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GUSTAVO RATTI DA SILVA

# PROPRIEDADES FARMACOLÓGICAS, TOXICOLÓGICAS E EFEITOS CARDIOMETABÓLICOS DA *Baccharis dracunculifolia*: UMA INVESTIGAÇÃO PRÉ-CLÍNICA

Umuarama
2025

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Tese apresentada ao Programa de Pós-Graduação em Ciência Animal com Ênfase em Produtos Bioativos da Universidade Paranaense como parte dos requisitos para obtenção do título de Doutor em Ciência Animal com área de concentração em Terapêutica Experimental de Produtos Bioativos Aplicados a Clínica e Reprodução Animal.

Orientação: Dra. Francislaine Aparecida dos Reis Lívero

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# PROPRIEDADES FARMACOLÓGICAS, TOXICOLÓGICAS E EFEITOS CARDIOMETABÓLICOS DA *Baccharis dracunculifolia*: UMA INVESTIGAÇÃO PRÉ-CLÍNICA

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3 COPG: Coordenadoria da Pós-Graduação

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GUSTAVO RATTI DA SILVA

# PROPRIEDADES FARMACOLÓGICAS, TOXICOLÓGICAS E EFEITOS CARDIOMETABÓLICOS DA *Baccharis dracunculifolia*: UMA INVESTIGAÇÃO PRÉ-CLÍNICA

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"*Os grandes feitos são conseguidos não pela força, mas pela perseverança*."

(Samuel Johnson, 1759)

SILVA, Gustavo Ratti da. **Propriedades farmacológicas, toxicológicas e efeitos cardiometabólicos da** *Baccharis dracunculifolia***: uma investigação pré-clínica**. Orientadora: Francislaine Aparecida dos Reis Lívero. 2025. 101 f. Tese (Doutorado em Ciência Animal com Ênfase em Produtos Bioativos) - Universidade Paranaense, Umuarama, 2025.


RESUMO

A *Baccharis dracunculifolia* DC., também conhecida por "alecrim-do-campo" é popularmente usada para tratar distúrbios gástricos e hepáticos e se destaca nas pesquisas científicas por suas propriedades antioxidante, anti-inflamatória e hepatoprotetora. Visando validar esses efeitos, esta pesquisa investigou a segurança e a eficácia da planta. O primeiro estudo explorou aspectos toxicológicos (*in vitro* e *in vivo*), bem como o potencial antioxidante e anti-inflamatório do extrato etanólico das partes aéreas de *B. dracunculifolia*. A análise fitoquímica identificou compostos fenólicos e flavonoides. Os ensaios antioxidantes *in vitro,* como os métodos de sequestro do radical livre 2,2-difenil-1-picrilhidrazil e poder antioxidante redutor férrico indicaram uma atividade antioxidante significativa ($IC_{50}$ 0,77 mg/mL e 1,24 μM sulfato ferroso/mg, respectivamente). Em modelos celulares, os testes de inibição de hemólise e fagocitose indicaram um potencial anti-inflamatório promissor sendo a concentração de 600 μg/mL a que apresentou os melhores resultados (58,60% e 60,84% respectivamente). No estudo de citotoxicidade, o extrato manteve a viabilidade celular acima de 70%, mesmo quando testado em altas concentrações. Por fim, os testes de toxicidade aguda em ratas Wistar foi conduzido de acordo com o protocolo 423 da Organização para a Cooperação e Desenvolvimento Econômico e corroboraram a segurança do extrato, não alterando parâmetros hematológicos, bioquímicos, clínicos e celulares. Os achados do primeiro estudo apontam para um perfil seguro do extrato de *B. dracunculifolia* e destacam seu potencial antioxidante e anti-inflamatório, importantes componentes em doenças metabólicas crônicas. Visando avaliar a eficácia do extrato, o segundo estudo avaliou o potencial terapêutico da planta em um modelo animal contemplando condições metabólicas complexas, como a síndrome cardiometabólica. O modelo de 4 semanas foi estabelecido com a associação de 3 fatores de risco: diabetes (induzido por estreptozotocina 30 mg/kg, i.p.), dislipidemia (induzida pelo consumo de ração enriquecida com 0,5% de colesterol) e o tabagismo (desencadeado pela inalação de 9 cigarros/dia, 1h/dia, 5 dias/semana) em ratas Wistar. Nas últimas 2 semanas, os animais foram tratados com o extrato de *B. dracunculifolia* (30, 100 e 300 mg/kg) por via oral. Observou-se redução de lipídios plasmáticos e hepáticos,

reversão da esteatose hepática e melhora de transaminases hepáticas, sendo a dose de 30 mg/kg a mais promissora. Além disso, o tratamento com o extrato aumentou os níveis de glutationa reduzida e superóxido dismutase e reduziu a lipoperoxidação, destacando a capacidade antioxidante da planta. Ambos os estudos ressaltam o potencial terapêutico de *B. dracunculifolia* para doenças metabólicas e inflamatórias. Dado o bom perfil de segurança e a capacidade de atuar em diversos alvos, a *B. dracunculifolia* pode ser uma abordagem inovadora para a tratamento de condições complexas, como a síndrome cardiometabólica. Essas descobertas não apenas validam cientificamente o uso tradicional de *B. dracunculifolia*, mas também abrem caminho para o desenvolvimento de novos medicamentos baseados em produtos naturais, promovendo tratamento mais eficaz e com menor incidência de efeitos adversos.

Palavras-chave: Alecrim-do-campo. Antioxidante. Anti-inflamatório. Síndrome metabólica. Toxicidade. Viabilidade celular.

SILVA, Gustavo Ratti da. **Pharmacological, toxicological, and cardiometabolic effects of** *Baccharis dracunculifolia*: **a preclinical investigation**. Advisor: Francislaine Aparecida dos Reis Lívero. 2025. 101 p. Thesis (Doctorate degree in Animal Science with Emphasis on Bioactive Products) - Universidade Paranaense, Umuarama, 2025.

ABSTRACT

*Baccharis dracunculifolia* DC., commonly known as "field rosemary," is traditionally used to treat gastric and hepatic disorders and has garnered scientific attention for its antioxidant, anti-inflammatory, and hepatoprotective properties. To validate these effects, this study investigated the safety and efficacy of the plant. The first study explored toxicological aspects (*in vitro* and *in vivo*) as well as the antioxidant and anti-inflammatory potential of the ethanolic extract from the aerial parts of *B. dracunculifolia*. Phytochemical analysis revealed the presence of phenolic compounds and flavonoids. *In vitro* antioxidant assays, such as the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method and ferric reducing antioxidant power (FRAP), indicated significant antioxidant activity (IC50 0.77 mg/mL and 1.24 µM ferrous sulfate/mg, respectively). In cellular models, hemolysis inhibition and phagocytosis assays indicated a promising anti-inflammatory potential, with the concentration of 600 µg/mL yielding the best results (58.60% and 60.84%, respectively). Acute toxicity tests in Wistar rats, conducted in accordance with Protocol 423 of the Organization for Economic Cooperation and Development, confirmed the safety of the extract, as it did not alter hematological, biochemical, clinical, or cellular parameters. These findings indicate that the extract of *B. dracunculifolia* has a favorable safety profile and highlights its antioxidant and anti-inflammatory potential, key factors in addressing chronic metabolic diseases. The second study assessed the therapeutic efficacy of the extract in an animal model of complex metabolic conditions, including cardiometabolic syndrome. This model of 6 weeks was established by combining three risk factors: diabetes (induced by intraperitoneal administration of streptozotocin at 30 mg/kg), dyslipidemia (induced by a diet enriched with 0.5% cholesterol), and smoking (induced by inhalation of nine cigarettes per day, for one hour per day, five days per week) in Wistar rats. Treatment orally with *B. dracunculifolia* extract at doses of 30, 100, and 300 mg/kg for 2 weeks resulted in significant reductions in plasma and hepatic lipid levels, reversal of hepatic steatosis, and improvement in hepatic transaminase levels, with the 30 mg/kg dose showing the most promising results. Additionally, the extract increased levels of reduced glutathione and superoxide dismutase while reducing lipid peroxidation, further demonstrating its antioxidant capacity. Both studies underscore the

therapeutic potential of *B. dracunculifolia* in addressing metabolic and inflammatory diseases. With its favorable safety profile and ability to target multiple pathways, *B. dracunculifolia* represents a promising approach for managing complex conditions such as cardiometabolic syndrome. These findings not only provide scientific validation for the traditional use of *B. dracunculifolia* but also open avenues for the development of new drugs based on natural products, offering more effective treatments with fewer adverse effects.

Keywords: Alecrim-do-campo. Anti-inflammatory. Antioxidant. Cell viability. Metabolic syndrome. Toxicity.

## LISTA DE ILUSTRAÇÕES

**Revisão bibliográfica - Síndrome cardiometabólica e *Baccharis dracunculifolia*: uma revisão de literatura sobre potenciais terapêuticos**



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**LISTA DE SIGLAS**

| | |
|---|---|
| CAPES | Coordenação de Aperfeiçoamento de Pessoal de Nível Superior |
| DL50 | Dose letal mediana |
| OECD | Organização para a Cooperação e Desenvolvimento Econômico |
| COPG | Coordenadoria de Pós-Graduação |
| UNIPAR | Universidade Paranaense |
| FRAP | Ferric Reducing Antioxidant Power |
| DPPH | 2,2-difenil-1-picrilhidrazil |
| GSH | Glutationa reduzida |
| SOD | Superóxido dismutase |
| LPO | Lipoperoxidação |
| AST | Aspartato aminotransferase |
| ALT | Alanina aminotransferase |
| SIM | Simvastatin |
| INSU | Insulina |
| \|GAE | Equivalentes de ácido gálico |
| IC50 | Concentração inibitória mediana |
| PC | Controle positivo |
| NC | Controle negativo |
| HRBC | Estabilização da membrana de hemácias humanas |
| DMEM | Meio de Eagle modificado por Dulbecco |
| MTT | 3-(4,5-dimetiltiazol-2yl)-2,5-di- fenil brometo de tetrazolina |

| | |
|---|---|
| CONCEA | Conselho Nacional de Controle de Experimentação Animal |
| BD | *Baccharis dracunculifolia* |
| C- | Controle negativo |
| MCV | Volume Corpuscular Médio |
| MCH | Hemoglobina Corpuscular Média |
| MCHC | Concentração da Hemoglobina Corpuscular Média |
| GHS | Sistema de Classificação Globalmente Harmonizado |
| CMS | Síndrome cardiometabólica |

**LISTA DE SÍMBOLOS**

| | |
|---|---|
| ºC | Graus Celsius |
| ® | Marca registrada |
| ± | Mais ou menos |
| M | Molaridade |
| % | Porcentagem |
| mL | Mililitro |
| µg | Micrograma |
| mg | Miligramas |
| kg | Quilograma |
| n | Número |

SUMÁRIO

# CAPÍTULO 1

# REVISÃO BIBLIOGRÁFICA

# SÍNDROME CARDIOMETABÓLICA E *Baccharis dracunculifolia*: UMA REVISÃO DE LITERATURA SOBRE POTENCIAIS TERAPÊUTICOS

O capítulo 1 foi editado de acordo com as normas da Associação Brasileira de Normas Técnicas - ABNT.

# SÍNDROME CARDIOMETABÓLICA E *Baccharis dracunculifolia*: UMA REVISÃO DE LITERATURA SOBRE POTENCIAIS TERAPÊUTICOS

## 1.1 Introdução

A síndrome cardiometabólica é caracterizada por um conjunto de fatores de risco que, combinados, aumentam a probabilidade de desenvolvimento de doenças cardiovasculares e distúrbios metabólicos (Bovolini et al., 2021). Entre os principais fatores de risco destacam-se o diabetes tipo 2, a dislipidemia e o tabagismo, que estão associados a processos de inflamação, estresse oxidativo e alterações metabólicas (Berlin et al., 2012; Defronzo et al., 2015; Sandesara et al., 2019). No entanto, o tratamento dessa síndrome exige uma abordagem multifatorial. Os medicamentos disponíveis atualmente tratam os fatores isoladamente, mas o uso concomitante de múltiplos fármacos pode gerar interações medicamentosas e causar efeitos adversos (Thompson et al., 2016; Neeland et al., 2024).

Nesse contexto, a etnobotânica e a etnofarmacologia têm desempenhado um papel essencial no estudo da biodiversidade, sendo responsáveis pela descoberta de diversas substâncias e compostos biologicamente ativos. Essas áreas impulsionam a pesquisa, o desenvolvimento e a exploração racional de recursos naturais provenientes da flora de diferentes regiões (Sales; Sartor; Gentilli, 2015).

Uma espécie popularmente utilizada, mas com poucos estudos etnofarmacológicos, é a *Baccharis dracunculifolia* DC (Asteraceae), conhecida no Brasil como “alecrim-do-campo” ou “vassourinha”. Esse arbusto nativo, amplamente distribuído no país, apresenta rápido desenvolvimento e potencial para ser usado na recuperação de solos degradados (Bastos et al., 2011).

A espécie é utilizada popularmente para tratar doenças gástricas, inflamações e distúrbios hepáticos (Guimarães et al., 2012; Figueiredo-Rinhel et al., 2013). Estudos indicam que os extratos obtidos de suas folhas possuem propriedades anti-inflamatórias, antibacterianas, antifúngicas, imunomoduladoras, antigenotóxicas e antimutagênicas (Gazim et al., 2022). Em um modelo animal de obesidade, ratas Wistar tratadas com extrato metanólico da planta apresentaram aumento na secreção de insulina nas ilhotas pancreáticas (Hocayen et al., 2016). Entretanto, os possíveis efeitos hipoglicemiantes e/ou hipolipemiantes da espécie ainda não foram investigados cientificamente.

Dessa forma, este estudo propõe investigar a segurança pré-clínica *in vitro* e *in vivo*, bem como os efeitos terapêuticos da *B. dracunculifolia* em um modelo animal de síndrome cardiometabólica desencadeado por múltiplos fatores de risco, como diabetes, tabagismo e dislipidemia

## 1.2 Revisão da literatura

### 1.2.1 Síndrome cardiometabólica

A síndrome cardiometabólica é uma das principais causas de morbimortalidade no mundo, configurando-se como um problema de saúde global que atinge mais de um quarto da população adulta mundial (Saad et al., 2014; WHO 2018; Liang et al., 2023). A síndrome é caracterizada por um conjunto de fatores de risco, como obesidade, hipertensão, diabetes tipo 2, dislipidemia e resistência à insulina, que, combinados, aumentam a predisposição ao desenvolvimento de doenças cardiovasculares e distúrbios metabólicos (Sposito et al., 2007; Bovolini et al., 2021).

O diabetes mellitus tipo 2 é uma doença multifatorial que causa diversas alterações fisiopatológicas, como disfunção das células β, resistência à insulina e inflamação crônica. Essas alterações resultam em hiperglicemia crônica, que, por sua vez, provoca danos endoteliais e inflamação sistêmica, elevando o risco de desenvolvimento de doenças cardiovasculares. Por este motivo, o diabetes tipo 2 é um dos principais componentes da síndrome cardiometabólica (Defronzo et al., 2015; Dhondge et al., 2024).

Outro elemento relevante da síndrome cardiometabólica é a dislipidemia, que se caracteriza por alterações nas concentrações de lipídios plasmáticos. Há aumento nos níveis de triglicerídeos, colesterol total e lipoproteína de baixa densidade (LDL), e redução da lipoproteína de alta densidade (HDL). Essas alterações contribuem para a progressão de doenças cardiovasculares e metabólicas por meio de mecanismos relacionados à inflamação, estresse oxidativo e alterações no metabolismo lipídico e glicêmico (Kolovou; Anagnostopoulou; Cokkinos, 2005; Kopin; Lowenstein, 2017; Sandesara et al., 2019; Sottero et al., 2018; Toth; Banach, 2024).

O tabagismo também está associado à síndrome cardiometabólica devido à sua capacidade de desencadear ou agravar condições como dislipidemia, resistência à insulina, obesidade central, hipertensão arterial e inflamações sistêmicas (Sun; Liu; Ning, 2012; Pan et al., 2015). Além disso, o tabagismo promove estresse oxidativo, danificando o endotélio vascular e acelerando o processo de aterosclerose (Berlin et al., 2012; Liu et al., 2021; Messner; Bernhard, 2014).

O tratamento da síndrome cardiometabólica é desafiador, pois exige a abordagem simultânea de múltiplos fatores. Medicamentos tradicionais, como estatinas (para dislipidemia) e metformina (para diabetes tipo 2), são eficazes, mas frequentemente causam efeitos adversos, como elevações nas transaminases hepáticas e distúrbios gastrointestinais, comprometendo a adesão do paciente ao tratamento. Além disso, esses medicamentos não

tratam todos os fatores de risco associados à síndrome cardiometabólica (Thompson et al., 2016; Stoica et al., 2019; Ruscica et al., 2022; Neeland et al., 2024). Por essa razão, há uma crescente demanda por terapias mais seguras e abrangentes, capazes de atuar em diversas vias metabólicas, reduzindo a polifarmácia e melhorando a qualidade de vida dos pacientes (Csermely; Agoston; Pongor, 2005; Lu et al., 2012).

Neste sentido, as plantas medicinais são uma fonte tradicional e importante de medicamentos. A indústria farmacêutica contemporânea explora amplamente a diversidade de metabólitos secundários presentes nos vegetais para o desenvolvimento de novas abordagens terapêuticas. Esses compostos bioativos desempenham um papel relevante na prevenção de inúmeras doenças crônicas (Konan et al., 2007; Pacífico et al., 2016). Além disso, as plantas medicinais podem ser utilizadas como adjuvantes às terapias convencionais, potencializando sua ação e minimizando os efeitos adversos (Dehghani et al., 2023; Harvey, 2000).

### 1.2.2 Exploração etnofarmacológica como estratégia para a descoberta de novos agentes terapêuticos

A atual meta científica é explorar a biodiversidade mundial ainda não avaliada para qualquer atividade biológica, estimada em 95% da quantidade total de espécies de plantas, animais, fungos e microrganismos (Mishra; Tiwari, 2011; Paton et al., 2020). A evolução ao longo de milhões de anos de organismos terrestres e marinhos, adaptando-se a estresses abióticos e bióticos, gerou uma vasta gama de produtos naturais bioativos. Por isso, há séculos, esses produtos têm sido utilizados como reservatórios de compostos promissores para o desenvolvimento de fármacos e tratamentos (David; Wolfender; Dias, 2015; Calixto, 2019; Newman; Cragg, 2020).

