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Mestrado

BIOPROSPECÇÃO DA GEOPRÓPOLIS DE *Melipona fasciculata* Smith: ESTUDO QUÍMICO E AVALIAÇÃO DA ATIVIDADE ANTITUMORAL EM LINHAGENS DE CÂNCER DE PULMÃO

FRANCISCO ASSIS NASCIMENTO PEREIRA

São Luís
2022

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Defesa submetida ao Programa de Pós-Graduação em Ciências da Saúde, área de concentração, Biotecnologia Aplicada ao Estudo de Produtos Naturais e Sintéticos, como requisito parcial para obtenção de título de Mestre em Ciências da Saúde.

São Luís

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“Se cheguei até aqui foi porque me apoiei no ombro dos gigantes.”

Isaac Newton

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RESUMO

A abelha sem ferrão *Melipona fasciculata* Smith (tiúba) acumula pólen e produz mel, cera e geoprópolis, que é uma mistura de resinas vegetais, secreções salivares, cera e terra/ou barro. A geoprópolis tem atividades biológicas comprovadas, como anti-inflamatória, antioxidante, antimicrobiana, cicatrizante, leishmanicida, imunomoduladora e anti-helmíntica. Entretanto, há escassez de referências de ação anticâncer da geoprópolis da espécie. Considerando que mundialmente, o câncer de pulmão possui grande mortalidade, e os fármacos convencionais de tratamento de câncer vem apresentando resistência, torna-se importante a busca de novas substâncias a partir dos produtos naturais. Dessa forma, a pesquisa objetivou fazer uma revisão bibliográfica de estudos pré-clínicos de própolis e geoprópolis de espécies de abelhas sem ferrão na área de câncer e estudos de bioprospecção da geoprópolis de *Melipona fasciculata* Smith em modelo de avaliação *in vitro* de atividade em células de câncer de pulmão, assim como identificação química das subfrações bioativas. Amostras de geoprópolis de tiúba foram coletadas em meliponário na cidade de Viana, Maranhão, as quais foram secas, trituradas e extraídas por maceração com etanol 70%, obtendo extrato hidroetanolico da geoprópolis e depois liofilizado (EHGV). EHGV foi submetido a partição líquida-líquida, obtendo-se as frações hexânica (HF), acetato de etila (EAF) e hidrometanólica (HMF). Fracionamento biodirecionado da EAF por cromatografia em coluna resultou em 13 subfrações (FRGE-1 a FRGE-13). As subfrações bioativas foram identificadas por LC e LC–ESI/IT–EM/EM. A citotoxicidade *in vitro* de frações e subfrações foi avaliada em linhagens celulares de câncer de pulmão (H460 e A549) e células normais (HUVEC) pelo ensaio de brometo de 3-4,5-dimetiltiazol-2,5-difenil (MTT) e os índices de seletividade determinados. Os resultados da revisão da literatura estão publicados na revista *Pharmaceuticals*, demonstrando a citotoxicidade da própolis e geoprópolis de espécies de abelhas sem ferrão em diferentes linhagens tumorais. Confirmando os resultados da revisão bibliográfica, HF, EAF e HMF inibiram a proliferação celular de linhagens celulares de câncer de pulmão (H460 e A549) em 48h. As subfrações FRGE-9 e FRGE-11 são três vezes mais ativas em células tumorais de pulmão (A549) do que em células normais. As subfrações bioativas são constituídas de ácido dihidroxibenzóico, apigenina 6-C-glicosídeo e luteolina-5-O-beta-glicosídeo (FRGE-9), diosmetina e miricetina-3-galactosídeo (FRGE-11). Diosmetina, apigenina 6-C-glicosídeo, luteolina-5-rutinosídeo e miricetina-3-galactosídeo estão sendo identificados pela primeira vez na geoprópolis de *M. fasciculata*. Sugerimos que a composição química das subfrações bioativas estão relacionadas com ação antitumoral contra H460 e A549. Os resultados encontrados estimulam a continuidade das pesquisas na perspectiva de buscar novos agentes contra câncer de pulmão partir da geoprópolis de *Melípona fasciculata.*

Palavras-chaves: câncer; composição química; neoplasia pulmonar; abelhas sem ferrão.

