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NATHÁLIA ALVES BENTO

POTENCIAL ANTICANCERÍGENO, ANTIOXIDANTE E ANTIPLASMÓDICO DOS EXTRATOS DE SEMENTE E CASCA DE MAPATI (*Pourouma cecropiifolia*): UMA INVESTIGAÇÃO BIOQUÍMICA E CELULAR.

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Orientador: Profª Drª Luciana Azevedo
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A mente que se abre a uma nova ideia jamais volta ao seu tamanho original.

(Albert Einstein)

RESUMO

O Mapati (*Pourouma cecropiifolia* Martius) é uma fruta tropical endêmica da Floresta Amazônica e constitui uma fonte promissora de compostos fenólicos, cujas propriedades antimaláricas e antiproliferativas ainda permanecem pouco exploradas. Tradicionalmente, cascas e sementes de frutos são descartadas, mas sua bioprospecção pode representar uma fonte sustentável de compostos bioativos, ao mesmo tempo em que valoriza e amplia o conhecimento sobre a flora nativa. Neste estudo, foram investigados aspectos de sua composição química e seu potencial biológico. As análises por UPLC-QToF/MS e HPLC- DAD dos extratos etanólicos da casca e da semente revelaram um perfil rico em polifenóis, incluindo ácido clorogênico e seus isômeros, quercetina e derivados glicosilados. Tais compostos estão associados a elevada capacidade antioxidante, especialmente no extrato da semente, como demonstrado pelos ensaios antioxidantes. Os testes biológicos correlacionaram esse potencial antioxidante com a redução das espécies reativas de oxigênio (EROs) em células A549 e em eritrócitos humanos. Ademais, ambos os extratos apresentaram efeito citotóxico sobre linhagens celulares tumorais em concentrações mais elevadas (50,6 μg EAG/mL para a semente e 104 μg EAG/mL para a casca), enquanto concentrações mais baixas (12,5 μg EAG/mL e 20 μg EAG/mL) demonstraram atividades antigenotóxica e antimutagênica, evidenciadas pelos ensaios de fragmentação de DNA e de alterações cromossômicas. Ambos os extratos também exibiram atividade antiplasmodial frente a todos os estágios intraeritrocíticos de *Plasmodium falciparum* (cepas 3D7 e W2). Destaca-se que o extrato da semente potencializou a eficácia do artesunato contra a cepa 3D7, enquanto o extrato da casca aumentou a atividade desse fármaco frente à cepa W2. Esses achados evidenciam os extratos da semente e da casca de mapati como fontes promissoras de compostos bioativos com propriedades antioxidantes, genoprotetoras e antimaláricas, ressaltando o potencial de subprodutos de frutas amazônicas para aplicações nas áreas alimentícia e farmacêutica.

Palavras-chave: Compostos; Bioativos; Fenólicos; Antigenotóxico; Antimutagênico; *Plasmodium Falciparum.*

ABSTRACT

Mapati (Pourouma cecropiifolia Martius) is a tropical fruit endemic to the Amazon rainforest and represents a promising source of phenolic compounds, whose antimalarial and antiproliferative properties remain largely unexplored. Traditionally, fruit peels and seeds are discarded; however, their bioprospecting may represent a sustainable source of bioactive compounds while also enhancing the valorization and knowledge of native flora. In this study, we investigated the chemical composition and biological potential of this fruit. UPLC- QToF/MS and HPLC-DAD analyses of ethanol extracts from the peel and seed revealed a polyphenol-rich profile, including chlorogenic acid and its isomers, quercetin, and glycosylated derivatives. These compounds were associated with high antioxidant capacity, particularly in the seed extract, as demonstrated by antioxidant assays. Biological tests correlated this antioxidant potential with reduced reactive oxygen species (ROS) in A549 cells and human erythrocytes. Moreover, both extracts exhibited cytotoxic effects on tumor cell lines at higher concentrations (50.6 μg GAE/mL for seed and 104 μg GAE/mL for peel), while lower concentrations (12.5 μg GAE/mL and 20 μg GAE/mL) demonstrated antigenotoxic and antimutagenic activities, as evidenced by DNA fragmentation and chromosomal alteration assays. Both extracts also displayed antiplasmodial activity against all intraerythrocytic stages of *Plasmodium falciparum* (3D7 and W2 strains). Notably, the seed extract enhanced the efficacy of artesunate against the 3D7 strain, while the peel extract potentiated its activity against the W2 strain. These findings highlight seed and peel extracts of mapati as promising sources of bioactive compounds with antioxidant, genoprotective, and antimalarial properties, underscoring the potential of Amazonian fruit by-products for applications in the food and pharmaceutical industries.

Keywords: Bioactive; Compounds; Phenolics; Antigenotoxic; Antimutagenic; *Plasmodium falciparum.*

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1 INTRODUÇÃO GERAL

A crescente demanda por soluções sustentáveis tem impulsionado o aproveitamento de subprodutos vegetais como fontes estratégicas de biomoléculas, com aplicações potenciais nas indústrias alimentícia, farmacêutica e biotecnológica (Rajesh Banu *et al*., 2020; Romero-Perdomo; González-Curbelo, 2023). Diversos estudos têm demonstrado que compostos bioativos extraídos de resíduos vegetais apresentam propriedades antioxidantes, anti-inflamatórias, antibacterianas, antivirais e até mesmo antiparasitárias (Välimaa *et al*., 2020; Jyske *et al*., 2023).

A Floresta Amazônica constitui um dos biomas mais biodiversos do planeta, com uma grande variedade de espécies frutíferas nativas de elevado potencial econômico, nutricional e tecnológico, ainda pouco explorado (Zanirato, 2021; Miranda *et al*., 2024). O aproveitamento de subprodutos amazônicos, tradicionalmente descartados, fortalece a estratégia de valorização de fontes alternativas de compostos bioativos, além de contribuir para o desenvolvimento sustentável, a segurança alimentar e a inovação em produtos agroindustriais (Duarte-Casar *et al*., 2024).

A *Pourouma cecropiifolia*, espécie explorada neste trabalho, popularmente conhecida como Mapati ou Uva amazônica, é uma árvore frutífera encontrada na Amazônia brasileira, Acre, Bolívia, Equador, Venezuela, Colômbia e Peru. Seus frutos, de polpa doce e suculenta, são consumidos na culinária local e utilizados na produção de vinhos e geleias; entretanto, a casca fibrosa e as sementes são frequentemente descartadas, embora possam ser reaproveitadas como fontes de compostos bioativos, como os polifenóis (Pedrosa; Clement; Schietti, 2018).

Classe de fitoquímicos abrangentes que englobam flavonoides, ácidos fenólicos, lignanas e estilbenos, conhecidos por exercerem atividades antioxidantes, anti- inflamatórias e antimicrobianas (Fraga *et al*., 2019; H. Al Mamari, 2022). Além de suas propriedades redox, esses compostos também apresentam mecanismos intracelulares relevantes, atuando na interação com a membrana plasmática, na estimulação de vias de sinalização e na regulação de fatores de transcrição além de modularem a produção de citocinas e mediadores inflamatórios (Kim; Quon; Kim, 2014; Fraga *et al*., 2019). Tais características têm direcionado novos estudos para o uso de polifenóis como agentes coadjuvantes no combate a parasitas como *Plasmodium* sp., agente etiológico da malária, reforçando o potencial desses compostos na estratégia de terapias combinadas (Sumbe; Barkade, 2023).

Tais evidências reforçam que a investigação de subprodutos vegetais, em especial cascas e sementes tradicionalmente descartadas, pode revelar novas fontes sustentáveis de compostos

bioativos de interesse farmacêutico e alimentar. No caso do fruto mapati (*Pourouma cecropiifolia*), a prospecção de seus resíduos apresenta um duplo potencial: contribuir para a valorização da biodiversidade amazônica e ampliar o conhecimento sobre polifenóis ainda pouco explorados quanto às suas propriedades antimaláricas e antiproliferativas.

Diante desse contexto, o presente trabalho teve como objetivo principal produzir extratos hidroalcoólicos da casca e da semente de *P. cecropiifolia*, analisar sua composição química, com ênfase na identificação e quantificação de compostos fenólicos, e investigar suas propriedades biológicas em modelos *in vitro*. Especificamente, buscou-se: (i) realizar a extração da casca e da semente utilizando solução hidroalcoólica; (ii) caracterizar os compostos fenólicos presentes; (iii) avaliar a atividade antioxidante, anti-hemolítica, citotóxica, apoptótica, genotóxica e mutagênica dos extratos; e (iv) verificar o potencial antimalárico contra *Plasmodium falciparum* em ensaios *in vitro*.

2 REVISÃO DA LITERATUA

2.1 SUBPRODUTOS AMAZÔNICOS COMO FONTE DECOMPOSTOS BIOATIVOS

A Floresta Amazônica é uma das regiões florestais mais importantes e conhecidas do mundo, abrangendo oito países da América do Sul e abrigando um terço do estoque genético do planeta. Estima-se que existam aproximadamente

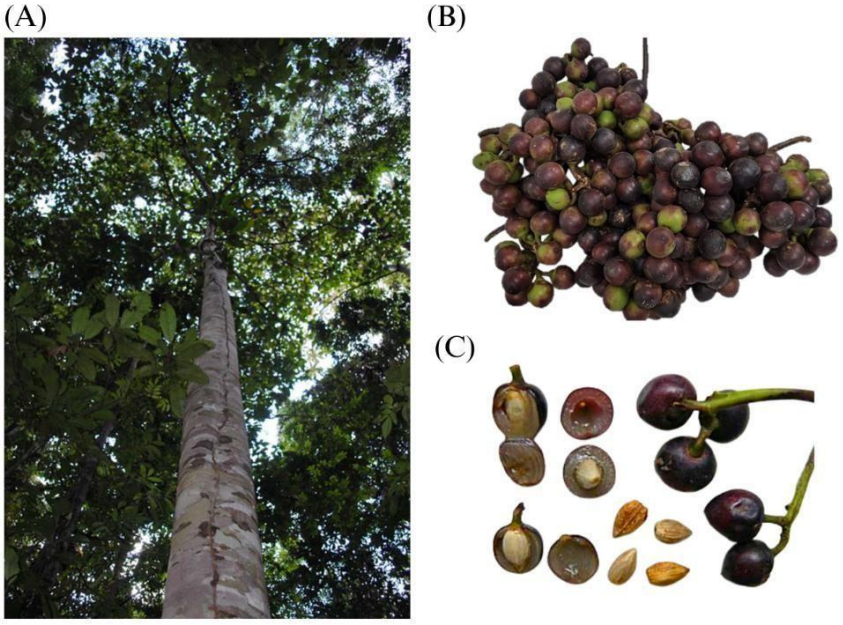
60.000 espécies de plantas nesse ecossistema, entre elas, inúmeras espécies frutíferas nativas com potencial econômico, tecnológico e nutricional. No entanto, essas espécies permanecem pouco exploradas e os estudos de bioprospecção ainda são escassos, portanto, mais pesquisas são essenciais para o completo entendimento do valor nutricional e das propriedades biológicas (Zanirato, 2021; Miranda *et al*., 2024).

As plantas são fontes ricas em compostos bioativos, e muitas de suas partes comestíveis têm sido historicamente integradas à dieta de populações locais, além de serem utilizadas no tratamento de diversas doenças infecciosas (Lima; De Carvalho; Conte-Junior, 2023; Sun; Shahrajabian, 2023). No entanto, partes tradicionalmente consideradas subprodutos, como cascas, folhas, bagaços e sementes sem valor comercial, também apresentam elevado potencial para aplicações nas indústrias alimentícia, farmacêutica e medicinal. A valorização desses subprodutos como fontes acessíveis de compostos bioativos contribui não apenas para o desenvolvimento sustentável, mas também para a segurança alimentar e a inovação tecnológica. Além disso, a incorporação de alimentos tradicionais fortalece a agricultura local, preserva o patrimônio cultural e amplia o valor econômico dessas espécies vegetais (Duarte-Casar *et al*., 2024).

Pourouma cecropiifolia Martius, pertencente à família Moraceae, é uma árvore de rápido crescimento que pode atingir de 3 a 15 metros de altura. Seus frutos, verdes quando imaturos e roxos na fase de maturação, são amplamente comercializados em mercados regionais da Amazônia brasileira, onde são popularmente conhecidos como embaúba, mapatí ou uva-caimarona. Podem ser consumidos *in natura* ou utilizados na produção de geleias e bebidas alcoólicas. Devido ao seu rápido desenvolvimento, essa espécie apresenta-se como uma matéria-prima promissora para aplicações agroindustriais, favorecendo o desenvolvimento de produtos processados. O epicarpo, geralmente descartado como subproduto, representa cerca de 20% da massa total do fruto e, em conjunto com a semente e

casca, constitui uma fonte potencial de compostos bioativos, especialmente polifenóis, cujas características químicas ainda demandam investigação mais aprofundada (Falcão, 1980; Barrios *et al*., 2010).

Figura 1 – (A) Árvore de *Pourouma cecropiifolia*, (B) Cachos de frutos maduros e (C) Detalhes dos frutos e sementes.



Fonte: (A) Elaborado pela autora a partir de GAGLIOTI *et al*. (2013); (B) Acervo pessoal de Jaqueline de Araújo Bezerra. Manaus, 2023; (C) COLECIONANDO FRUTAS. *Pourouma cecropiifolia*. Disponível em: https://www.colecionandofrutas.com.br/pouroumacecropiaefolia.htm. Acesso em: (20/06/2025).

2.2 ESTRESSE OXIDATIVO E IMPLICAÇÕES BIOLÓGICAS

Em condições fisiologicamente ótimas, as espécies reativas de oxigênio (EROs) desempenham um papel crucial na sinalização redox, mantendo a homeostase celular por meio da regulação do metabolismo, transdução de sinais, da defesa imunológica, da proliferação. No entanto, um desequilíbrio na produção de EROs pode interromper a sinalização redox fisiológica, levando ao estresse oxidativo, à ativação de mecanismos de dano celular e, consequentemente, ao desenvolvimento de doenças (Chen *et al*., 2020; Do Carmo; Granato; Azevedo, 2021).

Os radicais livres são definidos como espécies que contêm um ou mais elétrons desemparelhados, o que os torna altamente reativos e capazes de abstrair elétrons ou átomos de hidrogênio de outras moléculas, propagando assim reações em cadeia que causam danos

celulares (Lobo *et al*., 2010). Os radicais livres derivados do oxigênio englobam diversas espécies reativas, como ânion superóxido, radical peroxil, óxido nítrico, peróxido de hidrogênio, oxigênio singleto, radical hidroxila, ácido hipocloroso, radical hipoclorito, peroxinitrito e peróxidos lipídicos, os quais podem ser formados por processos endógenos, como o metabolismo celular, ou por exposição a fatores exógenos (Rudrapal *et al*., 2022).

Quando a geração dessas espécies reativas excede a capacidade dos sistemas antioxidantes de neutralizá-las, ocorre um desequilíbrio redox que pode resultar em danos significativos em proteínas, carboidratos, lipídios, RNA, DNA levando a lesões em tecidos e estruturas celulares (Xu; Hu; Liu, 2012). Esse cenário de estresse oxidativo prolongado está fortemente associado à gênese de doenças crônicas e degenerativas, ao processo de envelhecimento, bem como a condições agudas, tais como traumas e acidentes vasculares cerebrais (Law *et al*., 2017).

Os genes antioxidantes codificam diversas enzimas essenciais, como heme oxigenase, glutamato-cisteína ligase, NADPH quinona desidrogenase, superóxido dismutase, catalase, além da família das glutationa peroxidases, glutationa S-transferase e glutationa sintetase, que atuam de forma coordenada no controle do estresse oxidativo celular, na modulação de respostas inflamatórias e na regulação da apoptose (Liang; Kitts, 2018). No entanto, quando a geração de espécies reativas de oxigênio (EROs) ocorre em excesso, essas enzimas antioxidantes podem ser sobrecarregadas, resultando no acúmulo de EROs e na ativação de cascatas de sinalização, como a via JNK (via das quinases N-terminais de c-Jun), capazes de desencadear a morte celular programada, seja por mecanismos intrínsecos, envolvendo a liberação de sinais mitocondriais, seja por rotas extrínsecas mediadas por receptores de morte celular (Do Carmo *et al*., 2018).