A prática de usar plantas como recurso terapêutico remonta a tempos antigos, quando as pessoas confiavam em conhecimento intuitivo e empírico para identificar tratamentos na flora ao seu redor. Essa sabedoria foi transmitida de geração em geração e permanece valorizada até os dias atuais (Ferreira et al., 2019; Fischer; Stumpf; Mariot, 2019). Nesse contexto, a etnofarmacologia representa uma abordagem científica para investigar as atividades biológicas de preparações terapêuticas utilizadas por seres humanos, abrangendo qualquer estudo que documente o uso de plantas medicinais e realize pesquisas experimentais sobre drogas botânicas (Heinrich et al., 2009; Fitzgerald; Heinrich; Booker, 2020). Essa disciplina é intrinsecamente interdisciplinar, combinando elementos de antropologia, farmacologia e toxicologia para analisar os remédios populares (Heinrich, 2014).

Qualquer metodologia que envolva o uso de uma planta, aliada à sua avaliação científica para novas aplicações, pode ser considerada um estudo etnofarmacológico. Assim, o principal desafio é compreender e descrever os aspectos botânicos e fitoquímicos das espécies, além de aplicar metodologias rigorosas, reprodutíveis e direcionadas à solução de problemas específicos (Leonti, 2011; Heinrich, 2014; Fitzgerald; Heinrich; Booker, 2020).

A etnofarmacologia, que combina pesquisas de campo e experimentos laboratoriais, tem contribuído significativamente para o conhecimento sobre plantas medicinais utilizadas em diversas culturas, resultando no desenvolvimento de inúmeros fármacos (Fabrican; Farnsworth, 2001; Buenz; Verpoorte; Bauer, 2018). Muitos medicamentos disponíveis no mercado atualmente têm origem em plantas medicinais, reafirmando a relevância dessas espécies como fonte essencial para a descoberta de novas drogas. Estudos etnobotânicos indicam que plantas usadas tradicionalmente por longos períodos frequentemente apresentam potencial terapêutico (Atanasov et al., 2015; Buenz; Verpoorte; Bauer, 2018). Além disso, as plantas medicinais com histórico de uso tradicional têm maior probabilidade de demonstrar eficácia em sistemas biológicos, otimizando tempo e custos no desenvolvimento de novos medicamentos (David; Wolfender; Dias, 2015; Mohs; Greig, 2017; Jayasundara et al., 2019).

De acordo com dados da Organização Mundial da Saúde, aproximadamente 80% da população de países em desenvolvimento, incluindo o Brasil, recorre à medicina baseada em extratos vegetais (World Health Organizatio, 2018**)**. Em muitas comunidades, o conhecimento sobre plantas medicinais constitui a única opção terapêutica disponível (Silveira; Bandeira; Arrais, 2008; Ferreira et al., 2019). Apesar da rica biodiversidade brasileira, grande parte das plantas é consumida sem evidências científicas de suas propriedades farmacológicas, o que pode resultar em uso inadequado, intoxicações ou interações medicamentosas (Junior; Pinto; Maciel, 2005; Vendrusculo; Mentz, 2006).

Por fim, um dos maiores benefícios dos estudos etnofarmacológicos é a validação científica dos efeitos terapêuticos e a identificação de possíveis efeitos adversos, garantindo o uso seguro e eficaz das plantas medicinais pela população (Bhardwaj; Verma; Gupta, 2018). Com base nessas investigações, plantas medicinais podem ser incorporadas como terapias adjuvantes nos sistemas de saúde, reduzindo custos com medicamentos e ampliando o acesso a tratamentos, não apenas em unidades de saúde, mas também para usuários da medicina tradicional (Brasil, 2006; Enioutina et al., 2017).

### 1.2.3 *Baccharis dracunculifolia*: dos usos populares aos estudos farmacológicos

Uma espécie com potencial medicinal popularmente utilizada, mas ainda com poucos estudos etnofarmacológicos, é a *Baccharis dracunculifolia* DC, um arbusto lenhoso da família Asteraceae amplamente distribuído pela América do Sul (Figura 1). A planta pode atingir entre 2 e 5 metros de altura, apresentando galhos bem ramificados e folhas de formato lanceolado com ápice agudo (Budel et al., 2004; Coradin; Siminski; Reis, 2011). Devido ao seu rápido crescimento, a *B. dracunculifolia* pode ser empregada na recuperação de áreas degradadas, auxiliando no desenvolvimento de outras espécies vegetais nessas regiões (Ramón et al., 2022).

Figura 1. Espécie adulta da *Baccharis dracunculifolia*, com destaque para as folhas e flores.

Fonte: https://encurtador.com.br/glAK6

No Brasil a *B. dracunculifolia* é conhecida popularmente como ‘alecrim-do-campo’ ou ‘vassourinha' (Bastos et al., 2011). O óleo essencial derivado de suas folhas e flores é amplamente utilizado pelas indústrias alimentícia e cosmética, devido ao seu aroma característico de mel (Belini et al., 2016; Cazella et al., 2020). Já as folhas e flores são utilizadas popularmente em forma de chá para tratar doenças gástricas, inflamações e distúrbios hepáticos (Guimarães et al., 2012; Figueiredo-Rinhel et al., 2013; Salazar et al., 2018). Além disso, outras partes da planta são usadas para fabricar utensílios domésticos e agrícolas, em rituais religiosos e como lenha para cozinhar ou aquecer (Müller, 2006; Ritter et al., 2022).

As abelhas da espécie *Apis mellifera* utilizam a resina coletada dos ápices vegetativos da *B. dracunculifolia*, combinando-a com secreções salivares para produzir própolis verde. Esse produto é rico em compostos bioativos, como ácido clorogênico, ácido cafeico, ácidos isoclorogênicos (A, B e C) e artepellina C, triterpenoides, ácidos mono e di-cafeoilquínico e dos flavonoides kaempferol e luteolina (Belini et al., 2016; Ferreira; Negri, 2018; Sun et al., 2019; Chuang et al., 2022). O própolis verde destaca-se por suas múltiplas atividades biológicas, incluindo propriedades anti-inflamatórias (Miranda et al., 2019), antiulcerogênicas (Costa et al., 2020), antibacterianas (Barbosa et al., 2022), antioxidantes (Bittencourt et al., 2015), imunomoduladoras (Gazim et al., 2022) e antimutagênicas (Costa et al., 2018). Estudos relatam que no óleo essêncial da *B. dracunculifolia* há ácidos fenólicos, diterpenos, flavonoides, triterpenos, diversos tipos de glicosídeos, sesquiterpenos e monoterpenos, incluindo compostos como espatulenol, nerolidol e germacreno D, γ-cadineno e δ-cadineno (; Fabiane et al., 2008; Cazella et al., 2019; Minteguiaga et al., 2021; Tomazzoli et al., 2021). As atividades biológicas das folhas da *B. dracunculifolia* decorrem da diversidade de compostos bioativos presentes na planta, incluindo flavonoides (isosakuranetina, aromadendrin-40-metil éter), terpenos (bacarina) e ácidos fenólicos (artepelina C, ácido cafeico, ácido p-cumárico, ácido ferúlico) (Filho et al., 2004; Park et al., 2004; Loots; Van Der Westhuizen; Jerling, 2006; Minteguiaga et al., 2021).

Pesquisas *in vitro* mostram que o óleo essencial da *B. dracunculifolia* possui atividades antiparasitária (Parreira, 2010), antifúngica (Pedrotti; da Silva Ribeiro; Schwambach, 2019), antifitopatogênica (Luchesi et al., 2022), antioxidante (Tomazzoli et al., 2021), antiviral (Búfalo et al., 2009), acaricida (Cazella et al., 2020), antimicrobiana (Salazar et al., 2018), anti-inflamatória e imunomoduladora (Florão et al., 2012). Estudos *in vivo* com o óleo essencial são limitados, mas apresentam efeitos anti-inflamatório (Brandenburg et al., 2020) e antiulcerogênico (Massignani et al., 2009).

Estudo pré-clínicos *in vitro* realizados com extrato das folhas de *B. dracunculifolia* relatam propriedades antibacteriana (Bonin et al., 2020), antioxidante (Casagrande et al., 2018), hepatoprotetora (Da Silva et al., 2019), antifúngica (Silva Filho et al., 2008), antiparasitária (Da Silva Filho et al., 2009), antiviral (Búfalo et al., 2009), anticitotóxica (Bonin et al., 2020) imunomoduladora e anti-inflamatória (Bachiega et al., 2013). Já os estudos *in vivo* demonstraram ações anti-inflamatória (Lima et al., 2020), antiulcerogênica (Boeing et al., 2021), hepatoprotetora (Rezende et al., 2014), bem como efeitos antigenotóxico e antimutagênico (Roberto et al., 2016).

O estudo realizado por Hocayen et al. (2016) utilizou um modelo animal de obesidade induzida por glutamato monossódico em ratos Wistar, que foram tratados com extrato metanólico de *B. dracunculifolia* na dose de 400 mg/kg durante quatro semanas. Os resultados indicaram estímulo da secreção de insulina pelas ilhotas pancreáticas, apontando seu potencial no manejo terapêutico do diabetes mellitus.

Em relação à toxicidade, estudos conduzidos por Rodrigues et al. (2009) com camundongos tratados com extrato aquoso da *B. dracunculifolia* por três dias indicaram efeitos tóxicos em doses elevadas (1 g/kg e 2 g/kg), traduzidos por danos ao DNA e mutações cromossômicas. Munari et al. (2008) também relataram que altas concentrações (100 µg/mL) do extrato (fração acetato de etila) de *B. dracunculifolia* possuem efeito mutagênico em culturas de células de ovário além de demonstrar genotoxicidade em fibroblastos pulmonares, ambas provenientes de hamster chinês em concentrações de 50 e 100 µg/mL (Munari et al., 2010).

Frente ao exposto, a *B. dracunculifolia* apresenta um grande potencial para o desenvolvimento de novas abordagens terapêuticas mais eficazes e seguras. No entanto, seu uso ainda é pouco explorado em estudos que envolvem múltiplos fatores de risco metabólicos. Assim, o objetivo desta tese foi investigar os possíveis efeitos hipoglicemiantes e/ou hipolipemiantes dessa planta em um modelo animal de síndrome cardiometabólica, além de avaliar sua segurança toxicológica, por meio de testes *in vitro* e *in vivo*.

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## 1.4 Objetivo

Investigar o potencial antioxidante, anti-inflamatório e os efeitos toxicológicos *in vitro e in vivo* da *Baccharis dracunculifolia*, bem como seu potencial terapêutico em um modelo animal de síndrome cardiometabólica.

# CAPÍTULO 2

# ARTIGOS

# ARTIGO 1 - SAFETY AND BIOACTIVITY OF *BACCHARIS DRACUNCULIFOLIA* EXTRACT: ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIAL FOR FUNCTIONAL FOOD APPLICATIONS

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# *Safety and bioactivity of Baccharis dracunculifolia extract: antioxidant and anti-inflammatory potential for functional food applications*

**Running title:**

Safety and Bioactivity of *Baccharis dracunculifolia*

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**Abstract**

*Baccharis dracunculifolia*, a native South American plant and primary botanical source of Brazilian green propolis, is rich in phenolic compounds with recognized health-promoting properties. While traditionally used in folk medicine, its direct application as a functional food ingredient remains underexplored. This study aimed to evaluate the safety, antioxidant capacity, and anti-inflammatory potential of a hydroethanolic extract from the aerial parts of *B. dracunculifolia*, using both *in vitro* and *in vivo* approaches. The extract was analyzed for its antioxidant capacity using DPPH and FRAP assays, and its anti-inflammatory potential were assessed through hemolysis inhibition, macrophage treatments, and phagocytosis assays. Additionally, the total flavonoids and total phenolic content of the extract were determined, highlighting its significant bioactive potential. The results demonstrated important antioxidant and anti-inflammatory properties, supporting the therapeutic potential of *B. dracunculifolia* in managing oxidative stress and inflammation. *In vivo*, the extract was administered to Wistar rats at doses of 300 mg/kg and 2000 mg/kg. Biochemical analysis revealed significant reductions in triglycerides and creatinine levels, suggesting improvements in lipid metabolism and renal function. Histopathological evaluations of major organs, including the liver, heart, and kidneys, showed no significant toxic effects, indicating a favorable safety profile at the tested doses. Overall, the findings suggest that *B. dracunculifolia* has promising antioxidant and anti-inflammatory effects, with a favorable safety profile, positioning it as a potential candidate for the development of novel therapeutic approaches for managing chronic cardiometabolic and inflammatory diseases.

**Keywords:** anti-inflammatory activity; functional food ingredient; natural antioxidants; nutraceutical potential; phenolic compounds; safety assessment.

**Highlights**

*Baccharis dracunculifolia* extract shows significant antioxidant properties.

The extract demonstrated a favorable safety profile in histopathological tests.

No significant *in vitro* and *in vivo* cytotoxicity.

*B. dracunculifolia* reduced triglycerides and decreased creatinine levels.

## 1. Introduction

The search for natural bioactive ingredients derived from medicinal plants has significantly increased in recent decades, driven by the demand for safer, more sustainable alternatives and the identification of novel compounds with health-promoting properties. This growing interest reflects a broader shift toward preventive approaches to health and well-being, particularly through the consumption of functional foods and nutraceuticals enriched with naturally occurring antioxidants and anti-inflammatory agents. Plant-based compounds are increasingly recognized not only for their historical use in traditional medicine but also for their scientifically validated potential to contribute to the prevention of chronic diseases when incorporated into the human diet (Salmerón-Manzano et al., 2020; Najmi et al., 2022). Numerous studies have emphasized the potential of these compounds to serve as foundational ingredients in the development of functional food products (Aware et al., 2022; Dzobo, 2022; Nasim et al., 2022; Chaachouay & Zidane, 2024).

*Baccharis dracunculifolia* DC., a plant native to South America, has been extensively studied for its antioxidant and anti-inflammatory properties, attracting interest for both traditional and scientific purposes (Minteguiaga et al., 2021; Heiden, 2022). Commonly known as “vassourinha” or “alecrim-do-campo”, *B. dracunculifolia* belongs to the Asteraceae family and serves as the primary resin source for Brazilian green propolis produced by *Apis mellifera* bees (Sforcin et al., 2012; Dutra et al., 2016; Belini et al., 2016). Traditionally, its aqueous extracts have been used to alleviate gastrointestinal disturbances (Queiroga et al., 1990; Figueiredo-Rinhel et al., 2013), fevers (Rodrigues & Carvalho, 2001), fatigue, and appetite loss (Mors et al., 2000).

Phytochemical investigations of *B. dracunculifolia* reveal a rich composition of flavonoids, terpenes, and phenolic acids, particularly caffeic and p-coumaric acids, which are associated with its health-related properties (Bonin et al., 2020; Gazim et al., 2022). Various *in vitro* studies have demonstrated antioxidant (Casagrande et al., 2018), antibacterial (Bonin et al., 2020), hepatoprotective (da Silva et al., 2019), antifungal (Silva Filho et al., 2008), antiparasitic (da Silva Filho et al., 2009), antiviral (Búfalo et al., 2009), immunomodulatory, and anti-inflammatory effects (Bachiega et al., 2013). Complementary in vivo research has confirmed its anti-inflammatory (Figueiredo-Rinhel et al., 2019), antiulcerogenic (Boeing et al., 2021), hepatoprotective (Rezende et al., 2014), cardiometabolic (Silva et al., 2024), antigenotoxic, and antimutagenic activities (Rodrigues et al., 2009).

Although the pharmacological potential of *B. dracunculifolia* has been extensively documented, its applicability as a food-grade ingredient remains underexplored. Considering that Brazilian green propolis—obtained from its resin—is already widely used in dietary supplements and functional beverages, exploring the safety and functional properties of the plant extract itself is timely and relevant. To support its integration into food products or nutraceutical formulations, it is essential to assess its toxicological safety and biological activities under standardized and robust experimental conditions.

Therefore, this study adopts an integrated approach combining in vitro and in vivo assays to evaluate the safety, antioxidant capacity, and anti-inflammatory activity of a hydroethanolic extract of *B. dracunculifolia*. This study aims to generate scientific evidence to support the potential use of this native Brazilian plant as a functional ingredient in food systems.

## 2. Material and methods

### *2.1. Preparation of Baccharis dracunculifolia extract*

The aerial parts of *Baccharis dracunculifolia* were collected in the municipality of Maria Helena, Paraná, Brazil (23°39'49.7"S and 53°18'18.0"W), and was registered in the Brazilian National System for Management of Genetic Heritage and Associated Traditional Knowledge (SisGen, nº A1F489A). The plant material was air-dried at a temperature of 32°C and subsequently ground to a particle size of 850 μm. The powdered material underwent dynamic maceration with solvent renewal, using 96% ethanol (v/v) as the extraction solvent, until complete exhaustion of the plant material. The resulting extract was filtered and concentrated under reduced pressure using a rotary evaporator (Tecnal®, model TE-211, Brazil) at 40°C, yielding the crude extract of *B. dracunculifolia*. The extract was stored at −20°C until further analysis.

### *2.2 Chemical constituents of B. dracunculifolia extract*

#### *2.2.1 Determination of total phenolics*

The quantification of total phenolics was performed using the Folin-Ciocalteu method, with a gallic acid standard curve (0–2000 μM) as the reference, as described by Singleton and Rossi (1965). A fraction of the *B. dracunculifolia* extract was diluted in methanol to prepare the following concentrations: 0.25, 0.50, 0.75, and 1.0 mg/mL. An aliquot of 0.3 mL from each concentration (in triplicate) was mixed with 2.5 mL of 10% Folin-Ciocalteu solution and 2.0 mL of 7.5% sodium carbonate solution. The mixtures were kept in a dark environment for 60 minutes. Absorbance readings were then taken using a UV-Vis spectrophotometer (Kasuaki, model IL-593-BI, Japan) at 760 nm, with methanol used as the control. The results were expressed as micrograms of gallic acid equivalents (GAE) per milligram of sample (μg GAE/mg).

#### *2.2.2 Determination of total flavonoids*

The total flavonoid content was determined using the colorimetric method described by Alves and Kubota (2013), with aluminum chloride as the analytical control. The *B. dracunculifolia* extract was diluted in methanol/water (80:20, v/v) to prepare a stock solution at a concentration of 1 mg/mL. From this stock solution, three additional dilutions were prepared at concentrations of 0.25, 0.50, and 0.75 mg/mL. For each dilution, an aliquot of 0.5 mL was mixed with 0.5 mL of 2% aluminum chloride solution (w/v) and incubated at room temperature for 10 minutes. The absorbance variation was measured using a UV-Vis spectrophotometer (Kasuaki, model IL-593-BI, Japan) at a wavelength of

425 nm. Flavonoid concentration was calculated using a quercetin standard curve (5–40 µg/mL), and the aluminum chloride solution served as the analytical control. Results were expressed as micrograms of gallic acid equivalents (GAE) per milligram of sample (µg GAE/mg).