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ABSTRACT

The stingless bee *Melipona fasciculata* Smith (tiúba) accumulates pollen and produces honey, wax and geopropolis, which is a mixture of plant resins, salivary secretions, wax and earth/or clay. Geopropolis has proven biological activities, such as anti-inflammatory, antioxidant, antimicrobial, healing, leishmanicidal, immunomodulatory and anthelmintic. However, there is a lack of references to anti-cancer actions of the geopropolis of the species. Considering that worldwide, lung cancer has high mortality, and conventional cancer treatment drugs have been showing resistance, it is important to search for new substances from natural products. Thus, the research aimed to make a bibliographic review of pre-clinical studies of propolis and geopropolis of stingless bee species in the area of cancer and bioprospecting studies of geopropolis from *Melipona fasciculata* Smith in an in vitro evaluation model of activity in cells of lung cancer, as well as chemical identification of bioactive subfractions. The geopropolis samples of tiúba were collected in a meliponary in *the* city from Viana, Maranhão, which were dried, tritured and extracted by maceration with 70% ethanol, obtaining. hydroethanolic extract of geopropolis and later lyophilized (EHGV). EHGV was subjected to liquid-liquid partition, obtaining the hexane (HF), ethyl acetate (EAF) and hydromethanolic (HMF) fractions. Biodirected fractionation of EAF by column chromatography resulted in 13 subfractions (FRGE-1 to FRGE-13). Bioactive subfractions were identified by LC and LC–ESI/IT–EM/MS. The in vitro cytotoxicity of fractions and subfractions was evaluated in lung cancer cell lines (H460 and A549) and normal cells (HUVEC) by the 3-4,5-dimethylthiazol-2,5-diphenyl bromide (MTT) assay and the determined selectivity indices. The results of the literature review are published in the journal *Pharmaceuticals* 2021, demonstrating the cytotoxicity of propolis and geopropolis from stingless bee species in different tumor lineages. Confirming the results of the literature review, HF, EAF and HMF inhibited cell proliferation of lung cancer cell lines (H460 and A549) within 48h. The FRGE-9 and FRGE-11 subfractions are three times more active in lung tumor cells (A549) than in normal cells. The bioactive subfractions consist of dihydroxybenzoic acid, apigenin 6-C-glycoside and luteolin-5-O-beta-rutinoside (FRGE-9), diosmetin and myricetin-3-galactoside (FRGE-11). Diosmetin, apigenin-6-C-glucoside, luteolin-5-rutinoside and myricetin-3-galactoside are being identified for the first time in the geopropolis of *M. fasciculata.* We suggest that the chemical composition of the bioactive subfractions is related to the antitumor action of H460 and A549 lung cancer. The anticancer results found stimulate the continuity of research in the perspective of finding new agents against lung cancer from geopropolis of *Melipona fascicula.*

Keywords: cancer; chemical composition; lung neoplasm; stingless bees.

LISTA DE ABREVIATURAS

$AlCl_3$	Cloreto de alumínio
ALK	Receptor tirosina quinase
CD80	Grupo de diferenciação 80
CPCP	Câncer de pulmão de células pequenas
DPOC	Doença Pulmonar Obstrutiva Crônica
EGFR	Receptor do fator de crescimento epidérmico
HER-2	Receptor tipo 2 do fator de crescimento epidérmico humano
HepG2	Carcinoma hepatocelular humano
HIV	Vírus da imunodeficiência humana
HL-60	Leucemia promielocítica humana
CI_{50}	Concentração Inibitória 50%
IL-6	Interleucina 6
IL-10	Interleucina 10
IS	**Í**ndice de seletividade
K562	Leucemia mielocítica crônica humana
LUAD	Adenocarcinoma pulmonar
LUSC	Carcinoma escamoso de pulmão
MCF-7	Linhagem celular de câncer de mama
MET	Receptor de tirosina quinase
NSCLC	Câncer de pulmão de células não pequenas
ORR	Taxa de resposta geral
PARP	Poly (ADP-ribose) polymerase
P&D	Pesquisa e Desenvolvimento
PIK3CA	Subunidade catalítica alfa de fosfatidilinositol-4,5-bisfosfato 3-quinase
TKIs	Inibidores da tirosinase
TNF-α	Fator de necrose tumoral-alfa
UV	Ultravioleta

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1 INTRODUÇÃO

O câncer é um dos principais problemas de saúde pública no mundo e está entre as quatro principais causas de morte na maioria dos países. A incidência e a mortalidade por câncer vêm aumentando no mundo, em parte pelo envelhecimento, pelo crescimento populacional, como também pela mudança na distribuição e na prevalência dos fatores de risco de câncer, especialmente aos associados ao desenvolvimento socioeconômico (AMERICAN CANCER SOCIETY, 2018).

