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JOSÉ JOSENILDO BATISTA

CARACTERIZAÇÃO ESTRUTURAL E ATIVIDADES BIOLÓGICAS DO POLISSACARÍDEO DO EXSUDATO DE *Parkia pendula*

Recife
2025

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Tese apresentada ao Programa de Pós-graduação em Bioquímica e Fisiologia da Universidade Federal de Pernambuco, como requisito parcial para obtenção do título de Doutor em Bioquímica e Fisiologia.

Orientadora: Profa. Dra. Luana Cassandra Breitenbach Barroso Coelho

Coorientador: Prof. Dr. André de Lima Aires

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RESUMO

Parkia pendula (Fabaceae), popularmente visgueiro, é uma espécie comum no Brasil, conhecida pela produção de exsudato. Contudo, seus polissacarídeos ainda são pouco estudados. O presente estudo tem como objetivo caracterizar o polissacarídeo do exsudato de *Parkia pendula* (PePp) e investigar seus efeitos biológicos. O PePp foi obtido por extração aquosa e precipitação com álcool etílico (99,9%, 1:4), e submetido a caracterização química e estrutural. Em ensaios *in vitro*, avaliou-se a atividade prebiótica em cepas de *Lactobacillus* e *Bifidobacterium*, além da fermentação fecal pela microbiota humana. Em modelos *in vivo*, foram analisadas a toxicidade aguda (CEUA nº 127/2022), fermentação em camundongos BALB/C (CEUA nº 0052/2023) e o efeito gastroprotetor em úlcera gástrica (CEUA nº 128/2022). O rendimento do PePp foi de 48,2%, com 79,20% de carboidratos totais e 11% de ácido urônico, baixos teores de compostos fenólicos (2,72 mg/g GAE) e proteínas (1,20%). O peso molecular foi estimado em 7.08 x 10^4 g/mol, a composição monossacarídica constituída de arabinose (78%), galactose (10%) e ácido urônico (12%) e no FT-IR confirmou bandas características de polissacarídeos e ácido urônico (1309-1216 e 1410 cm^{-1}, respectivamente). *In vitro*, PePp apresentou atividade prebiótica, promovendo o crescimento de cepas probióticas, como *L. rhamnosus, L. brevis, L. plantarum, L. casei, L. paracasei, B. longum* e *B. adolescentis*. Durante a fermentação fecal, o consumo do PePp (de 79,20 para 33,56%) resultou na diminuição do pH (de 6,29 para 4,35), aumento da produção de ácido graxos de cadeia curta- AGCC (ácido acético, propiônico e isobutírico) e na proliferação de bactérias benéficas, como *Bacteroides*, *Bifidobacterium*, *Prevotella* e *Enterococcus*. Em teste de toxicidade aguda, a dose 2.000 mg/kg não provocou alterações no peso corporal, consumo de água e ração, nem nos parâmetros bioquímicos, hematológicos ou histopatológicos dos animais. *In vivo*, PePp modulou a microbiota intestinal dos camundongos, resultando na redução do pH fecal de 7,33 para 7,04, 7,10 e 7,03 nas doses de 50, 100 e 200 mg/kg, respectivamente. Esse processo também promoveu o aumento de AGCC e bactérias probióticas. Além disso, o pré-tratamento com PePp demonstrou efeito gastroprotetor em úlceras gástricas, inibindo a formação das lesões em 52%, 71% e 83% nas doses de 10, 25 e 50 mg/kg, respectivamente. Esse efeito foi acompanhado pela redução da peroxidação lipídica e dos níveis de citocinas pró-inflamatórias (IL-6 e TNF-α), além do aumento da superóxido dismutase, catalase e citocina anti-inflamatória IL-10. Conclui-se que PePp é um polissacarídeo do tipo galactoarabinano, seguro do ponto de vista toxicológico, com efeitos prebióticos, com capacidade de modular a microbiota intestinal e potencial gastroprotetor em modelo de úlcera gástrica.

Palavras-chave: *Parkia pendula*; galactoarabinano; prebiótico; microbiota intestinal; gastroprotetor.

ABSTRACT

Parkia pendula (Fabaceae), popularly known as visgueiro, is a common species in Brazil, known for its exudate production. However, its polysaccharides are still little studied. The present study aims to characterize the exudate polysaccharide of *Parkia pendula* (PePp) and investigate its biological effects. PePp was obtained by aqueous extraction and precipitation with ethyl alcohol (99.9%, 1:4), followed by chemical and structural characterization. *In vitro* assays evaluated the prebiotic activity on strains of *Lactobacillus* and *Bifidobacterium*, as well as fecal fermentation by human microbiota. *In vivo* models analyzed acute toxicity (CEUA nº 127/2022), fermentation in BALB/C mice (CEUA nº 0052/2023), and gastroprotective effects in gastric ulcer (CEUA nº 128/2022). The yield of PePp was 48.2%, with 79.20% total carbohydrates and 11% uronic acid, along with low levels of phenolic compounds (2.72 mg/g GAE) and proteins (1.20%). The molecular weight was estimated at 7.08 x 10^4 g/mol, with monosaccharide composition consisting of arabinose (78%), galactose (10%), and uronic acid (12%). FT-IR confirmed characteristic bands of polysaccharides and uronic acid (1309-1216 and 1410 cm^{-1}, respectively). *In vitro*, PePp demonstrated prebiotic activity, promoting the growth of probiotic strains such as *L. rhamnosus, L. brevis, L. plantarum, L. casei, L. paracasei, B. longum,* and *B. adolescentis*. During fecal fermentation, PePp consumption (from 79.20% to 33.56%) resulted in a decrease in pH (from 6.29 to 4.35), increased production of short-chain fatty acids (SCFA) (acetic, propionic, and isobutyric acids), and the proliferation of beneficial bacteria such as *Bacteroides*, *Bifidobacterium*, *Prevotella*, and *Enterococcus*. In an acute toxicity test, the dose of 2000 mg/kg caused no changes in body weight, water, or food intake, or in the biochemical, hematological, or histopathological parameters of the animals. *In vivo*, PePp modulated the gut microbiota of mice, resulting in a reduction of fecal pH from 7.33 to 7.04, 7.10, and 7.03 at doses of 50, 100, and 200 mg/kg, respectively. This process also promoted an increase in SCFA and probiotic bacteria. Furthermore, pretreatment with PePp demonstrated gastroprotective effects in gastric ulcers, inhibiting lesion formation by 52%, 71%, and 83% at doses of 10, 25, and 50 mg/kg, respectively. This effect was accompanied by a reduction in lipid peroxidation and pro-inflammatory cytokines (IL-6 and TNF-α), along with an increase in superoxide dismutase, catalase, and anti-inflammatory cytokine IL-10. It is concluded that PePp is an galactoarabinan-type polysaccharide, safe from a toxicological standpoint, with prebiotic effects, the ability to modulate the gut microbiota, and gastroprotective potential in a gastric ulcer model.

Keywords: *Parkia pendula*; galactoarabinan; prebiotic; gut microbiota; Gastroprotective..

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SUMÁRIO

1 INTRODUÇÃO

A microbiota intestinal (MI) desempenha papel crucial no funcionamento do sistema digestório, na saúde humana e de outros animais. Esse ecossistema complexo e dinâmico é constituído por bactérias (~90%), fungos, arqueias e vírus que podem ser benéficos ou prejudiciais, o que depende das interações complexas entre sua distribuição, diversidade, composição de espécies e metabólitos (Alhhazmi et al., 2023; Berg et al., 2020; Hou et al., 2022). Alterações na relação simbiótica entre a MI e o seu hospedeiro podem causar vários distúrbios, como diabetes, doença inflamatória intestinal e obesidade (Durack & Lynch, 2019; Carvalho et al., 2025). Assim, a pesquisa e o desenvolvimento de novos prebióticos a partir de polissacarídeos são recomendados (Zhang et al., 2023).

A úlcera gástrica (UG) é uma doença gastrointestinal que acomete cerca de 10% da população global, representando impactos na saúde pública e qualidade de vida dos acometidos (Dinat et al., 2023). A patogênese da UG pode estar associada ao aumento de ácido, pepsina e a presença de *Helicobacter pylori*, combinado com a redução de fatores defensivos, incluindo prostaglandinas, mucina, óxido nítrico e bicarbonato no estômago (Kuna et al., 2019). Além disso, o tabagismo, estresse, uso prolongado de anti-inflamatórios não esteroides e o consumo de álcool podem levar a danos na mucosa gástrica (Périco et al., 2020). Apesar das opções terapêuticas existentes, que inclui antibióticos, antiácidos e inibidores da bomba de prótons, o tratamento clínico convencional apresenta desafios notáveis devido a efeitos colaterais, entre eles, destaca-se lesão renal aguda, alterações na estrutura e função da mucosa gástrica e infecção intestinal (Carlotto et al., 2019; He et al., 2025). Nesse contexto, a pesquisa e desenvolvimento de novos compostos de origem vegetal, incluindo polissacarídeos com propriedade gastroprotetora, surge como uma alternativa promissora para o controle e/ou tratamento da UG.

Parkia é um gênero pantropical com aproximadamente 35 espécies reconhecidas e utilizadas na medicina popular, sendo suas diferentes partes processadas como pastas, decocção e/ou suco para o tratamento de diarreia, diabetes, úlceras cutâneas, feridas e hipertensão (Saleh et al., 2021). Estudos fitoquímicos revelaram a presença de lectinas, fenóis, flavonoides e polissacarídeos, nas sementes, vagens e cascas, os quais apresentaram atividade antioxidade (Gan et al., 2010a), imunorregulatória (Zou et al., 2014), anti-inflamatória (Ibraheem et al., 2022) e moluscicida (Batista et al., 2022). Apesar de conhecida, poucos estudos exploram a estrutura dos metabolitos primários, especialmente os polissacarídeos, e o potencial biológico de espécies do gênero *Parkia*. *Parkia pendula* é uma planta conhecida popularmente como “visgueiro”, “faveira” ou “favera-de-chorão” (Souza Filho et al., 2005).

No Brasil está espécie apresenta ampla distribuição, sendo comumente utilizada em programas de recuperação de áreas degradadas devido ao seu rápido crescimento (Cordeiro et al., 2021; Correa et al., 2021). Além disso, suas vagens produzem um exsudato rico em polissacarídeos, que têm demonstrado potencial para imobilização de lectinas, como a Concanavalina A, em matrizes magnéticas (Rêgo et al., 2014).

Os exsudatos de goma são compostos por polissacarídeos, que consistem em uma variedade de monossacarídeos interligados por ligações glicosídicas (Ren et al., 2019; Zhang et al., 2023). Estes são sintetizados e excretados pelas plantas como resultado de um mecanismo de defesa e suas principais características estão relacionadas à elevada viscosidade e solubilidade em água (Eghbaljoo et al., 2022). A utilização desses polissacarídeos é atribuída as suas propriedades químicas distintas, conferindo-lhes aplicações biotecnológicas e atividades biológicos, tais como antioxidante (Hamdani et al., 2018), antidiabética (Ganesan & Xu, 2019), anti-inflamatória (Zaheri-Abdevand & Badr, 2023), gastroprotetora (Lima et al., 2021), prebiótica (Rawi et al., 2021), baixa ou ausência de toxicidade (Mohanta et al., 2023). Dessa forma, é fundamental não apenas compreender as aplicações biológicas dos polissacarídeos presentes nos exsudatos, mas também adquirir conhecimento sobre suas propriedades físico-químicas e estruturais. Até o momento, não foram identificados estudos que relatem essas propriedades específicas no exsudato de *P. pendula*.

Portanto, torna-se relevante caracterizar estruturalmente o polissacarídeo do exsudato de *P. pendula* (PePp) e avaliar sua toxicidade aguda, efeito prebiótico e modulação da microbiota intestinal em modelos *in vitro* e *in vivo*, bem como atividade gastroprotetora em úlcera gástrica induzida por etanol. Nessa perspectiva, os resultados deste estudo contribuirão para o avanço do conhecimento científico sobre os potenciais benefícios do PePp, fornecendo bases para a aplicação desse polissacarídeo nas indústrias alimentícia e farmacêutica, com vista ao desenvolvimento de novos produtos voltados para a promoção da saúde humana.

2 OBJETIVOS

2.1 Objetivo geral

Extrair, caracterizar química-estruturalmente o polissacarídeo do exsudato de *Parkia pendula* e avaliar seu efeito toxicológico agudo e atividades biológicas *in vitro* e *in vivo*.

2.2 Objetivos específicos

• Extrair e caracterizar o polissacarídeo do exsudato de *Parkia pendula* (PePp);

• Avaliar a toxicidade aguda do PePp sobre os parâmetros comportamentais, hematológico, bioquímico e histopatológico;

• Avaliar a atividade prebiótica do PePp *in vitro* em cepas probióticas de *Lactobacillus* e *Bifidobacterium*;

• Avaliar o efeito do PePp na fermentação fecal humana e de camundongos, avaliando a produção de ácidos graxos de cadeia curta (AGCC), pH e alterações na composição da microbiota;

• Avaliar o efeito gastroprotetor do PePp em modelo de lesões gástricas induzidas por etanol em camundongos, analisando marcadores inflamatório e de estresse oxidativo.

3 FUNDAMENTAÇÃO TEÓRICA

3.1 Microbiota intestinal

A microbiota intestinal (MI) humana representa um ecossistema complexo e dinâmico, sendo predominantemente constituído por bactérias (Vemuri et al., 2020). Além das bactérias, a MI abriga outros microrganismos, como fungos, arqueias e vírus, cuja coexistência desempenha papel crucial na manutenção da integridade e bem-estar dos seus hospedeiros (Alhhazmi et al., 2023; Zhao et al., 2023). Esse cenário despertou interesse na pesquisa da relação entre MI e seus hospedeiros, com o objetivo de melhor entender sua influência na saúde e doença (Hou et al., 2022; Mohajeri et al., 2018).

Por muitas décadas, a ideia predominante era de que os fetos eram considerados estéreis, e a colonização bacteriana inicial do intestino do recém-nascido ocorria exclusivamente durante ou após o nascimento (Coscia et al., 2021). No entanto, estudos têm desafiado essa concepção ao documentar a presença de uma microbiota intrauterina, sem ocorrência de complicações ou episódios infecciosos (Aagaard et al., 2014; Gallo et al., 2023). Essa colonização abrange a placenta, o sangue do cordão umbilical e o fluido amniótico, evidenciando a existência de microrganismos, incluindo espécies dos filos *Bacillota*, *Pseudomonadota* e *Fusobacteriota* (Stout et al., 2013; Wang et al., 2020; Wu et al., 2021).

O processo de parto configura-se como um elemento importante para o desenvolvimento e colonização da MI da criança, sendo que o tipo de parto exerce uma influência na composição (Coelho et al., 2021; Jeong, 2022). Bebês que nascem de parto vaginal apresentam uma maior abundancia de bactérias do gênero *Lactobacillus*, *Atopobium* e *Sneathia,* ao passo que aqueles nascidos por cesariana apresentam uma prevalência de *Staphylococcus* e *Propionibactéria*, atualmente reclassificados como *Cutibacterium* (Hurley et al., 2019; Wang et al., 2018). Além disso, a exposição ao ambiente pós-parto também desempenha papel na colonização precoce (Azevedo et al., 2023; Y. Shao et al., 2019). Essas descobertas ressaltam a importância do tipo de parto e do ambiente circundante no estabelecimento do MI infantil, enfatizando a complexidade dos fatores que moldam essa comunidade microbiana.

Durante o desenvolvimento, que se estende ao longo de semanas e meses, a microbiota tende a se assemelhar á da mãe, e ao longo do tempo, uma maior diversidade bacteriana vai se colonizando (Kim et al., 2019). A amamentação é um dos fatores destacados nesse processo, pois o leite materno desempenha um papel crucial ao fornecer nutrientes e moléculas bioativas, incluindo células do sistema imunológico, oligossacarídeos, citocinas,

entre outros (Bardanzellu et al., 2020; Boix-Amorós et al., 2019). No final do primeiro ano de vida, os bebés apresentam um perfil microbiano individualmente distinto, e entre 2 e 5 anos de idade, a microbiota começa a se assemelhar à de um adulto em termos de composição e diversidade (Rodríguez et al., 2015). Esse período é considerado crucial para intervenções dietéticas, que desempenham papel no neurodesenvolvimento da criança (Schwarzenberg et al., 2018).

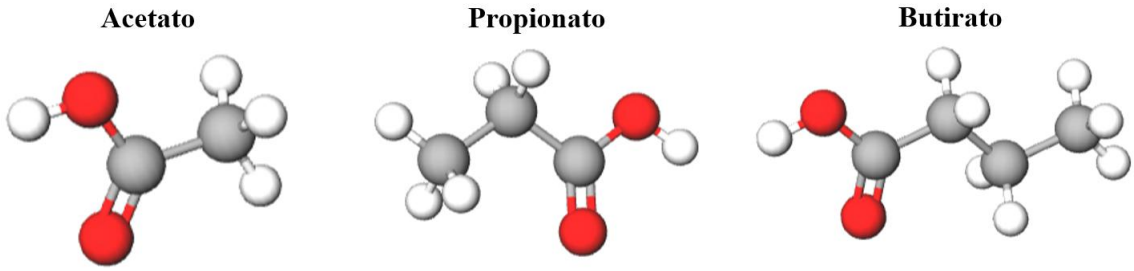
Em adultos saudáveis, a MI é predominantemente composta por Firmicutes (*Lactobacillus*, *Lachnospiraceae* e *Ruminococcaceae*), Bacteroidetes (*Bacteroidaceae*, *Prevotellaceae* e *Rikenellaceae*) e Actinobacteria (*Bifidobacteriaceae* e *Coriobacteriaceae*), formando um sistema estável ao longo da vida (Senghor et al., 2018). No entanto, alterações podem ocorrer em longo prazo devido a fatores como dieta, estilo de vida, infecções gastrointestinais, tratamentos com antibióticos ou cirurgia e até mesmo a localização geográfica, resultando em variações na diversidade bacteriana, que podem diminuir ou aumentar (Liu et al., 2023).

No que diz respeito a MI de idosos, observa-se uma característica de redução na diversidade bacteriana e na presença de microrganismos benéficos (Kim & Benayoun, 2020). Há também mudanças nas espécies dominantes, aumento de bactérias anaeróbias facultativas e diminuição na disponibilidade de ácidos graxos de cadeia curta - AGCC (Salazar et al., 2017). Essas alterações destacam a influência de fatores ao longo da vida na composição da MI e ressaltam a importância de compreender e abordar as particularidades da população em termos de saúde intestinal.

Os AGCC são produtos voláteis gerados pela MI, especialmente no intestino grosso, como resultado da fermentação de componentes alimentares não absorvidos ou digeridos no intestino delgado (Rasouli-Saravani et al., 2023). Caracterizam-se por terem menos de seis carbonos, apresentando cadeias lineares e ramificadas (Portincasa et al., 2022). Os principais AGCCs produzidos no cólon através da fermentação bacteriana de fibras alimentares e amido resistente são acetato, propionato e butirato (Figura 1). Esses compostos desempenham a função de fornecer energia, promovem proteção, mantêm a integridade da barreira intestinal, contribuem para a produção de muco e influenciam a motilidade gastrointestinal (Dalile et al., 2019; Wozniak et al., 2022). Além de suas funções metabólicas, esses AGCCs estão associados a diversas bioatividade, a destacar os efeitos anti-inflamatório e imunorregulador (Cait et al., 2018; Wen et al., 2021), bem como efeitos preventivos e terapêuticos em várias doenças, incluindo obesidade (Prentice et al., 2019), doenças cardiovasculares (Cao et al., 2019) e diabetes (Meng et al., 2019). A crescente compreensão do papel desses compostos

destaca a importância dos AGCCs não apenas na saúde intestinal, mas na sua influência sistêmica em diversos aspectos da saúde humana.

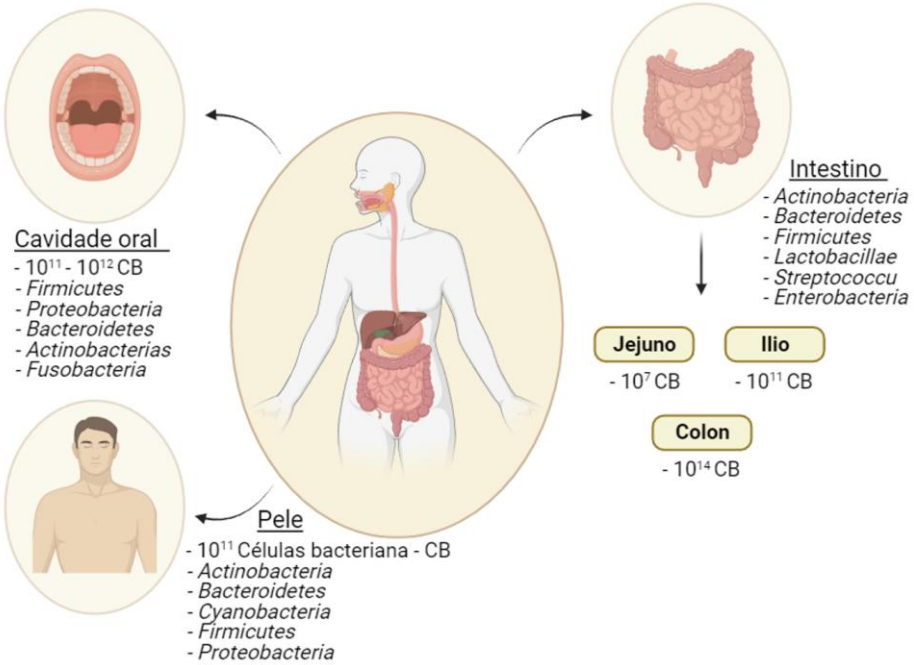
Figura 1. Estrutura 3D dos ácidos graxos de cadeia curta na fermentação bacteriana de fibras alimentares.



Fonte: Elaborado pelo autor (2025). Em branco hidrogênio, cinza carbono e vermelho oxigênio.

É relevante destacar que o corpo humano apresenta diversos locais de colonização bacteriana, como a cavidade oral, trato respiratório, pele, intestino, entre outros, diferenciando-se entre a quantidade e espécies bacterianas (Figura 2) (Vos et al., 2022; Hou et al., 2022). No entanto, o trato intestinal/intestino se destaca pelo predomínio, abrigando um número estimado de mais de 10^{14} células bacterianas (Kho & Lal, 2018; Thursby & Juge, 2017). Além disso, estudos metagenómico revelaram a presença de aproximadamente 3,3 milhões de genes no intestino, considerando, portanto, o segundo genoma humano (Zhu et al., 2010).

Figura 2. Abundância de bactérias em diferentes partes do corpo.



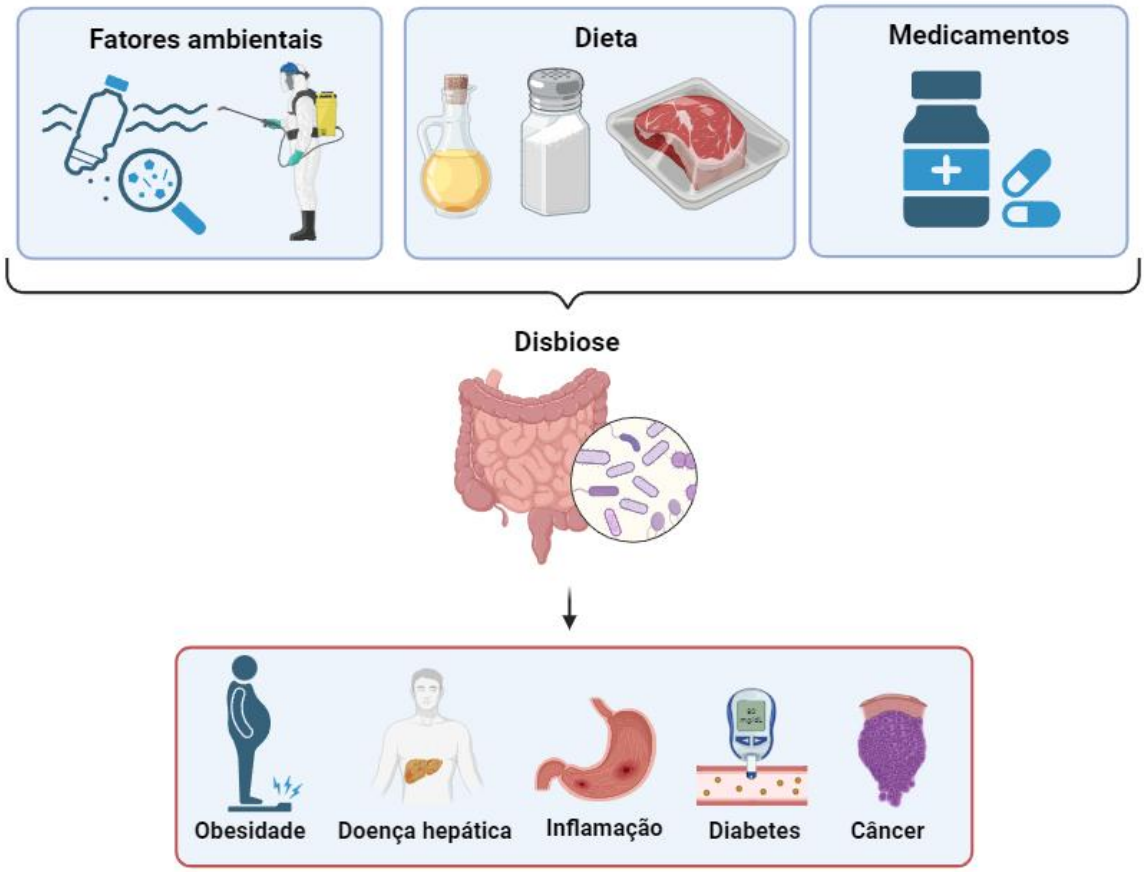
Fonte: elaborado pelo autor (2025).

Nesse contexto, a MI desempenha diversas funções essenciais, destacando-se seu papel vital na digestão, metabolismo (Rothschild et al., 2018), proteção contra patógenos por meio da atuação no sistema imunológico, através da colonização de superfícies mucosas e produção de substâncias antimicrobianas (Mills et al., 2019), controle da proliferação e diferenciação de células epiteliais (Wiley et al., 2017) bem como sua influência na comunicação cérebro-intestino (Zheng et al., 2019).

3.2 Disbiose da microbiota intestinal e fatores que influenciam

A homeostase da MI, tendo em vista que o desequilíbrio pode provocar danos à saúde conhecido como disbiose (Zhao et al., 2023; Ou et al., 2022). A disbiose pode ser definida como um desequilíbrio na MI, resultando na diminuição de simbiontes, aumento de patobiontes e perda da diversidade da microbiota (Chait et al., 2021; Singh et al., 2019). Pesquisas indicam que a disbiose está associada a diversos problemas de saúde, sendo sua incapacidade de manter o equilíbrio microecológico intestinal atribuído principalmente a fatores ambientais, hábitos alimentares e uso de medicamentos. A Figura 3 destaca os principais fatores que afetam a disbiose da MI e suas doenças associadas.

Figura 3. Principais fatores e doenças associadas a disbiose da microbiota intestinal.



Fonte: Elaborado pelo autor (2025).

3.2.1 Fatores ambientais

No corpo humano, o trato gastrointestinal e o sistema respiratório são as principais vias de entrada e processamento de substâncias tóxicas (Ahn et al., 2023). Estudos têm demonstrado que a exposição a metais pesados, partículas atmosféricas, microplásticos, pesticidas, produtos químicos perturbadores ou xenobióticos podem resultar em disbiose na MI (Osman et al., 2023; Tu et al., 2020).

Estudos utilizando animais experimentais, como camundongos e/ou ratos, como modelos experimentais, investigaram a composição microbiana desses animais após exposição a diferentes concentrações de chumbo. Os resultados indicaram que a exposição promoveu a produção de fatores inflamatórios, diminuição da expressão de Muc-2 colônico, comprometimento da barreira intestinal e alteração na composição microbiana (Wang et al., 2022; Yao et al., 2023). Além disso, relatos indicam que a exposição a outros metais, como cádmio, arsênico, está associado á diminuição na diversidade de bactérias (Wu et al., 2022; Yue et al., 2023; Zhao et al., 2023). Essas descobertas reforçam a interconexão entre a exposição a substâncias tóxicas e o equilíbrio do MI, destacando a sensibilidade da comunidade microbiana a fatores ambientais e seus potenciais efeitos adversos na saúde gastrointestinal.

Chen et al. (2023) destacam que camundongos C57BL/6J machos tratados com microplásticos e nanoplásticos por 60 dias apresentaram redução de bactérias benéficas como *Lachnoclostridium* e *Lactobacillus* e aumento de bactérias patogênicas, incluindo *Proteobacteria*, *Actinobacteria* e *Desulfovibrio*; além disso, houve redução da secreção de muco e aumento da permeabilidade intestinal. Kaikai et al. (2023), avaliando pesticida a base de metam-sódio e Liu et al. (2023) poluente químico aftatoxina B1, observaram que os animais expostos apresentavam menor diversidade bacteriana.

Esses contaminantes e produtos químicos, frequentemente utilizados em aplicações industriais e agrícolas, provocam poluição ambiental e, consequentemente, induzem a disbiose na microbiota, acarretando sérios problemas de saúde pública. Este cenário contribui para o desenvolvimento de diversas doenças metabólicas e imunológicas prevalentes, tais como obesidade (Meng et al., 2023), doenças inflamatórias intestinais (Guilloteau et al., 2022), diabetes (Lee et al., 2023) e doenças hepáticas (Wei et al., 2023). A associação desses contaminantes com o surgimento dessas condições ressalta a importância crítica de monitorar e mitigar a exposição a substâncias que impactam a qualidades na saúde da microbiota e, por conseguinte, o bem-estar humano.

3.2.2 Dieta

A dieta exerce uma influência na composição da MI, sendo que componentes dietéticos como vitaminas, minerais, aminoácidos, proteínas, fibras e lipídios desempenham papeis cruciais nesse processo (Zhao et al., 2023). Compreender como a disbiose da MI está associada à alimentação torna-se de suma relevância.

A escolha entre o leite materno (LM) ou introdução de alimentação complementar (AC) pode influenciar a colonização bacteriana. Laursen, (2021) destaca que o LM mantém a MI com baixa diversidade, sendo dominado por *Bifidobacterium*, que produz metabólitos capaz de reduzir o pH intestinal, criando um ambiente desfavorável para bactérias patogênicas. Por outro lado, a AC permite uma maior diversidade bacteriana, incluindo as patogênicas, como *Clostridium* e *Enterobacteriaceae*. Isso pode influenciar o risco de doenças infecciosas, metabólicas e imunomediadas, além de estar associado ao crescimento e ao desenvolvimento neuro, ósseo e imunológico. Assim, o LM é recomendado como o primeiro alimento para o bebê, fornecendo todos os nutrientes essenciais para apoiar seu crescimento e desenvolvimento.