Os danos ocasionados por níveis elevados em EROs em componentes celulares vitais, (DNA, proteínas e lipídios), podem resultar em mutações que comprometem genes essenciais, inclusive genes supressores de tumores, podendo assim desestabilizar os mecanismos de controle que restringem a proliferação celular desordenada (Iqbal *et al*., 2024). As EROs também podem intensificar a ativação de vias de sinalização oncogênicas, favorecem a sobrevivência e o crescimento celular anômalo, contribuindo para a progressão tumoral (Rizvi *et al*., 2021). Além disso, as células cancerígenas exibem uma taxa aumentada e localizada de geração de EROs, quando comparada às células não tumorais, o que fortalece a hiperativação de vias de sinalização pró- tumorigênicas, promovendo a proliferação, a resistência e a adaptação ao microambiente tumoral (Do Carmo; Granato; Azevedo, 2021)

Os antioxidantes desempenham um papel essencial devido às suas múltiplas

propriedades benéficas, principalmente pela habilidade de mitigar o estresse oxidativo e os danos moleculares deles decorrentes. Entre eles, os polifenóis destacam-se como uma classe relevante de compostos bioativos, atuando por diferentes mecanismos para neutralizar espécies reativas e preservar a integridade celular (Tošović *et al*., 2017).

2.3 EXTRATOS VEGETAIS E A MALÁRIA

A malária é uma enfermidade parasitária de ocorrência endêmica em áreas tropicais e subtropicais ao redor do mundo. A transmissão ocorre por meio da picada de mosquitos fêmeas do gênero *Anopheles*, vetores dos protozoários do gênero *Plasmodium* (Pan *et al*., 2018). Dentre as cinco espécies capazes de infectar seres humanos, *Plasmodium falciparum* e *P. vivax* são as responsáveis pela maior parte da carga global de casos, sendo o *P. falciparum* o mais comum e letal, especialmente devido ao aumento da resistência aos fármacos antimaláricos e à frequência de quadros graves (Fernández-Álvaro *et al*., 2016). Já o *P. vivax* impõe um desafio adicional por sua habilidade de permanecer em estágio latente no fígado do hospedeiro, na forma de hipnozoítos, que podem ser reativados posteriormente, causando recaídas da doença (Mamede *et al*., 2020).

Apesar das inúmeras estratégias de combate à malária, o rápido surgimento de resistência ao *Plasmodium falciparum* continua sendo um grande desafio. Portanto, o desenvolvimento de novas abordagens terapêuticas, como a combinação de compostos que aumentem a eficácia dos medicamentos existentes ou que atuem em vias metabólicas vitais, é essencial para superar a resistência aos medicamentos e melhorar os resultados do tratamento da malária (Crispim *et al*., 2025).

Durante a fase sintomática da malária no estágio eritrocítico, o parasito depende da manutenção de uma homeostase redox para sobreviver. A digestão de hemoglobina, o metabolismo mitocondrial e o sistema imune do hospedeiro geram espécies reativas de oxigênio, levando ao estresse oxidativo no interior do eritrócito infectado. Em contrapartida, o parasito conta com sistemas antioxidantes que envolvem proteínas como glutationa e enzimas como superóxido dismutase, capazes de minimizar a peroxidação lipídica, a inativação de enzimas, a oxidação de proteínas e a inibição da respiração mitocondrial (Isah; Ibrahim, 2014). Alguns fármacos antimaláricos atuam desestabilizando as defesas antioxidantes, levando a uma cascata de danos oxidativos, promovendo a oxidação de biomoléculas essenciais, danos genéticos que podem levar a morte do parasita. Alguns compostos fenólicos podem atuar como pró-oxidantes, intensificando o estresse oxidativo intracelular, desestabilizando o equilíbrio

redox do parasito (Mamede *et al*., 2020). Em contrapartida, alguns fenólicos podem atuar como agentes quelantes de ferro, restringindo a disponibilidade de ferro para o parasita. O ferro é essencial para a sobrevivência do *Plasmodium*, sendo crítico para processos como a respiração mitocondrial e a síntese de DNA (Egwu *et al*., 2021). Após a invasão eritrocitária, o parasita modifica a membrana celular do hospedeiro inserindo proteínas que aumentam sua permeabilidade a compostos essenciais ao metabolismo do parasito. Polifenóis, como flavonoides também podem atuar na inibição dessas vias de permeação, interferindo no metabolismo do parasito e favorecendo sua eliminação (Ziegler *et al*., 2004).

O medicamento mais amplamente utilizado atualmente no tratamento da malária é o artesunato, cuja descoberta tem raízes na medicina tradicional, que empregava o chá de *Artemisia annua*. Terapias combinadas, especialmente aquelas baseadas em derivados de artemisinina, trouxeram uma nova perspectiva para o tratamento da malária em regiões onde a resistência à cloroquina se tornou um obstáculo significativo (Jun *et al*., 2025). Nesse contexto, a associação de artemisinina com outros compostos naturais, configura- se como uma abordagem promissora que necessita ser mais explorada (Mishra; Bhattacharya; Bhasin, 2009). Sannella *et al*. (2007) apontam que flavonoides podem exercer ação aditiva ou sinérgica com artemisinínicos, auxiliando na obtenção de melhores desfechos terapêuticos (Puttappa; Kumar; Yamjala, 2017). Assim, explorar a combinação de artesunato com compostos fenólicos representa uma estratégia inovadora e viável para o desenvolvimento de novas terapias combinadas contra a malária.

2.4 COMPOSTOS POLIFENÓLICOS

Os compostos fenólicos são gerados como metabólitos secundários nas plantas para proteção contra herbívoria, microrganismos e até mesmo estresse oxidativo. São moléculas aromáticas ou alifáticas que contêm ao menos um anel aromático com um ou mais grupos hidroxila (-OH) ligados. Sua distribuição e concentração nas plantas variam significativamente dependendo do tecido vegetal, sendo influenciadas por seu estágio de maturação, período de coleta e outros fatores ambientais (Nurzynska-Wierdak, 2023). Atualmente, mais de 8.000 compostos fenólicos são conhecidos e reconhecidos por suas diversas bioatividades e aplicabilidades (Šamec *et al*., 2021). Essa diversidade estrutural pode ser classificada em subclasses como: ácidos fenólicos, estilbenos, lignanas, antocianinas, flavanóis, flavonóis, flavonas, flavanonas, isoflavonas e taninos. Os ácidos fenólicos, quantificados no extrato de semente do mapati, são derivados das vias biossintéticas do ácido chiquímico, como as rotas

fenilpropanóide e monolignol. Uma vez ingeridos, são absorvidos no trato gastrointestinal e passam por transformações metabólicas como metilação, glucuronidação e sulfatação, que modificam suas estruturas e influenciam seus efeitos biológicos. Alguns, como os ácidos cafeico e clorogênico, também sofrem metabolização pela microbiota intestinal. Além de sua origem dietética, os ácidos fenólicos podem surgir do metabolismo de outros polifenóis no cólon (Rashmi; Negi, 2020).

Os ácidos fenólicos possuem ação protetora contra diversas doenças crônicas, como câncer, diabetes, doenças cardiovasculares e inflamações, especialmente devido a sua habilidade, de neutralizar radicais livres. Essa ação antioxidante ocorre, principalmente, por meio da doação de átomos de hidrogênio, sendo modulada pela presença de substituintes no anel aromático, o que afeta a estabilidade da molécula e sua eficácia contra espécies reativas. As diferentes formas estruturais livres, esterificadas, glicosiladas ou não influenciam seu potencial antioxidante (Kumar; Goel, 2019).

Os taninos constituem uma classe de compostos polifenólicos caracterizados pela presença de anéis aromáticos, podendo ser subdivididos em dois grupos principais: hidrolisáveis e condensados(Pizzi, 2021), estes últimos também denominados proantocianidinas foram identificado neste trabalho por meio da técnica de UPLC no extrato de semente do mapati. Os taninos hidrolisáveis são formados por unidades de ácidos fenólicos e subdividem-se em galotaninos e elagitaninos, ao passo que os taninos condensados derivam da via biosintética dos flavonoides, apresentando dois anéis fenólicos conectados por um anel pirano central, tais estruturas correspondem a polímeros de flavan-3-óis, como as procianidinas, compostos por unidades com dois grupos hidroxila e as prodelfinidinas, que contêm três grupos hidroxila (Durazzo *et al*., 2019). Uma característica relevante dos taninos é sua capacidade de interagir com proteínas, promovendo sua desnaturação, fenômeno que fundamenta propriedades como adstringência e atividade antioxidante por redução de espécies reativas de oxigênio (EROs) (Melo *et al*., 2023). Ademais, distintas atividades biológicas e farmacológicas têm sido atribuídas aos taninos, incluindo efeitos antimicrobianos, antifúngicos, atividade contra cianobactérias, inibição de enzimas, propriedades antiobesidade, antidiabéticas, anti-helmínticas, nefroprotetoras e antivirais (Ogawa; Yazaki, 2018).

A quercetina é classificada como um flavonol, comumente encontrada em plantas na forma livre ou conjugada a carboidratos, desempenhando papel como pigmento responsável pela coloração de inúmeras frutas, sendo também identificada e quantificadas no extrato de casca do mapati. Sua estrutura química é composta por um núcleo de flavona formado por dois anéis aromáticos ligados a um anel pirano heterocíclico, sendo sua produção majoritariamente

decorrente da rota fenilpropanóide, que tem início na conversão da fenilalanina em ácido cinâmico (Deepika; Maurya, 2022; Mohammed *et al*., 2023). Este flavonoide exerce função antioxidante natural, protegendo as células por meio da neutralização de radicais livres gerados durante processos metabólicos, sendo essa atividade favorecida por sua afinidade com a bicamada lipídica, sua orientação na membrana e sua alta capacidade de interação molecular. Tais efeitos são atribuídos à sua configuração polifenólica, a qual viabiliza a ligação e modulação de enzimas, receptores, transportadores e sistemas de sinalização celular, conferindo-lhe múltiplas ações biológicas, especialmente a atividade antioxidante (Shabir *et al*., 2022). Na saúde humana, os compostos fenólicos desempenham um papel crucial nos mecanismos de defesa, exibindo propriedades antioxidantes, antimicrobiana, antiparasitários, anticancerígenos, antienvelhecimento, antiproliferativas, anti-inflamatórias e prebiótica(Sun; Shahrajabian, 2023)

3 ARTIGO 1 - Bioprospecting of the Amazonian mapati fruit (*Pourouma cecropiifolia* Martius): Antiproliferative and antimalarial potential of peel and seed hydroethanolic extracts

Food Research International

Bioprospecting of the Amazonian mapati fruit (Pourouma cecropiifolia Martius): Antiproliferative and antimalarial potential of peel and seed hydroethanolic extracts

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Abstract:	Mapati (Pourouma cecropiifolia Martius) is an endemic tropical fruit from the Amazon rainforest and a source of phenolic compounds, who's antimalarial and antiproliferative properties remain untapped. Herein, we provide insights into its composition and biological potential. The UPLC-QToF/MS and HPLC-DAD of the mapati peel and seed hydroethanolic extracts have uncovered a rich profile of polyphenols, including chlorogenic acid and its isomers, quercetin, and glycosylated derivatives. These compounds exhibit high antioxidant capacity, particularly in the seed extract, as demonstrated by DPPH, FRAP, and HRSA assays. The biological tests correlate this antioxidant potential with a reduction in reactive oxygen species (ROS) in A549 cells and human erythrocytes. Moreover, both extracts exhibited cytotoxic effects against cancer cell lines at 50.6 µg GAE/mL for seed and 104 µg GAE/mL for peel extract. Furthermore, lower concentrations (12.5 µg GAE/mL and 20 µg GAE/mL) showed antigenotoxic and antimutagenic activities, as demonstrated by DNA fragmentation and chromosomal abnormalities assays. Both extracts also showed antiplasmodial activity against all intraerythrocytic stages of Plasmodium falciparum (3D7 and W2). Notably, the seed extract enhanced the efficacy of artesunate against the 3D7 strain by reducing its IC_{50} by approximately 85% at 10µgGAE/mL, whereas the peel extract potentiated its activity against the W2 strain with a reduction of 38% at 5 and 10µgGAE/mL. These findings highlight mapati seed and peel extracts as promising sources of bioactive compounds with antioxidant, genoprotective, and antimalarial properties, underscoring the potential of Amazonian fruit.

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FEDERAL UNIVERSITY OF ALFENAS – UNIFAL/MG
In vivo Nutritional and Toxicological Analysis Laboratory

Alfenas (Brazil), August 2025

Dear **Prof. Dr. Anderson Sant'Ana**, editor in chief of Food Research International,

We are pleased to submit our manuscript entitled **"Bioprospecting of the Amazonian Mapati Fruit (*Pourouma cecropiifolia* Martius): Antiproliferative and Antimalarial Potential of Peel and Seed Hydroethanolic Extracts"** for consideration in *Food Research International*.

This study provides a comprehensive investigation of the chemical composition and biological potential of mapati peel and seed extracts, highlighting their rich phenolic profile, antioxidant capacity, genoprotective properties, selective cytotoxicity against cancer cells, and antimalarial activity, including synergistic effects with artesunate. Our findings reveal the potential of these Amazonian fruit by-products as promising sources of bioactive compounds for pharmaceutical and nutraceutical applications.

The novelty of this work lies in its multidisciplinary approach, integrating advanced chemical characterization (UPLC-QToF/MS and HPLC-DAD) with a broad spectrum of biological assays, including cytotoxic, antioxidant, genoprotective, and antiplasmodial evaluations. To the best of our knowledge, this is the first in-depth report on the combined antiproliferative and antimalarial potential of mapati peel and seed extracts.

This manuscript has not been published and is not under consideration elsewhere. All authors have approved the final version and declared no conflicts of interest. We believe that our findings align with the scope of *Food Research International*, particularly in the areas of functional foods, food bioactives, and valorization of agro-food by-products.

We kindly thank you for considering our manuscript for publication. We look forward to your evaluation and any additional information if needed.

On behalf of all authors,

Prof. Dr. Luciana Azevedo

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To Food Research International

Bioprospecting of the Amazonian mapati fruit (*Pourouma cecropiifolia* Martius): Antiproliferative and antimalarial potential of peel and seed hydroethanolic extracts

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Highlights

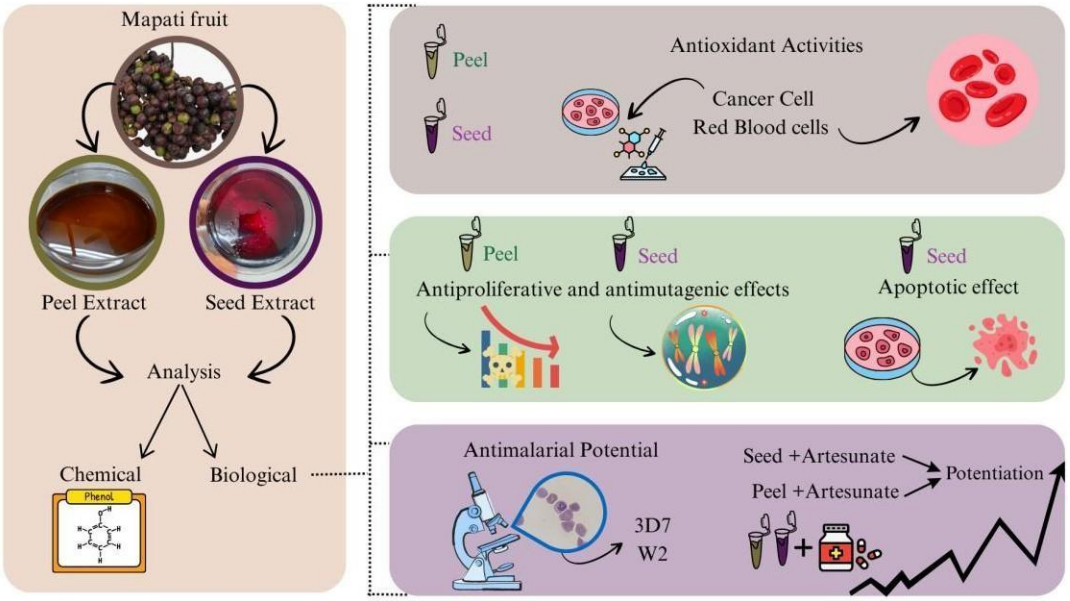
- Mapati seed extract had a high concentration of phenolic compounds and high chemical antioxidant capacity.
- The seed extract was more cytotoxic and selective to cancer cells than normal cells.
- The seed extract reduced intracellular ROS in A549 cells and human erythrocytes.
- Both extracts exhibited antimutagenic and antigenotoxic activity.
- Seed extract showed antimalarial properties, enhanced when combined with artesunate.

