, Extractivism and Conservation Needs. Plants. 11(13), 1685. https://doi.org/10.3390/plants11131685

Araujo, F. D., 1995. A review of *Caryocar brasiliense* (Caryocaraceae): an economically valuable species of the central Brazilian cerrados. Econ. Bot. 40-48.

Ascari, J., Takahashi, J. A., Boaventura, M.A.D., 2013. The phytochemistry and biological aspects of Caryocaraceae family. Rev. Bras. Plantas Med. 15, 293-308. https://doi.org/10.1590/S1516-05722013000200019

Bajaj, S., Singla, D., Sakhuja, N., 2012. Stability testing of pharmaceutical products. J. Appl. Pharm. Sci. 129-138. https://doi.org/10.7324/JAPS.2012.2322

Berman, J., Krysan, D. J., 2020. Drug resistance and tolerance in fungi. Nat. Rev. Microb. 18(6), 319-331. https://doi.org/10.1038/s41579-019-0322-2

Bezerra, J.W.A., Costa, A.R., da Silva, M.A.P., Rocha, M.I., Boligon, A.A., da Rocha, J.B.T., Barros, L.M., Kamdem, J. P., 2017. Chemical composition and toxicological evaluation of *Hyptis suaveolens* (L.) Poiteau (LAMIACEAE) in *Drosophila melanogaster* and *Artemia salina*. S. Afr. J. Bot. 113, 437-442. https://doi.org/10.1016/j.sajb.2017.10.003

Bezerra, J.W.A., Costa, A.R., de Freitas, M.A., Rodrigues, F.C., de Souza, M.A., da Silva, A.R. P., dos Santos, A.T.L., Linhares, K.V.A., Coutinho, H.D.M., Silva, J.R.L., Morais-Braga, M.F.B., 2019. Chemical composition, antimicrobial, modulator and antioxidant activity of essential oil of *Dysphania ambrosioides* (L.) Mosyakin & Clemants. Comp. Immunol. Microbiol. Infect. Dis., 65, 58-64. https://doi.org/10.1016/j.cimid.2019.04.010

Bhattacharya, S., Sae-Tia, S., Fries, B.C., 2020. Candidiasis and mechanisms of antifungal resistance. Antibiot. 9(6), 312. https://doi.org/10.3390/antibiotics9060312

Borges, O.M.A., Araújo, Í.M.S., Canuto, K.M., Carvalho, J.D.G., Magalhães, H.C.R., Rodrigues, T.H.S., Carioca, J.O.B., Gaban, S.V.F., 2022. Pequi pulp oil: Effect on the physicochemical, nutritional, and textural properties of cottage cheese. Food Sci. Technol. 42, e37221. https://doi.org/10.1590/fst.37221

Bu, Q. R., Bao, M.Y., Yang, Y., Wang, T.M., Wang, C.Z., 2022. Targeting Virulence Factors of *Candida albicans* with Natural Products. Food. 11(19), 2951. https://doi.org/10.3390/foods11192951

Carneiro, J.N.P., da Cruz, R.P., Campina, F.F., Costa, M.S., dos Santos, A.T.L., Sales, D.B., Bezerra, C.F., da Silva, L.E., de Araujo, J.P., do Amaral, W., Rebelo, R.A., Begnini, I.M., de Lima, L.F., Coutinho, H.D.M., Morais-Braga, M.F.B., 2020. GC/MS analysis and antimicrobial activity of the *Piper mikanianum* (Kunth) Steud. essential oil. Food Chem. Toxicol. 135(10), 1–8. https://doi.org/10.1016/j.fct.2019.110987

Carneiro, J.N.P., da Cruz, R.P., da Silva, J.CP., Rocha, J.E., Sales, D.B., Bezerra, C.F., Almeida, W.O., da Costa, J.G.M., da Silva, L.E., do Amaral, W., Rebelo, R.A., Begnini, I.M., Coutinho, H.D.M., Morais-Braga, M.F.B.,. *Piper diospyrifolium* Kunth.: Chemical analysis and antimicrobial (intrinsic and combined) activities. Microb. Pathog. 136(8), 1–9, 2019. https://doi.org/10.1016/j.micpath.2019.103700

Ciurea, C.N., Kosovski, I.B., Mare, A.D., Toma, F., Pintea-Simon, I.A., Man, A., 2020. Candida and candidiasis—opportunism versus pathogenicity: a review of the virulence traits. Microorganisms. 8(6), 857. https://doi.org/10.3390/microorganisms8060857

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

Costa, J.G.; Brito, S.A.; Nascimento, E.M.; Botelho, M.A.; Rodrigues, F.F.; Coutinho, H.D.M., 2011. Antibacterial properties of pequi Pulp oil (*Caryocar coriaceum*–Wittm.). Int. J. Food Prop. 14, 411–416. https://doi.org/10.1080/10942910903207744

Eix, E.F., Nett, J.E., 2020. How biofilm growth affects Candida-host interactions. Front. Microb. 11, 1437. https://doi.org/10.3389/fmicb.2020.01437

Figueiredo, P.R.L., Oliveira, I.B., Neto, J.B.S., de Oliveira, J.A., Ribeiro, L.B., de Barros Viana, G.S., Rocha, T.M., Leal, L.K.A.M., Kerntopf, M.R., Felipe, C.F.B., Coutinho, H.D.M., Menezes, I. R.A., 2016. *Caryocar coriaceum* Wittm.(Pequi) fixed oil presents hypolipemic and anti-inflammatory effects *in vivo* and *in vitro*. J. ethnopharmacol. 191, 87-94. https://doi.org/10.1016/j.jep.2016.06.038

Geris, R., Santos, N.A.C.D., Amaral, B.A., Maia, I.D.S., Castro, V.D., Carvalho, J.R.M., 2007. Biodiesel de soja: reação de transesterificação para aulas práticas de química orgânica. Quím. Nov. 30, 1369-1373. https://doi.org/10.1590/S0100-40422007000500053

Gupta, P.D., Birdi, T.J., 2017. Development of botanicals to combat antibiotic resistance. J. Ayurveda Integr. Med. 8(4), 266-275. https://doi.org/10.1016/j.jaim.2017.05.004

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

Jamiu, A.T., Albertyn, J., Sebolai, O., Gcilitshana, O., Pohl, C.H., 2021. Inhibitory effect of polyunsaturated fatty acids alone or in combination with fluconazole on *Candida krusei* biofilms *in vitro* and in *Caenorhabditis elegans*. Med. Mycol. 59(12), 1225-1237. https://doi.org/10.1093/mmy/myab055

Javadpour, M.M., Juban, M.M., Lo, W.C.J., Bishop, S.M., Alberty, J.B., Cowell, S.M., Becker, K.L., McLaughlin, M.L., 1996. De novo antimicrobial peptides with low mammalian cell toxicity. J. Med. Chem. 39(16), 3107–3113. https://doi.org/10.1021/jm9509410

Khan, F., Bamunuarachchi, N.I., Tabassum, N., Jo, D.M., Khan, M.M., Kim, Y.M., 2021. Suppression of hyphal formation and virulence of *Candida albicans* by natural and synthetic compounds. Biofouling. 37(6), 626-655. https://doi.org/10.1080/08927014.2021.1948538

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

Kumar, P., Lee, J.H., Beyenal, H., Lee, J., 2020. Fatty acids as antibiofilm and antivirulence agents. Trends Microbiol. 28(9), 753-768. https://doi.org/10.1016/j.tim.2020.03.014

Lee, Y., Puumala, E., Robbins, N., Cowen, L.E., 2020. Antifungal drug resistance: molecular mechanisms in *Candida albicans* and beyond. Chem. Rev. 121(6), 3390-3411. https://doi.org/10.1021/acs.chemrev.0c00199

Lemos, I.; Delmondes, G.; Santos, A.; Caprara, A.; Barbosa, R.; Menezes, I.R.; Coutinho, H.; Kerntopf, R.; Fernandes, G., 2016. Ethnobiological survey of plants and animals used for the treatment of acute respiratory infections in children of a traditional community in the municipality of Barbalha, Ceará, Brazil. Afr. J. Tradit. Complement. Altern. Med. 13, 166–175. https://doi.org/10.21010/ajtcam.v13i4.22