Na fase adulta, os hábitos alimentares da população tendem a variar consideravelmente, influenciados pelos estilos de vida e hábitos alimentares específicos de cada país, intrinsecamente associados às condições socioeconômicas (Derrien et al., 2019). Estudo revela que ratos submetidos a dieta rica em gordura e sal durante 12 semanas apresentaram aumento de peso e massa gorda. No entanto, ao substituir essa dieta por uma dieta proteica por 6 semanas, esses animais apresentaram perda de gordura, normalização dos níveis de glicose no sangue, manutenção da massa muscular e melhora na MI (Wang et al., 2017). Em outra pesquisa, uma dieta pobre em oligossacarídeos, dissacarídeos, monossacarídeos e polióis fermentados melhorou a qualidade de vida de pacientes com síndrome do intestino irritado e doença inflamatória intestinal (Bodini et al., 2019). Lin et al. (2023) reportaram em estudo randomizado com duração de 12 semanas que jovens/adultos submetidos a dieta rica em fibras e ao exercício físico do tipo pular corda, exibiram melhora nos indicadores de saúde cardiometabólica e na modulação da MI.

Diante disso, é evidente que as alterações transitórias na diversidade da MI estão associadas a cada macronutriente, afetando de forma específica espécies bacterianas. Por exemplo, a ingestão de fibras melhorou a frequência de bactérias produtoras de AGCC, resultando em um aumento na abundância de *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus*, *Lactobacillus*, *Akkermania* e *Roseburia* (Fu et al., 2022). A introdução de proteínas pelo hospedeiro, dependendo do tipo (vegetal ou animal) pode gerar efeitos variados

na MI. O consumo de proteínas de origem animal, como carne vermelha e produtos lácteos, causa o aumento de bactérias anaeróbias tolerantes à bile, como *Bacteroidetes*, *Alistipes* e *Bilophila*, enquanto o consumo de proteínas vegetais está associado ao aumento de *Bifidobacterium* e *Lactobacillus* (Piccioni et al., 2023). No caso de uma ingestão elevada de gorduras alimentares saturadas, observa-se diminuição de *Bacteroidetes* e aumento de *Firmicutes* e *Proteobacteria*, resultando em disbiose e alterações na barreira intestinal devido à abundância de bactérias redutoras de sulfato. Essas bactérias são capazes de reduzir as ligações dissulfeto no muco, causando danos e aumentando a inflamação intestinal (Rinninella et al., 2019).

3.2.3 Medicamentos

A utilização de medicamentos tem sido cada vez mais comum nos dias atuais, sendo a via oral a forma mais utilizada para tratamento em longo prazo. Essa preferência é atribuída à segurança, baixo custo, maior grau de flexibilidade e adesão do paciente (Javdan et al., 2020; Zhang et al., 2021). Diversos medicamentos têm alvo específico para as bactérias, como os antibióticos, enquanto outros estão relacionados à microbiota ou a sua composição, como a metformina e os inibidores da bomba de prótons - IBPs (Proffitt et al., 2020).

Os antibióticos desempenham um papel importante na medicina humana devido ao seu alto poder bacteriano. No entanto, o seu uso, seja a curto ou longo prazo, tem sido associado a efeitos nocivos à MI (Dahiya & Nigam, 2023; Patangia et al., 2022). Essas alterações incluem o aumento de espécies oportunistas e patogênicas (Huang et al., 2022), alteração da atividade metabólica (Bongers et al., 2022), desenvolvimento de resistência (Kavia et al., 2023) e incidência de infecções (Carrara et al., 2023). Além disso, o uso de diferentes classes químicas de antibióticos está associado em problemas de saúde, incluindo condições gastrointestinais (Issac & Kochuparambil, 2022), diabetes (Nuotio et al., 2022) e doenças neuroimune (Lynch et al., 2023).

A metformina, amplamente utilizada no tratamento do diabetes mellitus tipo 2, exerce seus efeitos ao suprimir a gliconeogênese hepática e aumentar a captação de glicose no tecido muscular esquelético (Rehani et al., 2019; Rena et al., 2017). Além de seu benefício reconhecido no controle da glicose, estudos indicam seus efeitos positivos como imunomodulador (Sutter et al., 2023), em doença óssea inflamatória (Hong et al., 2023) e potencial agente anticâncer (Shen et al., 2023). No entanto, a utilização da metformina está associada a efeitos adversos, como deficiência de vitamina B12 (Hurley et al., 2019), interferência na função plaquetária (Uematsu et al., 2023) e disbiose da MI em camundongos

ou pacientes diabéticos (Gravdal et al., 2023; Kim et al., 2021; Ma et al., 2022). Bryruo et al. (2019) demonstraram que indivíduos saudáveis apresentaram redução de *Intestinibacter* spp. e *Clostridium* spp., bem como aumento na abundância dos gêneros *Escherichia* e *Shigella* e da espécie *Bilophila wadsworthia* em resposta ao tratamento com metformina. Sabe-se que *B. wadsworthia* é uma bactéria anaeróbica Gram-negativa associada à inflamação, disfunção da barreira intestinal e alteração do metabolismo dos ácidos biliares (Natividad et al., 2018). Essas descobertas destacam a necessidade de cautela ao prescrever metformina, considerando que a disbiose pode ocorrer independentemente de o paciente apresentar diabetes ou não.

Os IBPs, como o omeprazol, lansoprazol e pantoprazol, são amplamente utilizados no tratamento de lesões ulcerativas e refluxo gastroesofágico (Sheele, 2017). Esses medicamentos atuam covalentemente nas bombas antiportadoras de hidrogênio, potássio ATPase das células parietais gástricas, inibindo a liberação de íons de hidrogênio na cavidade gástrica e elevando o pH (Bruno et al., 2019). Entretanto, o uso prolongado de IBPs está associado a efeitos colaterais em diferentes órgãos, como anemia (sangue), encefalopatia hepática (fígado), pneumonia (pulmão), rabdomiólise (músculo) (Kinoshita et al., 2018). Quanto às alterações na microbiota, estudos reportam que a utilização de IBPs promove redução de bactérias do gênero *Faecalibacterium*, enquanto aumenta *Streptococcaceae*, *Veillonellaceae*, *Acidaminococcaceae*, *Micrococcaceae* e *Flavobacteriaceae* (Burmeister et al., 2023; Kiecka & Szczepanik, 2023; Zhang, Ma, et al., 2023).

3.3 Modulação da microbiota intestinal

Como abordado anteriormente, a MI desempenha um papel fundamental na modulação das funções fisiológicas e está envolvida na patogênese de diversas doenças quando ocorre a disbiose. Diante desse cenário, torna-se necessário o desenvolvimento de estratégias terapêuticas que visem restabelecer o equilíbrio e a manutenção da MI. Nesse contexto, a utilização de probióticos e prebióticos surgiu como uma alternativa promissora.

3.3.1 Probióticos

Os probióticos são microrganismos não patogênicos que, quando administrados em quantidades adequadas, oferecem benefícios para a melhoria da saúde (Latif et al., 2023). Contudo, à medida que a compreensão em relação aos probióticos evolui, confirma-se que esses microrganismos atuam para além da simples modulação da microbiota, induzindo alterações fisiológicas e metabólicas no hospedeiro (Das et al., 2022; Fong; Li & Yu, 2020).

Dentre as bactérias probióticas da MI, destacam-se os gêneros *Lactobacillus* e *Bifidobacterium* (Tabela 1), conhecidas por suas propriedades antioxidade entre os micróbios intestinais. Além disso, esses probióticos têm capacidade de desintoxicar (Ibrahim et al., 2006) e excretar metais pesados nas remediações do intestino. Eles também se ligam a outros compostos tóxicos, como cianotoxinas e mutagênicos dietéticos (Abdel-Megeed, 2021; Arun et al., 2021). Curiosamente, bifidobactérias presentes no leite materno são conhecidas por ativar o sistema imunológico, reduzir os níveis de colesterol e produzir vitaminas B (Riasatian et al., 2023).

Tabela 1. Principais bactérias do gênero *Lactobacillus* e *Bifidobacterium* conhecidas como probióticos.

Gênero	Espécies de bactérias probióticas
Lactobacillus spp.	*L. acidophilus, L. rhamnosus, L. gasseri, L. casei, L. reuteri, L. plantarum, L. salivarius, L. johnsonii, L. gallinarum, L. plantarum, L. fermentum, L. helveticus, L. brevis, L. murinus, L. crispatus, L. amylovorus.*
Bifidobacterium spp.	*B. infantis, B. longum, B. lactis, B. adolascentis, B. bifidum, B. animalis, B. breve, B. thermophilum, B. pseudolongum.*

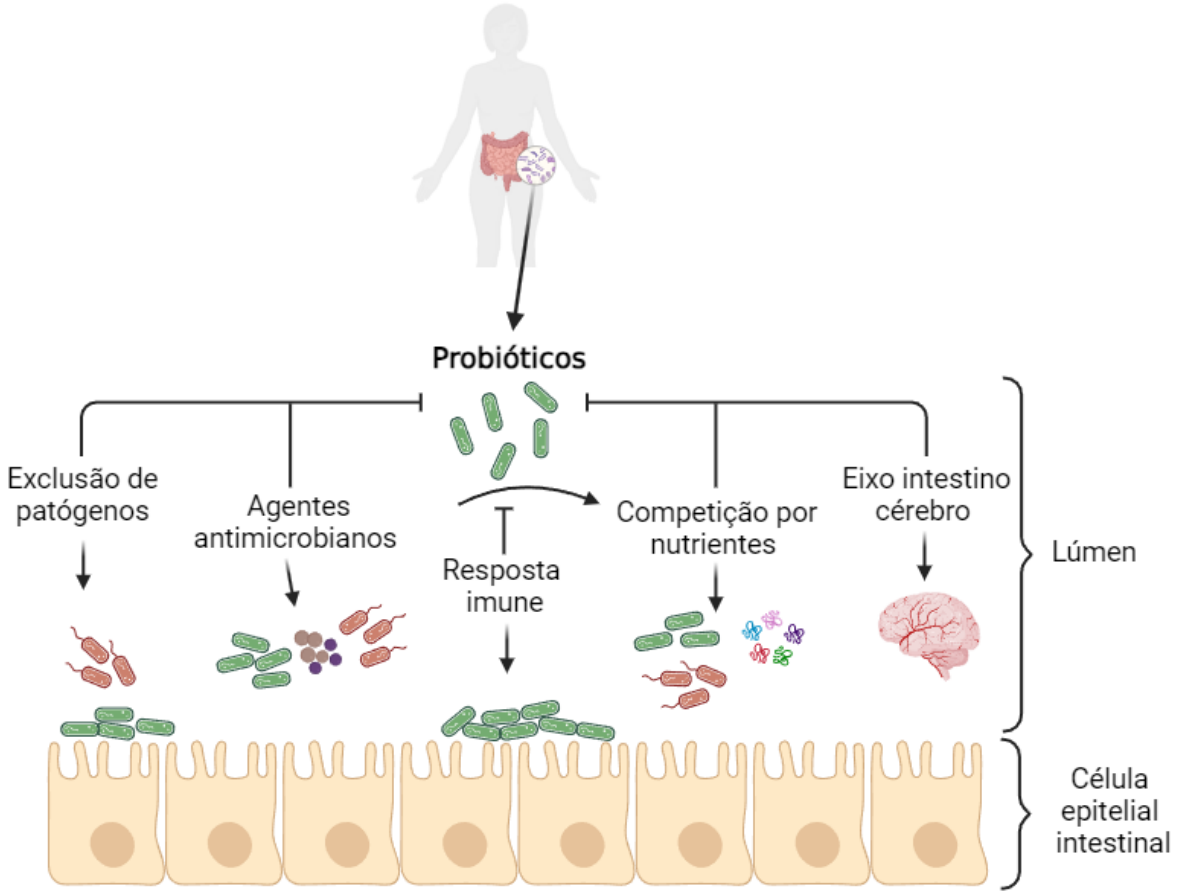
Fonte: Das *et al*. (2022).

Os probióticos apresentam uma ampla gama de efeitos à saúde, conforme extensivamente revisado em vários estudos (Lof et al., 2022; Manzoor et al., 2022; Soares et al., 2023; Tegegne & Kebede, 2022). Esses benefícios incluem a prevenção e redução de doenças alérgicas (Fiocchi et al., 2022), câncer (Huang et al., 2023; Kandati et al., 2022), intolerância à lactose (Oliveira et al., 2022), doença inflamatória intestinal (Bamola et al., 2022; Selvamani et al., 2022), diarreia (Grenov, 2023; Masuda et al., 2023) e síndrome do intestino irritável (Goodoory et al., 2023; Konstantis et al., 2023).

Embora não haja uma padronização clara de como os probióticos atuam, diferentes mecanismos de ação explicam a influência dessas bactérias na MI. Sua atuação provavelmente é multifatorial e específica da cepa, conforme ilustrado na Figura 4. Os probióticos atuam no corpo humano através da exclusão competitiva de patógenos, aprimorando as funções da barreira intestinal, imunomodulação, produção de neurotransmissores e competem com os patógenos por nutrientes e locais de ligação aos receptores (Plaza-Diaz et al., 2019). Eles também são como agentes antimicrobianos, gerando

AGCC, ácidos orgânicos, peróxido de hidrogênio e bacteriocinas (Ahire et al., 2021). Além disso, melhoram a função da barreira intestinal, estimulando a produção de proteínas mucinas (Chang et al., 2021), regulam a expressão de proteínas de junção estreita, resposta imune, inata e adaptativa (Bu et al., 2022; Ma et al., 2022), aumentam a síntese de citocinas anti-inflamatórias que interagem com células epiteliais intestinais e atraem macrófagos e células mononucleares (Petruzziello et al., 2023), e produzem neurotransmissores no intestino através do eixo intestino-cérebro (Gangaraju, Raghu & Gurudutt, 2022; Sajedi, Shabani & Elmieh, 2021).

Figura 4. Mecanismo de ação dos probióticos.



Fonte: elaborado pelo autor (2025).

3.3.2 Prebióticos

De acordo com especialistas em microbiologia, nutrição e investigação clínica da associação científica internacional de probióticos e prebióticos, o prebiótico (PRE) Um prebiótico é um substrato que é utilizado seletivamente por microrganismos hospedeiros conferindo um benefício à saúde. Essas substâncias têm a capacidade de induzir mudanças específicas na composição e/ou atividade da MI, conferindo benefícios à saúde do hospedeiro,

bem como não devem ser digeríveis, sendo capaz de resistir a absorção no intestino delgado (Gibson et al., 2017; You et al., 2022).

Com o aumento de estudos e interesse industrial, a utilização de PRE tornou-se cada vez mais comum. Para considerar seu uso seguro, características especificas foram estabelecidas e análises *in vitro* e *in vivo* devem ser desenvolvidas para avaliar a especificidade dessa substância. Dentre os critérios, destaca-se: (a) resistência à acidez gástrica, hidrólise por enzimas digestivas e absorção gastrointestinais; (b) fermentação pela MI, que pode ser avaliada *in vitro* através da adição dos respectivos PREs às suspensões de conteúdo do cólon ou culturas puras ou mistas de bactérias em sistema de fermentação anaeróbica e (c) promoção do crescimento de bactérias intestinais beneficamente relacionadas ao bem-estar e à saúde (Bamigbade et al., 2022).

Quanto aos tipos de PRE, destacam-se os carboidratos oligossacarídeos, incluindo principalmente xilooligossacarídeos (XOS), galacto-oligossacarídeos (GOS), inulina e seus frutose-oligossacarídeos derivados (FOS) (Khangwal & Shukla, 2019). Além disso, com a otimização contínua do processo de preparação, novas alternativas vêm sendo desenvolvidas, incluindo principalmente polissacarídeos, polifenóis, polipeptídeos, proteínas e glicoproteínas (Ashwini et al., 2019).

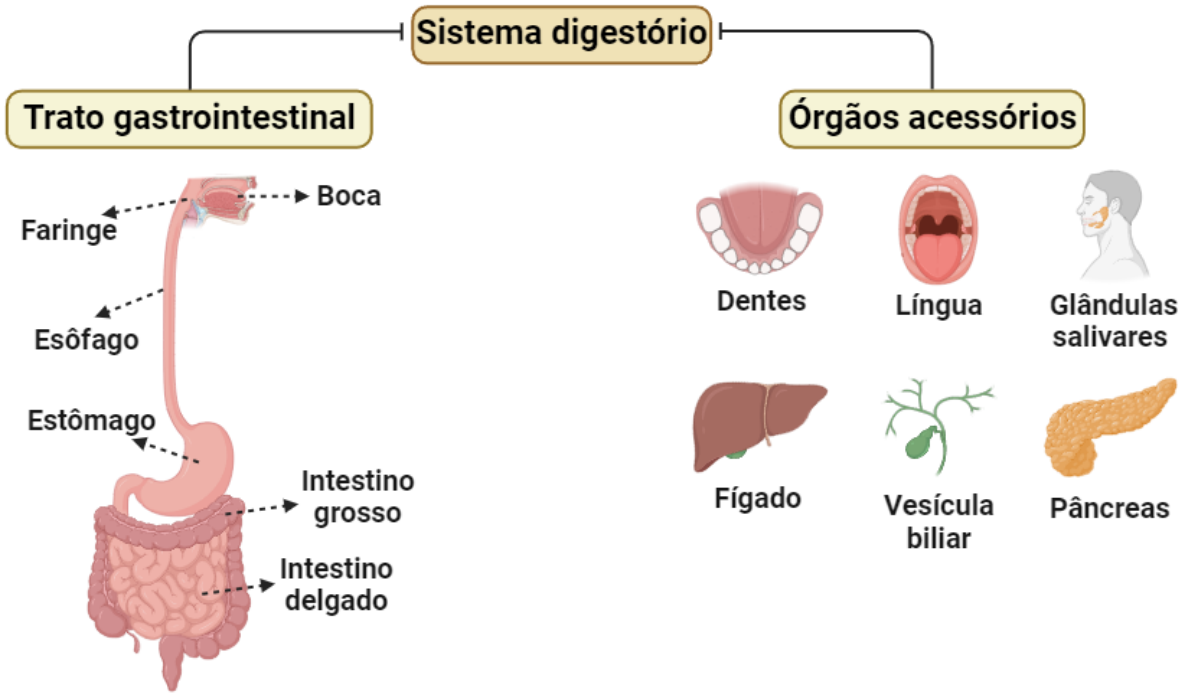
Os PREs atuam de maneira benéfica na MI, e estudos em modelos animais e ensaios clínicos confirmaram que sua ingestão pode alterar a composição e a abundância de certas bactérias. Durante o processo de fermentação, são gerados vários metabolitos, como AGCC, principalmente acetato, propionato e butirato (Rezende et al., 2021). Everard et al. (2014) demonstraram que camundongos tratados com PRE continha bactérias do gênero *Akkermansia*, *Dehalobacterium*, *Prevotella*, *Sutterella*, *Tannerella*, além de redução de *Alistipes*, *Allobaculum*, *Clostridium*, *Coprobacillus*, *Coprococcus*, *Eubacterium*, *Faecalibacteriume* e *Ruminococcus*. Em outra pesquisa, He *et al*. (2021) demonstraram que a adição de GOS a uma dieta rica em gordura pode estimular o crescimento de *Bifidobacterium*. A maioria das Bifidobactérias pode produzir ácido láctico, acidificando o ambiente intestinal, limitando o crescimento de bactérias patogênicas e melhorando a função da barreira mucosa (Mei et al., 2022).

Vale salientar que para os PREs atuarem beneficiando a saúde, é necessária uma dose diária de 3 g sendo tolerado até 20 g/dia (Gibson et al., 2017). Logo, a ingestão média recomendada de FOS e inulina está entre 2 e 12 g/dia (Nicolucci et al., 2017). No entanto, o consumo superior a esse nível pode resultar em efeitos colaterais, como gases e diarreia (Davani-Davari et al., 2019).

3.4 Fisiologia gástrica

O sistema digestório, conforme ilustrado na Figura 5, é composto por órgãos especializados que formam o trato gastrointestinal (TGI). Este trato inclui a boca, faringe, esôfago, estômago e intestinos (delgado e grosso), juntamente com órgãos acessórios como dentes, língua, glândulas salivares, fígado, vesícula biliar e pâncreas (Sensoy, 2021). Cada componente do TGI desempenha uma função específica, onde em conjunto são responsáveis por processar os alimentos em moléculas que podem ser absorvidas e utilizadas pelas células do corpo, enquanto os resíduos não aproveitados são excretados (Cheng et al., 2010).

Figura 5. Componentes do sistema digestório.



Fonte: Elaborado pelo autor (2025).

O estômago, posicionado no quadrante superior esquerdo do abdômen, desempenha diversas funções no processo digestivo ao receber os alimentos do esôfago. Suas reponsabilidades abrangem armazenamento, digestão, mistura, propulsão e proteção (Wilson & Stevenson, 2019). Anatomicamente, o estomago é divido em três regiões distintas: o fundo, responsável pelo armazenamento de ar engolido após as refeições; o corpo, a maior seção, responsável por armazenar os alimentos ingeridos; e o antro, localizado na parte inferior, onde ocorre a mistura dos alimentos com o suco gástrico (Brandstaeter et al., 2019; Hennig & Spencer, 2018). O revestimento interno do estomago é composto por quatro camadas teciduais, denominadas de serosa, muscular, submucosa e mucosa. A mucosa, em particular,

contém inúmeras glândulas, incluindo oxínticas e pilóricas, associadas a digestão química dos alimentos (Friis et al., 2023).

As glândulas oxínticas representam 80% do estômago (fundo e corpo) e é composta por células parietais produtoras de ácido clorídrico – HCl (Wood, 2019). O HCl desempenha funções cruciais, como eliminação de microrganismos patogênicos ingeridos durante a alimentação, facilita a digestão, promove a absorção de minerais (fosfato, cálcio e ferro) (Engevik et al., 2020) e ativa a pepsina em pepsinogênio (Blanchard & Czinn, 2021). A pepsina é uma enzima responsável pela quebra das ligações peptídicas presente nas proteínas (pH ideal de 2,0–3,0) (Alkalbani et al., 2022). As glândulas pilóricas, localizadas no antro do estômago, são responsáveis pela produção do hormônio gastrina, secretado por suas células G enteroendócrinas, que estimula a produção HCl (Schubert, 2017). Além disso, o muco secretado pelas células desempenha um papel significativo na proteção da superfície gástrica do meio ácido no estômago (Feher, 2017).

A mucosa gástrica produz diariamente aproximadamente de 1,2 a 1,5 litros de suco gástrico, composto por uma mistura variável de água, HCl, eletrólitos (sódio, potássio, cálcio, fosfato, sulfato e bicarbonato) e substâncias orgânicas (muco, pepsinas e proteínas) (Martinsen et al., 2019). O suco gástrico é responsável por tornar os alimentos em pequenos fragmentos solúveis, iniciando a digestão, principalmente das proteínas, e convertendo o conteúdo gástrico em uma massa semilíquida conhecida como quimo, que é posteriormente processado no intestino delgado (McKie et al., 2023; Whitman & O'Neil, 2018). É importante ressaltar que o estômago apresenta uma membrana de células epiteliais rica em lipoproteínas, revestindo o lúmen e protegendo a parede gástrica contra os efeitos do ácido e microrganismos (Luissint et al., 2016).

O processo de secreção gástrica pode ser dividido em três fases: cefálica, gástrica e intestinal, que se sobrepõem e são coordenados por complexos fatores neurais e hormonais (Wilson & Stevenson, 2019). A fase cefálica tem início antes da chegada do alimento no estômago e é mediada através do nervo vago, ocorrendo em resposta a sensações e/ou estímulos, incluindo visão, cheiro e sabor, o que provoca a secreção ácida (Smith & Morton, 2010). As fibras e os neurônios colinérgicos liberam acetilcolina, ativando as células parietais e induzindo a liberação de gastrina e histamina (Feher, 2017; Goo; Akiba; Kaunitz, 2010).

A fase gástrica é regulada pelo nervo vago e pela liberação de gastrina. Após uma refeição, a acidez é tamponada por proteínas, geralmente mantendo-as em torno do pH 3. O ácido continua a ser secretado em resposta à distensão causada pelos peptídeos e aminoácidos liberados durante a digestão das proteínas (Whitman & O'Neil, 2018). A ação química dos

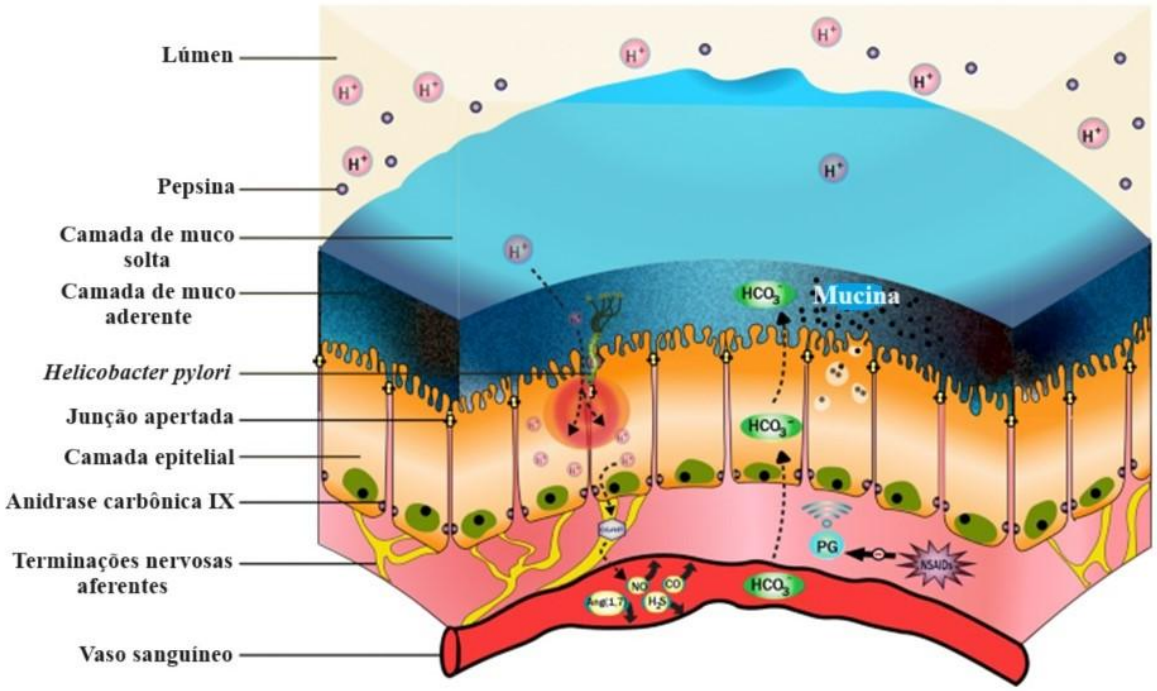
aminoácidos e peptídeos livres estimula a liberação de gastrina do antro para a circulação. Diversos fatores, como mecânicos, químicos e hormonais, contribuem para a resposta secretora gástrica, mantendo essa fase até que os alimentos deixem o estômago (Smith & Morton, 2010).

Na fase intestinal, desencadeia-se um processo intricado de estímulo e regulação. Aminoácidos e pequenos peptídeos induzem a secreção de ácido gástrico na corrente sanguínea quando o quimo chega o duodeno, sendo a secreção ácida estimulada pela distensão mecânica e pela presença de aminoácidos e peptídeos. As células G duodenais liberam gastrina para ativar as células parietais. Após um período, o duodeno exerce um efeito inibitório sobre a secreção gástrica, resultando em aumento da acidez à medida que ocorre um esvaziamento (Goo et al., 2010).

3.5 Fatores de proteção gástrica

A mucosa gástrica é exposta diariamente a diversas substâncias tóxicas, e a prevenção de sua integridade é um processo dinâmico, complexo e multifatorial, que envolve uma interação entre componentes fisiológicos e anatômicos (Galura et al., 2019). Portanto, para garantir a proteção gástrica, são necessários três mecanismos distintos, compreendo componentes pré-epitelial, epitelial e pós-epitelial (Figura 6).

Figura 6. Componentes que atuam para a proteção gástrica.



Fonte: Galura *et al*. (2019).

O componente pré-epitelial atua como primeira linha de defesa, compreendendo uma barreira de muco formada por mucinas (0,2-5%), água (95%), íons (sódio, potássio, cloro, bicarbonato, fosfato, magnésio, cálcio), lipídeos e proteínas, incluindo lisozimas e lactoferrina (Bansil & Turner, 2018; Mall et al., 2017). O componente epitelial constitui a segunda linha de defesa, caracterizada pela renovação contínua das células epiteliais. Esse processo é realizado pelas células progenitoras, regulado por fatores de crescimento e prostaglandinas (Galura et al., 2019). Por fim, a terceira linha de defesa é mantida pelo fluxo sanguíneo contínuo através da vasculatura da mucosa. Esse processo não fornece apenas oxigênio e nutrientes essenciais, mas também participa na produção de prostaglandinas e óxido nítrico, contribuindo para a regulação do ambiente gástrico (Yandrapu & Sarosiek, 2015). A cooperação eficaz desses três componentes é vital para garantir a proteção eficiente da mucosa gástrica contra agressões externas.

As prostaglandinas desempenham um papel fundamental no mecanismo de defesa e integridade da mucosa, exercendo regulação sobre a função de ácido gástrico, muco-bicarbonato e fluxo sanguíneo, enquanto os receptores 1–4 de prostaglandina tipo E são transferidos para a motilidade (Laine et al., 2008). Essas substâncias também exercem efeitos inibitórios sobre a ativação de mastócitos, a adesão de leucócitos e plaquetas ao endotélio vascular, assim como secreção de ácido clorídrico pelas células parietais (Cryer, 2001). Além disso, as prostaglandinas secretam muco e bicarbonato através dos receptores EP4 e EP1, bem como os fosfolipídios ativos de superfície, acelerando a restituição epitelial e contribuindo para a cicatrização da mucosa (Sáenz & Mills, 2018).

O óxido nítrico, produzido pela atividade da enzima óxido nítrico sintase, desempenha um papel importante na cicatrização de úlceras, contribuindo significativamente para a manutenção da integridade da barreira epitelial e estimulando a secreção de muco (Magierowski et al., 2015). Além de seus efeitos vasodilatadores, facilitando a entrega de bicarbonato, nutrientes e oxigênio (Tejero et al., 2019), o óxido nítrico também regula a secreção de ácido gástrico e inibe a agregação e adesão de neutrófilos, fornecendo assim os danos gástricos (Liang et al., 2021).

