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LAIS LUDMILA DE ALBUQUERQUE NERYS

**ATIVIDADES FOTOPROTETORAS, BIOLÓGICAS E COMPOSIÇÃO QUÍMICA
DO EXTRATO HIDROALCÓOLICO DE *Clarisia racemosa* COM APLICAÇÕES
COSMÉTICA E FARMACÊUTICA**

Recife

2022

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Tese apresentada ao Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal de Pernambuco, Centro de Ciências da Saúde, como requisito parcial para obtenção do título de doutor em Ciências Farmacêuticas. Área de concentração: Fármacos e Medicamentos.

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Dedico este trabalho aos amores da minha vida que do céu vão iluminando e abençoando meu caminho; minhas inspirações de força e fé: meus avós Antonio, Maria, Risoleta e painho Ronaldo Nerys (se dois corações eu tivesse só pra estar mais um dia com vocês, três eu daria).

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Muito obrigada

Laís Ludmila de Albuquerque Nerys

"Menor que meu sonho não posso ser."

(Bell, 1984, p. 128)

RESUMO

Clarisia racemosa Ruiz e Pav (Moraceae), popularmente conhecida como: “catruz”, “guariúba”, “guariúba-amarela”, é um arbusto que se desenvolve neotropical encontrada em florestas úmidas do Sul do México ao Sul do Brasil. Com o objetivo de avaliar o potencial fitoquímico e farmacológico da espécie, buscou-se avaliar o perfil fitoquímico do extrato hidroalcoólico da madeira do caule de *C. racemosa* e suas atividades biológicas. A análise da composição físico-química da madeira do caule mostrou teores de extrativos, hemicelulose, lignina e cinzas que foram de 10,7, 18,34, 29,40 e 0,79%, respectivamente. Através do estudo fitoquímico pode-se quantificar os teores de fenóis, flavonoides, flavonóis e taninos totais os quais apresentaram resultados significativos, estando em concordância com os resultados das atividades antioxidantes (DPPH•, ABTS⁺, HO•, NO •, fosfomolibdênio e redução de ferro). A atividade antimicrobiana mostrou-se moderada para bactérias (CMI = 512 µL/mL) e ineficaz para as leveduras. Os experimentos *in vitro* realizados em culturas de células de macrófagos J774 e eritrócitos murinos demonstraram que o extrato não possui potencial citotóxico nas concentrações estudadas que variaram de 3,9 a 500 µg/mL. Avaliando o fator de proteção solar, o extrato hidroalcoólico da madeira do caule de *C. racemosa* apresentou potencial de fotoproteção da radiação ultravioleta com variação de FPS igual 8. Os resultados obtidos, preliminarmente, revelam que o extrato hidroalcoólico de *C. racemosa* apresenta atividade promissora como antioxidante natural e fotoprotetora sem oferecer danos citotóxicos e reforçando o uso dessa planta para obtenção de informações relevantes para o desenvolvimento de novos fármacos.

Palavras chaves: Extrato vegetal; fitoquímica; antimicrobiano; antioxidante; citotocidade.

ABSTRACT

Clarisia racemosa Ruiz and Pav (Moraceae), popularly known as: “catruz”, “guariúba”, “guariúba-amarela”, is a neotropical shrub found in humid forests from southern Mexico to southern Brazil. In order to evaluate the phytochemical and pharmacological potential of the species, we sought to evaluate the phytochemical profile of the hydroalcoholic extract of wood from the stem of *C. racemosa* and its biological activities. The analysis of the physicochemical composition of the stem wood showed extractives, hemicellulose, lignin and ash contents that were 10.7, 18.34, 29.40 and 0.79 %, respectively. Through the phytochemical study, it is possible to quantify the levels of phenols, flavonoids, flavonols and total tannins, which presented significant results, in agreement with the results of antioxidant activities (DPPH•, ABTS+, HO•, NO•, phosphomolybdenum and reduction of iron). The antimicrobial activity was moderate for bacteria (CMI = 512 µL/mL) and ineffective for yeasts. In vitro experiments carried out in cell cultures of J774 macrophages and murine erythrocytes showed that the extract does not have cytotoxic potential at the concentrations studied, which ranged from 3.9 to 500 µg/mL. Evaluating the sun protection factor, the hydroalcoholic extract of *C. racemosa* stem wood showed photoprotection potential from ultraviolet radiation with a variation of SPF equal to 8. The results obtained, preliminarily, reveal that the hydroalcoholic extract of *C. racemosa* has promising activity as a natural antioxidant and photoprotector without causing cytotoxic damage and reinforcing the use of this plant to obtain relevant information for the development of new drugs.

Keywords: plant extract; phytochemistry; antimicrobial; antioxidant; cytotoxicity.

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1 INTRODUÇÃO

A utilização de plantas para fins medicinais é uma das práticas mais antigas da humanidade, iniciada nos primórdios da civilização e presente até hoje não apenas com finalidade de cura, mas para garantir uma melhor qualidade de vida (CRAGG; NEWMAN, 2013; DINIZ et al. 2018). De acordo com a Organização Mundial de Saúde (OMS), cerca de 85% da população mundial fazem uso de plantas medicinais como uma alternativa terapêutica e, por vezes, como único meio disponível para tratamento de alguma enfermidade (CHAKRABORTY, 2018; OMS, 2017; WANDERLEY et al. 2015).

Apesar da diversidade de organismos existentes (microrganismos, fungos, insetos e organismos marinhos) e dos avanços obtidos no campo medicinal e tecnológico, as plantas ainda constituem a principal matéria-prima para obtenção de compostos biologicamente ativos e modelos para síntese de novas substâncias de natureza sintética. Estes, por sua vez, possuem atividades biológicas diversas, como: atividade antibacteriana, antifúngica, anticancerígena, entre outros (COSTA-LOTUFO et al.2010; CRAGG; NEWMAN, 2013).

Os compostos bioativos das plantas podem interagir, de forma sinérgica ou isolada podendo promover diferentes atividades biológicas, como antibacteriana, antitumoral, antimalárica, etc. Estes compostos podem reduzir ou mesmo inibir o desenvolvimento de doenças como é o caso do câncer a exemplificar a camptotecina e Taxol potentes antineoplásicos. A baixa toxicidade e os baixos efeitos adversos são características importantes que tem levado ao aumento de pesquisas relacionadas a descoberta, isolamento e aplicação destes compostos bioativos. Sendo assim, o mercado vem sendo impulsionado devido a obtenção e venda de diferentes fitoterápicos (JAMSHIDI-KIA; LORIGOOINI; AMINI-KHOEI, 2018).

O Brasil possui um bioma vegetal com alta diversidade de espécies correspondendo a aproximadamente 15% de todas as espécies vivas do planeta que podem ser utilizadas como fontes de substâncias biologicamente ativas (VALLI; RUSSO; BOLZANI, 2018; MMA 2017). Podemos citar como exemplo, o alcalóide pilocarpina isolado de *Pilocarpus* sp (Rutaceae) isolado de uma espécie vegetal da biodiversidade brasileira e é usado no tratamento de xerostomia (HORIOT et al. 2000; VALLI; RUSSO; BOLZANI, 2018).

Dentre vegetação brasileira, destacam-se estudos com espécimes da família Moraceae, também chamada de família da amoreira ou família do figo, são plantas com flores que se desenvolve mem regiões tropicais e subtropicais (JUDD et al. 2009; SOMASHEKHAR; NAYEEM; SONNAD, 2013; THE PLANT LIST, 2013). Dentre as espécies relatadas na

literatura destaca-se a *Morus nigra* L devido as suas atividades biológicas, das quais podemos citar: anti-diabética, anti-hiperlipidêmica, anti-melanogênica, antiinflamatória, anticancerígena, antioxidante, antimicrobiana e organo-protetora (LIM; CHOI, 2019).

Na busca por maiores conhecimentos de plantas brasileiras e suas atividades biológicas, foi realizado o estudo das atividades biológicas do extrato hidroalcolico obtido dos caules de *Clarisia racemosa* Ruiz & Pav (Moraceae).

2 REVISÃO DE LITERATURA

2.1 PRODUTOS NATURAIS NO DESENVOLVIMENTO DE FÁRMACOS

O uso de produtos naturais para a prevenção, tratamento e cura de doenças é uma das práticas medicinais mais antigas realizadas pelo homem (CRAGG; NEWMAN, 2013). As plantas consideradas medicinais beneficiaram e ainda continuam beneficiando o homem, não só no tratamento de diversas enfermidades, além de serem utilizadas como elemento para descoberta e /ou produção de novos fármacos (BUENZ; VERPOORTE; BAUER, 2018; CRAGG; NEWMAN, 2013).

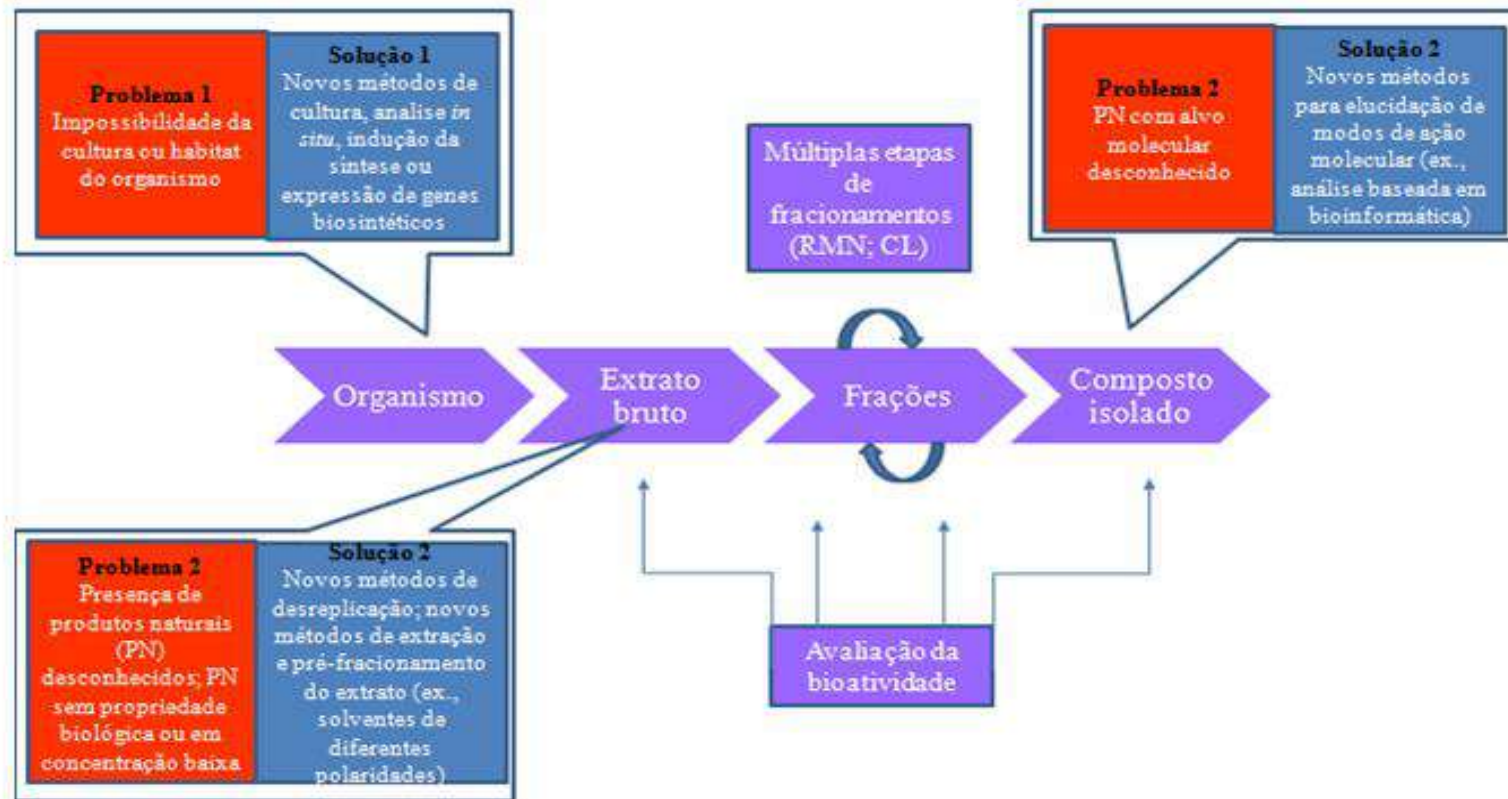
Na medicina popular a utilização de produtos naturais representa um recurso terapêutico de suma importância, visto que grande parcela da população vive em condições onde os cuidados médicos científicos e sanitários são precários (CALIXTO, 2000).

De um modo geral, os produtos naturais são responsáveis pela produção da maioria das substâncias orgânicas em uso terapêutico, contudo, é atribuído ao reino vegetal a maior parte da diversidade química dos metabólitos secundários, que só foram conhecidos e registrados na literatura graças aos aprimoramentos tecnológicos que reduziu os desafios relacionados ao processo de descoberta de medicamentos (Figura 1). Assim, foi reativado os interesses sociais e econômicos, estimulando pesquisa e desenvolvimento de fármacos (ATANASOV et al. 2021; BUENZ; VERPOORTE; BAUER, 2018; RIBEIRO et al. 2018).

O Brasil possui uma extensa flora, conhecida mundialmente, com exemplares vegetais considerados popularmente como medicinais, com princípios ativos responsáveis por efeitos terapêuticos, necessitando, contudo, de confirmação científica mais aprofundada e direcionada a uma aplicação específica (RIBEIRO et al., 2018; SANTOS et al., 2020; SILVA et al. 2021).

Dentre os vegetais, destacam-se espécimes da ordem Urticales, que apresentam propriedades emolientes e gastroprotetora, em função da mucilagem que as compõem, conforme relatos na literatura (UKU et al., 2020).

Figura 1 – Etapas do isolamento ao desenvolvimento de medicamentos de produtos naturais.

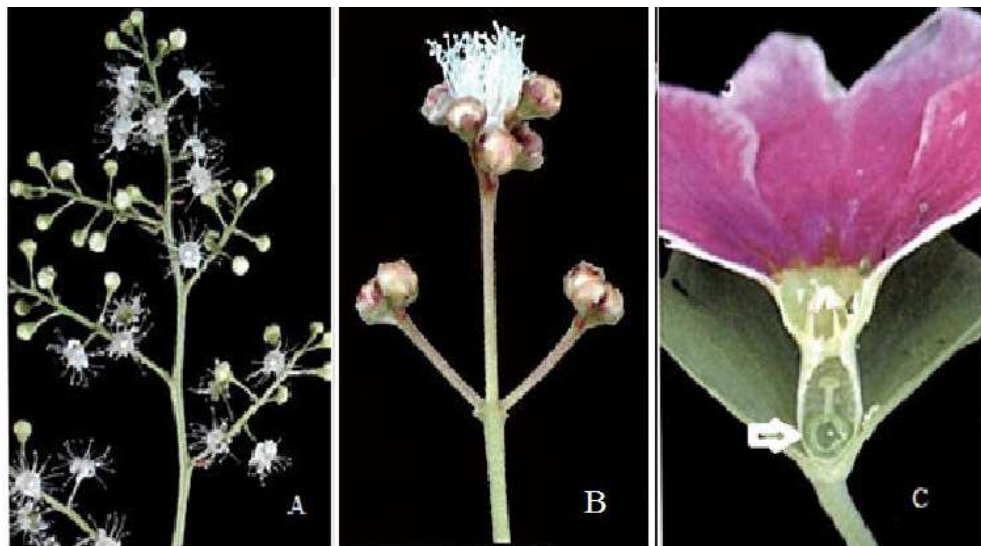


Em coloração roxa: etapas do processo; coloração vermelha: limitações de cada processo; coloração azul: soluções para cada limitações. RMN: Ressonância Magnética Nuclear; CL: Cromatografia Líquida. Fonte: Adaptado de Atanasov et al. (2021).

2.2 ORDEM URTICALES

A Ordem Urticales é composta por aproximadamente 2300 espécies de hábitos variados, sendo herbáceo, arbóreo e hemiepífitas mais comuns. As plantas representantes desta ordem possuem folhas inteiras, simples e alternadas, com estípulas ausentes ou presentes. As inflorescências podem apresentar-se cimosas ou racemosas, de flores aclamídeas ou monoclamídeas com gineceu de ovário súpero, formado por dois carpelos unilocular do ovário fértil e um só óvulo basal, apical ou lateral e fruto drupa (Figura 2). Outra característica dessas plantas é a presença de células secretoras de conteúdo laticífero ou mucilaginoso distribuídos por todas as partes da planta (CARAUTA, 1980; CRONQUIST, 1981; JOLY, 1998).

Figura 2 - Representação de inflorescência racemosa (A), cimosas (B) e ovário súpero (C).



Inflorescência racemosa: as flores pedunculadas inseridas ao longo de um eixo central alongado; inflorescência cimosas: o eixo principal termina com uma flor; ovário súpero: peças florais estão inseridas abaixo do ovário. Fonte: Gonçalves; Lorenzi (2007).

Os representantes da ordem Urticales podem ser encontrados nas zonas temperadas do Hemisfério Norte até subtropicais, na região central entre a Ásia e a Austrália, estendendo-se de Leste a Oeste na África e nas Américas Central e do Sul. No Brasil os 38 gêneros presentes estão distribuídos pelas famílias Ulmaceae, Cannabaceae, Urticaceae e Moraceae (CARAUTA, 1980; CRONQUIST, 1981; JOLY, 1998).

2.3 FAMÍLIA MORACEAE

A família Moraceae apresenta cerca de 40 gêneros e mais de 1000 espécies encontradas, em sua maioria, em regiões tropicais e subtropicais (JUDD et al. 2009; SOMASHEKHAR; NAYEEM; SONNAD, 2013; THE PLANT LIST, 2013). Podem ser encontradas como arbusto, árvore ou subarbusto com folhas alternadas monóicas ou dióicas. As sementes podem apresentar endosperma ou não. Uma das características da família Moraceae é a produção de látex, aplicada na indústria de borracha e a madeira que são utilizadas na indústria civil, mobiliária e na indústria têxtil (CORADIN et al. 2010; ROMANIUC-NETO et al., 2013; SOUZA; LORENZI 2005).

De acordo com Romaniuc-Neto e colaboradores (2013), no Brasil há 19 gêneros de Moraceae, 201 espécies e 23 subespécies em todo o território brasileiro, principalmente na região norte com destaque para os gêneros *Ficus*, *Dorstenia* e *Brosimum* (Figura 3) (JOLY, 2002; ROMANIUC-NETO et al., 2013). Espécies do gênero *Ficus* é o maior gênero da família Moraceae, com aproximadamente 800 espécies nas regiões tropicais e subtropicais no Brasil. Devido ao seu grande porte e beleza são usadas para fins ornamentais. Algumas espécies (*F. benghalensis*, *F. religiosa*, *F. glumosa*) demonstraram através de testes *in vivo* potencial antidiabético (DEEPA et al., 2018; PELISSARI; NETO, 2013).

As espécies gênero *Dorstenia* são usadas para obtenção de extratos obtidos por decocção com propriedades antiofídicas, anti-infecciosas e antirreumáticas em comunidades de países da África, América do Sul e América Central (ABEGAZ et al, 2002).

Já o gênero *Brosimum* evidencia-se por possuírem árvores de grande porte, podendo alcançar até quarenta metros de altura, pela madeira de grande interesse a indústria madeireira e pela produção de látex. O exsudato oriundo de *B. parinarioides*, por exemplo, é utilizado em algumas comunidades como substituto do leite bovino na alimentação humana em período de escassez (LIMA et al., 2013; ROMANIUC-NETO et al., 2012).

Figura 3 - Representantes dos gêneros *Ficus* (A), *Dorstenia* (B) e *Brosimum* (C) respectivamente.



Fonte: Flora do Brasil (2020).

O interesse no estudo de plantas desta família deu-se pela presença de furanocumarinas, compostos poli-heterocíclicos derivados da cumarina, com propriedades antimutagênico, antiretroviral e de uso na fotoquimioterapia e terapia fotodinâmica para o tratamento de psoríase e o vitiligo (ALAM et al. 2015; DALL' ACQUA, 1991; PIO, 1974). Além de furanocumarinas, os triterpenos, que representam 30% das substâncias predominantemente encontradas nas plantas pertencentes à família Moraceae, também se tornaram objetos de estudo, por apresentarem atividades antivirais e anticancerígenas. O ácido ursólico, por exemplo, é ativo em diferentes tipos de vírus como: herpes, adenovírus, *Chlorella* sp. e enterovírus 7-1, além de ser ativo contra linhagens de células leucêmicas (ALMEIDA et al., 2012; DZUBACK et al. 2006; PEREIRA, 1991).

2.4 GÊNERO *Clarisia* spp E ESPÉCIE *Clarisia racemosa*

O gênero *Clarisia* recebeu este nome em homenagem ao biólogo espanhol Miguel Barnades Clares e é constituído por 3 espécies, *C. biflora*, *C. ilicifolia* e *C. racemosa*, distribuídas, respectivamente, na América tropical e nas regiões sudeste, norte e centro-oeste do Brasil (CARAUTA, 1996).

Dentre as espécies do gênero destacaremos a espécie *C. racemosa* Ruiz & Pav. O enquadramento taxonômico de *C. racemosa*, segundo Cronquist (1981), baseia-se apenas em caracteres morfológicos, enquanto o de Engler, utiliza versão adaptada por Joly (1998) considerado mais moderno levando em consideração aspectos morfológicos, filogenéticos e a composição química vegetal (CRONQUIST, 1981; JOLY, 1998).

Quadro 1 – Enquadramento taxonômico de *Clarisia racemosa*.

	ENGLER (JOLY, 1998)	CRONQUIST (1981)
Divisão	Angiospermae	Magnoliophyta
Classe	Dicotyledoneae	Magnoliopsida
Subclasse	Archichlamydeae	Hamamelidae
Ordem	Urticales	Urticales
Família	Moraceae	Moraceae
Gênero	<i>Clarisia</i>	<i>Clarisia</i>
Espécie	<i>racemosa</i>	<i>racemosa</i>

Fonte: Joly (1998) e Cronquist (1981).

No Brasil a espécie pode ser encontrada como: “catruz”, “guariúba” e “oiti”. Na Bolívia é chamada de murere; na Colômbia, “arracacho”, “aji” e “caraco”; no Equador, “matapalo”, “moral-bobo” e “pituca”; e, no Peru como “capinuri”, “chichillica” e “turupay-amarillo” (COSTA, 1992; FERRAZ; VARELA, 2003; SANTOS, 2008).

C. racemosa é uma espécie neotropical encontrada em florestas úmidas do Sul do México ao Sul do Brasil. No Brasil, desenvolve-se bem em florestas com altitude variando entre 50 a 900 m com temperatura média anual de 23° C (COSTA, 1992; FERRAZ; VARELA, 2003; SANTOS, 2008). A Figura 4 apresenta a distribuição geográfica da espécie *C. racemosa*.

Figura 4 - Distribuição geográfica da espécie *Clarisia racemosa*.

Fonte: Melo (2015).

A árvore possui médio a grande porte, medindo até 40 m de altura e com 0,6-0,7 cm de diâmetro. O tronco é retilíneo e rico em tubos laticíferos. O córtex possui coloração castanho-escura a acinzentada, sendo vermelha internamente, e a superfície é verrucosa e estriada transversalmente, devido à presença de inúmeras lenticelas. As raízes também são de

tonalidade vermelha, facilmente reconhecíveis ao atingirem a superfície do solo. As folhas são alternas e simples, bastante variáveis em comprimento (4-15 cm), largura (2-6 cm) e formato (elíptica, ovalada ou oblonga) (SANTOS, 2008).

Os frutos de *C. racemosa* de coloração amarelada ou avermelhada servem de alimentação para diversos primatas e aves de médio e grande porte da floresta local. As sementes não se desenvolvem em ambientes secos. Assim como os frutos, as sementes que caem ao solo servem de alimento para fauna local que auxiliam na germinação ao enterrá-las. A Figura 5 apresenta as folhas, galhos e frutos pertencentes a espécie *C. racemosa*. (SANTOS, 2008).

Figura 5 – Arvore (A), caules folhas e frutos (B) de *C. racemosa*.



Fonte: <https://blog.brflor.com.br/clarisia-racemosa>; Santos (2009).

O vegetal destaca-se pela madeira de grande uso na indústria moveleira (compensados e laminados), na fabricação de instrumentos musicais (PAULA; ALVES, 1997), carpintaria, marcenaria, construção civil e naval e confecção de canoas (JANKOWSKY 1990; SANTOS, 2008). Dentre os estudos biológicos Santos et al. (2008) relataram a toxicidade do exsudato a cupins, revelando como potencial inseticida. Enquanto que a decocção do córtex foi utilizada para tratamento de doenças de pele e contra fungos de podridão da madeira (SANTOS, 2008).

Souza de Melo e Abreu (2014) avaliaram a toxicidade de extratos etanólicos das espécies vegetais *Brosimum rubescens*, *Dipteryx odorata* e *Clarisia racemosa* frente a *Artemia salina* e todos foram considerados tóxicos devido a seus respectivos valores de DL50.

Melo (2015) avaliou a atividade antineoplásica dos extratos e frações de *C. racemosa*. O extrato metanólico das folhas, a fração diclorometano e em acetato de etila das folhas e a fração hexânica da madeira apresentaram alta citotoxicidade frente à linhagem de células humanas do linfoma histiocítico. A fração diclorometânica destacou-se por apresentar resultados significativos frente as linhagens de células humanas adenocarcinoma de cólon, melanoma pouco metastático e células mononucleares do sangue periférico humano.

Em relação aos estudos químicos, Cunha, Pinto e Braz-Filho (1994) isolaram dois flavonoides, a artocarpina e isoartocarpina, das frações dos extratos hexânico e clorofórmico de *C. racemosa*. Ambos flavonoides possuem como propriedades biológicas atuando como anti-inflamatório, antioxidante, anticancerígeno e antimicrobiano, além de promoverem o aumento da deposição de colágeno, a reepitelização e a angiogênese acelerando o processo cicatricial (CHUNG JU et al, 2017; JAGTAP; BAPAT, 2010).

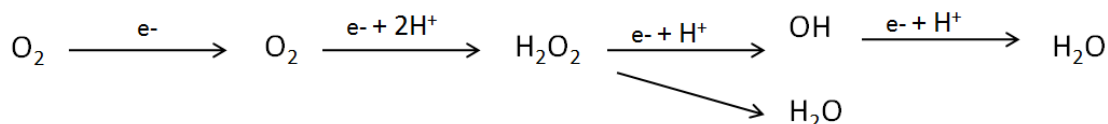
Apesar destes achados, ainda há pouco estudos relacionados as atividades farmacológicas e propriedades terapêuticas da espécie em questão.

2.5 ATIVIDADE BIOLOGICAS PROMOVIDAS POR DIFERENTES EXTRATOS DE PLANTAS

2.5.1 Atividade antioxidante *in vitro*

A denominação radical livre é utilizada para referir-se a um átomo ou molécula altamente reativo, que contém número ímpar de elétrons em sua última camada eletrônica, conferindo-lhes alta reatividade (Figura 6) (COTINGUIBA et al., 2013; MUNTEANU; APETREI, 2021; SILVA et al., 2010).

Figura 6- Reações químicas de formação de radicais livres.



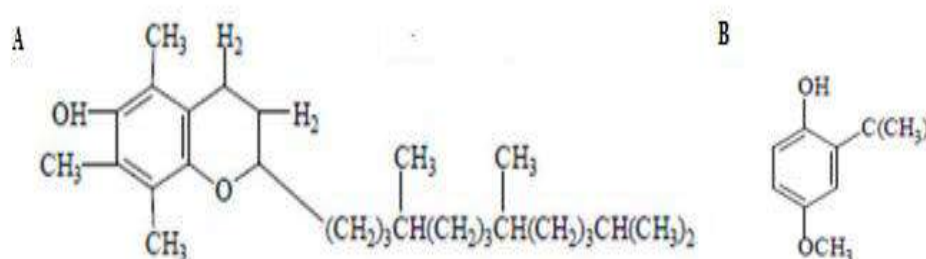
Fonte: Cotinguiba et al., (2013).