Lima, P.G., Souza, P.F., Freitas, C.D., Oliveira, J.T., Dias, L.P., Neto, J.X., Vasconcelos, I.M., Lopes, J.L.S., Sousa, D.O.B., 2020. Anticandidal activity of synthetic peptides: Mechanism of action revealed by scanning electron and fluorescence microscopies and synergism effect with nystatin. J. Pep. Sci. 26(6), 18. https://doi.org/10.1002/psc.3249

Lu, M., Li, T., Wan, J., Li, X., Yuan, L., Sun, S., 2017. Antifungal effects of phytocompounds on Candida species alone and in combination with fluconazole. I. J. Antimicrob. Agents. 49(2), 125-136. https://doi.org/10.1016/j.ijantimicag.2016.10.021

Magalhães, K.N., Guarniz, W.A.S., Sá, K.M., Freire, A.B., Monteiro, M.P., Nojosa, R.T., Bieski, I.G.C., Custódio, J.B., Balogun, S.O., Bandeira, M.A.M., 2019. Medicinal plants of the Caatinga, northeastern Brazil: Ethnopharmacopeia (1980–1990) of the late professor Francisco José de Abreu Matos. J. Ethnopharmacol. 237(8), 314–353. https://doi.org/10.1016/j.jep.2019.03.032

Maurya, I.K., Pathak, S., Sharma, M., Sanwal, H., Chaudhary, P., Tupe, S., Deshpande, M., Chauhan, V.S., Prasad, R., 2011. Antifungal activity of novel synthetic peptides by accumulation of reactive oxygen species (ROS) and disruption of cell wall against *Candida albicans*. Peptides. 32(8), 1732–1740. https://doi.org/10.1016/j.peptides.2011.06.003

Morais-Braga, M.F., Carneiro, J.N., Machado, A.J., Sales, D.L., Dos Santos, A.T., Boligon, A.A., Athayde, M.L., Menezes, I.R.A., Souza, D.S.L., Costa, J.G.M., Coutinho, H.D.M., 2017. Phenolic composition and medicinal usage of *Psidium guajava* Linn .: Antifungal activity or inhibition of virulence ? Saudi J. Biol. Sci., 24(2), 302–313. https://doi.org/10.1016/j.sjbs.2015.09.028

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

Muthamil, S., Prasath, K.G., Priya, A., Precilla, P., Pandian, S.K., 2020. Global proteomic analysis deciphers the mechanism of action of plant derived oleic acid against *Candida albicans* virulence and biofilm formation. Sci. Rep. 10(1), 1-17. https://doi.org/10.1038/s41598-020-61918-y

Nicola, A.M., Albuquerque, P., Paes, H.C., Fernandes, L., Costa, F.F., Kioshima, E.S., Abadio, A.K.R., Bocca, A.L., Felipe, M.S., 2019. Antifungal drugs: New insights in research & development. Pharmacol. Therapeut. 195, 21-38. https://doi.org/10.1016/j.pharmthera.2018.10.008

Oliveira, M.L.M., Nunes-Pinheiro, D.C.S., Tomé, A.R., Mota, E.F., Lima-Verde, I.A., de Melo Pinheiro, F.G., Campello, C.C., de Morais, S.M., 2010. *In vivo* topical anti-inflammatory and wound healing activities of the fixed oil of *Caryocar coriaceum* Wittm. seeds. J. Ethnopharmacol. 129(2), 214-219. https://doi.org/10.1016/j.jep.2010.03.014

Onyewu, C., Blankenship, J.R., Del Poeta, M., Heitman, J., 2003. Ergosterol biosynthesis inhibitors become fungicidal when combined with calcineurin inhibitors against *Candida albicans*, *Candida glabrata*, and *Candida krusei*. Antimicrob. Agents. Chemother. 47(3), 956-964. https://doi.org/10.1128/aac.47.3.956-964.2003

Pfaller, M.A., Castanheira, M., 2016. Nosocomial candidiasis: antifungal stewardship and the importance of rapid diagnosis. Med. Mycol. 54(1), 1-22. https://doi.org/10.1093/mmy/myv076

Pierce, C.G., Lopez-Ribot, J.L., 2013. Candidiasis drug discovery and development: new approaches targeting virulence for discovering and identifying new drugs. Expert. Opin. Drug. Discov. 8(9), 1117-1126. https://doi.org/10.1517/17460441.2013.807245

Prasath, K.G., Tharani, H., Kumar, M.S., Pandian, S.K., 2020. Palmitic acid inhibits the virulence factors of *Candida tropicalis*: Biofilms, cell surface hydrophobicity, ergosterol biosynthesis, and enzymatic activity. Front. Microbiol. 11, 864. https://doi.org/10.3389/fmicb.2020.00864

Pristov, K.E., Ghannoum, M.A., 2019. Resistance of *Candida* to azoles and echinocandins worldwide. Clin. Microbiol. Infect. 25(7), 792-798. https://doi.org/10.1016/j.cmi.2019.03.028

Roemer, T., Krysan, D.J., 2014. Antifungal drug development: challenges, unmet clinical needs, and new approaches. Cold Spring Harb. Perspect. Med. 4(5), a019703. https://doi.org/10.1101/cshperspect.a019703

Romo, J. A., Kumamoto, C.A., 2020. On commensalism of *Candida*. J. Fungi, 6(1), 16. https://doi.org/10.3390/jof6010016

Rosiana, S., Zhang, L., Kim, G.H., Revtovich, A.V., Uthayakumar, D., Sukumaran, A., Geddes-McAlister, J., Kirienko, N.V., Shapiro, R.S., 2021. Comprehensive genetic analysis

of adhesin proteins and their role in virulence of *Candida albicans*. Genet. 217(2), iyab003. https://doi.org/10.1093/genetics/iyab003

Sampaio, R.S.L., Pereira, R.L.S., Coutinho, H.D.M., Almeida-Bezerra, J.W., Morais-Braga, M.F. B., dos Santos Santana, M., Silva, M.E.P., Santos, A.T.L., Fonseca, V.J.A., Costa, A.R., Silva,V.B., Rodrigues, F.C., Bezerra, J.J.L., Raposo, A., Lima, J.P.M., Barros, L. M., 2023. Chemical composition and antimicrobial potential of *Acrocomia aculeata* (Jacq.) Lodd. ex Mart. and *Syagrus cearensis* Noblick (Arecaceae). Microb. Pathog. 106147. https://doi.org/10.1016/j.micpath.2023.106147

Silva, R.F., Barreto, A.S., Trindade, G.D.G.G., Lima, C.M., de Souza Araújo, A. A., Menezes, I. R.lA., Candido, E.A.F., Santana, E.T.N., Silva-Júnior, W.M., Quintans, J.S.S., Coutinho, H.D.M., Kim, B., Quintans-Júnior, L.J., 2022. Enhancement of the functionality of women with knee osteoarthritis by a gel formulation with *Caryocar coriaceum* Wittm ("Pequi") nanoencapsulated pulp fixed oil. Biomed. Pharmacother. 150, 112938. https://doi.org/10.1016/j.biopha.2022.112938

Simon, J.A.; Bedalov, A., 2004. Yeast as a model system for anticancer drug discovery. Nat. Rev. Cancer. 4(6), 481–487. https://doi.org/10.1038/nrc1372

Soliman, S., Alnajdy, D., El-Keblawy, A.A., Mosa, K. A., Khoder, G., Noreddin, A.M., 2017. Plants' natural products as alternative promising anti-Candida drugs. Pharmacogn. Rev. 11(22), 104. https://doi.org/10.4103/phrev.phrev_8_17

Spampinato, C., Leonardi, D., 2013. *Candida* infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. Biomed. Res. Int. 2013. https://doi.org/10.1155/2013/204237

Staniszewska, M., 2020. Virulence factors in *Candida* species. Curr. Protein Pept. Sci. 21(3), 313-323. https://doi.org/10.2174/1389203720666190722152415

Talapko, J., Juzbašić, M., Matijević, T., Pustijanac, E., Bekić, S., Kotris, I., Škrlec, I., 2021. *Candida albicans*—the virulence factors and clinical manifestations of infection. J. Fungi. 7(2), 79. https://doi.org/10.3390/jof7020079

Viana, E.S., de Oliveira Alves, J.V., da Silva Aguiar, I.F., da Silva, F.H.S., da Silva, R.L., de Arruda, L.G., Barbosa, M.F.S., Barbasa, B.V.D.R., de Amorim, L.C., da Silva, P.M., da Silva, M.V., 2022. Atividade antioxidante, caracterização físico-química e estudo da bioatividade do óleo fixo de Attalea speciosa Mart. ex Spreng (Arecaceae) contra agentes patogénicos

fúngicos. Res., Soc. Dev. 11(7), e37311730307-e37311730307. https://doi.org/10.33448/rsd-v11i7.30307

Vila, T., Sultan, A. S., Montelongo-Jauregui, D., & Jabra-Rizk, M.A., 2020. Oral candidiasis: a disease of opportunity. J. Fungi. 6(1), 15. https://doi.org/10.3390/jof6010015

Wikler, M.A., Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard. In: CLSI (NCCLS). [s.l: s.n.]. .