### *2.3 Antioxidant studies*

#### *2.3.1 Determination of antioxidant activity by DPPH radical scavenging assay*

The DPPH free radical scavenging assay was performed according to the methodology described by Rufino et al. (2007). Samples of *B. dracunculifolia* extract were diluted in methanol to prepare concentrations of 0.25, 0.50, 0.75, and 1 mg/mL. Then, 0.1 mL of each dilution was mixed with 3.9 mL of a methanolic DPPH solution (60 µM). The samples were kept in the dark for 30 minutes, after which absorbance readings were taken using a UV-Vis spectrophotometer (Kasuaki, model IL-593-BI, Japan) at a wavelength of 515 nm. A methanolic DPPH solution (60 µM) served as the analytical control. The total antioxidant capacity of the extract was calculated using a quercetin solution (60 µM) as a standard, representing 100% antioxidant activity. A plot of absorbance versus concentration was used to determine the sample concentration required to neutralize 50% of free radicals (IC50).

#### *2.3.2 Determination of antioxidant activity by ferric reducing antioxidant power (FRAP) assay*

The ferric reducing antioxidant power (FRAP) assay was conducted following the method described by Rufino et al. (2006). The *B. dracunculifolia* extract was diluted in methanol to concentrations of 0.25, 0.50, 0.75, and 1.00 mg/mL. From each dilution, 90 µL was mixed with 270 µL of distilled water and 2.7 mL of FRAP reagent [composed of 25 mL of acetate buffer (0.3 M), 2.5 mL of an aqueous solution of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ, 10 mM), and 2.5 mL of an aqueous solution of ferric chloride (20 mM)]. The mixtures were incubated at 37ºC for 30 minutes, and absorbance readings were taken using a UV-Vis spectrophotometer (Kasuaki, model IL-593-BI, Japan) at 595 nm. The ferric sulfate standard curve (0–1000 µM) was used to express antioxidant activity as micromoles of ferric sulfate per milligram of sample (µM $FeSO_4$/mg).

### *2.4 In vitro potential anti-inflammatory activity of B. dracunculifolia*

#### *2.4.1 Inhibition of hemolysis*

The human red blood cell (HRBC) membrane stabilization test was carried out following the method described by Ananthi and Chitra (2013). The reaction mixture consisted of 2 mL of hyposaline solution (0.18%), 1 mL of sodium phosphate buffer (0.1 M, pH 7.4), 1 mL of test samples containing *B. dracunculifolia* extract at concentrations of 200, 400, and 600 µg/mL, 40 µg/mL dexamethasone as the PC, 0.9% saline solution as the NC, and 0.5 mL of HRBC suspension. The hemoglobin content in the supernatant was determined using a spectrophotometer at 560 nm.

The percentage of protection against hemolysis was calculated using the following formula:

Protection (%) = E0 − ET/E0 × 100

Where E0 represents the mean absorbance value of the negative control group and ET represents the mean absorbance value in the treated groups

*2.4.1.1 Treatments of macrophages cell*

The murine macrophage cell line RAW 264 was used for both the spreading and phagocytosis inhibition assays. The crude extract of *B. dracunculifolia* was dissolved in distilled water at concentrations of 200, 400, and 600 μg/mL. Dexamethasone (40 μg/mL) was used as the positive control (PC) group, while the negative control (NC) group was treated with a saline solution (0.9%) (Azedo et al., 2011; Bastos et al., 2012).

*2.4.1.2 Inhibition of spreading*

The inhibition of cell spreading was evaluated using the method described by Bastos et al. (2012). Aliquots of 100 μL from each peritoneal lavage cell suspension, adjusted to a concentration of $5.0 \times 10^5$ cells/mL, were cultured in DMEM high-glucose medium supplemented with 10% fetal bovine serum and 1% Penicillin-Streptomycin-Amphotericin. The cell suspensions were placed onto glass slides and allowed to settle undisturbed for 15 minutes at 25 °C. After this period, the slides were washed with sterile saline solution to remove non-adherent cells, then incubated for 1 hour at 37 °C with 100 μL of DMEM high-glucose medium and 10 μL of the respective *B. dracunculifolia* extract solutions at concentrations of 200, 400, and 600 μg/mL. For the PC, 10 μL of dexamethasone solution (0.1 mg/mL) was used, and for the NC, 0.9% saline solution was applied instead of the extract. After incubation, the slides were washed again with sterile saline solution, and the adherent cells were fixed with 2.5% glutaraldehyde solution (Dinâmica, Brazil) for 5 minutes. Following fixation, the slides were stained with Wright's stain (Synth, Brazil) and mounted on permanent slides using Entellan for preservation. The prepared slides were examined under an optical microscope at 400× magnification, with a total of 100 cells counted. The assay was performed in triplicate.

The percentage of spreading inhibition was calculated as follows:

**Inhibition of spreading (%) = E0 − ET/E0 × 100**

Where E0 represents the mean number of spread cells in the negative control group, and ET represents the mean number of cells spread in the treated group.

*2.4.1.3 Phagocytosis*

The phagocytosis inhibition assay was conducted following the method described by Azedo et al. (2011). Initially, 200 µL of macrophage suspension were seeded onto 24-well plates containing glass coverslips, at a concentration of $3 \times 10^5$ cells/mL. After overnight adhesion, the adhered cells were washed with phosphate-buffered saline). Subsequently, the cells were incubated for 2 hours in culture medium with the respective treatments. The coverslips in each well were treated with the corresponding solutions of *B. dracunculifolia* extract at concentrations of 200, 400, and 600 µg/mL, and incubated for 2 hours in a 5% $CO_2$ incubator at 36 °C. For the PC group, 10 µL of dexamethasone solution (0.1 mg/mL) was used, and for the NC group, 0.9% saline solution was applied instead of the extract. After incubation, the adhered cells were washed three times with phosphate-buffered saline, fixed with 2.5% glutaraldehyde solution, and stained with Wright's stain. The coverslips were then mounted onto permanent slides for observation. The prepared slides were examined under an optical microscope at 400× magnification, with a total of 100 cells counted. The assay was performed in triplicate.

Inhibition of phagocytosis (IP) was calculated using the following formula:

$$\mathbf{IP\ (\%) = E0 - ET/E0 \times 100}$$

Where E0 represents the mean number of cells in the negative control group that phagocytosed the zymosan particles, and ET represents the mean number of cells in the treated groups that phagocytosed the zymosan particles.

*2.5 Toxicological studies*

*2.5.1 In vitro cytotoxicity of B. dracunculifolia*

The cytotoxicity of the *B. dracunculifolia* extract was assessed using the 3-(4,5-dimethylthiazol-2,5-diphenyl) tetrazolium bromide (MTT) assay, as described by Tsuboy et al. (2010). NIH/3T3 mouse dermal fibroblast cells (ATCC® CRL-1658™) were seeded into 96-well microtiter plates and cultured in medium for 24 hours at 37°C in a 5% $CO_2$ atmosphere. Once the cells reached approximately 75% confluence, they were treated with five different concentrations of the *B. dracunculifolia* extract (100, 200, 400, 800, and 1600 µg/mL). For the negative control (NC), cells were treated with a physiological solution, and for the positive control (PC), the medium was replaced with 2% (v/v) Tween 80. Treatments were conducted for 24, 48, and 72 hours. OECD guidelines (431 and 439), along with other standards such as ISO 10993-5:2009 (Biological evaluation of medical devices), and published studies such as Da Costa et al. (2023) and Fracasso et al. (2023), were used as parameters for interpreting potential cytotoxic effects based on the MTT methodology and the cellular line employed.

### *2.5.2 Acute toxicity evaluation of Baccharis dracunculifolia*

#### *2.5.2.1 Animals*

The acute toxicity of the *B. dracunculifolia* aerial parts extract was investigated in female Wistar rats weighing 200–250 g, obtained from the central animal facility of the Federal University of Grande Dourados. The animals were housed in the Experimental Neuroscience Laboratory of Universidade Paranaense under controlled environmental conditions (temperature: 22 ± 2°C; relative humidity: 50 ± 10%; light/dark cycle: 12 hours, with environmental enrichment) and had free access to liquid and solid diets. The experimental protocol was approved by the Ethics Committee on Animal Experimentation of UNIPAR (protocol no. 1003/2022), and all procedures followed the guidelines and recommendations of the National Council for the Control of Animal Experimentation (CONCEA, Brazil). The study adhered to the principles of Animal Research: Reporting of *In Vivo* Experiments (ARRIVE; Percie du Sert et al., 2020), ensuring animal welfare and minimizing the number of animals used in the experiments.

#### *2.5.2.2 Experimental design*

To determine the median lethal dose (LD50), female rats were divided into groups ($n = 6$), and an acute toxicity test was conducted following the guidelines outlined in Directive 423 of the Organisation for Economic Co-operation and Development (OECD, 2001). This protocol investigates toxicity at fixed doses of 5, 50, 300, and 2000 mg/kg. However, in this experiment, the initial dose was set at 300 mg/kg, as no prior data regarding the toxicity of *B. dracunculifolia* extract was available in the literature. Depending on the presence of toxic signs or mortality, subsequent doses were adjusted—reduced if toxicity or more than one death was observed or increased if no significant alterations or only one death occurred. After an 8-hour fasting period, the *B. dracunculifolia* extract was administered orally (gavage) as a single dose. Following treatment, the animals remained fasted for an additional 4 hours to prevent potential interactions. A control group ($n = 6$) was included as a baseline and received the vehicle (filtered water; Figure 1).

#### *2.5.2.3 Assessment of clinical signs*

After the administration of the *B. dracunculifolia* extract, the animals were initially observed at the following time points: 30 minutes, 1, 2, 3, and 4 hours post-procedure. All clinical alterations such as self-grooming, piloerection, dyspnea, abdominal contractions, diarrhea, prostration, ataxia, sedation, coma, and death were individually monitored and recorded. After the first 4 hours of observation, the animals were given water and food, continuing to be observed daily until the 14th day for any clinical signs or death, in accordance with the study by Silva et al. (2022).

#### *2.5.2.4 Euthanasia and material collection*

Euthanasia of the animals was performed on the 15th day by deepening anesthesia in a saturation chamber with isoflurane (1-3%). Subsequently, blood was collected by decapitation using guillotine for hematological and biochemical analyses. Following this, the liver, spleen, kidneys, and heart were carefully dissected and weighed on an analytical balance. To determine the relative weight (%), the weight of each organ was multiplied by 100 and divided by the body weight of the animal, with the weight of the kidneys being determined as the average between both kidneys. Then, small samples of each organ were cut, washed with saline solution, and separated for histological processing.

*2.5.2.5 Biochemical analyses*

The collected blood was centrifuged at 3,000 X g for 10 minutes, and then the plasma was stored at -80ºC. Biochemical analyses were carried out using commercial kits and various methods, including enzymatic-colorimetric (total cholesterol and triglycerides), kinetic-colorimetric (creatinine), kinetic-UV (urea), and colorimetric [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] in a semiautomatic analyzer (Global Analyzer®, model GTA-300, China).

*2.5.2.6 Hematological analyses*

The hematological profile was determined through the red blood cell series [erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)], the white blood cell series [leukocytes in their different morphological classes: neutrophils (bands and segmented), lymphocytes, and monocytes], and the platelet series (platelets) using an automatic hematological analyzer (ABX Micros ESV 60, Paris, France). The differential count of the white blood cell was performed through a smear stained with Giemsa and analyzed under an optical microscope (Leica®, model DM 2500, Germany).

*2.5.2.7 Microscopic analysis of tissue structure*

Fragments of the liver, spleen, heart, and kidneys were fixed in a solution composed of 1800 mL of distilled water, 200 mL of formaldehyde, 8 g of monosodium phosphate, and 13 g of disodium phosphate (10% buffered formalin). After standard histological processing (Behmer; Tolosa; Freitas Neto, 1976), the sections were stained with hematoxylin and eosin and analyzed histopathologically by a veterinary pathologist. The slides were examined under an optical microscope (Leica®, model DM 2500, Germany) to detect cellular alterations resulting from the treatments, as described by Lima et al. (2019) and Souza et al. (2020).

*2.6 Statistical analysis*

The data were initially analyzed for variance homogeneity and distribution normality. One-way ANOVA was performed, followed by Tukey's post hoc test for multiple comparisons. The

significance level was set at 95% ($p < 0.05$). For statistical analysis and graph creation, GraphPad Prism® version 9.0 software was used.

## 3 Results

### *3.1 Determination of phenolic compounds in Baccharis dracunculifolia extract*

The quantification of total phenols and flavonoids in the *B. dracunculifolia* extract at different concentrations (0.25–1.00 mg/mL) revealed a concentration-dependent increase in the phenolic content (Table 1). The total phenols ranged from 29.88 ± 7.17 µg GAE/mg at the lowest concentration (0.25 mg/mL) to 132.48 ± 2.13 µg GAE/mg at 1.00 mg/mL. In contrast, the flavonoid content exhibited a less consistent pattern. At 0.25 mg/mL, the flavonoid concentration was 404.82 ± 13.43 µg GAE/mg, but it decreased progressively to 355.75 ± 2.17 µg GAE/mg at 0.75 mg/mL before slightly increasing to 379.40 ± 1.74 µg GAE/mg at 1.00 mg/mL.

### *3.2 Determination of antioxidant capacity of Baccharis dracunculifolia extract*

The *in vitro* antioxidant activity of the *B. dracunculifolia* extract is presented in Table 2. In the DPPH assay, the extract exhibited a half-maximal inhibitory concentration ($IC_{50}$) of 0.771 ± 0.009 mg/mL, indicating moderate radical scavenging activity. For comparison, the positive control quercetin showed a much lower $IC_{50}$ value of 0.010 ± 0.001 mg/mL, demonstrating stronger antioxidant activity. The FRAP assay revealed that the *B. dracunculifolia* extract had a reducing power of 1.244 ± 0.223 µM ferrous sulfate/mg, which was significantly lower than the positive control Trolox, which demonstrated a higher reducing capacity of 9.175 ± 0.001 µM ferrous sulfate/mg.

### *3.3 In vitro anti-inflammatory screening of Baccharis dracunculifolia*

#### *3.3.1 Hemolysis inhibition*

Among the *B. dracunculifolia* concentrations evaluated, the highest anti-hemolytic response was observed at 600 µg/mL, with a percentage of hemolysis inhibition of 58.6 ± 0.23, indicating the most potent anti-hemolytic activity among the treatments tested. A concentration-dependent effect was evident, with increased anti-hemolytic activity corresponding to higher concentrations of the extract. Statistically significant differences were observed between all extract concentrations and the NC ($p < 0.05$), further supporting the concentration-dependent nature of the extract's efficacy in inhibiting hemolysis (Table 3).

#### *3.3.2 Inhibition of spreading*

The *B. dracunculifolia* extract exhibited a concentration-dependent increase in the percentage of inhibition of spreading, with the highest inhibitory activity observed at 600 µg/mL (Table 4). Statistical analysis confirmed significant differences between all extract-treated groups and the NC ($p < 0.05$).

### *3.3.3 Inhibition of phagocytosis*

Treatment with *B. dracunculifolia* extract led to a concentration-dependent increase in phagocytosis inhibition, with the highest inhibition observed at 600 µg/mL. Statistical analysis revealed significant differences between all extract concentrations and the NC (Table 5).

## *3.4 Safety assessment of Baccharis dracunculifolia extract*

### *3.4.1 Impact of Baccharis dracunculifolia on fibroblast cell viability*

Table 6 presents the results of the MTT cytotoxicity assay conducted on NIH/3T3 mouse dermal fibroblast cells to evaluate the effects of *B. dracunculifolia* extract on cell viability at different concentrations and time points. At concentrations of 100, 200, and 400 µg/mL, the extract displayed minimal cytotoxicity, maintaining approximately 100% cell viability after 24 hours, with slight increases of 4.98%, 2.19%, and 1.19%, respectively. Even at the highest concentrations tested, cell viability remained above 70%, a commonly accepted threshold for non-cytotoxic substances, suggesting the extract holds therapeutic potential. A dose-dependent decrease in cell viability was observed at the 48-hour and 72-hour time points, where viability decreased progressively with higher concentrations and longer exposure times. However, even at the 72-hour mark, despite a clear reduction in cell viability at higher concentrations, viability remained above 70%. This indicates that higher concentrations of the extract can be tested without significant risk of inducing cytotoxic effects.

### *3.4.2. Evaluation of clinical signs, relative organs weight and body weight of rats treated with Baccharis dracunculifolia extract*

The animals treated orally with a single dose of *B. dracunculifolia* extract at doses of 300 and 2000 mg/kg showed no behavioral and/or physiological changes analyzed throughout the observation period (30 minutes to 14 days) when compared to the baseline group (Table 7). Table 8 presents the body weight data for animals treated with two doses of *B. dracunculifolia* extract (300 mg/kg and 2000 mg/kg), compared to the basal group (no treatment). Throughout the 14-day observation period, animals in all groups showed a progressive increase in body weight. The basal group exhibited a steady gain, with the final body weight reaching 236.30 ± 1.89 g by day 14. Similarly, both treatment groups demonstrated weight gain over the course of the study. The 300 mg/kg and 2000 mg/kg treatment groups recorded final body weights of 228.70 ± 3.77 g and 221.50 ± 4.26 g, respectively. The weight increase in the treatment groups was consistent with that of the basal group, indicating that the extract did not negatively affect the body weight gain of the animals. No significant differences were observed in the body weight gain between the basal and treated groups. The relative organ weights of the liver, spleen, heart, and kidneys were evaluated in animals treated with *B. dracunculifolia* extract. No statistically significant differences were observed between the treated groups (300 mg/kg and 2000 mg/kg) and the basal group.

### *3.4.3. Biochemical serum parameters in Wistar rats treated with Baccharis dracunculifolia extract*

The biochemical analysis of serum parameters in Wistar rats treated with *B. dracunculifolia* extract highlighted significant changes in specific markers (Figure 2). Triglycerides levels were significantly reduced in the treated groups compared to the vehicle group, with values of 46.83 ± 4.84 mg/dL in the basal group, 32.50 ± 2.17 mg/dL in the 300 mg/kg group, and 33.50 ± 1.62 mg/dL in the 2000 mg/kg group (Figure 2B). Creatinine levels also showed a significant decrease at the highest dose, with values of 0.93 ± 0.02 mg/dL in the basal group, 0.87 ± 0.03 mg/dL in the 300 mg/kg group, and 0.82 ± 0.03 mg/dL in the 2000 mg/kg group (Figure 2F). No statistically significant differences were observed for the other parameters analyzed. Cholesterol levels showed a slight but non-significant increase, with a basal group value of 64.50 ± 2.88 mg/dL (Figure 2A). Urea levels remained stable across all groups, with a basal value of 39.17 ± 1.47 mg/dL (Figure 2E). Similarly, the liver enzymes AST and ALT showed no significant variations, with basal values of 135.50 ± 7.90 U/L for AST (Figure 2D) and 35.67 ± 2.47 U/L for ALT (Figure 2C).

