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**Óleo Essencial da Resina de *Protium heptaphyllum*: Uma
Revisão Sistemática do Potencial Biotecnológico com
Base em Evidências Experimentais**

BRASÍLIA
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Revisão Sistemática do Potencial Biotecnológico com
Base em Evidências Experimentais**

Dissertação apresentada à Faculdade de
Medicina da Universidade de Brasília para
obtenção do título de Mestre em Ciências
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de Ciências Médicas na área de
concentração Ciências Aplicadas em
Saúde

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VERSÃO CORRIGIDA

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A todos que estiveram presentes nos momentos mais árduos dessa trajetória, do fundo do meu coração, eu sei que não seria fácil, tampouco possível, chegar até aqui sem o amor, apoio e o acolhimento de cada um dos meus amigos e familiares. Um agradecimento especial à minha mãe Veridiana, que desde sempre me incentivou, ao meu pai Henrique, que sempre me inspira a buscar as respostas que mais pareciam distantes. À minha amiga Geisa, que esteve iluminando e direcionando o caminho desde o início, és uma grande inspiração. Aos meus amigos Cláudia, Felipe, Poliana, Cris, Ruy e Edu, por todo o apoio e momentos leves. Vocês enchem meu coração de saudades e sem vocês, nada disso seria possível. Obrigado por estarem ao meu lado e me recordarem do que realmente importa. Às minhas colegas do grupo de pesquisa, pela parceria, em especial Emãnuella, pela colaboração direta ao desenvolvimento do trabalho, Stefhani e Maria Vitória, pelo aprendizado. À minha orientadora, Prof^a Juliana Lott, por toda a compreensão e apoio, e à minha co-orientadora Prof^a Tais Gratieri, pela oportunidade e direcionamento. Agradeço à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – pelo apoio financeiro que possibilitou o desenvolvimento do presente trabalho – Código de Financiamento 001 – Processo 23106.045090/2023-80.

“僕はドレスを纏い踊ってみせよう 狂ってるかい教えて”

Buck-Tick - ドレス

RESUMO

Protium heptaphyllum, popularmente conhecido como “Breu-branco”, “Almécega” e “Almecegueira” é um importante componente da biodiversidade Amazônica, amplamente distribuído no território da América do Sul. Pertence à família botânica Burseraceae, fonte conhecida de resinas e exsudatos ricos em compostos aromáticos de aplicações diversas. Usos medicinais da resina e de outros derivados de *P. heptaphyllum* em comunidades tradicionais, incluindo suas aplicações como cicatrizante e alívios de dores de cabeça, corroboram sua investigação enquanto ativos para o desenvolvimento de novas abordagens farmacológicas, cosméticas e terapêuticas. Nesse contexto, a resina exsudada pelo tronco do vegetal é uma das principais fontes para a extração de óleos essenciais, derivados de grande interesse econômico devido à facilidade de extração e amplo potencial de exploração biotecnológica. Considerando o crescente interesse em derivados naturais, a presente dissertação objetiva sumarizar e analisar criticamente as atividades biológicas experimentalmente comprovadas do óleo essencial da resina de *P. heptaphyllum*. O total de 16 artigos foram incluídos na revisão sistemática da literatura – 15 provenientes de bases de dados científicas e 1 incluído manualmente. Foi verificada uma variação já esperada na composição dos óleos essenciais e evidências experimentais limitadas, corroborando a diversa aplicação biotecnológica e medicinal do óleo essencial da resina de *P. heptaphyllum* como potencial ativo antimicrobiano, inseticida, anti-inflamatório, antineoplásico, antinociceptivo, antioxidante, vasorrelaxante, antimutagênico e cicatrizante. As evidências mais convincentes apoiam suas propriedades antimicrobianas, inseticidas e anti-inflamatórias. Estudos adicionais ainda são necessários para estabelecer melhor o perfil de toxicidade do óleo essencial, os efeitos sinérgicos de seus componentes e suas contribuições específicas para as atividades biológicas em outros modelos experimentais, garantindo o uso seguro e eficaz do óleo essencial da resina de *P. heptaphyllum*.

Palavras-chave: Revisão Sistemática, *Burseraceae*, Óleo Essencial, *Protium heptaphyllum*, Oleorresinas, Atividades Biológicas.

ABSTRACT

Protium heptaphyllum Resin Essential Oil: A Systematic Review of Its Biotechnological Potential Based on Experimental Evidence

Protium heptaphyllum, popularly known as “Breu-branco,” “Almécega,” and “Almecegueira,” is widely distributed across South America and is an important component of Amazonian biodiversity. It belongs to the Burseraceae family, a well-known source of exudates and resins rich in aromatic compounds with diverse applications. The medicinal use of the resin and other *P. heptaphyllum* products by traditional communities, including their application for wound healing and headache relief, supports their investigation as ingredients for developing new pharmacological, cosmeceutical, and therapeutic approaches. In this context, the resin exuded from its bark serves as a primary source for essential oil extraction, a product of significant economic interest due to its ease of extraction and broad biotechnological potential. Given the growing interest in natural products and essential oils, this systematic review aims to summarize and critically analyze the experimentally proven biological activities of *P. heptaphyllum* resin essential oil. A total of 16 articles were selected—15 from scientific databases and one manually included. Variability in essential oil characterization and composition was detected across studies, as well as limited experimental evidence supporting its antimicrobial, insecticidal, anti-inflammatory, antineoplastic, antinociceptive, antioxidant, vasorelaxant, antimutagenic, and wound-healing potential. The most compelling evidence supports its antimicrobial, insecticidal, and anti-inflammatory properties. Importantly, further studies are needed to better establish the essential oil’s toxicity profile, the synergistic effects of its components, and their specific contributions to these biological activities across various experimental models, ensuring the safe and effective use of *P. heptaphyllum* resin essential oil.

Keywords: Systematic Review, *Burseraceae*, Essential Oil, *Protium heptaphyllum*, Oleoresins, Biological Activities.

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LISTA DE ABREVIATURAS E SIGLAS

JBI	Joanna Briggs <i>Institute</i>
OSF	<i>Center for Open Science</i>
PICOS	P – População de Interesse I – Intervenção C – Comparação O – Desfecho S – Tipos de estudos
PRISMA	<i>Preferred Reporting Items for Systematic reviews and Meta-Analyses</i>
RENTAS	Rede Nacional De Combate ao Tráfico de Animais Silvestres
SisGen	Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado

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1. INTRODUÇÃO

O Brasil compreende a maior biodiversidade do mundo, devido à extensão e à distribuição de biomas em seu território. O País abriga uma grande diversidade de espécies animais e vegetais de grande potencial de exploração biotecnológico (Valli, Russo, Bolzani, 2018), a se destacar o bioma da região Amazônica, presente, também, no território de outros países da América do Sul. A biodiversidade é um patrimônio natural de grande importância para o equilíbrio dos ecossistemas e estabelece a base de variadas atividades econômicas, como a agricultura, pecuária e extrativismo (Bruno, Mattos, 2021), além de ser um importante indicador de um ecossistema saudável. O grande interesse econômico associado à exploração da biodiversidade brasileira atrai não somente o interesse da comunidade científica enquanto uma importante estratégia de descoberta, estudo e desenvolvimento de novas abordagens farmacológicas e cosmetológicas, mas também a ação da biopirataria, sendo este um possível fator de perda da biodiversidade e à geração de impactos socioeconômicos às comunidades locais e à economia nacional, estabelecendo a necessidade de proteção dos ecossistemas (Pinter, Prist, Marrelli, 2022).

No contexto global, o Brasil foi um dos primeiros países a regulamentar a exploração da biodiversidade, sendo o primeiro país signatário da Convenção da Diversidade Biológica em 1992, e por meio da Medida Provisória nº 2.186-16/2001 (Brasil, 2001), a definir a primeira legislação específica sobre o tema, a fim de evitar e prevenir a biopirataria. Esta prática ilegal é caracterizada pela coleta de materiais provenientes da biodiversidade nacional para fabricação de produtos no exterior sem o pagamento de *royalties* ao país de origem e às comunidades afetadas, sendo considerada o terceiro maior tipo de tráfico do mundo (RENTAS, 2017; Valli, Russo, Bolzani, 2018). Em 2015, foi sancionada a Lei nº 13.123 de 20 de maio de 2015 (Brasil, 2015), também denominada Lei da Biodiversidade, que entrou em vigor no dia 20 de novembro de 2015 e revogou a Medida Provisória nº 2.186-16/2001, estabelecendo o novo Marco Legal sobre o acesso ao patrimônio genético, a proteção e o acesso ao conhecimento tradicional associado e sobre a repartição de benefícios para conservação e uso sustentável da biodiversidade (Valli; Russo; Bolzani, 2018; Bruno; Mattos, 2021).

Inúmeros produtos já foram desenvolvidos a partir da exploração da biodiversidade brasileira, por meio do estudo de derivados vegetais e animais. Dentre eles, é possível destacar dois medicamentos desenvolvidos a partir de derivados vegetais, como o Acheflan®, um antiinflamatório de aplicação tópica composto da mistura de sesquiterpenos do óleo essencial de *Cordia verbenaceae* (Boraginaceae), popularmente conhecida como erva-baleeira (Passos et al. 2007, Fernandes et al. 2007, Valli, Bolzani, 2019), e o Fitoscar®, um unguento que contém uma mistura de extratos fenólicos consistindo principalmente em

taninos condensados de *Stryphnodendron adstringens*, denominado popularmente como barbatimão, aplicado tradicionalmente na cicatrização de feridas (Souza et al. 2007, Minatel et al. 2010, Valli, Russo, Bolzani, 2018).

Os óleos essenciais, substâncias voláteis extraídos de vegetais (Donato et al., 2020; Al-Mijalli et al., 2022), se destacam enquanto derivados vegetais de interesse econômico, devido ao grau variável de reatividade de seus metabólitos, tornando a sua utilização relevante em abordagens terapêuticas diversas (Letseka et al., 2022; Perigo et al., 2022). Ademais, a lipofilicidade e o tamanho reduzido das moléculas presentes nos óleos os torna potenciais ingredientes para formulações de aplicação tópica, devido a maior facilidade para penetrar a pele e promover ação local (Sarkic; Stappen, 2018).

Dentre os vegetais componentes da biodiversidade Amazônica, está *Protium heptaphyllum*, popularmente denominado como “Breu-branco”, “Almécega” e “Almecegueira”, um importante componente da família botânica *Burseraceae*, fonte de resinas e exsudatos ricos em substâncias aromáticas de aplicações diversas (Rodrigues et al., 2022) (Figura 1). A família *Burseraceae* compreende 7 gêneros e aproximadamente 228 espécies (Batista; Carvalho, 2007), sendo *Protium* um de seus principais gêneros. Seus representantes estão amplamente distribuídos na América do Sul, no território brasileiro e em outros países do continente, como Colômbia, Paraguai, Suriname e Venezuela (Mobin et al., 2016; Mobin et al., 2017). A Figura 1 representa a árvore de *P. heptaphyllum*.

Figura 1 - Árvore de “Breu-branco” (*Protium heptaphyllum*), vegetal do qual se extrai a resina, uma das possíveis fontes de extração do óleo essencial.



Tarciso Leão

Fonte: Adaptado de Leão (2008).

Os derivados do Breu apresentam registros de seu uso para fins terapêuticos e medicinais principalmente nas comunidades tradicionais onde o vegetal ocorre naturalmente, o que gera maior interesse no estudo dos potenciais farmacológicos da planta (Mobin et al., 2017). A resina exsudada por *P. heptaphyllum* apresenta aplicações tradicionais como analgésico, antiinflamatório, expectorante e cicatrizante, sendo uma das principais fontes de extração do óleo essencial (Rodrigues et al., 2022). Alguns registros da aplicação tradicional da resina incluem o seu uso como contraceptivo, emoliente, analgésico, cicatrizante, expectorante, antisséptico, contra doença de Chagas, hemorragias, ansiedade, no tratamento de inflamações das vias aéreas (Rocha et al., 2022), e como repelente de insetos natural (Vieira-Júnior et al., 2007). Comunidades Quilombolas brasileiras também apresentam registros do uso da resina no tratamento de dores de cabeça por meio da inalação da fumaça gerada na combustão da resina (da Silva et al., 2017), e do seu uso tópico no tratamento de contusões e inflamações administrada como patch (MEB, 1993; Rodrigues, 1989). As atividades biológicas do óleo essencial da resina de *P. heptaphyllum* estão associadas à sua composição química, que varia de acordo com diferentes fatores, tais como a localização geográfica do vegetal do qual a resina é extraída, características relacionadas ao ambiente e às condições de coleta e armazenamento da resina (Mendes et al., 2019; de Melo et al., 2023).