Willems, H.M., Ahmed, S.S., Liu, J., Xu, Z., Peters, B.M., 2020. Vulvovaginal candidiasis: a current understanding and burning questions. J. Fungi. 6(1), 27. https://doi.org/10.3390/jof6010027

Wilson, D., 2019. *Candida albicans*. Trends Microbiol. 27(2), 188-189. https://doi.org/10.1016/j.tim.2018.10.010

Yu, Q., Zhang, B., Li, J., Zhang, B., Wang, H., Li, M., 2016. Endoplasmic reticulum-derived reactive oxygen species (ROS) is involved in toxicity of cell wall stress to *Candida albicans*. Free Radic. Biol. Med. 99, 572–583. https://doi.org/10.1016/j.freeradbiomed.2016.09.014

6 CONSIDERAÇÕES FINAIS

Nossa pesquisa revelou que o uso etnofarmacológico da polpa do fruto de *C. coriaceum* pelas comunidades da Chapada do Araripe, no Brasil, para tratar doenças infecciosas e parasitárias, foi em parte validado pelos nossos resultados. Embora o extrato de *C. coriaceum* não tenha demonstrado um efeito direto contra as bactérias patogênicas, ele mostrou a capacidade de potencializar a ação dos antibióticos contra microrganismos multirresistentes. No tocante à atividade antifúngica, o extrato foi eficaz na redução do crescimento de *Candida* spp., operando por meio da geração de espécies reativas de oxigênio. Adicionalmente, ele inibiu a transição morfológica das leveduras, um dos mecanismos de sua virulência. A contribuição do extrato de *C. coriaceum* também se estendeu à amplificação da atividade do fluconazol.

Demonstramos que o óleo fixo do mesocarpo interno de *C. coriaceum* é rico em ácido oleico. Esse óleo é frequentemente empregado pela população residente na Chapada do Araripe como um recurso para tratar distúrbios do sistema geniturinário, incluindo a candidíase. De forma que os achados deste estudo oferecem suporte à capacidade do óleo em combater infecções por *Candida*. Foi constatado que um dos fatores contribuintes para a atividade antifúngica deste óleo é a produção excedente de espécies reativas de oxigênio, que pode induzir a apoptose nas células de levedura. Além disso, o óleo apresentou a habilidade de inibir ou atenuar a transição morfológica tanto em *C. albicans* quanto em *C. tropicalis*, destacando-se como um elemento crucial na redução da virulência e, consequentemente, na limitação da capacidade de invasão tecidual dessas cepas. Adicionalmente, o óleo demonstrou a habilidade de potencializar os efeitos do fluconazol, revertendo a resistência antibiótica manifestada pelas cepas de *C. albicans* e *C. tropicalis*, tornando-as mais receptivas ao tratamento com a droga. Por fim, é importante ressaltar que, em concentrações clinicamente relevantes, o óleo extraído não demonstrou toxicidade para *D. melanogaster*, um organismo utilizado como modelo biológico no estudo.

REFERÊNCIAS

ABADI, A. T. B. *et al*. World Health Organization report: current crisis of antibiotic resistance. **BioNanoScience**, v. 9, p. 778-788, 2019.

AGRA, M. D. F. *et al*. Survey of medicinal plants used in the region Northeast of Brazil. **Revista brasileira de farmacognosia**, v. 18, p. 472-508, 2008.

AGRA, M. D. F.; FREITAS, P. F. D.; BARBOSA-FILHO, J. M. Synopsis of the plants known as medicinal and poisonous in Northeast of Brazil. **Revista Brasileira de Farmacognosia**, v. 17, p. 114-140, 2007.

ALMEIDA-BEZERRA, J. W. *et al. Caryocar coriaceum* Wittm.(Caryocaraceae): Botany, Ethnomedicinal Uses, Biological Activities, Phytochemistry, Extractivism and Conservation Needs. **Plants**, v. 11, n. 13, p. 1685, 2022.

ALVES, D. R. *et al*. Flavonoid composition and biological activities of ethanol extracts of *Caryocar coriaceum* Wittm., a native plant from Caatinga biome. **Evidence-Based Complementary and Alternative Medicine**, v. 2017, 2017.

AMORIM, W. R. D. *et al*. Estudo etnoveterinário de plantas medicinais utilizadas em animais da microrregião do Alto Médio Gurguéia–Piauí. **Pubvet**, v. 12, p. 131, 2018.

AMPARO, T. R. *et al*. Herbal medicines to the treatment of skin and soft tissue infections: advantages of the multi-targets action. **Phytotherapy Research**, v. 34, n. 1, p. 94-103, 2020.

ANGIOSPERM PHYLOGENY GROUP *et al*. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. **Botanical journal of the Linnean Society**, v. 181, n. 1, p. 1-20, 2016.

ARADA, J. M. G.; PEREZ, Z. C. Phytotherapy in dentistry: survey of products of plant origin for health oral. **Brazilian Journal of Implantology and Health Sciences**, v. 1, n. 3, p. 35-40, 2019.

ARARUNA, M. K. A et al. Phenolic composition and *in vitro* activity of the Brazilian fruit tree *Caryocar coriaceum* Wittm. **European Journal of Integrative Medicine**, v. 5, n. 2, p. 178-183, 2013.

ARARUNA, M. K. *et al*. Effect of pequi tree *Caryocar coriaceum* Wittm. leaf extracts on different mouse skin inflammation models: inference with their phenolic compound content. **African Journal of Pharmacy and Pharmacology**, v. 8, p. 629-637, 2014.

ARARUNA, M. K. *et al.* Phenolic composition and *in vitro* activity of the Brazilian fruit tree *Caryocar coriaceum* Wittm. **European Journal of Integrative Medicine**, v. 5, n. 2, p. 178-183, 2013.

ASCARI, J. *et al.* Phytochemical and biological investigations of *Caryocar brasiliense* Camb. **Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas**, v. 9, p. 20-28, 2010.

ASCARI, J.; TAKAHASHI, J. A.; BOAVENTURA, M. A. D. The phytochemistry and biological aspects of Caryocaraceae family. **Revista Brasileira de Plantas Medicinais**, v. 15, p. 293-308, 2013.

AUGUSTO, L. G. D. S.; Góes, L. Compreensões integradas para a vigilância da saúde em ambiente de floresta: o caso da Chapada do Araripe, Ceará, Brasil. **Cadernos de Saúde Pública**, v. 23, p. S549-S558, 2007.

AZEVEDO, F. R. D. *et al*. Larvicidal activity of vegetable oils against *Aedes aegypti* larvae. **Revista Facultad Nacional de Agronomía Medellín**, v. 74, n. 2, p. 9563-9570, 2021.

BATISTA, J. S. *et al*. Avaliação da atividade cicatrizante do óleo de pequi (*Caryocar coriaceum* wittm) em feridas cutâneas produzidas experimentalmente em ratos. **Arquivos do Instituto Biológico**, v. 77, p. 441-447, 2020.

BERMAN, J.; KRYSAN, D. J. Drug resistance and tolerance in fungi. **Nature Reviews Microbiology**, v. 18, n. 6, p. 319-331, 2020.

BEZERRA, J. C. B. *et al.* Molluscidal activity against *Biomphalaria glabrata* of Brazilian cerrado medicinal plantas. **Fitoterapia**, v. 73, p. 428-430, 2002.

BEZERRA, J. D. S. *et al*. Floristic and dispersion syndromes of Cerrado species in the Chapada do Araripe, Northeast of Brazil. **Research, Society and Development**, v. 9, n. 9, p. e864997934-e864997934, 2020.

BEZERRA, J. W. A. *et al.* Chemical composition, antimicrobial, modulator and antioxidant activity of essential oil of *Dysphania ambrosioides* (L.) Mosyakin & Clemants. **Comparative Immunology, Microbiology and Infectious Diseases,** v. 65, p. 58-64, 2019.