O câncer de pulmão é o segundo mais incidente no mundo, seguido pelo câncer colorretal, próstata e estômago (SUNG et al., 2021). No contexto brasileiro, há uma estimativa para cada ano do triênio 2020-2022, a incidência de câncer de mama e próstata (66 mil cada), cólon e reto (41 mil), pulmão (30 mil) e estômago (21 mil) (INCA, 2020).

Nessa perspectiva, os produtos naturais ao longo as história vem sendo pesquisados, como fontes de buscas de substâncias bioativas e têm contribuído para o desenvolvimento e produção de várias drogas utilizadas na clínica médica para os tratamentos de diferentes tipos de cânceres: vimblastina (Velban®) e vincristina (Oncovin®); os análogos vindesina (Eldisrine®) e vinorelbina (Navelbine®); paclitaxel (Taxol®) e o análogo docetaxel (Taxotere®); podofilotoxina e os análogos, etoposídeo (Etopophos®) e teniposídeo (Vumon®); e camptotecina (COSTA-LOTUFO et al., 2010; ALVARENGA et al., 2014; PAIER et al., 2018).

No entanto, apesar de já existirem muitos quimioterápicos efetivos para tratamento dos cânceres, os efeitos adversos produzidos por estes fármacos, visto que, agem de forma não-específica, lesando tanto células malignas quanto normais, assim como o surgimento de tumores resistentes e/ou a seleção de células resistentes em um tumor sob tratamento limitam o uso desses fármacos, aumentando a necessidade do desenvolvimento de novas drogas provenientes de produtos naturais mais eficazes (NEWMAN; CRAGG, 2020).

Produtos naturais, como os derivados de abelhas sem ferrão, podem ser promissores na busca de novas moléculas e/ou alternativas coadjuvantes na terapia do câncer (LAVINAS et al., 2018).

O extrato hidroetanólico da geoprópolis, produzida por *Melipona fasciculata* Smith, abelha sem ferrão, conhecida popularmente como tiúba, tem demonstrado atividade antitumoral em ensaios *in vitro* (CINEGAGLIA et al., 2013; ASSUNÇÃO,

2011; CUNHA, 2017; BARBOZA et al., 2020; PEREIRA, 2021). Extratos da geoprópolis de outras espécies de abelhas sem ferrão, como *Melipona scutellaris* inibem em baixas concentrações o crescimento total de várias linhagens tumorais (DA CUNHA et al., 2013), assim como *Melipona mondury*, possui atividade contra carcinoma hepatocelular humano (DOS SANTOS et al., 2017a).

Considerando que a maioria dos trabalhos descritos na literatura sobre citotoxicidade em diversas linhagens tumorais e de composição química de produtos de abelhas sem ferrão utilizaram apenas os extratos da geoprópolis, torna-se imperioso a continuação dos estudos relativos a separação em frações apolares e polares dos extratos, e os testes antitumorais destes produtos, assim como a identificação química.

Diante dos resultados preliminares do extrato bruto da geoprópolis de *M. fasciculata* em linhagens tumorais e a alta incidência e mortalidade do câncer de pulmão, essa pesquisa objetivou avaliar a atividade antitumoral em duas linhagens de câncer de pulmão das frações purificadas do extrato hidroetanólico da geoprópolis, bem como a identificação da composição química.

Com os resultados esperamos demonstrar o valor terapêutico das frações separadas do extrato hidroetanólico da geopropólis na perspectiva de sugerir futuros protótipos de moléculas para o tratamento do câncer a partir de um produto que é, normalmente, negligenciado pelos meliponicultores durante a etapa de asseio da colmeia de *Melipona fasciculata.*

2 REFERENCIAL TEÓRICO

2.1 Abelhas sem ferrão

As abelhas sem ferrão ou meliponíneos fazem parte da subfamília Meliponinae (Hymenoptera, Apidae) e são caracterizadas por possuírem ferrões atrofiados, facilitando o manejo na coleta de seus produtos. Esse comportamento mais dócil facilitou sua domesticação por indígenas. No entanto, não são totalmente indefesas, pois apresentam comportamentos de defesa, como a capacidade de se emaranhar nos cabelos e pelos do agressor e de construir ninhos com entrada estreita nas colmeias e a habilidade de se camuflarem (CAMARGO et al., 2017).