O crescente interesse em produtos naturais como bioativos vem impulsionando a pesquisa sobre óleos essenciais e suas aplicações farmacológicas, cosmeceúticas e terapêuticas. A resina de *P. heptaphyllum* é tradicionalmente utilizada por comunidades locais para diversas finalidades, como cicatrização de feridas e alívio de dores. Embora exista um rico conhecimento etnobotânico sobre o derivado, a validação científica de suas propriedades biológicas ainda é limitada e fragmentada. Dessa forma, uma revisão sistemática sobre o ativo se faz necessária para consolidar o conhecimento já existente relacionado ao óleo, oferecendo uma visão abrangente e baseada em evidências acerca de suas atividades biológicas, identificar as lacunas remanescentes na literatura, avaliando criticamente as metodologias empregadas, variações na composição do óleo e os desafios na padronização dos ensaios experimentais, apoiar suas aplicações biotecnológicas e, assim, contribuir para a valorização da biodiversidade Amazônica, ao destacar o potencial biotecnológico de uma espécie nativa, incentivando seu uso sustentável e, dessa maneira, promover a conservação dos recursos naturais. Portanto, a presente revisão sistemática visa não somente sintetizar o conhecimento atual sobre o óleo essencial extraído da resina de *P. heptaphyllum*, como também orientar futuras investigações científicas e tecnológicas, ressaltando sua relevância no que concerne óleos essenciais bioativos.

2. Objetivos

2.1. Objetivo geral

Reunir e avaliar criticamente as evidências acumuladas acerca das atividades biológicas experimentalmente comprovadas do óleo essencial extraído da resina de *P. heptaphyllum* por meio de uma revisão sistemática da literatura.

2.2. Objetivos específicos

- Definição da equipe de revisores, elaboração da pergunta de pesquisa e planejamento das atividades;
- Elaboração da estratégia PICOS da revisão sistemática, critérios de inclusão e exclusão de artigos e do conjunto de palavras-chave para seleção inicial dos artigos;
- Preparo e teste da estratégia de busca preliminar;
- Aperfeiçoamento da estratégia de busca e escolha das bases de dados;
- Busca final dos artigos;
- Exportação das referências das bases de dados para o software *EndNote* para remoção de duplicados e de artigos com arquivos inacessíveis, seleção dos artigos a partir da leitura dos títulos e resumos com o auxílio do software *Rayyan* e conferência com segundo e terceiro revisores;
- Seleção dos artigos com base nos critérios de elegibilidade por meio da leitura dos artigos com arquivos acessíveis e conferência com segundo e terceiro revisores;
- Resolução de discordâncias em relação aos artigos selecionados;
- Leitura do texto completo dos artigos incluídos na revisão sistemática e conferência com o segundo e terceiro examinador;
- Busca complementar de artigos com possibilidade de inclusão;
- Registro da Revisão Sistemática no OSF;
- Análise do risco de viés das evidências obtidas a partir dos artigos incluídos;
- Escrita do artigo de revisão.

3. METODOLOGIA

3.1. Seleção de Artigos

Esta revisão sistemática está estruturada de acordo com o protocolo *Preferred Reporting Items for Systematic reviews and Meta-Analyses* (PRISMA) (Page et al., 2021) para relatórios de revisões sistemáticas e meta-análises, e a seleção de palavras-chave para a busca definitiva nas bases de dados foi baseada na pergunta de pesquisa “Quais são as atividades biológicas do óleo essencial extraído da resina *Protium heptaphyllum*?” definida a partir da sigla PICOS, conforme visualizado na Tabela 1.

Tabela 1 – Estratégia PICOS da revisão sistemática da literatura.

P*	População de Interesse	Células, animais e humanos
I*	Intervenção	Óleo essencial da resina de <i>Protium heptaphyllum</i>
C*	Comparação	Grupos controle
O*	Desfecho	Atividade biológica do óleo essencial
S*	Tipos de Estudos	Experimentais

FONTE: Próprio do autor (2025). Adaptado de Garcez et al., 2023.

Legenda:

P* (*Population of Interest*) - População de Interesse; I* (*Intervention*) - Intervenção*; C (*Comparison*) - Comparação; O* (*Outcome*) - Desfecho; S* (*Studies*) - Tipos de Estudos.

As palavras-chave foram estruturadas e aplicadas de acordo com as especificidades de cada base de dados eletrônicas escolhidas para a seleção dos estudos, que foram *PubMed (MedLine)*, *Scopus*, *Embase*, *Web of Science*, *Cochrane*, *Proquest*, *Google Scholar* e *Lilacs*. A busca final foi realizada em 25 de julho de 2024, e foram selecionados artigos escritos em inglês e espanhol sem restrição no tempo de publicação. Uma busca adicional foi feita no dia 13 de dezembro de 2024, com o auxílio da ferramenta *Connected Papers* (<https://www.connectedpapers.com/>), resultando na adição manual de um artigo à revisão sistemática. A Tabela 2 representa o conjunto de palavras-chave selecionadas para a busca final nas bases de dados.

Tabela 2 – Conjunto de palavras-chave associadas para seleção dos artigos nas bases de dados selecionadas.

1º “*Protium heptaphyllum*” OR “Protium” OR “heptaphyllum” OR “P. heptaphyllum” OR “Resin *Protium heptaphyllum*” OR “Resina *Protium heptaphyllum*” OR “Aubl.” OR “Pitch” OR “*Protium heptaphyllum* Aubl.” OR “*Icica heptaphylla*” OR “*Icica*” OR “*heptaphylla*” OR “Almécega” OR “Almécega-brava” OR “Almécega-cheirosa” OR “Almecegueira” OR “Almecegueira-cheirosa” OR “Almecegueira-de-cheiro” OR “Almecegueira-vermelha” OR “Almecegueiro-bravo” OR “Almesca” OR “Almescla” OR “Almíscar” OR “Amescla” OR “Amescla-da-praia” OR “Amescla-de-cheiro” OR “Amesclão” OR “Amescla-seca” OR “Anime” OR “Arvore-do-incenso” OR “Anis verde” OR “Breu” OR “Breu-Almécega” OR

	“Breu-amescla” OR “Breu-branco” OR “Breu-branco-da-praia” OR “Breu-branco-do-campo” OR “Breu-branco-verdadeiro” OR “Breu-de-campina” OR “Breu-vermelho” OR “Cabata-de-leite” OR “Ciantaahua” OR “Cicantaa-ihua” OR “Cincataa-ilhua” NOT “Hydrogen”
2º	“Resins” OR “Plant” OR “Resin”
3º	“Essential Oils” OR “Essential Oil” OR “Volatile Oils” OR “Plant Oils” OR “Resin” OR “Extract” OR “Plant Extract”

FONTE: Próprio do autor (2025).

3.2. Critérios de Elegibilidade

Os artigos resultantes da busca final em cada base de dados foram inicialmente considerados para a seleção, porém, estudos de caso, estudos transversais, epidemiológicos, observacionais, de caso-controle e de coorte foram desconsiderados para inclusão. Estudos experimentais que não apresentavam grupos controle também foram excluídos. A Tabela 3 representa os critérios de exclusão utilizados para a seleção dos artigos.

Tabela 3 – Critérios de exclusão

1	Artigos de revisão
2	Artigos sobre outros derivados e extratos de <i>Protium heptaphyllum</i> que não o óleo essencial
3	Artigos sobre óleos essenciais extraídos de outras partes da planta (casca, frutos e folhas) que não a resina
4	Artigos sobre o óleo essencial da resina de <i>Protium heptaphyllum</i> sem relatos de atividades biológicas

FONTE: Próprio do Autor (2025).

Após a remoção dos artigos duplicados pelo software *Endnote*, a etapa de leitura dos títulos e resumos dos artigos foi feita por dois revisores na plataforma *Rayyan* (<https://new.rayyan.ai/>). Os critérios pré-estabelecidos de elegibilidade e exclusão foram considerados para a seleção ou descarte dos artigos, e as discordâncias foram discutidas entre os revisores e resolvidas por um terceiro pesquisador. Por fim, o texto completo dos artigos incluídos na revisão foi lido pelos revisores. Os revisores trabalharam independentemente em todas as etapas da revisão sistemática.

3.3. Extração de dados

Os dados foram extraídos dos artigos de acordo com as seguintes variáveis: título, autores, ano de publicação, concentrações/doses utilizadas, composição majoritária do óleo essencial, método de caracterização, origem da resina, modelos experimentais, vias de administração, efeitos demonstrados experimentalmente, métodos de análise e toxicidade do composto/relatos de reações adversas.

3.4. Análise do Risco de viés

A análise do risco de viés auxilia a interpretar se os dados obtidos através dos estudos incluídos são confiáveis para nortear as decisões clínicas, sendo crucial para garantir a validade interna dos estudos incluídos nesta revisão sistemática. O viés pode levar à subestimação ou superestimação dos verdadeiros efeitos das intervenções, e a presença de estudos com alto risco de viés impacta na confiança das estimativas de efeito da meta-análise. Neste trabalho, a despeito da existência de diversas ferramentas para avaliação do risco de viés, foi utilizada a ferramenta de avaliação crítica revisada do Joanna Briggs Institute (JBI) para avaliação do risco de viés em estudos quase experimentais (Barker et al., 2024).

3.5. Registro da Revisão Sistemática

A revisão sistemática foi registrada na plataforma *Center for Open Science* (OSF). O Registro pode ser visualizado em: <https://doi.org/10.17605/OSF.IO/8763N>. Por não incluir o acesso direto a amostras biológicas e ao patrimônio genético, não foi necessário o registro no Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen).

4. RESULTADOS E DISCUSSÃO

Como resultados obtidos nesta dissertação são apresentados a seguir os seguintes documentos que atendem aos requisitos do mestrado acadêmico no Programa de Pós-Graduação em Ciências Médicas (PPGCM) da Universidade de Brasília (UnB):

O artigo intitulado: “*Protium heptaphyllum* Resin Essential Oil: A Systematic Review of Its Biotechnological Potential Based on Experimental Evidence” submetido na revista *Journal of Essential Oil Bearing Plants*, no dia 11 de Fevereiro de 2025, de classificação Q2 no Web of Science; Fator de Impacto 2.1. Os dados são apresentados e discutidos no arquivo abaixo, que constitui a íntegra do artigo submetido.

***Protium heptaphyllum* Resin Essential Oil: A Systematic Review of Its Biotechnological Potential Based on Experimental Evidence**

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Abstract

Protium heptaphyllum, popularly known as “Breu-branco,” “Almecega,” and “Almecegueira,” is widely distributed across South America and is an important component of Amazonian biodiversity. It belongs to the Burseraceae family, a well-known source of exudates and resins rich in aromatic compounds with diverse applications. The medicinal use of the resin and other *P. heptaphyllum* products by traditional communities, including their application for wound healing and headache relief, supports their investigation as ingredients for developing new pharmacological, cosmeceutical, and therapeutic approaches. In this context, the resin exuded from its bark serves as a primary source for essential oil extraction, a product of significant economic interest due to its ease of extraction and broad biotechnological potential. Given the growing interest in natural products and essential oils, this systematic review aims to summarize and critically analyze the experimentally proven biological activities of *P. heptaphyllum* resin essential oil. A total of 16 articles were selected—15 from scientific databases and one manually included. Variability in essential oil characterization and composition was detected across studies, as well as limited experimental evidence supporting its antimicrobial, insecticidal, anti-inflammatory, antineoplastic, antinociceptive, antioxidant, vasorelaxant, antimutagenic, and wound-healing potential. The most compelling evidence supports its antimicrobial, insecticidal, and anti-inflammatory properties. Importantly, further studies are needed to better establish the essential oil's toxicity profile, the synergistic effects of its components, and their specific contributions to these biological activities across various experimental models, ensuring the safe and effective use of *P. heptaphyllum* resin essential oil.

Keywords: Systematic Review, *Burseraceae*, Essential Oil, *Protium heptaphyllum*, Oleoresins, Biological Activities.

Introduction

Protium heptaphyllum, popularly known as “Breu-branco”, “Almécega” and “Almecegueira”, is an important component of the Amazonian biodiversity. It belongs to the botanical family Burseraceae, a known source of resins and exudates rich in aromatic substances with various uses¹. The Burseraceae family has 7 genera and approximately 228 species², *Protium* being one of its main genera. Widely distributed in South America, *Protium* is present in Brazil and other countries of the continent, such as Colombia, Paraguay, Suriname and Venezuela^{3,4}.