BEZERRA, N. K. M. S.; BARROS, T. L.; COELHO, N. P. M. F. The effect of the pequi oil (*Caryocar brasiliense*) in the healing of skin lesions in mice. **Revista Brasileira de Plantas Medicinais**, v. 17, n. 4, p. 875-880, 2015.

BITU, V. D. C. N. *et al.* Ethnopharmacological study of plants sold for therapeutic purposes in public markets in Northeast Brazil. **Journal of Ethnopharmacology**, v. 172, p. 265-272, 2015.

BLAIR, J. *et al.* Molecular mechanisms of antibiotic resistance. **Nature Reviews Microbiology**, v. 13, n. 1, p. 42-51, 2015.

BOCCOLINI, P. M. M.; BOCCOLINI, C. S. Prevalence of complementary and alternative medicine (CAM) use in Brazil. **BMC complementary medicine and therapies**, v. 20, n. 1, p. 1-10, 2020.

BOTELHO, J.; GROSSO, F.; PEIXE, L. Antibiotic resistance in *Pseudomonas aeruginosa*– Mechanisms, epidemiology and evolution. **Drug Resistance Updates**, v. 44, p. 100640, 2019.

BRAGA, K. *et al.* Pequi Fruit Extract Increases Antioxidant Enzymes and Reduces Oxidants in Human Coronary Artery Endothelial Cells. **Antioxidants**, v. 11, n. 3, p. 474, 2022.

BRANDÃO, M. G. *et al.* Brazilian medicinal plants described by 19th century European naturalists and in the Official Pharmacopoeia. **Journal of Ethnopharmacology**, v. 120, n. 2, p. 141-148, 2008.

BRITO, R. M. *et al.* Bioactive compounds of pequi pulp and oil extracts modulate antioxidant activity and antiproliferative activity in cocultured blood mononuclear cells and breast cancer cells. **Food & Nutrition Research**, v. 66, 2022.

CALDEIRA, A. S. *et al.* Bioguided chemical characterization of pequi (*Caryocar brasiliense*) fruit peels towards an anti-diabetic activity. **Food Chemistry**, v. 345, p. 128734, 2021.

CAMPOS, L. Z. *et al*. Use of local ecological knowledge as phenology indicator in native food species in the semiarid region of Northeast Brazil. **Ecological Indicators**, v. 95, p. 75-84, 2018.

CAROLUS, H. *et al.* Amphotericin B and other polyenes—discovery, clinical use, mode of action and drug resistance. **Journal of Fungi**, v. 6, n. 4, p. 321, 2020.

CARTAXO, S. L.; SOUZA, M. M. D. A.; Albuquerque, U. P. D. Medicinal plants with bioprospecting potential used in semi-arid northeastern Brazil. **Journal of ethnopharmacology**, v. 131, n. 2, p. 326-342, 2010.

CAVALCANTI, M. C. B. T. *et al*. Pequi (*Caryocar coriaceum* Wittm., Caryocaraceae) oil production: A strong economically influenced tradition in the Araripe region, northeastern Brazil. **Ethnobotany Research and Applications**, v. 14, p. 437-452, 2015.

CHEUNG, G. Y.; BAE, J. S.; OTTO, M. Pathogenicity and virulence of *Staphylococcus aureus*. **Virulence**, v. 12, n. 1, p. 547-569, 2021.

CONCEIÇÃO, G. M. *et al*. Plantas do cerrado: comercialização, uso e indicação terapêutica fornecida pelos raizeiros e vendedores, Teresina, Piauí. **Scientia Plena**, v. 7, n. 12, 2011.

CONCEIÇÃO, G. M.; CASTRO, A. A. J. F. Fitossociologia de uma área de cerrado marginal, Parque Estadual do Mirador, Mirador, Maranhão. **Scientia Plena**, v. 5, n. 10, 2009.

COSTA, A. R. *et al.* Phytochemical profile and anti-*Candida* and cytotoxic potential of *Anacardium occidentale* L.(cashew tree). **Biocatalysis and Agricultural Biotechnology**, v. 37, p. 102192, 2021.

COSTA, I. R.; ARAÚJO, F. S. Organização comunitária de um encrave de cerrado *sensu stricto* no bioma Caatinga, chapada do Araripe, Barbalha, Ceará. **Acta Botanica Brasilica**, v. 21, p. 281-291, 2007.

COSTA, I. R.; ARAÚJO, F. S.; LIMA-VERDE, L. W. Flora e aspectos auto-ecológicos de um encrave de cerrado na chapada do Araripe, Nordeste do Brasil. **Acta Botanica Brasilica**, v. 18, p. 759-770, 2004.

COSTA, J. G. M. *et al*. Antibacterial properties of pequi pulp oil (*Caryocar coriaceum*– Wittm.). **International Journal of Food Properties**, v. 14, n. 2, p. 411-416, 2011.

CRAFT, K. M. *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA): antibiotic-resistance and the biofilm phenotype. **MedChemComm,** v. 10, n. 8, p. 1231-1241, 2019.

CRUZ, R. P. *et al.* Ethnopharmacology of the angiosperms of Chapada of Araripe located in Northeast of Brazil. **Journal of Environmental Analysis and Progress**, v. 6, n. 4, p. 326-351, 2021.

DUAVY, S. M. *et al*. Pequi enriched diets protect *Drosophila melanogaster* against paraquat-induced locomotor deficits and oxidative stress. **Journal of Toxicology and Environmental Health**, Part A, v. 82, n. 11, p. 664-677, 2019.

DUAVY, S. M. P. *et al*. Atividade biológica de extratos de folhas de *Caryocar coriaceum* Wittm.: Estudo *in vitro*. **Cadernos de Cultura e Ciência**, v. 11, n. 1, p. 13-19, 2012.

EIX, E. F.; NETT, J. E. How biofilm growth affects *Candida*-host interactions. **Frontiers in Microbiology**, v. 11, p. 1437, 2020.

FAGG, C. W. *et al.* Useful Brazilian plants listed in the manuscripts and publications of the Scottish medic and naturalist George Gardner (1812–1849). **Journal of Ethnopharmacology**, v. 161, p. 18-29, 2015.

FAIR, R. J.; TOR, Y. Antibiotics and bacterial resistance in the 21st century. **Perspectives in medicinal chemistry**, v. 6, p. 25-64, 2014.

FAST, D. *et al.* Commensal pathogen competition impacts host viability. **Proceedings of the National Academy of Sciences**, v. 115, n. 27, p. 7099-7104, 2018.

FEITOSA, I. S.; ALBUQUERQUE, U. P.; MONTEIRO, J. M. Knowledge and extractivism of *Stryphnodendron rotundifolium* Mart. in a local community of the Brazilian Savanna, Northeastern Brazil. **Journal of Ethnobiology and Ethnomedicine**, v. 10, n. 1, p. 1-13, 2014.

FERREIRA-JÚNIOR, W.S. *et al*. Check-list das plantas medicinais na Chapada do Araripe. In: Albuquerque, U.P., Meiado, M. V. (org.). **Sociobiodiversidade na Chapada do Araripe**, 1 ed, Recife: NUPEEA, 2015.

FIGUEIREDO, P. R. L. *et al*. *Caryocar coriaceum* Wittm.(Pequi) fixed oil presents hypolipemic and anti-inflammatory effects *in vivo* and *in vitro*. **Journal of ethnopharmacology**, v. 191, p. 87-94, 2016.

FIGUEREDO, C. A. D.; GURGEL, I. G. D.; GURGEL JUNIOR, G. D. A Política Nacional de Plantas Medicinais e Fitoterápicos: construção, perspectivas e desafios. **Physis: Revista de Saúde Coletiva**, v. 24, p. 381-400, 2014.

FISHER, M. C.; DENNING, D. W. The WHO fungal priority pathogens list as a game-changer. **Nature Reviews Microbiology**, v. 21, n. 4, p. 211-212, 2023.

FÜRNKRANZ, U.; WALOCHNIK, J. Nosocomial Infections: Do Not Forget the Parasites!. **Pathogens**, v. 10, n. 2, p. 238, 2021.

GARDNER, G. **Travels in the interior of Brazil, principally through the northern provinces, and the gold and diamond districts, during the years 1836-1841**. London: Reeve, Benham and Reeve, 2 ed., 1849.