Os meliponíneos são insetos sociais de grande diversidade e ampla distribuição geográfica, ocupando praticamente toda a América Latina e África, além do sudeste

asiático e norte da Austrália. Todavia, é nas Américas que grande parte da diversidade de espécies ocorre, com aproximadamente 400 tipos descritos (VILLAS-BÔAS, 2018). Elas podem ser divididas em 02 (duas) tribos distintas: Meliponini e Trigonini. De maneira geral, Meliponini, com espécies unicamente do gênero *Melipona*, são abelhas maiores, com aspecto robusto, de tamanho médio a grande (variando de 7 a 15 mm); popularmente conhecidas como: uruçus, jandaíras, tiúbas, mandaçaias e equivalentes. Já a tribo Trigonini é representada por espécies de todos os outros gêneros, representando abelhas menores, de aspecto mais esbelto, de tamanho pequeno a médio (variando de 2 a 11 mm); mais conhecidas popularmente como: jataís, iraís, mirins e canudos (KERR, 1987; NOGUEIRA-NETO, 1997; VILLAS-BÔAS, 2018).

As abelhas sem ferrão são insetos pantropicais (presentes apenas em regiões tropicais e subtropicais) e eussociais (vivem em colônias permanentes com divisões de castas). Organizam-se em colônias permanentes, que podem ser bastante numerosas, variando desde poucas dúzias a 100.000 ou mais operárias (SILVEIRA et al., 2002; MICHENER, 2007).

O cultivo de abelhas sem ferrão é denominado de meliponicultura, representando prática antiga, desenvolvida há muitos séculos, com relatos desde as civilizações antigas, como no Egito Antigo (PALAZUELOS BALLIVIAN, 2008) e o local de criação é denominado meliponário (Figura 1).

No Brasil, a meliponicultura foi inicialmente desenvolvida pelos índios e ao longo do tempo tem sido praticada de forma tradicional por pequenos e médios produtores, principalmente por aqueles que usavam mão de obra familiar nas atividades agropecuárias, sendo considerada atividade econômica complementar (COLETTO-SILVA, 2005; MAIA et al., 2017). Nos dias atuais é praticada por indígenas e comunidades tradicionais e camponesas, em diversas regiões do Brasil (MAIA et al., 2017).

A meliponicultura se destaca pelo seu fácil manejo, não interfere no tempo gasto de outras atividades agropecuárias e possui ampla aceitação popular. Isto se deve ao fato do mel de abelhas-sem-ferrão apresentar grande valor cultural e ser normalmente utilizado para fins medicinais. Além do mel, outros subprodutos das abelhas-sem-ferrão, como a geoprópolis, a própolis, o pólen e a cera, apresentam grande potencial como alternativa para auxiliar na subsistência em pequenas

propriedades rurais. Para outros a criação de abelhas se configura como atividade recreativa (PEREIRA et al., 2012).

Figura 1 – Meliponário, local de criação das abelhas sem ferrão.
Fonte: PEREIRA (2017)

Essa atividade é importante para conservação ambiental, pois permite a polinização de espécies vegetais e nativas e contribui para a diminuição do desmatamento e de danos ambientais. Para que a meliponicultura se desenvolva, são necessários alguns requisitos: ausência de ventos fortes, que dificultam o voo das abelhas e proximidade de vegetação que forneça alimento às colônias e de água corrente. Além disso, as colmeias devem ser colocadas à distância mínima de sessenta centímetros do solo e abrigadas do sol e das chuvas. Muitos meliponicultores utilizam galpões ou suportes individuais com telhado para instalação das colmeias (CARVALHO et al., 2014).

Dentre as abelhas sem ferrão, *Melipona fasciculata* Smith (Figura 2), conhecida como tiúba, tiúba do Maranhão, uruçu-cinzenta, tiúba-grande ou jandaíra-preta-da-Amazônia, é uma espécie nativa encontrada nas áreas de transição dos biomas Mata Atlântica e Cerrado. Distribui-se geograficamente nos estados de Tocantins, Pará, Piauí, Mato Grosso e Maranhão (CAMARGO; PEDRO, 2013).