Records of the traditional use of Breu derivatives for medicinal purposes can be found mainly where it occurs naturally, which intensifies the interest in the potential pharmacological uses of the plant⁴. Brazilian Quilombola communities have documented the use of the plant’s resin for headache treatment by inhaling the smoke produced from its combustion⁵, and there are also records of the topical use for wound healing and inflammation, when administered as a patch^{6,7}. The *P. heptaphyllum* resin is traditionally used for other applications as well, for its analgesic, anti-inflammatory, expectorant and wound healing activities, presenting several applications in traditional medicine. It is one of the main sources of extraction of the essential oil, which can come from other parts of the plant, such as leaves, stem, bark and fruits¹. Some records of the traditional uses of the resin include its use as a contraceptive, emollient, analgesic, healing, expectorant, antiseptic, against Chagas disease, hemorrhages, anxiety, in the treatment of airways inflammation⁸, and as an insect repellent⁹.

The study of natural products is an important strategy for developing new pharmacological and cosmeceutical approaches. Essential oils are plant-derived volatile compounds that stand out as natural products of pharmacological interest^{10,11}, due to the variable degree of reactivity of their metabolites. Their use is relevant for various therapeutic applications^{12,13}, as well as perfumery and cosmetics purposes¹⁴, as a result of their pleasant aroma. The lipophilicity and small size of the molecules present in essential oils also make them potential ingredients for topical formulations, due to their easier penetration on the cutaneous tissue and the possibility of exerting local effects¹⁵.

The traditional use of *P. heptaphyllum* resin essential oil among indigenous and Quilombola communities underscores its cultural and medicinal significance. However, we hypothesize that scientific exploration of its bioactive properties has been limited. This research gap is particularly notable given the growing global interest in natural products as sources of innovation for pharmacological, biotechnological, and cosmeceutical

development. Therefore, the present systematic review aims to synthesize and critically evaluate the existing experimental evidence on *P. heptaphyllum* resin essential oil, bridging the gap between traditional knowledge and modern scientific research, and paving the way for future studies and potential commercial applications in therapeutic and cosmetic products.

Methods

Article Selection

This systematic review was structured according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) protocol¹⁶ for systematic reviews and meta-analyses reports. The research question, based on the acronym PICOS (Table 1), was “What are the biological activities of the essential oil extracted from *Protium heptaphyllum* resin?”. Table 1 describes the PICOS strategy used for the systematic review.

Table 1 - Systematic review PICOS strategy

P*	Population of interest	Cells, animals and humans
I*	Intervention	<i>P. heptaphyllum</i> resin essential oil
C*	Comparison	Control groups
O*	Outcome	Essential oil biological activities
S*	Studies	Experimental

Based on Garcez et al., (17).

Legenda:

P* - Population of Interest; I* - Intervention C* Comparison

O* Outcome

S* Studies

Descriptors (Table 2) were applied in a structured way according to the specificity of each electronic database chosen for study selection, namely *PubMed*, *Scopus*, *Embase*, *Web of Science*, *Cochrane*, *Proquest*, *Google Scholar* and *Lilacs*. The final search was made on July 25th, 2024 and articles written both in English and Spanish were selected, with no time restriction. An additional search was made on December 13th, 2024 using the *Connected Papers* tool (<https://www.connectedpapers.com/>), resulting in the manual addition

of 1 article. Table 2 presents the set of keywords applied for the final search in each database.

Table 2 - Set of keywords applied for article selection in the selected databases.

1°	<p> <i>Protium heptaphyllum</i> OR "Protium" OR "heptaphyllum" OR "P. heptaphyllum" OR "Resin <i>Protium heptaphyllum</i>" OR "Resina <i>Protium heptaphyllum</i>" OR "Aubl." OR "Pitch" OR "<i>Protium heptaphyllum</i> Aubl." OR "<i>Icica heptaphylla</i>" OR "<i>Icica</i>" OR "<i>heptaphylla</i>" OR "Almécega" OR "Almécega-brava" OR "Almécega-cheirosa" OR "Almecegueira" OR "Almecegueira-cheirosa" OR "Almecegueira-de-cheiro" OR "Almecegueira-vermelha" OR "Almecegueiro-bravo" OR "Almesca" OR "Almescla" OR "Almíscar" OR "Amescla" OR "Amescla-da-praia" OR "Amescla-de-cheiro" OR "Amesclão" OR "Amescla-seca" OR "Anime" OR "Arvore-do-incenso" OR "Anis verde" OR "Breu" OR "Breu-Almécega" OR "Breu-amescla" OR "Breu-branco" OR "Breu-branco-da-praia" OR "Breu-branco-do-campo" OR "Breu-branco-verdadeiro" OR "Breu-de-campina" OR "Breu-vermelho" OR "Cabata-de-leite" OR "Ciantaahua" OR "Cicantaa-ihua" OR "Cincataa-ilhua" NOT "Hydrogen" </p>
2°	<p>"Resins" OR "Plant" OR "Resin"</p>
3°	<p> "Essential Oils" OR "Essential Oil" OR "Volatile Oils" OR "Plant Oils" OR "Resin" OR "Extract" OR "Plant Extract" </p>

Eligibility Criteria

The resulting articles from the final search were initially considered for selection; however, case studies, cross-sectional, epidemiological, observational, case-control, and cohort studies were disregarded for selection. Experimental studies that did not present control groups were also excluded. Table 3 presents the exclusion criteria used for article selection.

Table 3 - Exclusion criteria

1	Reviews
2	Articles regarding other <i>P. heptaphyllum</i> products and extracts that weren't the essential oil

-
- | | |
|----------|---|
| 3 | Articles regarding essential oils extracted from other parts of the plant that weren't the resin (bark, fruit and leaves) |
|----------|---|
-
- | | |
|----------|--|
| 4 | Articles about the <i>P. heptapyllum</i> resin essential oil that did not report any biological activity |
|----------|--|
-

After duplicate removal using the EndNote software, articles were evaluated according to title and abstract in the Rayyan platform (<https://new.rayyan.ai/>) by two reviewers (A.P.S. and E.M.G.). The pre-established eligibility and exclusion criteria were considered for the selection or discarding of the articles, and disagreements were discussed between the two reviewers and resolved by a third researcher (J.L.C.). The full text of the included articles was independently reviewed by the researchers at all stages of the systematic review.

Data Extraction

Data from the selected articles were extracted based on the following variables: title, authors, year of publication, concentration/dose used, major essential oil compounds, characterization method, resin origin, experimental model, route of administration, experimentally proven effects, analytical methods, and reported toxicity or adverse reactions.

Risk of bias

The risk of bias analysis helps understanding if the data obtained through the included studies are reliable to guide clinical decisions, being crucial to ensure their internal validity. Bias can lead to underestimation or overestimation of the intervention's true effects, as the presence of studies with high bias risk can impact the confidence in the effect estimates of the meta-analysis. There are several tools available to assess the bias risk, however, in this study, the revised critical appraisal tool of the Joanna Briggs Institute (JBI) was used to assess the risk of bias in quasi-experimental studies¹⁸.

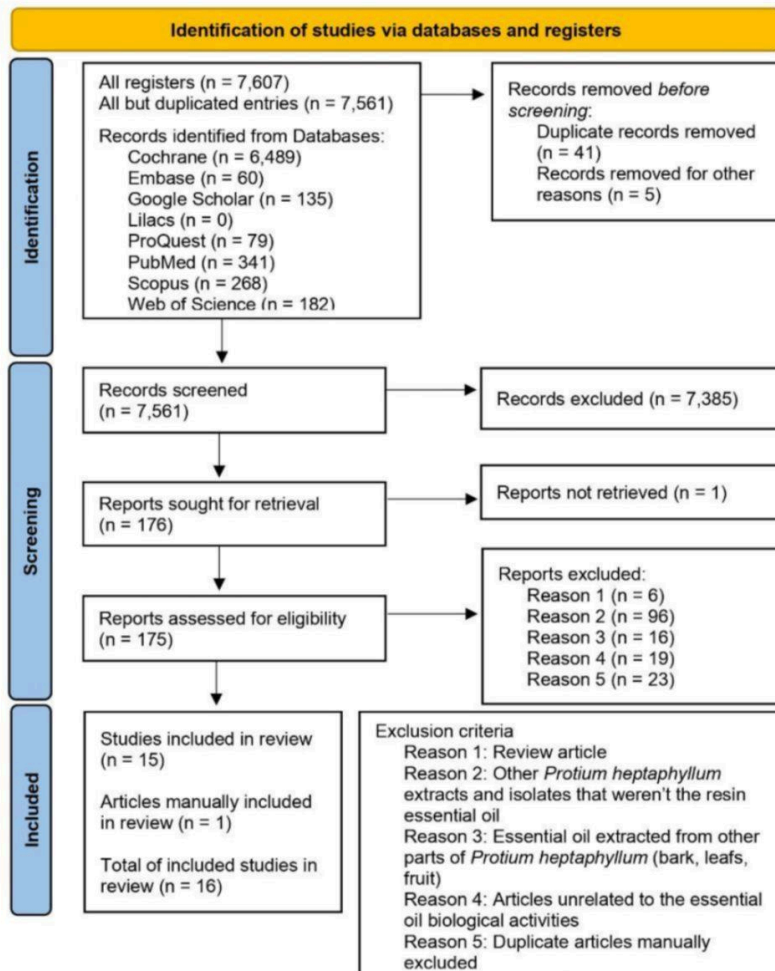
Systematic review registry

This systematic review was registered in the *Center for Open Science* (OSF) platform, and the registry can be accessed at: <https://doi.org/10.17605/OSF.IO/8763N>. This systematic review exclusively relies on previously published scientific literature and does not involve direct access to biological samples or genetic resources. Therefore, registration in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen) was not required.

Results

A total of 7,607 references were identified in the literature search, with 41 duplicates and 5 unavailable references removed during the initial selection phase using EndNote (Figure 1). In the second phase, 7,561 references were screened based on title and abstract, leading to the removal of 7,385 documents. The remaining 176 references were assessed for inclusion criteria, with 1 manually excluded due to retrieval issues, resulting in 175 eligible articles. Of these, 160 references were excluded: 96 for investigating other *P. heptaphyllum* products or extracts instead of essential oils, 23 as duplicates, 19 for lacking relevance to the resin essential oil's biological activities, 16 for focusing on essential oils from other plant parts, and 6 for being review articles. Ultimately, 15 articles were selected for full-text review, with 1 additional article manually included using the Connected Papers tool (<https://www.connectedpapers.com/>), totaling 16 articles in the systematic review. The exclusion criteria and their details are provided in Figure 1.

Figure 1 - Systematic review article identification, selection and inclusion flowchart, according to the PRISMA protocol.



Based on the PRISMA protocol¹⁶.

Study bias

A detailed bias risk assessment of the results from included studies showed that 75% of the studies presented low bias risk, according to the Joanna Briggs Institute (JBI) criteria. However, 25% of the studies raised concerns regarding the quality of the assessment and the description of results, as outlined in Figure 2.

Figure 2 - Studies bias risk assessment according to the Joanna Briggs Institute criteria.

Study	Risk of bias domains					Overall
	D1	D2	D3	D4	D5	
Siani et al. 1999	+	+	+	+	+	+
Bandeira et al. 2006	+	+	+	-	+	+
Rao et al. 2007	+	+	+	+	+	+
Amaral et al. 2009	+	+	+	+	+	+
Siani et al. 2011	+	+	+	+	+	+
Bernadi et al. 2015	+	+	-	-	+	-
Pinto et al. 2015	+	+	+	+	+	+
de Lima et al. 2016	+	+	+	-	+	+
Mobin et al. 2016	+	+	+	-	+	+
da Silva et al. 2017	+	+	-	-	+	-
Mobin et al. 2017	+	+	+	-	+	+
Mendes et al. 2019	+	+	+	-	+	+
Faustino et al. 2020	+	-	-	-	+	-
Faustino et al. 2021	+	+	+	+	+	+
Rodrigues et al. 2022	+	+	+	+	+	+
de Melo et al. 2023	+	-	-	-	+	-

Domains:
D1: Bias arising from the randomization process.
D2: Bias due to deviations from intended intervention.
D3: Bias due to missing outcome data.
D4: Bias in measurement of the outcome.
D5: Bias in selection of the reported result.

Judgement
- Some concerns
+ Low

Based on McGuinness et al., (19)

Main Characteristics of the Included Studies

The articles included in this systematic review were published between 1999 and 2023, spanning a period of 24 years. The majority of these articles were published in the last 20 years, categorizing them as recent studies. In terms of their primary objectives, 5 articles assessed multiple biological activities of the essential oil, while 11 studies focused on evaluating a single biological activity. The most frequently studied activity was the essential oil antimicrobial activity (n = 5) (antibacterial and antifungal), followed by the insecticidal (n = 3), antineoplastic (n = 2), antinociceptive (n = 2) and anti-inflammatory (n = 2) activities. Other essential oil activities studied by the articles include vasorelaxant (n = 1), antioxidant

(n = 1) and antimutagenic (n = 1) activities (Figure 3). Figure 3 presents the activities evaluated in the articles included in the systematic review.