### *3.4.4. Hematological profile of rats treated with Baccharis dracunculifolia extract*

Regarding the hematological parameters, no significant differences were observed between the groups for erythrocyte parameters such as erythrocyte count, hemoglobin, hematocrit, MCV, MCH, and MCHC. Hematocrit also showed no significant variation. Regarding leukocytes, no significant differences were observed between the groups. No significant differences were found in the levels of leukocyte subtypes, including segmented neutrophils, lymphocytes, and monocytes. Platelet counts also showed no significant changes (Table 9).

### *3.4.5. Baccharis dracunculifolia does not induce hepatic, cardiac and renal cellular alterations*

The histological examination of the liver, heart, and kidney of Wistar rats treated with *B. dracunculifolia* extract at doses of 300 mg/kg and 2000 mg/kg showed no significant pathological changes. In the liver, the tissue structure remained normal with no evidence of necrosis, inflammation, or degeneration across the different treatment groups. The heart showed no alterations in myocardial architecture, maintaining its regular structure without signs of damage or infiltration. Similarly, the kidneys displayed intact renal tubules and glomeruli, with no signs of interstitial inflammation or glomerular injury (Figure 3).

## 4. Discussion

This study provides a comprehensive assessment of the biological activity and safety of *Baccharis dracunculifolia* extract, integrating *in vitro* antioxidant and anti-inflammatory assays with *in vivo* toxicological evaluation in Wistar rats. The findings support its potential as a natural bioactive ingredient for functional food or nutraceutical applications, given its favorable safety profile and modulation of biomarkers associated with oxidative stress and inflammatory responses.

The quantification of total phenols and flavonoids in the *B. dracunculifolia* extract revealed a trend toward increased concentrations of total phenols, consistent with previous studies linking phenolic compounds to antioxidant activity (Fernandez-Panchon et al., 2008; Ketnawa et al., 2022; Shi et al., 2022). Notably, the flavonoid concentration exhibited a non-linear pattern, likely reflecting the intrinsic complexity of the extract's composition rather than variations in extraction efficiency. These findings align with the phytochemical characterization reported in our previous study (Silva et al., 2024), which identified a diverse profile of bioactive compounds. The antioxidant activity of the extract may therefore stem from a synergistic interplay among these constituents, rather than being solely attributable to flavonoids. Furthermore, the variability in flavonoid concentration suggests potential solubility-related interactions or compound-specific stability, emphasizing the importance of considering extract composition in food-grade applications.

The antioxidant activity of the extract was confirmed through DPPH and FRAP assays, demonstrating moderate but consistent activity when compared to quercetin and Trolox, used as positive controls. The IC50 observed in the DPPH assay aligns with literature indicating that *B. dracunculifolia* exhibits antioxidant properties due to its rich phenolic content (Gazim et al., 2022; Bonin et al., 2020; Casagrande et al., 2018). While FRAP values were lower than Trolox, they still confirm the extract's reducing power, which is relevant in preventing oxidative degradation in food systems. These results reinforce the *B. dracunculifolia* suitability as a natural antioxidant source for incorporation into functional food products aiming to enhance nutritional value and oxidative stability.

*In vitro* anti-inflammatory assays, including hemolysis inhibition, cell spreading, and phagocytosis, revealed promising bioactivity. The significant reduction in hemolysis at higher concentrations (600 µg/mL) and inhibition of cell spreading suggest membrane-stabilizing effects—an important mechanism in modulating inflammatory responses. Additionally, inhibition of phagocytosis supports the extract's anti-inflammatory potential, as excessive phagocytic activity can exacerbate chronic inflammation. These effects are consistent with previous findings on Baccharis species regulating inflammatory processes (Brandenburg et al., 2020; Lima et al., 2020; da Silva et al., 2022; França et al., 2022), and position *B. dracunculifolia* as a promising candidate for functional dietary interventions aimed at immune balance.

The biochemical analysis revealed favorable modulation of key metabolic markers, including reductions in triglyceride and creatinine levels, suggesting potential benefits in lipid metabolism and renal function. The reduction in triglycerides is particularly relevant in the context of dietary strategies targeting metabolic syndrome and cardiovascular risk. Similarly, the decrease in creatinine levels may indicate renal protective effects—important for food ingredients intended for health-supportive formulations. These findings are consistent with our previous study using a cardiometabolic rat model, in which *B. dracunculifolia* demonstrated beneficial effects on lipid profiles (Silva et al., 2024). Moreover, the absence of significant changes in cholesterol, urea, or liver enzymes (AST and ALT),

505 combined with unaltered histology of liver, heart, and kidneys, supports the extract's safety for use in
506 long-term dietary supplementation.
507 Hematological evaluation further reinforced the extract's safety, as no significant alterations
508 were observed in erythrocyte indices (erythrocyte count, hemoglobin, hematocrit, MCV, MCH,
509 MCHC), platelet count, or leukocyte profiles. These results suggest that *B. dracunculifolia* extract did
510 not interfere with erythropoiesis, thrombopoiesis, or immune cell dynamics under the tested conditions
511 (Kurata & Horii, 2004; Vo & Thompson, 2019; Schultze, Ennulat & Aulbach, 2022). Additionally,
512 body weight progression and organ-to-body weight ratios (liver, spleen, heart, kidneys) remained
513 within normal ranges, with no pathological signs, further confirming the extract's toxicological safety.
514 Despite the promising results, certain limitations must be acknowledged. The *in vitro* nature of
515 the antioxidant and anti-inflammatory assays limits direct extrapolation to complex food matrices or
516 human physiology. Future studies should explore the stability, bioavailability, and efficacy of the
517 extract when incorporated into actual food systems. Although histological analysis did not indicate
518 toxicity, additional evaluation of the gastrointestinal tract and central nervous system would provide a
519 more comprehensive safety profile. Moreover, the interaction of the extract with gut microbiota, a key
520 modulator of metabolic and inflammatory pathways, should be investigated in the context of
521 functional food development.
522 Another critical point concerns the variability in toxicity data found in the literature. Although
523 our findings indicate no toxicity at doses up to 2000 mg/kg, previous studies have reported conflicting
524 results, with some indicating genotoxic effects at higher concentrations (Rodrigues et al., 2009;
525 Shimizu et al., 2011; Lee et al., 2003; Hori et al., 2013). These discrepancies emphasize the
526 importance of standardized experimental protocols and long-term toxicological studies to ensure
527 regulatory acceptance for use as a food-grade ingredient.
528 This study contributes to the expanding knowledge on native South American plants with
529 nutraceutical potential, demonstrating that *B. dracunculifolia* extract possesses favorable antioxidant,
530 anti-inflammatory, and safety profiles. These characteristics support its suitability for development as
531 a natural ingredient in functional food products, beverages, or dietary supplements aimed at promoting
532 metabolic and immune health.
533
534 **Conclusion**
535 The findings of this study demonstrate that *Baccharis dracunculifolia* extract exhibits notable
536 antioxidant and anti-inflammatory activity, along with a favorable safety profile in both *in vitro* and *in*
537 *vivo* models. These properties highlight its potential as a natural source of bioactive compounds
538 suitable for incorporation into functional food products, nutraceuticals, or dietary supplements. While
539 the results are encouraging, further studies are needed to assess its stability in food matrices, evaluate
540 its bioavailability, and investigate its long-term effects in human populations. Overall, *B.*

*dracunculifolia* represents a promising candidate for the development of food-grade ingredients aimed at supporting metabolic and inflammatory health through dietary strategies.

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**Conflict of Interest Statement**

The authors declare that they have no conflicts of interest.

**Author contribution statement**

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**Declaration of AI-assisted technologies in the writing process**

During the preparation of this work the authors used ChatGPT in the writing process to improve the readability and language of the manuscript. After using this service, the authors reviewed and edited the content as needed and took full responsibility for the content of the published article.

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797 **Legends for figures**

798

799 **Figure 1.** Acute toxicity test conducted following OECD Guideline 423, illustrating the stepwise
800 process for determining the median lethal dose (LD50) cut-off value of *Baccharis dracunculifolia*
801 extract. This method involves sequential testing with three animals of the same sex per step. The acute
802 toxicity assessment is based on the mortality or moribund state of the animals, requiring 2–4 steps to
803 complete the evaluation. The initial dose (300 mg/kg in this study) is administered once. If 2–3
804 animals die, testing proceeds at a lower fixed dose. If one or no animals die, the initial dose is retested
805 twice, followed by testing at a higher fixed dose in subsequent steps until the appropriate Globally
806 Harmonized System toxicity category is identified. GHS: Globally Harmonized Classification System
807 (mg/kg).

808

809 **Figure 2.** Serum biochemical parameters of **(A)** cholesterol, **(B)** triglycerides, **(C)** ALT, **(D)**
810 AST, **(E)** Urea and **(F)** creatinine of Wistar rats treated with vehicle (filtered water) or
811 *Baccharis dracunculifolia* extract (300 or 2000 mg/kg) via oral administration as a single
812 dose. Statistical analysis was performed using one-way ANOVA followed by Tukey's post
813 hoc test. *$p < 0.05$ compared to the basal group. AST: aspartate aminotransferase; ALT:
814 alanine aminotransferase.

815

816 **Figure 3.** Histopathological analysis of the liver, heart, and kidney from Wistar rats treated with
817 *Baccharis dracunculifolia* extract (300 mg/kg and 2000 mg/kg), compared to the basal (vehicle)
818 group. Representative Hematoxylin & Eosin-stained tissue sections show no significant morphological
819 alterations in the liver, heart, or kidney at both dosages of the extract. Magnification: 20 *x*.

**Figures**

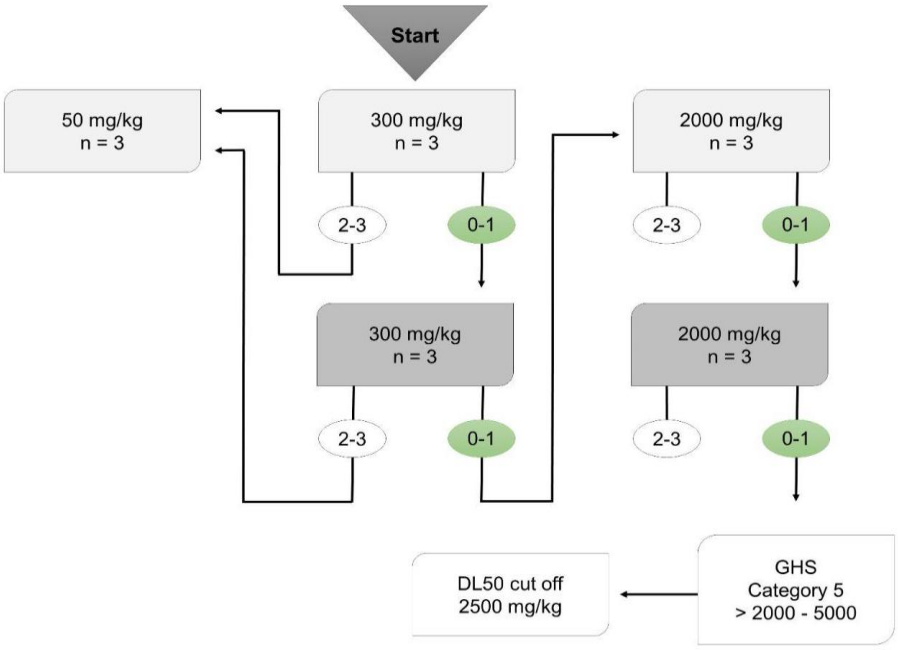


**Figure 1.** Silva et al.

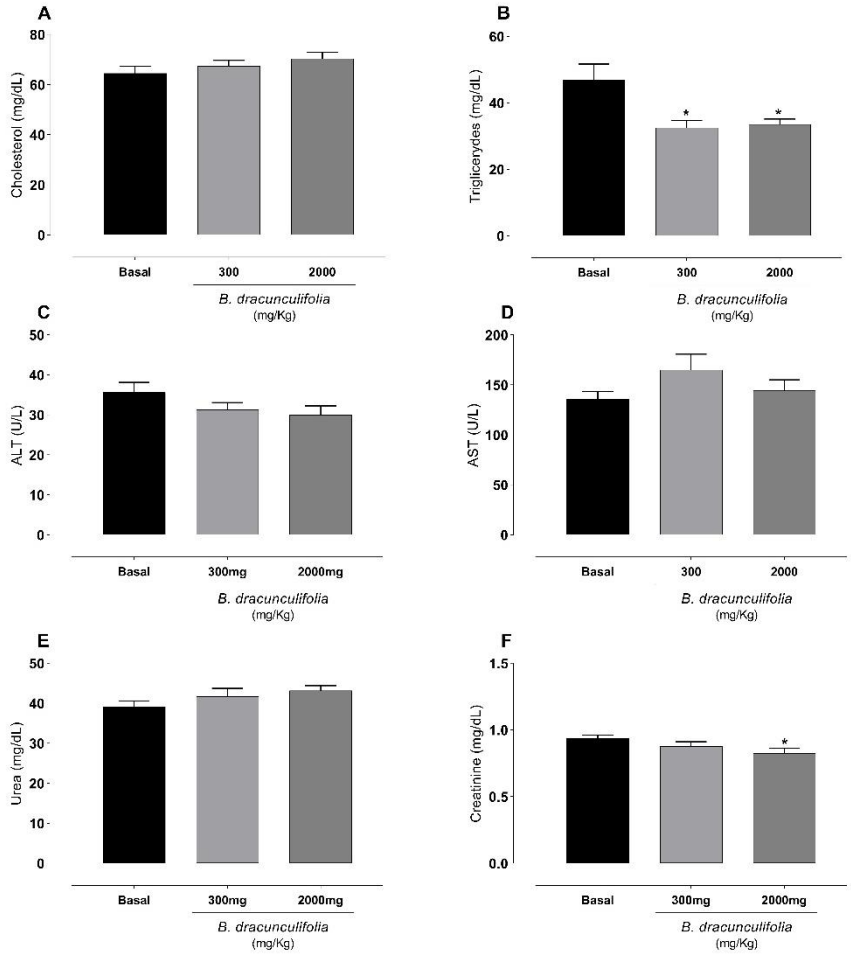


**Figure 2.** Silva et al.

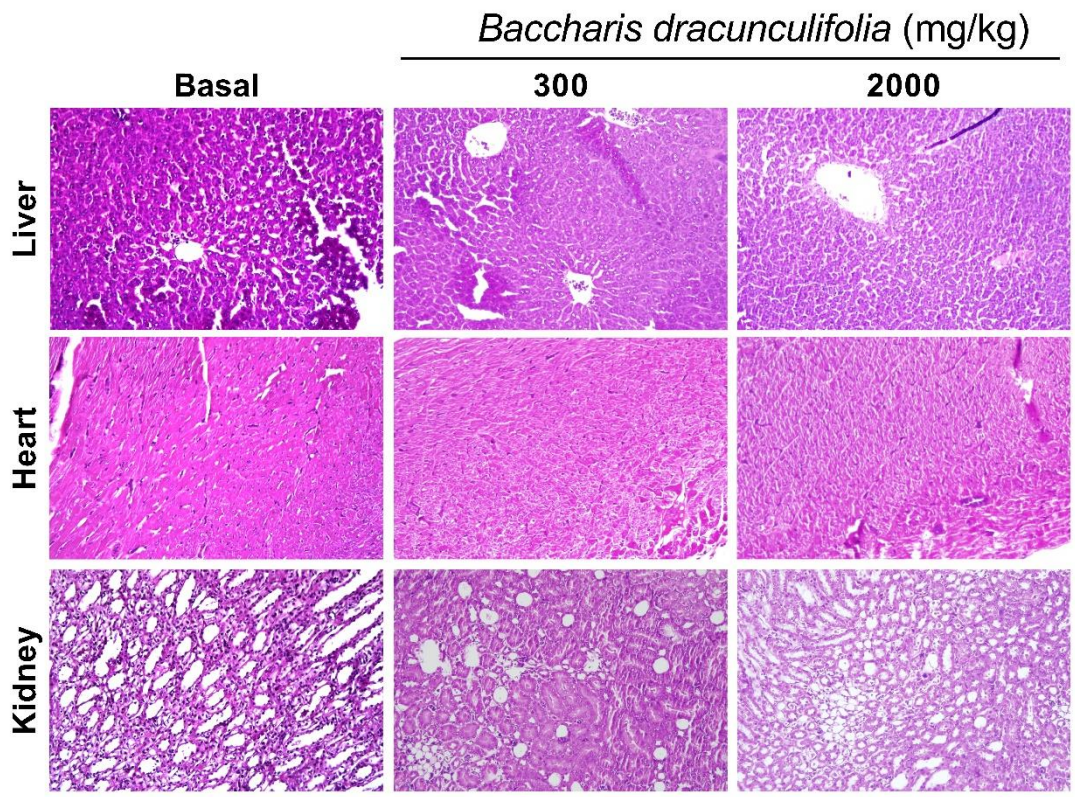


**Figure 3.** Silva et al.

## Tables

**Table 1**. Total phenolics and flavonoids of the *Baccharis dracunculifolia* extract.

| *B. dracunculifolia* (mg/mL) | **Total Phenols** (µg GAE/mg) | **Flavonoids** (µg GAE/mg) |
|---|---|---|
| 0.25 | $29.88 \pm 7.17^{d}$ | $404.83 \pm 13.44^{a}$ |
| 0.50 | $71.88 \pm 1.44^{c}$ | $397.12 \pm 2.86^{ab}$ |
| 0.75 | $102.15 \pm 1.54^{b}$ | $355.75 \pm 2.17^{c}$ |
| 1.00 | $132.48 \pm 2.13^{a}$ | $379.40 \pm 1.74^{b}$ |

Values are the mean ± standard deviation of triplicate assays. Data were subjected to analysis of variance (ANOVA), and differences between means were assessed by Tukey's test ($p \leq 0.05$). Values in the same column followed by different letters are significantly different ($p \leq 0.05$). GAE: gallic acid equivalents.

**Table 2.** Antioxidant activity, assessed by the elimination of the DPPH radical and FRAP assays of the *Baccharis dracunculifolia* extract.

| **Samples mg/mL** | **DPPH$^{\bullet}$ $IC_{50}$** (mg/mL) | **FRAP** (µM ferrous sulfate/mg) |
|---|---|---|
| *B. dracunculifolia* | $0.771 \pm 0.009^{b}$ | $1.244 \pm 0.223^{b}$ |
| Quercetin | $0.010 \pm 0.00^{a}$ | - |
| Trolox | - | $9.175 \pm 0.001^{a}$ |

Values are the mean ± standard deviation of triplicate assays. Data were subjected to analysis of variance (ANOVA), and differences between means were assessed by Tukey's test ($p \leq 0.05$). Values in the same column followed by different letters are significantly different ($p \leq 0.05$). Quercetin (0.0103 mg/mL) and Trolox (9.175 mg/mL) were used as positive controls in DPPH• and FRAP assays, respectively. Abbreviations: FRAP: ferric reducing antioxidant power; IC50: half-maximal inhibitory concentration; DPPH•: 2,2-diphenyl-1-picrylhydrazyl.