Abstract

Mapati (Pourouma cecropiifolia Martius) is an endemic tropical fruit from the Amazon rainforest and a source of phenolic compounds, who's antimalarial and antiproliferative properties remain untapped. Herein, we provide insights into its composition and biological potential. The UPLC-QToF/MS and HPLC-DAD of the mapati peel and seed hydroethanolic extracts have uncovered a rich profile of polyphenols, including chlorogenic acid and its isomers, quercetin, and glycosylated derivatives. These compounds exhibit high antioxidant capacity, particularly in the seed extract, as demonstrated by DPPH, FRAP, and HRSA assays. The biological tests correlate this antioxidant potential with a reduction in reactive oxygen species (ROS) in A549 cells and human erythrocytes. Moreover, both extracts exhibited cytotoxic effects against cancer cell lines at 50.6 μg GAE/mL for seed and 104 μg GAE/mL for peel extract. Furthermore, lower concentrations (12.5 μg GAE/mL and 20 μg GAE/mL) showed antigenotoxic and antimutagenic activities, as demonstrated by DNA fragmentation and chromosomal abnormalities assays. Both extracts also showed antiplasmodial activity against all intraerythrocytic stages of Plasmodium falciparum (3D7 and W2). Notably, the seed extract enhanced the efficacy of artesunate against the 3D7 strain by reducing its IC_{50} by approximately 85% at 10 μg GAE/mL, whereas the peel extract potentiated its activity against the W2 strain with a reduction of 38% at 5 and 10 μg GAE/mL. These findings highlight mapati seed and peel extracts as promising sources of bioactive compounds with antioxidant, genoprotective, and antimalarial properties, underscoring the potential of Amazonian fruit

Keywords: Phenolics; Antigenotoxic; Antimutagenic; Antioxidant; Cytotoxicity; Malaria.

Graphical abstract



1 Introduction

Mapati (*Pourouma cecropiifolia* Martius) is an endemic fruit of the Amazon rainforest belonging to the Moraceae family, and is popularly known as the Amazon grape, embaúba, and uva-caimarona (Zanirato, 2021; Duarte-Casar *et al*., 2024). Mapati are commercially available in regional markets and used for their medicinal properties and economic importance, even though their properties remain underexplored, and bioprospecting studies are still scarce (Miranda *et al*., 2024). Studies revealed that mapati represents a potential source of natural bioactive compounds, mainly found in b y-products such as peel and seed, which require further analysis for comprehensive characterization (Falcão,1980; Barrios *et al*., 2010). Still, no studies appear to have investigated in-depth the biological effects of peel and seed mapati extracts, which are considered potential sources of phenolic compounds.

Plants synthesize phenolic compounds as secondary metabolites to serve multiple defense functions, including antimicrobial activity and protection of oxidative damage (Duarte-Casar *et al*., 2024). In human health, these compounds exhibit antioxidant, anti-aging, antiproliferative, and anti-inflammatory properties. Moreover, their intake contributes to a healthy diet associated with antifungal, antibacterial, neuroprotective, and anticancer effects (Sun & Shahrajabian, 2023). Regarding antioxidant properties, the mitigation of oxidative stress through natural compounds is widely explored, mainly by increasing the global impact of oxidative damage-related disorders, such as malaria and cancer, infectious diseases that

highlight the need for new therapeutic strategies (Zeb, 2020).

Elevated levels of reactive oxygen species (ROS) are a characteristic of cancer cells that rely on oxidative signaling to sustain proliferation and survival pathways. Phenolic-rich extracts, with high antioxidant capacity, are able to mitigate this oxidative environment and are promising potential therapies (Do Carmo *et al*., 2021). On the other hand, ROS production can also serve a key mechanism to combat malaria-causing parasites. The most used drug for malaria nowadays is arsenate, whose discovery is rooted in traditional medicine, which utilizes. Artemisia annua tea. Currently, with the emergence of resistant strains, there is a growing need to search for new compounds with antimalarial potential to treat malaria symptoms (Egwu *et al*., 2021; Oliveira *et al*., 2022). Some studies explored plant extracts rich in polyphenols, such as green tea kombucha (Lacerda *et al*., 2024), camu-camu (Do Carmo *et al*., 2020), and Tapirira seed extract (Crispim *et al*., 2025) and found possible antimalarial properties by reducing the parasite proliferation in red blood cells (De Noronha *et al*., 2022).

Considering the importance of exploring new compounds for malaria treatment, as well as the potential of underexplored natural resources with antioxidative, antiproliferative, and antimutagenic properties for innovative and effective treatment options, the present study sought to evaluate mapati seed and peel extracts' potential as a source of therapeutic agents.

2 Materials and methods:

2.1 Sample Collection And Extract Preparation

Mapati fruits were collected in Manaus, Amazonas, Brazil, sanitized, and the peels and seeds were separated and lyophilized at -40°C and -700 mmHg for 72 h. The seeds were ground using a mortar and pestle, while the peels were triturated using a mixer. Subsequently, the samples were mixed with an extraction solution of 80% ethanol (Êxodo Científica, São Paulo, Brazil) at a solid-to-liquid ratio of 2:15. The mixture was subjected to ultrasonic bath (Mylabor, São Paulo, Brazil) extraction at room temperature (25–30 °C) for 30 min and then passed through filter paper Millipore (Sigma Aldrich, São Paulo, Brazil). After filtration, the residues were re-extracted two more times under the same conditions (Oliveira *et al*., 2025). The filtrates were combined, and the solvent was removed using a rotary evaporator to obtain the hydroethanolic extracts of the peels and seeds. Extraction yields obtained were 18.56% and 17.7%, respectively. These extracts were used for biological assays and chemical composition analysis.

2.2Chemical composition analysis

2.2.1 Solid-Phase Extraction

The purification of phenolic compounds from the hydroethanolic extracts of peels and seeds was performed using solid-phase extraction (SPE). Approximately 500 mg of each lyophilized hydroethanolic extract was accurately weighed and dissolved in 5 mL of ultrapure water acidified with 5% (v/v) formic acid (Sigma-Aldrich, São Paulo, Brazil). The mixtures were vortexed for 2 min and subsequently filtered through 0.22 µm membrane filters to remove insoluble particles prior to SPE processing.

The extraction procedure was carried out using STRATA-X polymeric reversed-phase cartridges (Phenomenex, California, USA) connected to a Visiprep 24 DL vacuum manifold (Supelco, Pennsylvania, USA), with a vacuum pump ECO-260 LAB (Biomec, Paraná, Brazil) maintained at approximately –5 inches of mercury (inHg) throughout the procedure. The cartridges were preconditioned with 3 mL of methanol, followed by equilibration with 3 mL of ultrapure water. Both steps were performed at a controlled flow rate of approximately two drops per second.

The acidified and filtered extracts were loaded in the SPE cartridges at a reduced flow rate of approximately one drop per second. Following sample percolation, the cartridges were washed with 3 mL of 5% (v/v) aqueous methanol solution to eliminate weakly retained polar compounds. Elution of the phenolic-rich fraction was carried out using 2 mL of pure methanol at the same flow rate.

The dried fractions obtained after SPE were reconstituted in methanol and filtered through polyvinylidene fluoride (PVDF) membranes (13 mm × 0.22 µm, Whatman, Massachusetts, EUA) directly into chromatographic vials (1.5 mL). The filtered solutions were subjected to brief sonication to eliminate entrapped air bubbles prior to chromatographic analysis by UPLC-qToF/MS and HPLC-DAD.

2.2.2 Phenolic composition

Total phenolic content (TPC) was determined using the Folin-Ciocalteu reducing capacity assay, as described by Cruz *et al*. (2024b). Total flavonols content was quantified using quercetin (Sigma Aldrich, São Paulo, Brazil). In this assay, extracts were mixed with ethanolic aluminum chloride solution (2%) (Dinâmica, São Paulo, Brazil), and sodium acetate (50 g/L) (Anidrol, São Paulo, Brazil). Absorbance was measured at 440 nm. The standard curve ranged from 7 to 70 mg/L ($R^2 = 0.9984$) (Cruz *et al*., 2024b). Condensed tannin content was estimated using the vanillin-H_2SO_4 method. In this assay, ethanol-diluted samples were

mixed with 4% vanillin solution and 32% (Dinâmica, São Paulo, Brazil) sulfuric acid (Fmaia, São Paulo, Brazil). Absorbance was measured at 500 nm. The standard curve ranged from 25 to 500 mg/L (R^2 = 0.9823) (Horszwald; Andlauer, 2011). Ortho-diphenol content was determined using an analytical curve of chlorogenic acid (2.6 – 160 mg/L, R^2 = 0.9835) (Sigma Aldrich, São Paulo, Brazil). Extracts were reacted with 5% sodium molybdate dihydrate solution (Reatec, Paraná, Brazil) and absorbance was measured at 370 nm (Maestro *et al*.,1991). The anthocyanins were quantified using the differential pH method (Lees and Francis 1972), employing two buffer solutions: pH 1.0 (0.025 M KCl) and pH 4.5 (0.40 M CH_3CO_2Na).

2.2.3 *Ultra-Performance Liquid Chromatography coupled with Quadrupole* Time-of- Flight Mass Spectrometry (UPLC-QToF/MS) for identification analysis

The analyses were performed on a Waters Xevo® G2-XS QTof mass spectrometer (Waters Co. Manchester, UK) coupled to an Acquity HClass UPLC and monitored with MassLynx® software (v. 4.1). Separation of the metabolites was achieved on an Acquity UPLC BEH C18 with reverse phase column (100 mm × 2.1 mm i.d, 1.7 m particle size) at 40°C (± 2 °C) and eluted with a gradient system of 0.1% formic acid aqueous solution (A) and 0.1% formic acid in acetonitrile (B) at a flow rate of 0.3 mL/min, using the following linear gradient elution program (A:B, in %: 0–15 min (98:2), 15–20 min (80:20), 20–25 min (60:40), 25–27 min (2:98) 27–27.10 min (98:2), 27.10–30 (98:2). The injection volume was 10 µL. The nebulization process operated in negative mode, with the mass range between 100 and 1500 amu, and a scan time of 0.2 s. The ESI source parameters were capillary voltage of 3.0 kV, desolvation temperature of 250 °C; source temperature of 100 °C; cone voltage 30 V; cone gas flow of 50 L h-1. The desolvation gas flow for negative polarity 700 L/h. Both low (MS1, 6 eV) and high (MS2, ramped from 20 to 35 eV) collision energy data were recorded by employing MSE continuum mode, acquisition time 0 to 30 min and mass correction during acquisition by an external reference (LockSprayTM), Leucine enkephalin (m/z 554.2615 [M-H]- and 556.2771[M+H]+) was used as the lock mass calibrant (Athayde *et al*., 2021; Xu *et al*., 2020).

2.2.4High-Performance Liquid Chromatography with Diode Array Detector (HPLC- DAD) for quantitative analysis

A reverse-phase HPLC system (Shimadzu, Kyoto, Japan) was used for the quantification of phenolic compounds (Irondi *et al*., 2023). The HPLC instrumentation consisted of an auto-sampler (SIL-20A), pumps (LC-20AD), attached to a degasser (DGU-

20A5) with a CBM 20A integrator, column module (CTO-20A), diode-array detector (SPD-M20A) and LC solution software (1.25 SP5 version). The analytical column was an XBridge BEH C18 (130 Å, 3.5 μm, 4.6 mm × 150 mm, Waters, USA). The flow rate and injection volume were 0.9 mL/min and 10 μL, respectively. The mobile phase consisted of 0.1% (v/v) aqueous formic acid (solvent A) and 100% methanol (solvent B). The samples and mobile phase were filtered through a 0.22 μm polyvinylidene difluoride (PVDF) membrane filter (47 mm diameter), (Millipore, Massachusetts, USA) and then degassed by ultrasonic bath prior to injection. The binary elution system used was as follows: a linear gradient of 10-60% B at 15 min, 60-80% B (20 min), 80-100% B (28 min), 100-10% B (30 min). The column and detector temperature were 35 and 40 °C, respectively.

Mixtures of phenolic acid standard solutions were prepared in the HPLC methanol reagent. The working standard solutions were freshly prepared, daily, by dilution of reference material methanol HPLC-grade solution. The standard curve was drawn by using a standard solution of epicatechin (270 nm), caffeic, chlorogenic acid and isoorientin (325 nm), rutin, quercetin and quercitrin (350 nm).

The wavelengths for detections were used for confirmation of chromatography peaks and quantification of their corresponding phenolic compounds achieved by comparing their retention time with those of reference standards and by DAD spectra (200 to 600 nm).

The chemical antioxidant capacity of mapati peel and seed extracts was assessed The chemical antioxidant capacity of mapati peel and seed extracts was assessed using three assays: DPPH radical-scavenging activity, hydroxyl radical-scavenging activity (HRSA), and ferric-reducing antioxidant power (FRAP). In the DPPH assay, the extract was incubated with a methanolic DPPH solution (0.10 mmol/L) (Sigma Aldrich, São Paulo, Brazil) and absorbance was measured at 571 nm. (Brand-Williams *et al*., 1995). For the HRSA assay, the sample was mixed with iron sulfate solution (1 mmol/L) (Synth, São Paulo, Brazil), hydrogen peroxide solution (15 mmol/L) (Synth, São Paulo, Brazil), and phenanthroline solution (1 mmol/L) followed by absorbance measurement at 536 nm (Mukhopadhyay *et al*., 2016). In the FRAP assay, the extract was combined with FRAP reagent containing TPTZ, sodium acetate, and ferric chloride, absorbance was recorded at 593 nm (Benzie & Strain, 1996). DPPH and FRAP results were expressed as milligrams of ascorbic acid equivalent per gram of dry extract (mg AAE/g), while HRSA results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

2.3 Evaluation in vitro

The cytotoxic The cytotoxic effects of mapati peel and seed extracts were evaluated, using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich São Paulo, Brazil) colorimetric method, in adenocarcinoma epithelial cells (A549), human ileocecal adenocarcinoma cells (HCT8), hepatocellular carcinoma (HepG2) and normal human umbilical vein endothelial cells (HUVEC) were obtained from Rio de Janeiro Cell Bank (Rio de Janeiro, Brazil). Briefly, the cells were seeded in 96-well plates at 1 × 104 cells/well density and incubated for 24 h. Then, the cells were treated with different concentrations of the extracts (0.5 - 200 µg GAE/mL) for 48 h. Following, MTT solution (0.5 mg/mL) was added to the wells and incubated for 4 h at 37 °C. The formazan crystals were dissolved in DMSO (Sigma Aldrich, São Paulo, Brazil), and the absorbance was measured at 570 nm. The IC50 (50% cell viability inhibition), GI50 (50% growth inhibition), LC50 (50% cell death), and the selective index (SI) were calculated (SI = IC50 normal cells/IC50 tumor cells), as described by Mosmann (1983) and Lima *et al*. (2024).

2.4 Intracellular ROS generation

Intracellular reactive oxygen species (ROS) generation was measured using the DCFH-DA assay. The A549, HepG2, and HUVEC cell lines were seeded into 96-well plates at 4.5 ×104 cells/well density and incubated for 24 h (37 °C). Afterward, cells were treated for 1 h with different concentrations of samples (6.25 - 50 µg GAE/mL for pell extract and 2.5 - 20 µg GAE/mL for seed extract), or 22.5 µM hydrogen peroxide (H2O2) as positive control, or culture medium (DMEM HAM F-12) (Gibco, New York, USA) with 2% fetal bovine serum (Gibco, New York, USA) as negative control. Then, the plate was washed with PBS 1X (Sigma-Aldrich, São Paulo, Brazil), and H2O2 (22.5 µM) diluted in HANKS solution was added. The fluorescence intensity was measured (λemission = 538 nm and λexcitation = 485 nm) and the results are expressed as a percentage of DCF-fluorescence (Granato *et al*., 2022).

2.5 DNA fragmentation

To assess DNA fragmentation, A549 cells were seeded in 6-well plates and incubated for 24 h to allow adherence, followed by treatment with Mapati peel extract at concentrations of 50 and 100 µg GAE/mL for 48 h. After treatment, cells were detached with 0.25% trypsin, washed once with PBS, and resuspended again in PBS. DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen, Maryland, USA) following the manufacturer's instructions. Briefly, samples underwent proteinase K digestion and lysis at 56 °C, DNA binding and washing steps, and elution in 50 µL of distilled water. The DNA samples (approximately 15 –

45 ng/µL) were electrophoresed on a 1.5% agarose gel stained with SYBR Gold-based fluorescence (Invitrogen, Thermo Fisher Scientific, California, USA) at a final concentration of 1 µg/mL, to visualize DNA laddering indicative of apoptosis (Elkins, 2013).

2.6 Apoptosis detection using Annexin V/7-AAD

The Annexin V PE Kit apoptosis detection kit (cat. n°88-8102-72, Invitrogen, Thermo Fisher Scientific, California, EUA) to determine phosphatidylserine externalization. For this purpose, A549 cells were seeded in a 6-well plate at a density of 1 x 10^5 cells/well and incubated for 24h. Afterward, the cell were treated for 24 h with 50 µg GAE/mL of seed extract or culture medium (DMEM/ HAM F-12) (Gibco, New York, USA) with 10% fetal bovine serum (Gibco, New York, USA) for negative control.