Figure 3 - Schematic representation of the *P. heptaphyllum* resin essential oil biological activities evaluated in the literature.



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Created in <https://app.biorender.com/>

Before evaluating the essential oil biological activities, each essential oil experimental sample components were analyzed. Notably, there was considerable variation in both the geographic origins of the resin samples used for essential oil extraction and in the major compounds identified in their compositions. One article reported the use of a mixture of two different *Protium* species resin essential oils, being those *P. decandrum* and *P. heptaphyllum*⁵. The methods used in the articles to identify the main components of the essential oils were largely consistent, primarily involving Gas Chromatography coupled with Mass Spectrometry (GC-MS) and its variations for essential oil characterization (Table 4). The most frequently identified compounds in the *P. heptaphyllum* resin essential oil samples were *p*-Cymene, identified in 68.8% of the studies (n = 11), α -Pinene, present in 43.8% of the articles (n = 7), and, finally, Limonene, identified in 37.5% of the articles (n = 6).

Table 4 - Resin origin, method applied for essential oil characterization and major compounds identified in the essential oils of studies included in the systematic review.

Reference	Resin collection source	Method	Major Compounds
Siani et al., 1999	Primary collected	GC-MS	Terpinolene (21.8%), Dillapiole (16.1%), <i>p</i> -Cymen-8-ol (11.3%), <i>p</i> -Cymene (11.0%), Limonene (4.20%)
Bandeira et al., 2006	Primary collected	GC-MS	Terpinolene (28.5%), α -Phellandrene (16.7%), Limonene (16.9%), and α -Pinene (10.5%)
Rao et al., 2007	Primary collected	GC-MS	α -Phellandrene (10.4%), α -Terpinene (13.7%) and 1,8-Cineole (58.7%)
Amaral et al., 2009	Primary collected	GC-MS	Limonene (49.96%), Trans- β -Ocimene (11.81%), Eucalyptol (10.92%) and <i>p</i> -Cymene (10.78%)
Siani et al., 2011	Primary collected	GC-MS	Fresh resin^{**} : Myrcene (35%), α -Pinene (27%), Sabinene (11%) and β -Caryophyllene (7.2%) Tapped resin^{**} : Terpinolene (28%) and <i>p</i> -Cymene (16%)
Bernadi et al., 2015	Primary collected	GC-MS	α -Terpinolene (32.7%), Limonene (22.0%) and 3-Carene (15.0%)
Pinto et al., 2015	Primary collected	GC-MS	Terpinolene (35.1%), <i>p</i> -Cymene (26.66%), Tricyclene (11.05%) and <i>p</i> -Cymen-8-ol (10.12%)
Mobin et al., 2016	Primary collected	MDGC-MS	<i>L</i> -Limonene (36.01%), α -Terpineol (32.57%), <i>p</i> -Cineol (18.68%), <i>o</i> -Cymene (17.43%) and α -Phellandrene (11.38%)
de Lima et al., 2016	Primary collected	GC-MS	2009 Sample[*] : Terpinolene (32.70%), Limonene (22.00%) and 3-Carene (15.00%) 2011 Sample[*] : Terpinolene (35.81%), <i>p</i> -Cymene (26.66%), α -Tujeno (11.05%) and <i>p</i> -Cymen-8-ol (10.12%) 2013 Sample[*] : <i>p</i> -Cymene (38.08%), Terpinolene (37.78%) and <i>p</i> -Cymene-8-ol (6.6%)
Mobin et al., 2017	Primary	GC-MS	Commercial resin : Limonene (34.51%), Eucalyptol (20.64%),

	collected		and <i>p</i> -Cymene (17.04%) Natural resin: Limonene (28.88%), <i>p</i> -Cymene (26.87%), and α -Terpineol (18.39%)
da Silva et al., 2017	Primary collected	GC-FID	Mixture A ^{***} : δ -3-Carene Mixture B ^{***} : <i>p</i> -Cymene
Mendes et al., 2019	Commercially obtained essential oil	GC-MS	β -Phellandrene (60.68%), <i>p</i> -Cymene (13.63%), α -Pinene (4.47%), and α -Phellandrene (3.38%)
Faustino et al., 2020	Primary collected	GC-MS	<i>p</i> -Cymene (27.70%) and α -Pinene (22.31%)
Faustino et al., 2021	Primary collected	GC-MS	<i>p</i> -Cymene (27.70%) and α -Pinene (22.31%)
Rodrigues et al., 2022	Commercially obtained	GC-MS	<i>d</i> -Limonene (40.1%), α -Terpineol (17.2%), 1.8-Cineol (16.4%), <i>p</i> -Cymene (15.3%), α -Phellandrene (5.2%), δ -3-Carene (3.2%) and α -Pinene (3.5%)
de Melo et al., 2023	Primary collected	GC-MS	α -Pinene (39.65%), δ -Carene (21.42%) and β -Pinene (9.56%)

Legend:

GC-MS - Gas Chromatography-Mass Spectrometry

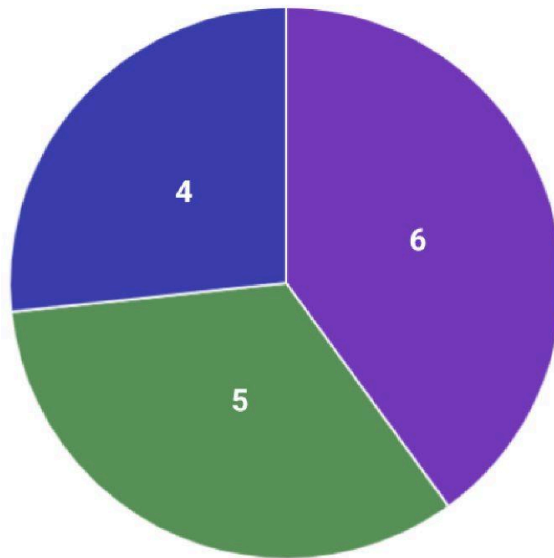
Siani et al., 2011^{**} - A fresh recently collected and an aged resin were evaluated in this study.

de Lima et al., 2016^{*} - Resins collected in 3 different years were evaluated in this study.

da Silva et al., 2017^{***} - Five different formulations consisting in mixtures of the essential oils derived from two different *Protium* species resins were evaluated in this study, being Mixture A and Mixture B the formulations containing the *Protium heptaphyllum* resin essential oil.

Regarding the experimental models used in the studies (Figure 4), most articles employed *in vivo* experimental models (n = 6), followed by those that applied both *in vivo* and *in vitro* experimental models (n = 5). Four articles utilized only *in vitro* experimental models (n = 4). Figure 4 represents the distribution of articles based on their experimental models.

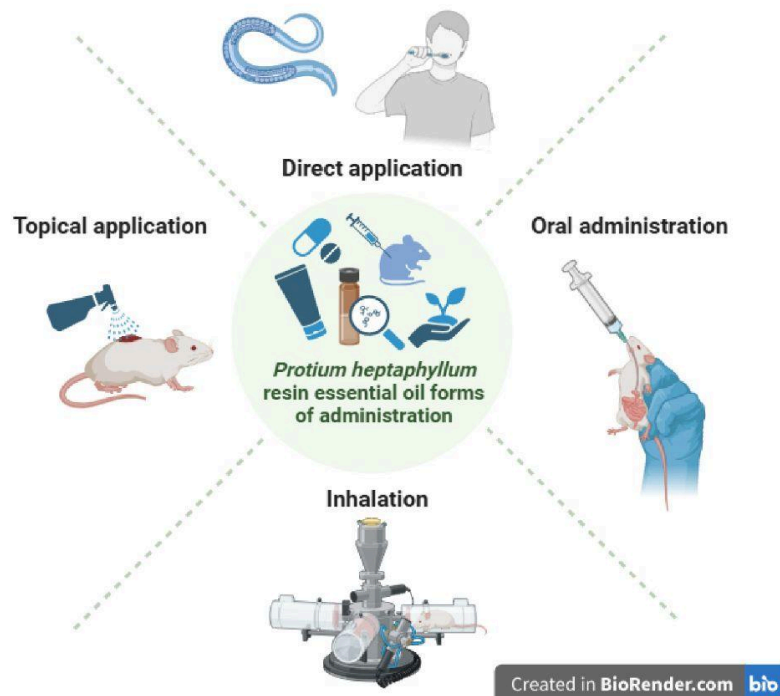
Figure 4 - Distribution of Experimental Models Used in the Included Studies.



- *In vivo* experimental model
- *In vitro* experimental model
- *In vitro* and *in vivo* experimental model

Five articles involving *in vivo* experimental models reported the direct application of the essential oil, while three report oral administration and one reports the use of inhalation (n = 1) as administration route (Figure 5).

Figure 5 - *P. heptaphyllum* resin essential oil administration routes in selected studies.



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The most frequently investigated property of the essential oil was its antimicrobial potential, confirmed in five independent studies (n = 5). The insecticidal effect was reported in three studies (n = 3). The antineoplastic, anti-inflammatory, and antinociceptive effects were each described in two studies. The essential oil's ability to limit oxidative stress and nitrite production was confirmed in one study each, as were its wound healing, vasorelaxant, and antimutagenic effects (Table 5).

Table 5 - Concentrations and experimental models used for proven effects of the essential oil by the articles.

Reference	Reported Effects	Concentration Used	Experimental Models used
Siani et al., 1999	Antineoplastic, inhibition of Nitrite production and anti-inflammatory	Antineoplastic: 0.1 mg/well inhibition of Nitrite: 0.1 mg/well Anti-inflammatory:	<i>In vitro</i> and <i>in vivo</i>

100 mg/kg			
Bandeira et al., 2006	Antimicrobial and antioxidant	Antimicrobial: MIC of 0.0125 mg/mL for <i>Candida albicans</i> and 0.025 mg/mL for <i>Staphylococcus aureus</i> Antioxidant: 1 mg/mL	<i>In vitro</i>
Rao et al., 2007	Antinociceptive	50 and 100 mg/kg	<i>In vivo</i>
Amaral et al., 2009	Anti-inflammatory	100 and 200 mg/kg	<i>In vivo</i>
Siani et al., 2011	Antineoplastic	0.1 mg/mL	<i>In vitro</i>
Bernadi et al., 2015	Skin wound healing	0.5%	<i>In vivo</i>
Pinto et al., 2015	Antimicrobial activity against <i>Streptococcus mutans</i>	0.000125 e 0.0024 mg/mL	<i>In vitro</i> and <i>in vivo</i>
de Lima et al., 2016	Antimutagenic	25, 50, and 100 mg/kg	<i>In vitro</i> and <i>in vivo</i>
Mobin et al., 2016	Antifungal activity against <i>Candida spp.</i>	0.1, 0.05 and 0.025 mg/L	<i>In vitro</i>
Mobin et al., 2017	Vasorelaxant	0.003 to 0.75 mg/mL	<i>In vitro</i>
da Silva et al., 2017	Antinociceptive effect of one mixture containing <i>Protium heptaphyllum</i> resin essential oil mixed with <i>Protium decandrum</i> resin essential oil	100 mg/mL	<i>In vivo</i>
Mendes et al., 2019	Antibacterial and antibiofilm activity against <i>Vibrio parahaemolyticus</i>	Antibacterial and antibiofilm: 16, 8, 4, 2, 1, 0.5, and 0.25 mg/mL Reducing sugar and cell constituents release: 2 and 4	<i>In vitro</i>

		mg/mL	
Faustino et al., 2020	Larvicidal and residual larvicidal effect against <i>Aedes aegypti</i>	0.00291 mg/mL	<i>In vivo</i>
Faustino et al., 2021	Ovicidal, pupicidal, adulticidal and repellent activities against <i>Aedes aegypti</i>	<p>Ovicidal assay: LC₅₀ = 0.000404 mg/mL in 24 h and 0.0000482 mg/mL in 48 h</p> <p>Pupicidal assay: 0.16 mg/mL reached 100% mortality in 24 h</p> <p>Adulticidal assay: 0.2 mg/mL (120 min)</p> <p>Repellency test: 0.2 mg/mL during 180 min of the test had a protection index of 77.67%</p>	<i>In vivo</i>
Rodrigues et al., 2022	Insecticidal and repellent activity against <i>Callosobruchus maculatus</i>	<p>Contact toxicity: 0.0005, 0.00075, 0.001, and 0.00175 mL/g</p> <p>LC₅₀ = 0.0007115 mL/g and an LC₉₅ = 0.0017875 mL/g</p> <p>Repellent effect: LC₅₀ = 0.0007115 mL/g and an LC₉₅ = 0.0017875 mL/g</p> <p>Fumigant toxicity: 0.125, 0.25, 0.375, and 0.4375 mL/L air</p> <p>LC₅₀ = 0.19128 mL/L air</p>	<i>In vivo</i>
de Melo et al., 2023	Synergic antibacterial activity with Amikacin against Polymyxin resistant <i>Klebsiella pneumoniae</i>	0.128 - 0.512 mg/mg	<i>In vitro and in vivo</i>

Legenda:

MIC - Minimum Inhibitory Concentration

LC - Lethal Concentration

Studies utilizing in vivo experimental models, excluding those focused on insecticidal activity, did not report any toxicity in the animals tested. Furthermore, no alterations related to essential oil toxicity or side effects were observed. However, it is noteworthy that three articles did not assess the potential toxicity of the oil treatment in the treated animals, with no mention of toxicity or adverse effects in their findings (Table 6).