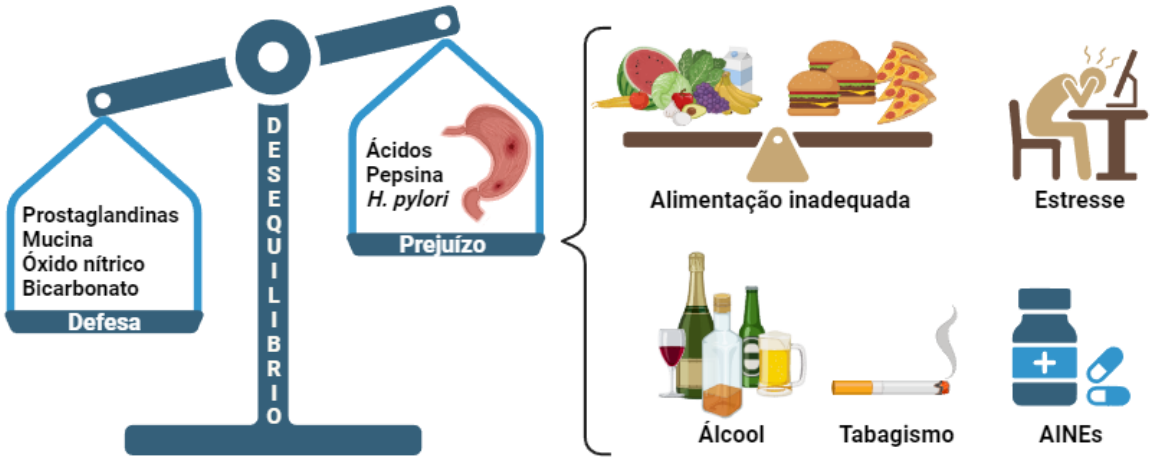
3.6 Úlcera gástrica

A úlcera é caracterizada por uma lesão no trato gastrointestinal que pode ocorrer na camada mucosa do estômago (úlcera gástrica – UG) ou no duodeno (úlcera duodenal), desencadeando um processo inflamatório agudo ou crônico (Jaswanth; Kumar; Venkatesh,

2022). Em 2019, a prevalência dessa doença na população geral foi de aproximadamente 8,09 milhões de casos (Xie et al., 2022).

O progresso da UG implica em uma interação complexa entre lesões, mecanismos de defesa e o processo de restauração da mucosa. A patogênese desta doença está ligada ao desequilíbrio entre fatores que promovem a defesa (FD) e aqueles que causam prejuízos (FP) (Figura 7). Entre os FD, incluem-se as prostaglandinas, mucina, óxido nítrico e bicarbonato, enquanto os FP englobam ácidos, pepsina e a infecção por *H. pylori* (Zhang *et al*., 2020). Além dos FP endógenos, o desenvolvimento da UG também está relacionado a fatores exógenos, como hábitos alimentares inadequados, estresse e agentes químicos, incluindo álcool, tabagismo e uso excessivo de anti-inflamatórios não esteroides – AINEs (Serafim et al., 2020; Tytgat, 2011).

Figura 7. Fatores prejudiciais e defensivos na patogênese da UG.



Fonte: Elaborado pelo autor (2025).

A nível histológico, a UG manifesta-se por quatro camadas distintas, compostas por tecido necrótico, comumente do tipo fibrinóide, localizada na parte inferior da lesão resultante da ação da digestão ácido péptica. A inflamação caracteriza-se pelo predomínio de neutrófilos, enquanto a camada subsequente é composta principalmente por tecido de granulação e tecido fibroso cicatricial (Braga et al., 2016). O processo de cicatrização desencadeado por uma úlcera envolve uma série de eventos, incluindo a proliferação celular, migração, reepitelização, formação de tecido de granulação, angiogênese e interações entre células e a matriz, resultando na formação da cicatriz e remodelação tecidual (Baatar et al., 2002; Vanwijck, 2001). Esses processos são regulados por fatores de crescimento, como

plaquetas, macrófagos, fator de crescimento epidérmico e nervoso, bem como por fatores de transcrição e citocinas (Tarnawski & Ahluwalia, 2021).

Quanto aos sintomas, a UG comumente se manifesta por dor epigástrica, frequentemente após as refeições, acompanhada de uma sensação de queimação. Menos frequentes, podem ocorrer náuseas, vômitos e perda de peso, embora alguns tipos de UG, especialmente as provocadas por AINEs podem ser assintomáticas (Ahmad et al., 2019; Mechu Narayanan, 2018). Nesse contexto, a restauração do equilíbrio da mucosa gástrica danificada é crucial, e essa estratégia tem sido desenvolvida por meio de uma variedade de tratamentos convencionais.

A abordagem terapêutica para a UG se concentra na neutralização da acidez gástrica ou no aumento da síntese dos fatores de proteção. As principais classes e o mecanismo de ação dos medicamentos utilizados no tratamento, como os inibidores da bomba de prótons, bloqueadores do receptor H2, bloqueador de ácido competitivo de potássio e agentes citoprotetores, estão resumidos na Tabela 2 (Kuna et al., 2019). De fato, é importante destacar que o tratamento convencional para UG tem sido associado a diversos efeitos adversos, como náuseas, vômitos, constipações, diarreia, efeitos sobre o sistema cardiovascular entre outros. Nesse contexto, a busca por novas alternativas terapêuticas é crucial. A necessidade de encontrar abordagens inovadoras para a prevenção e cicatrização da úlcera gástrica se justifica pela busca por tratamentos mais eficazes e com menor impacto de efeitos adversos. Além disso, considerando a complexidade da UG e a variabilidade de respostas aos tratamentos convencionais, a diversificação das opções terapêuticas pode oferecer soluções mais personalizadas e adaptadas às necessidades individuais dos pacientes. Portanto, a investigação e o desenvolvimento de novas alternativas terapêuticas para UG são essenciais para avançar na eficácia, tolerabilidade e abrangência em relação aos tratamentos já disponíveis.

Tabela 2. Medicamentos utilizados para o tratamento da úlcera gástrica.

Medicamentos		Mecanismo de ação	Efeitos adversos
IBP	Omeprazol, lansoprazol, rabeprazol, esomeprazol e pantoprazol	Inibição da atividade enzimática da bomba de prótons gástrico H^+/K^+ -ATPase.	Dor (na cabeça, abdominal), diarreia, náuseas, vômitos, prisão de ventre, flatulência, deficiência de vitamina B12 e osteoporose.
BBH2	Cimetidina, famotidina, nizatidina e ranitidina	Inibição da atividade dos receptores H2 da histamina nas células parietais, bloqueando sua ação.	Dor (na cabeça), ansiedade, depressão, tonturas, eventos cardiovasculares e trombocitopenia.
Antiácidos	Hidróxido de alumínio	Eleva o pH no ambiente gástrico para valores superiores a 4, ao mesmo tempo que inicia a atividade proteolítica da enzima pepsina	Náuseas, vômitos, hipofosfatemia, constipação, cólicas abdominais, diarreia e desequilíbrio eletrolítico.
	Hidróxido de magnésio	Induz a retenção osmótica de liquido.	
BACPs	Vonoprazan	Bloqueia a atividade da H+, K+-ATPase nas células parietais gástricas, interferindo na fase final da via de cartilagem ácida.	Nasofaringite, contusão, diarreia, inflamação do trato respiratório superior, eczema, constipação e dor nas costas.
AC	Misoprostol e sucralfato	Promove a síntese do muco e melhora o fluxo sanguíneo em toda a mucosa do trato gastrointestinal.	Diarreia, dor (abdominal e cabeça) e constipação.

IBP: Inibidores da bomba de prótons; BH2: Bloqueadores do receptor H2; BACPs: Bloqueador de ácido competitivo de potássio e AC: agentes citoprotetores. Fonte: Kuna *et al*., 2019.

3.7 Gênero *Parkia*

O gênero *Parkia* pertence à família Leguminosae, subfamília Mimosoideae, e compreende aproximadamente 35 espécies reconhecidas, descobertas em uma ampla distribuição pelos neotrópicos, Ásia e África (Saleh et al., 2021). Devido ao alto grau de simpatria entre as espécies, quatro áreas são endêmicas, a saber: América do Sul, África, Madagascar e região Indo-Pacífico (Luckow & Hopkins, 1995). No Brasil, a região amazônica destaca-se com riqueza de espécies para o gênero *Parkia*, 56% das espécies conhecidas; que se apresentam predominantemente como árvores ou arbustos de grande porte, variando de 3 a 45 metros de altura (Oliveira et al., 2021).

Na medicina popular, as espécies de *Parkia* têm sido tradicionalmente utilizadas para tratar diversas doenças e suas partes como cascas do caule e sementes são processadas e empregadas na forma de pasta ou decocção. Esses recursos são aplicados no tratamento de diversas condições, incluindo cicatrização de feridas (Ajaiyeoba, 2002), úlcera (Boye et al., 2014), diabetes (Mondal, 2013), e uma variedade de doenças relacionadas à pele, como eczema, sarampo, lepra, ferida, dermatite, varicela, sarna e micose (Singh, 2019; Srisawat et al., 2016); bem como para tosse e bronquite grave (Fotie et al., 2004; Kouadio et al., 2000; Ong et al., 2011). Essa utilização na medicina popular destaca o potencial terapêutico de espécies do gênero *Parkia* e reflete o conhecimento tradicional do seu uso acumulado ao longo do tempo.

Em revisões, Cavada et al. (2020) e Saleh et al. (2021) enfatizam que as espécies *P. biglobosa*, *P. speciosa, P. timoriana*, *P. bicolor*, *P. clappertoniana*, *P. discolor*, *P. roxburghii*, *P. filicoidea, P. pendula, P. platycephala* e *P. javanica* foram objetos de extensas investigações. Essas pesquisas resultaram no isolamento de uma variedade de componentes fitoquímicos, incluindo lectinas, fenóis, flavonoides, polissacarídeos, ácidos graxos, taninos, terpenóides, esteroides, saponinas, alcaloides, glicosídeos e antraquinonas, destacando a atividade antioxidade, imunorregulatória e anti-inflamatória.

Até o momento, polissacarídeos presentes nas espécies de *Parkia* são pouco explorados em estudos científicos. Segundo a Tabela 3, obtida por meio de uma pesquisa nas fontes de dados *Science Direct* e PUBMED, utilizando os descritores "polissacarídeo" e "*Parkia*", foram encontrados apenas cinco artigos através do cruzamento desses descritores. As informações sobre as características físico-químicas desses polissacarídeos foram das espécies *P. speciosa, P. biglobosa, P. timoriana* e *P. pendula*. Esses estudos exploraram as aplicações biológicas desses polissacarídeos em atividades antioxidantes (Buathongjan *et al*., 2020, Gan *et al*., 2010), imunomoduladoras (Zou *et al*., 2014), anti-inflamatórias (Ibraheem *et*

al., 2022) e moluscicida (Batista et al., 2022). Apesar do reconhecimento da diversidade de espécies vegetais de *Parkia* para uso popular, a investigação científica sobre as propriedades dos seus polissacarídeos precisa ser mais explorada.

Tabela 3. Estudos realizados com polissacarídeos de espécies do gênero *Parkia*.

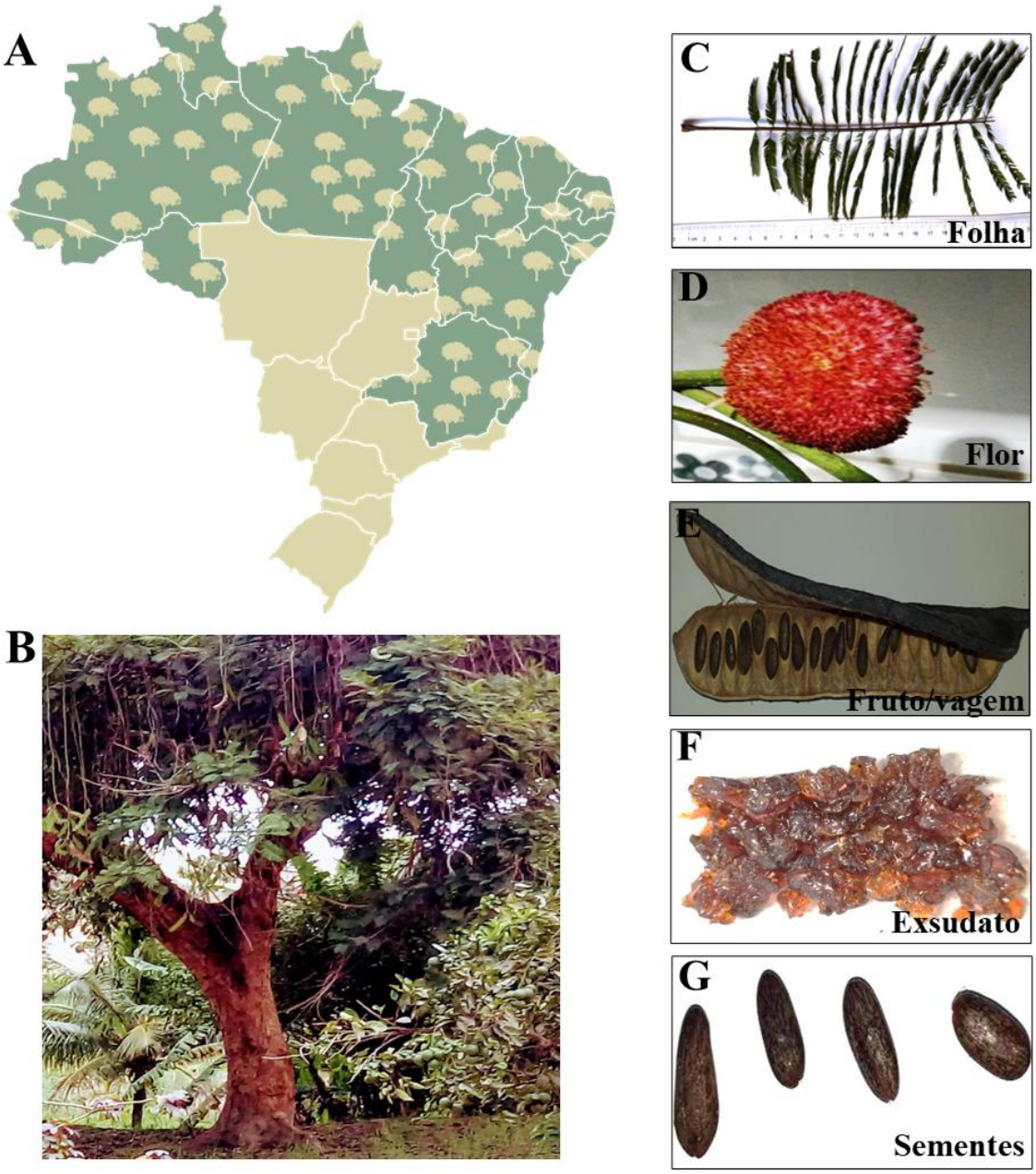
Obtenção			**Caracterização físico-química (%)**				**Atividades biológicas**
Espécie	**Parte utilizada**	**Extrato**	**CT**	**AU**	**PT**	**FE**	
P. speciosa	Vagens	Aquoso	-	97-99 mg/g	-	-	Antioxidade
P. speciosa	Sementes	Aquoso	-	60,9	-	-	Antioxidade
P. biglobosa	Cascas	HA	-	-	1,2	2,4	Imunomodulador
P. timoriana	Vagens	Aquoso	78,80	-	4,77	-	Antioxidade
P. timoriana	Pó/vagens	Aquoso	79,41	-	5,27	-	Antioxidade
P. biglobosa	Polpa	Aquoso	-	-	-	-	Anti-inflamatório
P. pendula	Semente	Salino	86	-	-	-	Moluscicida

CT (Carboidratos totais); AU (Ácido urônico); PT (Proteínas totais) e FE (Fenóis). HA: hidroalcóolico.

3.8 *Parkia pendula*

A espécie *Parkia pendula,* conhecida popularmente como “visgueiro”, “faveira” ou “faveira-de-chorão” apresenta ampla distribuição em diversas regiões do Brasil. Essa distribuição abrange a região Norte (Acre, Amazonas, Amapá, Pará, Rondônia, Roraima, Tocantins), Nordeste (Alagoas, Bahia, Ceará, Maranhão, Paraíba, Pernambuco, Piauí, Sergipe), Centro-Oeste (Mato Grosso) e Sudeste (Espírito Santo) (Figura 8A) (Oliveira & Hopkins, 2023).

Figura 8. Distribuição geográfica no Brasil e as diferentes partes de *Parkia pendula*.



Fonte: Elaborado pelo autor (2025).

Caracterizada como uma árvore de grande porte (Figura 8B) apresenta copa larga e plana, cascas avermelhadas, folhas grandes e folíolos finos (Figura 8C). Suas flores férteis estão localizadas na porção média e basal (Figura 8D) e os frutos são vagens não lenhosas e glabas, contendo de 10 a 25 sementes por vagem (Figura 8E). O tronco e as vagens são produtoras de goma/exsudato (Figura 8F) e suas sementes são viáveis anualmente (Figura 8G) (Salman et al., 2008; Oliveira et al., 2021; Samuichi et al., 2009).

Pesquisas confirmaram a existência de diferentes compostos e diversas propriedades biológicas de *P. pendula*, incluindo:

a) Folhas - o extrato revelou a presença de três aleloquímicos diferentes: ácido3,4,5-trimetoxibenzóico, ácido 3,4-dimetoxibenzóico e Blumenol A. Essas substâncias demonstram efeito inibitório moderado na germinação de sementes, com efeito alelopático dependente da concentração (Souza Filho, 2005).

b) Sementes - A partir do extrato salino (NaCl 0,15 M), fracionamento com sulfato de amônio e cromatografia de afinidade foi isolada uma lectina denominada PpeL (Coriolano et al., 2010). Esta foi utilizada na cristalização por método de difração de raios-X (Lombardi et al., 1998), marcador histoquímico na diferenciação de tumores meningotelial e diagnóstico clínico-patológico (Beltrão et al., 2002), infectividade por citomegalovírus humano, demonstrando ser uma alternativa para o desenvolvimento de droga antiviral (Favacho et al., 2007), caracterização morfológica de células animais normais e transformadas, principalmente tumor venéreo transmissível, auxiliando na formação do diagnóstico clínico (Rodrigues et al., 2008), efeitos significativos no processo de reparo de feridas cutâneas em camundongos saudáveis e imunossuprimidos (Coriolano et al., 2014), efeito inibitório no desenvolvimento de *Leismania infantum* (Carneiro et al., 2021) e atividade moluscicida sobre *Biomphalaria glabrata* (Batista et al., 2022).

c) Exsudato - O polissacarídeo do exsudato obtido das vagens apresenta capacidade adesiva e sua utilização como fonte alimentícia para diferentes espécies de vertebrados têm sido relatadas (Peres, 2000; Piechowski & Gottsberger, 2009b). Além disso, demonstrou ser uma matriz polissacarídica magnética promissora para a imobilização da lectina Concanavalina A e sua aplicação permite fácil recuperação no campo magnético (Rêgo et al., 2014).

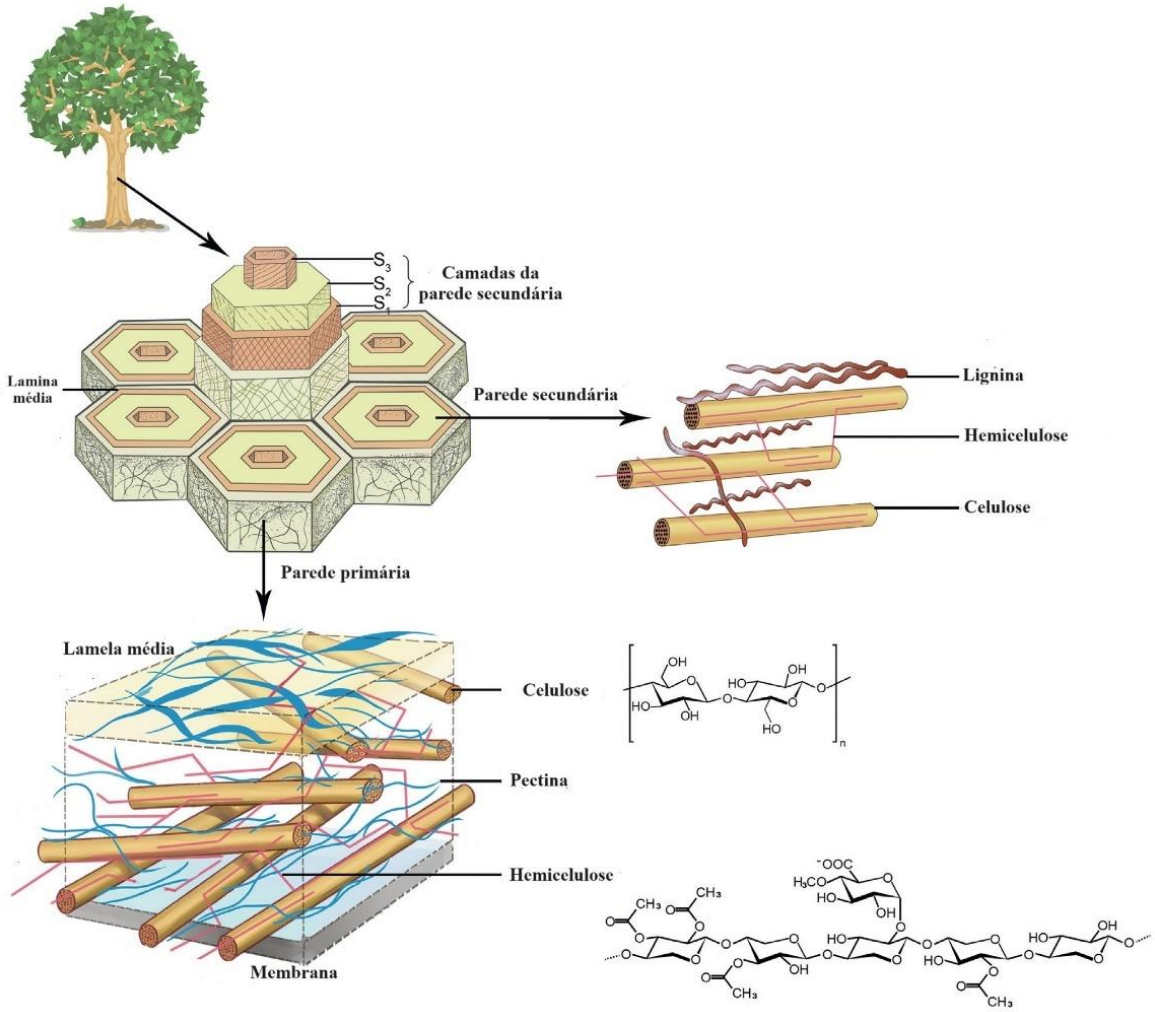
3.9 Polissacarídeos vegetais

Os polissacarídeos são cadeias poliméricas de carboidratos, compostos por monossacarídeos interligados por ligações glicosídicas, seja na conformação α ou β (Zhang et al., 2023). Esses polímeros exibem variações em sua massa molar média (Rao et al., 2024; Zhu et al., 2023) e são formados estruturalmente por um grupo heterogêneo de compostos ácidos ou neutros (Ren et al., 2019). Em vegetais, são amplamente distribuídos, podendo ser encontrados nas cascas do caule (Araujo et al., 2021), folhas (Campo-Grande et al., 2023), sementes (Sui & Xu, 2022), raízes (Liu et al., 2023) e frutos (Gao et al., 2023), sendo constituídos principalmente por pentoses (D-xilose, L-arabinose), hexoses (D-glucose, D manose, D-galactose), ácido urônico (ácido D-glucurônico, ácido D-galacturônico, ácido D-4-

O-metil-glucurônico) e, em menor quantidade, desoxi-hexoses (L-ramnose, L-fucose) (Navarro et al., 2019; Willför et al., 2009).

As plantas exibem uma organização em camadas, conhecidas como parede primaria e secundaria (Figura 9). As paredes primárias são formadas durante o crescimento e geralmente são estruturas relativamente finas, flexíveis e altamente hidratadas, sendo compostas de celulose (15–40%), xiloglucanos (hemicelulose) (20–30%), polissacarídeos pécticos (30–50%) e menores quantidades de arabinoxilanos e proteínas (Cosgrove & Jarvis, 2012). Por outro lado, as paredes secundárias conferem resistência aos tecidos vegetais, contendo maior proporção de celulose, com lignina, xilanas e glucomananas (Speck & Burgert, 2011).

Figura 9. Estrutura e composição da parede celular primária e secundária das plantas.



Fonte: Adaptado de Zhao *et al*. (2019).

A molécula de celulose é composta por unidades de glicose unidas por ligações β-1,4-glicosídicas, e as cadeias de glucano se associam por meio de ligações de hidrogênio e

forças de van der Waals, formando uma longa estrutura cristalina, denominada de microfibrila de celulose (Zhao et al., 2019). Além da importância biológica, a celulose desempenha um papel fundamental como matéria-prima para produção têxtil, papel, materiais de construção e derivados químicos de importância industrial (Rongpipi et al., 2019). Esse fato contribui para que a celulose seja considerada o carboidrato mais abundante do planeta, destacando-se como uma fonte promissora de energia renovável (Seddiqi et al., 2021).

A hemicelulose também é um polimero natural composto por arabinose, manose, galactose, glicose e xilose, caracterizado por possuir grupos ramificados e estruturas abertas (Benaimeche et al., 2020). Seu papel biológico crucial reside no fortalecimento da parede celular pela interação com a celulose e a lignina, representando aproximadamente um terço da biomassa lignocelulósica (Scheller & Ulvskov, 2010; Wan et al., 2021). Além de desenvolver essa função vital, a hemicelulose encontra ampla aplicação em diversos setores, incluindo a produção de filmes, bem como nas indústrias alimentícias e farmacêuticas (Berglund et al., 2020; Mikkonen et al., 2013).

A pectina é um polissacarídeo altamente complexo, caracterizado pela presença de ácido α-1,4-galacturônico na sua cadeia principal, desempenhando papel na integridade estrutural, resistência, flexibilidade à parede celular e atuando como barreira ao ambiente externo (Shin et al., 2021; Wan et al., 2021). Esse polímero tem sido amplamente explorado como fibra alimentar, uma vez que não é digerível pelos seres humanos devido à falta de enzimas digestivas específica para as pectinas (Roman-Benn et al., 2023). No entanto, microrganismos presentes no intestino grosso podem metabolizar facilmente essa molécula, convertendo-a em fibras solúveis (Vanitha & Khan, 2020). Dessa forma, os polissacarídeos pécticos têm sido alvo de pesquisas, por apresentarem baixa toxicidade (Jonker et al., 2020) e aplicações, incluindo biotecnologia, onde são utilizados como emulsificante, estabilizante e gelificante (Li et al., 2022). Além disso, destacam-se em atividades biológicas e farmacológicas, como imunomoduladora (Huang et al., 2021), antioxidante (Pak et al., 2023), anti-inflamatória (Liu et al., 2023), gastroprotetora (Oliveira et al., 2018) e prebiótica (Hou et al., 2022).

Quanto aos polissacarídeos naturais utilizados a nível industrial, as gomas destacam-se como polímeros de carboidratos sintetizados e excretados pelas plantas como parte do seu mecanismo de defesa, particularmente em condições de estresse, como danos físicos (ruptura do tecido) e ataque de insetos ou fungos (Bouaziz et al., 2016; Chen et al., 2022). Na natureza, esses compostos apresentam-se como gomas de sementes, exsudato ou mucilagem (Figura 10) (Barak et al., 2020).

Figura 10. Gomas de origem vegetal.

Goma Exsudato Mucilagem

Fonte: Elaborado pelo autor (2025).

Os exsudatos são facilmente removidos das árvores e destacam-se por sua especificidade e solubilidade em água (Gupta et al., 2020). Apresentam-se em formas de botões ou massas disformes, uma adaptação para evitar infecções e perda de água na área danificada, exibindo cores que variam de branco, cinza claro, âmbar ou marrom escuro (Eghbaljoo et al., 2022). A utilização desses biopolímeros é atribuída às propriedades atóxicas, composição química, biocompatibilidade e biodegradabilidade, conferindo efeitos biológicos e se destacando em aplicações na indústria médica e/ou ambiental (Licá et al., 2018, Mohammadinejad et al., 2020).

Dentre os exsudatos, destacam-se a goma arábica, karaya, ghatthi e tragacanta, que desempenham papeis cruciais na indústria alimentícia como ingredientes, influenciando a forma, textura, ligação à água e efeitos sensoriais (Gupta et al., 2018). Além disso, essas gomas encontram aplicação em diversas áreas industriais e biomédicas, desempenhando funções como estabilizantes, gelificantes, espessantes e emulsificantes (Ahmadian et al., 2023). Isso se deve à capacidade dessas substâncias de interagirem com a água, comportamento reológico e encapsulamento de diferentes materiais como sabores, aromas e agentes nutracêuticos, incluindo a capacidade de produzir filmes ou géis (Baranwal et al., 2022; Mittal et al., 2022; Yadav et al., 2023).