Os radicais livres podem ser gerados no citoplasma, nas mitocôndrias ou na membrana e o seu alvo celular (proteínas, lipídeos, carboidratos e DNA) está relacionado com o seu sítio de formação. A formação de radicais livres *in vivo* ocorre via ação catalítica de enzimas devido a fatores endógenos (respiração aeróbica, inflamações, peroxissomos, enzimas do citocromo P540) e pela exposição a fatores exógenos (ozônio, radiações, dieta, cigarro, etc.) (COTINGUIBA et al. 2013; MUNTEANU; APETREI, 2021; SILVA et al. 2010).

No entanto, a produção contínua de radicais livres durante os processos metabólicos leva ao estresse oxidativo acarretando danos celulares, comprometendo sua funcionalidade e a promoção no desenvolvimento de processos patológicos, como doenças cardiovasculares, neurológicas, alguns tipos de cânceres e envelhecimento precoce (MUNTEANU; APETREI, 2021; SILVA et al. 2010; VASQUES; FONSECA, 2018).

Uma substância antioxidante pode ser definida como uma substância química com capacidade de inibir o estresse oxidativo, quando presente em baixa concentração, sendo classificados como sintéticos ou naturais (SANTOS-SÁNCHEZ et al. 2019; SHAHIDI, F.; ZHONG, 2015; VASQUES; FONSECA, 2018). Os antioxidantes naturais são produzidos pelo próprio organismo ou ser oriundos da dieta, como as vitaminas, pigmentos naturais e metabólitos secundários. Já os sintéticos são caracterizados por apresentarem maior estabilidade e terem uma boa eficiência, como hidroxitoluenobutilado, β -hidroxiácido e terc-butil-hidroquinona (SANTOS-SÁNCHEZ et al. 2019; SHALABY; SHANAB, 2013). Na Figura 7 podemos observar exemplos de um antioxidantes natural e sintético

Figura 7 - Exemplos de antioxidantes naturais e sintéticos (A) o-tocoferol e (B) hidroxitoluenobutilado (BHA).



Fonte: Shalaby; Shanab (2013).

2.5.1.1 Antioxidantes Enzimáticos

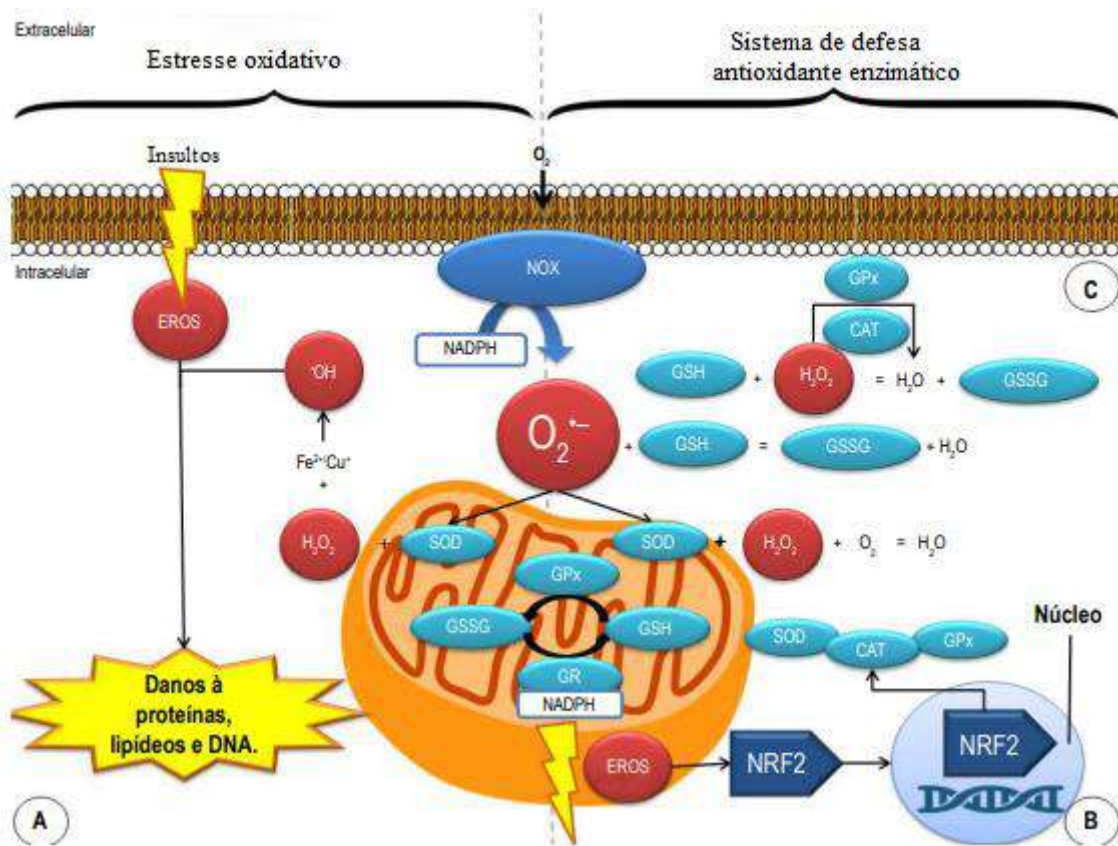
O estresse oxidativo tem seus danos minimizados pela ação do sistema de defesa enzimático. Esse sistema é composto por proteínas localizadas no interior das células que agem tanto na prevenção da oxidação gerada pelos radicais livres quanto na reparação dos danos causados pelos mesmos (FERREIRA; MATSUBARA, 1997; SANTOS-SÁNCHEZ et al., 2019; SHAHIDI; ZHONG, 2015).

Entre as principais enzimas antioxidantes estão a Superóxido Dismutase (SOD), Catalase (CAT) e Glutathione peroxidase. As enzimas SOD são metaloenzimas cuja principal é promover a dismutação do superóxido em peróxido de hidrogênio e oxigênio molecular (MUNTEANU; APETREI, 2021; PERRY et al., 2010; SARIKAYA; DOĞAN, 2020). Elas estão presentes essencialmente em todas as células do corpo e atualmente existem em três isoformas: isoforma Cu/ZnSOD (ou SOD1) dependente de cobre e zinco atuando no citosol, a isoforma MnSOD (ou SOD2) dependente de manganês de ação mitocondrial e a isoforma ECSOD (ou SOD3) que age no espaço extracelular (PERRY et al., 2010; SARIKAYA; DOĞAN, 2020).

A catalase é uma enzima homotetrâmera localizada principalmente nos peroxissomos das células de mamíferos, porém pode ser expressa em todos os órgãos do corpo, especialmente no fígado, nos rins, e nos eritrócitos (GLORIEUX; CALDERON, 2017; SANTOS-SÁNCHEZ et al., 2019). As catalases são classificadas em três grupos com base em sua estrutura e função sendo o primeiro e o segundo grupo de enzimas contendo grupamento heme (catalases típicas ou verdadeiras) e catalase-peroxidases, enquanto o terceiro grupo contém catalases de manganês (não heme) (GLORIEUX; CALDERON, 2017). A enzima atua evitando o acúmulo de peróxido de hidrogênio (H_2O_2) e sua presença em baixos níveis da expressão está relacionado a uma alta produção de H_2O_2 , o qual está envolvido na ativação de vias de sinalização para induzir a proliferação e invasão em células cancerosas (GLORIEUX; CALDERON, 2017; SARIKAYA; DOĞAN, 2020).

A glutathione-peroxidase (GSH-Px) é uma enzima tetramérica com um resíduo de seleno-cisteína incorporado no seu sítio ativo com capacidade de inativar H_2O_2 e peróxidos lipídicos pela redução da glutathione. Assim, protege os lipídios da membrana e a hemoglobina da oxidação dos peróxidos além de participar da desintoxicação de xenobióticos. A Figura 8 mostra esquematicamente o sistema formado pelas enzimas descritas (SARIKAYA; DOĞAN, 2020).

Figura 8 - Mecanismos de produção e controle de EROS intracelular.



Estresse oxidativo e sistema de defesa antioxidante enzimático em células renais diabéticas. CAT: catalase; EROS: espécies reativas de oxigênio; GPx: glutathione peroxidase; GSH: glutathione; GSSG: glutathione oxidada; GR: glutathione reduzida; H_2O_2 : peróxido de hidrogênio; NRF2: fator nuclear fator 2 relacionado ao eritróide 2; O_2 : oxigênio molecular; NOX: NADPH oxidase; $O_2^{\bullet-}$: ânion radical superóxido; $\bullet OH$: radical hidroxila; SOD: superóxido dismutase. Fonte: Amorim et al. (2019).

2.5.1.2 Antioxidantes não-enzimáticos

Os antioxidantes não enzimáticos podem ser de origem endógena ou exógena, inclui, especialmente, os compostos antioxidantes de origem dietética, entre os quais se destacam: vitaminas e compostos fenólicos. Independente da origem, tais substâncias apresentam uma elevada capacidade de defesa, atuando na neutralização dos radicais livres e no bloqueio das ações de espécies reativas, principalmente a de oxigênio, dentre estes existem as diferentes vitaminas (SANTOS-SÁNCHEZ et al., 2019; SARIKAYA; DOĞAN, 2020).

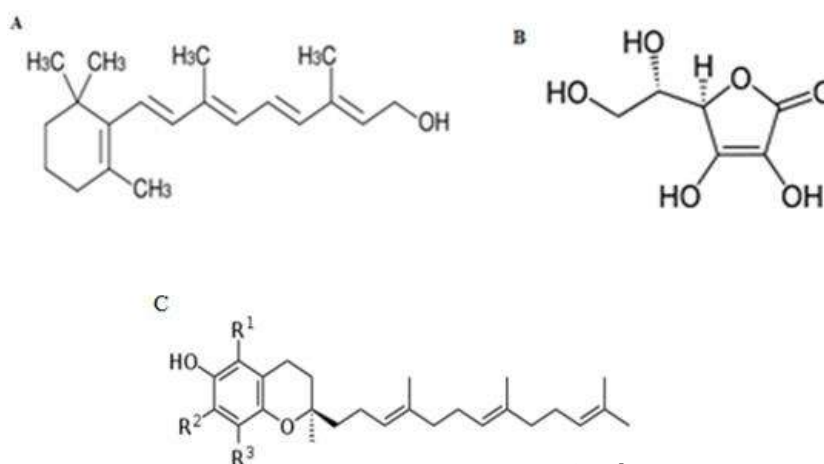
A vitamina A é uma vitamina lipossolúvel obtida de forma indireta a partir da dieta de origem animal. Nos vegetais é encontrada na forma de uma provitamina A ou carotenoides. A

vitamina e sua forma pró age desativando o oxigênio singlete ou sequestrando os radicais peroxila, reduzindo a oxidação do DNA e lipídios, que estão associados a doenças degenerativas. Além disso, a vitamina A participa do processo de formação da pele, unhas e cabelo, na queratinização e estimula a microcirculação cutânea. Dentre os carotenoides o mais importante é o β -caroteno e sua estrutura sofre uma clivagem para formar a vitamina A1, o retinol (ASLAM et al. 2017; KHADIM; AL-FARTUSIE, 2021).

A vitamina C (ácido L-ascórbico) é uma vitamina hidrossolúvel encontrada em nosso organismo na forma de ascorbato, sendo obtida a partir da dieta. Devido ao seu baixo peso molecular, é capaz de neutralizar EROS por transferência de elétrons ao inibir a peroxidação lipídica. Além de ser um poderoso antioxidante, a vitamina C atua promovendo regeneração da vitamina E, α -tocoferol, ao doar hidrogênio da sua estrutura na interface da membrana celular (ASLAM et al. 2017; KHADIM; AL-FARTUSIE, 22021).

A vitamina E, termo genérico para designar 8 compostos lipossolúveis naturais (tocoferóis) que apresentam a mesma atividade biológica, agindo como doadores de H^+ para o radical peroxila, interrompendo a reação radicalar em cadeia. Estudos também relatam sua ação contra danos ao DNA e na prevenção do envelhecimento precoce. A alta atividade dos tocoferóis contra a oxidação de lipídeos se explica por ser lipossolúvel atua protegendo a membrana celular ao proteger os ácidos graxos poli-insaturados da oxidação (ASLAM et al, 2017; KHADIM; AL-FARTUSIE, 2021). Na Figura 9 podemos observar as estruturas das vitaminas citadas.

Figura 9 - Estruturas químicas das vitaminas A (A), vitamina C (B) e vitamina E (C).



α -Tocotrienol: R1 = Me, R2 = Me, R3 = Me

β -Tocotrienol: R1 = Me, R2 = H, R3 = Me

γ -Tocotrienol: R1 = H, R2 = Me, R3 = Me

Fonte:Khadin; Al-Fartusie (2021).

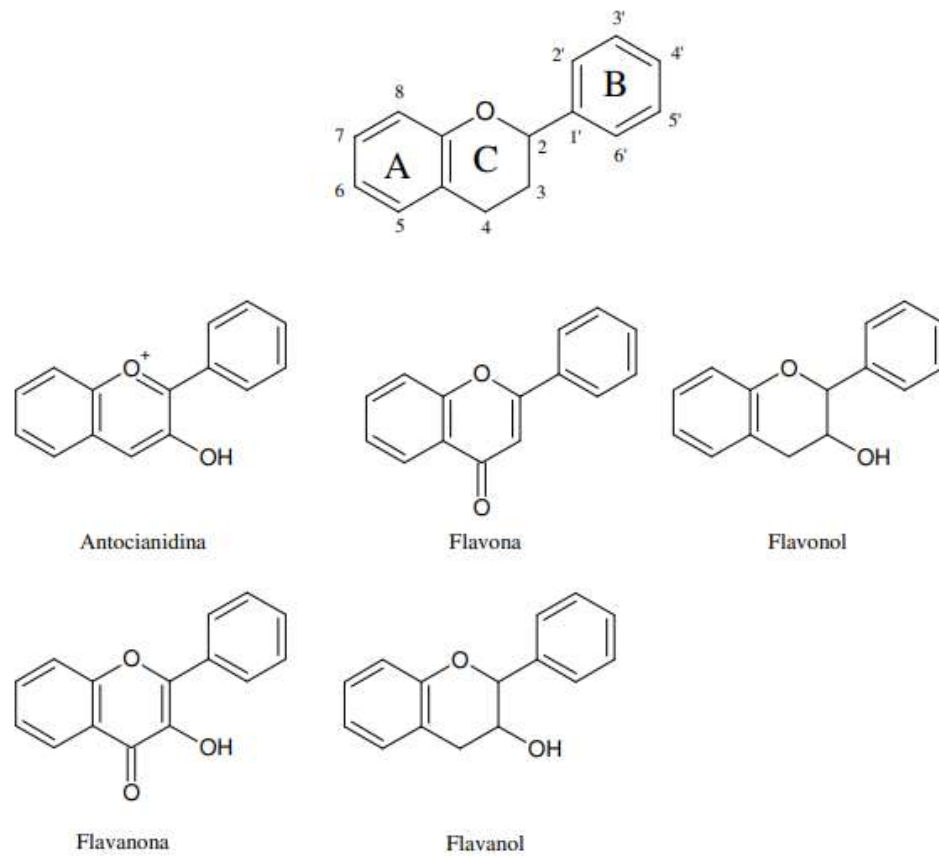
A) Compostos fenólicos.

Os compostos fenólicos são substâncias mais proeminente de compostos bioativos presentes em várias fontes vegetais. Eles são responsáveis pelas características organolépticas dos vegetais como também pela sua coloração (pigmentos). Os compostos fenólicos apresentam, em sua estrutura, vários grupos benzênicos característicos, tendo como substituintes grupamentos hidroxilas(KAURINOVIC; VASTAG, 2019; KUMAR; GOEL, 2019; RASHMI; NEGI, 2020).

Esta classe de compostos apresenta uma grande diversidade e divide-se em flavonoides (polifenóis) e não-flavonoides (fenóis simples ou ácidos). Os flavonoides compreendem um grupo de compostos fenólicos amplamente distribuídos nas frutas e nos vegetais, apresentando-se sob muitas variações como flavonóis, flavonas, flavanonas, catequinas, antocianinas, isoflavonas e chalconas. Na Figura10 podemos observar alguns exemplos das variações dos compostos fenólicos (KAURINOVIC; VASTAG, 2019; KUMAR; GOEL, 2019; RASHMI; NEGI, 2020).

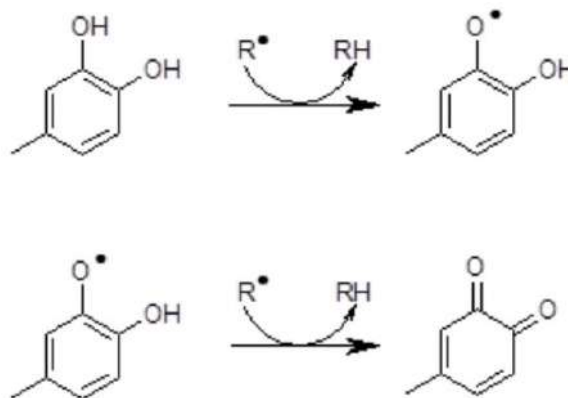
A configuração dos grupos hidroxilas do anel B consiste no fator mais significativo para o sequestro das espécies reativas e consequente o aumento da atividade antioxidante. Desta forma o grau de hidroxilação e a posição dos grupos hidroxilas no anel B tende a promover uma maior ação antioxidante uma vez que possui uma melhor capacidade de doação de elétrons através de uma deslocalização eletrônica (Figura 11), sendo assim o local ideal para a ligação dos metais de transição (BALASUNDRAM; SUNDRAM; SAMMAN, 2006; HEIM; TAGLIAFERRO; BOBILY, 2002).

Figura 10 - Estrutura de um composto fenólico e suas variações estruturais.



Fonte: Balasundram; Sundram; Samman (2006).

Figura 11- Mecanismo de remoção de radicais pelos flavonoides.



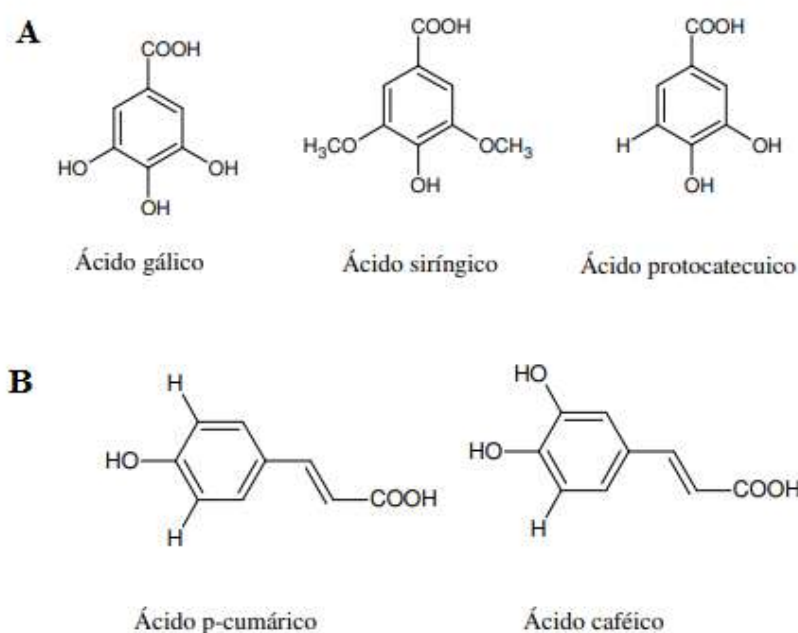
Fonte: Adaptado de Seabra et al. (2006).

Já a classe dos não-flavonoides estão os derivados dos ácidos hidroxicinâmico e hidroxibenzoico, por causa de suas duas estruturas de carbono distintas, bem como dependendo do posicionamento e do número de grupos hidroxila no anel aromático. Os ácidos hidroxicinâmicos são os derivados de ácidos cinâmicos apresentando a estrutura C6-C3. Entre os ácidos hidroxicinâmicos e seus derivados, são ferúlico, ácidos cafeico, p-cumárico e sinápico são os mais comuns, sendo o ácido clorogênico, o mais abundante (KAURINOVIC; VASTAG, 2019; KUMAR; GOEL, 2019; RASHMI; NEGI, 2020).

Os ácidos hidroxibenzoicos de forma livre são produtos da hidrólise química ou enzimática durante a extração. Os quatro ácidos hidroxibenzoicos comumente encontrados são os ácidos p-hidroxibenzoico, protocatecuico, vanílico e siringico (KAURINOVIC; VASTAG, 2019; KUMAR; GOEL, 2019; RASHMI; NEGI, 2020).

Ácidos hidroxicinâmicos exibem maior capacidade antioxidante quando comparado aos ácidos hidroxibenzoicos correspondente e pode estar relacionado ao grupo CH-CH-COOH, que garante maior capacidade de doação de H^+ e estabilização de radical do que o grupo -COOH nos ácidos hidroxibenzoicos (Figura 12) (BALASUNDRAM; SUNDRAM; SAMMAN, 2006).

Figura 12 - Alguns dos principais ácidos hidroxibenzoicos (A) e hidroxicinâmicos (B).



Fonte: Balasundram; Sundram; Samman (2006).

O nível de intensidade das atividades biológicas demonstradas pelos ácidos fenólicos está relacionado a sua biodisponibilidade. A qual determinará grau de absorção, digestão e metabolismo após entrar no sistema circulatório, podendo estes sofrer alterações em resposta aos fatores exógenos (a complexidade da matriz alimentícia, a forma química do composto de interesse) e endógenos (tempo de trânsito intestinal, possíveis interações com proteínas na circulação sanguínea) (BALASUNDRAM; SUNDRAM; SAMMAN, 2006; OLIVEIRA; BASTOS, 2011).

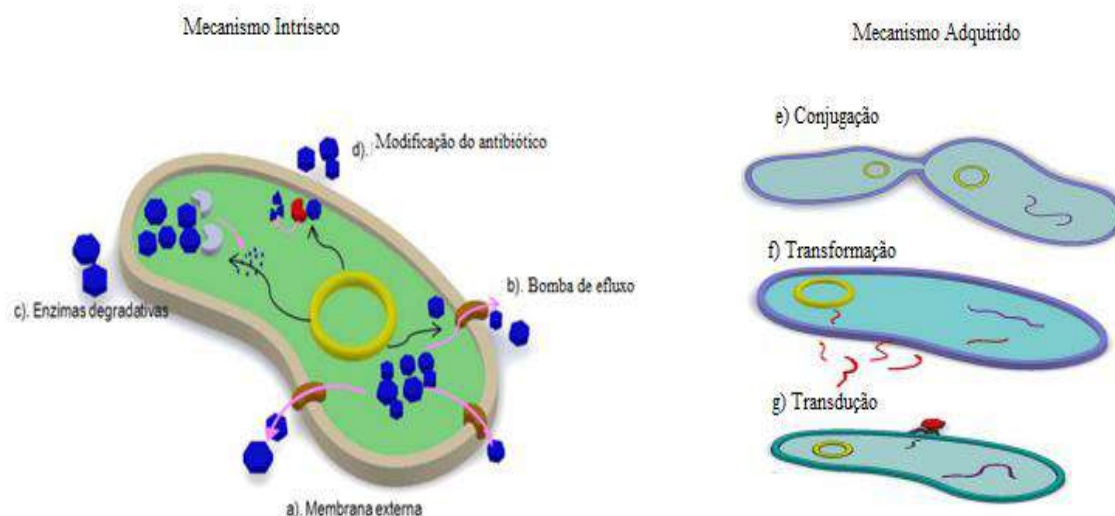
Na literatura podemos encontrar relatos científicos diversos que inferem o papel protetor dos ácidos fenólicos em doenças degenerativas, podendo ser citadas: doenças cardiovasculares, câncer, diabetes e inflamação (MACHADO et al., 2008).

2.5.2 Atividade antimicrobiana *in vitro*

Ao longo dos anos o uso indiscriminado de antibióticos levou a disseminação de microrganismos resistentes pondo em risco a eficácia da prevenção e do tratamento de um número cada vez maior de infecções por vírus, bactérias, fungos e parasitas (LOERA-VALENZUELA et al., 2016). Como resultado, houve um aumento crescente no desenvolvimento de cepas de bactérias resistentes, tornando o tratamento do que seria uma simples infecção mais onerosa (BANIN; HUGHES; KUIPERS, 2017; DA SILVA; AQUINO, 2018).

A resistência bacteriana pode ser classificada como intrínseca, onde os mecanismos de resistência ocorrem de forma natural em um gênero ou espécie bacteriana, ou adquirida, através de modificações genéticas ou aquisição de genes de resistência de outras bactérias através da conjugação, transdução ou transformação (Figura 12) (DZIDIC; SUSKOVIC; KOS, 2008; LOERA-VALENZUELA et al., 2016).

Figura 13 - Mecanismos de resistência microbiana intrínseca e adquirida.



Fonte: Loera-Valenzuela et al. (2016).

Destes citados, os mecanismos bioquímicos intrínsecos de resistência aos antibióticos reportados na literatura são:

- a) Alteração da permeabilidade da membrana: impedindo a difusão do fármaco através da alteração da estabilidade da membrana;
- b) Inativação enzimática (enzimas degradativas): o fármaco é degradado e/ou inativado através de reações enzimáticas (hidrólise, transferência de um grupo químico);
- c) Bombas de efluxo: onde os antibióticos atravessam proteínas nas membranas passando do meio intracelular para extracelular por transporte ativo;
- d) Modificação do antibiótico: modificações estruturais no alvo bacteriano evitando a ligação entre o mesmo e o antibiótico (DZIDIC; SUSKOVIC; KOS, 2008; LOERA-VALENZUELA et al., 2016).

A identificação de novos agentes que atuem fora dos mecanismos já existentes mostra-se uma solução capaz de reverter o problema relacionado a resistência aos medicamentos atuais. (DA SILVA; AQUINO, 2018; LOERA-VALENZUELA et al., 2018). Os vegetais possuem compostos múltiplos com potenciais benéficos para o desenvolvimento de novos antibióticos biocompatíveis, podendo controlar o mecanismo de resistência microbiana e trazer benefícios globais para a saúde (ANAND et al., 2019; LOERA-VALENZUELA et al., 2018). A Tabela 1 traz alguns exemplos de extratos vegetais com atividade antimicrobiana.

Tabela 1 - Exemplos de extratos vegetais com atividades antimicrobianas.

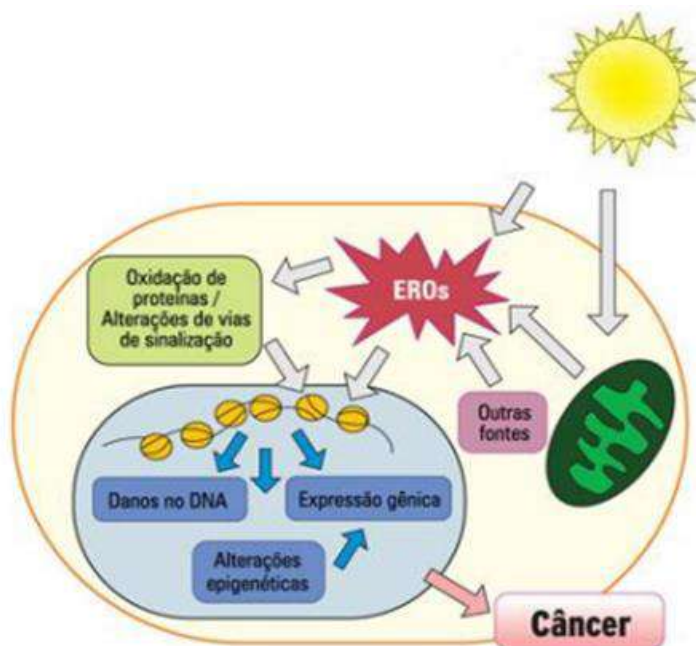
Planta	Tipo de extrato	Microrganismo	Referência
<i>Morus alba</i> L.	Etanólico (folhas)	<i>Staphylococcus aureus</i>	Sousa et al. (2020)
<i>Psidium cattleianum</i> Sabine	Hidroalcoólico (frutos)	<i>S. aureus</i>	Lima et al. (2020)
<i>Caesalpinia pulcherrima</i> (L.) Swartz	Salino (folhas)	<i>Candida albicans</i> , <i>C. parapsilosis</i> , <i>C. tropicalis</i>	Aguiar et al. (2019)
<i>Ficus vasta</i>	Hidroalcoólico (folhas)	<i>Escherichia coli</i>	Taviano et al. (2018)
<i>Genipa americana</i> L., <i>Dipteryx alata</i> Vog. e <i>Vitex cymosa</i> Bert.	Hidroalcoólico (casca, polpa e sementes, respectivamente)	<i>S. aureus</i> , <i>E. coli</i> , <i>Pseudomonas aeruginosa</i> , <i>C. albicans</i>	Santos, Ramos, Miyagaku (2017)
<i>Juglans regia</i> L.	Hidroalcoólico (casca do caule)	<i>S. aureus</i>	Bakhtiari et al. (2016)
<i>Mentha longifolia</i> L e <i>Zataria multiflora</i> Boiss	Acetoetilico aquoso e hidroalcoólico (folhas)	<i>P. aeruginosa</i> , <i>S. aureus</i> , <i>Shigella dysenteriae</i> , <i>Klebsiella pneumoniae</i> , <i>Salmonella typhi</i> , <i>Proteus mirabilis</i> , <i>Serratia marcescens</i> , <i>Enterobacter cloacae</i> , <i>Bacillus cereus</i> , <i>S. saprophyticus</i>	Saeide et al. (2014)
<i>Cannabis sativa</i>	Hidroalcoólico	<i>S. aureus</i> , <i>E. coli</i> , <i>P. aeruginosa</i> , <i>K. pneumoniae</i>	Sarmadyan et al. (2014)
<i>Astrocaryum aculeatum</i>	Etanólico (polpa e casca)	<i>E. faecalis</i> , <i>B. cereus</i> , <i>Listeria monocytogenes</i> , <i>C. albicans</i>	Jobim et al. (2014)

Fonte: Autor (2022).