Table 6 - Evaluation of *P. heptaphyllum* Resin Essential Oil Toxicity in Animal Experimental Models.

Reference	Toxicity evaluation	Concentration used
Siani et al., 1999	Oral administration of the essential oil at the tested concentration showed no toxic effects in mice.	100 mg/kg
Rao et al., 2007	Not evaluated	50 and 100 mg/kg
Amaral et al., 2009	Not evaluated	100 and 200 mg/kg
Bernadi et al., 2015	Necropsy did not show changes on organs that could be related to the use of the essential oil, demonstrating that the topical absorption of the compound does not lead to toxic effects. The animals tested had body weight gain of 10% during the experiment.	0.5%
Pinto et al., 2015	Toxicity was not evaluated in the study, based on previous work ³¹ (Siani et al., 1999), which reported the essential oil as non-toxic.	0.000125 and 0.0024 mg/mL
de Lima et al., 2016	Animals treated with all essential oil concentrations had normal cytotoxicity index in the PCE/NCE ratio.	25, 50, and 100 mg/kg
da Silva et al., 2017	Not evaluated.	100 mg/mL
de Melo et al., 2023	The tested concentrations of the essential oil did not affect the viability of the experimental model, suggesting its non-toxic effects on <i>C. elegans</i> .	0.128 - 0.512 mg/mL

Legend:

PCE/NCE - Polychromatic erythrocytes-micro nucleated/Normochromatic cytotoxicity index ratio

Discussion

Protium heptaphyllum holds significant economic potential, with its biotechnological applications supported by its traditional uses. Several studies carried out with the bulk resin, its derivatives and other *P. heptaphyllum* extracts, such as essential oils extracted from other plant parts, hint on its nervous, immune and gastrointestinal system activities and highlight its pharmacological potentials²⁰. Other *P. heptaphyllum* resin essential oil activities reported by the literature include antinociceptive^{21, 5}, antineoplastic^{22, 25, 30}, vasorelaxant⁴, antibacterial^{23, 24, 25, 3, 26}, anti-inflammatory^{31, 32}, antifungal³, antioxidant²³, wound healing²⁷ and insecticidal activities^{1, 28, 29}. Nevertheless, at this point, such knowledge has not yet been fully translated into clinical applications or therapeutic products. The lack of clinical studies and registered products corroborates the need for further research and development to confirm these potential therapeutic uses. Therefore, in this systematic review we consolidated and critically analyzed the current scientific literature regarding the *P. heptaphyllum* resin essential oil activities.

Variability in the Composition of *P. heptaphyllum* Resin Essential Oils

The biological activities of essential oils are closely linked to their chemical composition, which varies based on multiple factors. Such variations are inherent to any natural source of study material, the key factors influencing the composition of the plant's secondary metabolites including seasonality, geographic location, humidity, levels of ultraviolet radiation, altitude, average temperature, soil nutrients, plant defenses against herbivorous animals²⁹, intraspecific genetic variations^{29, 3}, resin collection methods and timing, air exposure, storage conditions²⁶, and resin aging. These factors significantly affect the monoterpene composition of the essential oils. As such, the inconsistencies in essential oil composition reported in the literature are expected and associated with these variables. All studies applied variations of the GC-MS technique to characterize their resin essential oil samples, an additional aspect that may also contribute to variations observed on the oil composition. Monoterpenes were the most representative group of molecules being detected in the samples.

Being composed mainly of monoterpenes and sesquiterpenes²⁸, the *P. heptaphyllum* resin essential oil can present components such as *p*-Cymene and β -Phellandrene (PHE), which have been associated with antimicrobial activity²⁰. Terpinolene, α -PHE, Limonene and α -Pinene are also components of the plant essential oil and have been related to potential antimicrobial and antioxidant activities²³. α -Pinene, δ -3-Carene and β -Pinene, have also showed synergism with Amikacin (AMK) against polymyxin-resistant *Klebsiella pneumoniae*²⁶.

Two studies demonstrated the variation in the essential oil composition according to the time and season of collection when analyzing resins collected from the same plant at different time periods. Siani et al. (22) analyzed the components of essential oils extracted from two different samples of resin, one being fresh and the other aged, identifying Mircene (35%), α -Pinene (27%), Sabinene (11%) e β -Caryophyllene (7.2%) as major components of the oil extracted from the fresh resin and Terpinolene (28%), and *p*-Cimene (16%) as major components in the aged resin. This variation is most likely related to the resin aging or to subspecific differences, such as the *P. heptaphyllum* ulei and *P. Heptaphyllum*. Analyzing the essential oils extracted from resins collected from the same plant in three different years, de Lima et al. (25) found Terpinolene (32.70%), Limonene (22.00%) and 3-Carene (15.00%) in the essential oil extracted from the resin collected in 2009, Terpinolene (35.81%), *p*-Cimene (26.66%), α -Tujene (11.05%) and *p*-Cimen-8-ol (10.12%) in the oil derived from the 2011 resin and *p*-Cimene (38.08%), Terpinolene (37.78%) and *p*-Cimene-8-ol (6.6%) in the oil extracted from the 2013 resin.

The *P. heptaphyllum* resin essential oil composition also varies according to the resin origin, as verified when analyzing the components of two essential oils extracted from resins of different sources, being one commercially acquired and the other extracted directly from the plant. There was a predominant monoterpene composition in both oils, being the essential oil derived from the commercial resin composed by Limonene (34.51%), Eucaliptol (20.64%) and *p*-Cimene (26.87%), e α -Terpineol (18.39%). Differences in the composition of essential oils extracted from resins of different origins are frequently reported, with oxygenated compounds being more present in commercially acquired resins, due to greater air exposure, a not so accentuated difference in the sample analyzed in the study⁴.

Finally, the analysis of the components of two mixtures of essential oils extracted from the *P. heptaphyllum* and *P. decandrum* resins resulted in δ -3-Carene as the main compound for the Mixture A and *p*-Cimene as a main compound in the Mixture B; however, the relative quantification was made from the volatile fraction of the essential oil mixtures injected inside the nebulized into the mice inhalation chamber and not directly from the essential oil mixtures⁵.

In general, the inherent variability identified in essential oils components, not just on the *P. heptaphyllum* resin essential oil, presents a challenge mainly when comparing compounds identified in natural products, being associated with the previously mentioned factors. In the same way, the variations also contribute to the diversity of biological activities associated with these metabolites.

Antimicrobial activity

Essential oils have various volatile compounds that exhibit several biological effects, such as antimicrobial activity. Accordingly, the most extensively studied activity of the *P. heptaphyllum* resin essential oil is its experimentally proven efficacy against bacterial and fungal pathogens^{23, 3, 20, 26}.

Generally, *P. heptaphyllum* resin essential oils predominantly comprise monoterpenes, and the lipophilic nature of these compounds affects the bacterial plasmatic membrane permeability to molecules, due to its affinity to such a structure^{20, 26}. Thus, in the same time that the essential oils affect membrane permeability to antimicrobials, they permeate easily into the intracellular medium, establishing their synergism to established antimicrobials²⁶. The membrane lysis caused by the destabilization of the membrane promotes toxicity, directly causing apoptosis, with that being one of the oil's potential mechanisms of action underlying its antimicrobial effects. Various essential oil components can be associated with the increase of membrane permeability to molecules, rendering it impossible to associate such activity to any singular component present in the oils. Besides, due to the unique components of each product, the singular mixture of components identified in each essential oil could also be associated with either the lack or the potentialization of this effect²⁰.

In a study examining the oil's anti-inflammatory effects, it was found to inhibit Nitric Oxide (NO) production by 74%. This effect occurred in an in vitro assay with murine macrophages, induced by 0.1 g/well of Lipopolysaccharides (LPS), and was strongly associated with its antimicrobial activity³⁰.

The essential oil has already been evaluated in regard to its activity against *Staphylococcus aureus*²³ (Bandeira et al., 2006; ²⁵de Lima et al., 2016), *Streptococcus mutans*^{24, 25}, *Serratia marcescens*²³, *Klebsiella pneumoniae*^{23, 26}, *Proteus mirabilis*²³, *Escherichia coli*^{23, 25}, *Pseudomonas aeruginosa*²³, *Enterococcus faecalis*²⁵ and *Vibrio parahaemolyticus*²⁰ bacterial species. As for the fungal species, the oil's activity was tested against the *Candida* genera, including *C. krusei*³, *C. albicans*^{23, 3}, *C. parapsilosis*, *C. metapsilosis*, *C. rugosa*, and *C. guilliermondii*³.

The resin essential oil showed moderate antimicrobial activity against *S. aureus*, with Minimum Inhibitory Concentration (MIC) of 0.0025 mg/mL, and against *C. albicans*, with a MIC of 0.00125 mg/mL²³. Another study tested the product against similar microorganism strains, in concentrations ranging from 0.3125 to 0.5 mg/mL, although the oil's antimicrobial activity was not observed in this work²⁵. The dissonance between the studies may be related to different oil compositions, as well as the different concentrations used.

Streptococcus from the *mutans* group are one of the bacteria most associated with dental caries. A toothbrush formulation containing *P. heptaphyllum* resin essential oil as the active ingredient was evaluated for its antimicrobial activity against *Streptococcus mutans*,

demonstrating a minimum inhibitory concentration (MIC) of 0.0013 mg/mL. The results confirmed both the antimicrobial efficacy of the essential oil and the effectiveness of the formulation. Importantly, no toxicity was not observed throughout the clinical study that included 21 children²⁴. The essential oil activity against *S. mutans* was also verified in another study, in which the active showed low antimicrobial activity, with a MIC of 0.5 mg/mL. For the other tested isolates (*E. coli*, *E. faecalis* and *C. albicans*) the essential oil showed no inhibitory or bactericidal activity²⁵, revealing, again, some discrepancies between different investigations.

When tested according to its activity against *Vibrio parahaemolyticus*, the essential oil showed a MIC of 2 mg/mL and Minimum Bactericidal Concentration (MBC) of 8 mg/mL, confirming the oil's bactericidal activity, due to its MBC/MIC ratio being ≤ 4 . The product also demonstrated the ability to prevent (4 mg/mL) and reduce (1 - 2 mg/mL) biofilm formation after 48 h of incubation. When used to treat 72h pre-formed biofilms, double and triple of MIC concentrations (i.e. 4 and 6 mg/mL) were able to reduce biofilm biomass. The underlying mechanism of action possibly involved extravasation of intracellular material and cell death induction through membrane lysis, according to a cellular constituent release assay. In the first 30 to 240 minutes of contact with the bacterial culture, 4 mg/mL of the essential oil increased relative electrical conductivity (%mV), indicating cell membrane damage. This effect was associated with increased membrane permeability, leading to intracellular material extravasation²⁰.

The use of essential oils as antifungals is studied not only for their natural antifungal potential but also due to the high cost of conventional antifungal agents, their associated side effects, and the resistance of some fungal species. When being tested to evaluate antifungal activity against *Candida* species isolates from onychomycosis lesions, the *P. heptaphyllum* resin essential oil (used at the concentration of 1 mg/mL) demonstrated antifungal activity with an inhibition halo of ≥ 14 mm against *C. albicans*, *C. krusei*, *C. parapsilosis*, *C. metapsilosis*, *C. rugosa* and *C. guilliermondii*. *C. rugosa*, *C. krusei*, *C. guilliermondii* and *C. albicans* were inhibited at all concentrations tested (1, 0.5 and 0.25 mg/mL), with an average inhibition halo of 12 to 14 mm. Despite being Fluconazole resistant, *C. krusei* showed sensitivity to the essential oil in all concentrations tested, and the reference strain was inhibited at 1 mg/mL concentration, with an inhibition halo of ≥ 14 mm. Similarly, the *C. parapsilosis* reference strain showed an average inhibition halo of 11 mm when treated with the same concentration of the essential oil. However, Fluconazole presented an average inhibition halo of 20 to 35 mm, indicating the lower efficacy of the essential oil compared to the reference antifungal³.