4 RESULTADOS E DISCUSSÕES

4.1 ARTIGO I – A SER SUBMETIDO

Parkia pendula exudate polysaccharide: Physicochemical and structural characterization and impact on human gut microbiota

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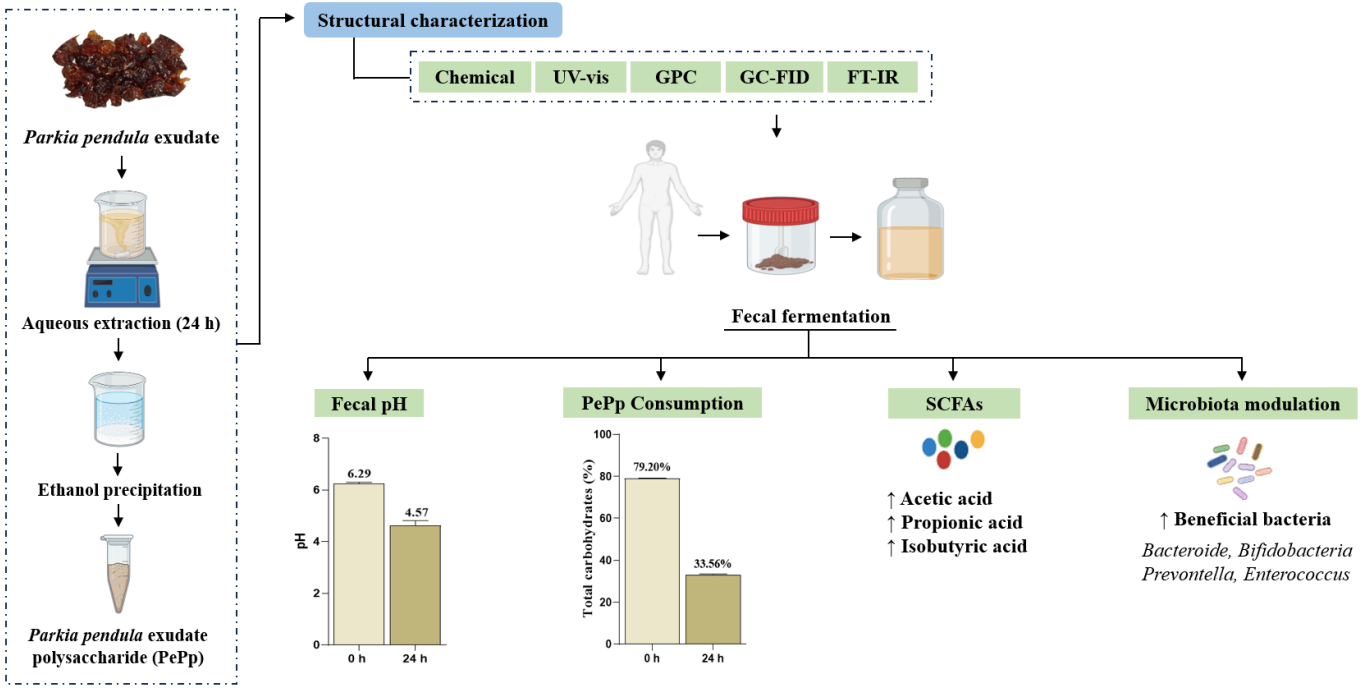
Highlights

- *Parkia pendula* polysaccharide (PePp) contains arabinose, galactose and uronic acid.
- PePp is a galactoarabinan.
- PePp is consumed by gut bacteria.
- PePp significantly increased the concentration of SCFAs.
- PePp modulates gut microbiota composition.

Graphical abstract



ABSTRACT

A polysaccharide from the exudate of *Parkia pendula* (PePp) was obtained by aqueous extraction and precipitation with ethyl alcohol, and its chemical and structural properties, as well as its *in vitro* fermentation by gut microbiota, were evaluated. Chemical analysis revealed a high percentage of total carbohydrates (79.20%) and uronic acid (11.0%), with a predominance of arabinose (78%), galactose (10%), and uronic acid (12%), indicating that PePp is an galactoarabinan. The molecular weight was estimated at 7.08 x 10^4 g/mol, and FT-IR analysis detected characteristic bands of polysaccharides and uronic acid (wavenumbers: 1309-1216 and 1410 cm^{-1}, respectively). During *in vitro* fecal fermentation, PePp significantly reduced fecal pH from 6.29 to 4.35 and promoted microbial growth (OD from 0.664 to 3.34). PePp was consumed by the gut microbiota (from 79.20 to 33.56%), with an increase in short-chain fatty acid production, including acetic, propionic, and isobutyric acids. Additionally, PePp stimulated the proliferation of beneficial bacteria belonging to the genera *Bacteroides*, *Bifidobacterium*, *Prevotella*, and *Enterococcus*. These findings highlight the prebiotic potential of PePp and its relevance for supporting gut health.

Keywords: *Parkia pendula* exudate; galactoarabinan; prebiotic; gut microbiota.

1. Introduction

The human gut microbiota is a highly diverse and dynamic microbial community that inhabits the gastrointestinal tract (Qadri et al., 2024). These microorganisms play essential roles in host physiology, including energy metabolism, nutrient absorption, immune modulation, and maintenance of gut homeostasis (Dasriya et al., 2024). Given their central importance to human health, increasing attention has been directed toward bioactive compounds capable of modulating the composition and function of the gut microbiota. Among these, carbohydrate polymers stand out due to their structural diversity and functional potential.

Polysaccharides, high molecular weight macromolecules composed of long chains of monosaccharide units linked by glycosidic bonds (Benalaya et al., 2024), have attracted considerable interest due to their ability to act as fermentable substrates for beneficial gut bacteria, such as probiotics, promoting their growth and suppressing harmful microorganisms (Cho et al., 2020; Liu et al., 2021). Polysaccharides contribute to the establishment of a balanced microbial profile and the enhancement of essential physiological functions, including immune regulation and gut barrier integrity (Yang et al., 2024). The fermentation of these polysaccharides results in the production of short-chain fatty acids (SCFAs), which have garnered particular interest for their positive effects on epithelial barrier preservation and immune response modulation (Facchin et al., 2024). Consequently, the interaction between polysaccharides and the gut microbiota has emerged as a promising avenue for improving host health.

The genus *Parkia* (Fabaceae) comprises approximately 35 pantropical species, many of which are traditionally used in folk medicine in the form of pastes, decoctions, or juices for the treatment of diarrhea, diabetes, skin ulcers, wounds, and hypertension (Phumthum & Balslev, 2018; Saleh et al., 2021; Srisawat et al., 2016). *Parkia pendula* is widely distributed, especially in the Amazon region of Brazil, and is frequently used in ecological restoration due to its rapid growth, nitrogen-fixing ability, and ecological role in attracting wildlife (Cordeiro et al., 2021; Correa et al., 2021). Ethnobotanical records also describe the use of its bark and leaves in the treatment of infections and malaria (Andel et al., 2007; Ferreira et al., 2017). The exudate produced by the pods of *P. pendula* is particularly rich in polysaccharides, which have shown potential as a magnetic polysaccharide matrix for Concanavalin A immobilization (Rêgo et al., 2014).

Despite its promising bioactivities, the structural characteristics of the *P. pendula* polysaccharide and its interaction with the human gut microbiota remain unexplored. In this

context, *in vitro* gastrointestinal fermentation models offer a controlled and reliable approach to evaluate, under anaerobic conditions, how complex polysaccharide structures are metabolized by the human gut microbiota. These models allow for a detailed analysis of microbiota–carbohydrate interactions and provide information into how structural complexity influences microbial composition and metabolic output (Chen et al., 2024; Jia et al., 2024; Liu et al., 2024).

In the present study, we investigated the structural characteristics of the polysaccharide extracted from the exudate of *P. pendula* (PePp) and evaluated its modulatory effects on the human gut microbiota using an *in vitro* fermentation model. The results of this study aim to advance our understanding of the relationship between polysaccharide structure and microbial metabolism, and to highlight the potential of PePp as a functional dietary component with prebiotic properties.

2. Materials and methods

2.1 Plant material

The exudate of *Parkia pendula* was collected from the pods of the species in the municipality of Novo Lino, Alagoas, Brazil (8°58′34.9”S, 35°40′29.2”W). The collection was carried out by the authors in December 2023. The specimen (N° 92,516) was deposited in the IPA—Dárdano de Andrade Lima Herbarium of the Agronomic Institute of Pernambuco.

2.2 Preparation of the PePp

A quantity of 15 g of crude exudate was weighed on an analytical balance (Bel M214-AIH) and suspended in 100 mL of distilled water at room temperature (25 ± 2 °C) for 24 h, allowing the solubilization of water-soluble components. After this period, the suspension underwent a sequential filtration process to remove insoluble particles, using “voil” and a screen-printing screen (90 threads), ensuring the retention of larger fragments and impurities. The obtained filtrate, corresponding to the aqueous extract, was subjected to ethanol precipitation at a 1:4 ratio (v/v) (extract: absolute ethanol, 99%) and kept under refrigeration at 4 °C for 18 h. The precipitated material was transferred to 50 mL tubes and centrifuged (LMC-4200R, Biosan) at 3000 rpm for 15 min, separating the supernatant from the pellet containing the polysaccharides. The supernatant was discarded, and the precipitate underwent three sequential washes with 10 mL of acetone (≥99%) for 30 min each, removing low molecular weight compounds and residual impurities. The material was then dried in an oven at 55 °C until a completely dry solid residue was obtained. After drying, the material

was ground in a blade mill (IKA® A11 basic), and the final product was designated as Parkia pendula exudate polysaccharides (PePp) (Batista et al., 2025).

2.3 Chemical analysis

The chemical composition of PePp was analyzed by spectrophotometry to determine the total carbohydrate content (Dubois et al., 1956), uronic acid (Blumenkrantz & Asboe-Hansen, 1973), phenolic compounds (Singleton & Rossi, 1965) and proteins (Bradford, 1976). D-galactose (A_{490} nm), D-galacturonic acid (A_{520} nm), gallic acid (A_{765} nm) and bovine serum albumin (A_{595} nm) were used as standards, respectively.

2.4 Ultraviolet (UV) spectroscopy analysis

PePp was dissolved in distilled water at a concentration of 2 mg/mL and analyzed using a UV-1800 spectrophotometer (SHIMADZU, Japan) at room temperature over a wavelength range of 200 to 400 nm (Peng et al., 2023).

2.5 Homogeneity and molecular weight (Mw) analysis

The molecular weight of PePp was determined by gel permeation chromatography (GPC) using a Shimadzu LC-20AD chromatograph equipped with a refractive index detector (RID-10A). PePp was solubilized in 0.1 mol/L sodium nitrate, which was also used as eluent. A 50 µL PePp solution was eluted at a flow rate of 1 mL/min through a PolySep column (7.8 x 300 mm), at 25 °C. Molecular weight was calculated using the calibration curve equation (LogMp = 14.2852 – 1.1677Ve; Mp: molar mass; Ve: elution volume), with pullulan polymer of different molecular masses ranging from 10^3 at 10^6 g/mol (MW of 5.9 x 10^3, 1.18 x 10^4, 4.73 x 10^4, 2.12 x 10^5 and 7.88 x 10^5 g/mol) as standards (Pereira Júnior et al., 2021).

2.6 Monosaccharide composition analysis

The monosaccharides composition was determined by analyzing the alditol acetate content using gas chromatography with flame ionization detection (GC-FID) and 2-deoxyglucose as an internal standard. PePp (2 mg) was first pre-hydrolyzed in 72% (v/v) sulfuric acid (H_2SO_4) (0.2 mL) for 3 h at room temperature, then hydrolyzed with 1 M H_2SO_4 for 2.5 h, at 100 ºC. After 1 h of hydrolysis, 500 µl of the solution was collected for uronic acid analysis. The remaining solution was reduced with sodium borohydride (15% in 3 M ammonia, for 1 h, at 30 °C), and acetylated with acetic anhydride (3 mL) and 1-methylimidazole (450 µL), for 30 min at 30 °C. The alditol acetates were separated by liquid-liquid extraction using water and dichloromethane. After evaporation of the organic solvent,

they alditol acetates were solubilized in anhydrous acetone and analyzed using a Perkin Elmer-Clarus 400 GC-FID equipped with a DB-225 capillary column (30 m long, 0.25 mm internal diameter and 0.15 μm film thickness). The oven temperature was programmed to increase from 200 ºC to 220 ºC at a rate of 40 ºC/min (held for 7 min), then to 230 ºC at a rate of 20 ºC/min (held for 1 min). The injector temperature was set to 220 ºC and the detector to 230 ºC, with hydrogen as the carrier gas (flow rate: 1.7 mL/min) (Blakeney et al., 1983; Selvendran et al., 1979).

The uronic acid content was determined using the m-phenylphenol method, with galacturonic acid as the standard (Blumenkrantz & Asboe-Hansen, 1973). To test tubes containing hydrolyzed PePp (500 μL, 1:4), 3 mL of 50 mM boric acid and 98% (w/w) H_2SO_4 were added, then the mixture was stirred and heated to 100 °C for 10 min. After cooling, 100 μL of m-phenylphenol was added, and the reaction was allowed to proceed in the dark for 30 min. The absorbance was then measured at 520 nm using a spectrophotometer.

2.7 Infrared (IR) spectrum analysis

The FT-IR spectrum of PePp was recorded using a Fourier transform infrared spectrophotometer IR-Tracer 100 (SHIMADZU, Japan). Briefly, 2 mg of the sample was mixed with 100 mg of potassium bromide (KBr) powder and pressed into 1 mm thick pellets for measurement. The test conditions were as follows: a frequency range 4000–400 cm^{-1} with a resolution 4 cm^{-1}, using 16 scans to acquire the FT-IR spectra.

2.8 *In vitro* fermentation of fecal inocula

2.8.1 Collection and preparation of fecal inoculum

Fresh feces were obtained from five healthy donors aged 21 to 45 years. An informed consent form was distributed among anonymous donors. Participants had not undergone antibiotic treatment, probiotic or prebiotic supplementation for at least 6 months prior to fecal sample donation and did not have gastrointestinal diseases. Fecal samples were collected in sterile vials, kept under anaerobic conditions, and used within 2 hours after collection. Fecal inoculum (FI) was prepared following the methodology proposed by Roupar et al. (2022). Briefly, FI was prepared by diluting feces in reduced physiological saline solution (RSS). The SFR solution consisted of cysteine-HCl (0.5 g/L) and NaCl (8.5 g/L) to obtain a concentration of 100 g of feces/L of RPS with a final pH of 6.8.

2.8.2 Preparation of the fermentation medium

In vitro fermentation of fecal inocula was conducted based on an established method with minor modification (Roupar et al., 2022). The basal medium consisted of trypticase soy broth without dextrose (5.0 g/L), bactopeptone (5.0 g/L), cysteine-HCl (0.5 g/L), saline solution A at 1.0% (v/v) [NH_4Cl 100.0 g/L, $MgCl_2•_6H_2O$ 10.0 g/L, $CaCl_2•_2H_2O$ 10.0 g/L], trace element solution at 1.0% (v/v), saline solution B at 0.2% (v/v) [$K_2HPO_4•_3H_2O$ 200.0 g/L], and resazurin solution at 0.2% (v/v) (0.5 g/L), with the pH adjusted to 6.8 using 1 M HCl. The basal medium (50 mL) was then distributed into anaerobic flasks, which were sealed with aluminum caps, and bubbled with a gas mixture of 10% CO_2, 5% H_2, and 85% N_2 before sterilization in an autoclave at 121°C for 15 min.

2.8.3 Fecal fermentations

In vitro fermentations were conducted using a static batch model under anaerobic conditions (85% N_2, 10% CO_2, and 5% H_2). For each donor, a fecal slurry (1 mL) was inoculated into 9.0 mL of basal nutrient medium without a carbon source (negative control – NC), basal medium supplemented with inulin (INU, positive control), or basal medium containing PePp. All experimental groups were incubated at 37 °C, for 48 h. Samples were collected at 0, 6, 12, and 24 h to evaluate changes in pH microbial growth, the latter assessed by measuring optical density at 600 nm (OD_{600}), sugar content and short-chain fatty acids (SCFAs) (Roupar et al., 2022). Prior to analysis, samples were centrifuged at 4,000 rpm for 6 min. The supernatants were used to assess total carbohydrate content and SCFA concentrations. The resulting pellets collected at 0 and 24 h fermentation were reserved for genomic DNA extraction, which was subsequently used for intestinal microbiota analysis.

2.8.4 Carbohydrate and organic acids analysis

Neutral sugars from the PePp sample were released by chemical hydrolysis using 200 µL of 72% (w/w) H_2SO_4 for 3 h at room temperature with occasional stirring, followed by further hydrolysis with 1.0 mol/L H_2SO_4 for 2.5 h at 100 °C in a heating block. The hydrolysates were neutralized with 25% NH_3 and subsequently reduced using acetic anhydride (3 mL) in the presence of 1-methylimidazole (450 µL) for 30 min at 30 °C. The resulting alditol acetates were extracted with dichloromethane and analyzed by gas chromatography with flame ionization detection (GC-FID) using a Perkin Elmer–Clarus 400 system equipped with a 30 m DB-225 capillary column (J&W Scientific, Folsom, CA, USA) with an internal diameter of 0.25 mm and a film thickness of 0.15 µm. All analyses were performed in triplicate (Hamed et al., 2022).

2.8.5 Analysis of SCFAs during *in vitro* fermentation

To determine the organic acid content, the collected samples were centrifuged (5000 rpm, 10 min) and the supernatant was filtered through cellulose acetate membranes (0.22 μm) prior to analysis. The aliquots were diluted with an internal standard (crotonic acid) in 8:2 ratio and analyzed using an HPLC system equipped with a Rezex ROA-Organic Acid H+ column (300 × 7.8 mm, Phenomenex). The mobile phase was 2.5 mM sulfuric acid at a flow rate 0.6 mL/min. Detection was performed at 210 nm, with average retention time of 15.83 min for lactic acid, 17.01 min for formic acid, 18.50 min for acetic acid, 21.77 min for propionic acid, 24.56 min for isobutyric acid, and 26.58 min for n-butyric acid. Short-chain fatty acid (SCFA) concentrations were determined by interpolating the sample areas according to a calibration curve generated with standard solutions (Parente et al., 2022).

2.8.6 DNA extraction and microbiota identification

Genomic DNA (gDNA) was extracted and purified from human fecal samples using the NZY Tissue gDNA Isolation Kit (NZYTech, Portugal), following the manufacturer's protocol with minor modifications. Briefly, fecal pellets were homogenized in Tris-EDTA buffer (10 mM Tris/HCl; 1 mM EDTA; pH 8.0) and centrifuged at 4000 × g for 10 min. This washing step was repeated until the supernatant became colorless. Subsequently, 180 μL of lysozyme solution (10 mg/mL in NaCl-EDTA, 30 mM:10 mM) was added, and samples were incubated at 37 °C for 1 h with periodic mixing. The pellets were then resuspended in 350 μL of NT1 buffer and incubated at 95 °C for 10 min. After cooling, samples were centrifuged at 11,000 × g for 10 min at 4 °C, and 200 μL of the supernatant was mixed with 25 μL of proteinase K, followed by incubation at 56 °C for 1 h. Subsequent steps followed the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

The taxonomic analysis of the microbial communities in each fermentation sample were analyzed by sequencing the 16S rRNA gene (V4 region) using the prokaryotic universal primer pair 515f/806r, by MiSeq Illumina sequencing.

2.9 Statistical analysis

All experimental procedures were performed in triplicate, and results were expressed as mean±standard deviation. Means were compared using Analysis of Variance (ANOVA) followed by a one-way test using GraphPad Prism software version 8.0 (Inc., California,

USA). Statistical significance was considered when $p < 0.05$, indicating the presence of significant differences.

3. Results and discussion

3.1 Extraction and chemical composition of the polysaccharide

The polysaccharide extracted from the exudate of *Parkia pendula*, referred as PePp, was obtained through aqueous extraction, followed by precipitation with ethyl alcohol and subsequent drying. The yield of PePp was 48.2%, corresponding to 7.23 g of dry powder (Table 1). This yield is comparable to the polysaccharide extracted from *Prunus persica* exudate (45%) (Qian et al., 2011), lower than that of *Prunus domestica* (73.05%) (Sharma et al., 2020) and higher than that of *Amygdalus scoparia* (8.21%) (Molaei & Jahanbin, 2018). The chemical composition analysis (Table 1) revealed that PePp contains a high content of total carbohydrates (79.20 ± 0.77) and uronic acid (11.00 ± 0.22), along with low levels of polyphenols (2.72 ± 0.06 mg/g in GAE) and proteins (1.20 ± 0.62). The combination of aqueous extraction and alcohol precipitation proved to be an effective method for obtaining polysaccharides with high carbohydrate content, consistent with studies that used similar approaches for exudate polysaccharide extraction (Chaudhari & Annapure, 2021; Malsawmtluangi et al., 2014; Mohebbi et al., 2022; Pachuau et al., 2012; Sharma et al., 2020).

Table 1. Yield, chemical composition, and monosaccharide profile of the polysaccharide extracted from *Parkia pendula* exudate (PePp).

Analysis	PePp
Yield (%)	48.2 ± 0.84
Chemical composition	
Total carbohydrates (%)	79.20 ± 0.77
Uronic acid (%)	11.00 ± 0.22
Polyphenols (mg/g in GAE)	2.72 ± 0.06
Proteins (%)	1.20 ± 0.62
Composition of monosaccharides (% mol)	
Arabinose	78
Galactose	10
Uronic Acid	12

Yield and chemical composition values were represented as the mean ± SD (n = 3).

3.2 Molecular weight and monosaccharide composition analysis

The relative molecular weight distribution and homogeneity of PePp were analyzed by GPC. The results revealed that PePp shows a single symmetrical peak with an elution volume of 8.08 mL. From the linear equation of the calibration curve, generated with pullulan

standards (LogMp = 14.2852 – 1.1677Ve), the molar mass of PePp was estimated to be 7.08 x 10^4 g/mol (Fig. 1). These results indicated that PePp has high purity and molecular weight. However, studies on polysaccharides from other species, such as Citrus × latifolia (Ribeiro et al., 2023) and *Prunus persica* (Wei et al., 2023) have also reported similar molecular weights.

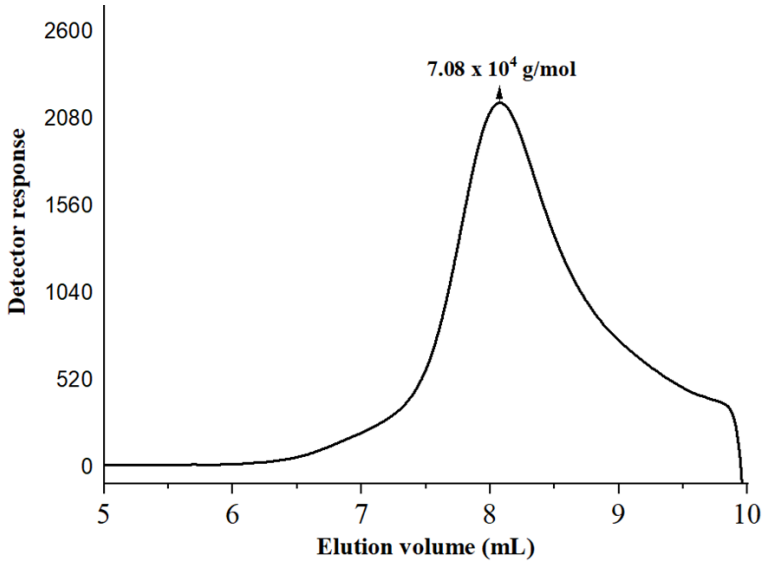


Fig. 1. Elution profile of PePp by GPC-RID chromatogram.

The monosaccharide composition of PePp was analyzed by GC-FID. The results indicated the presence of three monosaccharides, arabinose, galactose, and uronic acid, with percentages of 78%, 10%, and 12%, respectively (Table 1). The high percentage of arabinose and galactose suggests that PePp is an galactoarabinan. This monosaccharide composition is consistent with other polysaccharides, which also showed arabinose and galactose as predominant components (Li et al., 2023; Wang et al., 2024).

3.3 UV and FT-IR analysis

The UV-vis spectrum of PePp, illustrated in Fig. 2A, showed no evident absorption peaks at wavelengths between 260 and 280 nm, suggesting the absence of nucleic acids and free or residual bound proteins (Zhu et al., 2024). This finding is consistent with the chemical composition data presented in Table 1, which indicates a low protein content in PePp.

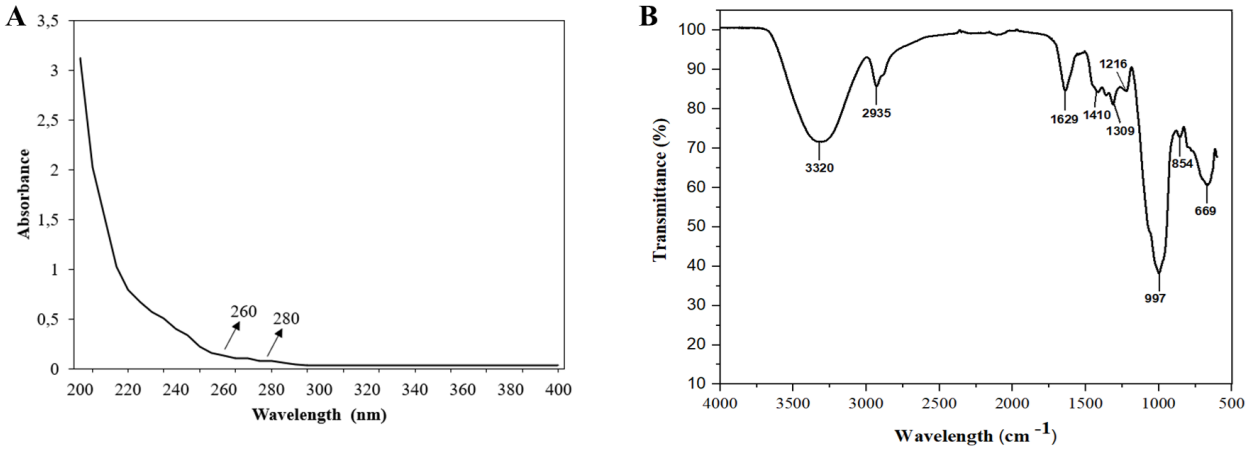


Fig. 2. Ultraviolet (UV-vis) spectrum (A) and FT-IR spectrum (B) of PePp.

The functional groups and chemical bonds illustrated in the FT-IR spectrum of PePp (Fig. 2B). The intense and broad absorption band at 3320 cm^{-1} was attributed to the stretching vibration of hydroxyl groups present in the polysaccharide (Jing et al., 2017). The bands at 2935 cm^{-1} and 1629 cm^{-1} are indicative of the stretching of CH- and C=C bonds or the vibration of the H-O-H linkage, respectively (Li et al., 2020). The absorption around 1410 cm^{-1} suggests the presence of carboxyl groups (-COOH) in the sugar chain, indicating that the polysaccharide contains uronic acid (Su & Li, 2020), which is consistent with the chemical and monosaccharide composition data of PePp. The bands at 1309 cm^{-1} and 1216 cm^{-1} are characteristics of polysaccharides (Li et al., 2022), while the band at 854 cm^{-1} indicates the presence of α- and β-glycosidic bonds (Guo et al., 2022). The band at 669 cm^{-1} is characteristic of -CH bending, O-C=O bending, O-C-O bending, and CO=O (Al-Ajalein et al., 2023). Thu, the FT-IR spectrum of PePp confirms the presence of the main functional group's characteristic of polysaccharides.

3.4 Changes in pH and optical density during fermentation

The interaction between the gut microbiota and dietary nutrients can influence the host's eating behavior (Yu et al., 2024). To monitor the fecal fermentation process of PePp over 24 h, indicators such as pH and optical density (OD_{600}) were used (Fig. 3). As shown in Fig. 3a, at the initial time (0 h), the pH values in the PePp and INU groups were similar to the negative control (NC) group, with a pH of around 6.29. The latter group recorded pH values of 6.56 and 6.74 after 12 and 24 h, respectively. In contrast, the PePp and INU groups showed a significant reduction in fecal pH to 5.87 and 4.57 after 12 h, and to 4.53 and 4.35 after 24 h, respectively. We believe that PePp can modulate the composition of the human gut

microbiota, promoting the production of acidic compounds, as the reduction in pH favors a more acidified gut environment, which benefits the growth of SCFA-producing bacteria and inhibits the proliferation of pathogenic microorganisms in the gut tract (Kong et al., 2021). Furthermore, our results are consistent with polysaccharides isolated from *Pleurotus eryngii* (Ma et al., 2022) and *Ficus carica* (Xu et al., 2024), showing a reduction in pH and consequent alteration of the bacterial population and variations in SCFA proportions.

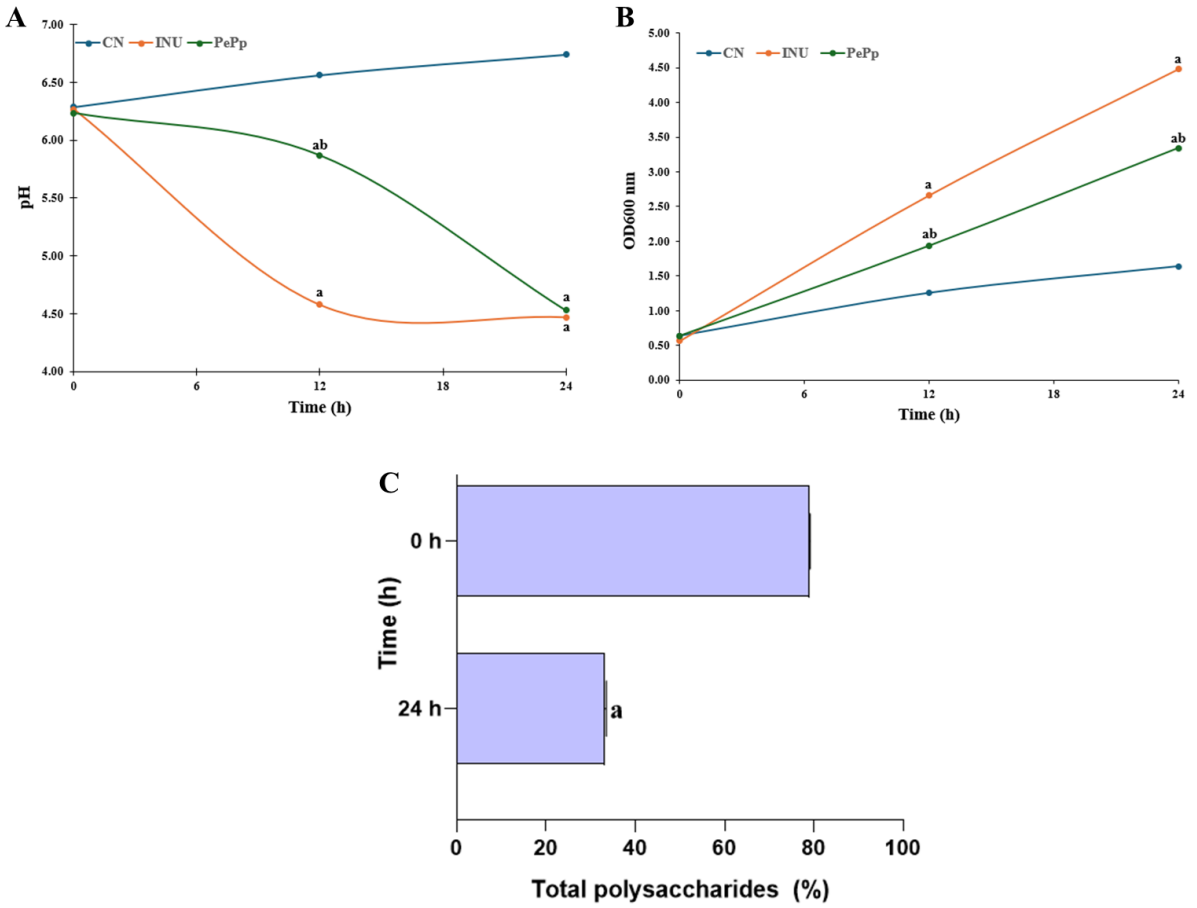


Fig. 3. Changes in indicators during fecal fermentation. (A) pH values, (B) optical density (OD) and (C) PePp consumption. The data presented indicate significant differences with [a]$p<0.0001$ compared to the negative control (NC) group and [b]$p<0.0001$ compared to inulin.