2.5.3 Atividade fotoprotetora *in vitro*

As radiações solares são fontes necessárias para diversos processos biológicos nos seres vivos (humano, plantas e animais), no entanto, elas também podem causar sérios danos à pele humana em resposta a exposição excessiva. A radiação ultravioleta (UV) gera reações fotoquímicas ao interagir de forma direta, com substratos moleculares como o DNA ou indiretamente, levando a produção de espécies reativas de oxigênio (EROs) e, conseqüentemente, promovendo o desenvolvimento de patologias diversas como as queimaduras, envelhecimento precoce e até mesmo o câncer (Figura 14) (ARRUDA et al. 2021).

Figura 14 - Resposta celular aos efeitos das Espécies Reativas de Oxigênio (EROs).



Fonte: Silva; Jasiulionis (2014).

Uma das formas de prevenir o desenvolvimento da doença e de outras patologias cutâneas gerados pela radiação inclui a redução a exposição solar, o uso de roupas adequadas e fotoprotetores. Os fotoprotetores, seja oriundo de substâncias naturais ou sintéticas, se encontram no grupo dos cosméticos com finalidade principal de proteger a pele contra a radiação. Seu mecanismo de ação varia de acordo com a composição do filtro, sendo este dividido em orgânicos e inorgânicos (ARRUDA et al., 2021; PRASANTH et al., 2020).

Os filtros orgânicos têm como característica absorver a radiação UV. Entre os filtros orgânicos, há os derivados de p-aminobenzoatos, salicilatos, cinamatos e cânfora com capacidade de absorção de UVB, e, os benzoenos, antranilatos e dibenzometanos com capacidade de absorção de UVA. Por apresentarem a capacidade de absorção em uma faixa estreita dos raios UV, é comum a associação de diferentes filtros para oferecer uma maior eficácia de proteção (JALLAD, 2016; NASCIMENTO et al., 2014). A Figura 15 apresenta alguns exemplos de filtros orgânicos.

Figura 15- Filtros solares orgânicos.

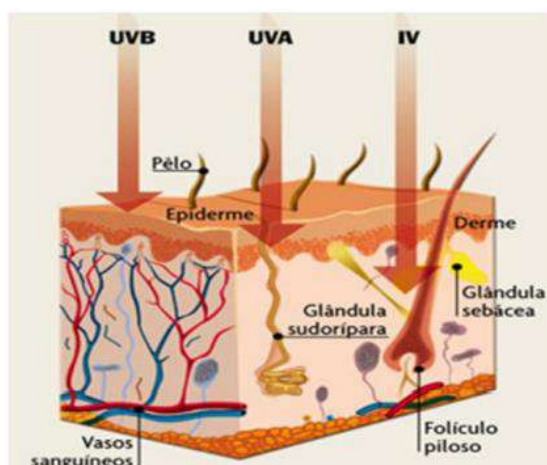


Fonte: Nascimento et al. (2014).

Já os filtros inorgânicos atuam como bloqueadores físicos refletindo a radiação. As substâncias mais comuns utilizadas nas formulações são dióxido de titânio e óxido de zinco. Uma característica destes filtros são a coloração de tonalidade opaca ou branca deixadas sobre a pele, o que é desfavorável esteticamente (NASCIMENTO et al., 2014).

Levando em consideração a importância na redução de riscos a pele e a saúde, a indústria farmacêutica tem explorado diferentes alternativas para formulação de novos fotoprotetores, adicionando a essas formas farmacêuticas compostos naturais que intensifiquem sua ação contra os raios UV, que é subdividida em radiação UVA (320-400nm), UVB (280-320nm) e UVC (100-280nm) de acordo com o comprimento de onda em que se encontra (ARRUDA et al., 2021; NASCIMENTO et al., 2014; PRASANTH et al., 2020) e sua capacidade de penetrar na derme, como mostrado na Figura 16.

Figura 16 - Capacidade de penetração da radiação ultravioleta na pele humana.



Fonte: Leger (2015).

Os organismos vivos são dotados de estruturas e/ou substâncias capazes de absorver a energia luminosa e, entre elas, a melanina pode ser citada como exemplo. A melanina é uma macromolécula derivada de quinonas e fenóis podendo ser encontrada tanto no homem como em insetos, fungos e bactérias respondendo a fotoproteção do DNA nuclear da radiação UVB (D'ISCHIA et al., 2015). Nas plantas, os compostos fenólicos, tais como derivados de ácido hidroxicinâmico, ácido p-cumárico, ferúlico e caféico, por conterem apenas um anel aromático, possuem uma banda de absorção na região UV, entre os comprimentos de onda 280 e 320 nm e, sendo assim, a maioria, atenuadores de UVB (ARRUDA et al., 2021; PRASANTH et al., 2020).

Dentre os produtos naturais, os extratos vegetais detêm o maior número de relatos na literatura que confirmam a atividade fotoprotetora. Os extratos de plantas possuem uma complexidade de moléculas que podem atuar, simultaneamente, intensificando a ação contra a radiação UV, como a ação antioxidante. Vale ressaltar que os fotoprotetores sintéticos podem causar efeitos adversos, tendo como exemplo, a irritação da pele, reações alérgicas de contato fazendo que os vegetais constituam uma alternativa promissora para o mercado de cosméticos voltados ao efeito fotoprotetor e rejuvenescedor (ARRUDA et al., 2021; PRASANTH et al., 2020).

2.5.4 Atividade citotóxica em células animais

Os produtos naturais, principalmente de vegetais e microrganismos, são utilizados como fonte de novos fármacos para o tratamento de diferentes combates de patologias que acometem o homem (COSTA-LOTUFO et al., 2010; CRAAG; NEWMAN, 2013).

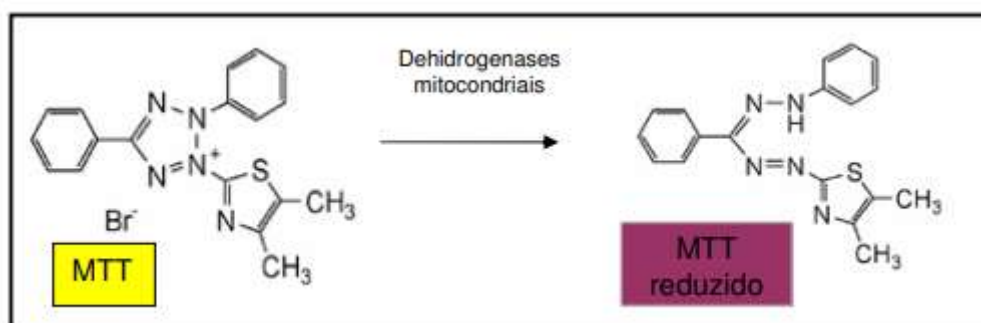
Por apresentarem metabólitos complexos, e alguns desconhecidos, a citotoxicidade *in vitro* é uma prática comum na avaliação biológica de produtos para uma análise inicial da biocompatibilidade dos mesmos. Além disso, através do teste é possível também avaliar a habilidade intrínseca do composto em causar alterações e/ou morte celular, como consequência de dano das funções celulares básicas após exposição ao determinado produto, podendo este ser descartado logo de início ou não eda necessidade da realização de ensaios em animais (ASLANTÜRK, 2018).

O composto dito como citotóxico é aquele que libera quantidade suficiente de elemento/ composto químico que causa a morte celular. A dose a ser absorvida capaz de ser citotóxica podem ser mensuradas por diferentes métodos diretos e indiretos em cultura celulares possibilitando, respectivamente, a avaliação qualitativa e quantitativa da presença e

severidade de efeito citotóxico ou apenas a avaliação qualitativa da citotoxicidade após ensaio (ASLANTÜRK, 2018; ALVES; GUIMARÃES, 2010).

Um dos métodos indiretos mais utilizados em estudos é o ensaio de redução do MTT (3- [4, 5-dimethyl-2-thiazolyl] -2, 5-diphenyl-2H-tetrazolium bromide, um sal de coloração amarela e solúvel em água) a formazan (sal de coloração púrpura e insolúvel em água) pela enzima mitocondrial succinato desidrogenase (Figura 17). Sendo assim, pela medição da atividade mitocondrial é possível estabelecer o número de células viáveis que está associada com a quantidade de cristais de formazan (MOSMANN, 1983).

Figura 17 - Redução do MTT por enzimas mitocondriais.



Fonte: Embrapa (2018).

Diante do exposto, o teste de citotoxicidade torna-se necessário para avaliar e definir a concentração de uma substância antes da realização testes em animais e/ou humanos a fim de se determinar uma resposta biológica eficaz e segura.

A Tabela 2 traz alguns exemplos de extratos vegetais com atividade citotóxica em diferentes linhagens celulares.

Tabela 2 - Exemplos de extratos vegetais com potencial citotóxico.

Planta	Tipo de extrato	Células	Referência
<i>Licania rigida</i>	Aquoso (folhas)	CHO-K1 (ovário de hamster chinês)	Batista et al. (2021)
<i>Mori cortexradicis</i>	Hidroalcoólico (raízes)	J774.A1 (macrófagos murinos)	Bayazid et al. (2020)
<i>Angelica archangelica</i> (Linn.)	Hidroetanólico (raízes)	4T1 (carcinoma mamário murinho), CCD1072Sk (fibroblastos humanos), MCF-7 (carcinoma mamário)	Oliveira et al. (2019)
<i>Cymbopogon citratus</i> e <i>C. nardus</i>	Hidroetanólico (partes aéreas)	A549 (adenocarcinoma de pulmão), VERO (rim do macaco verde africano)	Chiamenti et al. (2019)
<i>Baccharis trinervis</i> (Lam, Persoon)	Aquoso e frações etanólica e butanólica (partes aéreas brasileira e colombiana)	CHO (ovário de hamster chinês)	Jamarillo-Garcia et al. (2018)
<i>Garcinia brasiliensis</i> (Mart.)	Acetoetilico, diclorometano e hexânico (folhas)	HeLa (carcinoma cervical), HepG2 (carcinoma hepatocelular), MCF-7, NCI-H460 (carcinoma de pulmão),	Zan et al. (2018)
<i>Sapindus saponaria</i> L.	Hidroetanólico (casca do fruto)	J774.A1 (macrófagos murinos)	Moreira et al. (2017)

Fonte: Autor, (2022)

3 OBJETIVOS

3.1 OBJETIVOS GERAIS

- Avaliar o perfil fitoquímico e biológico do extrato hidroalcólico da madeira do caule de *Clarisia racemosa*.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar a composição química da madeira de *C. racemosa*;
- Avaliar o perfil fitoquímico do extrato hidroalcólico de *C. racemosa*;
- Identificar os compostos presentes no extrato;
- Determinar a atividade antioxidante através dos testes DPPH, ABTS, fosfomolibdênio, captura de radicais hidroxila, captura de radicais de óxido nítrico e redução e de íons férricos;
- Avaliar ação citotóxica *in vitro* frente as células eritrocitárias e macrófagos;
- Avaliar a ação antimicrobiana *in vitro*;
- Avaliar a atividade fotoprotetora *in vitro*;
- Avaliação do potencial antiglicante;
- Avaliação da toxicidade *in vivo* aguda intraperitoneal e oral do extrato hidroalcólico de *C. racemosa*;
- Realização de cálculos de predição de atividade biológica por meio de ferramentas *in silico*.

4 METODOLOGIA

4.1 OBTENÇÃO DO MATERIAL VEGETAL.

As madeiras do caule foram cedidas pela Mil Madeiras Preciosas uma filial do grupo suíço Precious Woods (<http://preciouswoods.com.br/>). A coleta do caule de *C. racemosa* foi realizada no distrito de Itacoatiara Manaus, Amazonas/Brasil, na seguinte localização 03°08'31'' e 58°26'33''W de longitude e latitude. Para autorização da coleta e estudo do caule, a espécie foi cadastrada no SisGen (Sistema Nacional do Patrimônio Genético e do Conhecimento Tradicional Associado), nº AAF588D.

4.1.1 Análise da composição química da madeira de *Clarisia racemosa*

As madeiras do caule de *Clarisia racemosa* obtido foram secas em estufa (Tecnal, TE-393/1) a 45°C por 48 horas, em seguida foram moídos em moinho de facas (FRITSCH – Pulverisette 14) e tamisados numa faixa granulométrica 80 mesh. Estes foram caracterizados quanto à composição química (celulose, hemicelulose, lignina, extratos e cinzas) de acordo com a metodologia analítica proposta por Gouveia et al. (2009). Os ensaios foram realizados em triplicata e os resultados foram expressos em porcentagem.

4.2 OBTENÇÃO DO EXTRATO HIDROALCÓOLICO

A madeira de *Clarisia racemosa* seca (100g) foi utilizada para preparação do extrato através do método de maceração exaustiva com etanol 70% por 7 dias a 37 °C. Em seguida, todo o extrato hidroalcólico foi filtrado em papel filtro e concentrado em rotaevaporador para posteriormente ser seco em spray dryer. O rendimento de extrato seco bruto foi determinado conforme descrito por Moghadamnia et al. (2019) a partir da relação da massa de extrato seco por massa do vegetal expresso em porcentagem conforme Equação 1.

$$\text{Rendimento (\%)} = \left(\frac{\text{Massa de extrato seco (g)}}{\text{Massa da madeira seca (g)}} \right) * 100 \quad (1)$$

4.3 CARACTERIZAÇÃO FITOQUÍMICA

4.3.1 Determinação do teor de fenólicos totais

O conteúdo de fenólicos totais foi determinado de acordo com Köprü et al. (2020). O extrato hidroalcólico de *C. racemosa* numa concentração de (1000µg/mL) foi adicionado a 1 mL do reagente FolinCiocalteu (1/10 v/v) deixando reagir por 1 minuto. Posteriormente, 2 mL de carbonato de sódio (2% w/v) foram adicionados ao sistema (extrato + reagente)

homogenizados em vórtex e incubados por 2 horas na ausência de luz (25 °C). Os valores de absorvância foram determinados a 765 nm em espectrofotômetro UV-vis (Hewlett-Packard®, modelo 8453). O branco do equipamento foi formado nas mesmas proporções da amostra utilizando água destilada no lugar do extrato. Após a incubação, o conteúdo fenólico total do extrato foi calculado utilizando uma curva preparada com o padrão ácido gálico em diferentes concentrações (7.8 –500 µg /mL) da qual obteve-se a equação, $y = 0.0065x + 0.042$, $R^2 = 0.9978$, onde y representa os valores de absorvância e x os valores de concentração. O teor de fenólicos totais foi expresso como mg de EQ (equivalente de ácido gálico) por g de extrato. Os ensaios foram realizados em triplicata.

4.3.2 Determinação do teor de flavonoides totais

O ensaio de determinação do teor de flavonoides totais foi realizado segundo metodologia proposta e adaptada por Mbaebie et al. (2012). O extrato foi diluído em etanol 70% numa concentração de 1000 µg/mL. Em um balão volumétrico de 10 mL, foram adicionados 1,0 mL da solução do extrato, 1,0 mL de reagente etanol-cloreto de alumínio a 2%, e o volume completado com etanol. As leituras foram realizadas após 30 minutos, a 425 nm em espectrofotômetro (Hewlett-Packard®, modelo 8453). O branco do equipamento foi formado nas mesmas condições da amostra utilizando água no lugar do extrato. Após a incubação, o conteúdo de flavonoides totais presentes no extrato foi calculado utilizando uma curva como padrão quercetina em diferentes concentrações (7.8 –500 µg /mL) da qual obteve-se a equação, $y = 0.0033x + 0.0972$, $R^2 = 0.9906$, onde y representa os valores de absorvância e x os valores de concentração. O teor de flavonoides totais foi expresso como mg de EQ (equivalente de quercetina) por g de extrato. Os ensaios foram realizados em triplicata.

4.3.3 Determinação do teor de flavonóis totais

O teor de flavonóis totais no extrato hidroalcólico foi previamente determinado utilizando a metodologia proposta e adaptada por Mbaebie et al. (2012). O extrato foi diluído em etanol 70% numa concentração de 1000 µg/mL. O ensaio foi constituído por 2 mL do extrato, 2 mL de $AlCl_3$ (2%) /etanol e 3 mL de acetato de sódio (50 g/L). A mistura foi agitada e incubada durante 2,5 horas a 20 °C. Após este período as absorvâncias foram determinadas a 440 nm em espectrofotômetro (Hewlett-Packard®, modelo 8453). O branco do equipamento foi formado nas mesmas proporções da amostra utilizando água no lugar do extrato. Após a incubação, o conteúdo de flavonóis totais presentes no extrato foi calculado utilizando uma curva de quercetina em diferentes concentrações (7.8 –500 µg /mL) da qual

obteve-se a equação, $y = 0.0019x + 0.0414$, $R^2 = 0.9927$, onde y representa os valores de absorbância e x os valores de concentração. O teor de flavonóis totais foi expresso como mg de EQ (equivalente de quercetina) por g de extrato. Os ensaios foram realizados triplicata.

4.3.4 Determinação do teor de taninos totais

O teor de taninos totais no extrato hidroalcóolico foi previamente determinado utilizando a metodologia proposta e adaptada por Mbaebie et al. (2012). O extrato foi diluído em etanol 70% numa concentração de 1000 $\mu\text{g/mL}$. O ensaio foi constituído por 2 mL do extrato, 3 mL de água destilada, 0,5 mL do reagente Folin Ciocalteau, o sistema reagiu por 3 minutos. Após esse período, acrescentou-se 1,5 mL de carbonato de sódio (Na_2CO_3) a 17% e 3 mL de água destilada, totalizando 10 mL. As amostras foram homogeneizadas e incubadas na ausência de luz por 2 horas. Após este período, a absorbância das amostras foi determinada em espectrofotômetro Uv/Vis (Hewlett-Packard®, modelo 8453) em um comprimento de onda de 725 nm. O branco do equipamento foi formado nas mesmas proporções da amostra utilizando água no lugar do extrato. Após a incubação, o conteúdo taninos totais presentes no extrato foi calculado utilizando uma curva utilizando o padrão ácido tânico preparada por quercetina padrão em diferentes concentrações (7.8 – 500 $\mu\text{g/mL}$) da qual obteve-se a equação, $y = 0.0122x + 0.055$ $R^2 = 0.99$, onde y representa os valores de absorbância e x os valores de concentração. O teor de taninos foi expresso como mg de EAT (equivalente de ácido tânico) por g de extrato. Os ensaios foram realizados em triplicata.

4.4 IDENTIFICAÇÃO DOS PRINCIPAIS COMPOSTOS POR CROMATOGRAFIA LÍQUIDA DE ULTRA PERFORMANCE (UPLC)

A identificação dos compostos foi realizada utilizando metodologia proposta por Barboza et al. (2018) com poucas modificações. A Cromatografia Líquida de Ultra Performance (UPLC) foi realizada com uma Acquity H-Class (Waters) empregando uma coluna BEH de 2.1 x 100 mm com um tamanho de partícula de 1.7 μm . A coluna foi mantida sob temperatura constante de 40 °C e o auto injetor a 10°C. Foi utilizada uma solução aquosa (eluente A) contendo 2% de metanol (MeOH), 5Mm de formato de amônio e 0.1% de ácido fórmico e uma solução de metanol (eluente B) contendo 0,1% de ácido fórmico, que foram bombeados a uma vazão de 0.3 mL/min. Dez microlitros do extrato foram injetados. A eluição foi realizada em modo gradiente e a condição inicial (98% A / 2% B) foi mantida por 5.0 minutos. A relação B aumentou linearmente para 99% em 15 minutos, permanecendo em 99% B por um minuto, seguido de diminuição imediata para 2% B, onde foi mantido por até

0 minutos. O sistema UPLC foi acoplado a um único espectrômetro de massa quadripolar SQ Detector 2 (Waters®).

Os dados foram obtidos no modo fullscan, analisando massas entre 100 e 1000 Da, em ionização negativa. A aquisição do cromatograma foi obtida através do software MassLynx TM (Waters®). A identificação dos componentes presentes no extrato hidroalcolólico baseou-se na comparação dos dados espectrais de massa por correspondência computacional com as Bibliotecas NIST. Além disso, Informações da literatura sobre a família botânica (Moraceae) foram consideradas úteis no processo de caracterização.

4.4.1 Estudo *in silico*: predição do perfil farmacocinético e toxicológico dos compostos identificados do extrato hidroalcolólico de *Clarisia racemosa*

Os compostos identificados no extrato foram submetidos a análise do perfil físico-químico e farmacocinético. Inicialmente as estruturas químicas foram obtidas através do banco de dados PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Em seguida os compostos foram submetidos à web server PkCSM (<http://structure.bioc.cam.ac.uk/pkcsml>) (Pires et al. 2015), onde as características de absorção, distribuição, metabolismo, excreção e toxicidade foram preditas. O perfil de predição foi avaliado segundo metodologia proposta por Hassan et al. (2017) e Taskin et al. (2021)

4.5 ATIVIDADE ANTIOXIDANTE *IN VITRO*

4.5.1 Método de captura de radicais DPPH• (2,2 difenil-1-picril-hidrazil)

O ensaio de captura de radical do radical estável 2,2-difenil-1-picrilhidrazil (DPPH) foi realizado segundo metodologia proposta e adaptada por Akbaribazm et al. (2020) e Lekouaghet et al. (2020). Este método baseia-se uma reação de oxirredução, onde o radical DPPH, que apresenta coloração violeta, é reduzido para DPPH-H (DPPH reduzido e estável), alterando a coloração da mistura de roxo para amarelo (AKBARIBAZM et al. 2020; ALAM et al. 2013; LEKOUAGHET et al. 2020). Inicialmente o extrato foi diluído em metanol em diferentes concentrações (7.8 - 1000 µg/mL) em seguida 0.32 mL de cada concentração foi adicionada a 2.0 mL da solução de metanol DPPH em 1 mM. Os ensaios foram incubados a 25 °C por 25 minutos com ausência de luz. Ao final da reação as absorbâncias foram determinadas em espectrofotômetro (Hewlett-Packard®, modelo 8453) em um comprimento de onda de 517 nm. Como controle experimental foi utilizada a solução de DPPH e o branco do equipamento foi o metanol. Os padrões utilizados neste experimento foram o ácido

ascórbico e o hidroxitoluenobutilado. Todos os experimentos foram realizados em triplicata. As porcentagens de sequestro para cada concentração foram determinadas pela Equação 2.

$$\text{DPPH (\%)} = \left(\frac{\text{ABS controle} - \text{ABS amostra}}{\text{ABS controle}} \right) * 100 \quad (2)$$

Onde: ABS controle = absorbância do controle; ABS Amostra = absorbância da amostra contendo o extrato após ensaio.

O coeficiente de inibição (EC_{50}) ou seja, concentração mínima da substância antioxidante necessária para reduzir em 50% a concentração inicial do radical livre DPPH foi determinada ajuste de regressão linear da capacidade antioxidante versus as concentrações de extrato bruto.

4.5.2 Método de redução do radical ABTS⁺ [2,2'- azinobis(3-etilbenzotiazolina-6-ácido sulfônico)]

Este método baseia-se na produção do radical ABTS⁺ (azul esverdeado) com a adição de um antioxidante ocorre a redução do radical promovendo a perda da coloração no meio reacional (ALAM et al. 2013; ILYASOV et al. 2020). O ensaio de captura de radicais cátions 2,2'-azinobis-3-etilbenzotiazolina-6-ácido sulfônico (ABTS⁺) foi determinada de acordo com metodologia proposta e adaptada por Köprüet al.(2020). A solução de ABTS⁺ foi preparada pela reação de ABTS 7 mM (5 mL) e 2.45 mM (88 µL) de persulfato de potássio, após incubação à temperatura ambiente no escuro, durante 16 horas. Posteriormente foi diluída com etanol a 80% para se obter uma absorbância de 0.70 a 734 nm. A solução ABTS⁺ (2.7 mL) foi cuidadosamente misturada com 0.3 mL e diferentes concentrações (7.8 - 1000 µg/mL) do extrato. O sistema foi incubado a 25°C durante 5 minutos na ausência de luz. Ao final da reação as absorbâncias foram determinadas em espectrofotômetro (Hewlett-Packard®, modelo 8453) em um comprimento de onda de 734 nm.

Como controle experimento foi utilizada a solução de ABTS⁺ e o branco do equipamento foi o metanol. Os padrões utilizados neste experimento foram o ácido ascórbico e o hidroxitoluenobutilado (BHT). Os experimentos foram realizados em triplicata. As porcentagens de sequestro para cada concentração foram determinadas pela Equação 3.

$$\text{ABTS (\%)} = \left(\frac{\text{ABS controle} - \text{ABS amostra}}{\text{ABS controle}} \right) * 100 \quad (3)$$

Onde: ABS controle = absorbância do controle; ABS Amostra = absorbância da amostra contendo os extratos após ensaio.

O coeficiente de inibição (EC_{50}), ou seja, concentração mínima da substância antioxidante necessária para reduzir em 50% a concentração inicial do radical ABTS⁺ foi determinada ajuste de regressão linear da capacidade antioxidante *versus* as concentrações de extrato bruto.

4.5.3 Método de redução do complexo fosfomolibdênico (RCF)

Esse método baseia-se na redução do Mo(VI) (esverdeado) em Mo(V) (azulado) e subsequente formação do complexo fosfato de Mo(V) em pH ácido (Alam et al. 2013). Os ensaios foram realizados segundo metodologia proposta e adaptada por Murugan et al. (2020). Para isto foi realizada uma solução de fosfomolibdênio (com fosfato de sódio 0,1 mol/L (28 mL), molibdato de amônio 0,03 mol/L (12 mL) e ácido sulfúrico 3 mol/L (20 mL), sendo o volume final ajustado H₂O para 100 mL). O extrato foi diluído em diferentes concentrações (7.8 - 1000 µg/mL). As reações foram realizadas com 1 mL da solução de fosfomolibdênio e 0.1 mL em cada diluição de extrato e incubadas a 95°C durante 90 minutos. Ao final da reação as amostras foram resfriadas e as absorbâncias foram determinadas em espectrofotômetro (Hewlett-Packard®, modelo 8453) em um comprimento de onda de 695 nm. O branco do equipamento foi uma solução de fosfomolibdênio (1 mL de solução e 0,1 mL de água). Os padrões experimentais utilizados foram o ácido ascórbico e o hidroxitoluenobutilado. Os experimentos foram realizados em triplicata. Os resultados de atividade para cada concentração foram determinados pela Equação 4.

$$RCF (\%) = \left(\frac{ABS_{amostra} - ABS_{branco}}{ABS_{padrão} - ABS_{branco}} \right) * 100 \quad (4)$$

Onde: ABS amostra = absorbância da amostra, ABS branco = absorbância do branco e ABS padrão = absorbância de ácido ascórbico.