**Table 3.** Percentage of hemolysis inhibition promoted by *Baccharis dracunculifolia* extract at concentrations of 200, 400, and 600 µg/mL, compared to the positive and negative control groups.

| **Treatments** | **Hemolysis inhibition (%)** |
|---|---|
| NC | 00.00 ± 0.00 |
| PC | 98.96 ± 0.34* |
| *B. dracunculifolia* 200 | 45.43 ± 0.48* |
| *B. dracunculifolia* 400 | 51.14 ± 0.86* |
| *B. dracunculifolia* 600 | 58.60 ± 0.23* |

The results were expressed in mean ± S.E.M. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. *$p < 0.05$ compared to NC. *B. dracunculifolia*: µg/mL. NC: negative control (0.9% physiological solution); PC: positive control (dexamethasone, 40 µg/mL).

**Table 4.** Percentage of inhibition of spreading induced by *Baccharis dracunculifolia* extract, compared to the positive and negative control groups.

| Treatments | Inhibition of spreading (%) |
|---|---|
| NC | 0.00 ± 0.00 |
| PC | 91.97 ± 2.12* |
| *B. dracunculifolia* 200 | 52.41 ± 0.69* |
| *B. dracunculifolia* 400 | 54.22 ± 0.40* |
| *B. dracunculifolia* 600 | 60.84 ± 0.40* |

The results were expressed in mean ± S.E.M. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. *p < 0.05 compared to NC. *B. dracunculifolia*: µg/mL. NC: negative control (0.9% physiological solution); PC: positive control (dexamethasone, 40 µg/mL).

**Table 5.** Percentage of phagocytosis inhibition promoted by *Baccharis dracunculifolia* extract compared to the positive and negative control groups.

| Treatments | Phagocytosis inhibition (%) |
|---|---|
| NC | 0.00 ± 0.00 |
| PC | 70.43 ± 2.87* |
| *B. dracunculifolia* 200 | 44.96 ± 2.57* |
| *B. dracunculifolia* 400 | 52.83 ± 1.35* |
| *B. dracunculifolia* 600 | 57.89 ± 2.96* |

The results were expressed in mean ± S.E.M. One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. *p < 0.05 compared to NC. *B. dracunculifolia*: µg/mL. NC: negative control (0.9% physiological solution); PC: positive control (dexamethasone, 40 µg/mL).

**Table 6.** Percentage of cell viability promoted by *Baccharis dracunculifolia* extract at concentrations of 100, 200, 400, 800, and 1600 µg/mL, compared to the positive and negative controls groups.

| Treatment | 24 h | 48 h | 72 h |
|---|---|---|---|
| NC | 100.00 ± 0.00 | 100.00 ± 0.00 | 100.00 ± 0.00 |
| PC | 1.84 ± 0.14* | 1.84 ± 0.14* | 1.37 ± 0.06* |
| *B. dracunculifolia* 100 | 104.98 ± 0.14* | 96.87 ± 0.09 | 92.07 ± 0.06 |
| *B. dracunculifolia* 200 | 102.19 ± 0.02 | 96.90 ± 0.02 | 87.13 ± 0.02* |
| *B. dracunculifolia* 400 | 101.19 ± 0.02 | 92.37 ± 0.03* | 85.57 ± 0.03* |
| *B. dracunculifolia* 800 | 95.93 ± 0.02 | 87.80 ± 0.01* | 80.86 ± 0.01* |
| *B. dracunculifolia* 1600 | 80.23 ± 0.11* | 81.69 ± 4.30* | 78.63 ± 1.31* |

911 The results were expressed in mean ± S.E.M. One-way ANOVA followed by Tukey's post hoc test
912 was used for statistical analysis. *p < 0.05 compared to NC. *B. dracunculifolia*: µg/mL. NC: negative
913 control (0.9% physiological solution); PC: positive control (0.1% Tween 80).
914
915 **Table 7.** Assessment of clinical signs in Wistar rats treated with vehicle or *Baccharis*
916 *dracunculifolia* extract (300 or 2000 mg/kg), orally, in a single dose.

| | | Clinical sign | Time | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 30 min | 1h | 2h | 3h | 4h | 14 days |
| **Basal (vehicle)** | | *Self-cleaning absence* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Piloerection* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Dyspnea* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Abdominal constriction* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Diarrhea* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Prostration* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Ataxia* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Sedation* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Coma* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Death* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| **Extract of *Baccharis dracunculifolia*** | **300 mg/kg** | *Self-cleaning absence* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Piloerection* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Dyspnea* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Abdominal constriction* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Diarrhea* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Prostration* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Ataxia* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Sedation* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Coma* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Death* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | **2000 mg/kg** | *Self-cleaning absence* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Piloerection* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Dyspnea* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Abdominal constriction* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Diarrhea* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Prostration* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Ataxia* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Sedation* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Coma* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |
| | | *Death* | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 |

**Table 8.** The body weight and relative organ weight of Wistar rats treated with vehicle or *Baccharis dracunculifolia* extract (300 or 2000 mg/kg), orally, in a single dose.

| **Day** | **Basal** | **Extract of** *Baccharis dracunculifolia* | |
|---|---|---|---|
| | | **300 mg/kg** | **2000 mg/kg** |
| | *Body weight (g)* | | |
| 1 | 216.20 ± 1.93 | 202.70 ± 3.53 | 193.80 ± 3.77 |
| 2 | 216.30 ± 2.04 | 203.50 ± 3.60 | 193.70 ± 3.73 |
| 3 | 218.70 ± 1.82 | 204.00 ± 3.96 | 196.00 ± 3.56 |
| 4 | 221.50 ± 1.91 | 206.30 ± 4.12 | 198.80 ± 3.51 |
| 5 | 223.20 ± 1.96 | 207.50 ± 3.83 | 199.30 ± 3.93 |
| 6 | 224.50 ± 2.51 | 210.50 ± 3.90 | 202.70 ± 3.62 |
| 7 | 225.50 ± 2.26 | 212.00 ± 3.16 | 206.00± 3.83 |
| 8 | 227.20 ± 2.16 | 214.70 ± 3.49 | 209.00 ± 4.25 |
| 9 | 229.80 ± 2.16 | 216.50 ± 3.77 | 212.20 ± 4.82 |
| 10 | 230.70 ± 1.83 | 219.00 ± 3.73 | 214.20 ± 3.90 |
| 11 | 223.00 ± 2.35 | 222.50 ± 3.04 | 216.00 ± 3.80 |
| 12 | 232.50 ± 2.34 | 222.20 ± 3.52 | 217.00 ± 3.79 |
| 13 | 235.50 ± 1.66 | 225.20 ± 3.38 | 219.50 ± 4.28 |
| 14 | 236.30 ± 1.89 | 228.70 ± 3.77 | 221.50 ± 4.26 |
| | *Relative organ weight (%)* | | |
| Liver | 3.80 ± 0.069 | 2.75 ± 0.072 | 2.69 ± 0.091 |
| Spleen | 0.19 ± 0.005 | 0.18 ± 0.003 | 0.20 ± 0.006 |
| Heart | 0.25 ± 0.004 | 0.28 ± 0.013 | 0.27 ± 0.009 |
| Kidneys | 0.64 ± 0.009 | 0.60 ± 0.011 | 0.61 ± 0.018 |

Values are expressed as mean ± S.E.M., $n = 6$. Data are presented as mean ± S.E.M., $n = 6$. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

**Table 9**. Hematological parameters of Wistar rats treated with vehicle or *Baccharis dracunculifolia* extract (300 or 2000 mg/kg), orally, single dose.

| Parameter | Basal | Extract of *Baccharis dracunculifolia* | |
|---|---|---|---|
| | | 300 mg/kg | 2000 mg/kg |
| *Red blood cells ($10^6$ mm/µL)* | 9.12 ± 0.21 | 9.20 ± 0.21 | 8.89 ± 0.28 |
| *Hemoglobin (g/dL)* | 17.47 ± 0.81 | 17.80 ± 0.45 | 17.80 ± 0.55 |
| *Hematocrit (%)* | 53.75 ± 1.50 | 53.73 ± 1.05 | 53.17 ± 1.73 |
| *MCV (fl)* | 58.80 ± 0.33 | 57.72 ± 0.64 | 59.68 ± 0.45 |
| *MHC (pg)* | 19.05 ± 0.54 | 19.08 ± 0.38 | 19.97 ± 0.21 |
| *MCHC (g/dL)* | 32.28 ± 0.79 | 33.08 ± 0.50 | 33.50 ± 0.38 |
| *Leukocyte ($10^3$ mm/µL)* | 4.97 ± 0.56 | 4.50 ± 0.42 | 3.97 ± 0.22 |
| *Rods ($10^3$ mm/µL)* | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| *Segmented ($10^3$ mm/µL)* | 2.52 ± 0.32 | 2.29 ± 0.18 | 1.97 ± 0.18 |
| *Lymphocyte ($10^3$ mm/µL)* | 2.50 ± 0.31 | 2.23 ± 0.26 | 1.96 ± 0.11 |
| *Monocyte ($10^3$ mm/µL)* | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.01 ± 0.00 |
| *Platelets ($10^5$ mm /µL)* | 5.22 ± 0.80 | 5.30 ± 0.39 | 5.80 ± 0.25 |

Values are expressed as mean ± S.E.M., $n$ = 6. Data are presented as mean ± S.E.M., $n$ = 6. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. MCHC: Mean corpuscular hemoglobin concentration; MCH: Mean corpuscular hemoglobin; MCV: Mean corpuscular volume.

# ARTIGO 2 - EFFECTS OF *Baccharis dracunculifolia* DC ON AN INNOVATIVE ANIMAL MODEL OF CARDIOMETABOLIC SYNDROME

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# Effects of *Baccharis dracunculifolia* DC on an Innovative Animal Model of Cardiometabolic Syndrome

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**Abstract: Background/Objective:** Cardiometabolic syndrome (CMS) is a complex clinical condition that encompasses metabolic dysregulation, cardiovascular disease, and diabetes risk factors. Worldwide, CMS is underdiagnosed, and its occurrence significantly increases cardiovascular morbimortality. Despite available pharmacological treatments, the approach is fragmented, and the associated clinical conditions are treated independently. This approach may be partially due to limited preclinical models to mimic the clinical conditions of CMS. Therefore, our study aims to present an innovative animal model of cardiometabolic syndrome and evaluate the effects of *Baccharis dracunculifolia* on the set of clinical alterations associated with the condition. **Methods:** Female Wistar rats were induced to develop diabetes, fed a cholesterol-enriched diet, and exposed to the smoke of 9 cigarettes/day for 6 weeks. During the last 2 weeks, the rats were treated with vehicle, *B. dracunculifolia* (30, 100, and 300 mg/kg), or a combination of simvastatin and insulin. At the end of the treatment, plasma lipid levels were measured, and the liver was analyzed histologically for hepatic lipid quantification and oxidative stress assessment. **Results:** Phytochemical analysis revealed seven phenolic acids and six flavonoids in the extract. *B. dracunculifolia* showed significant hepatoprotective effects, reducing AST and ALT levels and lowering both plasma and hepatic lipid levels. The extract also reversed hepatic steatosis and demonstrated antioxidant properties. **Conclusions:** These findings suggest that *B. dracunculifolia* may be a therapeutic option for the metabolic dysregulation present in CMS.

**Keywords:** alecrim-do-campo; diabetes; dyslipidemia; metabolic syndrome; preclinical study; smoking; traditional medicine



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## 1. Introduction

Cardiometabolic syndrome has become a significant public health that encompasses a range of interrelated risk factors, including obesity, hypertension, type 2 diabetes, dyslipidemia, and insulin resistance, which together significantly increase the risk of cardiovascular diseases and metabolic disorders [1–3]. This syndrome presents a growing challenge to global public health, with its complexity lying in the synergistic interactions among these risk factors, leading to a cascade of detrimental effects on multiple organs, particularly the heart and blood vessels [4].

The pathogenesis of cardiometabolic syndrome involves intricate mechanisms, including chronic inflammation, oxidative stress, endothelial dysfunction, and alterations in lipid and glucose metabolism. Among the key drivers of these processes are diabetes, smoking, and dyslipidemia, which not only exacerbate cardiovascular risks but also contribute to the systemic metabolic imbalance that characterizes the syndrome. Understanding the interconnection between these risk factors is crucial for developing comprehensive therapeutic approaches [1,5].

Cardiometabolic syndrome affects more than one-quarter of the global adult population and up to 48.6% of individuals aged 60 years and over, contributing significantly to cardiovascular morbidity and mortality [6–8]. This alarming trend underscores the urgent need for comprehensive and effective preclinical models that replicate the complex, multifactorial nature of cardiometabolic syndrome, thus facilitating the development of targeted therapeutic interventions. Traditional animal models often fail to capture the full spectrum of cardiometabolic disturbances, as they tend to isolate individual risk factors rather than simulating the multifactorial nature of the disease. The need for innovative experimental models that mirror the complexity of human cardiometabolic syndrome is essential for advancing therapeutic strategies [5,9]. Such models allow for the assessment of interventions that target multiple metabolic pathways, reflecting the real-world conditions of the syndrome [9,10].

In recent years, there has been growing interest in exploring natural products with potential therapeutic benefits in the treatment of cardiometabolic conditions. Consequently, there is increasing interest in exploring alternative and complementary therapies, particularly those derived from natural products known for their safety profiles and containing bioactive compounds with potential therapeutic effects [11–13].

A species with potential popular use but few ethnopharmacological studies is *Baccharis dracunculifolia*, a shrub belonging to the Asteraceae family, commonly known in Brazil as 'alecrim-do-campo' or 'vassourinha' [14]. This plant is commonly used in the form of tea to treat gastric diseases, inflammation, and liver disorders [3]. Preclinical studies conducted with crude extracts of *B. dracunculifolia* have demonstrated various biological effects, such as anti-inflammatory [15], antiulcerogenic [16], antibacterial [17], antioxidant [18], anticancer [19], immunomodulatory [20], antigenotoxic, and antimutagenic properties [21].

The present study aims to investigate the therapeutic effects of *B. dracunculifolia* extract in an innovative animal model that simulates the combined conditions of diabetes, dyslipidemia, and smoking-induced metabolic dysfunctions. By addressing the multifactorial aspects of the syndrome, this study seeks to evaluate the plant's potential in modulating key metabolic parameters, thereby contributing to the development of novel strategies for managing cardiometabolic diseases.

## 2. Materials and Methods

### *2.1. Preparation of Baccharis dracunculifolia Extract and Phytochemical Composition*

The aerial parts (leaves and flowers) of *Baccharis dracunculifolia* were collected in a rural area of the municipality of Maria Helena, Paraná, Brazil (23°39′49.7″ S and 53°18′18.0″ W). The plant material was dried at room temperature (32 °C). Then, it was ground to a particle size of 850 µm. The resulting powder was subjected to a dynamic maceration process with solvent renewal using 96% ethanol (*v*/*v*) until the plant material was exhausted. The filtrate was then concentrated under reduced pressure in a rotary evaporator (Tecnal®,

model TE-211, Piracicaba, Brazil) at 40 °C, until obtaining the crude ethanolic extract of *B. dracunculifolia*, which was subsequently stored at −20 °C. The analysis of the extracts was carried out using ultra-high performance liquid chromatography coupled with high resolution mass spectrometry (UHPLC-ESI-QTOF-MS/MS) following the methodology described by Silva et al. [22]. The chemical analysis was biodirected toward the investigation of phenolic compounds, and identification was performed as proposed in review studies on the genus *Baccharis* [23–25] by calculating the mass error and comparing the results with data from MassBank (http://www.massbank.jp/, accessed on 25 May 2021) and the Human Metabolome Database (http://www.hmdb.ca/, accessed on 25 May 2021).

### 2.2. Pharmacological Studies

#### 2.2.1. Animals

The experimental model was developed using female Wistar rats, weighing between 200 and 250 g, obtained from the Central Animal Facility of the Federal University of Grande Dourados. The choice of female rats was based on evidence suggesting a greater susceptibility to dyslipidemia under dietary modification, ensuring consistent model induction [26]. The animals were housed in the Animal Facility of the Neuroscience Laboratory at Paranaense University (UNIPAR), provided free access to liquid and solid diet, and maintained under controlled environmental conditions (temperature: 20 ± 2 °C; relative humidity: 50 ± 10%; light/dark cycle: 12 h; and environmental enrichment). After randomization, the rats were divided into 6 experimental groups ($n$ = 6–8). They were weighed weekly on an analytical balance. The entire experimental protocol was approved by the Ethics Committee on Animal Experimentation of UNIPAR under protocol number 1003/2022. All regulations and recommendations of the National Council for the Control of Animal Experimentation (CONCEA, Brazil) were followed in accordance with the guidelines of the Animal Research Reporting of In Vivo Experiments [27], aiming at animal welfare and a reduction in the number of animals used for experimentation.

#### 2.2.2. Experimental Design and Treatments

The model was established based on the associations among diabetes, dyslipidemia, and smoking. For the induction of diabetes, the animals received streptozotocin (30 mg/kg, i.p.) diluted in citrate buffer (10 mM, pH 4.5) as described by Souza et al. [28] and Vit et al. [29]. The rats were considered diabetic when their blood glucose levels were equal to or greater than 250 mg/dL.

Dyslipidemia was induced in the animals by providing a diet enriched with 0.5% cholesterol. This diet was prepared by mixing 150 g of standard rodent chow with one egg yolk and 13.5 mL of corn oil. The blend was combined with water, baked at 50 °C for 36 h, and stored in vacuum-sealed bags. Each 150 g portion of this diet contained 225 mg of cholesterol, 1.8 g of saturated fat, 2.16 g of monounsaturated fats, and 0.72 g of polyunsaturated fats [30].

The animals were exposed to 9 cigarettes (containing 0.8 mg nicotine, 10 mg tar, and 10 mg carbon monoxide) for 1 h per day, 5 days a week. Basal animals underwent the same conditions without smoke exposure. The setup included a smoke capture and distribution system adapted for smoking studies, with five cubic metal cages (24 cm × 17 cm × 15 cm) that each held three animals. These cages were spaced equally within a sealed acrylic box (70 cm × 70 cm × 20 cm). Cigarette smoke was drawn through an external holder using an electric pump with an automatic timer, allowing intermittent air intake to prevent asphyxiation. Inside the box, the tubing branched out to direct smoke into each cage, dispersing it evenly across the chamber [30].