Gut bacteria utilize carbohydrates as an energy source (Mora-Flores et al., 2023). Thus, variations in the density of these bacteria in fermented cultures can be evaluated through optical density (OD), allowing for the estimation of bacterial growth based on the availability of carbon sources. As shown in Fig. 3b, the NC, PePp, and INU groups presented average OD values of 0.630, 0.664, and 0.618, respectively, at time 0. After 12 and 24 h of fermentation, the NC group recorded a slight increase in OD to 1.25 and 1.64, respectively; this may be associated with the absence of available carbon sources, limiting the proliferation

of microorganisms (Ge et al., 2024). In contrast, the PePp and INU groups, compared to the NC, showed a significant increase in OD, reaching 1.93 and 2.66 after 12 h, and 3.34 and 4.48 after 24 h of fermentation, respectively. The OD value of the PePp group was significantly higher than that of the NC group, suggesting that this polysaccharide may serve as a nutrient source for the growth of gut microorganisms, as within a specific concentration range, the OD value in the fermentation medium is directly proportional to the concentration of bacteria in the solution (Guo et al., 2022). Furthermore, this result reinforces the previously demonstrated prebiotic potential of PePp and highlights the similarity to the *in vitro* fecal fermentation observed in other polysaccharides (Duan et al., 2023; Ye et al., 2024).

3.5 Changes in total carbohydrates after fermentation

The ability of the gut microbiota to degrade polysaccharides and utilize them as an energy source is fundamental for human health (Yang et al., 2024). In this context, we investigated the changes in total carbohydrate content after 24 h of fecal fermentation. As illustrated in Fig. 3c, the initial total carbohydrate content in the fermentation of PePp was 79.20%, reducing to 33.56% after 24 h of fermentation. This result highlights the efficiency of the microbiota in metabolizing PePp, suggesting high bioavailability. The significant degradation of carbohydrates can be attributed to the production of active enzymes by the microbiota, which break the glycosidic bonds of the polysaccharide, facilitating the utilization of the resulting fragments (Huang et al., 2020). Beyond demonstrating the microbial capacity for degradation, these findings suggests that PePp serves as a promising fermentable substrate with potential benefits for, gut health.

3.6 SCFA production during fermentation

SCFAs are the main metabolites resulting from the fermentation of carbohydrates by the gut microbiota, playing an important role in maintaining gut health (Zhang et al., 2023). As shown in Table 2, the initial concentrations (T0) of total SCFAs (sum of acetic, propionic, and isobutyric acid) and individual SCFAs were similar among the NC, INU, and PePp groups. However, after 12 and 24 h of fermentation, the PePp group showed a significant increase in total SCFA concentrations, reaching 3.22 and 5.11 g/L, respectively, values higher than those observed in the INU (2.25 and 3.43 g/L) and NC (1.75 and 2.11 g/L) groups. The concentrations of acetic acid (1.01 and 1.97 g/L), propionic acid (1.52 and 1.87 g/L), and isobutyric acid (0.69 and 1.27 g/L) in the PePp group after 12 and 24 h of fermentation were also significantly greater than those recorded in the INU and NC groups, highlighting the

potential of this polysaccharide to promote SCFA production by gut bacteria. These findings suggest that PePp may impact the modulation of the gut microbiota and SCFA production, surpassing inulin, a widely recognized prebiotic

The production of SCFAs is closely related to the adaptation of gut bacterial communities, being strongly influenced by the availability and type of substrate available for fermentation (Fusco et al., 2023). Different monosaccharides are metabolized into specific SCFAs through complex metabolic pathways. Studies conducted by Harris et al. (2020) and Wu et al. (2021) demonstrated that the fermentation of polysaccharides, such as arabinogalactan, by the gut microbiota leads to increaseds concentrations of acetic acid, propionic acid, and isobutyric acid, findings that are consistent with the results of the present study. The structure and composition of polysaccharides, especially the presence of monosaccharides like arabinose and galactose in arabinogalactan, play a crucial role in the selectivity of fermentative pathways and, consequently, in the production of specific SCFAs (Wu et al., 2022). Thus, PePp demonstrated a remarkable ability to promote the differential production of SCFAs, which has relevant implications for human physiology. Acetic acid is widely used by muscles as an important energy source (Cifuentes et al., 2024); propionate is taken up by the liver and contributes to gluconeogenesis (Ding et al., 2019); while butyric acid serves as the main energy supplier for colonic epithelial cells (Fu et al., 2019). These findings suggest that PePp may modulate the gut microbiota and influence specific metabolic pathways in the human body.

Table 2. Concentration of short-chain fatty acids (SCFAs) during *in vitro* fecal fermentation.

Group	Time (h)	SCFAs (g/L)			
		Acetic Acid	Propionic acid	Isobutyric acid	Total SCFAs
White	0	0.17 ± 0.08	0.69 ± 0.05	0.18 ± 0.04	1.04 ± 0.17
	12	0.42 ± 0.14	0.75 ± 0.19	0.58 ± 0.05	1.75 ± 0.38
	24	0.74 ± 0.21	0.66 ± 0.07	0.71 ± 0.13	2.11 ± 0.41
Inulin	0	0.33 ± 0.04	0.88 ± 0.09	0.20 ± 0.03	1.41 ± 0.16
	12	0.47 ± 0.05	1.16 ± 0.16[a]	0.62 ± 0.21	2.25 ± 0.42[a]
	24	0.85 ± 0.12[a]	1.14 ± 0.25[a]	1.44 ± 0.16[a]	3.43 ± 0.53[a]
PePp	0	0.13 ± 0.02	0.83 ± 0.03	0.30 ± 0.08	1.26 ± 0.13
	12	1.01 ± 0.02[ab]	1.52 ± 0.21[ab]	0.69 ± 0.02	3.22 ± 0.25[ab]
	24	1.97 ± 0.08[ab]	1.87 ± 0.07[ab]	1.27 ± 0.22[a]	5.11 ± 0.37[ab]

Values expressed as mean ± deviation.
PePp: Polysaccharide from *P. pendula* exudate.
[a]$p < 0.0001$ significant difference when compared to the blank group at the same time.
[b]$p < 0.0001$ significant difference when compared to the inulin group at the same time.

3.7 Changes in the composition of the gut microbiota

To evaluate the species richness and uniformity of the microbiota after fermentation with PePp, both α (Shannon and Simpson indices) and β (Principal Coordinates Analysis - PCoA and Bray-Curtis analysis) diversities were analyzed, as illustrated in Fig. 4. Both the initial microbiota of the donors (T0) and the NC group showed high Shannon (Fig. 4a) and Simpson (Fig. 4b) indices, indicating a diverse microbiome. However, after intervention with PePp and INU, a reduction in gut microbiota diversity was observed, suggesting that these treatments influenced the composition of bacterial species compared to the initial state. The PCoA analysis (Fig. 4c) and the clustering dendrogram (Fig. 4d) of β diversity revealed distinct groupings in the microbiota compositions among the NC, PePp, and INU groups, highlighting clear differences between the groups. Similarly, previous studies with polysaccharides from *Coix lacryma-joby* (Ge et al., 2024) and *Lycium barbarum* (Liang et al., 2024) also reported reductions in diversity indices after specific interventions. The reduction in diversity observed in the PePp and INU groups, compared to the initial inoculum, may be associated with the addition of a single carbon source in the medium during *in vitro* simulations, in contrast to the human diet, which contains a variety of complex components (Xie et al., 2024).

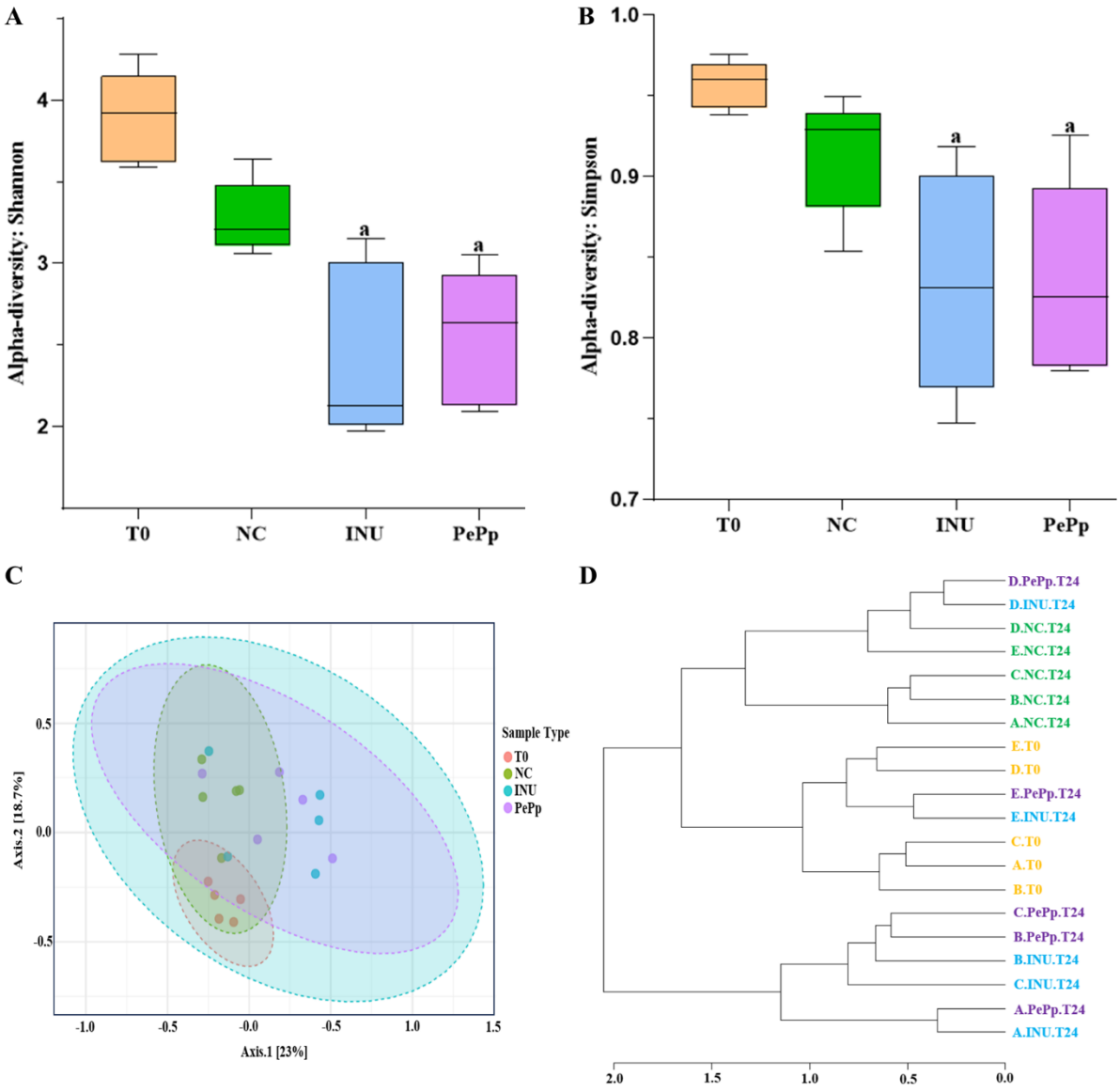


Fig. 4. Composition of α- and β-diversity of gut microbiota after 24 h of fermentation. (A) Shannon index, (B) Simpson index, (C) PCoA (principal component analysis) microbiota analysis, and (D) Hierarchical clustering tree using the Bray-Curtis index. Different fecal donors (=5) are highlighted by colors and letters (A-E). T0: initial fecal inoculum; INU: inulin; PePp: Polysaccharide from *P. pendula* exudate.

The bacterial composition at the phylum level for each donor is illustrated in Fig. 5a. In the initial fecal inoculum, the predominant phyla were Firmicutes, Actinobacteriota, Bacteroidota, and Proteobacteria, which together represent over 99% of the total microbiota in each donor. This distribution is consistent with previous reports describing the typical composition of the human gut microbiota (Zhou et al., 2023). After fermentation with PePp, significant changes in the microbiota composition were observed, with a decrease in the

abundance of Firmicutes from 34.15% to 11.51%, accompanied by an increase in the abundance of Bacteroidota (from 12.27% to 48.71%), Proteobacteria (from 3.76% to 23.98%), and Actinobacteriota (from 26.47% to 31.13%) (Fig. 5b). On the other hand, the group treated with INU showed increases of 19.06%, 35.53%, and 22.24% in these respective phyla. These results indicate that PePp and INU modulate the gut microbiota differently, highlighting their potential to alter bacterial populations. The observed increase in Bacteroidetes is particularly relevant, as these bacteria are known to hydrolyze polysaccharides through CAZyme enzymes, such as glycoside hydrolases, glycosyltransferases, carbohydrate esterases, and polysaccharide lyases (Bedu-Ferrari et al., 2022; Zafar & Saier, 2021). Furthermore, the reduction of Firmicutes and the increase of Bacteroidota have been associated with weight loss in experimental models with obese animals fed high-polysaccharide diets (Crovesy et al., 2020; Palmas et al., 2021; Stojanov et al., 2020). The increase in the abundance of Proteobacteria has been suggested as a potential marker for gut and extraintestinal diseases with inflammatory characteristics (Rizzatti et al., 2017), while Actinobacteriota plays an essential role in maintaining gut barrier homeostasis (Binda et al., 2018).

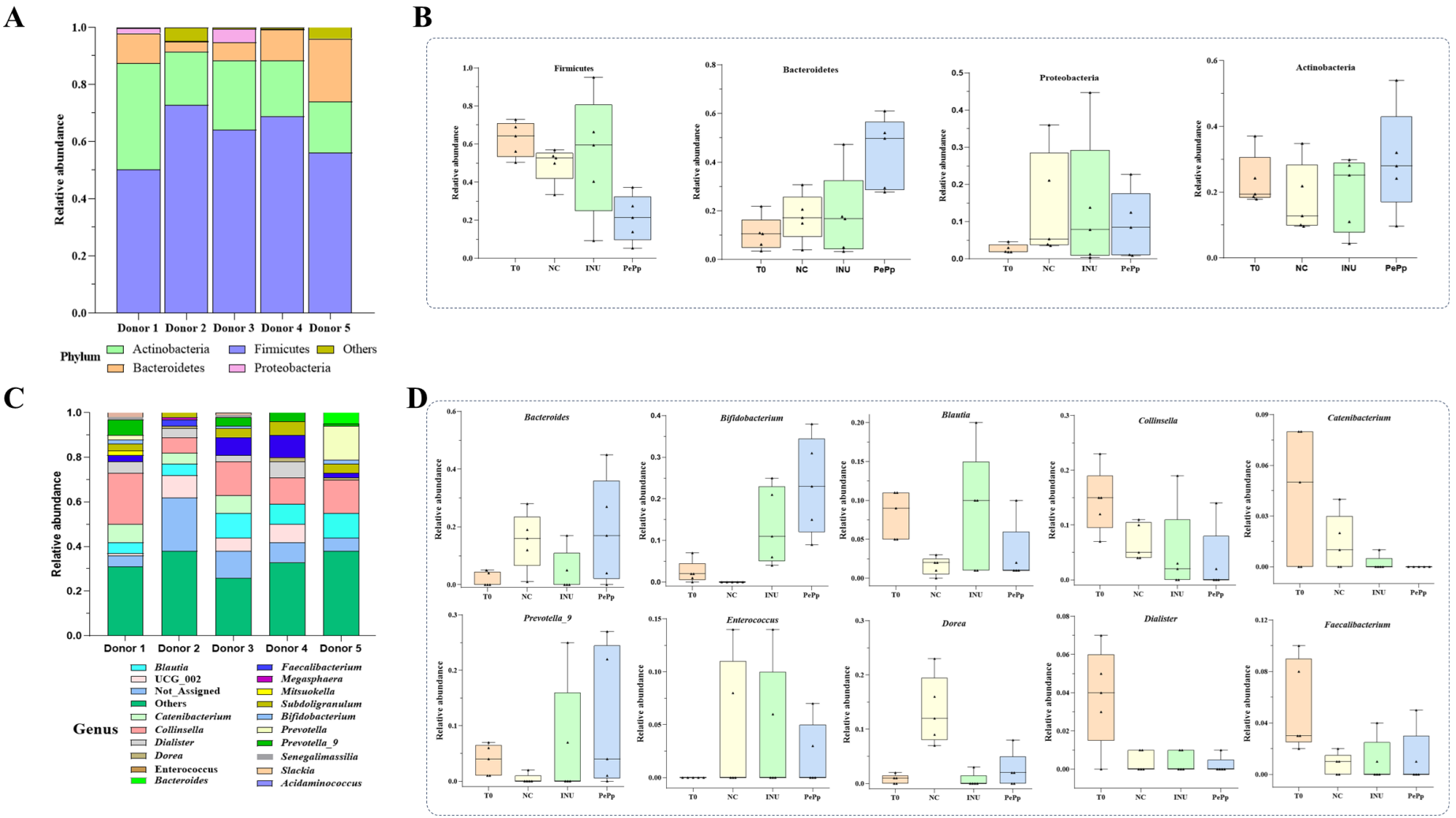


Fig. 5. Effect of *P. pendula* exudate polysaccharide (PePp) on gut microbiota after 24 h of fermentation. (A-B) Distribution of bacterial phyla, and (C-D) bacterial composition at genus level. T0: initial fecal inoculum; INU: inulin.

The bacterial composition at the genus level for each donor is illustrated in Fig. 5c. In the initial inoculum, the predominant genera are *Collinsella*, *Blautia*, *Faecalibacterium*, *Catenibacterium*, *Dialister*, *Prevotella* 9, and *Subdoligranulum*. After the intervention with PePp, there was an increase in the genera *Bacteroides*, *Bifidobacterium*, *Pr*evotella_9, and *Enterococcus*, accompanied by a reduction in the abundance of *Blautia*, *Collinsella*, *Catenibacterium*, *Dialister*, and *Faecalibacterium* (Fig. 5d).

Several studies highlight the importance of these bacterial genera for human health. (1) The genus *Bacteroides* has an arsenal of enzymes that metabolize SCFAs, such as acetate, propionate, and butyrate, from carbohydrates. These SCFAs play important roles in modulating gut inflammation, immunity, and maintaining the balance of the gut microbiota (Portincasa et al., 2022). (2) *Bifidobacterium* is an important probiotic for human health and acts in the hydrolysis of arabinogalactan-type carbohydrates through enzymes such as β-galactanases, β-galactosidases, α-arabinofuranosidase, and β-L-arabinopyranosidase, producing SCFAs like acetate, butyrate, and propionate (Ge et al., 2024; Gullón et al., 2013). It has been reported that monosaccharides such as arabinose and galactose can promote the growth of *Bifidobacterium* (Wang & LaPointe, 2020). (3) *Prevotella* plays an important role in maintaining health and is known for its ability to withstand adverse conditions in the gastrointestinal tract. It has enzymes that act on the degradation of polysaccharides, leading to the production of acetic acid, which is important in regulating postprandial glycemia and modulating gut inflammation (Vandeputte et al., 2017). (4) *Enterococcus* is a microorganism capable of resisting bile acids and the simulated digestion conditions of the stomach with a low pH environment. Although it is a common member of healthy microbiota, it can cause infections and antibiotic resistance in situations of imbalance (Kao & Kline, 2019). (5) *Blautia* has probiotic properties, producing acetic acid and has been associated with improvements in diabetes and obesity (Qu et al., 2024). (6) *Collinsella* and *Faecalibacterium* are capable of metabolizing carbohydrates to produce butyrate (Kang et al., 2020). (7) *Catenibacterium* has been associated with coronary atherosclerosis, increasing the risk of heart disease (Jiang et al., 2024). (8) *Dialister* can stimulate the production of propionate and is associated with the metabolism of butyrate and dopamine (Wei et al., 2022).

To evaluate the variation in gut microbiota and its correlation, a histogram of LDA values and an evolutionary branching profile were used based on LEfSe analysis (Fig. 6). As shown in Fig. 6a, LDA values greater than 4.0 indicated statistically significant differences between the experimental groups. The T0 group was predominantly composed of the taxa *Firmicutes*, *Clostridia*, *Oscillospirales*, *Lachnospiraceae*, *Ruminococcaceae*, *Blautia*,

Oscillospiraceae, *Subdoligranulum*, and *Eubacterium coprostanoligenes*. In particular, the PePp group showed a significant increase in the abundance of *Bacteroides*, *Bifidobacterium*, and *Actinobacteria*, while the INU group was characterized by an increase in *Enterobacteriaceae* and *Escherichia-Shigella*. Fig. 6b presents the results of the LEfSe analysis, highlighting the hierarchical arrangement of taxonomic levels (kingdom, phylum, class, order, family, and genus). The lines connect the different taxonomic levels, symbolizing their evolutionary relationships, and each circular node represents a distinct species. Yellow nodes indicate no significant differences between groups, while colored nodes indicate the species' predominance in the corresponding group. The colored sectors represent the lower taxonomic divisions of the identified microorganisms. These findings demonstrate that both PePp and INU acted as substrates for bacterial growth, with distinct effects on the diversity and composition of the gut microbiota, which may be attributed to the different characteristics of the polysaccharides. Therefore, we believe that PePp has the potential to modulate the gut environment by reducing fecal pH, increasing SCFA production, and promoting the growth of beneficial bacteria (Fig. 6c).

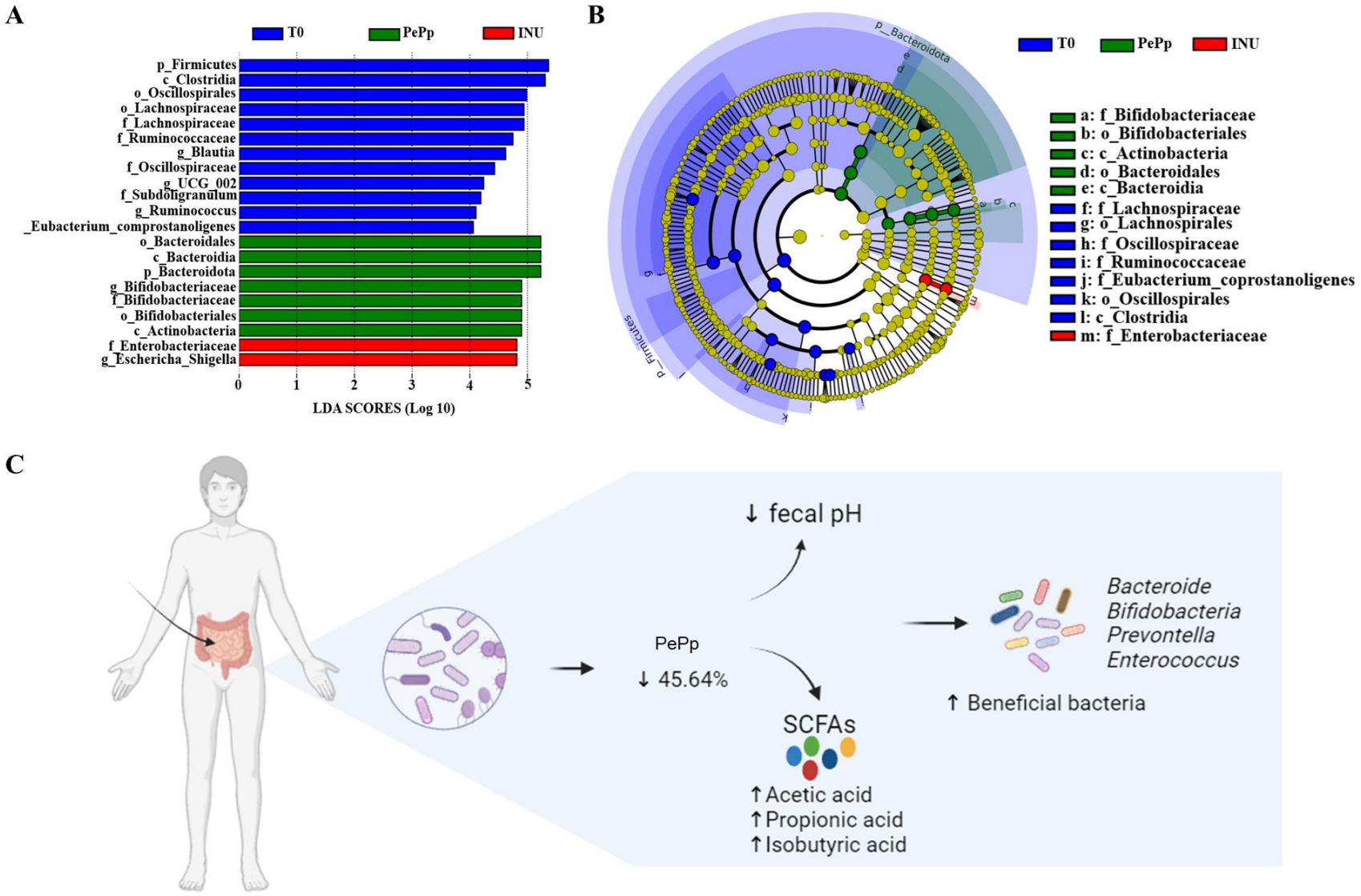


Fig. 6. Effect of *P. pendula* exudate polysaccharide (PePp) on gut microbiota. (A) Histogram of LDA scores computed for abundance difference traits, (B) Taxonomic cladogram obtained from LEfSe analysis of gut microbiota for different groups, and (C) schematic diagram of the effect of PePp on microbiota. T0: initial fecal inoculum, INU: inulin.

4. Conclusion

In this study, the polysaccharide from the exudate of *Parkia pendula* was evaluated for its structural characteristics and its effect on *in vitro* fecal fermentation. PePp is an galactoarabinan obtained through aqueous extraction and ethanol precipitation, with a homogeneous characteristic. In the microbiota analysis, the results showed that PePp was consumed during fermentation, the pH decreased over time, while the production of total SCFAs and acetic, propionic, and isobutyric acids increased significantly. PePp has potential prebiotic effects, modulating the composition of the gut microbiota and increasing the beneficial bacterial population, such as *Bacteroides*, *Bifidobacterium*, *Prevotella*, and *Enterococcus*. Therefore, considering biotechnological applications targeted at the gut microbiota may be a viable strategy for the future use of PePp as a prebiotic.

Author contributions:

José J. Batista: Research, methodology, writing - proofreading and editing; **Maria I.F Araújo**: Research, methodology; **Abigail González**: Research, methodology; **Marcos F. da Silva:** Research, methodology; **Soraia Silva**: Research, methodology; **Elisabete Coelho**: Research, Supervision, fundraising, writing - original draft; **Manuel A. Coimbra**: Supervision, fundraising, writing - original draft; **José A. Texeira**: Supervision, fundraising, writing - original draft; **André L. Aires**: Research, supervision, writing - original draft; **Paulo A.G. Soares**: Research, supervision, writing - original draft; **Clarisse Nobre**: Research, supervision, writing - original draft; **Luana C.B.B. Coelho**: Research, supervision, writing - original draft.

Declaration of interest

The authors declare that there is no conflict of interest that could influence the work reported in this article.

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4.2 ARTIGO II – A SER SUBMETIDO

Prebiotic activity and effect of *Parkia pendula* exudate polysaccharide on mouse fecal microbiota and SCFA production

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Highlights

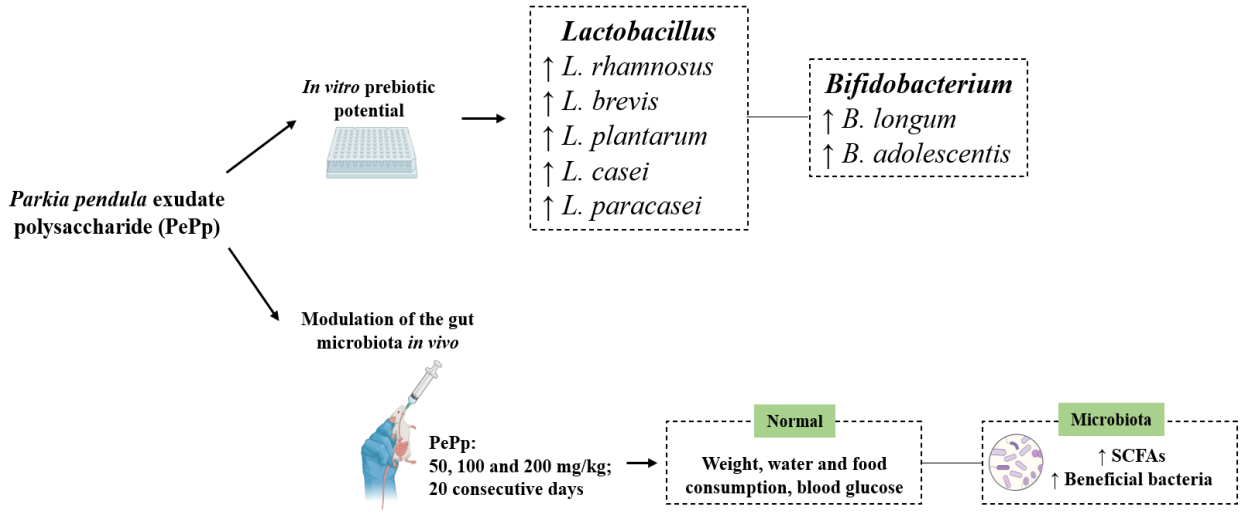
• PePp exhibits a prebiotic effect on *Lactobacillus* and *Bifidobacterium*.

• PePp did not affect body weight, water, or feed consumption *in vivo* during fermentation.

• PePp increased the production of short-chain fatty acids (SCFAs).

• PePp promotes the growth of beneficial bacteria in the gut microbiota of mice.

Graphical abstract



Abstract

This study evaluated the prebiotic potential of the polysaccharide from the exudate of *Parkia pendula* (PePp) in strains of *Lactobacillus* and *Bifidobacterium*, as well as its impact on the gut microbiota of mice after 20 days of administration. PePp demonstrated a prebiotic effect, stimulating the growth of *L. rhamnosus*, *L. brevis*, *L. plantarum*, *L. casei*, *L. paracasei*, *B. longum*, and *B. adolescentis*. No signs of morbidity, mortality, or behavioral changes were observed in mice treated with PePp at doses of 50, 100, and 200 mg/kg. There were no significant differences in body weight, feed and water consumption, or glycemic index among the groups. The fecal pH decreased from 7.33 to 7.04, 7.10, and 7.03 at doses of 50, 100, and 200 mg/kg, respectively, while the concentrations of short-chain fatty acids, such as acetic, propionic, and butyric acids, increased following the administration of PePp. Qualitative analysis of fecal bacteria using a culture-dependent method revealed a significant increase in the proportion of anaerobic bacteria (92.7%) and greater bacterial diversity, favoring the growth of lactic acid bacteria such as *Lactobacillus* and *Bifidobacterium*. These results suggest that PePp has a prebiotic effect and may beneficially modulate the gut microbiota of mice.