4.5.4 Método de captura de radicais hidroxila (HO•)

A capacidade de captura do radical hidroxila foi determinada de acordo com a metodologia proposta e adaptada por Smirnoff e Cumbes (1989). Os radicais HO• foram gerados a partir de FeSO₄·7H₂O e H₂O₂, e detectados a partir de sua habilidade de hidroxilar o salicilato (Alam et al. 2013). O extrato foi diluído em diferentes concentrações (7.8 - 1000 µg/mL). Ao extrato diluído em diferentes concentrações 1.0 mL foi adicionado 0,5 mL de FeSO₄·7H₂O (1,5 mM), 0,35 mL de H₂O₂ (6 mM), 0,15 mL de salicilato de sódio (20 mM). Este sistema foi incubado na ausência de luz a 37°C por 1 hora. Os padrões utilizados foram o ácido ascórbico e o hidroxitoluenobutilado. Após o período de incubação, as absorbâncias

foram determinadas em espectrofotômetro (Hewlett-Packard®, modelo 8453) em um comprimento de onda de 562 nm. O branco experimental utilizado foi: 1.0 mL de água destilada adicionado de 0,5 mL de $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1,5 mM), 0,35 mL de H_2O_2 (6 mM), 0,15 mL de salicilato de sódio (20 mM). Os padrões experimentais utilizados foram o ácido ascórbico e o hidroxitoluenobutilado (BHT). Todos os experimentos foram realizados em triplicata. A porcentagem de radicais sequestrados ($\text{OH}^\bullet$) foi determinada pela Equação 5.

$$\% \text{OH sequestro} = \left[1 - \frac{(\text{ABS amostra} - \text{ABS branco})}{\text{ABS padrão}} \right] * 100 \quad (5)$$

Onde, ABS amostra = absorbância da amostra dos extratos; ABS branco = absorbância do branco; ABS padrão = absorbância dos padrões em estudo.

O coeficiente de inibição (EC_{50}), ou seja, concentração mínima da substância antioxidante necessária para reduzir em 50% a concentração inicial do radical OH foi determinada ajuste de regressão linear da capacidade antioxidante versus as concentrações de extrato bruto.

4.5.5 Método de captura de radicais óxido nítrico ($\text{NO}^\bullet$)

Para avaliação do sequestro do radical óxido nítrico (NO) *in vitro* foi utilizada solução de nitroprussiato de sódio seguindo método descrito por Alam et al. (2013) com poucas modificações. O ensaio constituiu em 1,0 mL de extrato em diferentes concentrações (7,8 - 1000 $\mu\text{g/mL}$) foi adicionado a 1,0 mL de uma solução de nitroprussiato de sódio (5mM), o sistema foi incubado a 25 °C por 150 minutos na ausência de luz. Após a incubação, alíquotas de 0,5 mL foram adicionadas a 0,5 mL de reagente de Griess (1% sulfanilamida, 2% ácido o-fosfórico e 0,1% naftiletlenodiamina). A absorbância do cromóforo formado foi determinada em espectrofotômetro (Hewlett-Packard®, modelo 8453) em um comprimento de onda de 546 nm. Os padrões utilizados foram o ácido ascórbico e o hidroxitoluenobutilado (BHT). O branco do equipamento foi constituído por 0,5 mL de água destilada e 0,5 mL do reagente de Griess). Os experimentos foram realizados em triplicata. A porcentagem de radicais sequestrados (NO) foi determinada pela Equação 6.

$$\% \text{NO sequestro} = \left[1 - \frac{(\text{ABS amostra} - \text{ABS branco})}{\text{ABS padrão}} \right] * 100 \quad (6)$$

Onde, ABS amostra = absorbância da amostra dos extratos; ABS branco = absorbância do branco; ABS padrão = absorbância dos padrões em estudo.

O coeficiente de inibição (EC_{50}), ou seja, concentração mínima da substância antioxidante necessária para reduzir em 50% a concentração inicial do radical NO foi

determinada ajuste de regressão linear da capacidade antioxidante versus as concentrações de extrato bruto.

4.5.6 Método de determinação de redução de íons férrico (RIF)

O poder redutor do íon férrico baseia-se na redução de Fe^{3+} a Fe^{2+} monitorada pela formação de complexo formado denominado azul da Prússia (ALAM et al. 2013). Os experimentos foram realizados segundo metodologia adaptada e proposta por Köprü et al. (2020) com poucas modificações. Para isto um volume de 2,5 mL do extrato em diferentes concentrações (concentração final de 7.8 a 1000 $\mu\text{g/mL}$) foram adicionados a 2,5 mL tampão fosfato (0,2 M, pH 6,6) e 2,5 mL de ferricianeto de potássio. O sistema foi então incubado a 50 °C durante 20 minutos. Posteriormente, foram adicionados 2.5mL de ácido tricloroacético, para então serem centrifugado a 3000 rpm durante 30 minutos. Finalmente, uma alíquota 2,5 mL do sobrenadante foi adicionada a 2,5 mL de água destilada e a 0,5 mL de cloreto férrico e as absorbâncias foram determinadas em espectrofotômetro (Hewlett-Packard®, modelo 8453) em um comprimento de onda de 700 nm. O ácido ascórbico e hidroxitoluenobutilado (BHT) foram usados como padrão e tampão de fosfato como solução em branco. Os resultados foram realizados em triplicata. Os resultados foram expressos percentagem de inibição do Fe (II) calculado da seguinte Equação 7.

$$\text{RIF (\%)} = \left(\frac{\text{ABS controle} - \text{ABS amostra}}{\text{ABS controle}} \right) * 100 \quad (7)$$

Onde: ABS controle = absorbância do controle; ABS Amostra = absorbância da amostra contendo o extrato após ensaio.

4.6 ATIVIDADE ANTIGLICANTE PROMOVIDA PELO EXTRATO HIDROALCÓOLICO DE *Clarisia racemosa*

4.6.1 Sistemas: BSA-glicose/frutose e colágeno-glicose/frutose

O ensaio de atividade antiglicante foi realizado segundo Xavier et al. (2017) e Oliveira et al. (2021) com poucas modificações. Foram preparadas as soluções de BSA (2 mg/mL), frutose (200 mM) e glicose (200 mM) e as soluções das amostras preparadas em concentrações diferentes (7.8 -1000 $\mu\text{g/mL}$) para o extrato e os padrões (rutina, ácido gálico, ácido tânico e aminoguanidina). As amostras foram diluídas em tampão fosfato(pH 7,4). O sistema reacional consistiu na adição de 1000 μL da mistura de frutose e glicose, a 1000 μL das diferentes concentrações de amostra, em seguida adicionou-se 1000 μL da solução de

BSA. O sistema foi incubado a 37°C na ausência de luz sem agitação durante 7 dias. Para o controle negativo, na ausência da amostra, utilizou-se tampão fosfato (pH 7.4). As amostras foram analisadas utilizando um espectrofluorímetro (λ excitação = 360 e λ emissão = 440 nm). O branco do equipamento foi o tampão fosfato. A atividade antiglicante foi determinada pela porcentagem de inibição utilizando a Equação 8.

$$\% \text{ inhibition} = ((F \text{ negative control} - F \text{ of the sample}) / (F \text{ negative control})) \quad (8)$$

Onde: As fluorescências (F) da amostra (F da amostra) e do controle negativo (F controle negativo).

Os ensaios para o sistema colágeno-glicose/frutose foram realizados utilizando a mesma metodologia utilizado no sistema BSA-glicose/frutose (utilizando colágeno ao invés de BSA).

A determinação do CI_{50} (concentração necessária do extrato para inibir em 50% as reações de glicação) foi realizada utilizando-se uma equação de reta, em que o eixo x correspondeu à capacidade antiglicante (%) e o eixo y correspondeu à concentração do extrato ($\mu\text{g/mL}$). Para o cálculo do IC_{50} o valor de x foi substituído por 50 (%).

4.7 ATIVIDADE CITOTÓXICA E DETERMINAÇÃO DE ÓXIDO NÍTRICO *IN VITRO* E *IN VIVO*

4.7.1 Atividade hemolítica

Amostras de sangue foram coletadas de Hamster *Mesocricetus auratus*, peso corporal médio de 30 g e idade de 3 a 4 semanas. O ensaio foi realizado segundo a metodologia adaptada e proposta por Shubha et al. (2017) e Lima Viana et al. (2018). O extrato foi avaliado quanto à atividade hemolítica por ensaio realizado em microplacas de 96 poços. Cada poço recebeu 100 μL de uma solução de NaCl a 0,85% contendo 10 mM de CaCl_2 . Amostras (100 μL) de extrato foram adicionadas ao primeiro poço do respectivo tratamento, do qual 100 μL foram transferidos para o segundo para realizar uma diluição seriada (concentrações finais variando de 3.9 a 500 $\mu\text{g/mL}$ em solução salina). Em seguida, foram adicionados 100 μL de uma suspensão a 2% (v/v) de eritrócitos de hamster em solução salina contendo 10 mM de CaCl_2 . O controle negativo, foi constituído por 100 μL de solução salina mais 100 μL de suspensão de eritrócitos. O controle positivo continha 80 μL de solução salina mais 20 μL saponina Quillaja 0,0025% em solução salina. Após centrifugação durante 1 hora, seguida de incubação por 1 hora a 27 °C, o sobrenadante foi descartado e a hemoglobina

liberada foi determinada por absorbância a 540 nm. Os resultados de atividade hemolítica foram expressos pela seguinte Equação 9.

$$\text{Hemólise (\%)} = \left[\left(\frac{\text{ABS amostra} - \text{ABS branco}}{\text{ABS saponina} - \text{ABS branco}} \right) \right] * 100 \quad (9)$$

Onde: ABS amostra = Absorbância da amostra; ABS branco = absorbância do controle negativo; ABS saponina = absorbância controle positivo.

A concentração efetiva que promove 50% da hemólise (CI₅₀) foi determinada. Três experimentos independentes foram realizados em triplicata. O estudo foi aprovado pela Comissão de Ética no Uso de Animais do Instituto Aggeu Magalhães/Fundação Oswaldo Cruz número de protocolo 164/2020.

4.7.2 Atividade citotóxica em células de macrófagos J774. A1

A fim de avaliar os efeitos tóxicos em células de macrófagos J774.A1 foi realizado o teste de citotoxicidade pelo método do MTT (3- (4,5-dimetiltiazol-2-il) -2,5-difeniltetrazólio) de acordo com a metodologia proposta por Pandey e Khan, (2020) com modificações. As células foram semeadas em placas de 96 poços contendo meio RPMI com vermelho de fenol suplementado e incubadas em uma atmosfera a 5% de CO₂ e 37 °C. Após 24 horas, as células foram incubadas na presença de várias concentrações extrato (3.9 a 500 µg/mL) dissolvido em água destilada por 72 horas. A leitura da absorbância dos cristais de formazan foi determinada por meio de ELISA Benchmark Plus® (Bio-Rad, Califórnia, EUA) em comprimento de onda 540 nm. O controle experimental constituiu em apenas células crescidas em meio de cultura. Cada experimento foi conduzido em triplicata e réplica biológica. A viabilidade celular foi calculada utilizando a Equação 10.

$$\text{Viabilidade Celular (\%)} = \left(\frac{\text{VC}}{\text{TC}} \right) * 100\% \quad (10)$$

Onde VC é a quantidade de células em diferentes concentrações, TC é concentração de células no controle a qual representa viabilidade de 100%.

A concentração efetiva que promove 50% da hemólise (CI₅₀) foi determinada. Três experimentos independentes foram realizados em triplicata.

4.7.3 Determinação de óxido nítrico pelas células durante os ensaios de citotoxicidade *in vitro*

Os sobrenadantes de culturas de células contendo as diferentes concentrações de extrato foram submetidos a análise de nitrito pelo método colorimétrico de Griess (DING et

al. 1988). A concentração de óxido nítrico foi estimada usando uma curva padrão (3,12-100 $\mu\text{mol/ mL}$). A leitura foi realizada num espectrofotômetro (Bio-Rad® 3550, Hercules, CA) a 595 nm.

4.7.4 Ensaios de toxicidade *in vivo* promovida pelo extrato de hidroalcóolico de *Clarisia racemosa*

4.7.4.1 Animais: camundongos albinos suíços

Os camundongos albinos suíços machos (*Mus musculus*) com 50 dias pesando em torno de $30 \pm 0,5$ g foram obtidos no biotério do Laboratório de Imunopatologia Keizo Asami (LIKA) da Universidade Federal de Pernambuco (UFPE). Os animais passaram por um período de adaptação de duas semanas e em seguida foram submetidos aos testes realizados no Laboratório de Experimentação Animal do Departamento de Bioquímica da UFPE. Os camundongos foram diariamente alimentados com ração (Purina; Nestlé Brasil Ltda., Brasil) e água e mantidos a 22 °C sob condições controladas de luminosidade a fim de simular um ciclo de luz/escuridão natural de 12/12 horas. Os procedimentos experimentais descritos neste estudo foram aprovados pela Comissão de Ética no Uso de Animais do Instituto Ageu Magalhães/Fundação Oswaldo Cruz número de protocolo 164/2020.

4.7.4.2 Avaliação da toxicidade *in vivo* aguda intraperitoneal e oral: análises hematológicas, bioquímicas, histológicas e de estresses oxidativo.

Os experimentos de toxicidade aguda foi avaliado de acordo com was evaluated according to guidelines from the Organization for Economic Cooperation and Development, protocol 423 (OECD, 2001) e Silva et al. (2020) com poucas modificações. Camundongos fêmeas foram separados aleatoriamente em três grupos ($n = 5/\text{grupo}$). O grupo controle de camundongos recebeu apenas o veículo (água), o segundo e o terceiro grupo receberam o extrato hidroalcóolico diluído em água a 2000 mg/kg de peso corporal (b.w.), por administração intraperitoneal e oral (gavagem) respectivamente. Os animais do grupos oral foram privados da alimentação 3 horas antes da ingestão do extrato. As mudanças comportamentais foram avaliadas durante os primeiros 60 minutos e durante 14 dias após a administração do extrato hidroalcóolico diluído. Ao decorrer deste período foram avaliados os seguintes parâmetros: piloereção, sensibilidade ao som e ao toque, mudanças o aspecto das fezes, mobilidade e comportamento agressivo. Além destes o peso corporal, a ingestão de água e de alimentos foram diariamente avaliados.

No 15º dia ,todos os animais foram sacrificados com altas doses de anestésicos (cetamina 300 mg / kg e xilazina30 mg/kg) segundo os protocolos de cuidado, pesquisa e sacrifício de animais sugeridos pelos princípios e diretrizes adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA). Em seguida foi coletado o sangue da veia cava inferior e alíquotas em três tubos, dois tubos um contendo anticoagulante (ácido etilenodiaminotetracético, EDTA) e outro sem anticoagulante, que foram submetidos a análises hematológicas e bioquímicas.

Os ensaios hematológicos foram realizados em analisador automatizado (Animal BloodCounter: ABC Vet, Montpellier, França) e microscopia de luz foram usados para avaliar os seguintes parâmetros hematológicos: número de eritrócitos, hemoglobina, hematócrito, volume corpuscular médio (MCV), hemoglobina corpuscular média (MCH), corpuscular médio concentração de hemoglobina (MCHC), plaquetas (PLT), glóbulos brancos (WBC), segmentados (SEG), linfócitos (LYM), monócitos (MON), basófilos (BAS), eosinófilos (EOS). Também analisamos o coagulograma por meio de: tromboplastina parcial ativada (APTT), tempo de protrombina (PT) e tempo de trombina (TT).

A análise bioquímica foi realizada para avaliar a albumina (ALB), alanina aminotransferase (ALT), aspartato aminotransferase (AST), fosfatase alcalina (ALP), bilirrubina (BIL), gama glutamil transferase (GGT), proteína total (TP), uréia no sangue (UR), creatinina (CRE), colesterol total (TC), lipoproteína-colesterol de alta densidade (HDL), não-HDL (triglicerídeos (TG), lipoproteína-colesterol de baixa densidade (LDL), Colesterol de densidade muito baixa (VLDL), foram avaliados por meio de kits comerciais específicos (Diagnostica Stago, França; LabtestDiagnostic, Lagoa Santa, Brasil; analisador COBAS Mira Plus (Roche Diagnostics Systems, Basel, Suíça)) de acordo com as instruções do fabricante.

A avaliação do estresse oxidativo foi determinado segundo Silva et al. (2020). Homogenatos de fígado foram preparados em Tris-HCl 50 mM pH 7.4 contendo EDTA 1 mM, ortovanadato de sódio 1 mM e fluoreto de fenilmetanossulfonil 2 mM. Esses homogenatos foram avaliados em relação a peroxidação lipídica (MDA), atividade da catalase (CAT) e por fim a atividade da superóxido dismutase total do tecido (SOD).

Para avaliar os efeitos do extrato em nível histológico, fígado, rim, baço e estômago dos animais dos grupos controle e tratamento foram realizados por microscopia óptica. Parte dos órgãos foram fixados em formalina tamponada (10%, v/v) e então desidratados por meio de uma série graduada de etanol (70–100%), diafanizados em xilol e incluídos em parafina. Cortes histológicos (5µm) foram corados com hematoxilina-eosina e montados com lamínulas

com resina Entellan (Merck, Alemanha). Estes foram observados sob um microscópio Motic BA200 acoplado com uma câmera digital Moticom 1000 1.3 MP (Motic Incorporation Ltd, Causeway Bay, Hong Kong).

4.8 ATIVIDADE ANTIMICROBIANA *IN VITRO* PROMOVIDA PELO EXTRATO HIDROALCÓOLICO DE *Clarisia racemosa*

4.8.1 Micro-organismos e padronização do inóculo

Os micro-organismos utilizados neste trabalho foram obtidos da Coleção e Cultura de Micro-organismos, do Departamento de Antibióticos da Universidade Federal de Pernambuco (UFPEA). As cepas bacterianas utilizadas foram *Staphylococcus aureus* UFPEA-709, *Enterococcus faecalis* UFPEA-138, *Pseudomonas aeruginosa* UFPEA-416, *Enterococcus faecalis* UFPEA-69, *Pseudomonas aeruginosa* UFPEA-261, *Acinetobacter baumannii* UFPEA-1024. Além de cepas bacterianas foram também estudadas diferentes cepas de leveduras, sendo estas: *Candida albicans* UFPEA-1007, *Candida albicans* URM 95, *Candida albicans* 4664, *Candida glabrata* UFPEA-6393 e *Candida guilliermondii* UFPEA-6390.

A padronização do inóculo, tanto para bactérias como para as leveduras, foi realizado seguindo as recomendações dos documentos do *Clinical Laboratory Standards Institute*, M100 (CLSI, 2017a) e M27 (CLSI, 2017b), respectivamente. Para as bactérias, colônias isoladas foram inoculadas em caldo Mueller Hinton para atingir turvação equivalente a escala 0,5 de Mc Farland ($1,5 \times 10^8$ unidades formadoras de colônia/mL - UFC/mL). Em seguida, as suspensões bacterianas foram diluídas em solução fisiológica para obtenção do inóculo final ($1,5 \times 10^7$ UFC/mL). Já o inóculo para as leveduras, colônias isoladas foram inoculadas em solução salina estéril até turvação equivalente a 0,5 da escala de Mc Farland ($1,0 \times 10^6$ a $5,0 \times 10^6$ UFC/mL).

4.8.2 Determinação da concentração inibitória mínima (CIM) e concentração bactericida ou fungicida mínima (CBM/CFM)

A CIM do extrato hidroalcóolico e dos padrões Gentamicina, Oxacilina e Ampicilina+Sulbactam, frente as bactérias, foi determinada através da técnica de microdiluição em caldo Mueller Hinton em placas de 96 poços, de acordo com o documento M100 do CLSI (CLSI, 2017a). Após a microdiluição do extrato e dos padrões em placa, alíquotas de inóculo previamente preparados e padronizados das bactérias foram adicionados

aos poços a fim de obter uma concentração final de $1,5 \times 10^5$ UFC/poço. As placas foram incubadas a 37 °C por 24 horas.

Para as leveduras foi utilizado como padrão a Micafungina, também foi realizada a técnica de microdiluição em caldo RPMI 1640 em placa de 96 poços, seguindo as recomendações do documento M27 do CLSI (CLSI, 2017b). Após a microdiluição do extrato e da micafungina, alíquotas de inóculo foram adicionadas aos poços a fim de obter uma concentração final de $2,5 \times 10^3$ UFC/poço. As placas foram incubadas a 30 °C por 48 horas. A CIM foi definida como a menor concentração capaz de inibir o crescimento microbiano em comparação com controle positivo de crescimento microbiano, sem tratamentos. Para determinar a CBM/CFM, alíquotas de 10 µL foram retiradas de cada poço contendo o extrato e os padrões utilizados e foram semeadas em placas de Petri contendo ágar Müller-Hinton ou Agar Sabourand. As placas foram incubadas durante 24 horas a 37 °C, para bactérias e para as leveduras durante 48 horas a 30 °C. A CBM ou CFM foi definida como a menor concentração de cada composto que resultou em redução de 99,9% do crescimento microbiano, quando comparado com um controle de crescimento sem tratamento. As concentrações de extrato utilizadas nesta etapa variaram de 1.024 a 4 µg/mL e foi considerada atividade diante do micro-organismo quando a CIM foi ≤ 1.024 µg/mL.

4.9 ATIVIDADE FOTOPROTETORA PROMOVIDA PELO EXTRATO *IN VITRO*

A atividade de fotoprotetora foi realizada segundo metodologia proposta por Mansur et al. (1986) com modificações. Alíquotas de 1mL do extrato foram retiradas preparando-se soluções com a concentração final de 0,5; 1,0; 2,0; 5,0; 10% (v/v extrato/ água). Um espectrofotômetro (Hewlett-Packard®, modelo 8453) com cubeta de quartzo de 1cm de caminho óptico foi usado para aquisição dos espectros. Varreduras de 290 a 390 nm com intervalos de 5 nm foram realizadas. Os cálculos foram realizados considerando os intervalos de λ determinados por Mansur et al. (1986). Os experimentos foram realizados em triplicata e os resultados expressos como média $\pm$ DP. Os resultados de FPS (fator de proteção solar) foram determinados pela Equação 11:

$$\text{FPS } in \text{ vitro} = CF \times \sum E\lambda \times S\lambda \times Abs\lambda \quad (11)$$

Onde CF (= 10) é um fator de correção empírico do método *in vitro*, $E\lambda$ é o parâmetro de eficácia espectral eritematosa, $S\lambda$ é a irradiância espectral solar e $Abs\lambda$ é a absorção de radiação UV para um determinado comprimento de onda. Os produtos $E\lambda \cdot S\lambda$ foram calculados conforme mostrado na Tabela 3.

Tabela 3 - Relação entre intensidade da radiação e o efeito eritematogênico em cada comprimento de onda (MANSUR et al. 1986).

λ (nm)	EE (λ) x I (λ)
290	0,0150
295	0,0817
300	0,2874
305	0,3278
310	0,1864
315	0,0839
320	0,0180

5 RESULTADOS E DISCUSSÃO

Os resultados deste trabalho encontram-se apresentados em forma de artigo intitulado *Photoprotective, biological activities and chemical composition of the non-toxic hydroalcoholic extract of Clarisia racemosa with cosmetic and pharmaceutical applications* dispostos no Apêndice A.

6 CONCLUSÕES OBTIDAS

Diante dos resultados, foi possível concluir que:

- A análise físico-química da madeira do caule de *Clarisia racemosa* evidencia valores compatíveis com outros relatados na literatura para a mesmas espécies, com pequenas diferenças, podendo estas estarem relacionadas a sazonalidade e condições climáticas no período da coleta, método de extração e obtenção do extrato;
- O extrato hidroalcoólico da madeira do caule de *C. racemosa* demonstrou a presença de compostos fenólicos, flavonoides, flavonóis e taninos, conferindo-lhe potencial biológico;
O extrato analisado também se mostrou excelente perante a atividade antioxidante por diferentes mecanismos, como capacidade redutora (complexo fosfomolibdênio), capacidade de sequestro de radicais livres (DPPH; ABTS; OH; NO), sugerindo ação estabilizadora de radicais livres dos compostos presentes no extrato;
- O extrato apresentou citotoxicidade nas concentrações testadas dose-dependente com ativação de NO nos macrófagos, porém sem causar celulares;
- Não foi identificada atividade antimicrobiana em leveduras nas concentrações testadas e nas bactérias, apenas *Staphylococcus aureus* foi sensível ao extrato hidroalcoólico do caule de *C. racemosa*;
- A atividade citotóxica do extrato testado apresentou baixo percentual de inibição celular para as linhagens tumorais NCI-H292, MCF-7, Hep-2 e moderado para HL-60, o que confere potencial para novas investigações;
- O extrato mostrou potencial efeito fotoprotetor *in vitro* superior ao estabelecido pela legislação brasileira, podendo ser incorporado em formulações em concentrações adequadas ao uso.

Dentre as perspectivas futuras espera-se obter resultados positivos nos testes a serem realizados (avaliação em células tumorais, avaliação antiglicante em albumina e colágeno, s de toxicidade *in vivo*) para complementar o conhecimento biológico deste vegetal agregando-lhe valores científicos e econômico.

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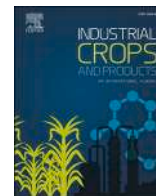
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Photoprotective, biological activities and chemical composition of the non-toxic hydroalcoholic extract of *Clarisia racemosa* with cosmetic and pharmaceutical applications

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ABSTRACT

Clarisia racemosa (guariúba), Moraceae, is a native tree of the Amazon region, with timber value in the local market and for export. Aiming to contribute to the biological study of the crude extract, we carried out its hydroalcoholic extraction and physicochemical characterization. The results showed that the wood had the following chemical composition: cellulose (40.1%), hemicellulose (18.34%), lignin (29.40%), extractives (10.7%), and ash (0.79%). The extract obtained showed a high content of phenolics and through liquid chromatography tests, it was possible to determine 17 compounds. Therefore, we evaluated its biological properties (antioxidant, antiglycant, *in vitro* and *in vivo* toxicity, antitumor, antimicrobial and photoprotective) to use it as a raw material in cosmetic and pharmaceutical formulations. The extract showed moderate antioxidant activity, it was also able to inhibit antiglycant reactions. And it showed no toxic effects *in vitro* and *in vivo* assays. Highlighting antiproliferative activity against MCF-7, T47D, DU-145, and Jurkat tumor cells. As reported in the literature, these models are still being used as a predictor of cytotoxicity. Regarding the *in vitro* antimicrobial assays, the extract showed activity against *Staphylococcus aureus* with a minimal inhibitory concentration similar to the oxacillin pattern. Finally, it was able to promote photoprotective activity *in vitro* at non-toxic concentrations. This study shows the potential of the hydroalcoholic extract of *Clarisia racemosa* to be used as a component in cosmetic and pharmaceutical formulations.

1. Introduction

The use of plants for medicinal purposes is one of the oldest practices of humanity, which began in the dawn of civilization and is still present today not only for curative purposes but to ensure a better quality of life (Carvalho and Conceição, 2015). According to data from the World Health Organization (WHO), about 85% of the world population uses the plant as a therapeutic alternative and, sometimes, as the only available means to treat diseases (Barbosa et al., 2010; Wanderley et al.,

2015; Chakraborty, 2018).

Brazil has a highly diverse plant biome, in addition to the largest plant genetic variety in the world (Ferreira Rodrigues Sarquis et al., 2019). It is estimated that about 75% of all forest species in the two main formations, Atlantic Forest and Amazon Forest, are in Brazil, which has attracted many researchers (Costa et al., 2019; Ferreira Rodrigues Sarquis et al., 2019). Despite this, there are few examples of phytotherapies or even chemical products from Brazilian plant species. Evidencing the lack of studies in the area to regularize medicinal plants and including

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the country in the bioeconomic sector (Valli et al., 2018). Among the vast diversity of plant species present in the Amazon Forest, the species *Clarisia racemosa* Ruiz has stood out for being a wood of great economic importance for the timber sector (Araujo et al., 2020a, 2020b).

Clarisia racemosa Ruiz & Pav is a Neotropical species found in humid forests from southern Mexico to southern Brazil. In Brazil, it thrives in forests with altitudes ranging between 50 and 900 m with an average annual temperature of 23 °C (Figueiredo and Figueiredo, 2019). The tree is medium to large, measuring up to 40 m in height and 0.6–0.7 cm in diameter (Corrêa, 1990; Frazão, 1990; Santana; Printes, 2004; Santos, 2015; Inga and Castillo, 2016).

We found few works related to the use of the species *C. racemosa* Ruiz & Pav. The wood of this species stands out for its wide use in the furniture industry (plywood and laminates) (Araujo et al., 2020a, 2020b), in the manufacture of musical instruments, carpentry, carpentry, civil and naval construction, and canoeing (Meza and Armenteras, 2021). Among the biological studies, we can mention those proposed by Welch (2010) who reported the toxicity of latex exudate to termites, revealing it as a potential insecticide. While bark decoction was used to treat skin diseases and against wood fungi.