Subsequently, the cells were washed with PBS, harvested via enzymatic digestion using 0.25% trypsin, and centrifuged (3 min at 151 xg). The pellet was washed with a 1× binding buffer apoptosis detection kit (cat. n°00-0055-43, Invitrogen, Thermo Fisher Scientific, California, EUA), resuspended in a 1× binding buffer, and mixed with 5 µL of Annexin V-PE apoptosis detection kit (cat. n°12-8102-45, Invitrogen, Thermo Fisher Scientific, California, EUA), followed by a 10 min incubation. Next, the cells were rewashed with 1× binding buffer, centrifuged (3 min 151 × g), resuspended in 1× binding buffer, and incubated with 5 µL of 7-aminoactinomycin D apoptosis detection (cat. n°00-6993-42, Invitrogen, Thermo Fisher Scientific, California, EUA) (Koopman *et al*., 1994). Finally, the samples were analyzed via flow cytometry using GuavaSoft 2.7 software.

2.7 Protection against chromosomal aberration

The *in vitro* chromosomal abnormalities assay was used to evaluate the protective or genotoxic effects of mapati samples following the protocol preconized by Do Carmo *et al*. (2019). The selection of the cell line (A549) was primarily based on its growth characteristics, and the concentrations of seed and peel mapati extracts were chosen based on the cytotoxicity results (Section 3.2).

Cells were seeded in 25 cm^2 flasks at a density of 5 × 10^5 cells per flask. The positive control was treated with 4 µM of cisplatin (Sigma Aldrich, São Paulo, Brazil) while the negativecontrol received only the culture medium. The treatment groups were exposed to

mapati peel (12.5, 25, and 50 µg GAE/mL) and seed (5, 10, and 20 µg GAE/mL) extracts in combination with 4 µM of cisplatin. Additionally, to assess the potential independent genotoxic effects of the extract, two groups were treated exclusively with peel (50 and 100 µg

GAE/mL) and seed (20 and 50 μg GAE/mL) extracts. To ensure chromosome visualization in metaphase, 0.0016% colchicine (Sigma Aldrich, São Paulo, Brazil) solution was added to each group for 6 h. Following, cells were incubated with 75 mM KCl hypotonic solution for 10 min at 37 °C, promoting chromosome spreading. Then, the cells were fixed and stained for further analysis, based on breakage criteria. The chromosomal abnormalities rate (%) was calculated as the percentage of chromosome breaks observed relative to the total number of chromosomes analyzed (Auerbach *et al*., 1989).

2.8 Erythrocyte cellular antioxidant activity and protection

The oxidative stress of human erythrocytes was induced by 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) (Sigma-Aldrich, Rheinland-Pfalz, Germany), followed by evaluation of hemolysis and intracellular ROS generation (Cruz *et al*., 2024a). First, blood (O+) was collected in heparinized tubes, from a female volunteer, after signing an informed consent form (Federal University of Alfenas - Ethics approval n° 6.910.474). The blood was washed with PBS three times and diluted until it reached 20% hematocrit. Mapati peel and seed extracts or PBS (negative control and positive control) or quercetin (standard) were mixed with hematocrit (20%) and incubated for 20 min (37 °C, 151 xg). After, samples and positive control were allowed to react with AAPH (200 mmol/L, Sigma-Aldrich, São Paulo, Brazil) for 2 h (37 °C, 151 xg) to complete the oxidation. Then, the mixture was centrifuged at 1200 xg for 10 min, to obtain the supernatant (SN) and the precipitate (PT).

The hemolysis rate (%) was measured at 523 nm by 100 μL of SN transferred to a 96-well microplate and mixed with 200 μL of PBS (Sigma Aldrich, São Paulo, Brazil), and the hemoglobin oxidation rate (%) was calculated as the ratio between the 630 nm and 540 nm absorbances. For the Intracellular ROS generation, the PT was washed with 400 μL PBS, and centrifuged (1200 xg, 3 min). Then, 400 μL of DCFH-DA solution (10 μmol/L, Sigma Aldrich, São Paulo, Brazil) was added and 300 μL was transferred to a 96-well microplate. The fluorescence intensity was measured at 485 and 520 nm for excitation and emission, after incubating in the dark for 20 min at 37 °C. The results were expressed as a percentage ofnintracellular ROS generation (%), compared to the negative control.

2.9 In vitro antiplasmodial test

The anti-plasmodial activity of mapati peel and seed extracts was assessed using W2 (resistant to chloroquine) and 3D7 (sensitive to chloroquine) strains of Plasmodium falciparum. In

summary, parasites were maintained at 4% hematocrit (Erythrocytes type O+) in Petri dishes including RPMI 1640 culture medium (Gibco, New York, USA) with 10% Albumax II (Gibco, New York, USA) at 37 °C, using the candle jar method (Trager & Jensen, 1976). The parasitemia was checked with smears using a panoptic staining kit (Renylab, Minas Gerais, Brazil) and the parasites were synchronized with sorbitol solution (5%) (Sigma Aldrich, Missouri, EUA) to obtain ring forms (Lambros & Vanderberg, 1979).

In 96-well plates, different concentrations (160 - 2.5 µg GAE/mL) of peel and seed extracts were prepared by serial dilution (1:1 v/v). 100 µL of the parasitic solution (RPMI 1640 culture medium, 2% hematocrit, 1% parasite) was incorporated into the positive controls and the treatment wells, while 200 µL of 2% red blood cell solution for negative control. Aliquots were taken at 18 h rings, 26 h trophozoites, and 38 h schizonts and treated as previously described by Do Carmo *et al*., (2020). After 48 h, 100 µL culture was added to a 96-well plate with 100 µL of lysis buffer solution and 0.1 µL/mL Sybr gold nucleic acid gel stain (cat. S11494, Sigma Aldrich, São Paulo, Brazil). The fluorescence intensity was measured at 485 nm excitation and 520 nm emission. The selective index (SI) was calculated by the ratio between the IC50 normal cell (HUVEC) and the IC50 of each P. falciparum strain (3D7 and W2).

2.10 Potentiation of artesunate by mapati extracts

To investigate whether mapati peel or seed extracts enhance the antiplasmodial activity of artesunate, combination experiments were performed against P. falciparum strains (3D7 and W2). The IC50 of artesunate was determined in the presence or absence of fixed concentrations of Mapati peel or seed extracts (5 or 10 µg GAE/mL). Parasites synchronized at the ring stage were incubated with varying concentrations of artesunate combined with a constant concentration of each extract for 48 h. Parasite viability was assessed using the SYBR Gold-based fluorescence assay. The IC50 values of artesunate alone and in combination with extracts were determined and compared to evaluate possible synergistic or additive interactions (Sannella *et al*., 2007).

2.11 Statistical analysis

The experiments were conducted in quadruplicate, with data reported as mean ± standard deviation (SD). Dose-response relationships were analyzed via nonlinear regression (curve fitting). Differences among groups were assessed using one-way ANOVA followed by Tukey's post hoc test. All statistical analyses were performed using GraphPad Prism® software (version 8.0, USA), and results with a p-value ≤ 0.05 were considered statistically significant.

3 Results and discussion

3.1 Chemical characterization

The scientific interest in the bioprospecting of natural products, particularly underexplored native fruit species from Brazilian biomes, has grown substantially driven by investigations into their chemical composition, potential applications, and beneficial biological effects on human health. Such studies unveil an innovative potential for both food and pharmaceutical industries, while also carrying significant economic, ecological, and nutritional relevance (Do Carmo *et al*., 2019; Crispim *et al*., 2025). In this context, the present work focused on the phenolic extraction from mapati seeds and peels, followed by chemical characterization and subsequent biological evaluation. For this purpose, during the extract preparation stage, solid-phase extraction (SPE) was employed, yielding an extraction efficiency of 16.58% for phenolic compounds from the seed extract.

Indeed, our findings underscore this fact, as the seed extract showed a higher content of the bioactive compounds compared to the peel extract (Table 1). Similar behavior was observed in the different classes of phenolic compounds studied, revealing that the seed extract presented higher levels of flavonols (8 ± 0.4 mg CE/g), anthocyanins (1 ± 0.02 mg CGE/g), condensed tannins (155 ± 11 mg CE/g), and ortho-diphenols (74 ± 4 mg CAE/g). Interestingly, condensed tannins and anthocyanins were the predominant phenolic compounds and were detected exclusively in the seed extract. The same result was observed in a previous study by our group, in which camu-camu seed extract presented the highest amount of condensed tannins (Do Carmo *et al*, 2019), pointing out that the seeds, although not as extensively studied as the peel, contribute to the fruit's overall polyphenolicprofile.

Table 1. Chemical composition and antioxidant activity of mapati peel and seed extracts.

Compounds	**Peel extract**	**Seed extract**
Total Phenolic Content (mg GAE/g)	3 ± 0.2	198 ± 5*
Total Flavonols Content (mg QE/g)	3 ± 0.1	8 ± 0.4*
Total Anthocyanins (mg CGE/L)	-	1 ± 0.02*
Condensed Tannin Content (mg CE/g)	-	155 ± 11*
Ortho-diphenols (mg CAE/g)	3 ± 0.7	74 ± 4*
Antioxidant activity		
DPPH (mg AAE/g)	4 ± 0.2	301 ± 8*
FRAP (mg AAE/g)	4 ± 0.3	117 ± 3*

HRSA (mg GAE/g)	2 ± 0.2	92 ± 4*

Note: GAE, gallic acid equivalent; CE, quercetin equivalent; CGE, cyanidin-3- glucoside equivalents; CAE, chlorogenic acid equivalent; AAE, ascorbic acid equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; HRSA, Hydroxyl radical scavenging activity; *represent significant differences ($p \leq 0.05$) using the t-test.

Interestingly, condensed tannins were the predominant phenolic compounds and were detected exclusively in the seed extract. The same result was observed in a previous study by our group, in which camu-camu seed extract presented the highest amount of condensed tannins (Do Carmo *et al*, 2019), showing that the seeds, although

To gain deeper insight into the chemical composition, UPLC-QToF/MS (Table 2) and HPLC-DAD (Table 3) were performed, and the results are discussed below and structured based on the major groups of phenolics such as tannins, phenolic acids, and epicatechins in the seed extract, followed by quercetin and derivatives profile in the peel extract. In this regard, compounds 5, 6, 9, 10, and 11 (condensed tannins) were identified in seed extract as B- and A-type proanthocyanidins, varying from dimers to pentamers (Figure 1). Compound 5 was assigned as a B-type proanthocyanidin dimer, based on its fragment ions at m/z 289.0684 (epicatechin monomer), m/z 407.0764, 425.0843, and 451.1027, associated with internal dehydration and hydroxyl group losses, typical of oligomeric flavonoids (Yue *et al*., 2022). Compound 6 was proposed as Procyanidin B1, with a $[M–H]^-$ ion at m/z 577.1342 and a low mass error (0.85 ppm). Its later elution compared to its structural isomer is consistent with known reversed-phase retention behavior (Toro-Uribe *et al*., 2020).

The compound 9 was identified as a type A proanthocyanidin trimer, with $[M–H]^-$ atm/z865.1984 ($C_{45}H_{34}O_{18}$). Fragmentation revealed ions at m/z 577.1342 (dimer) and 289.0684 (epicatechin), confirming its trimeric structure and A-type interflavan linkage (Tsamo *et al*., 2018). Compound 10, was identified as a B-type proanthocyanidin tetramer, based on $[M–H]^-$at m/z 1153.2589 ($C_{60}H_{50}O_{24}$). Its MS/MS profile showed sequential losses yielding m/z 865.1917 (trimer), 577.1342 (dimer), and 289.0684 (monomer), all supporting the tetrameric and B-type nature (Mesquita *et al*., 2018). Compound 11 showed $[M–H]^-$ at m/z 1441.3257, consistent with a B-type proanthocyanidin pentamer ($C_{75}H_{62}O_{30}$). MS/MS fragmentation yielded m/z 1153.2589, 865.1917, 577.1342, and 289.0684, indicating stepwise cleavage of flavan-3-ol subunits and confirming the pentameric structure (Tsamo *et al*., 2018). UPLC analysis revealed that compounds 1, 2, 3, and 4 are chlorogenic acid derivatives, primarily belonging to the class of caffeoylquinic acids and caffeoyl-hexoside. Compound 1 was assigned as 3-O-caffeoylquinic acid (3-CQA) based on its fragmentation pattern, particularly the base peak at

m/z 191.0533, indicative of quinic acid, and the ion at m/z 179.0327, corresponding to caffeic acid. The high relative intensity of m/z 191 aligns with the known fragmentation of 3-CQA. Compound 3, detected in both seed and peel, was identified as 5-O-caffeoylquinic acid (5-CQA). Its fragmentation profile included key ions at m/z 191.0533 (quinic acid, resulting from loss of the caffeoyl group), m/z 179.0346 (caffeic acid), and secondary ions at m/z 173.0417, 161.0217, and 135.0434, all typical of hydroxycinnamic acid derivatives. These patterns confirm the assignment and distinguish 5-CQA from its positional isomers. Compound 4, with the longest retention time among the three, showed a richer fragmentation pattern with dominant ions at m/z 173.0447, 179.0327, 191.0533, and 135.0434. The predominance of m/z 173, associated with rearrangements from 4-CQA, and the elution order (later than 3- and 5-CQA on C18) support its identification as 4-O-caffeoylquinic acid (4- CQA). Compound 2, detected in the seed, was identified as a caffeoyl-hexoside based on its $[M–H]^-$ ion at m/z 341.0882. Its fragmentation generated m/z 179.0354 (caffeic acid), 161.0230 (loss of H_2O), and 135.0433 (decarboxylated caffeic acid), consistent with typical breakdown patterns of glycosylated caffeic acid derivatives (Wang *et al*., 2023).

The HPLC-based profiling of the seed extract revealed predominantly derivatives of caffeic acid, such as caffeic acid 3-O-glucoside (51 mg/100g of extract), 3-CQA (83 mg/100g of extract), and 5-CQA (130 mg/100g of extract), followed by 4-CQA (35 mg/100g of extract). These findings are consistent with the results reported by Xia *et al*. (2011), who also identified higher levels of these isomers in the Prunus mume seed extract. Similarly, Amakura *et al*. (2024) identified 3-CQA, 4-CQA, and 5-CQA as the major constituents in the seed extract of Helianthus annuus, supporting the notion that seeds may serve as rich sources of caffeoylquinic acids. Furthermore, epicatechin (compound 8 [8 min]) was also identified in the seed extract based on comparison with a reference standard by coinjection under the same chromatographic conditions. The deprotonated molecular ion $[M–H]^-$ was observed at m/z 289.0723 (3.81 ppm). The MS/MS spectrum obtained in negative ionization mode (ESI–) showed a predominan fragment ion at m/z 289.0684 (100%), ion at m/z 245.0810 (20%) characteristic neutral loss of 44 Da. Additionally, a dimeric ion was detected at m/z 579.1525 (25%), attributed to a $[2M–H]^-$ species (Toro-Uribe *et al*., 2020). This compound has been frequently reported as a major phenolic present in seed matrices (Ma *et al*., 2022; Usman *et al*., 2016; Da Silva *et al*., 2024), and was consistently linked to antioxidant activity, reinforcing their functional significance in seeds, and further supports the contribution to the bioactivity as demonstrated by our in vitro tests.

From another perspective, the peel extract exhibited a predominantly phenolic profile

enriched in quercetin and its derivatives. Compounds 13, 15, 16, 17, 18, and 19 were identified as glycosylated flavonoids derived from quercetin, differing in sugar type and glycosylation position on the aglycone. Compound 13 showed a $[M–H]^-$ ion at m/z 755.2031. Fragmentation revealed sequential losses of rhamnose (–146 Da) and a rutinoside moiety (–308 Da), resulting in fragments at m/z 609.1431, 447.0918, and aglycone ions at m/z 301.0327, 300.0271, and 271.0223. This fragmentation pattern confirmed its identity as Manghaslin (quercetin 3-(2G-rhamnosylrutinoside)), as previously described by Chen *et al*. (2025). Compounds 15 and 16 detected as isomers in the peel, shared a $[M–H]^-$ ion at m/z 609.1431, consistent with quercetin rutinosides. Compound 16, exhibited a fragment at m/z 463.0864, corresponding to the loss of rhamnose and characteristic of rutin (quercetin-3-O-rutinoside). Further fragmentation generated aglycone ions at m/z 301.0327 and 300.0271. Co-injection with an authentic standard confirmed this identity. In contrast, compound 15 did not produce the m/z 463 fragment, suggesting an alternative glycosylation site, possibly corresponding to quercetin-7-O- rutinoside (Cvetković *et al*., 2025). Compound 17, also from the peel, was identified as quercetin-3-O-galactoside (hyperoside), with a $[M–H]^-$ ion at m/z 463.0864. The loss of a galactose unit (–162Da) yielded fragments at m/z 301.0327 and 300.0271, along with additional fragments at m/z 271.0223 and 255.0270, indicative of structural rearrangements and further cleavage within the flavonoid backbone (Mighri *et al*., 2019). Compound 18, with a $[M–H]^-$ ion at m/z 433.0761, was assigned as quercetin-3-O-arabinoside. The loss of an arabinose unit (– 132 Da) resulted in a base peak at m/z 300.0271, accompanied by fragments at m/z 271.0223 and 255.0270, consistent with monosaccharide glycosides of quercetin (Yu *et al*., 2024). Finally, compound 19 exhibited a $[M–H]^-$ ion at m/z 447.0918. The loss of a rhamnose unit (–Da) produced an aglycone at m/z 301.0327, with a base peak at m/z 300.0271 and additional fragments at m/z 271.0323 and 255.0270. Its identity as quercitrin (quercetin-3-O- rhamnoside) was confirmed by co-injection with an analytical standard (Calassara *et al*., 2021).