Keywords: *Parkia pendula*; Polysaccharide; Prebiotic; Gut microbiota; *In vivo* fermentation.

1. Introduction

The gut microbiota is a dynamic and complex ecosystem, composed of thousands of bacterial species that colonize the gastrointestinal tract, playing key roles in maintaining host homeostasis and health (Alhhazmi et al., 2023; Zhao et al., 2023). These bacteria are not only involved in nutrient digestion but also modulate the immune system and influence the integrity of the gut barrier. Disruptions in this ecosystem are associated with diseases such as gut inflammation, autoimmune diseases, allergies, and metabolic conditions, including type 2 diabetes and obesity (Maciel-Fiuza et al., 2023). Therefore, maintaining a healthy balance in the gut microbiota is essential for human well-being.

Non-digestible polysaccharides have a positive impact on the composition of the gut microbiota, promoting the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*. These microorganisms ferment carbohydrates, producing short-chain fatty acids (SCFAs), which play important roles in energy supply, maintaining gut barrier integrity, and modulating immune responses (Zheng et al., 2022). Moreover, SCFAs possess anti-inflammatory properties and regulate energy homeostasis, participating in cholesterol metabolism and contributing to disease prevention (Dalile et al., 2019; Wozniak et al., 2022). Given the importance of these interactions for human health, the study of polysaccharides as modulators of the gut microbiota holds significant therapeutic implications.

The genus *Parkia*, belonging to the Fabaceae family, includes several species, among them *Parkia pendula* (Oliveira & Hopkins, 2023). Commonly known as “visgueiro”, “faveira”, or “favera-de-chorão”, this species is widely used in programs for the recovery of degraded areas due to its rapid growth (Cordeiro et al., 2021; Correa et al., 2021). Additionally, its pods produce an exudate rich in polysaccharides, which have shown potential for lectin immobilization, such as Concanavalin A, in magnetic matrices (Rêgo et al., 2014). In our previous study, we demonstrated that the exudate of *P. pendula* (PePp) is an arabinogalactan, a promising polysaccharide, and does not present toxicity in animal models at a dose of 2000 mg/kg, indicating its safety for use. Furthermore, PePp exhibited significant gastroprotective activity in a model of ethanol-induced gastric ulcer. In a human fecal fermentation model, we observed that PePp can modulate the gut microbiota, stimulating the growth of beneficial bacteria such as *Bacteroides*, *Bifidobacterium*, *Prevotella*, and *Enterococcus*, reinforcing its potential as a prebiotic agent and contributing to gut health.

Despite the prebiotic potential demonstrated by PePp in *in vitro* conditions, no studies have yet evaluated its effects in animal models with prolonged exposure. To fill this gap, we investigated the impact of PePp on BALB/c mice, focusing on its ability to modulate

the gut microbiota and promote the growth of beneficial bacteria. Initially, the prebiotic activity of PePp was evaluated *in vitro* against *Lactobacillus* and *Bifidobacterium*, followed by an analysis of *in vivo* fermentation in an animal model. This study aims not only to broaden the understanding of PePp prebiotic properties but also to offer new perspectives on the use of bioactive compounds in therapeutic strategies targeting gastrointestinal health.

2. Materials and methods

2.1 Plant material

Parkia pendula exudate was obtained in the municipality of Novo Lino (geographically, Novo Lino is at 8°58′34.9′′S, 35º, 40′29.2′′W) in Alagoas, Brazil. The species exsicata (n° 92516) were deposited in the Herbarium IPA-Dárdano de Andrade Lima of the Agronomic Institute of Pernambuco.

2.2 Obtaining and isolating the polysaccharide from *P. pendula*

A quantity of 15 g of crude exudate was weighed on an analytical balance (Bel M214-AIH) and suspended in 100 mL of distilled water at room temperature (25 ± 2 °C) for 24 h, allowing the solubilization of water-soluble components. After this period, the suspension underwent a sequential filtration process to remove insoluble particles, using "voil" and a screen-printing screen (90 threads), ensuring the retention of larger fragments and impurities. The obtained filtrate, corresponding to the aqueous extract, was subjected to ethanol precipitation at a 1:4 ratio (v/v) (extract: absolute ethanol, 99 %) and kept under refrigeration at 4 °C for 18 h. The precipitated material was transferred to 50 mL tubes and centrifuged (LMC-4200R, Biosan) at 3000 rpm for 15 min, separating the supernatant from the pellet containing the polysaccharides. The supernatant was discarded, and the precipitate underwent three sequential washes with 10 mL of acetone (≥99 %) for 30 min each, removing low molecular weight compounds and residual impurities. The material was then dried in an oven at 55 °C until a completely dry solid residue was obtained. After drying, the material was ground in a blade mill (IKA® A11 basic), and the final product was designated as Parkia pendula exudate polysaccharide (PePp) (Batista et al., 2025).

2.3 Prebiotic activity of PePp

The bacterial strains *Lactobacillus rhamnosus* (CECT 278), *Lactobacillus brevis* (CECT 216), *Lactobacillus plantarum* (CECT 748) and *Lactobacillus casei* (ATCC 334 were obtained from the Laboratory of Microorganism Genetics at the Federal University of Pernambuco (UFPE), Brazil. *Lactobacillus paracasei* (CECT 277) and *Bifidobacterium*

adolescentis (CECT 5781) were sourced from the Spanish Collection of Type Culture (CECT), Spain, while *Bifidobacterium longum* (DSMZ 2019) was acquired from the German Collection of Microorganisms and Cell Cultures GmbH, Germany. The probiotic *Lactobacillus* strains were activated in Man, Rogosa, and Sharpe (MRS) broth, and the *Bifidobacterium* strains in *Bifidobacterium* broth (MI395-500G) (37 °C, 48 h).

MRS medium was prepared with the following formulation: peptone (10 g/L), meat extract (10 g/L), yeast extract (5 g/L), dipotassium phosphate (2 g/L), Tween 80 (1.08 g/L), sodium acetate (5 g/L), ammonium citrate (2 g/L), magnesium sulfate (0.2 g/L), and manganese sulfate (0.05 g/L). Samples of inulin (INU), glucose (GL), and PePp were added separately to the MRS medium to achieve a final concentration of 2% (w/v). Subsequently, the MRS medium containing different carbon sources was inoculated with bacteria, and aliquots (300 μL) were transferred to a 96-well microplate. The microplate was incubated at 37 °C in a microplate reader, and optical density (A_{600} nm) was recorded at 0, 12, 24, and 48 h. The assay was performed in triplicate, and bacterial growth results were expressed as CFU/mL (Santos et al., 2021).

2.4 *In vivo* fermentation of PePp

The experimental procedures involving animals were conducted in accordance with the guidelines set forth by the National Council for the Control of Animal Experimentation (CONCEA, Brazil) and were approved by the Ethics Committee on Animal Use (CEUA) at UFPE, under protocol number 0052/2023. Female BALB/c mice (8 weeks old, 19-23 g) were obtained from the Keizo Asami Institute (iLIKA) at UFPE. The animals underwent a 7-day acclimatization period in the Animal Facility of the Department of Biochemistry at UFPE. During this period, they were housed in cages with *ad libitum* access to sterilized food and water, maintained at an average temperature of 22 ± 2 °C, and on a 12-h light/dark cycle.

Following the acclimatization period, thirty-five female BALB/c mice were evenly divided into five experimental groups, each consisting of seven animals, as described below and summarized in Fig. 1. All animals underwent a 3-h fasting period (on day 0 and on day 20), with free access to water, for glycemic index analysis and fecal collection. Subsequently, they were administered a daily dose of inulin and PePp for 20 consecutive days. Daily water and food consumption, as well as body mass, were monitored throughout the experimental period (Su et al., 2023).

• Group 1 (G1): Saline solution (negative control, 10 mL/kg).
• Group 2 (G2): inulin (model positive control, 400 mg/kg).
• Group 3 (G3): PePp at a low dose of 50 mg/kg.
• Group 4 (G4): PePp at an average dose of 100 mg/kg.
• Group 5 (G5): PePp at a high dose of 200 mg/kg.

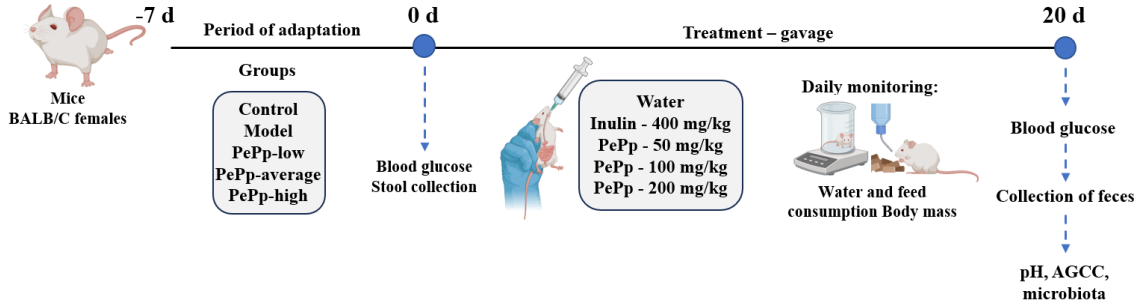


Fig. 1. Timeline of the PePp *in vivo* fermentation experiment.

2.5 Determination of the glycemic index

Following the 3-h fasting period, the glycemic index of the animals was assessed on day 0 (before administration) and on day 20 of the experiment. Blood glucose levels were measured by collecting blood samples from the tail vein using glucose meter strips (ACCU-CHEK).

2.6 Determination of fecal pH

Fecal samples (~100 mg) for fermentation analysis were collected on day 0 and on day 20 after PePp administration, as described in section 2.4. The samples were diluted in cold water at a ratio of 1:7 (weight/volume), vortexed for 5 min to facilitate solubilization, and centrifuged at 8.000 g for 20 min at 4 °C. This process was performed twice. Subsequently, the pH values of the supernatants were measured using a pH meter (model pH 2, Labmeter).

2.7 Determination of Short-Chain Fatty Acids (SCFAs)

Fecal samples were diluted in Ringer's phosphate solution (RPS), centrifuged at 5.000 rpm for 10 min, and the supernatant was filtered through cellulose acetate membranes (0.22 µm). Aliquots of the samples were diluted with an internal standard (crotonic acid) and analyzed by high-performance liquid chromatography (HPLC) using a Phenomenex Rezex ROA-Organic Acid H^+ column (300 × 7.8 mm). The mobile phase consisted of 2.5 mM sulfuric acid, with a flow rate of 0.6 mL/min. The samples were detected at 210 nm, with different retention times for acetic acid, propionic acid, iso-butyric acid, iso-valeric acid, and

n-valeric acid. SCFA concentrations were determined by interpolating the sample areas based on a calibration curve constructed from standard solutions.

2.8 Culture-dependent method for microbiota evaluation

Fecal samples collected on day 0 and day 20 after PePp administration were weighed and diluted in saline solution for microbiological investigation. After solubilization, aliquots were removed and subjected to serial dilutions from 10^{-1} to 10^{-7}. Then, 10 µl of each dilution were cultured in petri dishes containing enriched, selective or differential media, as described below: MRS medium (under aerobic and anaerobic conditions), MacConkey agar – MAC (under anaerobic conditions), glucose, yeast extract and peptone – GYP (under anaerobic conditions), and blood agar medium (under aerobic and anaerobic conditions). After a 3-day incubation period at 37 °C, colony-forming units (CFU) were counted and the results expressed as the mean ± standard error of the mean (SEM) of CFU per milligram of feces (Rungue et al., 2021a).

2.9 Statistical analysis

Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) combined with Tukey's analysis or Student's t-test was performed to compare differences between experimental groups.

3. Results and discussion

3.1 PePp presents prebiotic effect on *Lactobacillus* and *Bifidobacterium* strains

Polysaccharides, as carbon sources, can be metabolized by probiotic bacteria, promoting their growth and viability (You et al., 2022). In this study, we investigated the effect of different carbon sources, including PePp, inulin, and glucose, on the growth of *Lactobacillus* and *Bifidobacterium* strains in basal broth over 24 h of incubation. Inulin was used as a positive control due to its well-characterized and widely applied use as a prebiotic ingredient, while glucose, an easily metabolized carbohydrate, served as a non-prebiotic control. As shown in Fig. 2, the negative control group (NC), which did not receive a carbon source, exhibited only a small increase in bacterial population during the incubation period (0-24 h), suggesting that the growth observed in the other groups is directly related to carbon supplementation. The PePp group significantly promoted the growth of *L. rhamnosus* (Fig. 2A), *L. brevis* (Fig. 2B), *L. plantarum* (Fig. 2C), *L. casei* (Fig. 2D), *L. paracasei* (Fig. 2E), *B. longum* (Fig. 2F), and *B. adolescentis* (Fig. 2G) compared to the NC group, particularly during the 12 and 24-h incubation periods. These results suggest that PePp was utilized as a

carbon source, as evidenced by the increase in optical density (OD), which is directly proportional to the bacterial concentration in the solution (Guo et al., 2022). In comparison, inulin showed a significantly greater effect ($p<0.0001$) on the growth of all bacterial strains compared to the PePp and NC groups. As expected, the glucose group was rapidly metabolized, resulting in the highest growth levels across all strains, with a significant difference ($p<0.0001$) compared to PePp and inulin at 6, 12, and 24 h.

In this study, it was demonstrated that PePp exerts a significant prebiotic effect, as evidenced by the increased growth of *Lactobacillus* and *Bifidobacterium* strains. These results are consistent with previous studies investigating other polysaccharides of plant origin (Kang & Chang, 2024; Kansandee et al., 2024; Song et al., 2022). The possible reasons for the prebiotic effect observed in PePp can be discussed based on the following factors. First, the monosaccharide composition present in PePp may positively influence the growth of probiotics, as bacteria have an affinity for certain molecules found in carbohydrates. Previous studies have shown that polysaccharides containing mannose, arabinose, and/or galactose strongly influence prebiotic activity, stimulating the growth of beneficial strains (Cano et al., 2020; Chen et al., 2023). In our previous study, we demonstrated that PePp contains arabinose and galactose in its composition but not mannose, which may explain the proliferative effect observed in the selected probiotic strains. Second, the molecular weight of polysaccharides also plays a determining role in prebiotic activity. Low or moderate molecular weight polysaccharides are more easily metabolized by probiotics, resulting in greater stimulation of bacterial growth. For example, it has been shown that the polysaccharide from *Chimonobambusa quadrangularis* with a molecular weight of 117.5 kDa promoted better growth of *Lactobacillus* and *Bifidobacterium* compared to higher molecular weight polysaccharides (135.1 and 133.9 kDa) (Chen et al., 2020). In this context, PePp, with a molecular weight of 70.8 kDa, may be even more efficient, considering its low molecular mass, which favors its use as a carbon source by probiotic bacteria. Third, the glycosidic linkages of polysaccharides are another essential factor that can influence prebiotic activity. Previous studies have shown that polysaccharides from *Dimocarpus longan* with the linkage →5)-α-L-Araf-(1→ exhibit higher prebiotic activity (Huang et al., 2019). This linkage structure is consistent with the primary linkage found in PePp, which may partly explain its efficacy in promoting probiotic growth. Given this evidence, the significant prebiotic effect observed in PePp can be attributed to a combination of factors, including its monosaccharide composition, low molecular weight, and the presence of specific glycosidic linkages that

favor prebiotic activity. These factors collectively explain the positive impact of PePp on the growth of the seven probiotic strains demonstrated in this study.

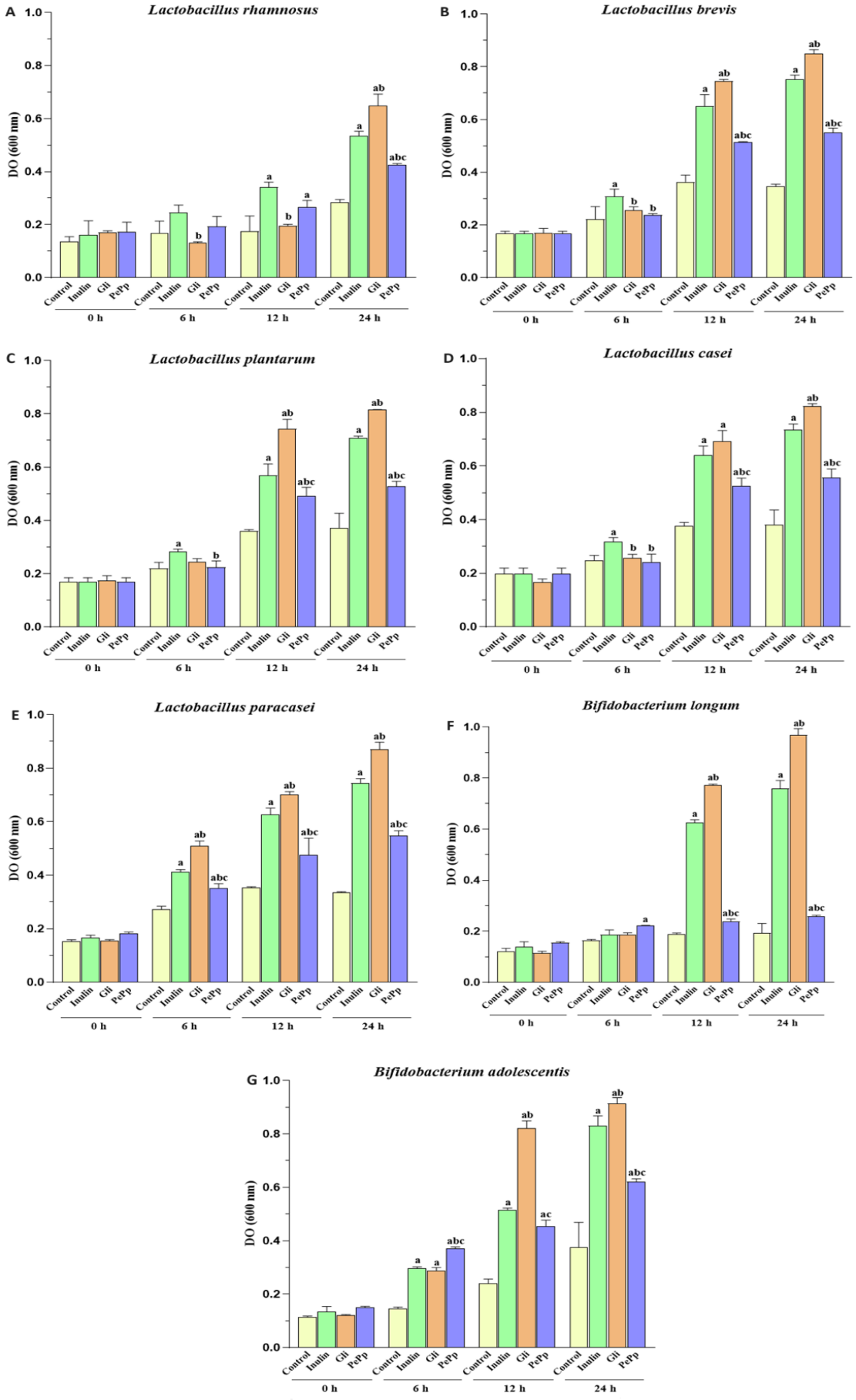

A
Lactobacillus rhamnosus
B
Lactobacillus brevis
C
Lactobacillus plantarum
D
Lactobacillus casei
E
Lactobacillus paracasei
F
Bifidobacterium longum
G
Bifidobacterium adolescentis
DO (600 nm)
Control
Inulin
Gli
PePp
0 h
6 h
12 h
24 h

Fig. 2. Effect of PePp on the growth of *Lactobacillus* sp. and *Bifidobacterium* sp. in MRS medium. (A) *L. rhamnosus*, (B) *L. brevis*, (C) *L. plantarum*, (D) *L. casei*, (E) *L. paracasei*, (F) *B. longum*, (G) *B. adolescentis*. Control: MRS medium without glucose; Inulin: MRS medium without glucose + inulin; Gli: MRS medium without glucose + glucose; PePp: MRS medium without glucose + PePp. Data are represented as mean ± SEM (n=3) and analyzed for significant differences $^{a}P < 0.0001$ between the control group, $^{b}P < 0.001$ between the inulin group, and $^{c}P < 0.0001$ between the glucose group (Gli).

3.2 PePp demonstrated no toxicity and no impact on glycemia in mice

To evaluate the gastrointestinal and digestive characteristics of PePp, an *in vivo* model using BALB/c mice was employed, based on doses previously established in the literature. The animals received oral administration of PePp at doses of 50 mg/kg (low), 100 mg/kg (medium), and 200 mg/kg (high), while the control group received inulin (400 mg/kg). During 20 consecutive days of administration, no clinical signs of morbidity were observed, such as weight loss, changes in exploratory activity, abnormal posture (such as hunching or lethargy), irregular fur, or alterations in food and water intake. No mortality or behavioral changes were observed in any group. Physiological parameters, such as body weight, food consumption, water intake, and glycemic index, were monitored as potential indicators of toxicity. The animals treated with PePp showed continuous body mass gain, with no significant differences compared to the negative control (NC) group or the inulin group, suggesting an absence of toxicity at the tested doses. Additionally, there were no significant differences in food and water intake between the NC, PePp, and inulin groups (Table 1). These results indicate that PePp is safe at the evaluated doses, corroborating the findings of previous studies with other polysaccharides, which also demonstrated beneficial effects (Silva et al., 2020; Ezeonwumelu et al., 2011; Hu et al., 2014).

Table 1. Body weight, food consumption and water intake during 20 consecutive days of oral administration of PePp in mice.

Sample	Parameters			
	Body weight (g)[a]		Feed consumption (g)[b]	Water consumption (mL)[b]
	Day 0	Day 20		
NC	22.00 ± 1.86	23.00 ± 1.98	21.00 ± 1.07	30.00 ± 0.09
Inulin	19.00 ± 0.89	22.00 ± 1.11	22.00 ± 1.66	31.00 ± 0.08
PePp50	21.00 ± 1.13	21.00 ± 1.21	22.00 ± 1.68	31.00 ± 1.81
PePp100	22.00 ± 1.41	23.00 ± 0.91	23.00 ± 0.48	32.00 ± 1.57
PePp200	23.00 ± 1.29	23.00 ± 1.50	23.00 ± 0.91	32.50 ± 1.05

Values expressed as mean ± deviation.
NC: negative control.
PePp: Polysaccharide from *P. pendula* exudate.
[a]Average weight of animals on day 0 and day 20 of the experiment.
[b]Average consumption during 20 days of experiments.

The glycemic index is a useful tool for assessing the glycemic response following food intake, providing an understanding of carbohydrate digestion and utilization by the body (Li & Hu, 2022). The effect of PePp administration on the glycemic index is illustrated in Fig. 3. On day 0, the NC, PePp, and INU groups exhibited similar glycemic levels, around 97 mg/dL. After 20 days of administration, no significant changes were observed in the glycemic levels of these groups, with values ranging between 97 and 99 mg/dL. These results suggest that PePp did not induce significant modifications in glucose levels, indicating an absence of adverse effects related to glucose metabolism. Such results are consistent with previous studies demonstrating that polysaccharides can regulate glucose absorption in the gastrointestinal tract (Huang et al., 2023; Song et al., 2021; Zhou et al., 2020).

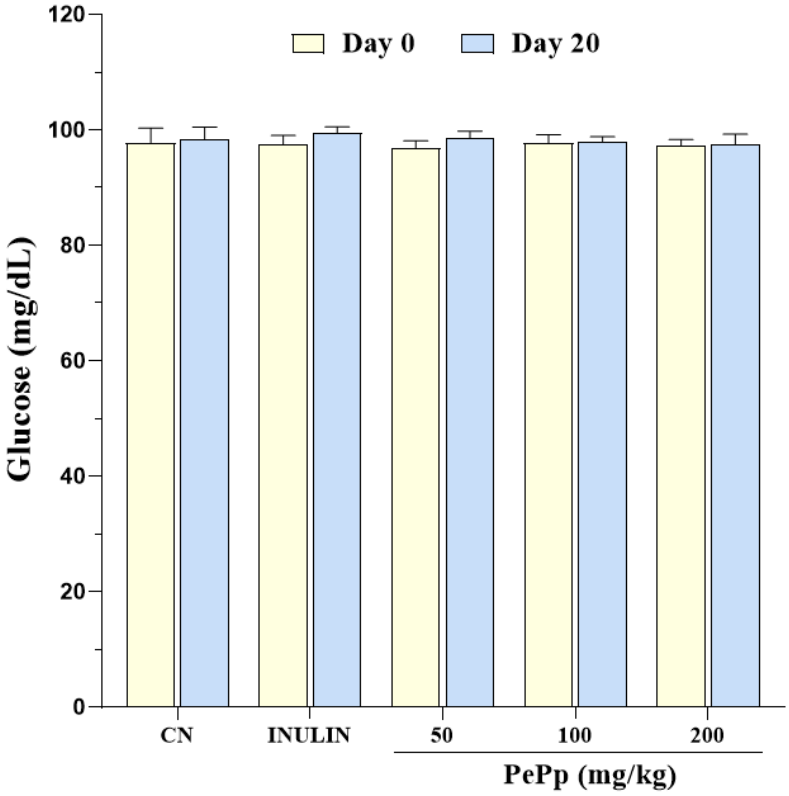


Fig. 3. Blood glucose values of BALB/c mice before and after oral administration of PePp for 20 consecutive days. CN - Negative control; PePp – Polysaccharide from *P. pendula* exudate.

3.3 PePp acts in the modulation of the gut microbiota of mice

The variation in pH levels is an important indicator of the polysaccharide fermentation process, reflecting microbial activity and the production of organic acids in the intestine. As shown in Fig. 4, there was no significant difference in the initial pH values (t=0 h) between the NC group (pH 7.33), inulin (pH 7.60), and PePp (pH 7.46, 7.67, and 7.59). On the 20th day of treatment, a reduction in pH levels was observed in the groups that received PePp at doses of 50, 100, and 200 mg/kg, with pH values of 7.04, 7.10, and 7.03, respectively, as well as in the INU group (pH 7.17). This decrease in pH may be associated with the fermentation of PePp by gut microorganisms, leading to the production of SCFAs. Therefore, PePp consumption may reduce gut pH, creating favorable conditions for the growth of beneficial bacteria.

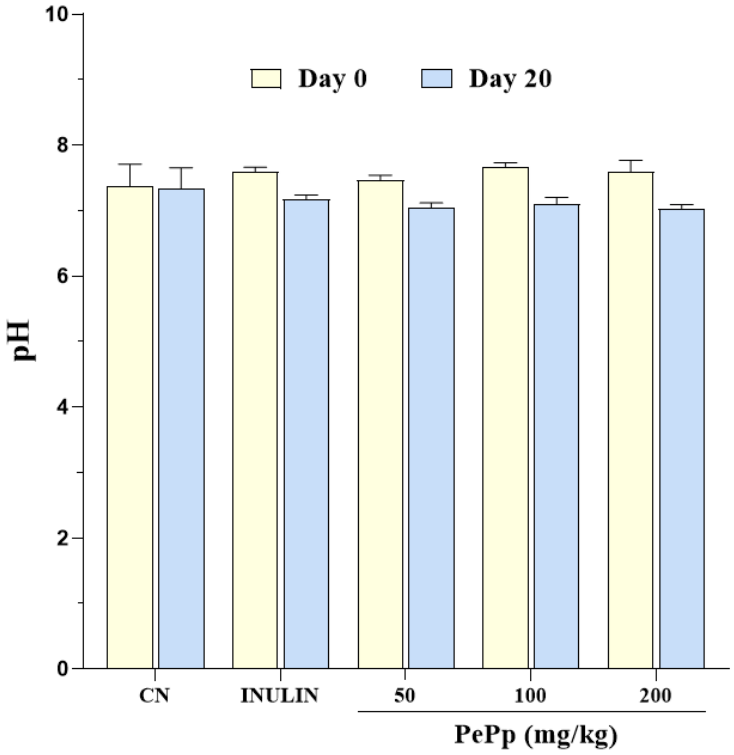


Fig. 4. Fecal pH values of BALB/c mice before and after oral administration of PePp for 20 consecutive days. CN - Negative control; PePp – Polysaccharide from *P. pendula* exudate.

SCFAs are the main products of carbohydrate fermentation by the gut microbiota and play a crucial role in the modulation of various diseases. As presented in Fig. 5, the initial levels of total and individual SCFAs in the fecal material of the animals (day 0) showed no significant differences among the NC, inulin, and PePp groups. On the 20th day, an increase in total SCFAs was observed, rising from 1.63 ± 0.06 to 3.82 ± 0.12, 5.71 ± 0.41, 5.23 ± 0.14, 5.31 ± 0.43, and 7.14 ± 0.20 in the NC, inulin, and PePp groups at doses of 50, 100, and 200 mg/kg, respectively (Fig. 5a). These results suggest that both inulin and PePp significantly

promote the increase in SCFA production compared to the NC group. Additionally, the levels of acetic acid (Fig. 5b), propionic acid (Fig. 5c), and isobutyric acid (Fig. 5d) produced by PePp at all doses were higher than those observed in the NC group. Notably, the dose of 200 mg/kg of PePp resulted in significantly higher levels of these acids compared to the inulin and NC groups, suggesting that PePp was efficiently metabolized by the gut microbiota, demonstrating a prebiotic effect comparable to or greater than that of inulin in SCFA production.

The high concentration of acetic acid can be attributed to the fermentation of PePp by strains of *Bifidobacterium* and *Lactobacillus*, as these bacteria possess enzymes capable of degrading complex polysaccharides into fermentable monosaccharides (Chen et al., 2022; Zhang et al., 2024). As the most abundant SCFA in peripheral circulation, acetic acid is rapidly absorbed by the epithelial cells of the colon, providing energy to peripheral tissues such as the brain and heart (Gao et al., 2024). Propionic acid, metabolized in the liver, plays an important role in regulating cholesterol biosynthesis and has shown a positive correlation with the abundance of *Bacteroides* genus bacteria (Fang et al., 2024; Liao et al., 2022). Butyric acid, in turn, is widely known for its beneficial effects, such as improving insulin sensitivity, inhibiting apoptosis, preventing colon cancer, reducing bacterial migration and inflammation, as well as contributing to the integrity of the gut barrier (Chen et al., 2023). These results indicate that the efficacy of PePp is closely related to the modulation of the gut microbiota and the increase in SCFA production, reinforcing its potential as a prebiotic agent. Furthermore, the observed metabolic properties suggest that PePp could be explored as a therapeutic tool in metabolic and inflammatory disorders, opening new possibilities for the development of dietary supplements and nutritional interventions.