Melo (2015) evaluated the antineoplastic activity of extracts and fractions of *C. racemosa* Ruiz & Pav. The leaf extract, the dichloromethane fraction from the leaves, the ethyl acetate fraction from the leaves, and the hexane fraction from the wood showed high cytotoxicity against the human histiocytic lymphoma cell line. The dichloromethane fraction stood out for presenting significant results against human colon adenocarcinoma cell lines, low metastatic melanoma, and human peripheral blood mononuclear cells.

Cunha and Braz-Filho (1994) isolated two flavonoids, artocarpin, and isoartocarpin, from hexane fractions and chloroform extracts of *C. racemosa* Ruiz & Pav.

In the context of sustainable exploitation of Amazon Forest resources. This work proposed the physical-chemical characterization of the hydroalcoholic extract obtained from the wood of *Clarisia racemosa*. In addition to its various biological properties (antioxidant, antiglycemic, toxicity, antitumor, antimicrobial and photoprotective). The assays discussed here potentiate the use of the hydroalcoholic extract of the species *Clarisia racemosa*, a resource still little explored, as a promising component to be used in cosmetic and pharmaceutical formulations.

2. Materials and methods

2.1. Reagents

The reagents used in this study were: ascorbic acid (CAS 50–81–7), 2,2-diphenyl-1-picrylhydrazyl (DPPH; CAS 1898–66–4), 2,2'-azino-bis acid (3-ethylbenzothiazoline-6-sulfonic) (ABTS; CAS 30931–67–0), sulfuric acid (H₂SO₄, CAS 7664–93–9), potassium persulfate (CAS: 7727–21–1), potassium ferrocyanide [K₄ Fe(CN)₆·3 H₂O] (CAS: 14459–95–1), methanol (CH₃OH, CAS: 67–56–1), trisodium phosphate (Na₃PO₄, CAS: 7601–54–9), butylated hydroxytoluene (BHT, CAS: 128–37–0), ammonium molybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·4 H₂O, CAS: 12054–85–2), ferrous sulfate heptahydrate (FeSO₄·7 H₂O, CAS: 7782–63–0), hydrogen peroxide (H₂O₂, CAS: 7722–84–1), sodium salicylate (HOC₆H₄COONa, CAS: 54–21–7), ferric chloride (FeCl₃, CAS: 7705–08–0), sodium nitroprusside (Na₂[Fe(CN)₅NO]·2 H₂O, CAS: 13755–38–9), griess reagent, gallic acid ((HO)₃C₆H₂CO₂H, CAS: 149–91–7), calcium carbonate (Na₂CO₃, CAS: 497–19–8), Folin-Ciocalteu phenol reagent, tannic acid (C₇₆H₅₂O₄₆, CAS: 1401–55–4), quercetin (C₁₅H₁₀O₇, CAS: 117–39–5), MTT (CAS: 298–93–1). All of these were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, EUA). In addition to these, antimicrobials were used: gentamicin (Medley), Oxacillin (Medley) and Ampicillin+Sulbactam (Novafarma). Mueller Hinton culture media (Kasvi) and RPMI 1640 medium (CBasalab).

2.2. Plant: *Clarisia racemosa* wood

The stalks used in this work were kindly provided by Mil Madeiras Preciosas, a subsidiary of the Swiss group Precious Woods (<http://preciouswoods.com.br/>). The stem collection of *C. racemosa* was carried out in the district of Itacoatiara Manaus, Amazonas, Brazil, at the following location: 03°08'31" and 58°26'33"W in longitude and latitude. To authorize the collection and study of the stem, the species was registered in SisGen (National System of Genetic Heritage and Associated Traditional Knowledge), No. AAF588D.

2.2.1. Analysis of the chemical composition of *Clarisia racemosa* wood

The stems of *Clarisia racemosa*, 20 cm long and 5 cm thick, were dried in an oven (Tecnal, TE-393/1) at 105 °C for 48 h. Then they were ground in a knife mill (FRITSCH – Pulverisette 14) and sieved in a granulometry range of 80 mesh. The ground material was again dried in an oven (Tecnal, TE-393/1) at 105 °C for 48 h (time required to obtain constant mass). These were characterized by chemical composition (cellulose, hemicellulose, lignin, extracts, and ash) according to the analytical methodology proposed by Arruda et al. (2021). For moisture determination, 3 g of ground wood were dried to constant weight at 105 °C for 48 h. Moisture determination was done by gravimetry.

For the determination of extractive, 15 g of wood was extracted consecutively in a Soxhlet extractor with the following solvents: Ethanol/toluene (1:1 v/v, for 24 h). The material was dried in an oven at 105 °C for 48 h. The extractive content was determined gravimetrically.

The determination of polysaccharides was performed after acid hydrolysis (2 g of wood, 72% H₂SO₄, 45 °C, 12 min). Cellulose and hemicellulose contents depend on the concentrations of carbohydrate and organic acids present in the hydrolyzate. After hydrolysis, the reaction mixture was filtered through previously dried filter paper. Carbohydrate and organic acid concentrations were determined by high-performance liquid chromatography (HPLC) (acid hydrolyzate).

The solid material retained on the filter paper corresponds to insoluble lignin. Soluble lignin was determined by HPLC and spectrophotometer (acid hydrolyzate). The total ash content was determined using 2 g of wood, which were calcined in a muffle furnace at 800 °C for 3 h. Total ash results were determined gravimetrically. Assays were performed in triplicate and results were expressed as a percentage.

2.2.2. Obtaining the hydroalcoholic extract

The dry wood of *Clarisia racemosa* (100 g) was used to prepare the extract by the method of exhaustive maceration with 70% ethanol for 7 days at room temperature. Then, aliquots (0.5 L) of the hydroalcoholic extracts from the wood were submitted to the process of solvent removal by rotary evaporation (Biovera, model IKA RV3). The extract was transferred to 0.5 L reaction flasks and kept at – 20 °C for 24 h to evaluate the freezing and the efficiency of the solvent evaporation process. Then, aliquots (20 mL) were lyophilized under a vacuum pressure of 0.024 mBar and a temperature of – 40 °C in a lyophilizer (model L-101, brand Liotop) for 24 h, then frozen at – 20 °C.

The dry extract yield was determined as described by Moghadamnia et al. (2019) and Mota et al. (2020) of the ratio between the dry extract mass and the plant mass expressed as a percentage according to Eq. 1.

$$\text{Yield} = \left(\frac{\text{Extract dry mass(g)}}{\text{Dry Wood Mass(g)}} \right) * 100\% \quad (1)$$

2.3. Phytochemical characterization

2.3.1. Determination of total phenolic content

The content of total phenolics was determined according to Köprü et al. (2020), Mota et al. (2020) and Chen et al. (2021) with modifications. The hydroalcoholic extract of *C. racemosa* (1 mL) at a concentration of 1000 µg/mL was added to 1 mL of the Folin-Ciocalteu reagent (1/10 v/v) and allowed to react for 1 min. Subsequently, 2 mL of

sodium carbonate (2% w/v) was added to the system (extract + reagent), homogenized by vortex and incubated for 2 h in the absence of light at ambient conditions (25 °C). Absorbance values were determined at 765 nm in a UV-vis spectrophotometer (Hewlett-Packard, model 8453). The equipment blank was formed in the same proportions as the sample, using distilled water instead of the extract. After incubation, the total phenolic content of the extract was calculated using a curve prepared with the standard gallic acid at different concentrations (3.12–500 µg/mL) from which the equation, $y = 0.0065x + 0.042$, $R^2 = 0.9978$, was obtained, where y represents the absorbance values and x the concentration values. The total phenolic content was expressed as mg of GAE (gallic acid equivalent) per g of extract. The tests were carried out in triplicate.

2.3.2. Determination of total flavonoid content

The test to determine the content of total flavonoids was carried out according to the methodology proposed by Mbaebie et al. (2012) with few modifications. The extract was diluted in 70% ethanol at a concentration of 1000 µg/mL. In a 10 mL volumetric flask, 1.0 mL of the extract solution, 1.0 mL of 2% ethanol-aluminum chloride reagent were added, and the volume was made up with ethanol. The readings were taken after 30 min, at 425 nm in a spectrophotometer (Hewlett-Packard, model 8453). The equipment blank was formed under the same conditions as the sample, using water instead of the extract. After incubation, the total flavonoid content present in the extract was calculated using a curve with the quercetin standard at different concentrations (3.12–500 µg/mL) from which the equation, $y = 0.0033x + 0.0972$, $R^2 = 0.9906$, was obtained, where y represents the absorbance values and x the concentration values. The total flavonoid content was expressed as mg of QE (quercetin equivalent) per g of extract. The tests were carried out in triplicate.

2.3.3. Determination of total flavonol content

The content of total flavonols in the hydroalcoholic extract was previously determined by Mbaebie et al. (2012) with modifications. The extract was diluted in 70% ethanol at a concentration of 1000 µg/mL. The assay consisted of 2 mL of extract, 2 mL of AlCl₃ (2%)/ethanol and 3 mL of sodium acetate (50 g/L). The mixture was shaken and incubated for 2.5 h at 20 °C. After this period, absorbances were determined at 440 nm in a spectrophotometer (Hewlett-Packard, model 8453). The equipment blank was formed in the same proportions as the sample, using water instead of the extract. After incubation, the total flavonol content present in the extract was calculated using a curve of quercetin at different concentrations (3.12–500 µg/mL) from which the equation, $y = 0.0019x + 0.0414$, $R^2 = 0.9927$, was obtained, where y represents the absorbance values and the concentration values. The content of total flavonols was expressed as mg of QE (quercetin equivalent) per g of extract. The tests were carried out in triplicate.

2.3.4. Determination of total tannin content

The content of total tannins in the hydroalcoholic extract was previously determined by Mbaebie et al. (2012) with modifications. The extract was diluted in 70% ethanol at a concentration of 1000 µg/mL. The test consisted of 2 mL of extract, 3 mL of distilled water, 0.5 mL of Folin-ciocalteu reagent. The system reacted for 3 min. After this period, 1.5 mL of 17% sodium carbonate (Na₂CO₃) and 3 mL of distilled water were added, totaling 10 mL. Samples were homogenized and incubated in the dark for 2 h. After this period, the absorbance of the samples was determined by a UV/Vis spectrophotometer (Hewlett-Packard, model 8453) at a wavelength of 725 nm. The equipment blank was formed in the same proportions as the sample, using water instead of the extract. After incubation, the total tannin content present in the extract was calculated using a curve using the standard tannic acid prepared with standard quercetin at different concentrations (3.12–500 µg/mL) from which the equation, $y = 0.0122x + 0.055$, $R^2 = 0.99$, was obtained, where y represents the absorbance values and x the concentration

values. The tannin content was expressed as mg of TAE (tannic acid equivalent) per g of extract. The tests were carried out in triplicate.

2.3.5. Identification of the main compounds by Ultra Performance Liquid Chromatography (UPLC)

The identification of compounds was performed using the methodology proposed by Barboza et al. (2018) with few modifications. Ultra-Performance Liquid Chromatography (UPLC) was performed with an Acquity H-Class (Waters) employing a 2.1 × 100 mm BEH column with a particle size of 1.7 µm. The column was kept at a constant temperature of 40 °C and the autoinjector at 10 °C. An aqueous solution (eluent A) containing 2% methanol (MeOH), 5 mM ammonium formate, and 0.1% formic acid and a methanol solution (eluent B) containing 0.1% formic acid were used, which were pumped. At a flow rate of 0.3 mL/min. Ten microliters of the extract were injected. Elution was performed in gradient mode and the initial condition (98% A / 2% B) was maintained for 5.0 min. The B ratio linearly increased to 99% in 15 min, remaining at 99% B for one minute, followed by an immediate decrease to 2% B, where it was maintained for up to 30 min. The UPLC system was coupled to a single SQ Detector 2 quadrupolar mass spectrometer (Waters®).

Data were obtained in full scan mode, analyzing masses between 100 and 1000 Da, in negative ionization. The acquisition of the chromatogram was obtained using the MassLynx™ TM (Waters®). Software. The identification of the components present in the hydroalcoholic extract was based on the comparison of mass spectral data by computational correspondence with the NIST Libraries. In addition, information from the literature on the botanical family (Moraceae) was considered useful in the characterization process.

2.4. In silico study: prediction of the pharmacokinetic and toxicological profile of identified compounds from the hydroalcoholic extract of *Clarisia racemosa*

The compounds identified in the extract were subjected to physical-chemical and pharmacokinetic profile analysis. Initially, the chemical structures were obtained through the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>). The compounds were then submitted to the PkCSM web server (<http://structure.bioc.cam.ac.uk/pkcsml>) (Pires et al., 2015), where absorption, distribution, metabolism, excretion, and toxicity characteristics were predicted. The prediction profile was evaluated according to the methodology proposed by Hassan et al. (2017) and Taskin et al. (2021).

2.5. In vitro antioxidant activity

2.5.1. DPPH• (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method

The radical capture assay of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was carried out according to the methodology proposed by Akbaribazm et al. (2020) and Lekouaghet et al. (2020) with few modifications. This method is based on an oxidation-reduction reaction, where the DPPH radical, which has a violet color, is reduced to DPPH-H (reduced and stable DPPH), changing the color of the mixture from purple to yellow (Alam et al., 2013; Akbaribazm et al., 2020; and Lekouaghet et al., 2020). Initially, the extract was diluted in methanol at different concentrations (7.8–1000 µg/mL) then 0.32 mL of each concentration was added to 2.0 mL of 1 mM DPPH methanol solution. Assays were incubated at 25 °C for 25 min in the absence of light. At the end of the reaction, absorbances were determined in a spectrophotometer (Hewlett-Packard, model 8453) at a wavelength of 517 nm. As an experimental control, DPPH solution was used and the equipment blank was methanol. The standards used in this experiment were ascorbic acid and butylated hydroxytoluene. All experiments were performed in triplicate. The sequestration percentages for each concentration were determined by Eq. 2.

$$\text{DPPH}(\%) = \left(\frac{\text{ABS control} - \text{ABS sample}}{\text{ABS control}} \right) * 100 \quad (2)$$

Where: ABS control = control absorbance; ABS sample = absorbance of the sample containing the extract after testing.

The inhibition coefficient (EC_{50}), that is, the minimum concentration of the antioxidant substance necessary to reduce the initial concentration of the DPPH free radical by 50%, was determined by linear regression adjustment of the antioxidant capacity versus the crude extract concentrations.

2.5.2. ABTS⁺ radical reduction method [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]

This method is based on the production of the ABTS⁺ radical (greenish-blue) with the addition of an antioxidant, the radical is reduced, promoting loss of color in the reaction medium (Alam et al., 2013; Ilyasov et al., 2020). The 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺) cation radical scavenging assay was determined according to the methodology described by Köprü et al. (2020) with few modifications. The ABTS⁺ solution was prepared by reacting 7 mM ABTS (5 mL) and 2.45 mM (88 µL) potassium persulfate, after incubation at room temperature in the dark for 16 h. It was then diluted with 80% ethanol to obtain an absorbance of 0.70–734 nm. The ABTS⁺ solution (2.7 mL) was carefully mixed with 0.3 mL and different concentrations (7.8–1000 µg/mL) of the extract. The system was incubated at 25 °C for 5 min in the absence of light. At the end of the reaction, absorbances were determined in a spectrophotometer (Hewlett-Packard, model 8453) at a wavelength of 734 nm.

As a control experiment, the ABTS⁺ solution was used and the equipment blank was methanol. The standards used in this experiment were ascorbic acid and butylated hydroxytoluene. The experiments were carried out in triplicate. The sequestration percentages for each concentration were determined by Eq. 3.

$$\text{ABTS}(\%) = \left(\frac{\text{ABS control} - \text{ABS sample}}{\text{ABS control}} \right) * 100 \quad (3)$$

Where: ABS control = control absorbance; ABS sample = absorbance of the sample containing the extract after testing.

The inhibition coefficient (EC_{50}), that is, the minimum concentration of the antioxidant substance necessary to reduce the initial concentration of the ABTS⁺ radical by 50%, was determined by linear regression adjustment of the antioxidant capacity versus the crude extract concentrations.

2.5.3. Phosphomolybdenum complex (RCF) reduction method

This method is based on the reduction of Mo^{6+} (greenish) to Mo^{5+} (bluish) and subsequent formation of the phosphate complex of Mo^{5+} at acidic pH (Alam et al., 2013). The tests were carried out according to the methodology proposed by Murugan et al. (2020) with few modifications. For this, a phosphomolybdenum solution (with 0.1 mol/L sodium phosphate (28 mL), 0.03 mol/L ammonium molybdate (12 mL) and 3 mol/L (20 mL) sulfuric acid was carried out-H₂O to 100 mL). The extract was diluted at different concentrations (7.8–1000 µg/mL). Reactions were performed with 1 mL of phosphomolybdenum solution and 0.1 mL of each extract dilution and incubated at 95 °C for 90 min. At the end of the reaction, the samples were cooled, and the absorbances were determined in a spectrophotometer (Hewlett-Packard, model 8453) at a wavelength of 695 nm. The equipment blank was a phosphomolybdenum solution (1 mL of solution and 0.1 mL of water). The experimental standards used were ascorbic acid and butylated hydroxytoluene. The experiments were carried out in triplicate. The activity results for each concentration were determined by Eq. 4.

$$\text{RCF}(\%) = \left(\frac{\text{ABS sample} - \text{ABS blank}}{\text{ABS standard} - \text{ABS blank}} \right) * 100 \quad (4)$$

Where: ABS sample = sample absorbance, ABS blank = blank absorbance and ABS standard = ascorbic acid absorbance.

2.5.4. Hydroxyl radical ($\text{HO}\bullet$) scavenging method

The hydroxyl radical scavenging capacity was determined according to the methodology proposed by Smirnov and Cumbe (1989) with adaptations. $\text{HO}\bullet$ radicals were generated from $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ and H_2O_2 , and detected from their ability to hydroxylate salicylate (Alam et al., 2013). The extract was diluted at different concentrations; 7.8–1000 µg/mL. To the extract diluted at different concentrations, 1.0 mL was added 0.5 mL of $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ (1.5 mM), 0.35 mL of H_2O_2 (6 mM), 0.15 mL of sodium salicylate (20 mM). This system was incubated in the absence of light at 37 °C for 1 h. The standards used were ascorbic acid and butylated hydroxytoluene. After the incubation period, absorbances were determined in a spectrophotometer (Hewlett-Packard, model 8453) at a wavelength of 562 nm. The experimental blank used was: 1.0 mL of distilled water added with 0.5 mL of $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$ (1.5 mM), 0.35 mL of H_2O_2 (6 mM), 0.15 mL of sodium salicylate (20 mM). The experimental standards used were ascorbic acid and butylated hydroxytoluene. All experiments were performed in triplicate. The percentage of scavenged radicals ($\text{OH}\bullet$) was determined by Eq. 5.

$$\% \text{OH} = \left[1 - \frac{(\text{ABS sample} - \text{ABS blank})}{\text{ABS standard}} \right] * 100 \quad (5)$$

Where, ABS sample = sample absorbance of extract; ABS blank = blank absorbance; ABS standard = absorbance of the standards under study.

The inhibition coefficient (EC_{50}), that is, the minimum concentration of the antioxidant substance necessary to reduce the initial concentration of the $\text{OH}\bullet$ radical by 50%, was determined by linear regression adjustment of the antioxidant capacity versus the crude extract concentrations.

2.5.5. Nitric oxide ($\text{NO}\bullet$) radical capture method

To evaluate the scavenging of the nitric oxide (NO) radicals in vitro, a solution of sodium nitroprusside was used following the method described by Alam et al. (2013) with few modifications. The assay consisted of 1.0 mL of extract at different concentrations (7.8–1000 µg/mL) was added to 1.0 mL of a solution of sodium nitroprusside (5 mM), the system was incubated at 25 °C for 150 min in the absence of light. After incubation, 0.5 mL aliquots were added to 0.5 mL of Griess reagent. The absorbance of the formed chromophore was determined by a spectrophotometer (Hewlett-Packard, model 8453) at a wavelength of 546 nm. The standards used were ascorbic acid and butylated hydroxytoluene (BHT). The equipment blank consisted of 0.5 mL of distilled water and 0.5 mL of Griess reagent). The experiments were carried out in triplicate. The percentage of scavenged radicals of nitric oxide (NO) radicals was determined by Eq. 6.

$$\% \text{NO} = \left[1 - \frac{(\text{ABS sample} - \text{ABS blank})}{\text{ABS standard}} \right] * 100 \quad (6)$$

Where, ABS sample = absorbance of the extract sample; ABS blank = white absorbance; ABS standard = absorbance of the standards under study.

The inhibition coefficient (EC_{50}), that is, the minimum concentration of the antioxidant substance necessary to reduce the initial concentration of the nitric oxide radical by 50%, was determined by linear regression adjustment of the antioxidant capacity versus the crude extract concentrations.

2.5.6. Method of determination of ferric ion reduction (RIF)

The reducing power of the ferric ion is based on the reduction of Fe^{3+} to Fe^{2+} monitored by the formation of a formed complex called Prussian blue (Alam et al., 2013). The experiments were carried out according to

the methodology proposed by Köprü et al. (2020) with few modifications. For this, a volume of 2.5 mL of extract at different concentrations (final concentration from 7.8 to 1000 µg/mL) was added to 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide. The system was then incubated at 50 °C for 20 min. Subsequently, 2.5 mL of trichloroacetic acid were added and then centrifuged at 3000 rpm for 30 min. Finally, a 2.5 mL aliquot of the supernatant was added to 2.5 mL of distilled water and 0.5 mL of ferric chloride, and the absorbances were determined in a spectrophotometer (Hewlett-Packard, model 8453) at a wavelength of 700 nm. Ascorbic acid and butylated hydroxytoluene (BHT) was used as standard and phosphate buffer as a blank solution. The results were performed in triplicate. The results were expressed as percent inhibition of Fe (II) calculated from the following Eq. 7.

$$\text{RIF}(\%) = \left(\frac{\text{ABS control} - \text{ABS sample}}{\text{ABS control}} \right) * 100 \quad (7)$$

Where: ABS control = control absorbance; ABS sample = absorbance of the sample containing the extract after testing.

2.6. Antiglycant activity promoted by the hydroalcoholic extract of *Clarisia racemosa*

2.6.1. Systems: BSA-glucose/fructose and collagen-glucose/fructose

The antiglycant activity test was carried out according to Xavier et al. (2017) and Oliveira et al. (2021) with few modifications. Solutions of BSA (2 mg/mL), fructose (200 mM) and glucose (200 mM), and sample solutions prepared at different concentrations (7.8–1000 µg/mL) for the extract and standards (rutin, acid) were prepared. gallic acid, tannic acid and aminoguanidine). Samples were diluted in phosphate buffer (pH 7.4). The reaction system consisted of adding 1000 µL of the mixture of fructose and glucose to 1000 µL of different sample concentrations, then 1000 µL of the BSA solution. The system was incubated at 37 °C in the absence of light without shaking for 7 days. For the negative control, in the absence of the sample, phosphate buffer (pH 7.4) was used. The samples were analyzed using a spectrofluorometer (Jasco, model FP-8250) (λ excitation = 360 and λ emission = 440 nm). The blank equipment was the phosphate buffer. Antiglycant activity was determined by the percentage of inhibition using Eq. 8.

$$\% \text{inhibition} = \left(\frac{F \text{ negative control} - F \text{ of the sample}}{F \text{ negative control}} \right) \quad (8)$$

Where: The fluorescence (F) of the sample (F of the sample) and the negative control (F negative control).

Assays for the collagen-glucose/fructose system were performed using the same methodology used in the BSA-glucose/fructose system (using collagen instead of BSA). The determination of the IC₅₀ (required concentration of the extract to inhibit glycation reactions by 50%) was performed using a straight-line equation, in which the x-axis corresponded to the antiglycant capacity (%) and the y-axis corresponding to the extract concentration (µg/mL). To calculate the IC₅₀ the value of x was replaced by 50 (%).

2.7. Evaluation of in vitro toxicity promoted by the hydroalcoholic extract of *Clarisia racemosa*

2.7.1. Hemolytic activity

Blood samples were collected from Hamster Mesocricetus auratus, mean body weight of 30 g and age of 3–4 weeks. The test was carried out by Shubha et al. (2017) and Lima et al. (2018) with some modifications. The extract was evaluated for hemolytic activity by assay performed in 96-well microplates. Each well received 100 µL of a 0.85% NaCl solution containing 10 mM CaCl₂. Samples (100 µL) of extract were added to the first well of the respective treatment, from which 100 µL were

transferred to the second to perform a serial dilution (final concentrations ranging from 3.9 to 500 µg/mL in saline solution). Then, 100 µL of a 2% (v/v) suspension of hamster erythrocytes in saline containing 10 mM CaCl₂ was added. the control consisted of 100 µL of saline solution plus 100 µL of erythrocyte suspension. After centrifugation for 1 h, followed by incubation for 1 h at 27 °C, the supernatant was discarded, and released hemoglobin was determined by absorbance at 540 nm. The results of hemolytic activity were expressed by the following Eq. 9.

$$\text{Hemolysis}(\%) = \left[\frac{(\text{ABS sample} - \text{ABS blank})}{(\text{ABS saponin} - \text{ABS blank})} \right] * 100 \quad (9)$$

Where: ABS sample = sample absorbance; ABS blank = negative control absorbance; ABS saponin = positive control absorbance.

The effective concentration that promotes 50% of hemolysis (IC₅₀) was determined. Three independent experiments were performed in triplicate. This study was approved by the Animal Use Ethics Committee of Institute Aggeu Magalhães/Oswaldo Cruz Foundation, protocol number 164/2020.

2.7.2. Cytotoxic activity on J774 macrophage cells A.1

To evaluate the toxic effects on J774 macrophage cells, the cytotoxicity test was performed by the MTT method (3-(4,5-dimethylthiazol-2-yl) – 2,5-diphenyltetrazolium) according to the methodology proposed by Mota et al. (2020) and Oliveira et al. (2021) with modifications. Cells were seeded on 96-well plates containing RPMI medium supplemented with phenol red and incubated in an atmosphere at 5% CO₂ and 37 °C. After 24 h, cells were incubated in the presence of various extract concentrations (3.9–500 µg/mL) dissolved in distilled water for 72 h. The absorbance reading of formazan crystals was determined using the Benchmark Plus ELISA (Bio-Rad, California, USA) at a wavelength of 540 nm. The experimental control consisted of only cells grown in a culture medium. Each experiment was carried out in triplicate and biological replica. Cell viability was calculated using Eq. 10.

$$\text{Cellular viability}(\%) = \left(\frac{\text{VC}}{\text{TC}} \right) * 100\% \quad (10)$$

Where: VC is the number of cells in different concentrations, TC is the cell concentration in control, which represents 100% viability.

The effective concentration that promotes 50% of cell viability (IC₅₀) was determined. Three independent experiments were performed in triplicate.

2.7.3. Determination of nitric oxide by cells during in vitro cytotoxicity assays

Cell culture supernatants containing the different extract concentrations were subjected to nitrite analysis by the Griess colorimetric method (Ding et al., 1988). Nitric oxide concentration was estimated using a standard curve (3.12–100 µmol/mL). The reading was performed on a spectrophotometer (Bio-Rad 3550, Hercules, CA) at 595 nm.

2.8. In vivo toxicity tests promoted by the hydroalcoholic extract of *Clarisia racemosa*

2.8.1. Animals: Swiss albino mice

Male Swiss albino mice (*Mus musculus*) aged 50 days weighing around 30 ± 0.5 g were obtained from the vivarium of the Keizo Asami Immunopathology Laboratory (LIKA) of the Federal University of Pernambuco (UFPE). The animals underwent an adaptation period of two weeks and then underwent tests carried out at the Animal Experimentation Laboratory of the Department of Biochemistry at UFPE. The mice were fed daily with chow (Purina; Nestlé Brasil Ltda., Brazil) and water and kept at 22 °C under controlled light conditions to simulate a natural

12/12-hour light/dark cycle. The experimental procedures described in this study were approved by the Animal Use Ethics Committee of Instituto Aggeu Magalhães/Oswaldo Cruz Foundation, protocol number 164/2020.