GOMES, A. B.; RIBEIRO, I. A. Evaluation of antifungal potential of oil of the species *Caryocar coriaceum* front of *Candida* species isolated in the oral cavity of oncological pediatric patients in antineoplastic treatment. **International Journal of Pediatric Research and Reviews**, v. 2, 2019.

GONÇALVES, C. U. Os piquizeiros da Chapada do Araripe. **Revista de Geografia**, v. 25, n. 1, p. 88-103, 2008.

GUERRA, M. D. F.; DE SOUZA, M. J. N.; SILVA, E. V. Veredas da Chapada do Araripe: subespaços de exceção no semiárido do estado do Ceará, Brasil. **Ateliê Geográfico**, v. 14, n. 2, p. 51-66, 2020.

GUPTA, P. D.; BIRDI, T. J. Development of botanicals to combat antibiotic resistance. **Journal of Ayurveda and integrative medicine**, v. 8, n. 4, p. 266-275, 2017.

HEALEY, K. R.; PERLIN, D. S. Fungal resistance to echinocandins and the MDR phenomenon in Candida glabrata. **Journal of fungi**, v. 4, n. 3, p. 105, 2018.

HERZOG-SOARES, J. D. *et al.* Atividade tripanocida *in vivo* de *Stryphnodendron adstringens* (barbatimão verdadeiro) e *Caryocar brasiliensis* (pequi). **Revista Brasileira de Farmacognosia**, v. 12, p. 11-15, 2002.

IBE, C.; MUNRO, C. A. Fungal cell wall: An underexploited target for antifungal therapies. **PLoS Pathogens**, v. 17, n. 4, e1009470, 2021.

JAMSHIDI-KIA, F.; LORIGOINI, Z.; AMINI-KHOEI, H. Medicinal plants: Past history and future perspective. **Journal of herbmed pharmacology**, v. 7, n. 1, p. 1-7, 2018.

JOHNSON, L. R.; MANGEL, M. Life histories and the evolution of aging in bacteria and other single-celled organisms. **Mechanisms of ageing and development**, v. 127, n. 10, p. 786-793, 2006.

KERNTOPF, M. R. *et al*. Óleo de pequi (*Caryocar coriaceum* W.) e a potencial atividade cardioprotetora. **Ensaios e Ciência C Biológicas Agrárias e da Saúde**, v. 17, n. 4, 2013.

KHAN, F. *et al*. Suppression of hyphal formation and virulence of *Candida albicans* by natural and synthetic compounds. **Biofouling**, v. 37, n. 6, p. 626-655, 2021.

KHAN, H. Medicinal plants in light of history: Recognized therapeutic modality. **Journal of Evidence-Based Integrative Medicine**, v. 19, p. 216-219, 2014.

KHAN, R.; PETERSEN, F. C.; SHEKHAR, S. Commensal bacteria: an emerging player in defense against respiratory pathogens. **Frontiers in Immunology**, v. 10, p. 1203, 2019.

KHOURI, J. *et al.* Anticlastogenic potential and antioxidant effects of na aqueous extract of pulp from the pequi tree (*Caryocar brasiliense* Camb.). **Genetics and Molecular Biology**, v. 30, n. 2, p. 442–448, 2007.

KINDAICHI, T.; ITO, T.; OKABE, S. Ecophysiological interaction between nitrifying bacteria and heterotrophic bacteria in autotrophic nitrifying biofilms as determined by microautoradiography-fluorescence in situ hybridization. **Applied and Environmental Microbiology**, v. 70, n. 3, p. 1641-1650, 2004.

KORNITZER, D. Regulation of *Candida albicans* hyphal morphogenesis by endogenous signals. **Journal of Fungi**, v. 5, n. 1, p. 21, 2019.

LACERDA NETO, L. J. *et al.* Modulation of antibiotic activity by the hydroalcoholic extract from leaves of *Caryocar coriaceum* Wittm. **Natural Product Research**, v. 32, n. 4, p. 477-480, 2017.

LACERDA-NETO, L. J.; RAMOS, A. G. B.; VIDAL, C. S. Serviços ecossistêmicos: o caso do *Caryocar coriaceum* WITTM.(pequi) na chapada do araripe. **Rev. Biol. Farm**. v. 9, n. 2, p. 34-40, 2013.

LEE, Y. *et al.* Antifungal drug resistance: molecular mechanisms in *Candida albicans* and beyond. **Chemical Reviews**, v. 121, n. 6, p. 3390-3411, 2021.

LEITE, G. O. et al. Gastroprotective effect of medicinal plants from Chapada do Araripe, Brazil. **Journal of Young Pharmacists**, v. 1, n. 1, p. 54, 2009.

LEMOS, I. C. S. *et al*. Ethnobiological survey of plants and animals used for the treatment of acute respiratory infections in children of a traditional community in the municipality of Barbalha, Ceará, Brazil. **African journal of traditional, complementary and alternative medicines**, v. 13, n. 4, p. 166-175, 2016.

LI, Y. *et al.* A genome-scale phylogeny of the kingdom Fungi. **Current Biology**, v. 31, n. 8, p. 1653-1665, 2021.

LI, Y. *et al*. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. **Plant Physiology and Biochemistry**, v. 148, p. 80-89, 2020.

LOZANO, A. *et al*. The apparency hypothesis applied to a local pharmacopoeia in the Brazilian northeast. **Journal of Ethnobiology and Ethnomedicine**, v. 10, p. 1-17, 2014.

MACÊDO, D. G. *et al.* Versatility and consensus of the use of medicinal plants in an area of cerrado in the Chapada do Araripe, Barbalha-CE-Brazil. **Journal of Medicinal Plants Research**, v. 10, n. 31, p. 505-514, 2016.

MACIEL, T. C. M. *et al*. Pequi (*Caryocar coriaceum* Wittm.) extrativism: situation and perspectives for its sustainability in Cariri Cearense, Brazil. **Acta Agronómica**, v. 67, n. 2, p. 238-245, 2018.

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

MATOS, F. J. A. **Plantas medicinais: guia de seleção e emprego de plantas usadas em fitoterapia no Nordeste do Brasil**. 3 ed, Fortaleza: Imprensa Universitária, 2007.

MEDEIROS, M. B.; WALTER, B. M. T. Composição e estrutura de comunidades arbóreas de cerrado stricto sensu no norte do Tocantins e sul do Maranhão. **Revista Árvore**, v. 36, p. 673-683, 2012.

MEDEIROS, M. B.; WALTER, B. M. T.; SILVA, G. P. Fitossociologia do cerrado *stricto sensu* no município de Carolina, MA, Brasil. **Cerne**, v. 14, n. 4, p. 285-294, 2008.

MIRANDA-VILELA, A. L. *et al.* Antigenotoxic activity and antioxidant properties of organic and aqueous extracts of pequi fruit pulp (*Caryocar brasiliense* Camb.). **Genetics an Molecular Biology**, v. 31, n. 4, p. 956-963, 2008.

MONTENEGRO, A. P. *et al*. Risk of developing Candida not albicans in patients in intensive care unit with previous use of fluconazole. **Medicina Crítica**, v. 31, n. 2, p. 55-59, 2017.

MORAIS, H. L. M. D. N. *et al*. Hydroalcoholic extract of *Caryocar brasiliense* Cambess. leaves affect the development of *Aedes aegypti* mosquitoes. **Revista da Sociedade Brasileira de Medicina Tropical**, v. 53, p. e20200176, 2020.

MORO, M. F. *et al.* Vegetação, unidades fitoecológicas e diversidade paisagística do estado do Ceará. **Rodriguésia**, v. 66, p. 717-743, 2015.

NASCIMENTO-SILVA, N. R. R.; NAVES, M. M. V. Potential of whole Pequi (*Caryocar* spp.) fruit—pulp, almond, oil, and shell—as a medicinal food. **Journal of Medicinal Food**, v. 22, n. 9, p. 952-962, 2019.

NICOLA, A. M. *et al.* Antifungal drugs: New insights in research & development. **Pharmacology & therapeutics**, v. 195, p. 21-38, 2019.

NUNES, R. *et al.* Caryocaraceae Voigt (Malpighiales): a Synthesis Based on Science Mapping and Systematic Review. **The Botanical Review**, p. 1-21, 2020.

OKWU, M. U. *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA) and anti-MRSA activities of extracts of some medicinal plants: A brief review. **AIMS microbiology**, v. 5, n. 2, p. 117, 2019.