Quercetin and its glycosylated derivatives are commonly concentrated in fruit peels, where they contribute to bioactivity and play protective roles in plant defense. Suleria *et al*. (2020) reported that mango peel extract exhibits high levels of quercetin and its derivatives, particularly quercetin-3-galactoside and quercetin-3-glucoside, based on a comprehensive flavonoid profiling. Similarly, HPLC-DAD and gas chromatography analyses detected quercetin in the hydroethanolic extract of Citrus reticulata peel (Ali *et al*., 2020), as well as the peel of Opuntia ficus-indica has been reported to contain quercetin as a major phenolic component (Elkady *et al*., 2020). These results are supported by the composition and biological function of seeds, which, as essential plant organs, serve as key sites for the biosynthesis and

storage of diverse specialized metabolites, including a wide variety of phenolic compounds, accumulated in seed tissues, where they play important protective roles for the developing embryo (Corso *et al*., 2020). Highlighting this observation, Kupe *et al*. (2021) reported that grape seeds exhibit higher total phenolic content and antioxidant activity compared to peel and pulp, reinforcing the biological potential of the seed matrix, as will be discussed in the following sections

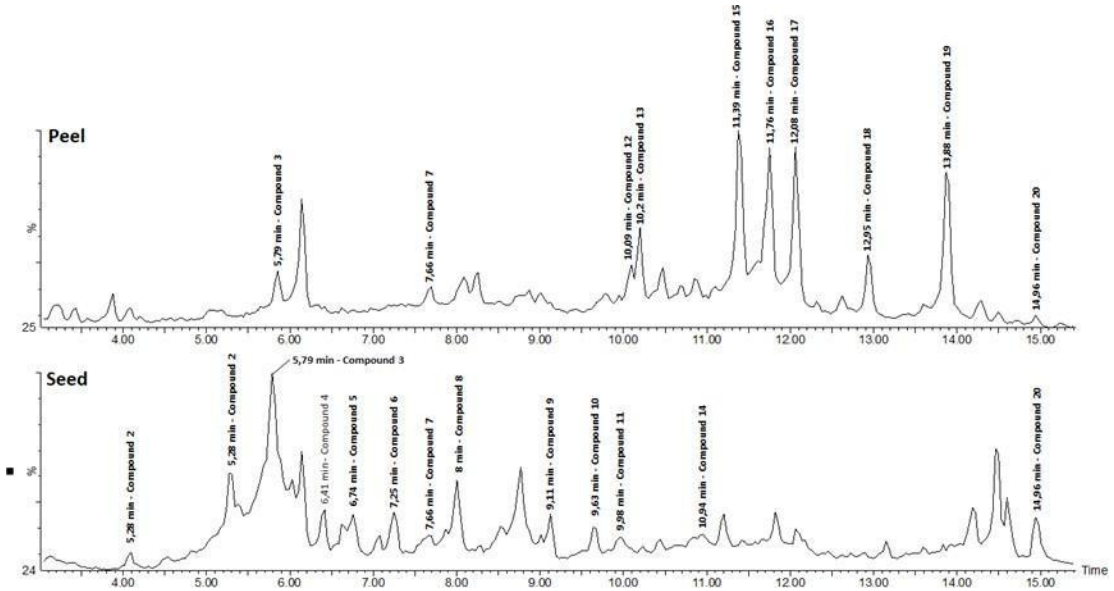


Figure 1 Comparative total ion chromatograms (TICs) of mapati peel and seed hydroethanolicextracts obtained by UPLC-qToF-MS analysis in negative ion mode.

Table 2. Chromatographic and spectrometric data obtained by UPLC-qToF-MS in ESI(–) mode for compounds identified in mapati *Pourouma cecropiifolia* peel and seed extracts

continued

N	RT (min)	Observed ion $[M-H]^-$ (m/z)	Mass error (ppm)	Fragments MS/MS (m/z, Relative Intensity)	Compound, Molecular formula	Matrix	Reference for Identification
1	4.09	353.0876	0.85	191.0533 (100), 135.0434 (40), 179.0327 (30)	3-*O*-caffeoylquinic acid (3-CQA), $C_{16}H_{18}O_9$	Seed	(Santanatoglia *et al*., 2024)
2	5.28	341.0882	−2.78	341.0882(100), 179.0354 (50), 161.0230(45, 135.0433 (40)	Caffeic acid 3-*O*-glucoside, $C_{16}H_{18}O_7$	Seed	(Wang *et al*., 2023)
3	5.79	353.0876	0.85	191.0533 (100), 353.0876 (10)	5-*O*-caffeoylquinic acid (5-CQA), $C_{16}H_{18}O_9$	Peel and Seed	Standard
4	6.41	353.0876	0.85	173.0447 (100), 179.0327 (70), 191.0533 (50), 135.0434 (50)	4-*O*-caffeoylquinic acid (4-CQA), $C_{16}H_{18}O_9$	Seed	(Santanatoglia *et al*., 2024)

continued

5	6.74	577.1342	0.85	577.1342 (100), 289.0684 (50), 407.0764 (40), 425.0843 (20), 451.1027 (10)	Procyanidin B2/B5 (proanthocyanidin type B), $C_{30}H_{26}O_{12}$	Seed	(Yang *et al.*, 2022)
6	7.25	577.1342	0.85	577.1342 (100), 1155.2760 (80), 289.0684 (50), 407.0764 (35), 425.0843 (20), 451.1027 (5)	Procyanidin B1 (proanthocyanidin type B), $C_{30}H_{26}O_{12}$	Seed	(Toro-Uribe *et al.*, 2020)
7	7.66	337.0936	3.77	191.0533 (100), 163.0371 (25), 119.0487 (10)	4-p-coumaroylquinic acid, $C_{16}H_{18}O_8$	Peel and Seed	(Li *et al.*, 2024)
8	8.00	289.0723	3.81	289.0684 (100), 579.1525 (25), 245.0810 (20)	Epicatechin, $C_{15}H_{14}O_6$	Seed	Standard
9	9.11	865.1984	–3.2	865.1984 (100), 287.0530 (20), 577.1342 (15), 289.0684 (10), 605.2193 (8)	Procyanidin trimer type A, $C_{45}H_{34}O_{18}$	Seed	(Tsamo *et al.*, 2018)

continued

10	9.63	1153.259	2.9	1153.2589 (100), 577.1342 (15), 865.1917 (5), 289.0684 (25)	Procyanidin tetramer type B, $C_{60}H_{46}O_{24}$	Seed	(De Souza Mesquita *et al.*, 2018)
11	9.98	1441.326	5.4	1441.3257 (80), 289.0684 (50), 577.1342 (20), 865.1917 (15), 1153.2589 (10)	Proanthocyanidin pentamer type B, $C_{75}H_{62}O_{30}$	Seed	(Tsamo *et al.*, 2018)
12	10.09	447.0918	-2.08	447.0918 (100), 327.0568 (80), 357.0613 (50), 285.0369 (15)	Isorientin (Luteolin-6-*C*-glucoside), $C_{21}H_{20}O_{11}$	Peel	Standard and (Marzouk *et al.*, 2023)
13	10.20	755.2031	-0.48	755.2031 (100), 447.0918 (40), 300.0271 (35), 301.0327 (20), 609.1431 (10), 271.0223 (5)	Manghaslin (Quercetin 3-(2G-rhamnosylrutinoside)), $C_{33}H_{40}O_{21}$	Peel	(Chen *et al.*, 2025)
14	10.94	367.1037	2.26	135.0434 (100), 179.0327 (80), 161.0246 (70), 367.1037 (50)	Chlorogenic acid methyl ester, $C_{16}H_{18}O_{10}$	Seed	(Wu *et al.*, 2009)

continued

15	11.39	609.1431	−4.03	609.1431 (100), 300.0271 (42), 301.0327 (30)	Quercetin-7-*O*-rutinoside, $C_{27}H_{30}O_{16}$	Peel	(Tsamo *et al*., 2018)
16	11.76	609.1431	−4.03	609.1431 (100), 463.0864 (10), 301.0327 (35), 300.0271 (50)	Quercetin-3-*O*-rutinoside (Rutin), $C_{27}H_{30}O_{16}$	Peel	Standard and (Cvetković *et al*., 2025)
17	12.08	463.0864	−2.59	300.0271 (100), 463.0864 (90), 301.0327 (62), 271.0223 (12), 255.0270 (5)	Quercetin-3-*O*-galactoside (Hyperoside), $C_{27}H_{28}O_{16}$	Peel	(Mighri *et al*., 2019)
18	12.95	433.0761	−2.31	300.0271 (100), 271.0223 (50), 433.0761 (30), 255.0270 (15)	Quercetin-3-*O*-arabinoside (guaijaverin), $C_{22}H_{24}O_{16}$	Peel	(Yu *et al*., 2024)
19	13.88	447.0918	−2.01	300.0271 (100), 301.0327 (90), 271.0323 (15), 255.0270 (5)	Quercitrin, $C_{21}H_{20}O_{11}$	Peel	Standard and (Calassara *et al*., 2021)
20	14.96	515.1189	−0.09	173.0447 (100), 179.0327 (70), 353.0876 (50),	4,5-Dicapeoylquinic acid,	Peel and Seed	(Zhou *et al*., 2018)

	191.0533 (40), 135.0434 (20), 515.1189 (10)	$C_{25}H_{24}O_{12}$

Note: Retention time (RT) in minutes. Mass error (ppm) calculated as the difference between theoretical and observed m/z values. Fragment ions were obtained in ESI mode using qTOF-MS. Compound identification was supported by MS/MS fragment patterns and literature data.

Table 3: Concentration of compounds determined in samples of Mapati seed and peel in mg/100 g of hydroethanolic Extracts.

Compound Molecular Formula	**HPLC-DAD Analysis** **Retention time (min)**	**Concentration (mg/100g extract)** **Peel**	**Seed**
3-*O*-caffeoylquinic acid (3-CQA), $C_{16}H_{18}O_9$	4.96		83 ± 0.6
Caffeic acid 3-*O*-glucoside, $C_{16}H_{18}O_7$	6.18		51 ± 0.6
5-*O*-caffeoylquinic acid (5-CQA), $C_{16}H_{18}O_9$	6.95	0.23 ± 0.001	130 ± 6
4-*O*-caffeoylquinic acid (4-CQA), $C_{16}H_{18}O_9$	7.39		35.4 ± 1.4
Epicatechin, $C_{15}H_{14}O_6$	8.16		86.4 ± 2
Isorientin (Luteolin-6-*C*-glucoside), $C_{21}H_{20}O_{11}$	10.28	0.15 ± 0.001	
Quercetin 3-(2G-rhamnosylrutinoside), $C_{33}H_{40}O_{21}$	10.4	0.32 ± 0.003	
Quercetin-7-*O*-rutinoside, $C_{27}H_{30}O_{16}$	12.12	0.74 ± 0.003	
Quercetin-3-*O*-rutinoside (Rutin), $C_{27}H_{30}O_{16}$	12.23	0.44 ± 0.01	
Quercetin-3-*O*-galactoside (Hyperoside), $C_{27}H_{28}O_{16}$	12.74	0.52 ± 0.001	
Quercitrin, $C_{21}H_{20}O_{11}$	13.46	1.33 ± 0.003	
4,5-Dicaffeoylquinic acid, $C_{25}H_{24}O_{12}$	15.34		12 ± 0.2
Sum of Total Phenolic Compounds		3.745	396.7

Note: Retention time (min) determined by HPLC-DAD analysis. Concentrations expressed as mean ± standard deviation (mg per 100 g of extract). Compounds were identified by comparison with authentic standards and literature data.

3.2 Antioxidant capacity -

In line with chemical assays, the presence of the major components phenolics in the seed extract mirrored in higher antioxidant activity, proved according to DPPH (301 ± 8 mg AAE/g), FRAP (117 ± 3 mg AAE/g), and HRSA (92 ± 4 mg GAE/g) (Table 1). It is well known that phenolic compounds are the main contributor to the antioxidant effect, mostly due to their capacity for single electron-proton transfer (DPPH) and reduction of iron (FRAP), especially related to the hydroxyl groups on the A and B rings in their structure (Serrano *et al*., 2009; Mohammadi *et al*., 2024).

We hypothesize that the antioxidant capacity observed in the DPPH, FRAP, and HRSA assays for the seed extract is attributed to the high content of phenolic compounds identified by UPLC-qToF-MS and HPLC-DAD, including procyanidins, chlorogenic acid (3-CQA) and derivatives (4-CQA, 5-CQA). These compounds are known as a potent antioxidant agent that can act via hydrogen atom transfer under acidic or neutral pH, or through sequential proton loss electron transfer under basic conditions, thereby preventing oxidative damage (Tošović *et al*., 2017). Xu *et al*. (2012) emphasize that 3-CQA, 4-CQA, and 5-CQA exhibit antioxidant activity primarily due to their oxidizable ortho-diphenolic structure, which can chelate transition metals, preventing Fenton-type reactions. This feature enables them to act as hydrogen atom donors, thereby interrupting the propagation of radical chain reactions and neutralizing biological oxidants, such as hydrogen peroxide, as demonstrated in Section 3.3.