2.8.2. Assessment of acute intraperitoneal and oral in vivo toxicity: hematological, biochemical, histological and oxidative stress analysis

Acute toxicity experiments were evaluated according to guidelines from the Organization for Economic Cooperation and Development, protocol 423 (OECD) and Silva et al. (2020) with few modifications. Female mice were randomly divided into three groups ($n = 5/\text{group}$). The control group of mice received only the vehicle (water), the second and third groups received the hydroalcoholic extract diluted in water at 2000 mg/kg of body weight (b.w.), by intraperitoneal and oral administration (gavage), respectively. The animals in the oral group were deprived of food for 3 h before ingesting the extract. Behavioral changes were evaluated during the first 60 min and for 14 days after the administration of the diluted hydroalcoholic extract. During this period, the following parameters were evaluated: piloerection, sensitivity to sound and touch, changes in the appearance of feces, mobility, and aggressive behavior. In addition to these, body weight, water, and food intake were assessed daily.

On the 15th day, all animals were sacrificed with high doses of anesthetics (ketamine 300 mg/kg and xylazine 30 mg/kg) according to the care, research, and animal sacrifice protocols suggested by the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA). Then, blood was collected from the inferior vena cava and aliquoted into three tubes, two tubes, one containing anticoagulant (ethylenediaminetetraacetic acid, EDTA) and the other without anticoagulant, which was submitted to hematological and biochemical analyses.

Hematological assays were performed in an automated analyzer (Animal Blood Counter: ABC Vet, Montpellier, France) and light microscopy was used to evaluate the following hematological parameters: number of erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), corpuscular hemoglobin mean (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), white blood cells (WBC), segmented (SEG), lymphocytes (LYM), monocytes (MON), basophils (BAS), eosinophils (EOS). We also analyzed the coagulogram using: activated partial thromboplastin (APTT), prothrombin time (PT), and thrombin time (TT).

Biochemical analysis was performed to evaluate albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), bilirubin (BIL), gamma-glutamyl transferase (GGT), total protein (TP), urea in blood (UR), creatinine (CRE), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), non-HDL (triglycerides (TG), low-density lipoprotein cholesterol (LDL), High-density cholesterol low (VLDL), were evaluated using specific commercial kits (Diagnostica Stago, France; Labtest Diagnostic, Lagoa Santa, Brazil; COBAS Mira Plus analyzer (Roche Diagnostics Systems, Basel, Switzerland)) according to the manufacturer's instructions.

The assessment of oxidative stress was determined according to Silva et al. (2020). Liver homogenates were prepared in 50 mM Tris-HCl pH 7.4 containing 1 mM EDTA and 1 mM sodium orthovanadate. These homogenates were evaluated for lipid peroxidation (MDA), catalase activity (CAT) and, finally, total tissue superoxide dismutase (SOD) activity.

To evaluate the histological effects of the extract, liver, kidney, and spleen of the animals in the control and treatment groups, they were performed by optical microscopy. Part of the organ was fixed in buffered formalin (10%, v/v) and subsequently dehydrated in a graded series of ethanol (70 – 100%), clarified in xylene, and embedded in paraffin. Histological sections (5 μm) were stained with hematoxylin-eosin and mounted with Entellan resin coverslips (Merck, Germany). These were observed under a Motic BA200 coupled microscope with a Moticam 1000 1.3 MP digital camera (Motic Incorporation Ltd, Causeway Bay,

Hong Kong).

2.9. Evaluation of in vitro antiproliferative activity

Cytotoxicity assays on tumor cells were determined by MTT assays (3-[4,5-dimethyl-thiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) as described by Statti et al. (2015) and Tizziani et al. (2017) with modifications. MCF-7, T-47D, DU-145, and Jurkat cancer cell lines were obtained from the Department of Antibiotics at the Federal University of Pernambuco, Brazil. Cells were cultured in RPMI 1640 medium (Gibco®), supplemented with 10% fetal bovine serum (SFB-Gibco®) and penicillin: streptomycin solution (1000 IU/mL:1000 $\mu\text{g/mL}$, 1%). The cells were kept in an oven at 37 °C at 5% CO_2 .

Assays were performed using 1×10^4 (MCF-7), 1×10^4 (T-47D), 1×10^4 (DU-145) and 1×10^4 (Jurkat) cells/well and three standard compounds: amsacrine, asulacrin and doxorubicin. The standards were solubilized in DMSO and the extract in water diluted and diluted in RPMI 1640 medium and then tested at $\mu\text{g/mL}$ ranging from 3.12 to 500 $\mu\text{g/mL}$ and incubated for 48 h in a humidified chamber with 5% CO_2 . After treatment, to determine cell viability and later calculate IC_{50} , 20 μL of MTT solution was added and incubated for 3 h. The formed formazan crystals were dissolved in DMSO. Absorbance was performed at 570 nm in a Varioskan model plate reader.

The experiments were performed in triplicate and two independent experiments, with IC_{50} values calculated by non-linear regression using the GraphPad Prism 7 software. In addition, the selectivity index (SI) for the ratio between the values of IC_{50} for normal cells and antitumor cells for the extract, amsacrine, asulacrine, and doxorubicin. These values were determined using Eq. 11.

$$\text{SI} = \frac{\text{IC}_{50} \text{ Normal cells}}{\text{IC}_{50} \text{ Tumor cells}} \quad (11)$$

2.10. In vitro antimicrobial activity promoted by the hydroalcoholic extract of *C. racemosa*

2.10.1. Microorganisms and inoculum standardization

The microorganisms used in this work were obtained from the Collection and Culture of Microorganisms, from the Department of Antibiotics, Federal University of Pernambuco (UFPEDA). The bacterial strains used were *Staphylococcus aureus* UFPEDA-709, *Enterococcus faecalis* UFPEDA-138, *Pseudomonas aeruginosa* UFPEDA-416, *Enterococcus faecalis* UFPEDA-69, *Pseudomonas aeruginosa* UFPEDA-261, *Acinetobacter baumannii* UFPEDA-1024. In addition to bacterial strains, different yeast strains were also studied, namely: *Candida albicans* UFPEDA-1007, *Candida albicans* URM 95, *Candida albicans* 4664, *Candida glabrata* UFPEDA-6393, and *Candida guilliermondii* UFPEDA-6390.

Inoculum standardization for both bacteria and yeast were performed following the recommendations of the Clinical Laboratory Standards Institute documents, M100 (CLSI, 2017) and M60 (CLSI, 2018), respectively. For bacteria, isolated colonies were inoculated in Mueller Hinton broth to achieve turbidity equivalent to 0.5 Mc Farland scale (1.5×10^8 colony-forming units/mL - CFU/mL). Then, the bacterial suspensions were diluted in saline solution to obtain the final inoculum (1.5×10^7 CFU/mL). As for the inoculum for yeasts, isolated colonies were inoculated in the sterile saline solution until turbidity equivalent to 0.5 of the Mc Farland scale (1.0×10^6 to 5.0×10^6 CFU/mL).

2.10.2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal or fungicidal concentration (MBC/MFC)

The MIC of the hydroalcoholic extract and the Gentamicin, Oxacillin, and Ampicillin+Sublactam standards, against bacteria, was determined using the microdilution technique in Mueller Hinton broth in 96-well plates, according to CLSI document M100 (CLSI, 2017). After microdilution of the extract and plate standards, aliquots of previously

prepared and standardized inoculum of the bacteria were added to the wells to obtain a final concentration of 1.5×10^5 CFU/well. Plates were incubated at 37 °C for 24 h.

For yeasts, Micafungin was used as standard, the microdilution technique in RPMI 1640 broth in a 96-well plate was also performed, following the recommendations of the CLSI document M100 (CLSI, 2017). After microdilution of the extract and micafungin, aliquots of inoculum were added to the wells to obtain a final concentration of 2.5×10^3 CFU/well. Plates were incubated at 30 °C for 48 h. MIC was defined as the lowest concentration capable of inhibiting microbial growth compared to a positive control for microbial growth, without treatments. To determine the MBC/MFC, 10 µL aliquots were taken from each well containing the extract and the standards used and were seeded in Petri dishes containing Müller-Hinton agar or Sabouraud agar. Plates were incubated for 24 h at 37 °C, for bacteria, and yeasts for 48 h at 30 °C.

MBC or MFC was defined as the lowest concentration of each compound that resulted in a 99.9% reduction in microbial growth when compared to untreated growth control. The extract concentrations used in this step ranged from 1024 to 4 µg/mL and activity against the microorganism was considered when the MIC was ≤ 1024 µg/mL.

2.11. Photoprotective activity promoted by the in vitro extract

The photoprotective activity was carried out according to the methodology proposed by Mansur et al. (1986), Mota et al. (2020), and Oliveira et al. (2021) with modifications. 1 mL aliquots of the extract were removed, preparing solutions with a final concentration of 20, 30, 50, 70, and 100 µg/mL. A spectrophotometer (Hewlett-Packard, model 8453) with a 1 cm optical path quartz cuvette was used to acquire the spectra. Scans from 290 to 390 nm with 5 nm intervals were performed. The calculations were performed considering the λ intervals determined by Mansur et al. (1986). The experiments were performed in triplicate and the results were expressed as mean $\pm$ standard deviation. The SPF (sun protection factor) results were determined by Eq. 12:

$$\text{SPF in vitro} = CF \times \sum E\lambda \times S\lambda \times \text{Abs}\lambda \quad (12)$$

Where CF (= 10) is an empirical correction factor of the in vitro method, $E\lambda$ is the erythematous spectral efficacy parameter, $S\lambda$ is the solar spectral irradiance and $\text{Abs}\lambda$ is the absorption of UV radiation for a given wavelength. The products $E\lambda \cdot S\lambda$ were calculated as shown in Table 1.

2.12. Statistical analysis

Results are expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using analysis of variance (ANOVA) with Tukey's test to assess significant differences between groups. Values of $p < 0.05$ were considered significant. All statistical analyzes were performed using the GraphPad application.

Table 1
Relationship between radiation intensity and erythemal effect at each wavelength (Mansur et al., 1986).

λ (nm)	EE (λ) x I (λ)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

3. Results and discussion

3.1. Analysis of the physical and chemical composition of *C. racemosa* wood and yield for obtaining the hydroalcoholic extract

The woods presented a complex chemical structure due to the intertwining of the main constituents, cellulose (hexoses) and hemicellulose (pentoses) and lignin (Lucejko et al., 2021). In addition to these, they have extractives and mineral salts in their composition (Lucejko et al., 2021; Kwon et al., 2021). Table 2 presents the results of wood stem composition of *C. racemosa* compared as well as the comparison between the compositions found by others.

Through the composition results found in the literature, it was possible to determine a possible percentage composition range. The cellulose values found ranged from 40.58% to 50.51%, hemicellulose from 16.54% to 24.1%, lignin 25–34.72%, extractives 3.82–16.01% and ash 0.3–1.67%. The chemical composition of wood can vary according to seasonality, climatic conditions, quantification methods, among others (Sluiter et al., 2010; Adeeyo et al., 2015). This justifies the small variations in the compositions between the works found in literature. Therefore, the composition values obtained in this study are in agreement or very close to the data obtained in the literature.

Among the constituents mentioned, extractives or extracts have stood out, as they presented cosmetic and pharmacological properties (Yahya et al., 2018; Veiga et al., 2020). This is because they have a varied and complex chemical composition (Govindappa, 2015). Extracts can be obtained by maceration, infusion, percolation, decoction, hot continuous extraction (Soxhlet), countercurrent extraction, microwave-assisted extraction, ultrasound, supercritical fluid, and turbolysis, alone or together (Azmir et al., 2013; Govindappa, 2015). According to the extraction method used, different yields can be obtained and these can be influenced by several factors, including part of the plant material used, the origin, the degree of processing, the particle size, the solvent used, the extraction time, temperature, polarity and solvent concentration (Tiwari et al., 2011).

The method used in this work, maceration using 70% ethanol, provided a yield of approximately 14.5%. The literature presents different yield results for hydroalcoholic extracts.

Araujo et al. (2020a, 2020b), obtained an extraction yield of 23.62% for an extract obtained from waste from the processing industry of black pepper (*Schinus terebinthifolia* Raddi). Assam et al. (2020) obtained extraction yields of 13.63%, 5.97%, and 10.97% for *Zingiber officinale*, *Vitellaria paradoxa*, *Alstonia boonei* respectively. Alain et al. (2015) obtained extraction yields of 15.2% and 15.6% for *Acacia polyacantha* and *Cissus quadrangulares* plants respectively.

Table 2

Results analysis of the composition of the wood trunk of *Clarisia racemosa* in this study and compared with other authors.

Cellulose (%)	Hemicellulose (%)	Lignin (%)	Extractives (%)	Ashes (%)	References
40.1 $\pm$ 0.1	18.34 $\pm$ 0.0	29.40 $\pm$ 0.2	10.7 $\pm$ 0.0	0.79 $\pm$ 0.0	This work
40.58	16.56	27.33	9.2	0.32	Corrêa (1990)
48	24.1	28	11	0.3	Santana and Okino (2007)
50.81	ND	~25	8.10	1.67	Printes (2004)
50.32	16.82	27.46	3.82	1.58	Inga and Castillo (2016)
40.58	16.56	27.33	7.39	0.32	Frazão (1990)
46.58	ND	34.72	16.01	ND	Santos (2015)

Mean $\pm$ Standard Deviation; ND: Not determined.

Ribeiro et al. (2021) obtained hydroalcoholic extracts from different parts of cashew trees obtained from different regions. For cashew leaves from different regions: Cashew Tree (7.2%), Lime (11.8%), Labino (5.8%), Armadillos (11.0%). For the bark: Cashew Tree (11.4%), Lime (11.0%), Labino (16.4%), Armadillos (12.4%). These findings further confirm the justification that the extraction method, origin, and plant parts influence the yield for obtaining the extract.

3.2. Phytochemical characterization of hydroalcoholic extract

3.2.1. Extract characterization by UV/Visible spectroscopy

Phytochemical characterization by UV/Visible spectroscopy of the extract of *C. racemosa* leaves. Through this analysis, it was possible to determine the presence of phenolic constituents. The results are shown in Table 3.

The content of phenolic compounds in the extract under study was 321.0 mg GAE/g, for flavonoids and flavonols they were 271.9 and 171.3 mg QE/g, respectively. Islam et al. (2019) when evaluating the hydroalcoholic extract of the bark of *Artocarpus lacucha* Buch-Ham obtained values for the phenolic and flavonoid contents equal to 363.34 and 292.06 mg QE/g of the extract, respectively. Studies carried out by Shukla and Kashaw (2019) with hydroalcoholic extract of the pulp of *Artocarpus heterophyllus* Lam obtained phenols and flavonoids equal to 53.8 mg GAE/g and 722.2 mg QE/g, respectively. Pokhriyal et al. (2021), when evaluating the hydroalcoholic extract of *Ficus subincisa* leaves, the results were in phenols and flavonoids equal to 78.67 mg GAE/g and 195.58 mg QE/g, respectively.

Regarding the tannin contents, a value equal to 47.6 mg TAE/g was found. The literature presents different results in relation to the tannin contents for hydroalcoholic extracts. Parimala and Shoba (2013) evaluated the tannin content in the hydroethanolic extract of the seeds of *Nymphaea stellata* and had, as a result, a value equal to 195.84 mg GAE/g. Baghel et al. (2018) obtained a tannin concentration equal to 14.54 mg equivalent of catechin/per g of extract for the hydroalcoholic extract of *Saccharum munja* roots. Popovici et al. (2019) obtained a value equal to 16.23 mg equivalent of catechin for the hydroalcoholic extract of the leaves of *Satureja hortensis* L.

The presence of phenolic compounds, as well as flavonoids in vegetables, is related to several biological properties protecting them from different organisms (Taviano et al., 2018; Suliman et al., 2021; Taher et al., 2021). Among these properties we can highlight the antioxidant activity and the antimicrobial activity; the latter, due to its ability to react with DNA and interfere with replication and, consequently, with microbial growth (Ghasemzadeh and Ghasemzadeh, 2011). Tannins, on the other hand, act in growth regulation, intra, and interspecific interactions, protection against ultraviolet radiation and infections, and herbivory (Monteiro et al., 2005).

It is noteworthy that the composition of secondary metabolites of a plant can be influenced by different factors, including altitude, seasonality, age of the plant, and quantification method (Ghasemzadeh and Ghasemzadeh, 2011; Monteiro et al., 2005).

Table 3

Contents of phenolics, flavonoids, flavonols and tannic acid determined by UV/Vis spectroscopy present in the hydroalcoholic extract from the leaves of *Clarisia racemosa*.

Constituents	Hydroalcoholic Extract (70% v/v)
Total Phenolic Content (mg of EGA/g of extract)	492.0 ± 2.3
Total Flavonoids Content (mg EQ/g extract)	271.9 ± 2.9
Total flavonols Content (mg EQ/g extract)	171.3 ± 1.3
Tannin Content (mg of ETA/g of extract)	47.6 ± 0.4

Mean ± Standard deviation. EAG: Gallic acid equivalent. EQ: Quercetin equivalent. ETA: tannic acid equivalent.

3.2.2. Identification of the main compounds present in the hydroalcoholic extract by ultra-high-performance liquid chromatography coupled with mass spectrometry (UHPLC-MS)

To identify individual phenolic compounds, present in the hydroalcoholic extract obtained from the stems of *Clarisia racemosa*, the analysis was carried out by UHPLC-MS, with ionization in electrospray (ESI) and scanning in negative mode. Through the negative mass values ($[M-H]^-$) it was possible to identify 17 compounds of phenolic nature present in the extract. These compounds were also found by other authors Foyet et al. (2017), Nwokocha et al. (2017), Pádúa et al. (2018), Taviano et al. (2018), Suliman et al. (2021), Taher et al. (2021) and Tewari et al. (2021) characterizing extracts from different plants belonging to the Moraceae family. The results obtained are shown in Fig. 1 (chromatogram) and Table 4 (chemical identification of compounds).

Major signs were determined by compounds 3 (Vanillic acid), 7 (Rutin), 10 (luteolin) and 15 (ferulic acid). The characterized compounds known to promote different biological activities, these being antioxidant (Zimila et al., 2020; Khanal and Patil, 2020), anti-inflammatory (Hmidani et al., 2020), antiglycans (Oliveira et al., 2021), antitumor (Anantharaju et al., 2016; Purnamasari et al., 2019), antimicrobial photoprotective (Silva et al., 2016) among others.

3.3. In silico study: prediction of absorption, distribution, metabolism, excretion and toxicity (ADMET) properties for the compounds identified in the hydroalcoholic extract of *Clarisia racemosa*

Prediction of pharmacodynamic and pharmacokinetic properties is an essential step for the use of different compounds, to assess whether they are safe and effective (Cao et al., 2012; Leahy, 2006; Roy et al., 2015). The literature has shown the use of ADMET to predict the pharmacodynamic and pharmacokinetic properties of phytoconstituents present in different extracts (Hassan et al., 2017; Taskin et al., 2021; Aouadi et al., 2021).

The 17 phytocomponents identified in the hydroalcoholic extract of *Clarisia racemosa*: shikimic acid (1), caffeoylquinic acid (2), vanillic acid (3), (-) epicatechin (4), syringic acid (5), p-coumaric acid (6), rutin (7), kaempferol (8), quercetin dihydrate (9), luteolin (10), quercetin-3-β-D-glycoside (11), apigenin (12), amentoflavone (13), myricetin 3,4'-diglucoside (14), ferulic acid (15), vitexin (16) and tricetin (17) were evaluated for their absorption, distribution, metabolism, excretion and toxicity (ADMET) properties through the free PkCSM platform (<http://structure.bioc.cam.ac.uk/pkcsfm>). The results obtained in this analysis can be seen in Table 5.

Plant extracts have mixtures of phytocomponents in different concentrations, which synergistically promote different biological activities (Foyet et al., 2017; Nwokocha et al., 2017; Pádúa et al., 2018; Taviano et al., 2018; Suliman et al., 2021; Taher et al., 2021; Tewari et al., 2021). Therefore, the results obtained in the evaluation of the properties of

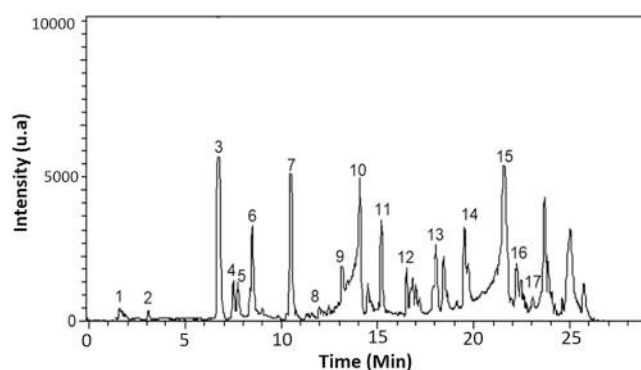


Fig. 1. Chromatogram obtained for the hydroalcoholic extract obtained from the stem of *Clarisia racemosa*.

Table 4

Main compounds identified for the hydroalcoholic extract obtained from the stem of *Clarisia racemosa*.

N	Compound	Tr (min)	Molecular formula	M _w (g/mol)	m/z [H-]
1	Shikimic acid	1.5	C ₇ H ₁₀ O ₅	174.15	173.15
2	Caffeoylquinic acid	3.58	C ₁₆ H ₁₈ O ₉	354.31	353.31
3	Vanillic acid	6.7	C ₈ H ₈ O ₄	168.14	167.14
4	(-) Epicatechin	7.03	C ₁₅ H ₁₄ O ₆	290.27	289.27
5	Syringic acid	7.48	C ₉ H ₁₀ O ₅	198.17	197.17
6	p-Cumaric acid	8.47	C ₉ H ₈ O ₃	164.04	163.04
7	Rutin	10.34	C ₂₇ H ₃₀ O ₁₆	610.517	609.517
8	Kaempferol	12.24	C ₁₅ H ₁₀ O ₆	286.23	285.23
9	Quercetin Dihydrate	13.03	C ₁₅ H ₁₄ O ₉	338.27	337.27
10	Luteolin	14.1	C ₁₅ H ₁₀ O ₆	286.24	285.24
11	Quercetin-3-β-D-glycoside	15.31	C ₂₁ H ₂₀ O ₁₂	464.4	463.4
12	Apigenin	16.28	C ₁₅ H ₁₀ O ₅	270.24	269.24
13	Amentoflavone	18.1	C ₃₀ H ₁₈ O ₁₀	538.45	537.45
14	Myricetin 3,4'-diglucoside	19.7	C ₂₇ H ₃₀ O ₁₈	642.5	641.5
15	Ferulic acid	21.8	C ₁₀ H ₁₀ O ₄	194.18	193.18
16	Vitexin	22.5	C ₂₁ H ₂₀ O ₁₀	432.281	431.08
17	Tricetin	23.2	C ₁₅ H ₁₀ O ₇	302.23	301.23

Tr. retention time in minutes.

absorption, distribution, metabolism, excretion and toxicity (ADMET) in silico are a prediction (Hassan et al., 2017; Taskin et al., 2021; Aouadi et al., 2021). The analysis was performed in a majority way, that is, the evaluation of all phytocomponents presented in the crude extract together.

Regarding the absorption properties, the extract is soluble, presents permeability in caco2 cells and moderate intestinal absorption, and high permeability in the skin. Furthermore, the extract is not able to inhibit the P-glycoprotein (P-gp), and it can be a substrate for this glycoprotein. Substrates enter intestinal mucosal cells by passive diffusion, and only a portion of the substrate is transported out of the cell and into the intestinal lumen by P-gp.

The steady-state volume of distribution (VDSS values) for the extract was considered moderate. Also, according to the prediction in the study, the extract could be considered partially permeable to the CNS and to the blood-brain barrier (BBB). The unbound fraction will cross the membranes, becoming available for interactions with receptors. The phytocomponents have a low unbound fraction, a property confirmed by permeability in cells.

Metabolism was assessed by some cytochrome P-450 isoforms, also identified as CYP, found in the liver, which promotes the biotransformation of different drugs and bioactive compounds. The extract is not able to inhibit different CYP isoforms. Furthermore, it is not able to be used as a substrate.

Regarding toxicity, the extract does not have a high mutagenic potential. The recommended maximum tolerated dose determines the dosage to be used in the first phase of clinical trials. Values for the extract are considered from moderate to low. In addition, it has the potential to inhibit hERG I. and II. Thus, the extract showed low acute and chronic oral toxicity in rats. The extract is non-hepatotoxic and does not irritate the skin. The extract was considered toxic against the protozoan *Tetrahymena pyriformis*; however, it was not considered toxic to the cells of boring Minnows. This prediction shows that the hydroalcoholic extract of *C. racemosa* is a potential candidate that can be used in different cosmetic and pharmaceutical formulations. However, in vitro and in vivo experimental analysis will be necessary to confirm this assertion.

3.4. In vitro antioxidant activity promoted by the hydroalcoholic extract of *C. racemosa*

Antioxidants play an important role in inhibiting and scavenging free

radicals (Krishnaiah et al., 2011). These tests have been widely used in plant extracts due to the number of phenolic compounds they have (Krishnaiah et al., 2011; Al-Jaber et al., 2011). Currently, several tests are developed to evaluate the antioxidant capacity of samples, in which the ability of antioxidants to scavenge reactive species generated in the reaction medium or promote the reduction of ionic complexes can be determined (Al-Jaber et al., 2011; Alam et al., 2013).

Fig. 2 shows the results of antioxidant activity for the different assays performed in this study. Table 6 presents the EC₅₀ results (minimum concentration for radical capture or complex reduction) for the tests performed in this study.

The results presented in Fig. 2A and Table 6 showed that the extract promoted greater antioxidant activity for the DPPH assays. An increase in activity was observed with increasing concentration. The value shown is 4.7 times greater than ascorbic acid and only 1.9 times greater than BHT, proving to be very promising for this activity. The DPPH radical capture assay is considered one of the most used to assess antioxidant activity (Alam et al., 2013). From the color conversion, purple to yellow, it is possible to determine the reduction of DPPH free radical in solution due to the formation of more stable species of DPPH free radical, present in pure substances or complex mixtures, generally phenolic (Alam et al., 2013; Akbaribazm et al., 2020; Lekouaghet et al., 2020).

Fig. 2B and Table 6 present the results of molybdenum complex reducing activity. An increase in activity was observed with increasing concentration, the value shown is 5.9 times greater than ascorbic acid and 3.5 times greater than BHT. The phosphomolybdenum complex reduction method was commonly used to identify vitamin E (Prieto et al., 1999; Sethiya et al., 2014). This test evaluates the antioxidant capacity of a complex mixture of compounds, both lipophilic and hydrophilic components (Alam et al., 2013; Sethiya et al., 2014). The reaction mechanism is through the transfer of electrons between the reducing agent and the molybdenum complex that undergoes reduction from molybdenum VI to molybdenum V (Alam et al., 2013). The polyphenolic ring structure is primarily responsible for the activity (Prieto et al., 1999).

The ABTS test is widely used to quantify the antioxidant capacity because it is simple and easy to reproduce (Alam et al., 2013). Furthermore, the ABTS+ radical can be solubilized in either a hydrophilic or lipophilic medium, without being affected by the ionic strength of the medium (Alam et al., 2013; Ilyasov et al., 2020; Köprü et al., 2020). Regarding the ABTS+ radical capture assay (Fig. 2C), an increase in activity was also observed with increasing concentration. The results showed that the EC₅₀ value promoted by the extract was 1.9 times higher than ascorbic acid and 5.0 times higher than BHT.

Other radicals that deserve attention in the oxidative process are the hydroxyl radicals (HO•) (Fig. 2D) and nitric oxide (NO•) (Fig. 2E). In the body, these radicals are involved in important biological processes, such as phagocytosis, cell growth regulation and intercellular signaling (Farag, and Alagawany, 2018; Mameri et al., 2021). In excess, however, both radicals damage DNA, RNA, proteins and lead to membrane lipid peroxidation (Smirnoff and Cumbes, 1989; Alam et al., 2013; Köprü et al., 2020; Mameri et al., 2021). Among the metals, iron is evident due to its greater bioavailability and, being found in the body, complexed to transport proteins (eg. transferrin) or in storage (eg. ferritin and hemosiderin) (Smirnoff and Cumbes, 1989; Alam et al., 2013; Köprü et al., 2020). In this case, the extract of the present study showed low capture of nitric oxide radicals (Fig. 2E) and was not able to promote the capture of hydroxyl radicals (Fig. 2D). The extract was not able to reduce iron ions (Fig. 2F).