Another interesting study showed that, despite the resin essential oil isolated failing to reduce polymyxin-resistant *K. pneumoniae* viability, it showed a synergistic potential when

combined with Amikacin (AMK) at concentrations ranging from 0.128 to 0.512 mg/mL and 0.002 to 0.008 mg/mL, respectively²⁶.

The same study reported positive synergism between the resin essential oil and AMK in a *Caenorhabditis elegans in vivo* infection model, showing the capacity of inhibiting the polymyxin-resistant *K. pneumoniae* Kp20 strain *in vivo*. Worm survival increased significantly with essential oil treatment at 0.0001 mg/mL and AMK at 0.0002 mg/mL, when compared to the untreated control group. The lipophilic nature of the terpenoid compounds identified in the essential oil composition present a certain affinity to the bacterial plasma membrane, affecting its permeability to molecules. It has been proven that the presence of α -Pinene increases cell membrane permeability and causes increased inhibition of efflux pumps in *Campylobacter jejuni* and *S. aureus*, promoting antimicrobials influx in these microorganisms. Furthermore, α - and β -Pinene demonstrated synergy with Ciprofloxacin, reducing its MIC while maintaining their effectiveness and reducing toxicity. In that way, the potential use of natural compounds as antimicrobials is recognized in the sense of potentially broadening the spectrum of antimicrobials against bacteria and reducing the toxicity associated with high antibiotic doses²⁶.

In summary, current evidence mostly confirms the antimicrobial potential of the *P. heptaphyllum* resin essential oil against bacterial^{23, 24, 25, 20} and fungal^{23, 3} agents, in addition to the synergy of the oil with antimicrobial agents²⁶. These findings are consistent with the existing knowledge related to the essential oil's components and corroborate its use in medicine as well as other applications, such as a natural preservative. Despite the variations observed in the essential oil compositions and consequently in its activities, the evidences suggests some essential oil components exhibit antimicrobial activity, which still have to be further explored in future studies. The synergy presented by the essential oil with antimicrobial agents²⁶ suggests the future evaluation of essential oil synergy with other antimicrobials in future works, as well as its effectiveness against other microorganisms. Regarding remaining knowledge gaps related to the resin essential oil antimicrobial activities, it is notable that there's a higher number of papers regarding the essential oil antibacterial effects than its antifungal effects, requiring more in-depth studies of the antifungal activity of the product, against other fungal species, besides its proven activities against *Candida* species, which are the only species screened in the included studies. The oil also showed lower reactivity than the reference antifungal used in one of the articles³, suggesting a lower essential oil antifungal activity and a possible study topic, for evaluating the activity in association with other antifungal molecules in order to enhance drug activity. The product activity against biofilm formation has not been completely explored, being reported by only one study and regarding only one bacterial isolate²⁰. Generally, it was observed that only one paper applied an *in vivo* infection model to evaluate toxicity and

synergistic activity of the essential oil with AMK, establishing the base for future works regarding other *in vivo* infection models to assess toxicity and prove the antimicrobial activity of the product previously reported in *in vitro* models and verify its safety, since the essential oil toxicity remains not properly described in the literature.

Insecticidal activity

Other studied activity of the *P. heptaphyllum* resin essential oil is the insecticidal activity against *Aedes aegypti*^{28, 29}, known popularly as Dengue mosquito, and *Callosobruchus maculatus*, popularly denominated as cowpea weevil or cowpea seed beetle¹. Essential oils are a viable option for the development of insecticide products for various uses, such as against disease vectors, such as *A. aegypti*, vector of Dengue viruses^{28, 29}, and *C. maculatus*, a common pest that can cause losses up to 70% in *Vigna unguiculata* grain stocks, popularly known as cowpea¹. The essential oil's insecticidal activity is due to its compounds alone or in association²⁹.

The Burseraceae essential oils are a natural alternative to synthetic molecules for novel insecticidal agents development, being a viable option for disease vectors control, due to their lower environmental toxicity and its lipophilic nature^{28, 29}.

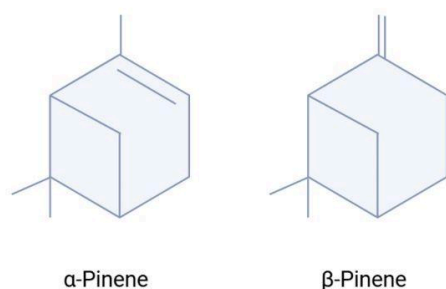
One of the oils components that stand out for insecticidal activity is *d*-Limonene, which presents insecticidal activity against other agricultural pests, such as *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae), *Sitophilus zeamais* Mots. (Coleoptera: Bostrichidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Lasioderma serricorne* F. (Coleoptera: Anobiidae), and *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae)¹.

The *P. heptaphyllum* resin essential oil biocidal activities against *A. aegypti* reported by the literature include ovicidal, pupicidal, larvicidal and repellent effects, evaluated through a nanoemulsion containing the essential oil and proving its activity against different *A. aegypti* development stages. Essential oil major composition consisted in *p*-Cymene (27.70%) and α -Pinene (22.31%)^{28, 29}.

In the larvicidal activity assay, the essential oil containing nanoemulsion presented Lethal Concentration (LC₅₀) of 0.000291 mg/mL in 24 h and 0.000017 mg/mL in 48 h and a LC₉₀ of 0.001244 mg/mL in 24h and 0.000887 mg/mL in 48 h, proven its activity. The effect was time and dose dependent, showing a 48 h LC₅₀ 17 times lower than the 24 h LC₅₀, stating the essential oils cumulative action. In comparison to the positive control LC₅₀ in 48 h of exposure, the essential oil nanoemulsion was 51 times more efficient against the larvae, showing its high larvicidal effect against *A. aegypti*. The residual larvicidal effects were observed for a 11 day period after the initial larvae exposure to the essential oil nanoemulsion in the 0.002, 0.004, 0.006, 0.008 and 0.01 mg/mL concentrations. In the 48 h

interval, the 0.002 mg/mL concentration showed 20% of mortality rates. The larvicidal effects of said concentration decreased by 78% when compared to larval mortality in 24 h of exposure. After 96 h, the 0.002 mg/mL did not cause larval mortality 96 h after solution preparation, indicating that this concentration did not release essential oil from the nanoemulsion after this time period. According to the literature, the larvicide activity of the terpenes found in the *P. heptaphyllum* resin essential oil, such as α -Pinene, are related to molecular structural characteristics, like the exocyclic double bound (Figure 6). Figure 6 presents the α - and β -Pinene molecular structures and the exocyclic double bounds associated with essential oil larvicide action²⁸.

Figure 6 - Visual representation of α - and β -Pinene molecular structures, frequently identified components of the *P. heptaphyllum* resin essential oil.



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Another minor compound identified in the nanoemulsion essential oil was γ -Muurolene (1.49%), a sesquiterpene with a exocyclic double bound in its structure that could also be related to the nanoemulsion larvicidal action. Thus, the larvicidal effect of the nanoemulsion containing the *P. heptaphyllum* resin essential oil could be associated with the presence of compounds of known insecticidal activity, such as α -Pinene and γ -Muurolene and their possible synergy as well. Essential oil larvicidal mechanisms of action could be related to their action in digestive and/or neurological enzymes, in addition to possible interactions with the integumentary system, being possible to come to the conclusion that the increase of lipophilicity of chemical constituents enhances the molecule permeation in the integumentary system, since the identified compounds present high lipophilicity and corroborate such action, while the residual larvicidal action results of the controlled release of the essential oil compounds present in the nanoemulsion throughout time²⁸.

The same nanoemulsion was evaluated in regards to its ovicidal, pupicidal, adulticidal and repellent actions against *A. aegypti*. For the ovicidal activity, the nanoemulsion had a LC₅₀ of 0.0000404 mg/mL, as for the pupicidal activity assay, the concentration of 0.016 mg/mL led to 100% mortality rate after 24 h of exposure. Regarding the adulticidal activity, it was determined the concentration of 0.02 mg/mL in the 120 minutes time exposure. Finally, in the repellent effect assay, the 0.02 mg/mL nanoemulsion concentration showed a protection index of 77.67%²⁹. Thus, the essential oil nanoemulsion had its activity against different *A. aegypti* life stages proven regarding its larvicidal²⁸, ovicidal, pupicidal, adulticidal and repellent action²⁹.

Regarding its insecticidal activities against *C. maculatus*, the *P. heptaphyllum* resin essential oil alone had its effects proven with a LC₅₀ of 0.0007115 mL/g and a LC₉₅ of 0.0017875 mL/g in the contact toxicity assay, classifying the oil as repellent when the emergent insects were quantified after LC₉₅ exposure in the repellent effect assay. Oviposition was reduced when the insects were exposed to the essential oil treatment, with an average egg count of 3.4 eggs, while the isolated essential oil major compound, *d*-Limonene, tested along with the essential oil had an average egg count corresponding to 4.8 eggs. At last, the essential oil also presented higher fumigant toxicity than *d*-Limonene, with an LC₅₀ = 0.19128 mL/L of air. Briefly, the essential oil showed higher toxicity than its major compound, *d*-Limonene, tested in parallel by the study. Different essential oil major and minor compounds were evaluated in the efforts of elucidating which components are responsible for insecticidal activity. Contact toxicity results, for instance, are possibly related to the combination of the essential oil's components, as well as the major compounds' possible synergistic effects. Due to the oil's higher toxicity when compared to its major component alone, it is possible that the insecticidal mechanism is probably related to the combination of the essential oil's compounds, since the activity of its major compounds can be modulated by minor oil compounds¹.

Therefore, the *P. heptaphyllum* resin essential oil insecticidal activity against *A. aegypti*^{28, 29} and *C. maculatus*¹ reported in the literature corroborates the resin traditional uses as an insect repellent, making the compound a viable alternative for the development of sustainable approaches in disease vectors and agricultural pests control, as well as for other arthropod control, despite the difference observed in the studies methods and in the concentrations used, since different species were evaluated and the studies conditions were carried out accord to each species specificities. The methods applied are reference methodologies, and, as a result, establish the reliability of the reported evidence. However, due to the reduced number of articles addressing the essential oils insecticidal activities, future studies are required for the understanding of the action mechanism of the active, taking into account the different composition of the essential oils studied. Studies evaluating

the oil activity against other insects can also be carried out, with the future possibility of using the essential oil as an active ingredient in repellent and insecticidal formulations.

Anti-inflammatory activity

Traditionally, the *P. heptaphyllum* resin is applied for the treatment of skin diseases and ulcers, and as an analgesic. Studies showed the essential oil anti-inflammatory activity in animal models, both in acute and chronic inflammation models^{30,31}, proving the efficacy of its traditional uses.

The acute inflammation model used by one of the studies was the pleural cavity Zymosan injection, that induces inflammation in a 4 h time period, defined by neutrophil migration and proteic extravasation (plasma). In this experimental model, the essential oil pre-treatment with 100 mg/kg was capable of reducing proteic material extravasation without changing leucocyte count in the pleural cavity. The essential oil effect in the late LPS-induced inflammation model injected in the pleural cavity, that lasts 24 h, was also evaluated, being a model characterized by significant cell migration and eosinophil migration, without protein extravasation. The migration of total leukocytes, mononuclear cells and neutrophils showed a reduction of 41, 42 and 62% in animals treated with the essential oil, while eosinophil migration was inhibited by 60%. The accumulation of eosinophils is an important component of allergic diseases and bacterial and parasitic infections, and is considered one of the most important causes of lung injury resulting from these processes. The essential oil demonstrated efficiency in the cell migration late inflammation model, in addition to also being involved in the inhibition of an acute inflammation important marker, in the Evans blue extravasation assay, related to the protein extravasation induced by Zymosan quantification³⁰.

When administered orally to mice 1 h before injection of carrageenan or albumin, the essential oil caused significant dose-dependent paw edema inhibition at concentrations of 100 and 200 mg/kg. Maximum inhibitory effect was reached with the 200 mg/kg dose 2 h to 3 h after carrageenan and albumin injection, respectively, suggesting effective exudative phase of acute inflammation inhibition by the oil. The effect of the compound was also evaluated in the vascular permeability model induced by acetic acid and carrageenan. Vascular permeability induced by acetic acid increases mediators such as serotonin, histamine and prostaglandins, leading to vasodilation and increased vascular permeability. The essential oil demonstrated dose-dependent inhibition of peritoneal vascular permeability induced by acetic acid at 100 to 200 mg/kg concentrations, suggesting its role in the release of inflammatory mediators in the first phase. As it is an acute inflammation model, fluid extravasation and leukocyte migration involved in the inflammatory response can be easily detected³¹.