Procyanidins, characterized by ortho-dihydroxy (–OH) groups on the aromatic B ring, readily chelate Fe^{2+} and Fe^{3+} ions via their catechol and galloyl moieties, forming stable metal–polyphenol coordination complexes. This chelation capacity substantially enhances the antioxidant potential of the seed extract. These dehydroxylated groups (–OH) are essential for electron donation, allowing tannins to reduce Fe^{3+} to Fe^{2+} (Unusan *et al*., 2020). Bosso *et al*. (2020) found a strong positive correlation between the antioxidant properties of grape seeds and their condensed tannin content. This interaction corroborates the association between the high tannin content and the elevated FRAP antioxidant activity identified in our analysis. Bearing in mind, the differences observed in chemical analysis behavior between seed and peel extracts are reflected in the antioxidant activity evaluated, as presented in the in vitro cellular tests and antiplasmodial assays in the next sections

3.3 Cytotoxicity in cell culture

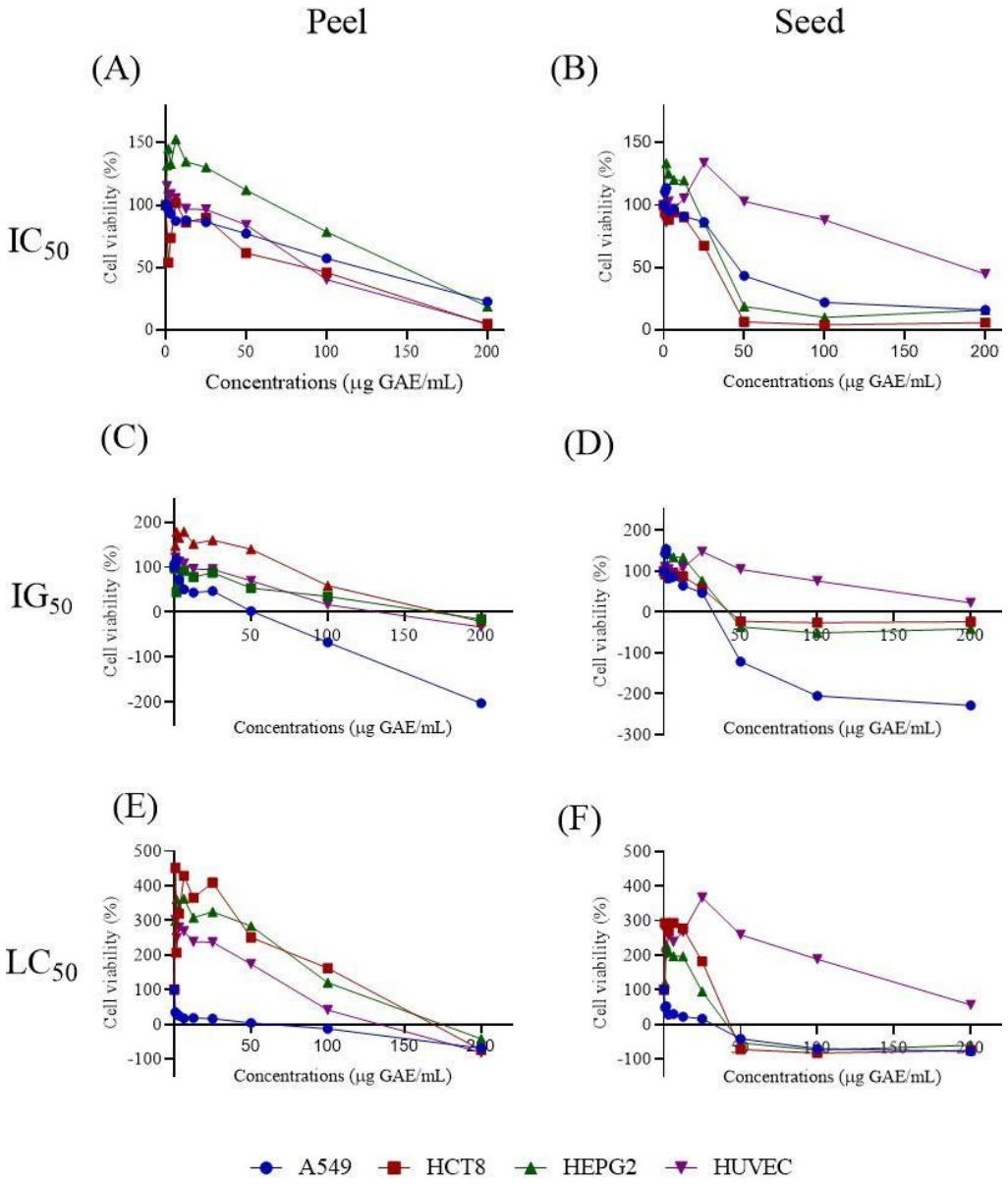
The cytotoxicity of mapati extracts was evaluated in normal (HUVEC) and cancer

(A549, HepG2, HCT8) cell lines (Figure 2A-F), using the MTT colorimetric assay (Lima *et al*., 2024). The relative cytotoxicity potential of seed extracts revealed that all concentrations tested reduced the cells viability in lower concentrations, especially in cancer cells (A549 = 51μg GAE/mL, HepG2 = 37 μg GAE/mL, and HCT8 = 29 μg GAE/mL). Similarly, GI50 values were also reduced to A549 (17 μg GAE/mL), HepG2 (26 μg GAE/mL), and HCT8 (25 μg GAE/mL), possibly indicating an antiproliferative effect. In contrast, the seed extract exhibited higher IC50 (188 μg GAE/mL), GI50 (141 μg GAE/mL), and LC50 (> 200 μg GAE/mL) values in HUVEC cells, indicating that higher concentrations were required and thereby suggesting low cytotoxicity toward normal cells. This behavior, characterized by a greater susceptibility of malignant cells to mapati seed extract compared to normal cells, suggests a degree of selectivity and safety in vitro, indicating a potential therapeutic window for its development as an antiproliferative agent. The selective targeting of cancer cells is reflected in the SI values, ranging from 3.7 to 6.4 (Figure 2G), confirming the classification proposed by Crispim *et al*. (2025), which considers values greater than 3 as high selectivity to cancer cells.

Similar cytotoxic effects of hydroethanolic seed extracts of Tapirira guianensis and Myrciaria dubia have previously been reported by our group. These findings were attributed to the intermediate polarity of the hydroethanolic solvent system, which may enhance the extraction efficiency of bioactive compounds and facilitate their permeation through cellular membranes, ultimately impacting cell viability (Do Carmo *et al*., 2019; Crispim e*t al*., 2025). In this sense, considering that the main compound of seed extracts analyzed by the HPLC was phenolic acids, we hypothesize that part of the cytotoxicity observed in cells is associated with these compounds. According to Ramli *et al*. (2025), chlorogenic acid exhibited a dose-dependent cytotoxic effect on adenocarcinoma epithelial cells (A549) and hepatocellular carcinoma (HepG2). In addition, Wang, Du, and Chen *et al*. (2020) report that chlorogenic acid inhibits cell viability in a dose-dependent manner in A549 cell by negatively regulating the protein ANXA2 responsible for the suppression of the expression of anti-apoptotic genes.

Furthermore, although peel extract exerts a lesser cytotoxic effect on cancer cells than seed extract, we observed a moderate reduction in the HUVEC viability (IC50 = 87 μg GAE/mL). This result was confirmed by the intracellular ROS generation presented in the next section, in which peel extract potentialized the oxidation caused by the hydrogen peroxide. It is well-established that the rise in the oxidative cell environment leads to less cell viability, confirming our results (Do Carmo *et al*., 2021). Taken together, the results demonstrate that,

compared to the peel extract, the seed extract exhibits greater selectivity toward cancer cells, reflecting a chemopreventive potential



(G)

Cell lines	IC50 (µg GAE/mL) Seed	SI HUVEC/ Cancer cells	IC50 (µg GAE/mL) Peel	SI HUVEC/ Cancer cells	IG50 (µg GAE/mL) Seed	IG50 (µg GAE/mL) Peel	LC50 (µg GAE/mL) Seed	LC50 (µg GAE/mL) Peel
HUVEC	187.6		87.2		140.6	61.8	> 200	188.9
A549	50.6	3.7	104	0.8	16.5	8.5	70.7	162.7
HCT8	29.2	6.4	72.4	1.2	25.3	52.7	48.3	192.5
HEPG2	37.2	5	140.4	0.6	25.9	101.3	50	> 200

Figure 2**.** Cytotoxic effects of mapati peel and seed extracts on normal (HUVEC) and cancer cell lines (A549, HCT8, HepG2) after 48 h of treatment. A–B: concentration of extracts that inhibit cell viability by 50 % (IC_{50}), C–D: concentration of extracts that inhibit cell growth by 50 % (GI_{50}), and E–F:concentration of extracts that result in 50% loss of cells (LC_{50}), for peel (A, C, E) and seed (B, D, F) extracts. G: IC_{50}, GI_{50}, LC_{50} values (µg GAE/mL) and Selectivity Index (SI) for cancer cells, calculated by IC_{50} HUVEC/IC_{50} cancer cells ratio. All IC_{50}, GI_{50}, and LC_{50} values were determined by nonlinear regression using the least squares method**.**

3.4Intracellular Generation of Reactive Oxygen Species (ROS)

The Physiologically, ROS regulates multiple cell fates, including differentiation, maintenance, and survival, which are essential for maintaining normal cellular homeostasis. Otherwise, their excessive production triggers cellular damage mechanisms, including apoptosis and insufficient ROS generation (below the optimal range), can disrupt cell signaling pathways (Do Carmo *et al*., 2021; Villapando-Rodriguez & Gibson, 2021). In this study, A549 and HepG2 cells treated with both extracts showed a reduction in intracellular ROS, regardless of the presence of hydrogen peroxide (H_2O_2) (Figure 3A-D), suggesting a protective effect, with the most pronounced effect observed in lung cancer cells (A549). It is noteworthy that cancer cells are characterized by the necessity of an increased rate of ROS generation, which contributes to the biochemical and molecular changes essential for tumor initiation, promotion, and progression (Galadari *et al*., 2017). This protective effect that affected ROS production may have contributed to decrease the cancer cells viability, as we demonstrated previously.

Lage et al. (2020) highlighted that phenolic compounds potentially contribute to reducing oxidative stress, which may impair pro-tumorigenic signaling, cell growth, and tumor progression by negatively regulating the PI3K/Akt/mTOR pathway and the Sirt1/survivin pro-survival pathway, inducing apoptosis. These findings are consistent with those reported by Sun *et al*. (2017), who observed that proanthocyanidins regulate liver cancer progression by inhibiting the MAPK/AKT and PI3K/AKT pathways and key enzymes that promote cell proliferation. In this sense, we consider that the higher presence of phenolic compounds in the seed extract may reduce ROS production below basal levels, leading to an imbalance and subsequent apoptosis, as demonstrated in Section 3.4.

Regarding the HUVEC normal cells, we observed that seed and peel extracts do not exhibit antioxidant activity, increasing ROS when alone or in the presence of H_2O_2, keeping the oxidation levels equal to those of the positive control (Figure 3E-F). The essence of the whole story is that while normal cells possess efficient repair systems under physiological conditions, they may have reduced tolerance to sudden and high levels of oxidative stress, mostly because of the lack of adaptive mechanisms commonly found in cancer cells, such as

the upregulation of Nrf2-mediated antioxidant pathways (Hayes *et al*., 2020). These processes may help us to understand the basic mechanisms of samples action, explaining our findings in the reduced HUVEC viability at 87 μg GAE/mL and cell growth at 67 μg GAE/mL

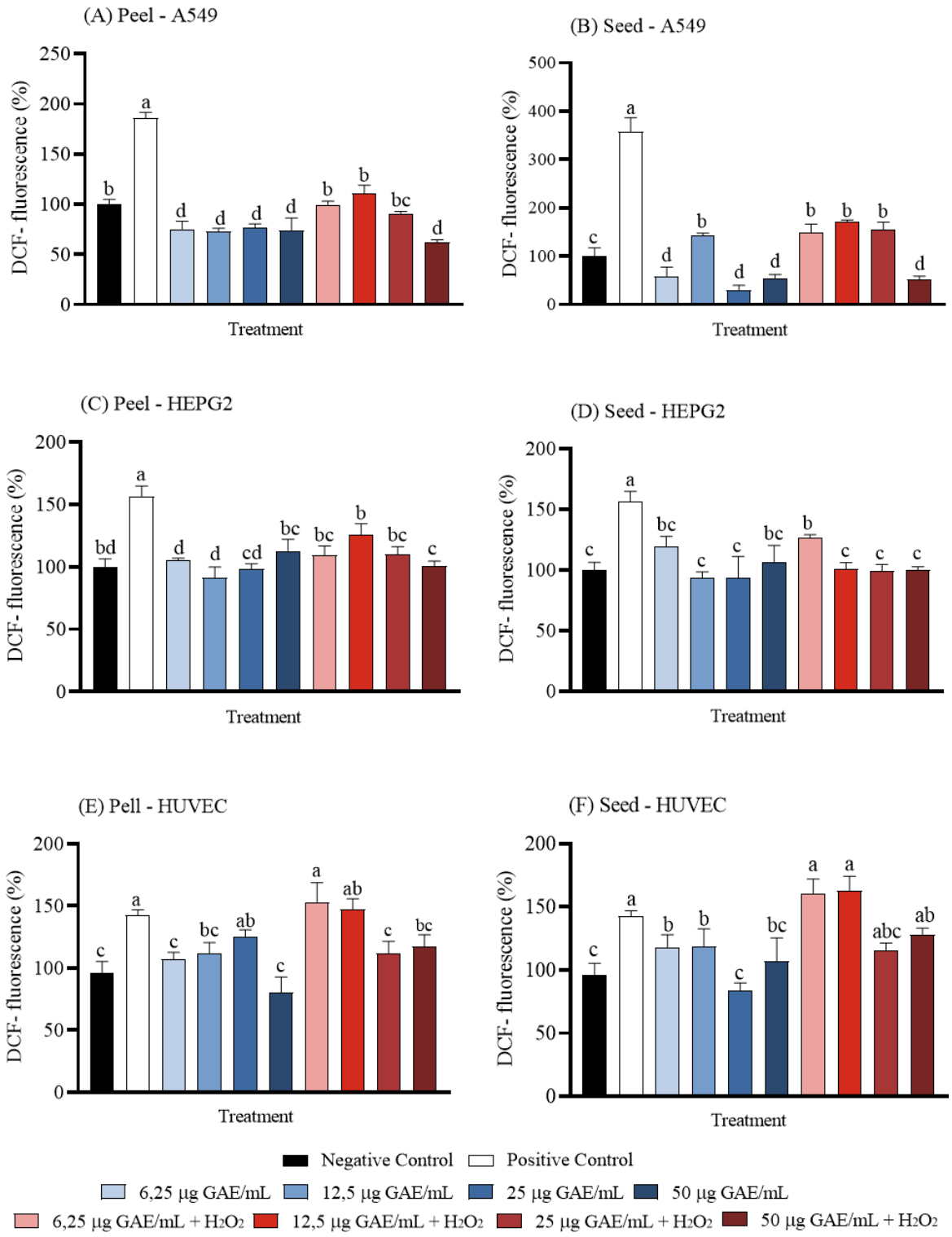


Figure 3. Intracellular ROS measurement in A549, HepG2, HUVEC cells by spectrofluorimetry. Treatment with extract mapati peel and seed extracts (6.25, 12.5, 25, and 50 µg GAE/mL for peel, and 2.5, 5, 10, and 20 µg GAE/mL for seed), and H2O2 (22.5 µM).Quantitative data are shown as mean ± standard deviation. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Different letters represent significant differences ($p \leq 0.05$).

3.5 Mapati extracts induce apoptosis in cancer cells

Flow cytometry analysis using Annexin V-PE revealed an increased frequency of Annexin V-positive cells in A549 cells treated with the seed extract at 50 μg GAE/mL, compared to the negative control (Figure 4A-G). This result suggests that the cytotoxic effect of the seed extract involves, at least in part, the induction of apoptosis through phosphatidylserine externalization.

It is well known that cancer cells rely on elevated ROS to maintain their high proliferation rate and metabolic activity, and antioxidant compounds, present in the seed extract, may disrupt this balance by reducing ROS levels, thereby interfering with intracellular signaling pathways (Do Carmo *et al*., 2019). Herein, a reduction in basal ROS levels was observed in A549 cells treated with the seed extract, which may modulate the activity of key regulators such as AKT and other kinases, commonly overexpressed in cancer cells. Inhibition of these survival pathways may lead to cell cycle arrest and trigger apoptosis (Sharma *et al*., 2018).

Several natural compounds, such as those investigated in this study, have shown promise in mitigating chemotherapy-related toxicity and resistance. Caffeic acid and procyanidins, identified in the seed extract via HPLC-DAD, are known to induce apoptosis through various mechanisms, such as the modulation of initiator and effector caspases, alteration of the BAX/BCL-2 ratio, and inhibition of the AKT/mTOR signaling pathway(Chang 2010; Miura *et al*., 2007; Zhang *et al*.,2016; Feriotto *et al*., 2021; Li *et al*., 2021). Caffeic acid has demonstrated low toxicity in normal cells and synergistic activity when combined with cisplatin in cancer cells (Raviadaran *et al*., 2021). This enhanced cytotoxicity of cisplatin in combination with caffeic acid is believed to result from the inhibition of detoxification enzymes (glutathione S-transferase and glutathione reductase), leading to increased intracellular accumulation of cisplatin and allowing for dose reduction, thereby minimizing its adverse effects (Sirota, Gibson, and Kohen 2017).

In contrast, treatment with the peel extract did not result in an increase in Annexin V-positive cells compared to the control (Figure 4G). In this extract, UPLC-QToF- MS analysis revealed the presence of quercetin glycosides and quercetin, compounds that are known for their cytoprotective and anti-apoptotic properties. These substances have been investigated for their potential protective effects against apoptosis and have been associated with benefits in conditions such as hypertension, hyperlipidemia, and other chronic metabolic diseases (Hu *et al*., 2015; Kim & Lee, 2021; Liu *et al*., 2022). Studies from Chow *et al*. (2005) and Zhang *et al*. (2023) provide evidence that quercetin can attenuate oxidative stress induced by H_2O_2, protecting against mitochondrial damage and preventing apoptosis. These findings are

consistent with our cytotoxicity and ROS generation results, in which the bark extract reduced ROS levels in A549 cells and showed a minor reduction in cell viability. Supported by the literature, these results indicate that polyphenol-rich plant extracts may exert either pro-apoptotic or cytoprotective effects depending on their composition and the cellular context.