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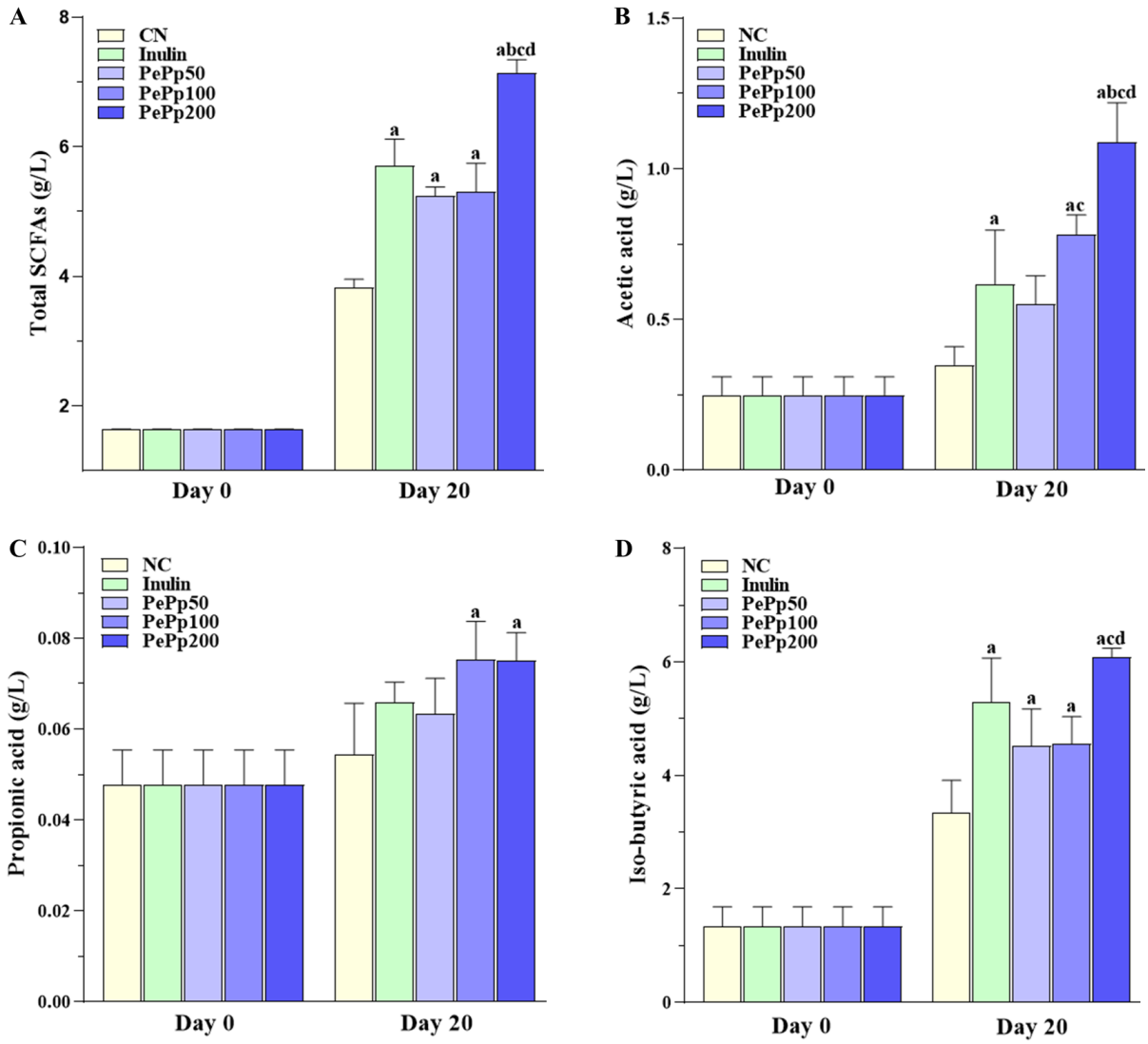

Fig. 5. Concentration of SCFAs. Total SCFAs (A), acetic (B), propionic (C) and iso-butyric (D) acids in fermentation products with *P. pendula* exudate polysaccharide (PePp) compared with NC and inulin group. Data are represented as mean ± SEM (n=7) and analyzed for a significant difference $^{a}P < 0.0001$ between NC group, $^{b}P < 0.001$ between inulin group; $^{c}P < 0.0001$ between PePp group at 50 mg/kg dose, $^{d}P < 0.0001$ between PePp group at 100 mg/kg dose.

Qualitative analysis of mouse fecal bacteria using culture-dependent methods is shown in Figure 6. On day 0, PePp administration revealed a predominance of bacteria under anaerobic conditions, which represented 57.89% of the microbiota, while aerobic bacteria constituted 42.11% (Figure 6a). However, after 20 days of PePp administration, there was a significant increase in the proportion of anaerobic bacteria, reaching 92.7%, with a corresponding decrease in aerobic bacteria, which were reduced to 7.73% (Figure 6b). The anaerobic environment, prevalent after PePp treatment, plays important physiological roles,

including epithelial barrier function (Gieryńska et al., 2022), nutrient absorption (Oliphant & Allen-Vercoe, 2019), immune regulation (Luca et al., 2018), and the production of essential metabolic compounds such as SCFAs (Portincasa et al., 2022). These findings highlight the potential of PePp to promote the modulation of a healthy gut microbiota by creating a favorable environment for the growth of beneficial bacteria.

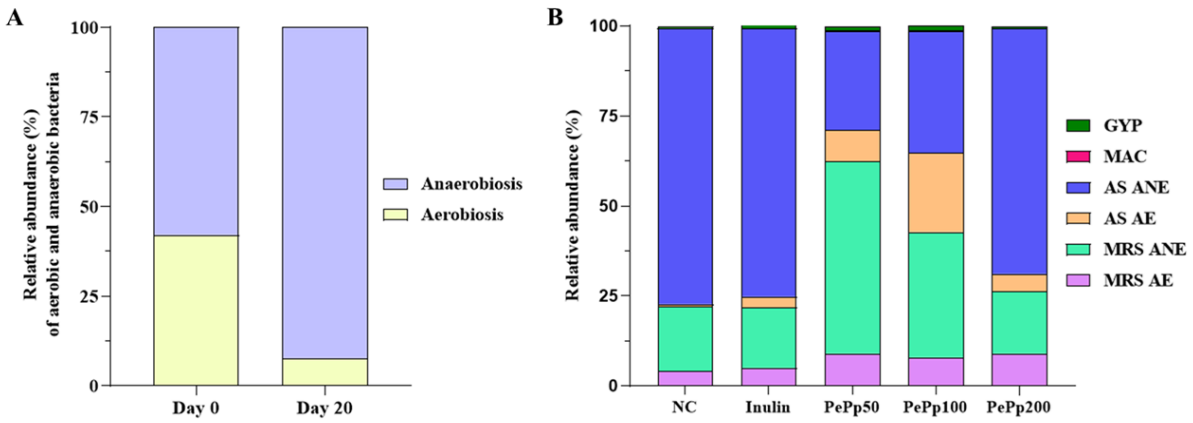

Fig. 6. Composition of the intestinal microbiota of mice before and after fermentation with *P. pendula* exudate polysaccharide (PePp). (A) Relative abundance (%) of anaerobic and aerobic bacteria. (B) Relative abundance (%) of different culture media in selective and differential agar in anaerobic and aerobic environments. NC: negative control; PePp50: *P. pendula* exudate polysaccharide at a dose of 50 mg/kg; PePp100: *P. pendula* exudate polysaccharide at a dose of 100 mg/kg; PePp200: *P. pendula* exudate polysaccharide at a dose of 200 mg/kg; MRS: Man, Rogosa and Sharp broth; AS: blood agar; MAC: MacConkey agar; GYP: glucose, yeast and peptone extract; EA: aerobic; ANE: anaerobic.

Qualitative analysis of the intestinal microbiota in mice using selective and differential agar culture media revealed distinct patterns of bacterial abundance among the experimental groups (Fig. 6b). Animals in the NC group and inulin group showed higher bacterial abundance in anaerobic blood agar, with percentages of 76.64% and 74.44%, respectively, and in anaerobic MRS, with 17.94% and 16.83%, respectively. After administration of PePp, there was an increase in bacterial diversity in the culture media. In the group that received the 50 mg/kg and 100 mg/kg dose of PePp, there was a predominance of bacteria in anaerobic MRS, with percentages of 53.46% and 34.70%, respectively. However, at a dose of 200 mg/kg of PePp, there was a higher presence of bacteria in anaerobic blood agar (68.12%). The anaerobic MRS culture medium favors the growth of lactic acid bacteria, such as Lactobacillus and Bifidobacterium, known for their probiotic properties (Rungue et al., 2021b). These bacteria acidify the intestinal environment, inhibiting the growth of

pathogens and promoting the health of the intestinal mucosa (Ayivi et al., 2020), and are also associated with the production of SCFA (Markowiak-Kopeć & Śliżewska, 2020). On the other hand, the anaerobic blood agar culture medium provides conditions for the growth of several microorganisms, including Streptococcus spp (Friedman et al., 2018; Rungue et al., 2021b). These results indicate that oral administration of PePp promoted the growth of specific bacteria, with increased bacterial populations on both anaerobic MRS agar and anaerobic blood agar. Both media are important for the cultivation of beneficial bacteria that play essential roles in the healthy functioning of the intestinal microbiota.

4. Conclusion

This study demonstrated the prebiotic potential of PePp and its positive impact on the gut microbiota of mice. The prebiotic activity of PePp was evidenced by a significant increase in the growth of beneficial strains of *Lactobacillus* and *Bifidobacterium*, including *L. rhamnosus*, *L. brevis*, *L. plantarum*, *L. casei*, *L. paracasei*, *B. longum*, and *B. adolescentis*. Furthermore, no signs of morbidity, mortality, or behavioral changes were observed at the tested doses, and no significant adverse effects were recorded concerning body weight, feed and water consumption, or glycemic index among the groups. PePp also demonstrated the ability to modulate the gut microbiota, resulting in a decrease in fecal pH and an increase in concentrations of SCFAs, such as acetic, propionic, and butyric acids. These results not only favored greater bacterial diversity but also indicated a healthier gut environment conducive to the growth of beneficial microorganisms. These findings establish a solid foundation for the broader application of the polysaccharide from *P. pendula* in promoting gut microbiota homeostasis and maintaining host gut health. Future research should explore the applicability of PePp, thereby expanding its potential as a functional ingredient in prebiotic therapies.

Author contributions:

J.J. Batista: Conceptualization, research, methodology, writing – review and editing; **M.I.F. Araújo:** Research, methodology; **F.C Oliveira:** Research, methodology; **P.A.G. Soares:** Supervision, writing – review and editing; **C. Nobre:** Supervision, validation, writing – review and editing; **Aires:** Supervision, validation, writing – review and editing; **L.C.B.B. Coelho:** Supervision, validation, writing – review and editing.

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Parkia pendula polysaccharides have no acute toxicity and prevent ethanol-induced gastric ulcers via downregulation of TBARS, IL-6, and TNF-α and upregulation of SOD, CAT, and IL-10

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ABSTRACT

We investigated the safety of *Parkia pendula* exudate polysaccharide (PePp) for acute toxicity and its gastroprotective effect on ethanol-induced gastric ulcers. In the acute toxicity test, animals were treated with a single dose of PePp (2000 mg/kg body weight) and evaluated for behavioral parameters, morbidity and mortality; also biochemical, hematological, and histopathological analysis. The mice were pretreated with a single dose of PePp (10, 25, 50 mg/kg) or ranitidine (80 mg/kg) before gastric ulcer induction with ethanol (99.9 %, orally). Subsequently, the stomachs were analyzed for macroscopic and histopathological parameters and the activity of catalase (CAT), superoxide dismutase (SOD), lipid peroxidation, and cytokines IL-6, TNF-α and IL-10. PePp did not cause any changes in acute toxicity parameters. Pretreatment with PePp inhibited the formation of gastric ulcers by 52 %, 71 %, and 83 % at doses of 10, 25 and 50 mg/kg, respectively. Macroscopic improvements and treating gastric tissue exudative inflammatory infiltrate reduction were dose-dependent on PePp. In gastric tissue, PePp presented antioxidative effects, evidenced by increasing CAT and SOD activities and reducing lipid peroxidation; it also showed anti-inflammatory effects, evidenced by reduced IL-6 and TNF-α levels and elevated IL-10 levels. PePp is toxicologically safe and has a gastroprotective effect on ethanol-induced gastric ulcers.

1. Introduction

Gastric ulcers are a digestive system disease that affected approximately 8 million people worldwide in 2019, becoming a public health problem and compromising the quality of life of those affected [1]. Gastric ulcers develop due to an imbalance between protective agents (production of mucus, bicarbonate, and prostaglandins) and aggressive agents (secretion of acid and pepsin), resulting in necrosis in the glands of the stomach tissue characterized by exudative inflammation and redox imbalance [2,3]. External agents such as alcohol consumption, smoking, stress, and the use of non-steroidal anti-inflammatory drugs can induce the formation of gastric ulcers [4,5].

Treating and controlling gastric ulcers involves protecting and/or healing the gastric mucosa through antacids, proton pump inhibitors, antibiotics, and histamine H2 receptor antagonists [6,7]. However, prolonged use of these medications is associated with complications such as acute kidney injury, changes in the structure and function of the gastric mucosa [6,8,9], and resistance to *Helicobacter pylori* [10,11]. This

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scenario highlights the importance of research into new pharmacological alternatives for treating and controlling gastric ulcers. Recently, natural products of plant origin have emerged as promising gastroprotectors [12,13].

Natural compounds obtained from medicinal plants play an important role in human health. These compounds are widely explored in research and discovery for producing new herbal medicines or as a basis for new drugs [14,15]. Polysaccharides are polymeric chains of carbohydrates composed of monosaccharides interconnected by glycosidic bonds [16]. These polymers have stood out for their pharmacological and biological applications, including antioxidant [17], immunomodulatory [18,19], anti-inflammatory [20,21] and gastroprotective [22].

Parkia is a pantropical genus with approximately 35 species recognized and used in folk medicine. Its plant parts are processed in the form of pastes, decoctions, and/or juice for treating diarrhea, diabetes, skin ulcers, wounds, and hypertension [23–25]. In the genus *Parkia*, the species *Parkia pendula* is found in Brazil, especially in the Amazon region, and is used in the recovery of degraded areas due to its rapid growth, ability to fix nitrogen in the soil and attract wild animals [26]. Studies by Andel [27] and Ferreira [28] report using *P. pendula* bark and leaves in folk medicine to treat infections and malaria. Furthermore, *P. pendula* polysaccharides demonstrated to be a promising magnetic polysaccharide matrix for immobilizing the lectin Concanavalin A, and its application allows easy recovery in the magnetic field [29].

Given the need for new therapeutic and safe biopolymers for the prevention of gastric ulcers, this study aims, for the first time, to evaluate the safety in acute oral toxicity and the gastroprotective effect of the polysaccharide from the exudate of *Parkia pendula* (PePp) in the ethanol-induced gastric ulcer model. Histopathological analysis and assays of SOD, CAT, and TBARS, as well as cytokines IL-6, IL-10, and TNF-α, were evaluated. The findings highlight that PePp did not present acute oral toxicity in mice and exhibited a gastroprotective effect, attributed to its antioxidant and anti-inflammatory activity on the gastric mucosa.

2. Materials and methods

2.1. Sample collection

The exudate of *Parkia pendula* was collected from the pods of the species in the municipality of Novo Lino, Alagoas, Brazil (8°58′34.9″S, 35°40′29.2″W). The collection was carried out by the authors in December 2023. The specimen (N° 92,516) was deposited in the IPA—Dárdano de Andrade Lima Herbarium of the Agronomic Institute of Pernambuco.

2.2. Extraction of polysaccharides

A quantity of 15 g of crude exudate was weighed on an analytical balance (Bel M214-AIH) and suspended in 100 mL of distilled water at room temperature (25 ± 2 °C) for 24 h, allowing the solubilization of water-soluble components. After this period, the suspension underwent a sequential filtration process to remove insoluble particles, using "voil" and a screen-printing screen (90 threads), ensuring the retention of larger fragments and impurities. The obtained filtrate, corresponding to the aqueous extract, was subjected to ethanol precipitation at a 1:4 ratio (*v*/v) (extract: absolute ethanol, 99 %) and kept under refrigeration at 4 °C for 18 h. The precipitated material was transferred to 50 mL tubes and centrifuged (LMC-4200R, Biosan) at 3000 rpm for 15 min, separating the supernatant from the pellet containing the polysaccharides. The supernatant was discarded, and the precipitate underwent three sequential washes with 10 mL of acetone (≥99 %) for 30 min each, removing low molecular weight compounds and residual impurities. The material was then dried in an oven at 55 °C until a completely dry solid residue was obtained. After drying, the material was ground in a blade mill (IKA® A11 basic), and the final product was designated as *Parkia pendula* exudate polysaccharides (PePp). For chemical analysis, the total carbohydrate content was analyzed [30], uronic acid [31], proteins [32] and phenolics [33]. In addition, Fourier transform infrared spectroscopy (FT-IR) analysis.

2.3. Experimental animals and ethical considerations

Female Swiss mice were obtained from the vivarium of the Keizo Asami Institute (iLIKA) at the Federal University of Pernambuco (UFPE) and maintained in the vivarium of the Department of Biochemistry at UFPE. The animals were kept under standardized breeding conditions, with access to water and food ad libitum, in a controlled environment (23 ± 2 °C and 12-h light-dark cycle). All experimental protocols were approved by the UFPE ethics committee (acute oral toxicity process n° 127/2022 and gastric ulcer process n° 128/2022).

2.4. Evolution of acute toxicity

PePp acute toxicity was performed according to the instructions of the Organization for Economic Co-operation and Development [34]. Female Swiss mice were randomly separated into two groups (G1 and G2), containing three animals in each cage ($n = 3$/group). Subsequently, a single oral dose of 0.9 % saline solution (G1 - control group) and PePp at a dose of 2000 mg/kg (G2 - treated group) were administered using gavage. After that, the animals were observed for signs of toxicity (piloerection, appearance of feces, sensitivity to touch, mobility, and aggressive behavior) and mortality within the first two hours and daily for 15 consecutive days. In addition, body weight, food consumption, and water intake were measured daily. On the 15th day, all animals were weighed and fasted for 3 h, with access to water ad libitum.

To evaluate the hematological and biochemical parameters, the animals were anesthetized (ketamine/xylazine; 90/15 mg/kg body weight, i.p.), and blood was collected by cardiac puncture. For hematological analyses, samples were collected in tubes containing EDTA K2 and analyzed in a hematological cell counter (LABMAX) to determine the following parameters: red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, hemoglobin concentration middle corpuscular, leukocytes, segmented, eosinophils, lymphocytes, monocytes and platelets. For biochemical analyses, samples were collected in tubes with a clot activator, with subsequent centrifugation (4000 rpm, 5 min) to evaluate serum glucose, urea, creatinine, and total cholesterol.

A pubic xiphoid incision was made to collect and subsequently determine the weight of the following organs: liver, kidneys, lungs, heart, spleen, thymus, and stomach. For each animal, three samples of liver, kidney, lung, and splenic tissues were fixed in formaldehyde (10 % buffered), processed for paraffin inclusion, and histological sections (5 μm) stained with hematoxylin-eosin were obtained. Three slides were made and evaluated from each sample under optical microscopes (LEICA ICC50 W).

2.5. Experimental animals and ethanol-induced gastric ulceration

Twenty-five Swiss mice (females, 25–30 g) were equally distributed into five experimental groups according to the therapeutic protocol with ranitidine or PePp, as described below:

- Group 1 (G1): Saline solution (ulcerated negative control; 1 mL/kg).
- Group 2 (G2): Ranitidine (positive control; 80 mg/kg).
- Group 3 (G3): PePp at 10 mg/kg dose.
- Group 4 (G4): PePp at 25 mg/kg dose.
- Group 5 (G5): PePp at 50 mg/kg dose.

All animals were fasted for 16 h (Fig. 1A), but with free access to water, and subsequently administered a single oral dose with ranitidine or PePp (Fig. 2B). Sixty min after administration, all animals, including

Fig. 1. Experimental design. Female mice were fasted (A) and distributed into five experimental groups according to the therapeutic protocol with ranitidine or Polysaccharide from *Parkia pendula* exudate (PePp) (B). All animals were induced to gastric ulcer with ethanol (99.9 %) (C), after 60 min anesthetized (D), euthanized, and the stomachs were removed for analysis.

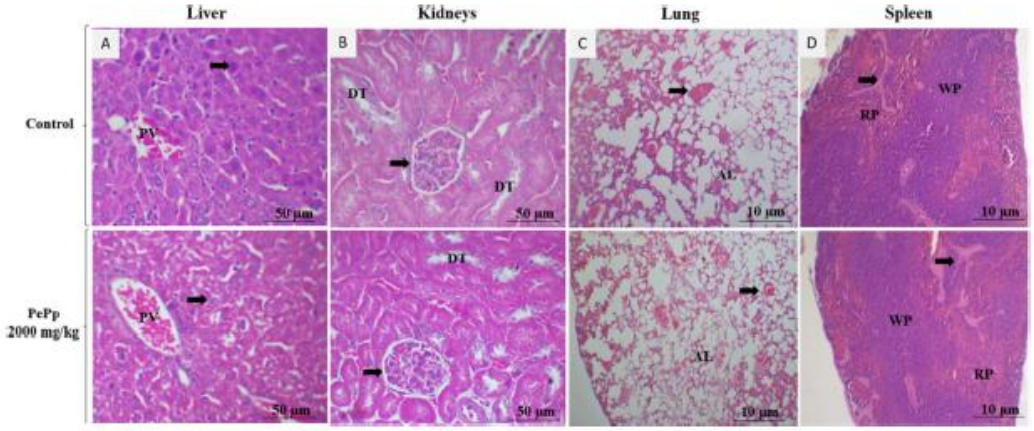

Fig. 2. Oral acute toxicity analysis of the treatment with Polysaccharides from *Parkia pendula* exudate (PePp). Histological evaluation of hepatic (A), renal (B), pulmonary (C), and splenic (D) tissues of female mice from the control and PePp (2000 mg/kg) groups. In both groups, the preservation of the evaluated tissues is observed. In the hepatic tissue, the preservation of the portal vein (PV) and hepatocytes (arrow) is noted. In the renal tissue, the glomeruli appear normocellular (arrow), and the ductal tubules maintain their integrity. In the pulmonary tissue, the preservation of blood vessels (arrows) and alveoli (AL) is observed. Splenocytes, trabeculae (arrows), white pulp (WP), and red pulp (RP) remained preserved in the splenic tissue.

the negative control, were induced to have gastric ulcers through oral administration of ethanol (99.9 %, 0.2 mL/kg) (Fig. 1C). Subsequently, after 60 min, all groups were anesthetized (ketamine: 90 mg/kg and xylazine hydrochloride 2 %, 15 mg/kg) (Fig. 1D), euthanized by cervical dislocation [35].

2.5.1. Macroscopic evaluation and area of the gastric ulcerative lesions

After the experiment, the stomach was removed and opened along the greater curvature, washed with saline solution, fixed between glass plates, and photographed. The area of gastric mucosal injury was measured using computerized planimetry software (Image J® 3.0). The percentage and inhibition were calculated according to the following formula:

Inhibition rate (%) = [Ulcerated area (treatment-model)/ulcerated area (model)] x 100 %.

2.5.2. Histopathology of the gastric mucosa

For all animals in the experimental groups, stomach tissue samples were removed, washed in PBS, and fixed in buffered formalin. Briefly, samples were dehydrated in increasing concentrations of ethanol (70–100 %), cleared in xylene, embedded in paraffin, and histological sections (5 μm) were obtained and stained with hematoxylin and eosin. For all samples, three slides from different sections were prepared for evaluating the composition of the inflammatory infiltrate and categorizing the damage. The scoring criteria for the histopathological evaluation of gastric tissue inflammation were based on inflammatory infiltration and necrosis [36–38]. Score I: mild inflammatory infiltrate and necrotic area < 20 %; Score II: moderate inflammatory infiltrate and necrotic area between 20 and 39 %; and Score III: intense inflammatory infiltrate, epithelial surface rupture, hemorrhage, and necrosis >40 %. Images were captured using an optical microscope (Leica®) connected to a digital camera (Nikon®) and a computer running analysis software (Motic Images Plus 2.0 ML™). Analyses were performed by two experienced pathologists who were blinded to the samples.

2.5.3. Obtaining stomach homogenate and measuring SOD, CAT, and TBARS levels

Stomach homogenate (10 % (*w/v*)) was obtained in a Potter-Elvehjem homogenizer in ice-cold buffer containing 0.02 M phosphate saline (pH 7.4), containing phenylmethanesulfonyl fluoride (PMSF - 1 mM) and trypsin inhibitor (0.0115 %). Subsequently, the homogenate was centrifuged (13.000 *g*, 4 °C for 10 min), the supernatant was carefully removed, and the protein content was determined by the Bradford method [32]. The protein content of the supernatant was adjusted to 1 mg/mL and used to evaluate the activity of the enzymes superoxide dismutase (SOD), catalase (CAT), as well as thiobarbituric acid reactive species (TBARS). All analyses were performed in triplicates in two independent experiments.

2.5.3.1. Superoxide dismutase (SOD) activity. SOD activity was

evaluated according to the ability of the sample to reduce the formation of the pink chromophore adrenochrome, which is produced from the oxidation of epinephrine [39]. Briefly, a gastric tissue sample (~1 mg/ml) was incubated in a flat-bottom 96-well plate containing 0.05 M sodium carbonate buffer (pH 10.2), and the reaction started with the addition of 1.5 mM epinephrine. The rate of adrenochrome formation resulting from epinephrine oxidation was measured at 15 s intervals for 2 min at a wavelength of 480 nm, using a multimode microplate reader (Varioskan Flash, Thermo Scientific, Loughborough, UK). Each sample was tested in triplicate and the results were expressed as U/mg of protein, where on U (unit) is the amount of enzyme required to decrease the adrenochrome formation by 50 %.

2.5.3.2. Catalase activity (CAT). CAT activity was evaluated by measuring the absorbance decay due to the reduction of H_2O_2 to H_2O, according to the Aebi [40]. Briefly, a gastric tissue sample (~1 mg/ml) was added to a reaction medium containing 50 mM potassium phosphate buffer (pH 7.0), supplemented with 10 mM H_2O_2. After homogenization, the decomposition rate of H_2O_2 was measured at an interval of 15 s for 2 min at 240 nm, using a multimode microplate reader (Varioskan Flash). Each sample was tested in triplicate and the results were expressed in mmol of H_2O_2 consumed per milligram of protein, using the H2O2 extinction coefficient for the calculation.

2.5.3.3. Assessment of lipid peroxidation (TBARS). The evaluation of lipid peroxidation was quantified by the production of malondialdehyde (MDA) in reaction with thiobarbituric acid (TBA, $C_4H_4N_2O_2S$), according to the method of Ohkawa [41]. The reaction was carried out by adding an aliquot of the tissue sample (1 mg/ml) with a solution containing 0.02 M phosphate-buffered saline (PBS, pH 7.4), supplemented by 1.0 mM PMSF and 0.0115 % trypsin inhibitor, in an ice bath. Then, a reaction medium containing thiobarbituric acid (0.3 %), sodium dodecyl sulfate (SDS) (0.4 %), and acetic acid (7.5 %) (pH 3.5) was added. The reaction tubes were sealed and incubated (95 °C, 60 min). After cooling, the mixture was analyzed at a wavelength of 535 nm. Each sample was tested in triplicate and the concentration was obtained by comparison with a standard curve of malondialdehyde (MDA), and the results were corrected for the protein concentration of the homogenate and expressed as nmol MDA per milligram of protein.

2.5.4. Measurement of inflammatory markers

The quantification of cytokines in the homogenates was determined using the Cytometric Bead Array (CBA) Human Th1/Th2 Cytokine Kit II (Becton Dickinson Biosciences, USA) for detection of interleukins (IL) 6 and 10, and tumor necrosis factor alpha (TNF-α), following the manufacturer's instructions. The data were acquired on the BD Accuri C6 flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Individual standard curves (0–5000 pg/mL) were determined and the data were analyzed using the BD Accuri C6 software. Cytokine measurements were performed in quadruplicate in two independent experiments.

2.6. Statistical analysis

Data were expressed as means±standard deviation (SD). The normal distribution of each data set was confirmed using the Shapiro-Wilk normality test and by observing the data distribution of each biological parameter on histograms. The homogeneity of variance was confirmed by Brown-Forsythe test. The differences between experimental groups was compared by one-way analysis of variance (ANOVA) combined with Tukey analysis or unpaired Student's *t*-test, according to the number of groups means been compared.

3. Results and discussion

3.1. Extraction and physical-chemical characteristics

The polysaccharide extracted from the exudate of *Parkia pendula* (PePp) was obtained by aqueous extraction, followed by precipitation with ethyl alcohol and subsequent drying. The PePp yield was 48.2 %, corresponding to 7.23 g of dry powder. It is known that there are variations in the yield of polysaccharides depending on the species and plant part and the extraction process. In our study the yield was similar to the polysaccharide extracted from *Prunus persica* exudate (45 %) [42], lower than that of *Prunus domestica* (73.05 %) [43] and higher than that of *Amygdalus scoparia* (8.21 %) [44]. Chemical composition analysis revealed that PePp contains high levels of total carbohydrates (79.20 %) and uronic acid (11.00 %), along with low levels of polyphenols (2.72 mg/g in GAE) and proteins (1.20 %). The combination of aqueous extraction and alcoholic precipitation proved to be an effective method for obtaining polysaccharides with high carbohydrate content, in agreement with studies that used this same type of extraction for polysaccharides from plant exudate [43,45–48]. Furthermore, the presence of polysaccharide and uronic acid was confirmed by Fourier transform infrared spectroscopy (FT-IR) analysis with intense absorption bands at 1309–1216 cm^{-1} and 1410 cm^{-1}, respectively. The extraction of polysaccharides from *Parkia pendula* exudate is sustainable, since the material is a product of plant excretion. Thus, there is no loss or damage to the species, which is advantageous when compared to other compounds that are extracted from plant parts, such as bark, leaves and roots. Furthermore, the use of PePp in processes developed in pharmaceutical products will contribute to the local production chain.