When evaluated in chronic inflammation models, the essential oil, at the same concentration, significantly reduced cell migration to the peritoneal cavity after carrageenan injection. In the cotton pellet-induced granuloma model, the proliferative and transudative components of chronic inflammation are evaluated, in which the pellet net weight relates to the transudative components and the pellet dry weight relates to granulomatous tissue amount. The essential oil administration (100 and 200 mg/kg) was effective in inhibiting both pellet weights in the granuloma model, suggesting the oil efficacy in inhibiting the inflammatory process proliferative phase. Mast cells are responsible for inflammatory mediators secretion, both in acute and chronic inflammation, causing vasodilation, plasma extravasation and granulocyte recruitment and activation. Mast cell degranulation is triggered by a series of positively charged substances, known as basic mast cell secretagogues. The most effective secretagogues are the synthetic compound 48/80, a mixed polymer of formaldehyde-crosslinked phenethylamine, known to stimulate only specific mast cell subtypes, such as mouse peritoneal mast cells, to induce inflammatory mediators via phospholipase D and heterotrimeric GTP-binding proteins. The essential oil showed the ability to inhibit degranulation at a concentration of 200 mg/kg, with an action comparable to the effect of ketotifen, used as a positive control for degranulation inhibition, in addition to inhibiting the edema response in the paws induced by 48/80 and dextran T40 in a murine model. Analyzed together, the results suggest the essential oil protective effect against mast cell degranulation, indicating that the action may be associated with the plant's anti-inflammatory effects. Limonene is one of the compounds present in the studied oil possibly involved in anti-inflammatory mechanisms, a monoterpene frequently identified in other essential oils that exhibits anti-inflammatory effects in the literature. Thus, the essential oil anti-inflammatory activity may be partially associated with Limonene presence in its composition, not excluding the possible synergy with other essential oil components³¹.

Regarding the essential oil anti-inflammatory activity evaluation, the methods used by the studies were in accordance, with both having been performed in mice. However, there's a difference in the experimental methods applied, since each study focuses on different acute and chronic inflammation inducing strategies. The second article also explored mechanisms not studied by the previous article, in addition to a higher concentration being used based on the absence of toxicity proven by the first study³⁰. The essential oils biological activities described by both studies suggest that the monoterpenes present in greater proportion in its composition can be associated with the oils anti-inflammatory mechanisms, not excluding the possible synergy between the essential oil components, nor the possibility of the compounds being able to affect other inflammatory mediators. Thus, the limitations of the currently described results regarding the *P. heptaphyllum* resin essential oil anti-inflammatory activities are noteworthy, considering the

reduced number of articles regarding the activity, as well as the models used for evaluation, which can be further investigated by future studies in order to clarify the anti-inflammatory mechanisms of the essential oil, the possibility of its compounds synergy and effects of the essential oil on other inflammatory mediators not yet evaluated by the literature. In addition, the essential oil toxicity was a pending matter in one of the studies, being a topic of utmost importance for the essential oil future application. Standardization of the used concentrations are also required to verify the animals direct treatment response.

Sedative and antinociceptive activity

The antinociceptive effect of *P. heptaphyllum* resin essential oil was already evaluated according to different administration routes, namely oral²¹ and via inhalation⁵. The inhalation route of administration was evaluated according to the traditional use of *P. heptaphyllum* resin by Quilombola communities in the northern region of Brazil, who use the inhalation of smoke generated by the combustion of plant's resin as a headache and migraines treatment⁵.

When evaluated for analgesic activity against chemical and thermal nociception, the *P. heptaphyllum* resin essential oil administered orally to mice at concentrations of 50 and 100 mg/kg in the formalin-induced nociception assay, compared with morphine at 7.5 mg/kg, suppressed only the second phase of the formalin response, which showed resistance to naloxone, an antagonist of μ -type opioid receptors, suggesting the involvement of a non-opioid nociceptor mechanism. The two phases of the animals' response to formalin were attributed to different mechanisms, both peripheral and central, and it was concluded that the analgesic activity in the second phase was due to the anti-inflammatory activity of the active ingredient. In the capsaicin test, on the other hand, the oil demonstrated intense nociceptive activity, as did morphine, as evidenced by the licking response in the hind paws. The extent of the reduction in the respective groups of animals was in the order of 55 to 74% for the essential oil, while for Morphine it was 97%. Unlike Morphine, the antinociceptive effect of the oil in the capsaicin assay was not reversed by Naloxone at 2 mg/kg. In the tail-flick assay, applied to evaluate the animals' response to pain, the essential oil significantly prolonged the response latency, suggesting the attenuation of peripheral nociception induced by capsaicin, possibly through desensitization of primary afferent sensory neurons. Finally, the essential oil at both doses was ineffective against thermal nociception in the plate heating test. While the tail-flick test measures spinal reflexes, the thermal nociception test predominantly measures supraspinal reflexes and, due to the efficiency of the treatment only in the first assay, its nociceptive action is possibly at the spinal level. No behavioral changes were observed in the animals, nor was there any influence on the sleep time induced by Pentobarbital, suggesting absence of central depressant and sedative activity. The results

suggest that the essential oil is an effective oral antinociceptive with peripheral and spinal action ²¹.

Based on the inhalation of smoke resulting from the combustion of the resin in the treatment of headaches and migraines by traditional communities, the possible analgesic and sedative effects of the mixture of essential oils of *Protium decandrum* and *P. heptaphyllum*, as well as other formulations consisting of the mixture of essential oils of plants of the genus *Protium*, were evaluated from inhalation administration. The antinociceptive activity was evaluated through the formalin-induced inflammation model, to evaluate the formulations for pain associated with the inflammatory process. The formalin assay is characterized by two distinct phases. The initial or neurogenic phase occurs in the first 5 minutes and is associated with direct chemical stimuli in the afferent fibers, mainly C fibers, with activation of Transient Receptor Potential Cation Channel Subfamily A Member 1 (TRPA1) channels and reflects the pain central mediation. The late or inflammatory phase occurs 15 to 20 minutes after formalin injection and is mediated by the release of a combination of inflammatory mediators and sensitization of central nociceptor neurons. Drugs with central action inhibit nociception in both phases, while drugs with peripheral action inhibit only the second phase. The formulations containing essential oil in the final concentration of 100 mg/mL, established based on the amount of resins used in combustion and inhalation by traditional communities, as well as the average yield of essential oils extracted from the resins. The formulations were dispersed in water before nebulization, generating a heterogeneous system known as liquid-liquid dispersion. Although the nature of the solution is heterogeneous, the compressed air injected by the nebulizer into the chamber generated a homogeneous nebulization of the air inside the chamber. In the heated plate test, in which the response to the nociceptive stimulus is related to supraspinal reflexes mediated by μ_1 and μ_2 opioid receptors, the antinociceptive activity, in general, is characterized by an increase in the animal's pain tolerance when in contact with the heated plate. None of the mice that inhaled the tested formulations showed a significant increase in response time when compared to animals that inhaled compressed air or vehicle, indicating absence of antinociceptive activity of the essential oil mixture, in agreement with the results previously obtained by Rao et al. (21). In the study, five formulations were tested, with formulations A and B containing the mixture of essential oils from *P. decandrum* and *P. heptaphyllum*, in addition to the vehicle group and the untreated control group. Formulation B reduced licking time by 36.9% only in the initial phase, suggesting a possible central nociceptive inhibition. The activity demonstrated by formulation B is related with the combined essential oil components, since the control, vehicle and formulation A groups have not presented a reduction in licking time in any of the phases. The predominance of monoterpenes in the composition of formulation B in the nebulized and inhaled air fraction is

possibly related to the antinociceptive activity of the formulation, since the main component of formulation B, *p*-Cymene, presents records of orofacial antinociceptive activity in intraperitoneal injection in nociception models induced by formalin, capsaicin and glutamate, without sedative activity. In addition to the antinociceptive activity, monoterpenes also exert anti-inflammatory activities, as previously discussed. Thus, the antinociceptive activity of the formulation exerts its effect by decreasing the release of inflammatory mediators or directly on different receptors present in the animals' paws, such as bradykinin, serotonin or opioid receptors, reducing licking time. In the rotarod test, used to measure the animals' motor coordination and balance, no behavioral changes were observed in the mice after inhalation of the formulations. The test makes it possible to differentiate the anti-inflammatory effects from the analgesic effects of the treatment, since non-specific muscle relaxation can reduce motor coordination and mask the mice's response to nociception. Even though there are records that monoterpenes induce convulsions, the study did not observe this effect in the treated animals. The absence of sedative effects and effects that depress motor coordination is a result that demonstrates that the anti-inflammatory and antinociceptive effects observed are not related to a depressant effect on the central nervous system. Some components of the oils, such as Myrcene and Linalool, demonstrate sedative and depressant activity on the motor system in high concentrations; however, in the study, such activities were not observed, possibly because their concentrations in the nebulized air inhaled by the animals were not high enough to trigger these actions. Finally, the formulation that presented the greatest nociceptive effect was the formulation containing the essential oil extracted from the *P. strumosum* resin, already demonstrating the other formulations lower efficacy when inhaled⁵.

The cited articles therefore present experimental evidence of the nociceptive action of the compound, while none observed sedative effects related to the oil and the formulations, even when administered by different routes (oral and inhalation). The essential oil demonstrated effective antinociceptive activity when administered orally, presenting peripheral and spinal action. Regarding the formulation containing the mixture of essential oils from *P. decandrum* and *P. heptaphyllum* resins administered by inhalation, an attenuated *in vivo* nociception effect was observed when compared to the oral and intraperitoneal routes, with the formulation of *P. strumosum* essential oil showing a more efficient nociceptive effect via inhalation, sustaining the hypothesis that when delivered orally or peritoneally, the *P. heptaphyllum* resin essential oil presents greater antinociceptive activity. However, due to the reduced number of studies regarding the activity, the reported results are established as limited evidence of the nociceptive potential of the active ingredient, which can be further explored by more robust experimental models in future studies.

Antineoplastic activity

Essential oils derived from *Protium* genus plants are frequently studied in regards of their antineoplastic activity by the literature ³⁰, with the evaluation of the essential oil antineoplastic potential being investigated in *in vitro* proliferation models, both in tumoral ^{30, 22, 25} and normal cell lines ²².

The antineoplastic effect of essential oils extracted from *P. heptaphyllum* resin and *P. strumosum*, *P. grandifolium*, *P. lewellyni* and *P. hebetatum* leaves were evaluated by means of the *in vitro* proliferation assay in murine cell line culture, namely SP2/0, a murine plasmacytoma, Neuro-2a, a murine neuroblastoma and J774, a murine monocyte cell lines. The results showed that all studied oils inhibited cell line proliferation of all cell types tested by more than 50%, with the *P. heptaphyllum* resin essential oil being the most efficient, inhibiting 100% of the proliferation of the Neuro-2a cell line and 75% of the SP2/0 and J774 cell lines at a concentration of 0.1 mg/well. The monoterpenes present in the oil composition were associated with this activity, due to the inhibition of specific isoprenylation of cellular proteins, related to neoplastic transformation, due to the ease of association of modified proteins with cell membranes, a functional requirement for cellular transformation. The *in vitro* antineoplastic activity of sesquiterpenes can also be inferred from the results obtained in the assay, since oxidized species of these compounds present marked antitumor and cytotoxic activity ³². Even though significant, these results did not indicate a correlation between the observed effects and the composition of the oils. The article did not evaluate the influence of factors such as the presence of hydrocarbons and oxidized species, such as alcohols, of mono and sesquiterpenes, in addition to the possible presence of arylpropanoids in the composition of the oils, also highlighting the importance of studying the synergistic and antagonistic effects between the components of the active ingredients ³⁰.

When comparing the effect of two essential oils of *P. heptaphyllum* extracted from different resins, one aged and the other freshly collected, both at a 0.1 mg/well concentration, in relation to the *in vitro* cell proliferation assay, the essential oil from the freshly collected resin demonstrated almost complete inhibition of SP2/0 lineage cell proliferation (91.4%), low inhibition for J774 (28%) and no effect on the Neuro-2a lineage. The normal cell line MK2 (Rhesus monkey kidney cells) proliferation was stimulated by both compounds, being the essential oil extracted from the aged resin less effective, inhibiting the neoplastic cell lines growth by 67% for the SP2/0 lineage, 63% for the Neuro-2a lineage and 59% for the J774 lineage. The inhibitory effect is possibly related to the major monoterpene oil composition, since these compounds antitumor and antimutagenic activity have already been described in other works ^{33,34,35}. The article also suggests further investigation of the possible synergistic effects of the oils compounds, a not yet explored topic by the literature ²².

In another study, the essential oil antineoplastic activity was not verified when tested at a concentration of 0.04 mg/mL against the MCF-7 cell line, isolated from human breast adenocarcinoma²⁵.