During the last 2 weeks of the study, the rats received oral treatments via gavage with either a vehicle (filtered water, 1 mL/kg, serving as the negative control [C−]) or varying doses of *B. dracunculifolia* extract (30, 100, and 300 mg/kg). The positive control group (SIM + INSU) was treated with simvastatin (2.5 mg/kg, orally) and insulin (6 IU, subcutaneously). The doses of both the drugs and the extract used in this study were

determined according to the guidelines proposed by Barbosa et al. [31], Zago et al. [32], and Auth et al. [33].

The selection of *B. dracunculifolia* dosages aimed to investigate potential dose–response relationships and to identify the optimal therapeutic window for this phytotherapeutic agent. Previous studies involving *Baccharis* spp. [28,31,34,35] have shown that low-to-moderate doses often yield maximal efficacy due to the biphasic effects commonly associated with natural products. By including this range of dosages, we were able to assess both subtherapeutic and potentially saturating doses, clarifying the most effective dose in terms of metabolic and hepatic effects. Additionally, a baseline group was included, consisting of rats that were not exposed to the risk factors and were treated solely with the vehicle (filtered water, 1 mL/kg; see Figure 1).

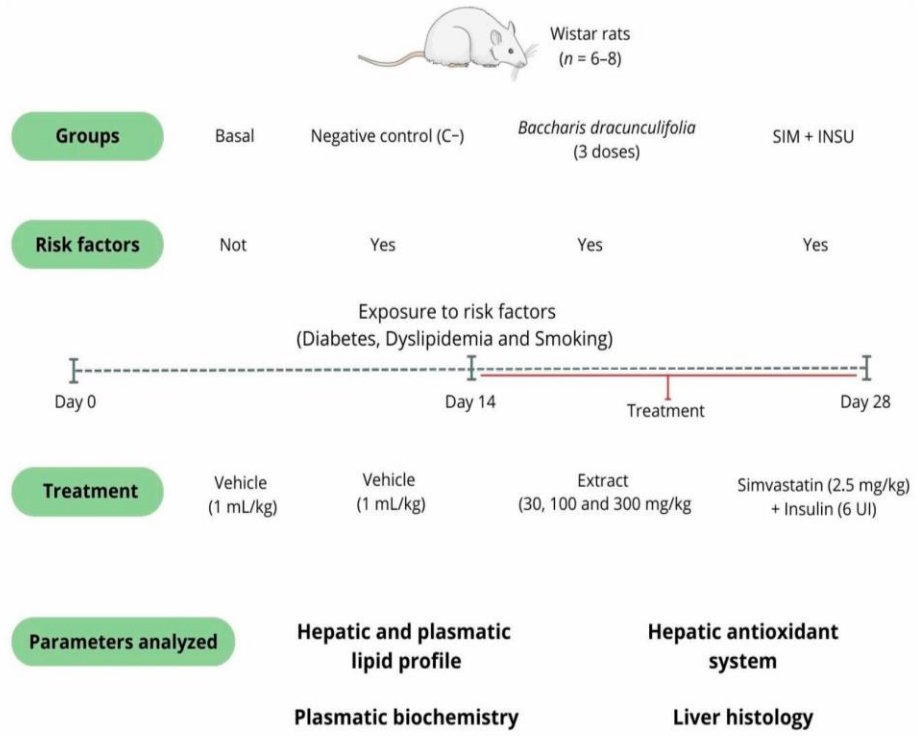



**Figure 1.** The model of fatty liver disease associated with metabolism was generated by inducing diabetes, dyslipidemia, and smoking conditions in Wistar rats. The study lasted for 4 weeks and consisted of a baseline group [normoglycemic rats not exposed to risk factors and treated with vehicle (filtered water, 1 mL/kg, $n = 6$)] and five other groups exposed to risk factors and treated orally (gavage) in the last two experimental weeks with vehicle (filtered water, 1 mL/kg, negative control [C−], $n = 8$), *Baccharis dracunculifolia* extract (30, 100, and 300 mg/kg, $n = 8$), and simvastatin (2.5 mg/kg) + insulin (6 IU, s.c., positive control group [SIM + INSU], $n = 8$). At the end of the treatment, the lipid-lowering and hepatoprotective effects of *B. dracunculifolia* were analyzed.

### 2.2.3. Euthanasia, Sample Collection, and Biochemical Analysis

At the end of the four-week period, following a 12 h fast, the animals were euthanized by deepening anesthesia in an isoflurane (1–3%) saturation chamber. Blood was then collected via decapitation, with glucose levels immediately measured using a blood glucose meter (Roche®, model Accu-Chek Active, Mannheim, Germany). The remaining blood was centrifuged at 1500× *g* for 10 min, and the plasma was stored at −80 °C for subsequent analysis. Plasma levels of cholesterol, triglycerides, glucose, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using colorimetric methods on a semi-automatic analyzer (Global Analyzer®, model GTA-300, Calgary, AB, Canada). After blood collection, abdominal laparotomy was performed to collect the liver, which was washed with saline and weighed on an analytical balance. A sample of liver tissue

was quickly collected and frozen in liquid nitrogen for evaluation of the tissue redox state. Another sample of the organ was stored for histological analyses.

#### 2.2.4. Measurement of Hepatic Cholesterol and Triglycerides

Hepatic cholesterol and triglycerides determination was performed following the methodology described by Lívero et al. [34]. For this purpose, liver samples were lyophilized, subjected to extraction with hexane (solvent, 1:10 [tissue:solvent]), and heated in an oven (80 °C, 12 h). After this period, the supernatant was transferred to a new container and allowed to evaporate naturally, with this extraction step being repeated three times. The percentage of hepatic lipids was calculated using the formula [lipids (%) = 100 × (final weight of the flask/initial weight of the flask)/0.3 g]. Additionally, the resulting material from the extractions was resuspended in chloroform (1 mL) and isopropanol (2 mL) to determine the levels of cholesterol and triglycerides by colorimetric enzymatic technique on a semi-automatic analyzer (Global Analyzer®, model GTA-300).

#### 2.2.5. Investigation of the Hepatic Antioxidant System

To evaluate the hepatic antioxidant potential of *B. dracunculifolia*, liver samples were initially homogenized in a potassium phosphate buffer solution (0.1 M, pH 6.5) at a 1:10 dilution. Next, 100 µL of the homogenate was separated and combined with 80 µL of 12.5% trichloroacetic acid. The solution was then vortexed and centrifuged at 6000 rpm for 15 min at 4 °C to measure reduced glutathione (GSH) levels, following the method outlined by Sedlak and Lindsay [36]. The remaining homogenate was further centrifuged at 9700 rpm for 20 min at 4 °C to assess superoxide dismutase (SOD) activity and lipid peroxidation (LPO) levels, according to protocols by Gao et al. [37] and Jiang et al. [38], respectively. Each assay was performed in triplicate, and results were normalized per gram of tissue.

#### 2.2.6. Histopathological Analysis

The hepatic tissue was fixed in 10% buffered formalin (1800 mL distilled water, 200 mL formaldehyde, 8 g monobasic sodium phosphate, and 13 g dibasic sodium phosphate). After fixation, the sample was dehydrated with alcohol and xylene, and subsequently embedded in paraffin. Sections were cut at a thickness of 6 µm and stained with hematoxylin and eosin for assessment of general morphology and Sudan Black for lipid visualization. For triglyceride-specific staining, an additional liver sample underwent a distinct preparation. The sample was gradually saturated in sucrose solutions of increasing concentrations (10%, 20%, and 30%), with each concentration maintained for 24 h. Following saturation, the sample was embedded in Tissue Tek (O.C.T. Sakura, Torrance, CA, USA) and rapidly frozen using liquid nitrogen. Frozen sections, cut at a thickness of 6 µm, were then stained with Sudan Black, as adapted from the methodology of Lívero et al. [34]. The slides were examined using an optical microscope (Leica®, model DM 2500, Wetzlar, Germany) to assess structures and cellular abnormalities. Hepatic lesions were classified on a scale from 0 to 3 according to the percentage of tissue affected by multiple risk factors (grade 0: less than 5% occurrence; grade 1: 6–33% occurrence; grade 2: 34–66% occurrence; and grade 3: 67–100% occurrence), following the classification by Lívero et al. [34] and Mendes et al. [39]. To enhance the robustness of our histological analysis, the number of fields assessed for each animal was standardized. Specifically, we analyzed five fields per animal to provide a comprehensive evaluation of the histological findings.

### *2.3. Blinding Clarification and Statistical Analysis*

To address potential bias, the study was conducted using a blind approach. Investigators responsible for data collection and analysis were unaware of the group assignments throughout the experimental process and data analysis. This blinding was strictly maintained until the conclusion of data analysis to minimize any potential bias. The data were first analyzed for homogeneity of variance and normality of distribution. One-way

ANOVA, followed by Bonferroni's post-test, was used. The level of significance adopted was 95% ($p < 0.05$). GraphPad Prism® version 9.0 was used for statistical analysis and graph preparation.

## 3. Results

### 3.1. Phytochemical Analysis

The phytochemical investigation indicated the presence of seven phenolic acids (quinic acid, chlorogenic acid, 4-hydroxybenzoic acid, caffeic acid, *p*-coumaric acid, ferulic acid, and protocatechuic acid) and six flavonoids (isoquercetin, quercetin, isokaempferide, kaempferol, 3-methoxy-quercetin, and apigenin). The compounds, theoretical mass *m*/*z* [M-H], experimental mass m/z [M-H], and retention time (min) are described in Table 1.

**Table 1.** Chemical composition of crude extract of *Baccharis dracunculifolia* aerial parts using UHPLC-ESI-QTOF-MS/MS.

| Compounds | Theoretical Mass *m*/*z* [M-H] | Experimental Mass *m*/*z* [M-H] | Retention Time (min) | Error |
|---|---|---|---|---|
| Phenolic acids | | | | |
| Quinic acid | 191.05 | 191.05 | 0.84 | −3.66 |
| Chlorogenic acid | 353.08 | 353.08 | 4.25 | 0 |
| 4-Hydroxybenzoic acid | 137.02 | 137.02 | 4.10 | −5.11 |
| Caffeic acid | 179.03 | 179.03 | 4.15 | 4.47 |
| *p*-Coumaric acid | 163.03 | 163.03 | 4.60 | −4.29 |
| Ferulic acid | 193.04 | 193.05 | 5.51 | −3.62 |
| Protocatechuic acid | 153.01 | 153.01 | 1.28 | −3.92 |
| Flavonoids | | | | |
| Isoquercetin | 463.08 | 463.08 | 4.40 | 2.59 |
| Quercetin | 301.03 | 301.03 | 5.16 | 0.33 |
| Isokaempferide | 299.05 | 299.05 | 5.45 | −2.01 |
| Kaempferol | 285.03 | 285.03 | 5.48 | 0 |
| 3-Methoxy-quercetin | 315.05 | 315.05 | 5.51 | 0.32 |
| Apigenin | 269.04 | 269.04 | 5.43 | −0.74 |

### 3.2. Baccharis dracunculifolia Did Not Alter the Body Weight and Chow Consumption of Rats

The average body weight of the basal group did not show a significant difference between the initial weight (274.30 ± 1.87 g) and the final weight after four weeks (287.90 ± 3.58 g). Also, no difference was observed in the average feed consumption between the experimental weeks. No statistical differences were found when analyzing the body weight gain or feed consumption of the groups exposed to risk factors and treated with different doses of *B. dracunculifolia* or SIM + INSU throughout the four weeks of the experiment (Table 2).

**Table 2.** Body weight and food consumption of rats exposed to diabetes, smoking, and dyslipidemia and treated with *Baccharis dracunculifolia*.

| Groups | Initial | At 2 Weeks | | At 4 Weeks | |
|---|---|---|---|---|---|
| | Body Weight (g) | Body Weight (g) | Chow Consumption (g) | Body Weight (g) | Chow Consumption (g) |
| Basal | 274.30 ± 1.87 | 283.70 ± 3.55 | 108.90 ± 2.93 | 287.90 ± 3.58 | 101.60 ± 4.98 |
| C− | 244.30 ± 5.57 | 245.40 ± 7.94 | 135.90 ± 3.23 | 254.50 ± 6.53 | 126.4 ± 6.43 |
| BD 30 | 240.30 ± 2.71 | 233.80 ± 5.83 | 142.80 ± 5.06 | 230.30 ± 10.53 | 136.4 ± 4.88 |
| BD 100 | 227.30 ± 4.25 | 228.90 ± 2.77 | 128.30 ± 8.57 | 230.40 ± 6.51 | 132.9 ± 8.53 |
| BD 300 | 205.90 ± 7.36 | 223.10 ± 7.35 | 112.20 ± 3.72 | 231.10 ± 8.77 | 103.7 ± 5.10 |
| SIM + INSU | 241.90 ± 5.13 | 246.50 ± 4.71 | 133.80 ± 7.16 | 287.00 ± 4.98 | 153.60 ± 1.17 |

Values are presented as mean ± SEM ($n$ = 6–8). Feed consumption in grams (g) per week. Legend: Basal, normoglycemic rats; C−, negative control; BD30, BD 100, and BD 300, *B. dracunculifolia* at doses of 30, 100, and 300 mg/kg, respectively; SIM + INSU, simvastatin (2.5 mg/kg) plus insulin (6 IU).

### 3.3. *Baccharis dracunculifolia* Exhibit a Median Hypoglycemic Effect and Demonstrated Significant Hepatoprotective Properties

After diabetes induction, a significant increase in blood glucose levels was noted in the C− group (636.20 ± 32.38 mg/dL) compared to the basal group (115.20 ± 5.07 mg/dL). The administration of SIM + INSU resulted in a decrease in blood glucose levels (222.00 ± 4.09 mg/dL), while treatment with *B. dracunculifolia* partially reversed these alterations (Figure 2A). Diabetes, dyslipidemia, and smoking led to a significant rise of approximately 132.53% in ALT and 182.92% in AST levels compared to the basal group (63.00 ± 3.73 U/L and 113.00 ± 6.71 U/L, respectively). However, the administration of 30 mg/kg *B. dracunculifolia* extract completely reversed the elevation in AST and ALT levels. Treatment with 100 and 300 mg/kg *B. dracunculifolia* extract partially reversed these alterations, and the combination of SIM+INS was ineffective (Figure 2B,C).

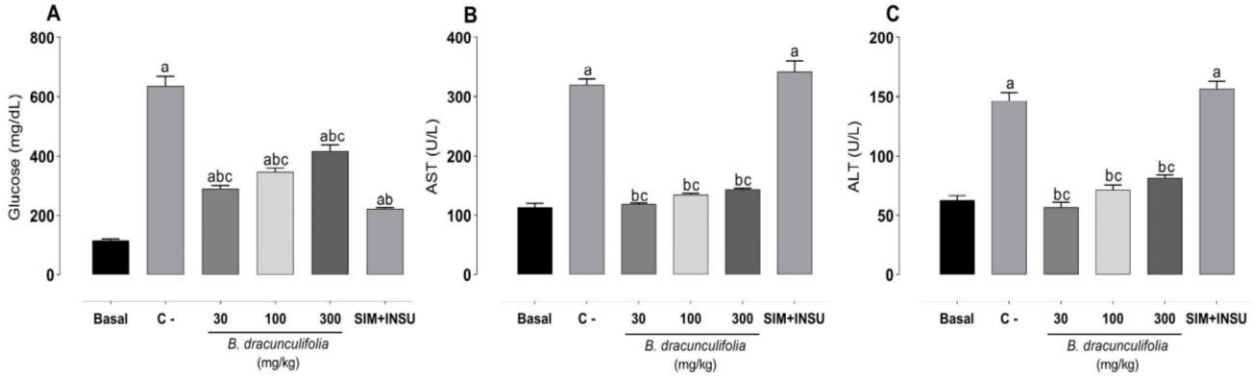



**Figure 2.** Plasma levels of (**A**) glucose, (**B**) aspartate aminotransferase, and (**C**) alanine aminotransferase in normoglycemic, non-dyslipidemic, and non-smoker Wistar rats (basal group, $n = 6$) and diabetic, dyslipidemic, and smoker Wistar rats that were treated with vehicle (negative control [C−], $n = 8$), *Baccharis dracunculifolia* (30, 100, and 300 mg/kg, $n = 8$), and simvastatin (2.5 mg/kg) + insulin (6IU; SIM + INSU, $n = 8$). The data are expressed as mean ± SEM. [a] $p < 0.05$, vs. basal; [b] $p < 0.05$, vs. C−; [c] $p < 0.05$, vs. SIM + INSU (one-way ANOVA followed by Bonferroni post hoc test).

### 3.4. *Baccharis dracunculifolia* Extract Exerted Lipid-Lowering Effects

The presence of diabetes, dyslipidemia, and smoking significantly elevated plasma levels of triglycerides (776.80 ± 18.14 mg/dL, Figure 3A) and cholesterol (924.00 ± 18.11 mg/dL, Figure 3B) compared to the basal group (72.50 ± 4.58 mg/dL and 94.17 ± 4.42 mg/dL, respectively). These risk factors also induced a substantial increase in hepatic triglyceride (237.36%, Figure 3C) and cholesterol (339.19%, Figure 3D) levels compared to the basal group (40.52 ± 2.32 mg/dL and 14.67 ± 1.25 mg/dL, respectively). However, treatment with 30 mg/kg *B. dracunculifolia* extract and SIM + INSU completely reversed these alterations in both plasma and liver. Treatment with 100 and 300 mg/kg *B. dracunculifolia* extract partially restored triglyceride and cholesterol levels in plasma and liver (Figure 3).

### 3.5. *Baccharis dracunculifolia* Reversed Hepatocellular Alterations

The results shown in Figure 4 reveal significant findings regarding liver weight, lipid percentage, and histopathological analyses. First, the relative liver weight in the C− group exhibited a significative increase (4.60 ± 0.08%) compared to the basal group (3.16 ± 0.14%). However, treatment with *B. dracunculifolia* (30 and 100 mg/kg) effectively normalized this parameter (Figure 4A), while a dose of 300 mg/kg showed intermediate results. Additionally, risk factors significantly elevated the hepatic lipid percentage by 283.62% in the C− group compared to the basal group (2.81 ± 0.19%). Conversely, treatment with 30 mg/kg of *B. dracunculifolia* extract successfully reversed these changes (Figure 4B), with doses of 100 mg/kg and 300 mg/kg showing intermediate improvement.