In the extract obtained in this study, there was a higher content of phenolic compounds. Phenolic compounds can reduce or inhibit free radicals by transferring a hydrogen atom, due to the presence of the hydroxyl group (OH) (Santos-Sánchez et al., 2019; Chrzęszcz et al., 2021). In this way, the reaction mechanism occurs between the phenolic compound with the peroxy radical (ROO•) through the combined transfer of hydrogen cations from the phenol with the radical and

Table 5

ADMET *Clarisia racemosa* hydroalcoholic extract.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Absorption																	
Water solubility (log mol/L)	-0.64	-2.77	-2.14	-3.19	-2.09	-2.32	-2.89	-3.04	-2.92	-3.09	-2.92	-3.32	-2.89	-2.88	-2.81	-2.84	-3.02
Caco2 permeability (log Pc cm/s)	-0.29	-0.86	0.34	-0.40	0.42	1.13	-0.94	0.03	0.24	0.09	0.24	1.00	0.14	-1.27	0.17	-0.95	-0.27
Intestinal absorption (% A)	33.01	20.78	82.8	72.6	82.4	99.7	23.4	74.29	67.3	81.1	47.9	93.2	84.3	7.94	93.6	46.6	78.3
Skin Permeability (log Kp)	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73	-2.73
P-glycoprotein substrate	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
P-glycoprotein I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No
P-glycoprotein II inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No
Distribution																	
VDssa	-1.36	-1.59	-1.53	0.21	-1.18	-1.67	1.66	1.27	1.57	1.15	1.84	0.82	-1.06	0.73	-1.36	1.07	0.93
Fraction unbound	0.74	0.39	0.57	0.13	0.60	0.47	0.18	0.17	0.19	0.16	0.22	0.14	0.26	0.24	0.34	0.24	0.20
BBB permeability (log BB)	-0.87	-1.78	-0.41	-1.06	0.06	-0.30	-1.89	-0.93	-1.11	-0.90	-1.68	-0.73	-1.65	-2.35	-0.23	-1.49	-1.38
CNS permeability (log PS)	-3.58	-4.7	-2.55	-3.40	-2.96	-2.37	-5.17	-2.22	-3.37	-2.25	-4.09	-2.06	-3.2	-5.74	-2.61	-3.83	-3.55
Metabolism																	
CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 substrate	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No
CYP1A2 inhibitor	No	No	No	No	No	No	No	Yes	No	Yes	No	Yes	No	No	No	No	Yes
CYP2C19 inhibitor	No	No	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No	No	Yes	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Excretion																	
Total clearance (log mL/min/kg)	0.68	0.33	0.62	0.29	0.66	0.67	-0.36	0.47	0.49	0.49	0.39	0.56	0.48	-0.20	0.62	0.44	0.51
Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Toxicity																	
AMES toxicity	No	No	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No
Maximum tolerated dosed	2.24	1.10	1.18	0.44	1.48	1.06	0.45	0.53	0.49	0.49	0.56	0.32	0.43	0.45	1.08	0.57	0.54
hERG I inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
hERG II inhibitor	No	No	No	No	No	No	Yes	No	No	No	Yes	No	Yes	Yes	No	No	No
Oral rat acute toxicity	1.31	1.91	2.13	2.12	2.01	2.13	2.49	2.44	2.46	2.45	2.54	2.45	2.52	2.47	2.28	2.59	2.42
Oral rat chronic toxicity	2.80	4.15	1.83	2.03	2.41	2.12	3.67	2.50	2.60	2.40	4.41	2.29	3.57	6.32	2.06	4.63	2.55
Hepatotoxicity	No	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Skin Sensitization	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
T. Pyriformis toxicity (log µg/L)	0.28	0.28	0.28	0.34	0.28	0.27	0.28	0.31	0.28	0.32	0.28	0.38	0.28	0.28	0.27	0.28	0.31
Minnow toxicity (log mM)	3.93	4.63	2.49	1.92	2.55	1.70	7.67	2.88	4.15	3.16	8.06	2.43	2.68	10.5	1.82	4.89	4.09

forming a transition state of HO bond with an electron (Santos-Sánchez et al., 2019).

The literature presents different results of in vitro antioxidant activity for hydroalcoholic extracts. Khanal and Patil (2020) evaluating the in vitro antioxidant activity of hydroalcoholic extracts from *F. benghalensis* and *D. repens* obtained EC₅₀ of 73.99 ± 2.22 µg/mL and 70.90 ± 2.13 µg/mL for the DPPH assay, 51.45 ± 1.23 µg/mL and 53.86 ± 2.421 µg/mL for the phosphomolybdenum complex reduction assay, 45.73 ± 1.17 and 53.72 ± 2.13 µg/mL for ABTS and finally obtained 69.02 ± 2.57 and 67.85 ± 4.25 for the nitric oxide elimination assay. Cruz et al. (2020) evaluated the antioxidant activity of different hydroalcoholic extracts obtained for the DPPH EC₅₀ assay that ranged from 42.3 ± 4.8 µg/mL to 207.8 ± 8.7 µg/mL and for the phosphomolybdenum complex reduction assay, they obtained maximum activity values of 141.9 ± 2.9–246.3 ± 4.1 µg/mL. Zimila et al. (2020) evaluating the hydroalcoholic extract from *Salacia kraussii* leaves obtained an EC₅₀ of 35.78 ± 0.09 for DPPH, 36.44 ± 0.67 µg/mL for phosphomolybdenum complex reduction, and 2.22 ± 0.25 µg/mL for the ABTS assay.

These different values are related to the levels of the different constituents present in the extract, which in turn are related to the extraction method and the levels of extracts present in the plant under study (Khanal and Patil, 2020; Cruz et al., 2020; Zimila et al., 2020).

3.5. Evaluation of in vitro antiglycant activity

The glycation mechanisms are still not well understood; however, it is known that this reaction is based on the addition of reducing sugars and/or their reactive products in primary or secondary groups in a non-enzymatic way (Ramkissoon et al., 2013; Yeh et al., 2017; Dil et al., 2019). This process occurs due to condensation between the carbonyl group of reducing sugar and a primary amino group (present in proteins), promoting the formation of a Schiff base. This base undergoes rearrangement for the formation of Amadori products (initial products of glycation) (Yeh et al., 2017; Dil et al., 2019). These can undergo autooxidation and generate different free radicals that, when formed, can contribute to the formation of advanced glycation products which in turn can promote changes in the chemical and functional properties of

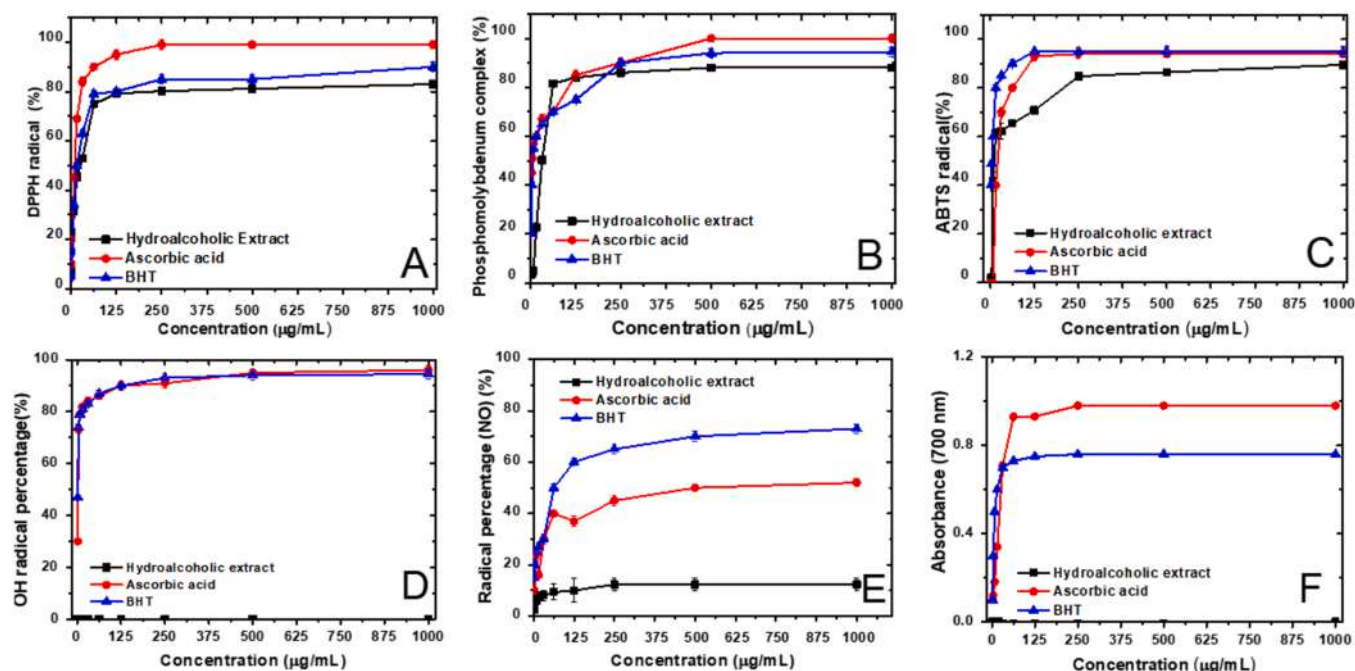


Fig. 2. Antioxidant activity promoted by the extract by different *in vitro* DPPH methods (A); reduction of the phosphomolybdenum complex (B); ABTS (C) scavenging of hydroxyl radicals (OH) (D) nitric oxide (NO) (E) and reduction of iron ions (F).

Table 6

Results of antioxidant activity in percentage at the highest experimental concentration (1000 µg/mL) and EC₅₀ values for *Clarisia racemosa* extract and for ascorbic acid and BHT standards.

	<i>Clarisia racemosa</i> extract		ascorbic acid		BHT	
	AA %	EC ₅₀ (µg/mL)	AA %	EC ₅₀ (µg/mL)	AA %	EC ₅₀ (µg/mL)
DPPH	78.0	36.5 ± 0.5	95.0	7.75 ± 0.1	90.0	18.97 ± 0.4
MO complex	83.0	31.3 ± 0.01	98.0	5.34 ± 0.5	94.0	8.85 ± 0.1
ABTS	84.0	26.34 ± 0.5	95.0	13.37 ± 0.0	95.0	5.24 ± 0.1
OH	0.0	ND	97.0	1.55 ± 0.3	97.0	2.34 ± 0.7
NO	0.0	ND	50.0	501.4 ± 1.3	70.0	67.23 ± 0.1
Iron	0.0	ND	90.0	26.49 ± 0.3	70.0	5.30 ± 0.1

Mean ± Standard deviation; DPPH: DPPH free radical capture; ABTS: ABTS free radical cation capture; MO complex: total antioxidant capacity; OH: hydroxyl radical scavenging; NO: nitric oxide radical scavenging; iron reduction; AA% percentage of antioxidant activity at a concentration of 1000 µg/mL ND: not determined under the conditions studied.

proteins (Yeh et al., 2017; Qais and Ahmad, 2019; Dil et al., 2019).

Fig. 3 shows the results of antiglycating activity for the hydroalcoholic extract of *C. racemosa*, gallic and tannic acids, and rutin at different concentrations against the BSA-glucose/fructose (A) and collagen-glucose/fructose (B) systems.

The results showed that the extract and the phenolic standards used showed an increase in response with increasing concentration. This profile was similar to the one obtained by Ardestani and Yazdanparast (2007), Antora et al. (2012), and Alsahli et al. (2021) also evaluating hydroalcoholic extracts. Through the curves obtained, it was possible to determine the IC₅₀ (concentration capable of inhibiting the formation of advanced glycation products by 50%) for the hydroalcoholic extract of *C. racemosa*, gallic and tannic acids, and rutin, these values were compared to the drug aminoguanidine. These results are shown in

Table 7.

The results show that the extract, when compared to the phenolic standards (gallic acid, tannic acid, and rutin) has a lower IC₅₀ of inhibition, generating greater antiglycating activity. This fact may be related to the different compounds present in the extract (Yeh et al., 2017; Dil et al., 2019). Thus, in descending order of greater antiglycating activity, extract > tannic acid > gallic acid > rutin is found. Compared to the drug aminoguanidine, the extract showed lower activity. This fact may be related to the mechanism of action, aminoguanidine is a very versatile molecule, being able to act by sequestering and/or inhibiting intermediate compounds involved in the formation of advanced glycation products, resulting in greater antiglycating activity (Yeh et al., 2017; Dil et al., 2019; Oliveira et al., 2021).

In the literature, some works present different IC₅₀ values of antiglycating activity for hydroalcoholic extracts. Gutiérrez et al. (2010), evaluating the activity of hydroalcoholic extracts of different types of spices (16) and pepper (21) from cooking, obtained IC₅₀ values ranging from 1330 and 620 µg/mL. Telapolu et al. (2018) obtained for a herbal formulation (extract obtained in ethanol/water system) an IC₅₀ of 1.03 ± 0.54 µg/mL. Arachchige et al. (2017) obtained for the hydroalcoholic extracts of bark and leaves of Ceylon cinnamon (*Cinnamomum zeylanicum* Blume) IC₅₀ values ranging from 94.33 and 107 µg/mL. Oliveira et al. (2021) obtained IC₅₀ values for the ethanolic extract of coconut husk fiber of 9.61 ± 1.12 µg/mL for the BSA - glucose/fructose system and 4.50 ± 0.32 µg/mL for the Collagen - glucose/fructose system. These differences are directly related to the content and type of constituents present in each extract (Gutiérrez et al., 2010; Arachchige et al., 2017).

The hydroalcoholic extract of *C. racemosa* showed antiglycating activity, being able to reduce the interaction of the carbohydrates undertaken with the different proteins (albumin and collagen). Glycation reactions promote damage to these proteins, causing them to lose functionality (Oliveira et al., 2021). The extract from this study showed promising properties for its use as a component in cosmetic and pharmaceutical formulations.

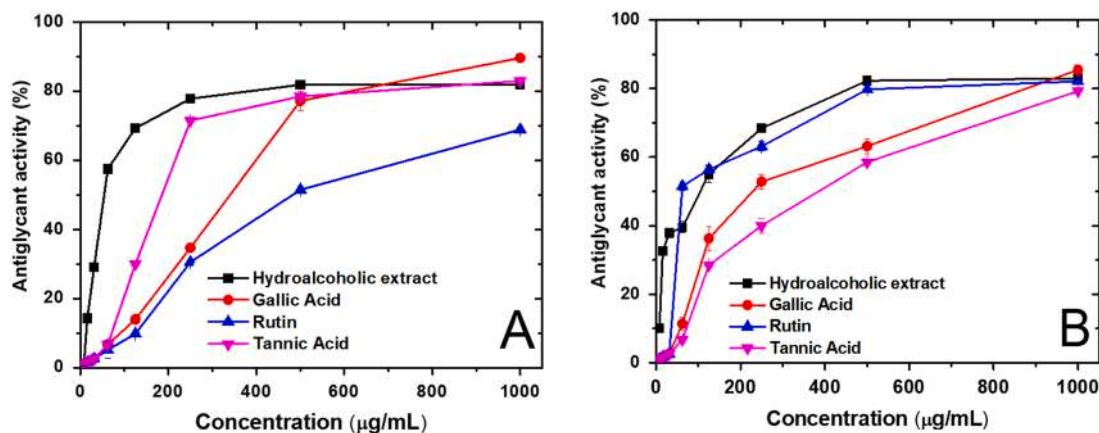


Fig. 3. Antiglycant activity results in percentage for the hydroalcoholic extract of *C. racemosa*, gallic and tannic acids, and rutin at different concentrations against the BSA-glucose/fructose (A) and collagen-glucose/fructose (B) systems.

Table 7

Results of the antiglycant activity in percentage at the highest experimental concentration (1000 µg/mL) and IC₅₀ values for the extract of *Clarisia racemosa* and for the standards of gallic acid, tannic acid and rutin compared to the drug aminoguanidine.

Samples	Albumin		Collagen	
	Antiglycant (%)	IC ₅₀ (µg/mL)	Antiglycant (%)	IC ₅₀ (µg/mL)
Extract <i>Clarisia racemosa</i>	81.8	145.7 ± 0.1	82.3	150.4 ± 0.01
Gallic acid	89.63	467.62 ± 0.5	85.49	459.1 ± 2.5
Tannic acid	83.01	421.67 ± 0.9	79.30	527 ± 1.0
Rutin	68.9	639.79 ± 1.4	82.1	349.5 ± 1.9
Aminoguanidine	100	20.12 ± 1.1	100	90.34 ± 0.2

Mean ± Standard deviation.

3.6. Evaluation of the *in vitro* cytotoxic activity promoted by the hydroalcoholic extract of *C. racemosa*

3.6.1. *In vitro* cytotoxicity in normal cells

In vitro cytotoxicity assays using animal cells are important to verify the toxicity of different organic extracts, especially when evaluating their applicability as a therapeutic and cosmetic agent. For this last application, the use of animal models to assess toxicity has been decreasing, both *in vitro* (primary cultures) and *in vivo* (use of animals) due to the use of other experimental models (culture of immortalized cells and tissue culture (Kumar et al., 2011; Tsai et al., 2020; Mameri et al., 2021)).

Fig. 4 presents the results for cytotoxicity assays against Hamster erythrocyte cells (Fig. 4A) and J774 A.1 macrophages (Fig. 4B). In addition to the cytotoxicity assays, the levels of nitric oxide produced by erythrocytes (Fig. 4C) and macrophages (Fig. 4D) were determined during cultivation with different concentrations of the extract.

In vitro hemolysis assays are important tools in evaluating the cytotoxicity of natural compounds (Kumar et al., 2011). It is an activity that is easy to perform and gives quick results (Kumar et al., 2011; Shubha et al., 2017; Lima et al., 2018). Red blood cells or red blood cells are used in many studies related to membrane composition and behavior (Shubha et al., 2017; Lima et al., 2018). These assays contribute information to estimate the behavior of other cell membranes, mainly due to their availability and accessibility (Kumar et al., 2011; Shubha et al., 2017; Lima et al., 2018). These cells are used as models for cell studies for mechanisms of cytotoxicity and oxidative stress (free radical

production) (Farag and Alagawany, 2018; Mameri et al., 2021).

The hemolysis results (Fig. 4A) showed that the higher the concentration of the extract, the greater its hemolytic activity (dose dependent effect). However, even at the highest concentrations (125, 250 and 500 µg/mL) the extract was not able to promote 50% hemolysis of erythrocytes (IC₅₀). At the highest concentration (500 µg/mL) a percentage of hemolysis of 21.94 ± 1.6% was obtained. At concentrations lower than 125 µg/mL, the hemolysis activity was practically null. Other authors also evaluated the hemolytic effect promoted by different hydroalcoholic extracts. Luz et al. (2021), where they found a percentage of hemolysis of 40% for the concentration of 500 µg/mL for the hydroalcoholic extract of the leaves of *Licania rigida Benth.* Ramos et al. (2020) evaluated the hydroalcoholic extracts of the bark and leaves of *Erythrina velutina* and these showed a percentage of hemolysis varying between 27.01% and 66.84% for the concentration of 500 µg/mL. The extract from the leaves of *Microgramma vacciniifolia*, evaluated by Silva et al. (2020), showed a percentage of hemolysis of 10.23% for the concentration of 500 µg/mL.

Hemolytic processes with percentage values above 40% are considered high, and extracts are considered highly hemolytic (Ramos et al., 2020). Thus, the concentrations under study promote low or no hemolytic activity against erythrocytes.

The results shown in Fig. 4B show that during cell culture, there was an increase in nitric oxide without promoting significant cell death. In adverse conditions, erythrocytes or red blood cells are among the first cells in the body to be affected (Mameri et al., 2021). As they do not have a nucleus and mitochondria, the cellular response of this type of cell mainly considers the permeability of the compounds in the membrane, as well as the interaction with the antioxidant systems in the cytoplasm (Mameri et al., 2021). The structural preservation and function of the membrane is essential in maintaining adequate permeability and flexibility as well as the ionic balance between the intracellular and extracellular environment (Li et al., 2013). Chemically, the erythrocyte membrane consists mainly of phospholipids, forming a lipid bilayer, and proteins, which are considered the main targets for damage caused by excess reactive oxygen and nitrogen species in situations of oxidative stress (Kleinbongard et al., 2006; Li et al., 2013; Farag, e Alagawany, 2018; Mameri et al., 2021).

Macrophage cells as well as erythrocytes are also important models to assess the cytotoxicity promoted by different organic extracts (Tsai et al., 2020). It is known that macrophages are important cells for the activation of the immune response, since they induce the release of cytokines (Gordon, 1998). The results presented in Fig. 4C showed that the extract had IC₅₀ = 160.5 ± 1.8 µg/mL. That is, this concentration can promote cell death of macrophages by 50%.

The literature presents different results of cytotoxicity promoted by

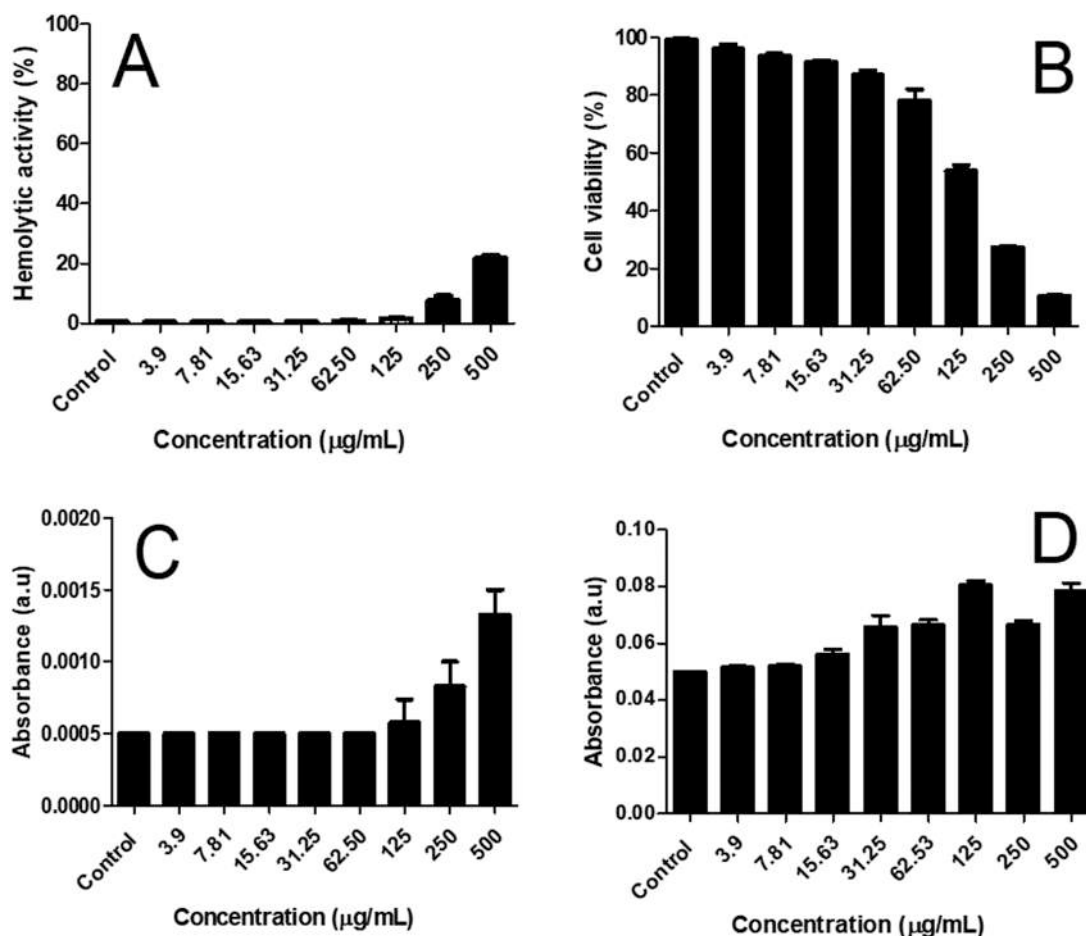


Fig. 4. Hemolytic activity in erythrocyte cells (A), cell viability in J774 A.1 macrophages (B) and nitric oxide production in erythrocyte cells (C) and J774 A.1 macrophages (D).

hydroalcoholic extracts. Bayazid et al. (2020) evaluated the antiproliferative effect of hydroethanolic and aqueous extracts of *Mori Cortex Radicis* both significantly inhibited LPS-induced cell death in RAW 264.7 macrophage cells. Adeniran and Ashafa (2020) analyzed the same effect using the hydroethanolic and ethanolic extract of the roots of *Hermannia geniculata* on RAW 264.7 macrophage cells, both also showed no significant difference in the percentage of cell viability at all concentrations of the extracts tested. Moreira et al. (2017) evaluated this antiproliferative effect of the hydroethanolic extracts of the fruits of *Sapindus saponaria* L which presented IC_{50} equal to 81.66 ± 2.88 μg/mL in macrophage cells. These results vary according to the type, constitution, origin and concentration of the extract. Fig. 4D shows the increase in the concentration of nitric oxide with the increase in the concentration of the extract.

Macrophages play an important role in the defense mechanism against pathogens (Cruz et al., 2007; Chou et al., 2013). After phagocytosis of the pathogen, macrophages degrade it in the phagosome, through reactive oxygen species (ROS), activating NADPH oxidase (Goyal et al., 2011; Chou et al., 2013). This process leads to electron transfer from cytosolic NADPH to molecular oxygen-releasing superoxide in the phagosomal lumen (Cruz et al., 2007; Chou et al., 2013). Inside the phagosome, the oxygen-free radical is rapidly converted to hydrogen peroxide (H_2O_2) via superoxide dismutase, which therefore reacts with iron to generate highly reactive hydroxyl radicals (HO) (Cruz et al., 2007; Goyal et al., 2011; Chou et al., 2013). Here, nitric oxide acts as an intercellular marker recognized in the immune system (Orsi et al., 2000). This is involved in both immunologically mediated diseases and inflammation, and it will be up to molecules, such as phenols and

flavonoids, the role of preventing the attack of ROS and reactive nitrogen species or regenerating the damage caused in biological systems (Barreiros et al., 2006).

3.7. In vivo tests: determination of single dose acute toxicity

Acute toxicity experiments were performed in three groups ($n = 5/\text{group}$). In the first group, the mice received only water (control), the second and third groups received the hydroalcoholic extract diluted in water at 2000 mg/kg of body weight (bw), by intraperitoneal administration and by gavage respectively, for 14 days. After acute treatment, orally (gavage) and intraperitoneally, with a dose of 2000 mg/kg in mice, there was no evidence of death or behavioral changes in the animals evaluated. At the end of the treatment, the animals were euthanized, then the organs were removed, weighed, and analyzed macroscopically. No signs of hemorrhage or necrosis were observed. Table 8 shows the values of water consumption, feed, and evolution of the average weight and weight of organs, kidneys, spleen, stomach, and liver during the 14 days of treatment.

The results show that no significant differences were found ($p > 0.05$) in the parameters of water consumption, feed, and weight of animals treated with *C. racemosa* extract via gavage or intraperitoneally when compared to the control (animals treated with water). In addition, organ weight did not change significantly ($p > 0.05$) during the treatment when compared to control.

The literature presents different hydroalcoholic extracts that do not present in vivo toxicity against mice. Bhandare et al. (2010) performed acute oral toxicity tests at a dose of 2000 mg/kg of the hydroalcoholic

Table 8

Evaluation of water and food consumption, average weight of animals and evaluation of relative weight (g/10 g of animal body weight) of organs in mice treated with the hydroalcoholic extract of *Clarisia racemosa*.

Parameters	Treatments		
	Control	Gavage	Intraperitoneal
Water consumption (mL)	26.79 ± 1.88	26.92 ± 1.38	26.67 ± 1.82
Food consumption (g)	17.35 ± 0.41	17.85 ± 0.51	17.32 ± 0.86
Average weight (g)	35.09 ± 0.85	34.81 ± 0.49	35.68 ± 0.92
Organs	Control	Gavage	Intraperitoneal
kidney (g)	0.54 ± 0.08	0.55 ± 0.09	0.55 ± 0.13
Spleen (g)	0.28 ± 0.03	0.28 ± 0.07	0.28 ± 0.09
Liver (g)	2.37 ± 0.58	2.36 ± 0.61	2.37 ± 0.65

Mean ± Standard deviation.

extract of *Areca catechu* L. nut and verified that the extract did not promote death in mice and rats for 14 days. The animals did not show signs of toxicity or change in general behavior or other physiological activities. Vyas et al. (2011) showed that acute toxicity studies with alcoholic extracts did not cause mortality in animals treated with concentrations of up to 2000 mg/kg being considered safe. Damasceno et al. (2016) evaluated the effect of the hydroalcoholic extract of *Solanum cernuum* leaves. They found that the extract showed no toxic effects in Swiss mice. Nureye et al. (2021), evaluating the in vivo toxicity promoted by the hydroalcoholic extract of *Zehneria scabra* roots, found that the extract did not promote toxicity at a concentration of 2000 mg/kg in 14 days. Therefore, the hydroalcoholic extract of *C. racemosa* is non-toxic and safe. Histological analysis confirms this result and awaits the result. However, the discussion is ready, as there were no significant changes during treatment.