OLIVEIRA, F. F. B. *et al*. Antinociceptive and anti-inflammatory effects of *Caryocar coriaceum* Wittm fruit pulp fixed ethyl acetate extract on zymosan-induced arthritis in rats. **Journal of ethnopharmacology**, v. 174, p. 452-463, 2015.

OLIVEIRA, M. E. B. *et al*. Aspectos agronômicos e de qualidade do pequi. **Embrapa Agroindústria Tropical-Documentos (INFOTECA-E)**, 2008.

OLIVEIRA, M. E. B. *et al*. Caracterização física de frutos do pequizeiro nativos da chapada do Araripe-CE. **Revista Brasileira de Fruticultura**, v. 31, p. 1196-1201, 2009.

OLIVEIRA, T. S. *et al.* Neuroprotective effect of *Caryocar brasiliense* Camb. leaves is associated with anticholinesterase and antioxidant properties. **Oxidative medicine and cellular longevity**, 2018.

PACHORI, P.; GOTHALWAL, R.; GANDHI, P. Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. **Genes & Diseases**, v. 6, n. 2, p. 109-119, 2019.

PANG, Z. *et al*. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and alternative therapeutic strategies. **Biotechnology advances**, v. 37, n. 1, p. 177-192, 2019.

PAPPAS, P. G. Opportunistic fungi: a view to the future. **The American journal of the medical sciences**, v. 340, n. 3, p. 253-257, 2010.

PASSOS, X. S. *et al.* Composition and antifungal activity of essential oils of *Caryocar brasiliensis*. **Pharmaceutical Biology**, v. 41, n. 5, p. 319-324, 2003.

PATINI, R. *et al.* The effect of different antibiotic regimens on bacterial resistance: A systematic review. **Antibiotics**, v. 9, n. 1, p. 22, 2020.

PATWARDHAN, B. *et al.* Reverse pharmacology and systems approaches for drug discovery and development. **Current Bioactive Compounds**, v. 4, n. 4, p. 201-212, 2008.

PAULA-JUNIOR, W. *et al.* Leismanicidal, antibacterial, and antioxidant activities of *Caryocar brasiliense* Camb. Leaves hydroethanolic extract. **Revista Brasileira de Farmacognosia**, v.16, p.625-630, 2006.

PESSOA, A. S. *et al*. Extraction of pequi (*Caryocar coriaceum*) pulp oil using subcritical propane: Determination of process yield and fatty acid profile. **The journal of supercritical fluids**, v. 101, p. 95-103, 2015.

PIRES, J. *et al.* Healing of dermal wounds property of *Caryocar brasiliense* oil loaded polymeric lipid-core nanocapsules: Formulation and *in vivo* evaluation. **European Journal of Pharmaceutical Sciences**, v. 150, p. 105356, 2020.

POOLMAN, J. T. *Escherichia coli.* **International Encyclopedia of Public Health**, v. 2, p. 585– 593, 2017.

PRADO, D. *Caryocar coriaceum*. **The IUCN Red List of Threatened Species**, 1998.

PRANCE, G. T. Caryocaraceae. In: K. KUBITZKI (ed.). Flowering Plants. Eudicots: Malpighiales (Vol. 11). Springer Berlin Heidelberg, 2014. 332 p.

PRISTOV, K. E.; GHANNOUM, M. A. Resistance of *Candida* to azoles and echinocandins worldwide. **Clinical Microbiology and Infection**, v. 25, n. 7, p. 792-798, 2019.

QUIRINO, G. S. *et al*. Healing potential of Pequi (*Caryocar coriaceum* Wittm.) fruit pulp oil. **Phytochemistry Letters**, v. 2, n. 4, p. 179-183, 2009.

RAMOS, K. M. C.; SOUZA, V. A. B. Características físicas e químico-nutricionais de frutos de pequizeiro (*Caryocar coriaceum* Wittm.) em populações naturais da região meio-norte do Brasil. **Revista Brasileira de Fruticultura**, v. 33, p. 500-508, 2011.

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

RIBEIRO, F. C. *et al.* Action mechanisms of probiotics on *Candida* spp. and candidiasis prevention: an update. **Journal of Applied Microbiology**, v. 129, n. 2, p. 175-185, 2020.

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

RIBEIRO-SILVA, S. *et al.* Angiosperms from the Araripe national forest, Ceará, Brazil. **Check list**, v. 8, p. 744, 2012.

RIBEIRO-SILVA, S. *et al*. Angiosperms from the Araripe national forest, Ceará, brazil. **Check list**, v. 8, n. 4, p. 744-751, 2012.

RICHARDS, T. A.; LEONARD, G.; WIDEMAN, J. G. What defines the “kingdom” fungi?. **Microbiology spectrum**, v. 5, n. 3, p. 5-3, 2017.

RODRIGUES, B. S. *et al*. Morphobiometry and ecophysiology of *Caryocar coriaceum* Wittm.(Pequi) in cerrado areas of Northeast Brazil. **Journal of Experimental Agriculture International**, v. 41, n. 4, p. 1-7, 2019.

RODRIGUES, F. C. *et al*. Antimicrobial activity, modulatory effect and phytochemical analysis of *Sida galheirensis* Ulbr.(Malvaceae). **South African Journal of Botany**, v. 147, p. 286-293, 2022.

ROEMER, T.; KRYSAN, D. J. Antifungal drug development: challenges, unmet clinical needs, and new approaches. **Cold Spring Harbor Perspectives in Medicine**, v. 4, n. 5, a019703, 2014.

ROLL, M. M. *et al.* The pequi pulp oil (*Caryocar brasiliense* Camb.) provides protection against aging-related anemia, inflammation and oxidative stress in Swiss mice, especially in females. **Genetics and Molecular Biology**, v. 41, p. 858-869, 2018.

ROMO, J. A.; KUMAMOTO, C. A. On commensalism of *Candida*. **Journal of Fungi**, v. 6, n. 1, p. 16, 2020.

ROSIANA, S. *et al.* Comprehensive genetic analysis of adhesin proteins and their role in virulence of *Candida albicans*. **Genetics**, v. 217, n. 2, iyab003, 2021.

RUIZ-HERRERA, J.; ORTIZ-CASTELLANOS, L. Cell wall glucans of fungi. A review. **The Cell Surface**, v. 5, 100022, 2019.

SANT'ANA, P. J. P. D.; ASSAD, A. L. D. Programa de pesquisa em produtos naturais: a experiência da CEME. **Química Nova**, v. 27, n. 3, p. 508-512, 2004.

SARAIVA, R. A. *et al*. Topical anti-inflammatory effect of *Caryocar coriaceum* Wittm.(Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. **Journal of ethnopharmacology**, v. 136, n. 3, p. 504-510, 2011.

SERRA, D. S. *et al*. Effects of fixed oil of *Caryocar coriaceum* Wittm. Seeds on the respiratory system of rats in a short-term secondhand-smoke exposure model. **Journal of Ethnopharmacology**, v. 252, p. 112633, 2020.

SILVA, F. D. J. *et al.* The effect of organic compost of *Caryocar brasiliense* waste against Meloidogyne javanica in two vegetable crops. **Nematology**, v. 23, n. 10, p. 1171-1178, 2021.

SILVA, L. F. B. P. *et al*. Anti-inflammatory action of pequi oil associated to ultrasound in tendinitis in rats: macroscopic and histological analysis. **Manual Therapy, Posturology & Rehabilitation Journal**, p. 1-6, 2016.

SILVA, M. A. P. *et al.* Fenologia de *Caryocar coriaceum* Wittm. Caryocaraceae, ocorrentes na Chapada do Araripe–Crato-CE-Brasil. **Cadernos de Cultura e Ciência**, v. 12, n. 2, p. 21-31, 2013.

SILVA, M. A. P.; MEDEIROS-FILHO, S. Morfologia de fruto, semente e plântula de piqui (*Caryocar coriaceum* Wittm.). **Revista Ciência Agronômica**, v. 37, n. 3, p. 320-325, 2006.

SILVA, N. F. *et al*. Local knowledge and conservation priorities of medicinal plants near a protected area in Brazil. **Evidence-Based Complementary and Alternative Medicine**, v. 2019, 2019.

SIMÕES, C. M. O.; SCHENKEL, E. P. A pesquisa e a produção brasileira de medicamentos a partir de plantas medicinais: a necessária interação da indústria com a academia. **Revista brasileira de farmacognosia**, v. 12, p. 35-40, 2002.