The study of the antineoplastic activity of the essential oil was carried out entirely in *in vitro* cell proliferation models, using different cell lines from mice and humans. It is also worth noting that only one study compared the essential oils effects on tumor and normal cell lines proliferation²², establishing the possibility of the oils inhibitory activity against exclusively tumoral cell types. However, when tested in a human cell line, the compound did not inhibit cell proliferation, which may be related to tested concentration, which differs from previous studies, being lower. The comparison of the essential oil effects on normal cell types proliferation to different tumoral cell cultures demonstrates the need to robustly evaluate the active ingredient effect on diverse normal cell types cultures, demonstrating the fragility of the data described. Thus, there is limited evidence of the *P. heptaphyllum* resin essential oil antineoplastic potential, requiring further investigation of the activity in subsequent studies. Thus, in the future, the evaluation of the essential oil effects on cell proliferation of different cell lines in comparison with tumor cell lines, and, finally, the evaluation of different concentrations are needed for better understanding the essential oil effects on cell proliferation of both normal and tumoral cell lines.

Antioxidant activity

The antioxidant activity of the *P. heptaphyllum* resin essential oil was evaluated using the *in vitro* 1,1-Diphenyl-2-Picryl-Hydrazyl (DPPH) stable free radical scavenging assay. The oil presented significant free radical scavenging capacity at 1 mg/mL concentration, when compared to the standard Butylated Hydroxytoluene (BHT), with radical inhibition equivalent to 83.2%. At 0.25 mg/mL, the oil inhibited free radicals by 18.6%. Such observations are possibly associated with the synergic relationship between the essential oil components, such as β -PHE and α -Pinene, which already have antioxidant activities described in the literature²³.

The study provides limited evidence of the essential oil's antioxidant activity, as it relies on a single report of findings derived from an *in vitro* assay. Further research is needed to confirm these findings, and to critically establish whether the oil concentration is in the ideal range for industrial applications, as well as clinical use.

Antimutagenic activity

The oil demonstrated antimutagenic activity in the micronucleus assay in an animal model, receiving the essential oil diluted in vehicle treatment orally at 25, 50 and 100 mg/kg concentrations twice a day for 3 days. The positive control group for mutagenic activity was

treated with cyclophosphamide at 45 mg/kg and the negative control group for mutagenic activity received vehicle treatment, which consisted of peanut oil. The penultimate essential oil dose was administered along with cyclophosphamide to all groups 24 h before euthanasia. After euthanasia, the animals bone marrow slides were prepared, and the results of the assay were expressed by the micronucleated polychromatic erythrocytes ratio frequency (PCE-MN), PCE/Normochromatic Erythrocytes (NCE) toxicity index. Thus, the micronucleus assay revealed a reduction in MN-PCE and cytotoxicity within normal limits according to the PCE/NCE index ratio in animals treated with the oil at all concentrations. The micronucleus assay is applied to evaluate antimutagenic activity due to its ability to detect damage to chromosomes and the mitotic machinery. The results obtained indicate the possibility of protection against genotoxicity induced by cyclophosphamide by the essential oil, suggesting its antimutagenic activity and the application of the oil as a chemopreventive agent for neoplastic diseases²⁵.

The essential oil antimutagenic activity described by the study is characterized as limited evidence of this effect of the active ingredient, making it necessary to further evaluate the essential oil antimutagenic activity, by replicating the study obtained results and considering the various mechanisms that could cause mutagenic changes during cell cycle, and, consequently, the essential oil's different mechanisms for possible DNA damage prevention, gaps that can be elucidated in the future in more complex experimental models evaluating different mechanisms of antimutagenicity of the product.

Wound healing activity

The healing activity of Breu essential oil has already been described in the literature²⁷, documenting its traditional medicinal use as a healing agent^{30, 31}. Nevertheless, direct experimental investigation supporting the traditional use is still needed to validate and better understand its therapeutic potential through scientifically controlled studies.

The formulation containing 0.5% of essential oil in 0.9% saline solution was studied in regards to its healing activity in surgically-induced wounds in mice, and the essential oil's healing effects were monitored throughout 21 days. A volume of 300 µL of the formulation containing the oil was applied to the wounds of the treated group, with the same volume of saline being applied to the control group. The wound area measurement was made 3, 7, 14 and 21 days after the surgical wound was induced. There was a significant wound reduction in both groups, with 94.77% contraction in the treated group, while the control group showed 93.91% contraction of the incisions at the end of the study. Despite the similarity in the lesion contraction, the reduction in the lesion area was faster in the treated group on the first two weeks of evaluation. Furthermore, the inflammatory response was more moderate in the oil-treated group, which is consonant with the reported anti-inflammatory potential of oil^{30, 31}.

The treated group intense epithelialization process was characterized by moderate inflammation, as well as the presence of blood vessels and fibroblasts in perpendicular positions. Histopathological analysis showed collagen fibers contraction and myofibroblasts presence, while the control group revealed presence of collagen matrix deposition and a very evident scar structural arrangement. The compounds possibly involved in the oil healing activity are monoterpenes, identified as its major components by GC-MS²⁷.

Thus, according to the results described in the selected study, the *P. heptaphyllum* essential oil positively influences the wound healing process *in vivo*, by promoting hyperplasia at the wound edges, accelerating lesion contraction, and reducing inflammation²⁷. The presented results constitute extremely limited evidence of the regenerative potential of the oil, given that such observations were not replicated at this point. Furthermore, the absence of a positive control group limits a critical evaluation of the superiority of the oil compared to reference treatments. The replication of these findings in subsequent studies involving other experimental models, such as porcine models, similar to human physiology, and those involving organotypic cultures of human skin^{36, 37, 38}, in addition to other injury induction methods, such as skin burns and diabetic wound models, will contribute to consolidate the oil's healing potential.

Vasorelaxant activity

Considering the multiple therapeutic indications of *P. heptaphyllum* derivatives, the cardiovascular activity of essential oils extracted from resins of two different origins, being one collected and the other commercially acquired, was studied in a vascular pre-contraction *in vitro* model induced by 10 $\mu\text{mol/L}$ Phenylephrine in annular sections of murine mesenteric arteries, with induction of pre-contractions in rings with and without endothelium. Results indicated that the oil induced a vasorelaxant response when added cumulatively in concentrations ranging from 0.003 to 0.75 mg/mL, applied directly to the sections, approximately 30 minutes after stabilization of the response to induction. The vasorelaxant effect was reduced in preparations containing the endothelium when compared to arterial rings without endothelium. Since phenylephrine-induced contractions are related to both voltage-sensitive Ca^{2+} channels and receptor-operated Ca^{2+} channels, it was observed that the effect of the essential oil was similar, yet inferior, to Verapamil, an L-type Ca^{2+} channel blocker. The vasorelaxant activity of different plant derivatives is often due to the blockade of Ca^{2+} channels, and thus, Ca^{2+} channel inhibitors are used as antihypertensive agents. Due to the observation of a greater vasorelaxant essential oil response in preparations without endothelium, the activity of its constituents may be increased in Ca^{2+} channels in the smooth vasculature membrane, evidencing that the oil may present more than one active secondary metabolite, leading to variations in the pharmacological response⁴.

The essential oil of the resin collected in this study was predominantly composed of Limonene, *p*-Cymene, and α -Terpineol, while the oil from the commercial resin contained Limonene, Eucalyptol, and *p*-Cymene. Among the 23 identified monoterpenes, five (*p*-Cymene, Eucalyptol, Linalool, α -Pinene, Limonene, and α -Terpineol) have been studied for their cardiovascular effects. *p*-Cymene has been associated with hypotension and bradycardia *in vivo*; Eucalyptol with a negative inotropic effect; Linalool with cardiovascular stimulation and depression; Limonene with the reduction and prevention of cardiovascular injury due to pulmonary hypertension; and α -Terpineol with hypotension, vasorelaxation, and antihypertensive effects. These findings suggest that essential oils containing these components may hold potential for preventing and treating cardiovascular diseases⁴.

In isolation, the results presented in the article demonstrate the need for further investigation of the vasorelaxant activity of the active ingredient, since they come from a single, *in vitro* observation. In subsequent studies, the evaluation of the vasorelaxant activity in an *in vivo* model can be considered, based on the results of the *in vitro* test of the vasorelaxant activity of the compound⁴. Thus, the result described constitutes limited evidence of the vasorelaxant activity of the essential oil, due to the existence of only one article related to the activity, the model applied for study, and the verification of lower pharmacological potency when compared to the model drug applied as a positive control of the vasorelaxant activity, establishing the need to obtain more robust data, in more complex experimental models, in addition to the possibility of evaluating the association of the oil with other vasorelaxant agents.

Toxicity

According to the selected studies and its evaluated concentrations, the *P. heptaphyllum* resin essential oil did not present toxic and adverse effects in animal models, demonstrating its low toxicity in the experimental systems and administration protocols studied, which include mice^{31, 32, 25, 27} and a bacterial infection model in helminths²⁶. However, there was no direct evaluation of the essential oil potential toxicity to the experimental models in some studies^{21, 31, 5}, while in other studies, the potential toxicity caused by the compound was discussed based on the performed tests parameters, general effects observed during the experiments^{31, 25, 26} and by necropsy after animals were euthanized²⁷. The essential oil samples did not show toxicity in the administration routes tested, which include oral administration^{25, 31, 32}, topical administration²⁸, direct application²⁶ and inhalation⁵. Therefore, the essential oil toxicity has not been properly clarified yet, with little evidence confirming the safety of its use, despite its current commercialization as aromatic oil.

Limitations

A critical assessment of the present systematic review highlights several limiting factors that impact the interpretation of findings. The primary limitation is the limited number of studies. The variability in resin and essential oil origins across the selected studies also hinders a more precise correlation between biological activities and active compounds, due to inherent differences in essential oil composition. Additionally, the diversity of experimental models, encompassing both in vitro and in vivo studies performed with different animal models and cell lines, contributes to heterogeneity in study design and reported outcomes. The methodological discrepancies and the limited number of articles precluded a quantitative meta-analysis of the selected articles.

Conclusions and future perspectives

This systematic review compiles the experimental evidence regarding the biological activities of the *P. heptaphyllum* resin essential oil, offering an overview of the studies involving the product using in vitro and in vivo experimental models. Despite the generally limited literature involving this essential oil, the antimicrobial, insecticidal, and anti-inflammatory potential constitute the most consistently reported and most compelling findings. The great variability in the composition of the resin essential oil reflects an intrinsic characteristic of the different resin origins, as well as their aging, stating the importance of a broad characterization of the essential oil compounds in every study carried out. The essential oil characterization allows it to be associated with the proven activities, and is of paramount importance for future commercial and clinical translation. However, it is noteworthy that several authors pointed that, even if the activities of the oil are associated with its composition, a remaining gap persists considering that the effect of the complete oil is more than the activity of specific active components. As knowledge regarding the biological activities of the *P. heptaphyllum* resin oil is set to expand, the compilation and critical analysis of the experimental evidence regarding the oil constitutes an excellent starting point for future studies. Importantly, those studies must evolve compared to the published ones, focusing on more complex and relevant experimental models, as well as the comparison between the oil samples and positive controls. Safety and efficacy must also be consistently established, with dedicated study designs, the investigation of different concentration ranges and different routes of administration. The low toxicity and varied biological activities of Breu oils render such efforts eagerly anticipated, as they are likely to build ground for the economic exploration for different purposes, ranging from therapeutic and pharmacological uses, to cosmetic and food industry applications.

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Conflict of Interest statement

Authors declare no conflict of interest.

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5. CONSIDERAÇÕES FINAIS

O presente trabalho reuniu as evidências experimentais acumuladas acerca do óleo essencial da resina de *P. heptaphyllum* ao longo do período de 1999 a 2023 em uma revisão sistemática da literatura, com o principal objetivo de sintetizar e analisar criticamente os resultados experimentais obtidos a partir dos artigos incluídos na revisão em relação às atividades biológicas associadas ao derivado natural descritas na literatura. Apesar de um extenso conhecimento etnobotânico do uso tradicional dos derivados do vegetal, estudos mais robustos e aprofundados se mostram necessários, devido às limitações associadas às evidências atuais das atividades do óleo encontradas na literatura. Embora as evidências das atividades biológicas do óleo essencial apresentem limitações, é possível destacar que as atividades antimicrobianas, inseticidas e anti-inflamatórias do metabólito são as mais promissoras dentre seus efeitos estudados. Por fim, há uma lacuna significativa no que se refere ao perfil de toxicidade do óleo essencial, apesar de sua comercialização como óleo aromático, estabelecendo a necessidade de sua investigação adequada. Assim, o estudo destas atividades em modelos experimentais mais robustos também se mostram pertinentes, não somente para verificação dos usos, como para assegurar a segurança e eficácia do uso do óleo essencial futuramente nas mais diversas aplicações possíveis.

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