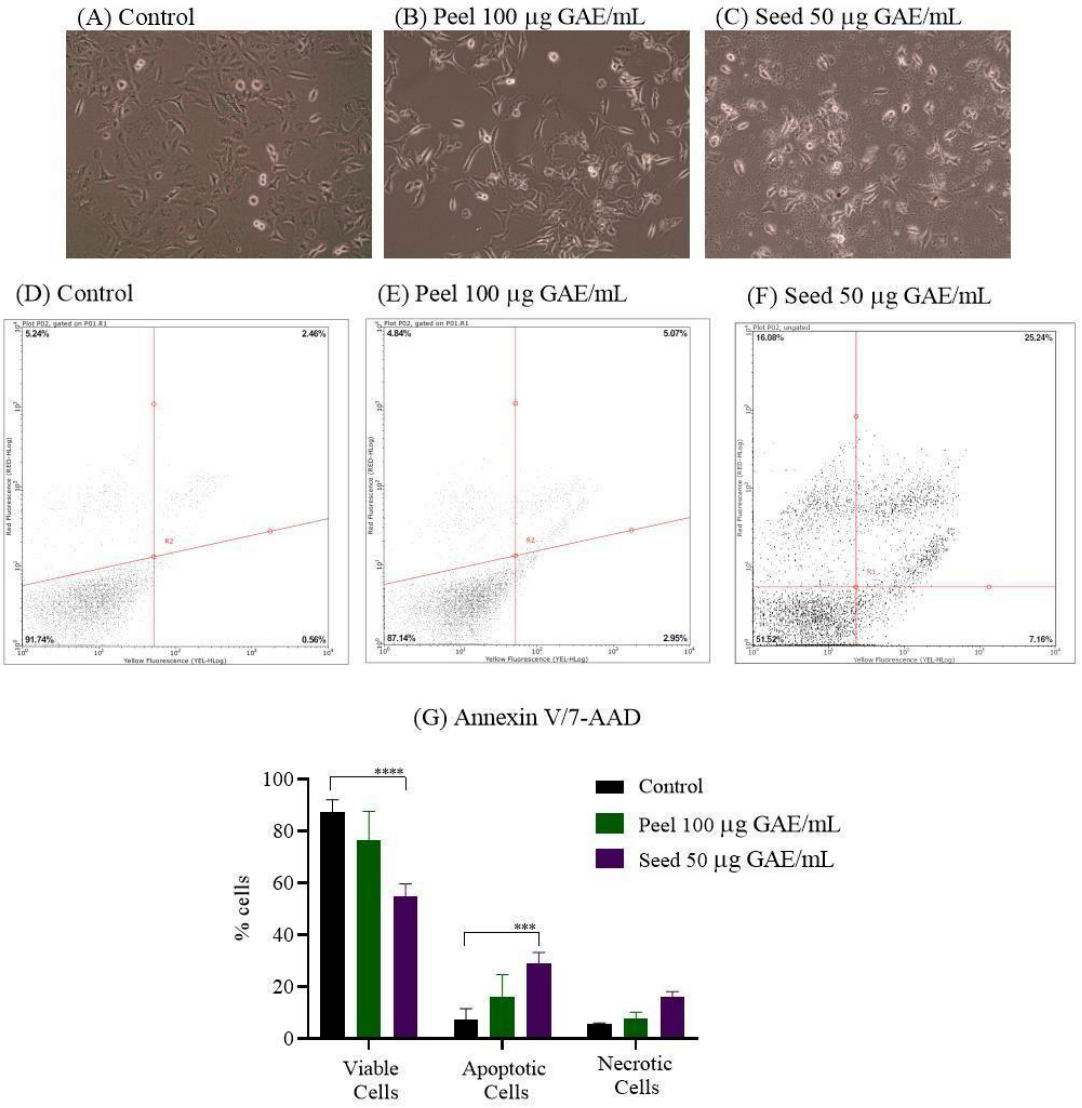


Figure 4. A-C: Representative microscopy electron images of A549 cells after 24 h of treatment: (A) control, (B) treated with 100 µg GAE/mL of peel extract, and (C) treated with 50 µg GAE/mL of seed extract. D-F: Representative dot plots of annexin V-PE assays were performed in A549 cultures after 24 h of treatment with 50 µg GAE/mL seed extract and 100 µg GAE/mL peed extract. Viable cells (lower left quadrants), early apoptosis (lower right quadrants), late apoptosis (upper right quadrants), and necrotic cells (upper left quadrants). **G:** Representative graph of viable, apoptotic and necrotic cells. Statistical analysis was performed using two- way ANOVA followed by Tukey's multiple comparisons test.

3.6 *Chromosomal abnormalities and DNA fragmentation*

Chromosomal abnormalities are a common consequence of exposure to genotoxic agents that cause chemical or physical damage to DNA. The exploration of new agents, such as mapati extracts, which have demonstrated non-toxic effects and the ability to reduce

chromosomal abnormalities, is crucial in screening strategies aimed at identifying substances with the potential to mitigate genotoxic damage induced by chemotherapeutic agents (Obe *et al*., 2002).

When combined with cisplatin, both peel and seed extracts appeared to attenuate cisplatin-induced mutagenic damage (Figure 5). The peel extract was effective at the lowest tested concentrations (12.5 μg GAE/mL and 25 μg GAE/mL), while the seed extract showed protective effects at the highest concentrations tested (10 μg GAE/mL and 20 μg GAE/mL). Furthermore, mapati extracts reduced the frequency of dicentric chromosomes (DCs) and rearrangements (REs), types of chromosomal abnormalities frequently observed in the positive control group (Figure 5C). These abnormalities are indicative of severe genomic damage and chromosomal instability, and their reduction suggests a potential antimutagenic effect of the extracts (Bonassi *et al*., 2008).

Cisplatin preferentially targets cells with high metabolic activity, such as cancer cells. Once inside the cell, it binds to DNA in its active form, altering its structure, inducing cellular stress, and impairing DNA function. These disruptions can lead to cell cycle arrest and apoptosis. Additionally, cisplatin stimulates the production of ROS by mitochondria, contributing to oxidative stress as part of its mechanism of action. The antioxidant activity demonstrated by the seed extract may help reduce cisplatin-induced oxidative stress, thereby mitigating DNA damage, as observed in the groups co-treated with cisplatin and the extracts (Dasari & Tchounwou, 2014; Nakamura & Takada, 2021).

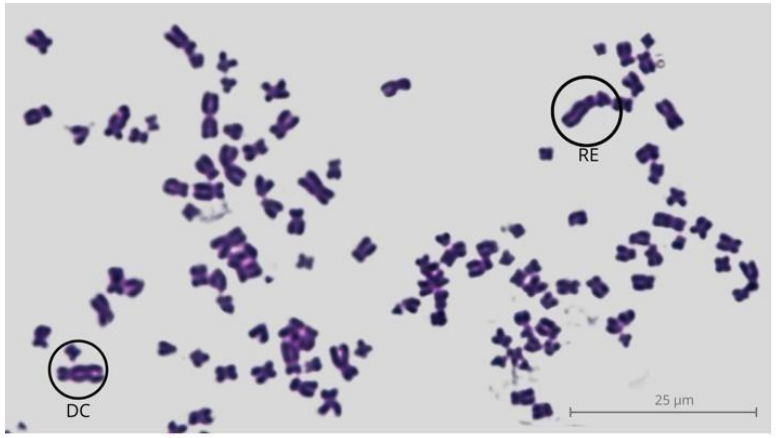
The DNA fragmentation assay evidenced the non-clastogenic activity of mapati extracts, as no increase in chromosomal breakage frequency was observed, highlighting their potential role as genoprotective agents. In addition, studies conducted by Xu, Hu, and Liu (2012) demonstrated that 3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, and 4-O- caffeoylquinic acid, found in the seed extract, offer protection against DNA damage. These compounds, known as chlorogenic acid isomers, have also been associated with antioxidants and genoprotective properties. According to Tomac, Šeruga, and Labuda(2020),theseisomers (3-CQA, 4-CQA, and 5-CQA) contribute to DNA repair through a rapid chemical mechanism involving electron transfer and hydroxyl radical scavenging. These findings lend further support to the hypothesis that the seed extract exerts both protective and reparative effects on DNA in cells co-treated with cisplatin.

To complement the absence of Annexin V labeling and the reduction in ROS levels observed in A549 cells treated with the peel extract, the DNA fragmentation assay, conducted after 24 and 48 h of treatment, did not reveal the characteristic DNA laddering pattern, providing

further evidence of the cytoprotective effects of the peel extract (Figure 5B). According to Çelik & Arinç (2010), quercetin was the most potent antioxidant in preventing DNA damage, significantly reducing the extent of DNA strand breaks in a dose-dependent manner. Similarly, Aherne & O'Brien (2000) reported that quercetin reduced DNA damage levels by 50%, while its glycoside form exhibited an 18% protective effect against genotoxic agents. These effects were attributed to their ability to scavenge ROS and chelate transition metal ions.

Together, these findings suggest that compounds such as quercetin and quercetin glycosides, present in the peel extract, may contribute to the maintenance of genomic stability by mitigating oxidative stress and enhancing DNA repair mechanisms. The cytoprotective effects observed may be related to the antioxidant properties of quercetin and its derivatives, which are abundant in the peel extract. In conclusion, the complex composition of plant extracts may allow compounds beyond phenolics to interact with multiple metabolic pathways, leading to diverse biological effects depending on their concentrations (Azqueta & Collins, 2016).

(A)



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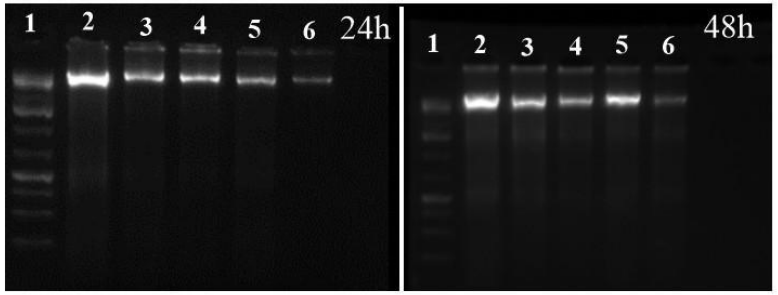

1 Ladder; **2** Control negative; **3** Peel 50 µg GAE/mL; **4** Peel 100 µg GAE/mL; **5** Seed 25 µg GAE/mL; **6** Seed 50 µg GAE/mL

(C)

Extracts	CIS (µM)	TC	Aberrant Type					TNCA	CA Rate (%)
Peel			R	DC	TC	QC	RE		
NC	-	4315	2	31	1	1	7	42[a]	1.0
PC	4	5239	8	40	3	5	22	78[b]	1.5
50	-	4235	5	26	1	3	2	37[a]	0.9
12.5	4	4690	0	23	3	7	4	37[c]	0.8
25	4	5038	3	25	0	7	5	42[c]	0.8
50	4	4164	1	22	3	13	5	44[bc]	1.0
Seed									
NC	-	4315	2	31	1	1	7	42[a]	1.0
PC	4	5239	8	40	3	5	22	78[b]	1.5
20	-	5395	2	9	0	11	23	45[a]	0.8
5	4	4822	1	14	1	9	31	56[c]	1.2
10	4	4753	3	26	1	10	4	45[c]	1.0
20	4	4178	0	26	2	4	2	34[bc]	0.8

Figure 5. (A): Photomicrographs (1000×) of metaphase plates of A549 cells, with representation of abnormalities, were RE: Rearrangements and DC: dicentric chromosomes; (B): DNA fragmentation using electrophoresis in 1.5% agarose gel stained with SYBR gold nucleic acid gel stain after 60 min of running at 80 volts in cell A549 treated with seed and peel extract. The marker used was 1Kb Plus DNA; **(C):** Results of the chromosome abnormalities test in A549 cells treated with mapati seed and peel extract (µg GAE/mL) *in vitro*. CIS = cisplatin; TC = total cells; R = rings; DC = dicentric chromosomes; TC = triradial chromosomes; QC = quadriradial chromosomes; RE = rearrangements; TNCA = total number of chromosome-abnormalities cells; CA = chromosomal abnormalities; NC = negative control; PC = positive control. Statistical analysis was performed using the chi-square test, and the letters indicate the significant difference when compared ($p < 0.05$).

3.7 Erythrocyte protection against AAPH oxidation

Erythrocyte oxidation induced by AAPH was performed to evaluate the antioxidant properties of mapati extracts in human red blood cells, considering three oxidative stress parameters: hemolysis, hemoglobin oxidation, and intracellular ROS generation. According to Cruz *et al*. (2024a), erythrocytes represent 40% (v/v) of blood and are cells that transport oxygen and carbon dioxide, with hemoglobin being the protein responsible for this transportation. Moreover, these cells are a promising model to use for screening the antioxidant capacity of food-derived extracts rich in polyphenols in membrane protection against oxidative stress (Mohammadi *et al*., 2024).

Taking this into account and considering that red blood cells are vulnerable to oxidative damage, our study demonstrated that both seed and peel extracts exhibited anti- hemolytic and antioxidant effects, protecting the erythrocyte membrane from lysis and decreasing oxidative stress in a dose-dependent manner (Figure 6A-C). These anti-hemolytic and antioxidant activities are a good indicator of the cytotoxicity of the extract toward normal, healthy human red blood cells, ensuring their safety (Egwu *et al*., 2021), aligned with our previous results in normal and cancer cells. Extracts with higher concentrations of phenolic compounds, such as mapati seed extracts, present various degrees of antioxidant activity that diminished levels ofROS and less oxidative environment (Do Carmo *et al*., 2021).

Despite this promising result, our extracts did not show a protective effect against hemoglobin (Hb) oxidation (Figure 6B). This could be explained by the dramatic autoxidation rate of the Hb, which produces methemoglobin and H2O2, contributing to oxidative stress. Although it is common to associate hemoglobin oxidation with an increase in ROS levels, there are scenarios in which the cell can maintain ROS at reduced levels due to the action of antioxidant compounds, or cellular compensatory mechanisms, which explains the results found (Mohanty *et al*., 2014).

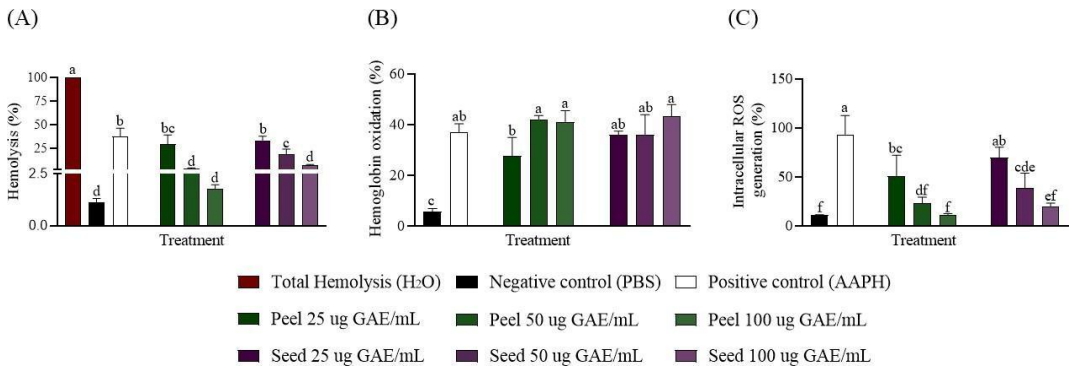

Figure 6. Effects of mapati peel and seed extracts on (A): hemolysis, (B): hemoglobin oxidation, and (C): intracellular reactive oxygen species (ROS) generation in human erythrocytes, induced by 2′-azobis(2-

amidinopropane) dihydrochloride (AAPH). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test. Different letters reveal statistical differences ($p < 0.05$).

3.8 Antimalarial Activity

Artemisinin- based combination therapies (ACTs Artemisinin-based combination therapies (ACTs) are recommended as the first-line treatment for uncomplicated P. falciparum malaria, which remains a public health challenge (Maiga *et al*., 2021; Rasmussen *et al*., 2022). However, the emergence of artemisinin-resistant parasites underscores the urgent need for new and alternative therapeutic strategies (Van der Pluijm *et al*., 2021). In this context, traditional medicinal knowledge represents a valuable resource for identifying novel antimalarial compounds (Ceravolo *et al*., 2021).

In assays using 3D7 and W2 strains, regardless of the intraerythrocytic stage at which treatment was initiated (rings, trophozoites, and schizonts), both peel and seed extracts effectively inhibited the growth of P. falciparum (Figure 7A-D). The seed extract exhibited the lowest IC_{50} value in the W2 strain (19.5 µg GAE/mL), suggesting enhanced efficacy against chloroquine-resistant parasites. Regarding the peel extract, it showed greater efficacy when applied at the schizont stage, which may be attributed to the higher expression of genes involved in merozoite maturation at this stage, reinforcing the stage- specific metabolic differences of the parasite (Do Carmo *et al*., 2020).