In addition to macroscopic and histological analyses, evaluations of hematological, biochemical, and oxidative stress parameters were performed. These results are shown in Table 9.

Regarding the hematological, biochemical, and oxidative stress parameters, the extract did not promote significant changes ($p > 0.05$) during the treatment. Other authors found results similar to those obtained in this study. Coelho et al. (2001), evaluating the effects of the hydroalcoholic extract of *Pterodon pubescens* seeds, verified that the extract was not able to change the hematological parameters. Hussain et al. (2012) evaluating the in vivo toxicity of the hydroalcoholic extract of *Tephrosia purpurea* verified that the extract did not promote significant changes in hematological and biochemical parameters in experimental groups of rats. Cossetin et al. (2020) verified that the hydroalcoholic extract of *Arachis hypogaea* leaf leaves did not promote significant changes in hematological and biochemical parameters by studying male and female Wistar rats.

Furthermore, we evaluated the liver oxidative profile of mice treated with the hydroalcoholic extract of *C. racemosa*. The liver plays an important role in xenobiotic metabolism, making it a target organ for studies of oxidative damage (Silva et al., 2020). The hydroalcoholic extract of *C. racemosa* did not promote any significant changes ($p > 0.05$) in the levels of MDA, SOD, and CAT in the mouse liver (Table 9), thus indicating the absence of induction of oxidative stress. These results are similar to those obtained by Silva et al. (2020) for extracts of *M. vaciniifolia*.

3.8. In vitro antiproliferative activity promoted by the hydroalcoholic extract of *C. racemosa*

The hydroalcoholic extract of *C. racemosa* showed low cytotoxicity in cells of erythrocytes and J774 A.1 macrophages, in addition to not promoting toxicity in vivo assays. Therefore, it's in vitro antiproliferative potential was investigated. Tumor cells have four characteristics that differentiate them from normal cells, namely uncontrolled proliferation, differentiation and loss of function, invasiveness, and ability to undergo metastases (Anantharaju et al., 2016; Wannes et al.,

Table 9

Hematological, biochemical and oxidative stress parameters for animals in the control group and treated with the hydroalcoholic extract of *Clarisia racemosa*.

Parameters	Treatments		
	Control	Gavage	Intraperitoneal
Hematological parameters			
RBC	5.21 ± 0.58	5.52 ± 0.42	5.31 ± 0.44
HCT	34.2 ± 2.09	36.4 ± 3.40	35.1 ± 3.32
HB	14.1 ± 0.26	14.4 ± 0.32	14.1 ± 0.29
MCV	46.4 ± 3.98	46.8 ± 4.08	47.9 ± 3.93
MCH	16.5 ± 1.31	17.5 ± 1.34	18.0 ± 1.95
MCHC	36.1 ± 3.16	37.7 ± 3.49	37.9 ± 3.86
WBC	7.65 ± 0.56	7.42 ± 0.48	7.28 ± 0.57
SEG	68.8 ± 4.66	70.1 ± 4.48	69.6 ± 5.86
LYM	27.9 ± 1.35	26.0 ± 1.83	27.1 ± 1.82
MON	3.43 ± 0.30	3.27 ± 0.39	3.15 ± 0.41
BOS	0.25 ± 0.05	0.27 ± 0.07	0.28 ± 0.05
EOS	1.34 ± 0.19	1.42 ± 0.19	1.43 ± 0.18
Biochemical parameters	Control	Gavage	Intraperitoneal
ALB	39.1 ± 3.76	39.9 ± 3.44	37.9 ± 4.12
ALT	67.3 ± 4.51	68.1 ± 4.27	67.4 ± 4.22
AST	89.1 ± 4.31	91.5 ± 4.78	90.4 ± 5.24
ALP	13.2 ± 0.45	13.2 ± 0.61	13.3 ± 0.37
BIL	0.42 ± 0.09	0.38 ± 0.10	0.40 ± 0.08
GGT	12.4 ± 0.41	12.2 ± 0.53	12.3 ± 0.64
TP	70.2 ± 5.14	69.1 ± 5.26	71.2 ± 5.73
UR	0.35 ± 0.04	0.38 ± 0.06	0.35 ± 0.03
CRE	4.57 ± 0.50	4.28 ± 0.42	4.19 ± 0.39
TC	70.6 ± 6.34	74.1 ± 5.11	73.1 ± 5.16
TG	90.1 ± 7.53	93.5 ± 7.14	90.4 ± 8.73
Oxidative stress	Control	Gavage	Intraperitoneal
MDA (nM/mg of protein)	6.19 ± 0.52	10.12 ± 1.25	3.10 ± 0.15
SOD (U/mg of protein)	6.45 ± 0.62	10.68 ± 1.19	3.18 ± 0.25
CAT (nM/mg of protein)	6.22 ± 0.51	10.51 ± 1.24	3.23 ± 0.30

Mean ± Standard deviation; RBC: Red Blood Cells (106/mm³); HCT: Hematocrit (%); HB: Hemoglobin (g/dL); MCV: Mean Corpuscular Volume (%); MCH: Mean Corpuscular Hemoglobin (%); MCHC: Mean Corpuscular Hemoglobin Concentration (%); PLT: Platelets (103/mm³); WBC: White Blood Cells (103/mm³); SEG: Segmented (%); LYM: Lymphocytes (%); MON: Monocytes (%); BOS: Basophil; EOS: Eosinophil. ALB: albumin (g/dL); ALT: alanine aminotransferase (U/L); AST: aspartate aminotransferase (U/L); ALP: alkaline phosphatase (U/L); BIL: bilirubin (mg/dL); GGT: gamma-glutamyl transferase; TP: total protein (g/dL); UR: blood urea (mg/dL); CRE: creatinine (mg/dL); TC: cholesterol total (mg/dL); TG: triglycerides (mg/dL); HDL: high-density lipoprotein-cholesterol; LDL-c: low-density lipoprotein-cholesterol; VLDL: Very low density-cholesterol. Lipid peroxidation (MDA), catalase activity (CAT) and total tissue superoxide dismutase (SOD) activity.

2018). Therefore, plant extracts can inhibit cancer cells by multiple mechanisms, such as the release of hydrogen atoms and electrons, induction of the release of protective conjugated enzymes, increase in apoptosis, inhibition of lipid peroxidation, inhibition of angiogenesis, and inhibition of oxidation of the DNA (Neto, 2007; Anantharaju et al., 2016; Wannes et al., 2018; Purnamasari et al., 2019).

The results of the inhibition of cancer cells promoted by the extracts may be related to the presence of high levels of phenolic groups (Neto, 2007; Anantharaju et al., 2016; Purnamasari et al., 2019). Phenolics with a greater number of hydroxylic groups promote greater anticancer activity compared to those without hydroxylic groups or compounds with -OCH₃ moieties (Anantharaju et al., 2016; Wannes et al., 2018). In addition, the presence of short unsaturated fatty acid chains in phenolic compounds also enhances the activity (Neto, 2007; Anantharaju et al., 2016; Wannes et al., 2018; Purnamasari et al., 2019). Fig. 5.

Fig. 6 and Table 10 show the results of in vitro antiproliferative activity promoted by the hydroalcoholic extract of *C. racemosa* against cancer cells, these being Jurkat (leukemia/lymphoma) (A), T47D (breast cancer) (B), MCF-7 (breast cancer) (C) and DU-145 (human prostate cancer cell) (D). The results obtained for the hydroalcoholic extract under study were expressed as IC₅₀ (concentration that inhibits the proliferation of cancer cells by 50%) and these were compared to the standards of doxorubicin, amsacrine, and asulacrine. In addition, the index of selectivity relationship between cancer cells and normal cells

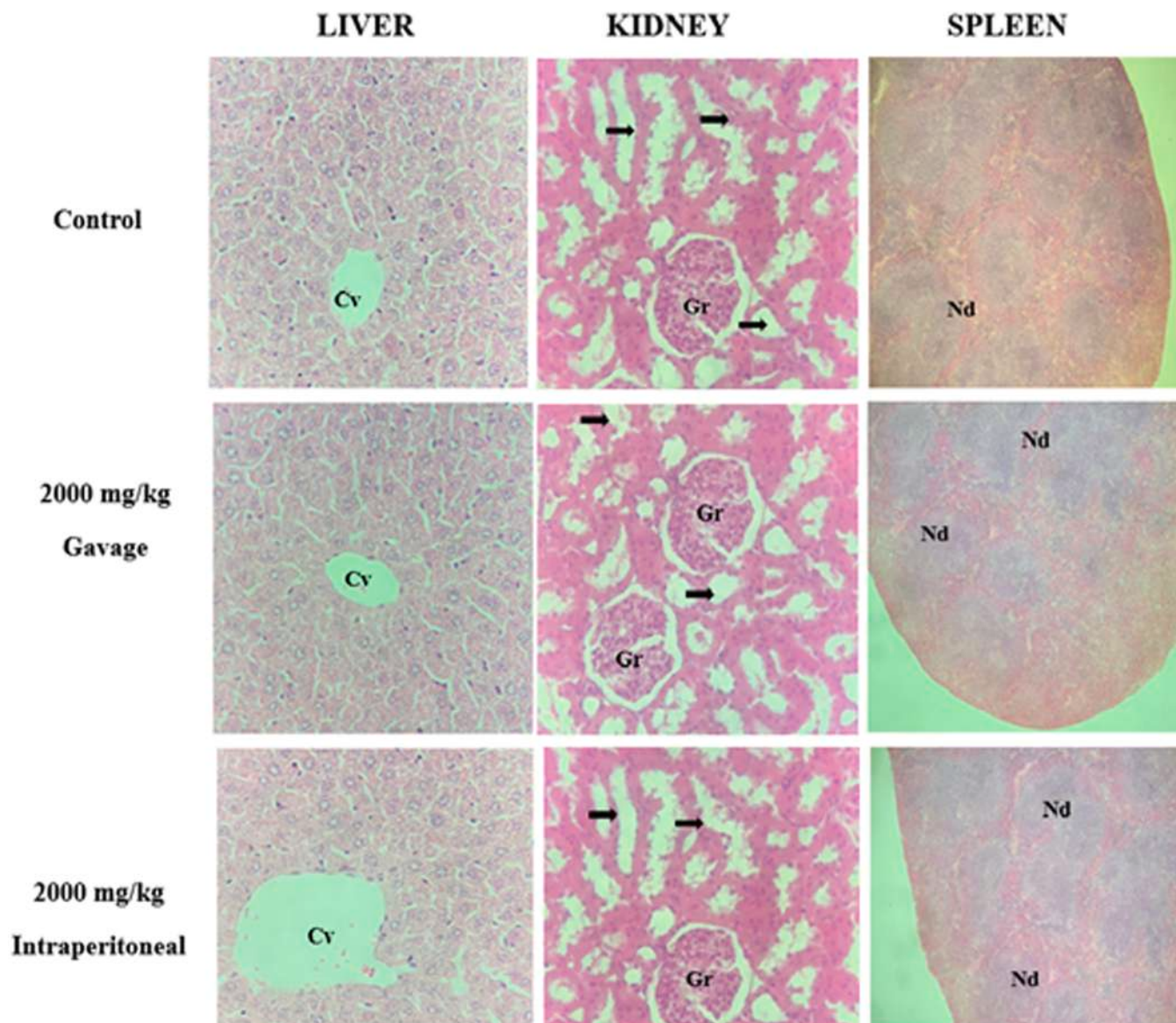


Fig. 5. Representative photomicrographs of liver, kidney and spleen of mice from control groups and those treated with a single dose with the hydroalcoholic extract of *C. racemosa* at a dose of 2000 mg/kg intraperitoneal and by gavage. Livers: the centrilobular vein (cv) is seen on all images with the presence of well-organized hepatocyte cords. Kidneys: Kidney glomeruli (Gr) and contorted tubes (arrowheads) preserved in order. Bowman's intracapsular space is well delimited and with a normal diameter. Spleen: Lymph nodes (Nd) are well defined in the control and treated groups. We can visualize the organ pulps without hyperactivation and with well-defined contours. Hematoxylin and eosin staining was used. Magnification: 400x for liver and kidney. 100x magnification for the spleen.

was determined (Macrophages J774 A.1). **Fig. 7.**

The results, presented by the curves found in **Fig. 6**, showed an increase in antiproliferative activity with increasing concentration. A similar profile was found for other hydroalcoholic extracts against different tumor cells, among which we can mention the work carried out by [Dermani et al. \(2021\)](#) evaluating the *Cuscuta chinensis* extract against MCF7 cells, a fact confirmed by [Mallick et al. \(2015\)](#) for the extract of *Picrorhiza kurroa*, by [Don and Yap \(2019\)](#) evaluating ethanol extract against MCF7 and Jurkat cells and by [Mosadegh et al. \(2021\)](#) evaluating the extract against MCF7 and T47D cells.

The IC₅₀ results obtained for the extracts are close to those obtained for the standards (m-amsacrine, asulacrinc, and doxorubicin). However, with the selectivity index, greater results were observed for the extract of *Clarisia racemosa*. Higher SI values indicate that the extract is more toxic to tumor cells when compared to normal cells (macrophages). The antitumor activity of extracts is related to their chemical composition (origin, types of constituents, and concentration) ([Neto, 2007](#); [Mallick](#)

[et al., 2015](#); [Anantharaju et al., 2016](#); [Don and Yap, 2019](#); [Purnamasari et al., 2019](#); [Dermani et al., 2021](#)).

3.9. In vitro antimicrobial activity promoted by the hydroalcoholic extract of *C. racemosa*

In vitro antimicrobial activity, assays serve to identify potential antimicrobials that act as new anti-infective agents ([Nwonuma et al., 2020](#)). **Table 11** presents the results of in vitro antimicrobial activity promoted by the hydroalcoholic extract of *C. racemosa* against Gram-positive, Gram-negative bacteria and yeasts, as well as the activity results for commercial antimicrobials, which were used as controls, in addition, to determine certain resistance patterns of the tested strains.

It can be observed that the extract of *C. racemosa* showed antibacterial activity against the species of gram-positive cocci tested, *Enterococcus faecalis* and *Staphylococcus aureus*, with MIC ranging from 512 to 1024 µg/mL, with emphasis on the strain *S. aureus* UFPEDA-709, in front

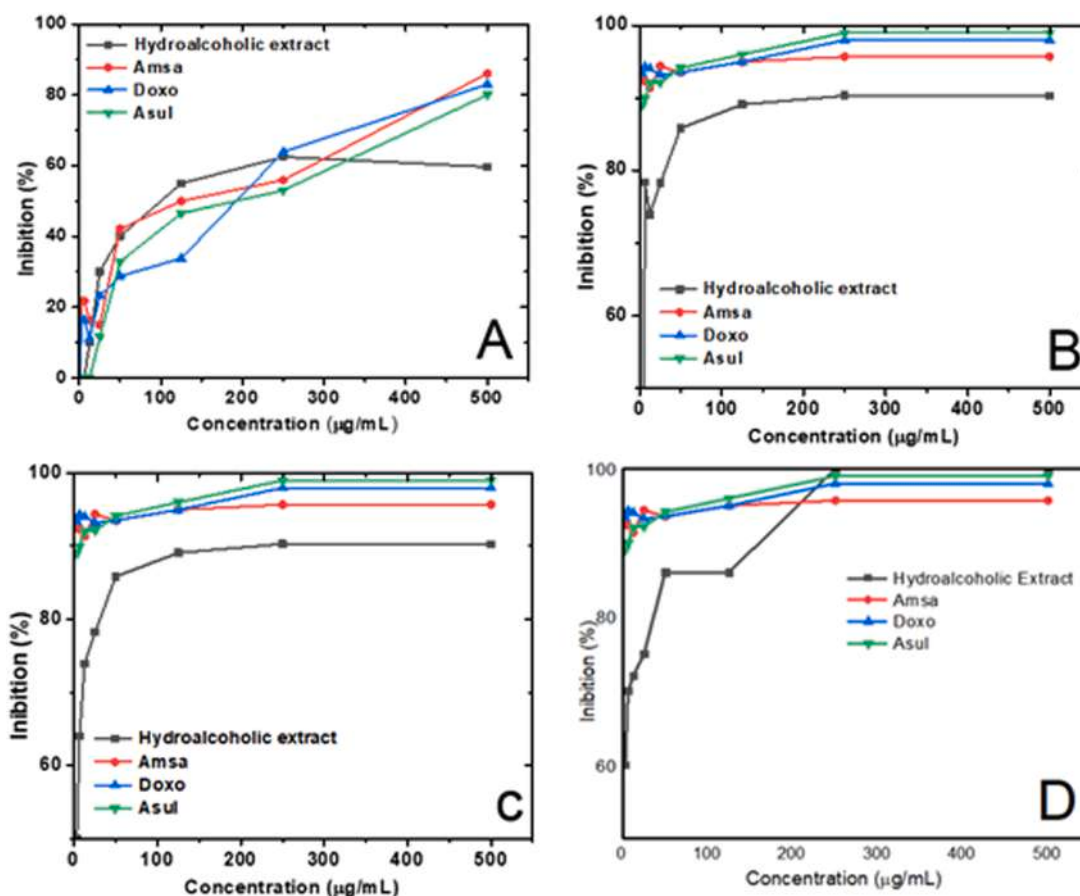


Fig. 6. Results of in vitro antiproliferative activity promoted by the hydroalcoholic extract of *Clarisia racemosa* and different standards (Doxo: doxorubicin m-Amsa: amsacrine and Asul: asulacrine against Jurkat (A), T47D (B), MCF-7 (C) and DU-145 (D) cancer cells.

Table 10

In vitro antitumor activity assays promoted by the hydroalcoholic extract of *Clarisia racemosa* compared to different standards and selectivity index (SI) results.

Samples	Jurkat IC ₅₀ (µg/mL)	SI	T47IC ₅₀ (µg/mL)	SI	MCF-7 IC ₅₀ (µg/mL)	SI	DU-145 IC ₅₀ (µg/mL)	SI
Extract of <i>Clarisia racemosa</i>	1.06 ± 0.4	51.4	1.01 ± 0.1	158.9	0.96 ± 0.1	167.1	0.31 ± 0.1	517.7
Doxo	0.74 ± 0.0	76.6	1.03 ± 0.3	55.0	1.11 ± 0.8	51.1	0.76 ± 0.0	74.6
m-Amsa	1.41 ± 0.1	67.7	1.25 ± 0.4	76.4	1.14 ± 0.1	83.7	0.80 ± 0.0	119.4
Asul	1.33 ± 0.1	82.7	1.26 ± 0.4	87.3	1.18 ± 0.1	93.3	0.66 ± 0.2	166.8

Mean ± Standard deviation; IC₅₀ for Macrophages J774 A.1 for the different Doxo standards: doxorubicin (IC₅₀ 56.7 µg/mL); m-Amsa: amsacrine (IC₅₀ 95.5 µg/mL) and Asul: asulacrine (IC₅₀ 110.1 µg/mL).

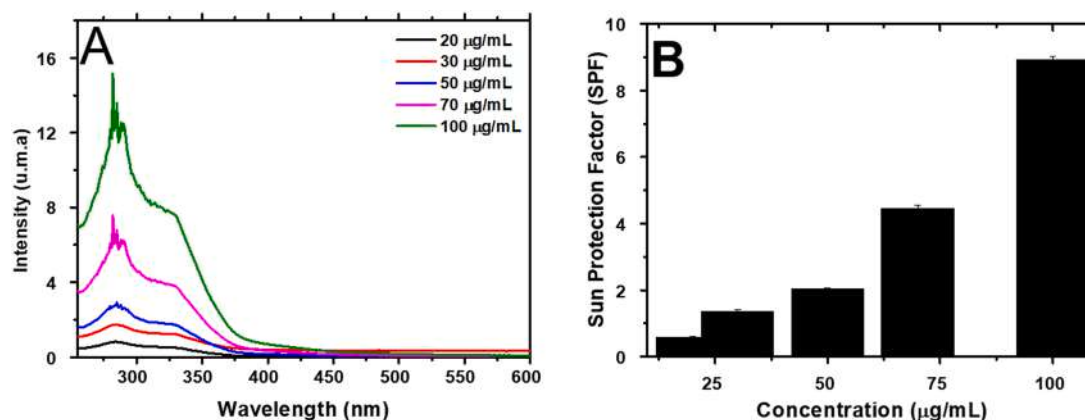


Fig. 7. Absorption curves (A) and SPF values (B) for the hydroalcoholic extract of *C. racemosa* at different concentrations.

Table 11

Results of in vitro antimicrobial activity promoted by the hydroalcoholic extract of *Clarisia racemosa*.

Microorganisms	Extract of <i>Clarisia racemosa</i>		Control - antimicrobial	
Bacteria	MIC (µg/mL)	MBC (µg/mL)	MIC (µg/mL)	
<i>Enterococcus faecalis</i> UFPEDA-69	1024	> 1024	Amikacin	8 (S)
<i>Enterococcus faecalis</i> UFPEDA-138	1024	> 1024	Amikacin	8 (S)
<i>Staphylococcus aureus</i> UFPEDA-709	512	> 1024	Oxacillin	512 (R)
<i>Acinetobacter baumannii</i> UFPEDA-1024	> 1024	> 1024	Amp.+ Sulbac.	16 (I)
<i>Pseudomonas aeruginosa</i> UFPEDA-261	> 1024	> 1024	Amikacin	32 (I)
<i>Pseudomonas aeruginosa</i> UFPEDA-416	> 1024	> 1024	Amikacin	8 (S)
Yeasts	MIC (µg/mL)	MFC (µg/mL)	MIC (µg/mL)	
<i>Candida albicans</i> UFPEDA-1007	> 1024	> 1024	Micafungin	0.0625 (S)
<i>Candida albicans</i> URM 95	> 1024	> 1024	Micafungin	0.0312 (S)
<i>Candida albicans</i> 4664	1024	> 1024	Micafungin	0.0156 (S)
<i>Candida glabrata</i> UFPEDA-6393	1024	> 1024	Micafungin	0.0156 (S)
<i>Candida guilliermondii</i> UFPEDA-6390	> 1024	> 1024	Micafungin	0.0156 (S)

MIC – Minimum inhibitory concentration; MBC – Minimum bactericidal concentration; MFC – Minimum fungicidal concentration; R – Resistant; S - Sensitive; I – Intermediate resistance; Amp.+Sulbac – Ampicillin + Sulbactam

of which the extract had a MIC of 512 µg/mL, in addition, this strain was classified as methicillin resistant *S. aureus* (SARM), by determining the MIC of the antimicrobial oxacillin. Given the Gram-negative bacilli represented by the species *Acinetobacter baumannii* and *Pseudomonas aeruginosa*, no antimicrobial activity of the extract was observed, as evidenced by the MIC value greater than 1024 µg/mL, against the strains of these species.

Activity of products and extracts against gram-positive cocci is generally expected when compared to activity against gram-negative bacilli, since gram-positives are more sensitive to antibiotics than gram-negative ones, especially non-fermenters (Morais-Braga et al., 2016; Vergara et al., 2019). This characteristic difference is mainly due to the protective outer membrane of Gram-negative bacteria that usually prevents the entry of molecules, in addition to the periplasmic space formed between this membrane and the cell wall, which contains protective enzymes against external molecules, especially antibiotics (Vergara et al., 2019).

The antimicrobial activity is related to the type and contents of different metabolites present in the extract (Nwonuma et al., 2020). The extract from this study had a higher content of phenolic compounds. The mechanisms of action of phenolic compounds are not fully elucidated. However, it is known that hydroxyl groups can reduce cell wall rigidity with loss of integrity due to different interactions with the cell membrane (Miklasinska-Majdanik et al., 2018). In addition to promoting inhibition of enzymes responsible for microbial growth (Miklasinska-Majdanik et al., 2018; Bouarab-Chibane et al., 2019).

The literature presents different hydroalcoholic extracts that showed antimicrobial activity against *Staphylococcus aureus* strains, Gram-positive bacteria considered to be highly pathogenic (Dimech et al., 2013; Lima et al., 2020a, 2020b). Moori et al. (2016) obtained results for the hydroalcoholic extract of the stem bark of *Juglans regia* with inhibitory (MIC) and minimal bactericidal (MCB) concentrations of 7.81 mg/mL and 3.9 mg/mL, respectively. Saeidi et al. (2014) obtained activity for the hydroalcoholic extract of *Zataria multiflora* MIC and MCB of 2.5 mg/mL, 5.0 mg/mL. Lima et al. (2020a, 2020b),

evaluating the in vitro antimicrobial activity promoted by the hydroalcoholic extract of *Psidium cattleianum* Sabine obtained MIC and MCB values of 9 mg/mL and 18 mg/mL, respectively. However, Sarmadyan et al. (2014) evaluated the hydroalcoholic extract of *Cannabis sativa* that obtained MIC of 25 µg/mL. These results show the potential of the extract against *Staphylococcus aureus* strains.

The activity of the extract of *C. racemosa* against the strains: *Candida albicans* 4664 and *Candida glabrata* UFPEDA-6393, with MIC of 1024 µg/mL for both strains, was observed in the tested yeasts. All tested yeasts showed a sensitivity profile to the antifungal micafungin, as evidenced by the MIC of this antifungal determined in the microdilution tests in broth.

3.10. Photoprotective activity promoted by the in vitro extract

Fig. 6A shows the absorption curves of the hydroalcoholic extract at different concentrations (20; 30; 50; 70 and 100 µg/mL). Fig. 6B presents the results of the sun protection factor (SPF) at the same concentrations, as follows: 0.59 ± 0.0 ; 1.36 ± 0.0 ; 2.03 ± 0.0 ; 4.46 ± 0.1 and 8.93 ± 0.5 respectively.

Similar results were found by Almeida et al. (2019a, 2019b) evaluating fifteen species from the Caatinga region, from which *Erythrina velutina* stood out, obtained an SPF of 9.71 at a concentration of 100 µg/mL. Carvalho et al. (2019a, 2019b) analyzed the photoprotection of the hydroalcoholic extract of *Pterodon emarginatus* fruits and this presented SPF equal to 8. Andrade et al. (2021) evaluated the hydroalcoholic extract of grape pomace from the Bordeaux, Isabel, and Merlot cultivars for photoprotection, and the best SPF was observed with the Bordeaux extract (SPF = 12). Almeida et al. (2019a, 2019b) evaluating the effect of the hydroalcoholic extract of propolis at the same concentrations as those used in our study, obtained SPF ranging from 2.29 to 12.3 for the extract obtained with 70% ethanol at room temperature, 2.25–11.2 for 70% heated ethanol. Seregheti et al. (2020) used the ethanol extract of *Calea fruticosa* obtained in concentrations of 30–100 µg/mL SPF ranging from 2.77 to 9.6.

Although the methodology is in vitro, it has a good correlation with in vivo tests as it relates the absorbance of the test substance with the erythematogenous effect of radiation and the light intensity at wavelengths specific to the UVB region (Mansur et al., 1986; Almeida et al., 2019a, 2019b).

4. Conclusion

The results obtained in the chemical characterization tests of the hydroalcoholic extract of *C. racemosa* showed a high content of phenolic phytocomponents. The ADMET predictive study showed that the extract can be used safely in biological assays. And the high content of phenolics showed that the extract has potential activity as an antioxidant agent (inhibit free radicals), antiglycemic (decrease damage to skin proteins, collagen), antimicrobial (capable of inhibiting the proliferation of *Staphylococcus aureus*, a microorganism that causes infections of skin) and photoprotective (capable of protecting the skin against ultraviolet rays). In addition, the results also revealed that the hydroalcoholic extract of *C. racemosa* does not present toxicity in the experimental conditions in vitro and in vivo evaluated. Thus, the results obtained with the extract of *C. racemosa* suggest application as a component in cosmetic and pharmaceutical formulations.

Declaration of Competing Interest

None.

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