3.2. Parkia pendula exudate polysaccharide (PePp) did not present acute oral toxicity in mice

Mice subjected to oral treatment with a single dose of PePp (2000 mg/kg) and evaluated after 2 h and daily for 15 consecutive days showed no signs of morbidity, mortality, or behavioral changes. Additionally, at the end of the experiment, no significant changes in body weight, food and water consumption were observed (Table 1). Thus, according to OECD testing guidelines, PePp has toxicological safety considering the absence of mortality at the dose recommended for the acute toxicity test [34]. This result is important since, according to Choudhury [49] the assessment of acute oral toxicity in a murine model of compounds of plant origin is a parameter for the safety of new herbal medicines.

Treatment with PePp did not change the weight of the organs (heart, lung, liver, kidney, spleen, thymus, and stomach) compared to the control group (Table 2). Furthermore, the animals subjected to treatment with PePp did not reveal changes in the colour or texture of the organs evaluated.

In screening new phytopharmaceuticals, analyzing hematological and serum biochemical parameters is important since they are sensitive and reliable indicators of hematopoietic and physiological functions of

Table 1
Average body weight and food and water consumption during 15 days of experiments in mice subjected to acute treatment with PePp (2000 mg/kg).

Treatment	Parameters		
	Body weight[a] (g)	Feed consumption[b] (g)	Water consumption[b] (mL)
Control	30.00 ± 0.81	28.00 ± 0.45	59.5 ± 0.11
PePp	28.25 ± 1.45	28.00 ± 1.31	63.0 ± 0.79

Values were expressed as mean ± standard deviation ($n = 3$).
PePp: Polysaccharide from the exudate of *P. pendula*.
[a] Average weight during 15 days of experiments.
[b] Average consumption during 15 days of experiments.

Table 2
Average organ weight of mice after 15 days subjected to acute treatment with PePp (2000 mg/kg).

Organ	Organ weight (g)	
	Control	PePp
Heart	0.14 ± 0.08	0.14 ± 0.03
Lung	0.19 ± 0.06	0.19 ± 0.04
Liver	1.54 ± 0.05	1.70 ± 0.18
Kidney	0.19 ± 0.03	0.18 ± 0.07
Spleen	0.16 ± 0.01	0.14 ± 0.04
Thymus	0.05 ± 0.01	0.05 ± 0.06
Stomach	0.26 ± 0.03	0.26 ± 0.02

Values were expressed as mean ± standard deviation.
PePp: Polysaccharide from the exudate of *P. pendula*.

body systems, respectively. In our study, PePp did not change any hematological and biochemical parameters (glucose, urea, creatinine, and total cholesterol), as shown in Table 3. These results are important as they highlight the safety of using PePp at a high dose (2000 mg/kg).

In Fig. 2, microphotographs of the liver, kidney, lung, and splenic tissues of mice treated with PePp are shown. In the histological evaluation, all animals exhibited preservation of tissue architecture when compared to the control group. The liver tissue showed no evidence of inflammation or apoptosis and preserved portal vein (PV) and hepatocytes (arrow) (Fig. 2A). The renal tissue showed the presence of normocellular glomeruli (arrow), suggesting the absence of changes and normality of the ductal tubes (Fig. 2B). In Fig. 2C, it is possible to visualize preserved blood vessels (arrows) and pulmonary alveoli (AL). In the spleen, splenocytes, trabeculae (arrows), white pulp (WP), and red hoopoe (RP) also exhibited preservation (Fig. 2D).

The results of the acute oral toxicity assessment of PePp at a single dose of 2000 mg/kg affirm its safety, as evidenced by the maintenance of body and organ weights and the absence of macroscopic, hematological, and biochemical alterations. These results corroborate studies that investigated the acute toxicity of plant exudates, such as *Terminalia elliptica*, *Buchanania lanzan*, *Albizia labback* [50], *Sterculia ffoetida* [51] and *Anogeissus latifolia* [52]. In our study, acute oral toxicity was assessed to validate the safety of PePp in the acute model of ethanol-induced gastric ulcer prevention. In clinical and experimental models, the prevention and/or treatment of gastric ulcers can involve a single dose, multiple doses, or chronic use. There are still no studies on the long-term toxicity of PePp treatment, nor on the evaluation of its effects on metabolism, bioavailability, and potential risks of accumulation. However, studies report chronic toxicological safety of plant-derived polysaccharides, including *Astragalus membranaceus* [53], *Acacia Senegal* [54], *Cussonia arborea* e *Cordia millenii* [55]. Future investigations are needed to assess the long-term toxicity (subchronic and chronic) of PePp, followed by an evaluation of its effects on prevention and/or treatment in chronic models of gastric ulcer.

Table 3
Effect of PePp on hematological and biochemical parameters.

Hematological parameters	Control	PePp
Red blood cells	7.77 ± 0.86	9.13 ± 1.04
Hemoglobin (g/dL)	13.6 ± 1.00	14.85 ± 1.51
Hematocrit (%)	44.6 ± 3.84	45.15 ± 1.75
Mean corpuscular volume (%)	56.1 ± 4.10	52.7 ± 2.11
Mean corpuscular hemoglobin	17.1 ± 1.01	16.4 ± 0.04
Average corpuscular hemoglobin concentration (g/dL)	30.05 ± 2.26	31.45 ± 0.38
Leukocytes (10/mm^3)	5.3 ± 1.23	4.9 ± 1.07
Segmented (%)	22.0 ± 1.15	25.5 ± 1.94
Typical lymphocyte (%)	68.00 ± 0.67	70.00 ± 0.92
Monocytes (%)	2.30 ± 0.51	2.00 ± 0.63
Platelets (thousands/mm^3)	742.00 ± 47.64	702.00 ± 45.25

Biochemical parameters (mg/dL)	Control	PePp
Glucose	69.25 ± 0.49	77.75 ± 0.78
Urea	32.20 ± 0.90	29.15 ± 0.93
Creatinine	0.44 ± 0.09	0.66 ± 0.15
Total cholesterol	114.5 ± 2.30	119.7 ± 4.65

Values were expressed as mean ± standard deviation.
PePp: Polysaccharide from the exudate of *P. pendula*.

3.3. PePp reduces gastric ulcer formation and improves histopathological parameters in ethanol-induced gastric ulcer model

Macroscopic analysis of the stomach of animals in the control group, free of therapeutic intervention, revealed extensive hemorrhagic lesions, ulcerated erosion, and asymmetrical reddish spots along the stomach. In the ranitidine group, there was a significant reduction in the formation of these ulcerative lesions (Fig. 3A). On the other hand, pre-treatment with PePp promoted, in a dose-dependent manner, the inhibition of lesions, since macroscopic analysis revealed less intensity of tissue damage, as seen in Fig. 3A (10 mg/kg x 25 mg/kg x 50 mg/kg).

In animals that received only saline solution, the gastric ulcer area induced by ethanol was 47.40 ± 2.76 (Fig. 3B). Animals that received ranitidine showed 2.03 ± 0.96 and 95.71 % area and inhibition of gastric ulcer formation, respectively (Fig. 3B and C). Pre-treatment with PePp significantly reduced ($p < 0.001$) the gastric ulcer area and the ulcer inhibition rate by 22.89 ± 0.81, 13.94 ± 3.82, and 7.86 ± 1.03 and by 51.71, 70.58 and 83.41 % for doses of 10, 25 and 50 mg/kg, respectively (Fig. 3B and C). In the histopathological evaluation, the model group exhibited profound alterations in tissue architecture, highlighted by lesions with severe rupture of the superficial epithelium, hemorrhage, necrotic lesions in the mucosa (arrow), and exudative inflammatory infiltrate (*) (Fig. 3D). The pretreatment with PePp (dose-dependent) or ranitidine improved the lesions mentioned above, which suggests their protective effect. As shown in Table 4, the histological scores of ethanol-induced gastric mucosa damage were assessed. The control group presented a score of III, characterized by intense inflammatory infiltration, epithelial surface rupture, hemorrhage, and necrosis exceeding 40 %. In contrast, the group treated with ranitidine and the groups treated with PePp at doses of 25 and 50 mg/kg exhibited mild inflammatory infiltrate and necrotic areas smaller than 20 % (score I). Meanwhile, the group treated with PePp at a dose of 10 mg/kg showed moderate inflammatory infiltration and necrotic areas ranging from 20 % to 39 % (score II).

The area and inhibition of gastric ulcers induced by ethanol, as well as the histopathological parameters in the groups that received only saline or ranitidine, were similar to the results obtained by Ren [42] and Sistani Karampour [43] Thus, the present study demonstrated the uniformity of the gastric ulcer model.

3.4. PePp prevents gastric ulcers via antioxidative and anti-inflammatory activity

Fig. 4 A-C shows that the gastric ulcer model induced by ethanol caused oxide-redox imbalance through a reduction in SOD and CAT levels and an increase in TBARS, corroborating other studies [56,58–61]. The pre-treatment with PePp at all studied concentrations significantly increased ($p < 0.0001$) SOD levels, with no statistical difference compared to the reference drug, ranitidine. Moreover, there was no difference between the doses of 10 (64.53 ± 5.14), 25 mg/kg (63.33 ± 5.60), and 50 mg/kg (74.43 ± 3.15) of PePp, reaching up to twice the increase compared to the untreated group (Fig. 4A). There was no significant difference in CAT levels between the control group animals and animals treated with ranitidine ($p < 0.5$). On the other hand, PePp at a dose of 10 mg/kg (30.53 ± 2.83) showed a significant difference ($p < 0.0001$) compared to the control group, but when compared to the dose of 25 mg/kg (24.08 ± 3.16), there was no statistical difference. At a dose of 50 mg/kg, PePp (39.05 ± 4.87) showed a significant difference when

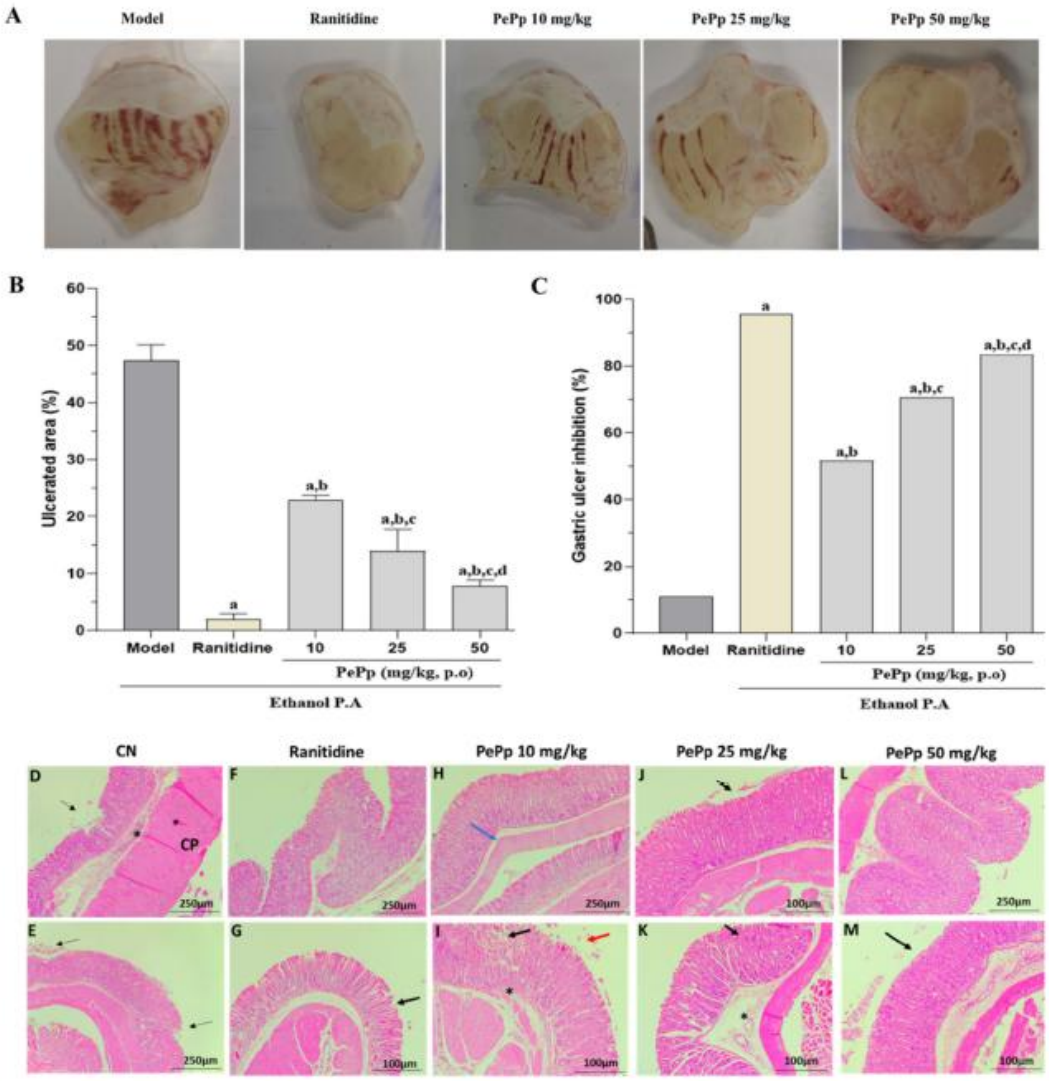

Fig. 3. Effect of *Parkia pendula* exudate polysaccharide (PePp) on the reduction of ethanol-induced gastric ulcer in mice. (A) Macroscopic image of gastric lesions: the control group presents hemorrhagic lesions, ulcerated erosion, and asymmetrical reddish areas throughout the stomach. Ranitidine (80 mg/kg) and PePp, at all concentrations, reduced gastric lesions. Percentage of ulcerative area (B) and percentage of gastric ulcer inhibition (C). Data are expressed as mean ± standard deviation; where $^{a}p < 0.0001$ (significant difference from the control group); $^{b}p < 0.0001$ (significant difference from ranitidine); $^{c}p < 0.0001$ (significant difference from the 10 mg/kg dose); $^{d}p < 0.001$ (significant difference from the 25 mg/kg dose). Gastric ulcer models of the negative control (D-E), showing lesion with moderate to severe rupture of the superficial epithelium and necrotic lesions penetrating deep into the mucosa (arrow) and inflammation of the submucosal layer (*). (F-G) Ranitidine group in (F) shows intact appearance of the histological structure of the epithelium and mucosal layer in (G) mild lesions on the surface (arrow). (H—I) Group treated with 10 mg/kg dose showing lesion (black arrow) and inflammation of the submucosal layer (*), edema (blue arrow) and loss of epithelial cells (red arrow). (J-K) Group treated with 25 mg/kg dose the arrows indicate mild rupture of the superficial epithelium showing reduced damage to the mucosa and inflammation (*). (L-M) Group treated with 50 mg/kg shows better gastroprotective effect than the previous doses. And does not show rupture of the gastric architecture or loss of epithelial cells (H&E staining).

compared to the control group ($p < 0.0001$), ranitidine, and the doses of 10 mg/kg ($p < 0.001$) and 25 mg/kg ($p < 0.0001$) (Fig. 4B). Similar to ranitidine, PePp at all doses significantly ($p < 0.0001$) reduced TBARS levels, with no difference between the studied doses (Fig. 4C).

Fig. 4 D-E shows the levels of the cytokines IL-6 (Fig. 4D), TNF-α (Fig. 4E), and IL-10 (Fig. 4F) of mice-induced gastric ulcers by ethanol. According to Fig. 4, the model group showed an increase in the pro-inflammatory cytokines IL-6 and TNF-α and reduced IL-10, an anti-inflammatory cytokine. These results indicate ulcerative development in the gastric mucosa, since these cytokines are related to the inflammatory process and are widely explored in studies in the screening of new gastroprotective drugs [62–64]. In contrast, animals that received ranitidine showed a reduction in IL-6 and TNF-α and an increase in IL-10. Similar to the control group treated with ranitidine, pretreatment with PePp dose-dependently modulated the reduction in IL-6 and TNF-α levels and the increase in IL-10. In the present study, the dose of 50 mg/

Table 4
Histological scores of ethanol-induced gastric lesions in mice treated with *Parkia pendula* exudate polysaccharide (PePp).

Groups	Scores
Control	III
Ranitidine	I
PePp 10 mg/kg	II
PePp 25 mg/kg	I
PePp 50 mg/kg	I

Score I: Mild inflammatory infiltrate and necrosis area < 20 %.
Score II: Moderate inflammatory infiltrate and necrosis area between 20 and 39 %.
Score III: Intense inflammatory infiltrate, rupture in the epithelial surface, hemorrhage and necrosis >40 %.

kg of PePp showed an immunoregulatory effect for the cytokines IL-6, TNF-α and IL-10 with no statistical difference when compared to ranitidine.

According Huang [61] and Raafat [62] ethanol metabolism induces the formation of free radicals, reduction of the antioxidative reserve, and changes in pro-inflammatory cytokines. These elements are responsible for causing vascular endothelial damage of the gastric mucosa, disruption of microcirculation, and ischemia, promoting the development of gastric ulcers. In this scenario, searching for new gastroprotective drugs with antioxidant and anti-inflammatory is desired, since eliminating free oxygen radicals increases antioxidative reserve and a pro-inflammatory microenvironment.

The reduction of TBARS promoted by PePp demonstrates its antioxidant activity. TBARS is widely used to quantify lipid peroxidation, a marker of cellular damage caused by lipid oxidation, which compromises cell fluidity and permeability, including the gastric mucosa [65]. Additionally, PePp increased the levels of the antioxidant enzymes SOD and CAT. It is known that SOD catalyzes the superoxide anion ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2), which is converted into H_2O and O_2 via CAT [66]. This reaction is important because H_2O_2 reacts with lipid chains in cell membranes, increasing lipid peroxidation.

Furthermore, the notable reduction in pro-inflammatory cytokines, IL-6 and TNF-α, in PePp-treated groups, along with the increase in the anti-inflammatory cytokine IL-10, strongly suggests that PePp exerts its gastroprotective effects, at least in part, through modulating the inflammatory response. In our study, the reduction of TNF-α and IL-6 promoted by pre-treatment with PePp may have contributed to the reduction of exudative inflammation in gastric tissue (Fig. 3D – PePp 50 mg/kg) since these cytokines are responsible for cell activation, including macrophages, lymphocytes and nuclear polymorphs, during the inflammatory process, triggering oxidative pathways with the production of reactive oxygen species and contributing to the progression of tissue damage in gastric ulcer [57,67]. Furthermore, pre-treatment with PePp increased IL-10, a cytokine that contributes to the control of inflammation, establishes an anti-inflammatory environment and inhibits the synthesis of TNF-α.

The complete mechanism of action of PePp in the acute model of protection against ethanol-induced gastric ulcer has not yet been elucidated. However, plant-derived polysaccharides have demonstrated gastroprotective activity through the reduction of gastric acid and pepsin secretion [68], increased mucus synthesis [69] and coating of gastric mucosal membranes [70]. Regarding the gastroprotective activity of ranitidine, it is suggested that its mechanism of action occurs through the blockage of H_2 receptors on the surface of acid-producing

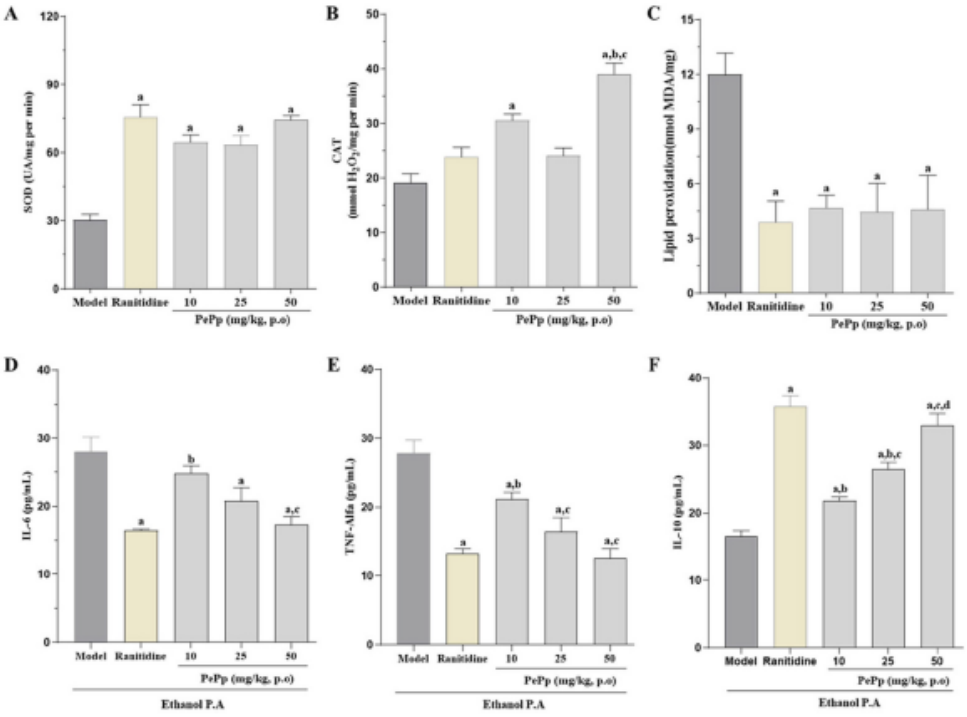

Fig. 4. Effect of *Parkia pendula* exudate polysaccharide (PePp) on SOD (A), CAT (B), and TBARS (C) levels, as well as cytokines IL-6 (D), TNF-α (E), and IL-10 (F) in gastric tissue homogenate. Data are expressed as mean ± standard deviation, where: $^{a}p < 0.0001$ (significant difference from the control group); $^{b}p < 0.001$ (significant difference from ranitidine); $^{c}p < 0.001$ (significant difference from the 10 mg/kg dose); $^{d}p < 0.0001$ (significant difference from the 25 mg/kg dose).

cells in the stomach, reducing gastric acid and pepsin secretion, which may contribute to the prevention of gastric ulcer formation [71,72]. Our results show that PePp exhibited a similar effect to ranitidine concerning antioxidant and anti-inflammatory activity, and this effect can also be confirmed through macroscopic and histological evaluation.

Other studies corroborate our results regarding the antioxidant and anti-inflammatory properties of plant polysaccharides on ethanol-induced gastric ulcers. Polysaccharide obtained from *Evodiae fructus* [73], *Hericium erinaceus* [74], *Lycium barbarum* [64], *Momorcodia charantia* [75] e *Prunus cerasus* [62] increased SOD and CAT levels and reduced TBARS levels; in addition to causing a reduction in IL-6 and TNF-α and an increase in IL-10.

4. Conclusion

The results highlight the acute oral toxicological safety of PePp and its gastroprotective effect in the experimental model of ethanol-induced gastric ulcer. The gastroprotective effect was demonstrated by the reduction of gastric ulcer formation, in addition to the macroscopic and histological preservation of the gastric mucosa. This effect can, in part, be attributed to the antioxidant and anti-inflammatory action of PePp. Our experimental results are promising and encourage the search for new plant-based drugs with gastroprotective action. Furthermore, the present study contributes to the perspective of using pharmaceutical technologies to develop a final product based on PePp that can be made available for the prevention and treatment of pathologies associated with gastric ulcer formation.

CRediT authorship contribution statement

José Josenildo Batista: Writing – original draft, Methodology, Investigation, Conceptualization. **Francisca Crislândia Oliveira Silva:** Methodology, Investigation. **Maria Isabela Ferreira de Araújo:** Methodology, Investigation. **Pedro Henrique de Almeida Moura Nunes:** Methodology, Investigation. **Sílvio Assis de Oliveira Ferreira:** Methodology, Investigation. **Luzia Abilio da Silva:** Methodology, Investigation. **Leydianne Leite de Siqueira Patriota:** Methodology, Investigation. **Thiago Henrique Napoleão:** Supervision, Investigation. **Patrícia Maria Guedes Paiva:** Supervision, Investigation. **Jennyfer Martins de Carvalho:** Methodology, Investigation. **Leucio Duarte Vieira Filho:** Supervision, Investigation. **Jonh F. Kennedy:** Writing – review & editing, Investigation. **Paulo Antônio Galindo Soares:** Writing – review & editing, Supervision, Investigation. **André de Lima Aires:** Writing – review & editing, Supervision, Investigation. **Luana Cassandra Breitenbach Barroso Coelho:** Writing – review & editing, Resources, Project administration.

Declaration of competing interest

The authors declare that there is no conflict of interest that could influence the work reported in this article.

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Data availability

Data will be made available on request.

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5. CONCLUSÕES

• PePp apresenta alto rendimento, com uma composição rica em carboidratos totais e ácido urônico, além de se tratar de um polissacarídeo homogêneo, cuja cadeia principal é formada de arabinose e galactose.

• PePp é um polissacarídeo seguro, conforme demonstrado pela avaliação de toxicidade aguda, que não prevê sinais de mortalidade, alterações comportamentais ou mudanças nos parâmetros bioquímicos, hematológicos e histológicos.

• PePp apresenta atividade prebiótica, promovendo o crescimento de *L. rhamnosus*, *L. brevis*, *L. plantarum*, *L. casei*, *L. paracasei*, *B. longum* e *B. adolescentis*.

• Na fermentação fecal *in vitro*, PePp foi metabolizado pelas bactérias intestinais, resultando na redução do pH fecal ao longo de 24 h, enquanto a produção de AGCC, como ácido acético, propiônico e isobutírico, aumento significativamente. Além disso, esse polissacarídeo demonstrou a capacidade de modular a composição da microbiota intestinal, promovendo o crescimento de bactérias benéficas, como *Bacteroides*, *Bifidobacterium*, *Prevotella* e *Enterococcus*.

• Na fermentação *in vivo*, PePp modulou a microbiota intestinal de camundongos BALB/C, levando a redução do pH fecal e ao aumento das concentrações de SCFAs, incluindo ácidos acético, propiônico e butírico, além de promover o crescimento de bactérias benéficas.

• PePp apresentou efeito gastroprotetor em modelos de úlcera gástrica induzida por etanol, inibindo a formação de lesões gástricas. Esse efeito foi acompanhado pela redução da peroxidação lipídica e dos níveis de citocinas pró-inflamatórias, como IL-6 e TNF-α, além de promover o aumento da superóxido dismutase e catalase, e da citocina anti-inflamatória IL-10.

• PePp apresentou efeitos benéficos, demonstrando potencial para aplicações futuras no campo da saúde e no desenvolvimento de produtos funcionais. Seus efeitos prebióticos, gastroprotetor e sua capacidade de modular a microbiota intestinal indicam um impacto positivo na saúde digestiva.

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ANEXO A – Comissão de Ética no Uso de Animais

Universidade Federal de Pernambuco
Centro de Biociências
Av. Prof. Nelson Chaves, s/n
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Recife, 29 de dezembro de 2022

Ofício nº 115/22

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: Prof. Andre de Lima Aires
Departamento de Medicina Tropical/CCS
Processo nº127/2022

Certificamos que a proposta intitulada "**Caracterização estrutural, toxicidade aguda e subcrônica e avaliação da citotoxidade e genotoxicidade do polissacarídeo de *Parkia pendula***. Registrado como **nº127/2022** sob a responsabilidadeda **Prof . Andre de Lima Aires** Que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo CONSELHO NACIONAL DE CONTROLE DE EXPERIMENTAÇÃO ANIMAL (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DE PERNAMBUCO (UFPE), em reunião de 06/12/2022

Finalidade	() Ensino (x) Pesquisa Científica
Vigência da autorização	Julho de 2023 a dezembro de 2023
Espécie/linhagem/raça	Camundongo heterogênico
Nº de animais	61 animais
Peso/Idade	25-35g/ 35 dias
Sexo	Macho (35) e Fêmea (26)
Origem: Biotério de Criação	Bioterio do LIKA /UFPE
Destino: Biotério de Experimentação	Biotério Convencional do Instituto Keizo Asami (iLIKA/UFPE).

Atenciosamente

Prof. Sebastião R. F. Silva
Presidente CEUA/UFPE
SIAPE 2345691
UFPE

ANEXO B – Comissão de Ética no Uso de Animais

Universidade Federal de Pernambuco
Centro de Biociências
Av. Prof. Nelson Chaves, s/n
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Recife, 26 de setembro de 2023

Ofício nº 76/23

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: Prof. André de Lima Ayres
Departamento de Medicina Tropical/ CCS
Processo nº0052/2023

Certificamos que a proposta intitulada "**Atividade prebiótica e efeito do polissacarídeo de Parkia pendula na fermentação in vivo pela microbiota intestinal de camundongos.**" Registrado como **0052/2023** sob a Responsabilidade do **Prof. André de Lima Ayres** Que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo CONSELHO NACIONAL DE CONTROLE DE EXPERIMENTAÇÃO ANIMAL (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DE PERNAMBUCO (UFPE), em reunião de 29/08/2023

Finalidade	() Ensino (x) Pesquisa Científica
Vigência da autorização	31/08/2023 a 01/11/2026
Espécie/linhagem/raça	Camundongo heterogênico
Nº de animais	35
Peso/Idade	18-22g
Sexo	Femea (35)
Origem: Biotério de Criação	Biotério do Instituto Keizo Asami (LIKA).
Destino: Biotério de Experimentação	Biotério Convencional do Instituto Keizo Asami. (LIKA)

Atenciosamente

Prof. Sebastião R. F. Silva
Presidente CEUA/UFPE
SIAPE 2345691

ANEXO C – Comissão de Ética no Uso de Animais

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Recife, 26 de setembro de 2023

Ofício nº 77/23

Da Comissão de Ética no Uso de Animais (CEUA) da UFPE
Para: Prof. André de Lima Ayres
Departamento de Medicina Tropical/ CCS
Processo nº128/2022

Certificamos que a proposta intitulada "**Avaliação do efeito e mecanismo de ação do polissacarídeode parkia pendula em úlceras gástricas.**" Registrado como **128/2022** sob a Responsabilidade do **Prof. André de Lima Ayres** Que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo CONSELHO NACIONAL DE CONTROLE DE EXPERIMENTAÇÃO ANIMAL (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DA UNIVERSIDADE FEDERAL DE PERNAMBUCO (UFPE), em reunião de 29/08/2023

Finalidade	() Ensino (x) Pesquisa Científica
Vigência da autorização	Agosto de 2023 a dezembro de 2025
Espécie/linhagem/raça	Camundongo heterogênico/ Swiss Rato heterogênico/ Wistar
Nº de animais	235 Animais
Peso/Idade	25 - 35 g/ Adultos /Camundongo heterogênico 180- 200 g/ Adultos/ Rato heterogênico
Sexo	Macho (235)
Origem: Biotério de Criação	Biotério Convencional do Instituto Keizo Asami (LIKA).
Destino: Biotério de Experimentação	Biotério Convencional do Instituto Keizo Asami (LIKA)

Atenciosamente

Prof. Sebastião R. F. Silva
Presidente CEUA/UFPE
SIAPE 2345691