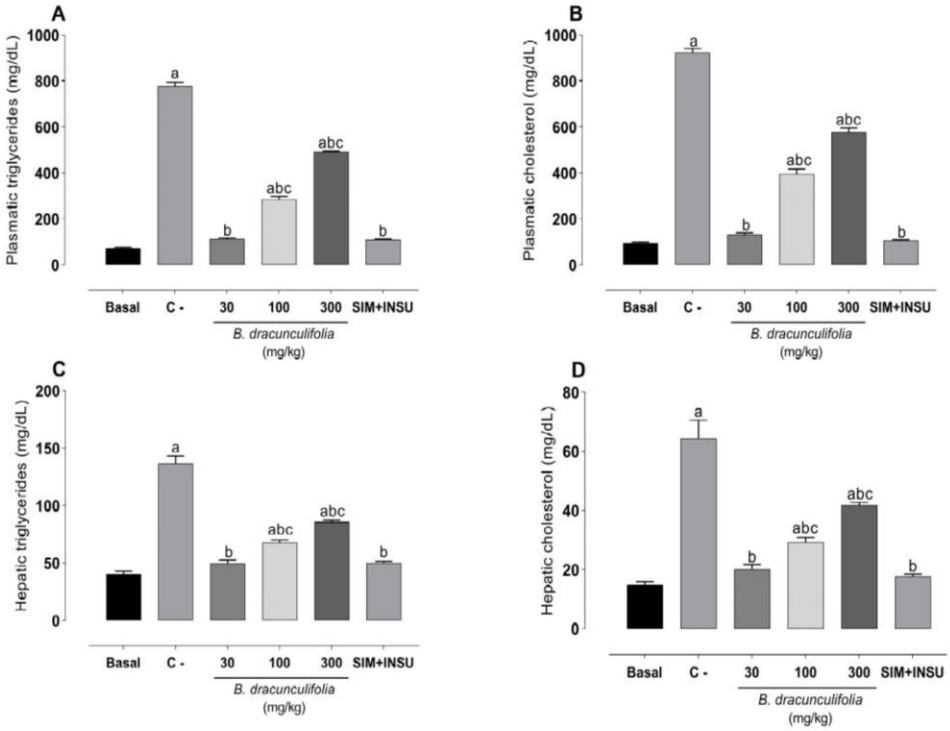



**Figure 3.** Plasma levels of (**A**) triglycerides and (**B**) cholesterol as well as hepatic levels of (**C**) triglycerides and (**D**) cholesterol in normoglycemic, non-dyslipidemic, and non-smoker Wistar rats (basal group, $n = 6$) and diabetic, dyslipidemic, and smoker Wistar rats that were treated with vehicle (negative control [C−], $n = 8$), *Baccharis dracunculifolia* (30, 100, and 300 mg/kg, $n = 8$), and simvastatin (2.5 mg/kg) + insulin (6IU; SIM + INSU, $n = 8$). n = 8/group. The data are expressed as mean ± SEM. [a] $p < 0.05$, vs. basal; [b] $p < 0.05$, vs. C−; [c] $p < 0.05$, vs. SIM + INSU (one-way ANOVA followed by Bonferroni post hoc test).

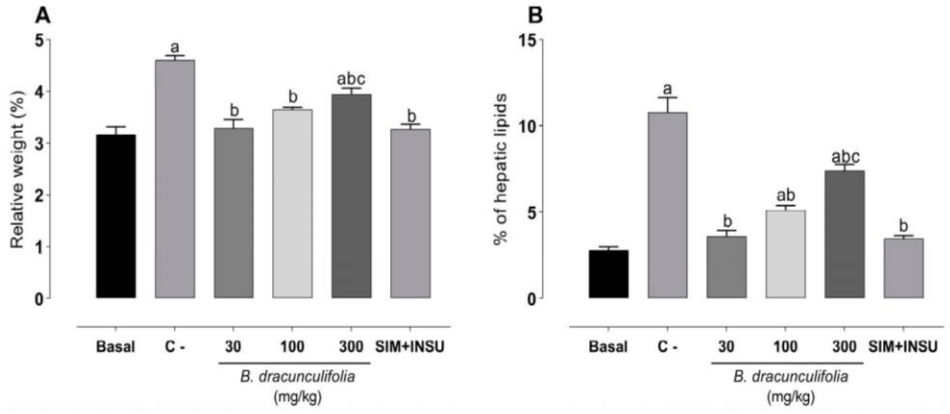
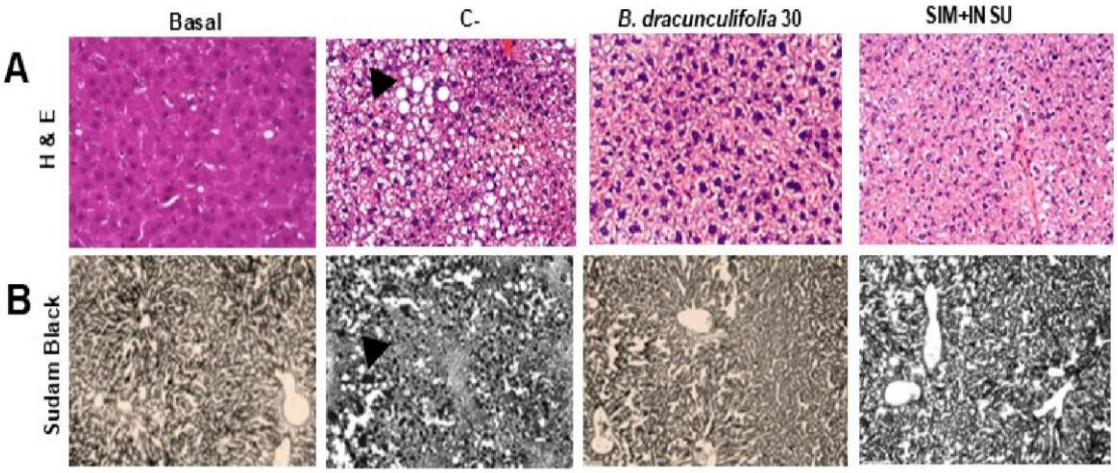



**Figure 4.** Relative (%) hepatic weight (**A**) and % of hepatic lipids (**B**). The study involved normoglycemic, non-dyslipidemic, and non-smoker Wistar rats (basal group, $n = 6$) as well as diabetic, dyslipidemic, and smoker Wistar rats treated with vehicle (negative control [C−], $n = 8$), *Baccharis dracunculifolia* (30, 100, and 300 mg/kg, $n = 8$), and simvastatin + insulin (SIM + INSU, $n = 8$). The data are shown as mean ± SEM. [a] $p < 0.05$, vs. basal; [b] $p < 0.05$, vs. C−; [c] $p < 0.05$, vs. SIM + INSU (one-way ANOVA followed by Bonferroni post hoc test).

To confirm the presence of steatosis and cellular alterations, liver samples were stained with hematoxylin and eosin and Sudan Black, revealing macroscopic liver alterations that indicate the successful induction of hepatic injury (Figure 5A,B). In the basal group, the absence of ballooning and microvesicular and macrovesicular steatosis was observed. In

contrast, group C− exhibited the highest grades across all three evaluated categories, with grade 2 ballooning, grade 3 microvesicular steatosis, and grade 3 macrovesicular steatosis. Groups treated with varying doses of *B. dracunculifolia* showed variations in the degrees of hepatic injury: grade 1 ballooning and steatosis at 30 mg/kg; grade 1 ballooning, grade 1 microvesicular steatosis, and grade 2 macrovesicular steatosis at 100 mg/kg; and grade 2 ballooning and grade 2 steatosis at 300 mg/kg. Treatment with SIM + INSU resulted in grade 1 ballooning and steatosis (Figure 5C).




C

| | Ballooning | Microvesicular steatosis | Macrovesicular steatosis |
|---|---|---|---|
| Basal | 0 | 0 | 0 |
| C- | 2 | 3 | 3 |
| BD 30 | 1 | 1 | 1 |
| BD 100 | 1 | 1 | 2 |
| BD 300 | 2 | 2 | 2 |
| SIM + INSU | 0 | 1 | 1 |

**Figure 5.** Hepatic histopathological analysis with hematoxylin/eosin (**A**), Sudan Black (**B**), and grades of liver injury (**C**). The study involved normoglycemic, non-dyslipidemic, and non-smoker Wistar rats (basal group, $n = 6$) as well as diabetic, dyslipidemic, and smoker Wistar rats treated with vehicle (negative control [C−], $n = 8$), *Baccharis dracunculifolia* (30, 100, and 300 mg/kg, $n = 8$), and simvastatin + insulin (SIM + INSU, $n = 8$). In the histopathological analysis, black arrows indicate hepatic fatty degeneration (steatosis). The images were taken at 40× magnification.

### *3.6. Effects of Baccharis dracunculifolia on Hepatic Redox State*

The oxidative stress analysis revealed significant variations among the experimental groups for the three parameters: GSH, SOD, and LPO. The basal group exhibited the highest levels of GSH (162.40 ± 7.68 µg GSH/g of tissue) and SOD (1258 ± 39.80 U SOD/mg of tissue) and the lowest levels of LPO (63.70 ± 3.60 mmol LPO/min/g of tissue). The C− group showed a sharp reduction in GSH (35.16 ± 2.42 µg GSH/g of tissue) and SOD (739.40 ± 30.80 U SOD/mg of tissue) and a substantial increase in LPO (186.90 ± 5.58 mmol LPO/min/g of tissue). Among the treated groups, treatment with 30 mg/kg *B. dracunculifolia* extract maintained GSH (160.60 ± 4.40 µg GSH/g of tissue) and SOD (1260 ± 29.45 U SOD/mg of tissue) levels close to basal levels, with low LPO levels (61.25 ± 1.34 mmol LPO/min/g of tissue). Higher doses of *B. dracunculifolia* extract (100 and 300 mg/kg) and the combination of SIM + INSU resulted in partially reduced GSH and SOD levels and increased LPO levels (Figure 6).

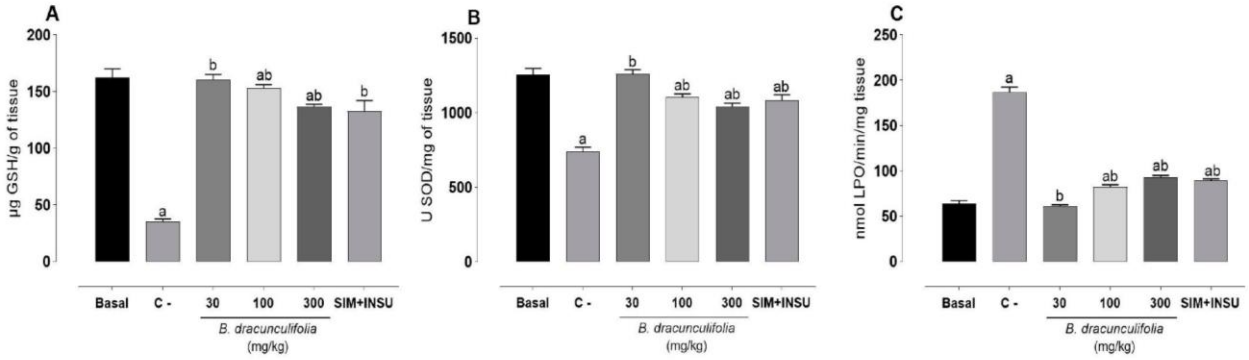



**Figure 6.** Reduced glutathione (**A**), superoxide dismutase (**B**), and lipoperoxidation (**C**). The study involved normoglycemic, non-dyslipidemic, and non-smoker Wistar rats (basal group, $n = 6$) as well as diabetic, dyslipidemic, and smoker Wistar rats treated with vehicle (negative control [C−], $n = 8$), *Baccharis dracunculifolia* (30, 100, and 300 mg/kg, $n = 8$), and simvastatin + insulin (SIM + INSU, $n = 8$). The data are shown as mean ± SEM. [a] $p < 0.05$, vs. basal; [b] $p < 0.05$, vs. C− (one-way ANOVA followed by Bonferroni post hoc test).

## 4. Discussion

Cardiometabolic syndrome is a multifactorial condition that requires an integrative approach to management [40,41]. Developing innovative and comprehensive models is essential to advancing our understanding and treatment of this complex syndrome. Our study contributes to this goal, showing that *B. dracunculifolia* effectively targets key metabolic disturbances associated with cardiometabolic syndrome, including hyperglycemia, dyslipidemia, and hepatic and cardiovascular dysfunction. Our findings reveal the multifaceted therapeutic potential of *B. dracunculifolia*, which stands out for its ability to modulate glycemic control, lipid profiles, hepatic markers, and oxidative stress—addressing the interconnected pathways that drive disease progression. *B. dracunculifolia* not only impacts isolated components of the syndrome but also appears to offer an integrative therapeutic approach by simultaneously influencing multiple risk factors. This holistic effect is especially valuable given the limitations of conventional treatments, which often target individual aspects of the disease without addressing the full range of metabolic dysfunctions. These promising findings underscore the need for further investigation into the underlying mechanisms and potential clinical applications of *B. dracunculifolia* as a complementary therapy for managing cardiometabolic syndrome.

Our study indicates that the therapeutic potential of *B. dracunculifolia* in managing cardiometabolic syndrome may be largely attributed to its complex array of bioactive compounds, including flavonoids and phenolic acids. These compounds possess a range of biological activities that may act synergistically, enhancing the plant's overall therapeutic effect. Flavonoids such as quercetin and rutin are well-documented for their antioxidant, anti-inflammatory [42–44], and lipid-lowering properties [45,46], which likely contribute significantly to reducing oxidative stress [47–49] and improving lipid profiles in the context of cardiometabolic syndrome. The reductions in lipid peroxidation and improvements in antioxidant markers, such as GSH and SOD observed in our study, support this mechanism. Additionally, phenolic acids like caffeic and p-coumaric acids enhance *B. dracunculifolia*'s bioactivity, promoting cardiovascular and metabolic health through their vasodilatory and lipid-regulating effects [50,51]. These compounds may interact with flavonoids, potentially amplifying the extract's therapeutic effects through synergistic actions that target multiple cardiometabolic risk factors simultaneously [52].

The dose-dependent response observed with *B. dracunculifolia* extract further underscores the complexity of its therapeutic action. While lower doses completely reversed hyperlipidemia and liver enzyme elevations, higher doses showed only partial effects. This phenomenon could be attributed to saturation, synergistic or antagonistic effects, or the presence of different bioactive compounds at varying concentrations, each exerting distinct

biological activities [53,54]. This warrants further investigation into the pharmacokinetics and pharmacodynamics of *B. dracunculifolia* to better understand its therapeutic window and optimize its dosing regimen.

The lack of results with the highest dose of *B. dracunculifolia* can be explained by the hormetic effect. This phenomenon is commonly observed when working with crude plant extracts, which contain various secondary metabolites. These compounds may act independently, synergistically, or even antagonistically on different biological targets. Typically, a substance—or a combination of substances—exhibits a dose at which its maximal effect is achieved. Beyond this dose, increasing the amount yields no further therapeutic benefit, a point referred to as the plateau. This type of dose–response curve is often observed because the biological tissue or function in question has already reached its maximum response.

The partial reversal of hyperglycemia by *B. dracunculifolia* suggests that this extract may contain bioactive compounds capable of modulating glucose metabolism. The hypoglycemic effects of *B. dracunculifolia* are corroborated by studies on other medicinal plants with similar properties. Several preclinical studies have demonstrated the hypoglycemic effects of plant flavonoids [55–57]. These results emphasize the role of natural products in managing diabetes and its complications. This is particularly significant in the context of cardiometabolic syndrome, where insulin resistance and hyperglycemia are pivotal contributors to disease progression.

The protective effects of *B. dracunculifolia* on cardiovascular and metabolic health, evidenced by the improvement in liver enzymes and metabolic markers, suggest its role in modulating oxidative stress, which is central to the pathophysiology of cardiometabolic syndrome. The normalization of markers such as GSH, SOD, and LPO levels following treatment with *B. dracunculifolia* indicates its capacity to mitigate oxidative stress, further supporting its use in addressing the oxidative burden linked to this syndrome [58]. The analysis of redox status revealed significant variations among the experimental groups for the GSH, SOD, and LPO parameters. The C− group showed substantial reductions in GSH and SOD levels, accompanied by a marked increase in LPO, indicative of pronounced oxidative stress associated with the studied live condition. Treatment with *B. dracunculifolia* extract demonstrated protective effects against hepatic oxidative stress. This treatment maintained GSH and SOD levels close to those of the basal group and reduced LPO levels, indicating the extract's ability to preserve liver antioxidant defenses and mitigate lipid peroxidation associated with the model studied. These findings are consistent with existing literature on the variable effects of botanical extracts of *Baccharis* genus on oxidative stress, depending on dosage and experimental conditions [28,31,34,35,39,59–61].

Elevated levels of ALT and AST enzymes are markers of hepatic injury, and their reduction indicates a protective effect on liver tissue. In the context of the *Baccharis* genus, Barbosa et al. [31] demonstrated that *B. trimera* extract reduced ALT and AST levels in rats exposed to smoking, dyslipidemia, and diabetes. Additionally, *B. trimera* exhibited a hepatoprotective effect in a mouse model of alcoholic liver disease. Lívero et al. [34] further reported that treatment with *B. trimera* reversed the increase in ALT and AST levels in mice exposed to ethanol and a low-protein diet, compared to those treated with a vehicle. In this study, the hepatoprotective effects of *B. dracunculifolia*, evidenced by the normalization of ALT and AST levels, are noteworthy. The extract's ability to mitigate liver damage induced by combined metabolic risk factors suggests its potential role in modulating inflammation and oxidative stress, which are critical pathways in the progression of metabolic associated fatty liver disease (MAFLD), a condition that has been considered the hepatic manifestation of metabolic syndrome [62].

The ineffectiveness of the combination of SIM + INS in normalizing liver enzymes suggests that standard pharmacological treatments may not address all the pathological mechanisms involved in MAFLD. This finding is supported by Nassir [63], who emphasized the limitations of conventional treatments in addressing the multifactorial nature of MAFLD and the need for alternative therapeutic approaches. This highlights the need for alternative or complementary therapies that can target the disease's multifactorial nature.

*B. dracunculifolia*'s broad spectrum of biological activities, including anti-inflammatory, antioxidant, and immunomodulatory effects, positions it as a promising candidate for such therapies.

Simvastatin is a widely used lipid-lowering agent effective in managing dyslipidemia associated with cardiometabolic conditions. It functions by inhibiting HMG-CoA reductase, the enzyme responsible for cholesterol synthesis in the liver, resulting in decreased levels of low-density lipoprotein cholesterol and triglycerides [64]. Simvastatin was selected as a control agent in our study due to its well-established hypolipemiant effects, providing a reliable comparative standard for evaluating the potential therapeutic effects of *B. dracunculifolia*. In addition to simvastatin, we also employed insulin as part of the positive control regimen. Insulin plays a critical role in glucose metabolism and is often used to manage hyperglycemia in patients with diabetes, which is a common comorbidity in cardiometabolic disorders [65]. The combination of simvastatin and insulin serves as a robust positive control, allowing us to assess the efficacy of *B. dracunculifolia* in comparison to established therapeutic approaches.