Our results suggest that phenolic-rich mapati extracts may be a potential source of antimalarial constituents. The combination of artesunate (1 and 2 nM) with peel extract (5 and 10 µg GAE/mL) enhanced the antiplasmodial activity of artesunate in the W2 strain (Figure 7E-H). This finding supports the idea that plant-derived compounds, in combination with synthetic antimalarials, can improve treatment efficacy. Besides, quercetin and its glycosylated derivatives, present in peel extract, can form stable complexes with free heme, thereby preventing β-hematin crystallization. This inhibition results in the accumulation of free heme in the digestive vacuole of the parasite, creating an intracellular environment that is irreversibly toxic (Ganesh *et al*., 2012). The formation quercetin-heme complex is attributed to multiple interaction forces, including coordination between the Fe^{2+} center of heme and quercetin hydroxyl or carbonyl groups, π–π stacking between aromatic rings, and hydrogen bonding (Abu-Lafi *et al*., 2011).

Moreover, modified quercetin has been shown to interact favorably with key parasitic enzymes such as Falcipain-2 and -3 and Plasmepsins I and II, by preventing their access to amino acids, which are essential for their survival, growth and replication, through interactions involve hydrogen bonding and hydrophobic contacts facilitated by the compound's carbonyl

and hydroxyl groups of quercetin (Garba *et al*., 2024). Other phenolic compounds, such as isoorientin, were also identified in the peel extract and have antimalarial potential, exhibiting a moderate effect against chloroquine-resistant (HB3) and multidrug-resistant (Dd2) strains (Atay *et al*., 2016; Ferreira *et al*., 2019).

On the other hand, the seed extract combined with artesunate exhibited the greatest efficacy against the 3D7 strain. This enhanced effect was evidenced by a reduction in the IC_{50} value from 8.7 µg GAE/mL to 1.3 µg GAE/mL (Table 4). Supporting our findings, Sannella *et al*. (2007) observed that epicatechin, which was also detected in the seed extract, moderately potentiates the antiplasmodial activity of artemisinin when administered at sublethal concentrations. Additionally, Tasdemir *et al*. (2006) demonstrated that epigallocatechin-3-gallate and epicatechin gallate have the potential to inhibit FabG, a critical enzyme in the parasite's fatty acid biosynthesis pathway. Inhibiting this enzyme can impair membrane synthesis and parasite growth. HPLC-DAD analysis of the seed extract also identified chlorogenic acid and its derivatives as well as caffeic acid and its glycosylated forms. These compounds have been associated with moderate antimalarial activity, likely through iron-chelating mechanisms (Fordjour *et al*., 2020). Given the parasite's dependence on iron metabolism, such chelation may contribute to the observed antiplasmodial activity.

The selective index (SI) is a critical parameter in the early-stage evaluation of antimalarial compounds. An SI ≥10 generally indicates a favorable therapeutic window, ensuring selective toxicity against the parasite while sparing host cells (Alves *et al*., 2021). The peel and seed extracts exhibited SI values ranging from 2.3 to 10.4, with the seed extract showing values above 10, suggesting significantly higher toxicity toward P. falciparum than toward normal HUVEC cells (Table 4). Microscopic analysis of P. falciparum-infected erythrocytes treated with an IC_{50} concentration of mapati peel and seed extracts for 48 h, compared to the culture medium as a negative control, revealed no observable differences in the development of surviving parasites, demonstrating that peel and seed extracts are effective regardless of the intraerythrocytic stage at which they are used (Figure 8).

Table 4. Antiplasmodial activity of mapati peel and seed extracts, alone and in combination with artesunate, against Plasmodium falciparum 3D7 and W2 strains after 48 h of treatment for each intraerythrocytic stage and corresponding selectivity index (SI).

Strains	Intraerythrocytic stage	Average of Replicas (µg GAE/mL)	SI A549/ *P. falciparum*	SI HCT8/ *P. falciparum*	SI HepG2/ *P. falciparum*	SI HUVEC/ *P. falciparum*
	Rings	31.7 ± 5	3.3	2.3	4.4	2.8

	Trophozoites	36.0 ± 8.4	2.9	2.0	3.9	2.4
Peel	Schizonts	37.8 ± 7.5	2.7	1.9	3.7	2.3
	Rings	37.1 ± 4	2.8	2.0	3.8	2.4
	Trophozoites	37.8 ± 1.3*	2.8	1.9	3.7	2.3
Peel	Schizonts	25.2 ± 2.5*	4.1	2.9	5.6	3.5
	Rings	34.5 ± 5.2	1.5	0.8	1.1	5.4
	Trophozoites	38.3 ± 0.7	1.3	0.8	1.0	4.9
Seed	Schizonts	22.2 ± 6	2.3	1.3	1.7	8.4
	Rings	18.0 ± 9.6	2.8	1.6	2.1	10.4
	Trophozoites	18.3 ± 6	2.8	1.6	2.0	10.2
Seed	Schizonts	22.2 ± 7.5	2.3	1.3	1.7	8.4

		Artesunate in combination with				
Strains	Artesunate	Peel 5 µg GAE/mL	Peel 10 µg GAE/mL	Seed 5 µg GAE/mL	Seed 10 µg	GAE/mL
3D7 - IC_{50}	8.7	7.4	8.7	6.9	1.3	
W2 - IC_{50}	2.1	1.3	1.3	2.2	2.1	

Note: SI = IC_{50} human cell / IC_{50} P. falciparum); 3D7 (chloroquine-sensitive) and W2

(chloroquine-resistant); *Indicate statistically significant differences between stages ($p \leq 0.05$). IC_{50} values were determined by nonlinear regression using the least squares method

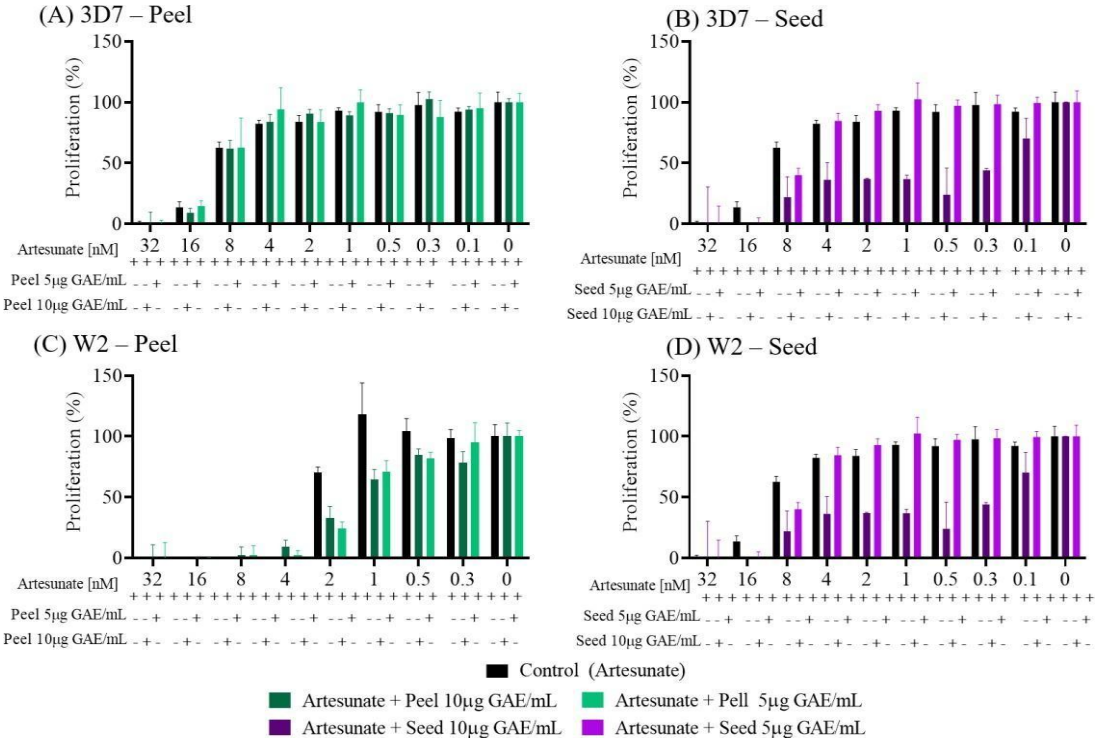

Figure 7. Proliferation of 3D7 and W2 P. falciparum strains under artesunate treatment in combination with peel (A-C) and seed (B-D) extracts at 5 and 10 µg GAE/mL. Values were determined by nonlinear regression.

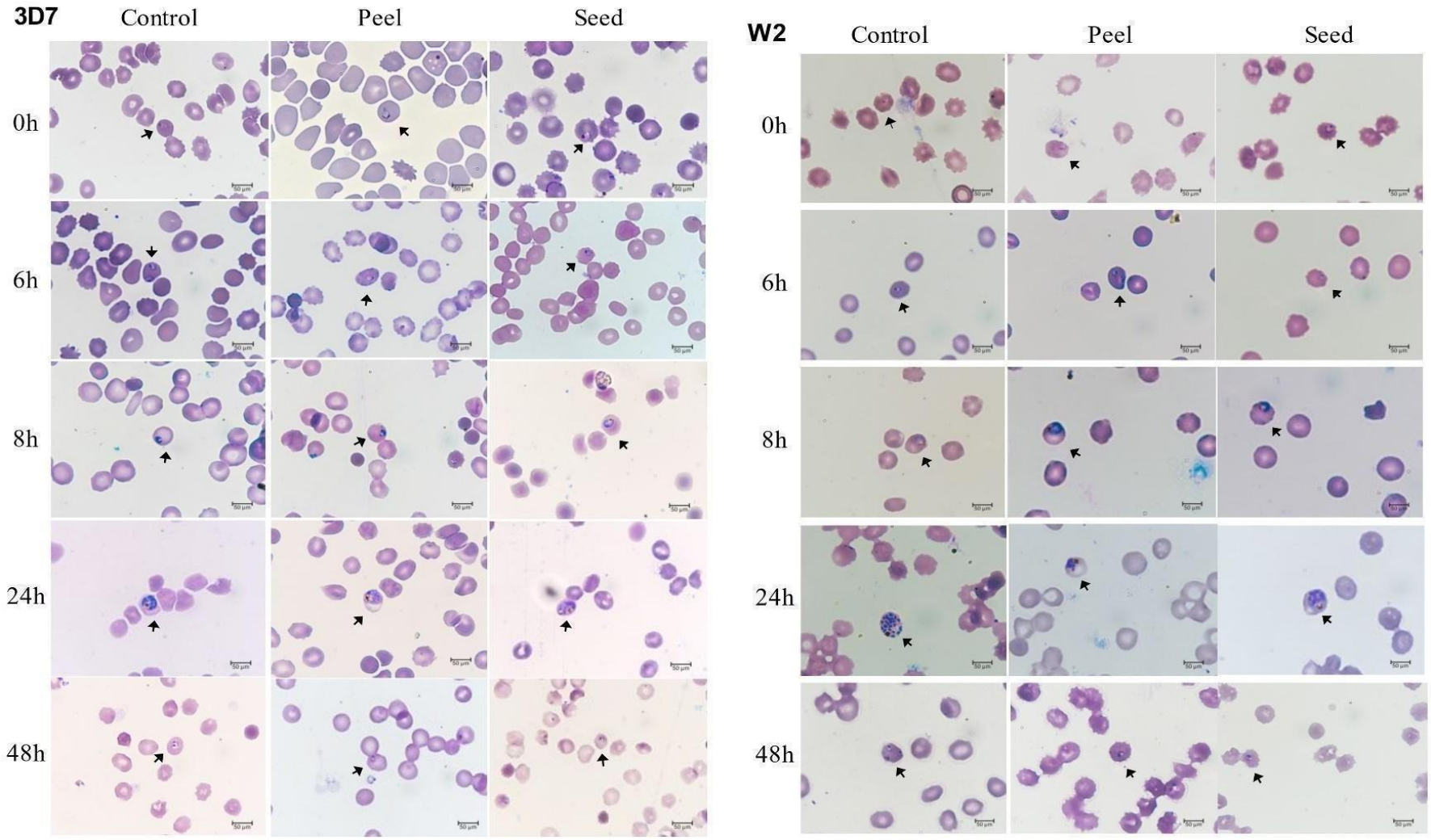

Figure 8. Effects of mapati peel and seed extracts on synchronized cultures of *P. falciparum* 3D7 and W2 strains. Parasite development was assessed at different intervals (0, 6 h, 8 h, 24 h, and 48 h) using microscopy and panoptic fast-stained smears in the presence of RPMI culture medium supplemented with 10% Albumax II (as a control)

4 Conclusion

Our studies explored the bioprospecting, chemical profile and bioactivity of peel and seed extracts from the Amazonian fruit mapati (Pourouma cecropiifolia). UPLC analysis revealed a distinct polyphenolic composition, with the peel characterized mainly by quercetin and its derivatives, and the seed enriched in phenolic acids and condensed tannins. HPLC quantification showed that the seed is a major source of polyphenols (397 mg/100 g extract), a feature associated with its enhanced cell antioxidant activity, reduction in ROS levels in A549 cell line leading to apoptosis. In addition, the seed extract also exhibited selective cytotoxicity toward cancer cells, with selectivity index (SI) values ranging from 3.7 to 6.4. Also, both extracts showed genoprotective and antimutagenic effects at lower concentrations (12.5 µg GAE/mL for peel and 20 µg GAE/mL for seed). Moreover, antiplasmodial assays demonstrated efficacy against all intraerythrocytic stages of Plasmodium falciparum, with a potential effect observed when artesunate was combined with peel extract for W2 strain (IC_{50} reduction from 2.3 to 1.3 µg GAE/mL), and seed extract against 3D7 strain (IC_{50} reduction from 8.7 to 1.3 µg GAE/mL). In conclusion, these results unveil the potential of Amazonian fruit by-products in pharmaceutical and nutraceutical applications, encouraging further in vivo studies to explore mechanisms of action.

Authors contributions

Nathália Alves Bento, Marcell Crispim, Amanda dos Santos Lima Formal analysis, Methodology, Writing – original draft, Writing – review and editing. Thaise Caputo Silva: Formal analysis, Writing – original draft, Writing. Giselly Karoline Paiva da Silva, Laura da Silva Cruz, Guilherme Álvaro Ferreira-Silva, Marisa Ionta, Josias Martins dos Anjos Cruz and Daniel de Queiroz Rocha: Formal analysis. Jaqueline de Araújo Bezerra: Formal analysis, Writing – original draft. Luciana Azevedo: review and editing, Project administration.

Declaration of Competing Interest

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

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4 CONSIDERAÇÕES FINAIS

A Floresta Amazônica abriga uma das maiores biodiversidades do planeta, com milhares de espécies vegetais de potencial econômico e biotecnológico. Entre elas, a *Pourouma cecropiifolia* Martius, conhecida como mapati, destaca-se como uma fruta endêmica ainda subutilizada, cuja exploração agroindustrial e científica permanece incipiente. Este estudo contribuiu para preencher essa lacuna ao caracterizar a composição química e o potencial biológico dos extratos etanólicos da casca e da semente por meio de UPLC-QToF/MS e HPLC-DAD, revelando um perfil expressivo de polifenóis, como ácido clorogênico, quercetina e taninos condensados. Esses compostos, descritos na literatura por suas múltiplas bioatividades, foram associados à elevada atividade antioxidante observada, especialmente no extrato da semente, comprovada pelos ensaios DPPH, FRAP e HRSA, além da redução significativa de espécies reativas de oxigênio em células A549 e em eritrócitos humanos.

O estresse oxidativo, um fator crítico na gênese de doenças crônicas, degenerativas e infecciosas, pode ser mitigado pela ação de polifenóis, que atuam como doadores de hidrogênio, quelantes de metais e moduladores de enzimas antioxidantes. Nesse contexto, os extratos de mapati demonstraram efeitos citotóxicos seletivos contra células tumorais, além de propriedades antigenotóxicas e antimutagênicas, reforçando o papel dos compostos fenólicos no controle do dano oxidativo e da proliferação celular desordenada.

Adicionalmente, foi evidenciada a atividade antimalárica frente a todas as fases intraeritrocíticas de *Plasmodium falciparum*, sugerindo potenciais mecanismos relacionados à desestabilização da homeostase redox do parasito e à possível ação quelante de ferro, estratégias descritas como promissoras no combate à resistência medicamentosa. Observou- se ainda um efeito sinérgico na combinação com artesunato, alinhando-se à tendência de desenvolvimento de terapias combinadas baseadas em compostos naturais.

Assim, os resultados obtidos indicam que os subprodutos do mapati, tradicionalmente descartados, constituem fontes sustentáveis e promissoras de biomoléculas com aplicações relevantes para as áreas de alimentos, saúde e biotecnologia, reforçando a importância da valorização de recursos amazônicos e evidenciando a necessidade de aprofundar estudos sobre sua biodisponibilidade, mecanismos de ação e potencial aplicação em terapias combinadas e formulações funcionais.

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