SOBRAL, A. *et al*. Conservation efforts based on local ecological knowledge: The role of social variables in identifying environmental indicators. **Ecological Indicators**, v. 81, p. 171-181, 2017.

SOUSA-JÚNIOR, J. R. *et al*. O pequi (*Caryocar coriaceum* Wittm. - Caryocaraceae) na Chapada do Araripe. In: Albuquerque, U.P., Meiado, M. V. (org.). **Sociobiodiversidade na Chapada do Araripe**, 1 ed, Recife: NUPEEA, 2015.

SOUSA-JÚNIOR, J. R.; Albuquerque, U. P.; Peroni, N. Traditional knowledge and management of *Caryocar coriaceum* Wittm.(Pequi) in the Brazilian savanna, Northeastern Brazil. **Economic Botany**, v. 67, p. 225-233, 2013.

SOUZA, R. K. D. *et al.* Ethnopharmacology of medicinal plants of carrasco, northeastern Brazil. **Journal of Ethnopharmacology**, v. 157, p. 99-104, 2014.

SPAMPINATO, C.; LEONARDI, D. *Candida* infections, causes, targets, and resistance mechanisms: traditional and alternative antifungal agents. **BioMed research international**, v. 2013, 2013.

TAN, S. Y.; TATSUMURA, Y. Alexander Fleming (1881–1955): discoverer of penicillin. **Singapore medical journal**, v. 56, n. 7, p. 366, 2015.

THI, M. T. T.; WIBOWO, D.; REHM, B. H. *Pseudomonas aeruginosa* biofilms. International **Journal of Molecular Sciences**, v. 21, n. 22, p. 8671, 2020.

TOMIOTTO-PELLISSIER, F. *et al. Caryocar coriaceum* extracts exert leishmanicidal effect acting in promastigote forms by apoptosis-like mechanism and intracellular amastigotes by Nrf2/HO-1/ferritin dependent response and iron depletion: Leishmanicidal effect of *Caryocar coriaceum* leaf extracts. **Biomedicine & Pharmacotherapy**, v. 98, p. 662-672, 2018.

TOMIOTTO-PELLISSIER, F. *et al*. *Caryocar coriaceum* extracts exert leishmanicidal effect acting in promastigote forms by apoptosis-like mechanism and intracellular amastigotes by Nrf2/HO-1/ferritin dependent response and iron depletion: Leishmanicidal effect of *Caryocar coriaceum* leaf exracts. **Biomedicine & Pharmacotherapy**, v. 98, p. 662-672, 2018.

VARGAS, S. H. *et al.* Phytotoxic activity and chemical composition of aqueous foliar extracts of Cerrado species. **Floresta e Ambiente**, v. 28, 2021.

WHALEY, S. G. *et al.* Azole antifungal resistance in *Candida albicans* and emerging non-*albicans Candida* species. **Frontiers in microbiology**, v. 7, p. 2173, 2017.

WILSON, D. *Candida albicans*. **Trends in Microbiology**, v. 27, n. 2, p. 188-189, 2019.

YAMAGUCHI, K. K. *et al*. HPLC-DAD profile of phenolic compounds, cytotoxicity, antioxidant and anti-inflammatory activities of the amazon fruit *Caryocar villosum*. **Química Nova**, v. 40, p. 483-490, 2017.

ANEXO A

PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NO PERIÓDICO ***PLANTS*** (ARTIGO 1)

Disponível em: https://doi.org/10.3390/plants11131685

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Review

Caryocar coriaceum Wittm. (Caryocaraceae): Botany, Ethnomedicinal Uses, Biological Activities, Phytochemistry, Extractivism and Conservation Needs

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Citation: Almeida-Bezerra, J.W.; Bezerra, J.J.L.; Silva, V.B.d.; Coutinho, H.D.M.; Costa, J.G.M.d.; Cruz-Martins, N.; Hano, C.; Menezes, S.A.d.; Morais-Braga, M.F.B.; Oliveira, A.F.d. *Caryocar coriaceum* Wittm. (Caryocaraceae): Botany, Ethnomedicinal Uses, Biological Activities, Phytochemistry, Extractivism and Conservation Needs. *Plants* **2022**, *11*, 1685. https://doi.org/10.3390/plants11131685

Academic Editor: Suresh Awale

Received: 18 May 2022
Accepted: 22 June 2022
Published: 25 June 2022

Abstract: *Caryocar coriaceum* is an endemic tree of Brazil, occurring mainly in the northeast region in the Cerrado environment. The species, popularly known as "pequi", produces fruits that are used in the manufacture of oil for food and medicinal purposes. This work reviewed studies conducted with the species, highlighting its ethnomedicinal use, its pharmacological potential, including its chemical constituents, and its cultural and socioeconomic importance. Information was obtained through the main scientific research platforms. The keyword "*Caryocar coriaceum*" was used as the main index for searching the following platforms: PubMed®, PubMed Central®, SciElo, Scopus® and Web of Science™. The compiled papers demonstrate that *C. coriaceum* has great medicinal, economic and cultural importance for northeastern Brazil. Popularly, the fruits of *C. coriaceum* are used to treat broncho-pulmonary diseases (bronchitis, colds and flu). The fixed oil is widely used to relieve pain from various causes in the treatment of inflammation, flu, eczema, burns, fever, rickets, indigestion, heart murmurs, fatigue and erectile dysfunction. Some of these uses are corroborated by pharmacological trials, which have demonstrated the antioxidant, healing, anti-inflammatory, gastroprotective, antinociceptive and antimicrobial properties of the species. Chemically, fatty acids and phenolic compounds are the main constituents recorded for the species. Due to its medicinal properties, the fruits and oil of *C. coriaceum* have a high commercial demand and are one of the main forms of subsistence activities for local populations. On the other hand, the extractive practice of the fruits, associated with anthropic factors and its physiological nature, makes the species threatened with extinction. Thus, public management policies are highly necessary in order to avoid its extinction.

Keywords: Oleic acid; Caryocaraceae; extractivism; flavonoids; Chapada do Araripe

ANEXO B

PRIMEIRA PÁGINA DO ARTIGO PUBLICADO NO PERIÓDICO ***MICROBIAL PATHOGENESIS*** (ARTIGO 2)

Disponível em: https://doi.org/10.1016/j.micpath.2023.106203

Microbial Pathogenesis 181 (2023) 106203

Contents lists available at ScienceDirect

Microbial Pathogenesis

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

Caryocar coriaceum fruits as a potential alternative to combat fungal and bacterial infections: *In vitro* evaluation of methanolic extracts

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ARTICLE INFO

Keywords:
Candidiasis
Chapada do Araripe
Infectious and parasitic diseases

ABSTRACT

Caryocar coriaceum, commonly known as 'pequi', is a medicinal species used traditionally for the herbal treatment of infectious and parasitic diseases in the Brazilian Northeast region. In this study, we investigated whether the fruits of *C. coriaceum* have bioactive chemical constituents against etiological agents of infectious diseases. The methanolic extract of the internal mesocarp of the fruits of *C. coriaceum* (MECC) was chemically analyzed and evaluated for its antimicrobial and drug-enhancing activity against multidrug-resistant pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*), and *Candida* spp. strains. The extract had flavones, flavonols, xanthones, catechins, and flavanones as major classes. A total of 11.26 mg GAE/g of phenolics, and 5.98 mg QE/g of flavonoids were found. No intrinsic antibacterial activity was observed; however, the extract was able to intensify the action of gentamicin and erythromycin against multi-resistant strains. The anti-*Candida* effect observed in this study was mainly due to the formation of reactive oxygen species. The extract was capable of causing damage to the plasmatic membrane of *Candida tropicalis* through pores formation. Our findings partially support the ethnopharmacological uses of the fruit pulp of *C. coriaceum* against infectious and parasitic diseases.

1. Introduction

The mortality as a result of infectious and parasitic diseases (IPDs) has grown in recent decades, mainly due to microbial resistance originating from the indiscriminate use of antibiotics. Pathogenic bacteria, fungi, and protozoa have been shown to overcome the action of commercially available drugs, and the discovery of new drugs has not kept pace with the adaptation speed of these microorganisms [1–3].