Srikanth and Deedwania [66] highlighted the importance of managing dyslipidemia in patients with diabetes and metabolic syndrome. The significant elevation in plasma and hepatic triglyceride and cholesterol levels in the C− group reflects the dyslipidemia commonly associated with metabolic syndrome. The normalization of these lipid levels with 30 mg/kg *B. dracunculifolia* extract and SIM+INS indicates the efficacy of these treatments in managing dyslipidemia. This effect is crucial as elevated triglycerides and cholesterol contribute to the progression of liver steatosis and subsequent liver injury. The partial restoration with higher doses of *B. dracunculifolia* suggests that while the extract is effective, its lipid-lowering capacity might be influenced by the dosage.

The increase in relative liver weight and hepatic lipid percentage in the C− group indicates the successful induction of liver steatosis, a hallmark of MAFLD. The normalization of liver weight and lipid content with 30 mg/kg *B. dracunculifolia* treatment further supports its hepatoprotective effects. Histopathological analysis revealed significant liver alterations in the C− group, including steatosis, which were reversed by *B. dracunculifolia* treatment. This finding is critical as it confirms the macroscopic and microscopic protective effects of the extract against hepatic injury.

In our study, the weight stability observed in the C− group despite significant hyperglycemia raises interesting considerations regarding metabolic adaptations and the temporal aspects of diabetes induction. Typically, sustained hyperglycemia in diabetes models leads to weight loss due to increased protein and fat catabolism associated with insulin deficiency and poor glycemic control [67]. However, in this study, the diabetes induction period was relatively short (four weeks), which may have been insufficient to trigger the marked weight loss often seen in chronic hyperglycemic states. Furthermore, the controlled dietary conditions in this model provided consistent calorie intake, which may have mitigated the typical weight fluctuations observed in prolonged diabetic states. Another plausible explanation for this stability could be attributed to adaptive metabolic compensations [68]. Acute or sub-chronic models of diabetes, particularly when induced in a well-nourished and monitored animal cohort, may prompt compensatory mechanisms in peripheral tissues to temporarily preserve body weight. This adaptation could involve an upregulation of alternative metabolic pathways, such as increased reliance on lipid oxidation and amino acid catabolism, which may contribute to maintaining baseline body weight in the short term [69,70]. Additionally, while diabetes and associated metabolic disturbances lead to significant weight changes over time, these effects may not manifest fully within the timeframe used here. Future studies could extend the induction period and explore additional metabolic markers, such as ketone body levels and proteolysis indicators, to assess whether weight stability persists under prolonged hyperglycemia and if other compensatory mechanisms are activated. These insights could provide a more comprehensive understanding of the complex metabolic adaptations in early diabetic

states and enhance the translational relevance of this model for studying cardiometabolic syndrome.

The study underscores the potential of *B. dracunculifolia* as a natural therapeutic agent for cardiometabolic syndrome, capable of addressing multiple facets of the disease, including hyperglycemia, dyslipidemia, and hepatic injury. Its efficacy at a lower dose suggests a favorable safety profile and encourages further research to optimize dosing strategies. Furthermore, the traditional use of *B. dracunculifolia* in treating various ailments supports its safety and efficacy; however, rigorous clinical trials are necessary to confirm these benefits in patients. The ethnopharmacological background provides a rich context for understanding how traditional knowledge can inform modern therapeutic strategies.

## 5. Conclusions

This study highlights the significant therapeutic potential of *Baccharis dracunculifolia* extract in alleviating the effects of cardiometabolic syndrome induced by diabetes, dyslipidemia, and smoking. The extract exhibited hypoglycemic properties and robust hepatoprotective effects, normalizing liver enzyme levels and lipid profiles. *B. dracunculifolia* shows promise as a complementary or alternative natural therapy, warranting further investigation to elucidate its molecular mechanisms, optimize dosing, and validate its clinical efficacy and safety.

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## 3. CONCLUSÃO

1 Os resultados obtidos indicam que o extrato de *Baccharis dracunculifolia* possui potencial antioxidante e anti-inflamatório significativo, além de apresentar um perfil de segurança favorável. Embora o estudo tenha demonstrado efeitos positivos em diversos parâmetros, são necessárias investigações adicionais para compreender melhor os mecanismos envolvidos e explorar o potencial terapêutico da planta no tratamento de doenças inflamatórias crônicas e metabólicas. Assim, o extrato de *B. dracunculifolia* representa um candidato promissor para o desenvolvimento de novas abordagens terapêuticas voltadas para o tratamento de distúrbios metabólicos e inflamatórios.

2 O estudo destacou o potencial terapêutico significativo do extrato de *B. dracunculifolia* em um modelo animal de síndrome cardiometabólica induzida por diabetes, dislipidemia e tabagismo. O tratamento com o extrato exerceu efeitos hipoglicemiantes e efeitos hepatoprotetores robustos, normalizando os níveis de enzimas hepáticas e perfis lipídicos. A *B. dracunculifolia* mostra-se promissora como uma terapia natural complementar ou alternativa, sendo necessário mais investigações para elucidar seus mecanismos moleculares, otimizar a dosagem e validar sua eficácia e segurança clínicas.

# 4 ANEXOS

ANEXO 1 - Normas do Revista *Food Research International*

## Food Research International

## Writing and formatting

### File format

We ask you to provide editable source files for your entire submission (including figures, tables and text graphics). Some guidelines:

- Save files in an editable format, using the extension .doc/.docx for Word files and .tex for LaTeX files. A PDF is not an acceptable source file.
- Lay out text in a single-column format.
- Use spell-check and grammar-check functions to avoid errors.

We advise you to read our Step-by-step guide to publishing with Elsevier.

### Title page

You are required to include the following details in the title page information:

- Article title. Article titles should be concise and informative. Please avoid abbreviations and formulae, where possible, unless they are established and widely understood, e.g., DNA).
- Author names. Provide the given name(s) and family name(s) of each author. The order of authors should match the order in the submission system. Carefully check that all names are accurately spelled. If needed, you can add your name between parentheses in your own script after the English transliteration.
- Affiliations. Add affiliation addresses, referring to where the work was carried out, below the author names. Indicate affiliations using a lower-case superscript letter immediately after the author's name and in front of the corresponding address. Ensure that you provide the full postal address of each affiliation, including the
- country name and, if available, the email address of each author.
- Corresponding author. Clearly indicate who will handle correspondence for your article at all stages of the refereeing and publication process and also post-publication. This responsibility includes answering any future queries about your results, data, methodology and materials. It is important that the email address and contact details of your corresponding author are kept up to date during the submission and publication process.
- Present/permanent address. If an author has moved since the work described in your article was carried out, or the author was visiting during that time, a "present address" (or "permanent address") can be indicated by a footnote to the author's name. The address where the author carried out the work must be retained as their main affiliation address. Use superscript Arabic numerals for such footnotes.

### Abstract

You are required to provide a concise and factual abstract which does not exceed 250 words. The abstract should briefly state the purpose of your research, principal results and major conclusions. Some guidelines:

- Abstracts must be able to stand alone as abstracts are often presented separately from the article.
- Avoid references. If any are essential to include, ensure that you cite the author(s) and year(s).
- Avoid non-standard or uncommon abbreviations. If any are essential to include, ensure they are defined within your abstract at first mention.

**Keywords**

You are required to provide 1 to 7 keywords for indexing purposes. Keywords should be written in English. Please try to avoid keywords consisting of multiple words (using "and" or "of").

We recommend that you only use abbreviations in keywords if they are firmly established in the field.

**Highlights**

You are required to provide article highlights at submission.

Highlights are a short collection of bullet points that should capture the novel results of your research as well as any new methods used during your study. Highlights will help increase the discoverability of your article via search engines. Some guidelines:

- Submit highlights as a separate editable file in the online submission system with the word "highlights" included in the file name.
- Highlights should consist of 3 to 5 bullet points, each a maximum of 85 characters, including spaces.

We encourage you to view example article highlights and read about the benefits of their inclusion.

**Graphical abstract**

You are required to provide a graphical abstract at submission.

The graphical abstract should summarize the contents of your article in a concise, pictorial form which is designed to capture the attention of a wide readership. A graphical abstract will help draw more attention to your online article and support readers in digesting your research. Some guidelines:

- Submit your graphical abstract as a separate file in the online submission system.
- Ensure the image is a minimum of 531 x 1328 pixels (h x w) or proportionally more and is readable at a size of 5 x 13 cm using a regular screen resolution of 96 dpi.
- Our preferred file types for graphical abstracts are TIFF, EPS, PDF or MS Office files.

We encourage you to view example graphical abstracts and read about the benefits of including them.

**Units, classifications codes and nomenclature**

This journal requires you to use the international system of units (SI) which follows internationally accepted rules and conventions. If other units are mentioned within your article, you should provide the equivalent unit in SI.

**Math formulae**

- Submit math equations as editable text, not as images.
- Present simple formulae in line with normal text, where possible.
- Use the solidus (/) instead of a horizontal line for small fractional terms such as X/Y.
- Present variables in italics.
- Denote powers of e by exp.
- Display equations separately from your text, numbering them consecutively in the order they are referred to within your text.

**Tables**

Tables must be submitted as editable text, not as images. Some guidelines:

- Place tables next to the relevant text or on a separate page(s) at the end of your article.
- Cite all tables in the manuscript text.
- Number tables consecutively according to their appearance in the text.
- Please provide captions along with the tables.
- Place any table notes below the table body.
- Avoid vertical rules and shading within table cells.

We recommend that you use tables sparingly, ensuring that any data presented in tables is not duplicating results described elsewhere in the article.

**Figures, images and artwork**

Figures, images, artwork, diagrams and other graphical media must be supplied as separate files along with the manuscript. We recommend that you read our detailed artwork and media instructions. Some excerpts:

When submitting artwork:

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- Number images according to the sequence they appear within your article.
- Submit each image as a separate file using a logical naming convention for your files (for example, Figure_1, Figure_2 etc).
- Please provide captions for all figures, images, and artwork.
- Text graphics may be embedded in the text at the appropriate position. If you are working with LaTeX, text graphics may also be embedded in the file.

**Artwork formats**

When your artwork is finalized, "save as" or convert your electronic artwork to the formats listed below taking into account the given resolution requirements for line drawings, halftones, and line/halftone combinations:

- Vector drawings: Save as EPS or PDF files embedding the font or saving the text as "graphics."
- Color or grayscale photographs (halftones): Save as TIFF, JPG or PNG files using a minimum of 300 dpi (for single column: min. 1063 pixels, full page width: 2244 pixels).
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- Combinations bitmapped line/halftones (color or grayscale): Save as TIFF, JPG or PNG files using a minimum of 500 dpi (for single column: min. 1772 pixels, full page width: 3740 pixels).

Please do not submit:

- files that are too low in resolution (for example, files optimized for screen use such as GIF, BMP, PICT or WPG files).
- disproportionally large images compared to font size, as text may become unreadable.

**Figure captions**

All images must have a caption. A caption should consist of a brief title (not displayed on the figure itself) and a description of the image. We advise you to keep the amount of text in any image to a minimum, though any symbols and abbreviations used should be explained.

Provide captions in a separate file.

**Color artwork**

If you submit usable color figures with your accepted article, we will ensure that they appear in color online.

Please ensure that color images are accessible to all, including those with impaired color vision. Learn more about color and web accessibility.

For articles appearing in print, you will be sent information on costs to reproduce color in the printed version, after your accepted article has been sent to production. At this stage, please indicate if your preference is to have color only in the online version of your article or also in the printed version.

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Please read our policy on the use of generative AI and AI-assisted tools in figures, images and artwork, which can be found in Elsevier's GenAI Policies for Journals. This policy states:

- We do not permit the use of Generative AI or AI-assisted tools to create or alter images in submitted manuscripts.

- The only exception is if the use of AI or AI-assisted tools is part of the research design or methods (for example, in the field of biomedical imaging). If this is the case, such use must be described in a reproducible manner in the methods section, including the name of the model or tool, version and extension numbers, and manufacturer.
- The use of generative AI or AI-assisted tools in the production of artwork such as for graphical abstracts is not permitted. The use of generative AI in the production of c
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**Supplementary material**

We encourage the use of supplementary materials such as applications, images and sound clips to enhance research. Some guidelines:

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- Submit supplementary materials at the same time as your article. Be aware that all supplementary materials provided will appear online in the exact same file type as received. These files will not be formatted or typeset by the production team.
- Include a concise, descriptive caption for each supplementary file describing its content.
- Provide updated files if at any stage of the publication process you wish to make changes to submitted supplementary materials.
- Do not make annotations or corrections to a previous version of a supplementary file.
- Switch off the option to track changes in Microsoft Office files. If tracked changes are left on, they will appear in your published version.

We recommend you upload research data to a suitable specialist or generalist repository. Please read our guidelines on sharing research data for more information on depositing, sharing and using research data and other relevant research materials.

**Video**

This journal accepts video material and animation sequences to support and enhance your scientific research. We encourage you to include links to video or animation files within articles. Some guidelines:

- When including video or animation file links within your article, refer to the video or animation content by adding a note in your text where the file should be placed.
- Clearly label files ensuring the given file name is directly related to the file content.
- Provide files in one of our recommended file formats. Files should be within our preferred maximum file size of 150 MB per file, 1 GB in total.
- Provide "stills" for each of your files. These will be used as standard icons to personalize the link to your video data. You can choose any frame from your video or animation or make a separate image.
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We publish all video and animation files supplied in the electronic version of your article.

For more detailed instructions, we recommend that you read our guidelines on submitting video content to be included in the body of an article.

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We are committed to supporting the storage of, access to and discovery of research data, and our research data policy sets out the principles guiding how we work with the research community to support a more efficient and transparent research process.

Research data refers to the results of observations or experimentation that validate research findings, which may also include software, code, models, algorithms, protocols, methods and other useful materials related to the project.

Please read our guidelines on sharing research data for more information on depositing, sharing and using research data and other relevant research materials.

For this journal, the following instructions from our research data guidelines apply.

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You are **required** to:

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- Cite and link to this dataset in your article.
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To foster transparency, you are required to state the availability of any data at submission.

Ensuring data is available may be a requirement of your funding body or institution. If your data is unavailable to access or unsuitable to post, you can state the reason why (e.g., your research data includes sensitive or confidential information such as patient data) during the submission process. This statement will appear with your published article on ScienceDirect.

Read more about the importance and benefits of providing a data statement.

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Linking to the data underlying your work increases your exposure and may lead to new collaborations. It also provides readers with a better understanding of the described research.

If your research data has been made available in a data repository there are a number of ways your article can be linked directly to the dataset:

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- Divide your article into clearly defined and numbered sections. Number subsections 1.1 (then 1.1.1, 1.1.2, ...), then 1.2, etc.
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The theory section should lay the foundation for further work by extending the background you provided in the introduction to your article. The calculation section should represent a practical development from a theoretical basis.

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Please provide definitions of field-specific terms used in your article, in a separate list.

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You may use system features that automatically build footnotes into text. Alternatively, you can indicate the position of footnotes within the text and present them in a separate section at the end of your article.

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It is not necessary to include detailed descriptions on the program or type of grants, scholarships and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.

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*This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.*

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- Give separate numbering to formulae and equations within appendices using formats such as Eq. (A.1), Eq. (A.2), etc. and in subsequent appendices, Eq. (B.1), Eq. (B. 2) etc. In a similar way, give separate numbering to tables and figures using formats such as Table A.1; Fig. A.1, etc.

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Any references cited within your article should also be present in your reference list and vice versa. Some guidelines:

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- We recommend that you do not include unpublished results and personal communications in your reference list, though you may mention them in the text of your article.
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- References cited as "in press" imply that the item has been accepted for publication.

Linking to cited sources will increase the discoverability of your research.

Before submission, check that all data provided in your reference list are correct, including any references which have been copied. Providing correct reference data allows us to link to abstracting and indexing services such as Scopus, Crossref and PubMed. Any incorrect surnames, journal or book titles, publication years or pagination within your references may prevent link creation.

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nic article referenced.

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Citations in the text should follow the referencing style used by the American Psychological Association. You are referred to the *Publication Manual of the American Psychological Association,* Seventh Edition (2020) ISBN 978-1-4338-3215-4.

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Examples:

**Reference to a journal publication:**

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Oguro, M., Imahiro, S., Saito, S., & Nakashizuka, T. (2015). Mortality data for Japanese oak wilt disease and surrounding forest compositions [dataset]. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

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We ask you to mark preprints clearly. You should include the word "preprint" or the name of the preprint server as part of your reference and provide the preprint DOI.

Where a preprint has subsequently become available as a peer-reviewed publication, use the formal publication as your reference.

If there are preprints that are central to your work or that cover crucial developments in the topic, but they are not yet formally published, you may reference the preprint.

**Reference management software**

Most Elsevier journals have their reference template available in popular reference management software products. These include products that support Citation Style Language (CSL) such as Mendeley Reference Manager.

If you use a citation plug-in from these products, select the relevant journal template and all your citations and bibliographies will automatically be formatted in the journal style. We advise you to remove all field codes before submitting your manuscript to any reference management software product.

If a template is not available for this journal, follow the format given in examples in the reference style section of this Guide for Authors.

ANEXO 2 - Comitê de Ética em Pesquisa Envolvendo Experimentação Animal (CEPEEA)

**UNIVERSIDADE PARANAENSE – UNIPAR**

*Reconhecida pela Portaria – MEC N.º 1580, de 09/11/93 – D.O.U. 10/11/93*

***Mantenedora: Associação Paranaense de Ensino e Cultura – APEC***

**Coordenadoria de Pós-Graduação - COPG**

**COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO EXPERIMENTAÇÃO ANIMAL (CEPEEA)**

# ***CERTIFICADO***

Certificamos que o projeto intitulado "**INVESTIGAÇÃO DOS EFEITOS HEPATOPROTETORES DA BACCHARIS DRACUNCULIFOLIA EM UM MODELO ANIMAL DE DIABETES, TABAGISMO E DISLIPIDEMIA"**, protocolo nº. **1003/2022**, sob a responsabilidade de **FRANCISLAINE APARECIDA DOS REIS LÍVERO** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da lei nº. 11.794, de 08 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 aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA). DO(A) Universidade Paranaense – UNIPAR, em reunião de 28/07/2022.

Umuarama, PR 28/07/2022.

***Prof. Salviano Tramontin Belettini***
Presidente CEPEEA/UNIPAR

***Lucilene N. Castilho Monteiro***
Secretária CEPEEA/UNIPAR