Another factor that significantly contributes to the increase in mortality due to infectious and parasitic diseases, is the lack of access to appropriate medicines by the population, especially in underdeveloped countries. On the other hand, medicinal plants have stood out as the therapeutic alternative for the treatment of IPDs, due to their easy access, low cost, and traditional and cultural usage by many communities [4,5]. Therefore, the investigation of the bioactive potential of plant species becomes important to formulate new medicines or drug enhancers [6,7].

In Brazil, medicinal plants are widely used by different communities, the country is one of the top ten consumers of *in natura* plant therapeutic resources [8–14]. Brazil has a huge diversity in terms of traditional communities which make use of herbal medicine (e.g., indigenous peoples, afro-descendant quilombos, rubber tappers, and traditional inhabitants of the coastal regions, among others). However, the use of medicinal plants is not only related to these cultural aspects but also to

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https://doi.org/10.1016/j.micpath.2023.106203
Received 19 May 2023; Received in revised form 10 June 2023; Accepted 14 June 2023
Available online 15 June 2023
0882-4010/

ANEXO C

NORMAS PARA SUBMISSÃO DE MANUSCRITO AO PERIÓDICO ***PLANTS***

Disponível em: https://www.mdpi.com/journal/plants/instructions

Instructions for Authors

Shortcuts

Submission Checklist

Please:

1. Read the **Aims & Scope** to gain an overview and assess if your manuscript is suitable for this journal;
2. Use the **Microsoft Word template** or **LaTeX template** to prepare your manuscript;
3. Make sure that issues about **publication ethics**, **research ethics**, **copyright**, **authorship**, **figure formats**, **data** and **references format** have been appropriately considered;
4. Ensure that all authors have approved the content of the submitted manuscript.
5. Authors are encouraged to add a **biography** (optional) to the submission and post it to **SciProfiles**.

ANEXO D

NORMAS PARA SUBMISSÃO DE MANUSCRITO AO PERIÓDICO ***MICROBIAL PATHOGENESIS***

ELSEVIER

MICROBIAL PATHOGENESIS

AUTHOR INFORMATION PACK

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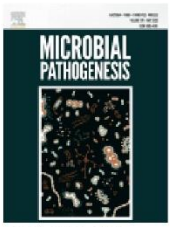

ISSN: 0882-4010

DESCRIPTION

Microbial Pathogenesis publishes original contributions and reviews about the molecular and cellular mechanisms of infectious diseases. It covers microbiology, host-pathogen interaction and immunology related to infectious agents, including bacteria, fungi, viruses and protozoa. It also accepts papers in the field of clinical microbiology, with the exception of case reports.

Research Areas Include: Pathogenesis Virulence factors Host susceptibility or resistance Immune mechanisms Identification, cloning and sequencing of relevant genes Genetic studies Viruses, prokaryotic organisms and protozoa Microbiota Systems biology related to infectious diseases Targets for vaccine design (pre-clinical studies)

The journal aims for rapid publication of articles of high quality and significance in an international forum. Please note that reviews are only accepted upon editorial invitation.

ABSTRACTING AND INDEXING

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EDITORIAL BOARD

Editor-in-Chief

J.-P. Gorvel, Immunology Centre Marseille-Luminy, Marseille, France

ANEXO E

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

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 A4848B1

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:	**A4848B1**
Usuário:	**José Weverton Almeida Bezerra**
CPF/CNPJ:	**603.251.523-06**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa e Desenvolvimento Tecnológico**

Espécie

Caryocar coriaceum

Título da Atividade:	**PROSPECÇÃO FITOQUÍMICA E AVALIAÇÃO DO POTENCIAL ANTIMICROBIANO E ANTIPARASITÁRIO DOS FRUTOS DE Caryocar coriaceum Wittm. (CARYOCARACEAE)**

Equipe

José Weverton Almeida Bezerra	**Universidade Federal de Pernambuco**

Data do Cadastro:	**03/04/2021 10:39:13**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **10:39** de **03/04/2021**.

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

ANEXO F

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

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: 77450-1	Data da Emissão: 27/01/2021 13:04:30	Data da Revalidação*: 27/01/2022
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: José Weverton Almeida Bezerra	CPF: 603.251.523-06
Título do Projeto: Prospecção Fitoquímica e Avaliação do Potencial Antimicrobiano e Antiparasitário dos Frutos de Caryocar coriaceum Wittm. (Caryocaraceae)	
Nome da Instituição: Universidade Federal de Pernambuco - UFPE	CNPJ: 24.134.488/0001-08

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Coleta de Frutos	03/2021	05/2021
2	Coleta de Folhas e Flores	09/2021	12/2021

Observações e ressalvas

1	O pesquisador somente poderá realizar atividade de campo após o término do estado de emergência devido à COVID-19, assim declarado por ato da autoridade competente.
2	Em caso de pesquisa em UNIDADE DE CONSERVAÇÃO, o pesquisador titular desta autorização deverá contactar a administração da unidade a fim de CONFIRMAR AS DATAS das expedições, as condições para realização das coletas e de uso da infraestrutura da unidade.
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5	As atividades de campo exercidas por pessoa natural ou jurídica estrangeira, em todo o território nacional, que impliquem o deslocamento de recursos humanos e materiais, tendo por objeto coletar dados, materiais, espécimes biológicos e minerais, peças integrantes da cultura nativa e cultura popular, presente e passada, obtidos por meio de recursos e técnicas que se destinem ao estudo, à difusão ou à pesquisa, estão sujeitas a autorização do Ministério de Ciência e Tecnologia.
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8	Este documento não dispensa o cumprimento da legislação que dispõe sobre acesso a componente do patrimônio genético existente no território nacional, na plataforma continental e na zona econômica exclusiva, ou ao conhecimento tradicional associado ao patrimônio genético, para fins de pesquisa científica, bioprospecção e desenvolvimento tecnológico. Veja maiores informações em www.mma.gov.br/cgen.

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Código de autenticação: 0774500120210127

ANEXO G

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

URCA webMail

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

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

4 mensagens

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

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

Abraços,

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

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

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

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

--

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

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

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

ANEXO H

COMPROVANTE DE AUTORIZAÇÃO DE USO DA FIGURA 3 DO REFERENCIAL TEÓRICO. NUNES ET AL., (2020)

URCA webMail

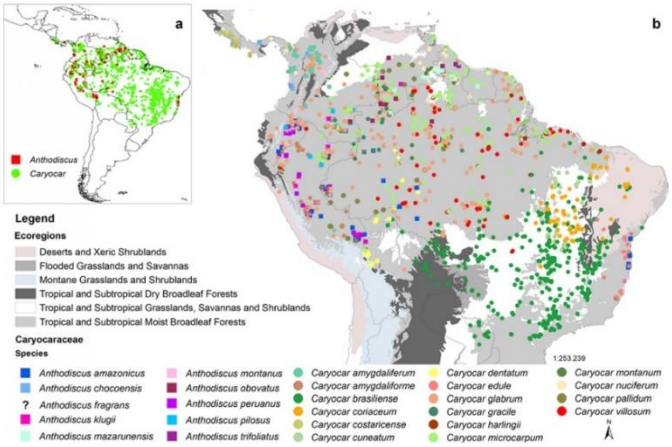
José Weverton Almeida Bezrrra - Aluno Curso de Biologia <weverton.almeida@urca.br>

Solicitação

3 mensagens

José Weverton Almeida Bezerra <weverton.almeida@urca.br> 4 de março de 2022 às 11:44
Para: rhewter@gmail.com

Caro professor Rhewter Nunes, estou entrando em contato com o senhor a fim de solicitar a permissão para usar a imagem anexa abaixo na revisão de literatura de minha tese, a qual trabalho com a espécie *Caryocar coriaceum*. A mesma será referenciada conforme o artigo "Caryocaraceae Voigt (Malpighiales): a Synthesis Based on Science Mapping and Systematic Review".
Desde já agradeço a sua atenção.



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Prof. José Weverton Almeida Bezerra
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Graduado em Ciências Biológicas - URCA
Universidade Regional do Cariri- URCA
Lattes
Orcid: 0000-0002-0966-9750

Rhewter Nunes <rhewter@gmail.com> 5 de março de 2022 às 13:07
Para: José Weverton Almeida Bezerra <weverton.almeida@urca.br>

Olá José Weverton, boa tarde.

Fique a vontade para utilizar desde referencie nosso paper como fonte da figura.

Abraços,
Rhewter.
[Texto das mensagens anteriores oculto]