Morfologicamente, a tiúba mede cerca de 12mm de comprimento, possui coloração predominantemente acinzentada. Segmentos abdominais com faixas

esbranquiçadas, contínuas ou interrompidas no centro, além disso, apresenta pilosidade esbranquiçada abundante pelo corpo e pilosidade avermelhada presente próxima a inserção das asas (COSTA, 2019).

Figura 2 - *Melipona fasciculata* Smith
Fonte: MESQUITA (2016)

A classificação taxonômica da espécie *Melipona fasciculata* Smith está demonstrada na Tabela 1.

Tabela 1. Classificação taxonômica da espécie *Melipona fasciculata* Smith

	Classificação taxonômica
Classe	Insecta
Ordem	Hymenoptera
Superfamília	Apoidea
Família	Apidae
Subfamília	Apinae
Tribo	Meliponini
Gênero	*Melipona*
Espécie	*Melipona fasciculata* Smith

Fonte: VILLAS-BÔAS (2018)

No estado do Maranhão, o cultivo de *Melipona fasciculata* Smith exerce uma importante atividade econômica para comunidade local com produção e comercialização do mel (HOLANDA, 2012). Outros produtos meliponículas da espécie possuem grandes potencialidades, como a geoprópolis, mas devido, o pouco conhecimento difundido, este produto torna-se subestimado.

A geoprópolis é formada por material resinoso coletado das plantas pelas abelhas, trazida para sua colméia, misturando com secreções salivares, cera e adicionando barro ou terra. A geoprópolis também é conhecida como batume, é uma

estrutura porosa de coloração cinza, marrom ou preta que protege a cavidade interna do ninho do meio externo, vedando frestas e delimitando o espaço ocupado pelo mesmo (Figura 3). Esta é construída para proteger a colmeia contra a invasão de insetos e serve como suporte no controle da temperatura. Pode ser constituído por uma camada única, fina e frágil que se quebra quando perturbado e permite a saída rápida de muitas abelhas para a defesa e que suaviza irregularidades das paredes de madeira ou da cavidade subterrânea. Entretanto, nas espécies do gênero *Melipona*, os batumes podem formar uma camada mais espessa para fechar o excesso de espaço nas cavidades, onde os ninhos são construídos (WITTER, 2014; VILLAS-BÔAS, 2018).

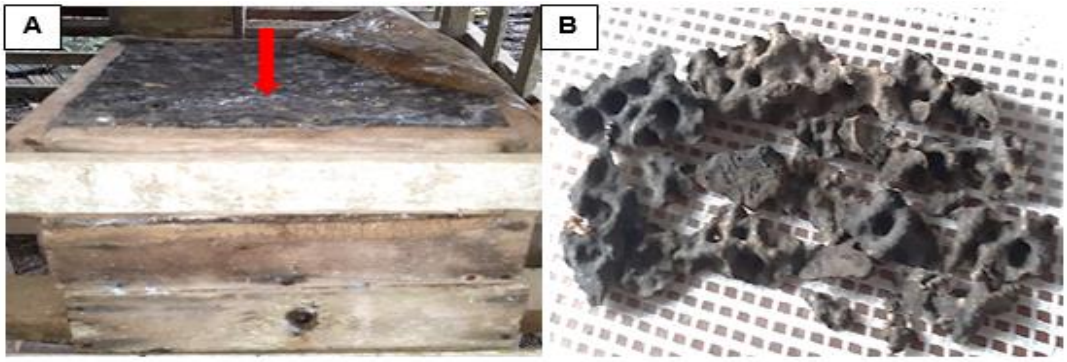

Figura 3 – (A) Destaque da geoprópolis na parte superior (seta vermelha) da caixa da colmeia de *Melipona fasciculata* Smith. (B) Geoprópolis de *Melipona fasciculata* Smith

Fonte: PEREIRA (2017)

Esse material apresenta uma composição química complexa que pode variar de acordo com as espécies vegetais de cada região. A mesma é utilizada tradicionalmente por índios e comunidades rurais para tratar doenças pulmonares, contra inapetência, infecção dos olhos, fortificantes e agentes bactericidas (CARVALHO-ZILSE et al., 2011).

Na literatura são descritas algumas atividades biológicas da geoprópolis, como: ação antinoceptiva (FRANCHIN et al., 2012), antioxidante (DUTRA et al., 2014; BATISTA et al., 2016; SILVA et al., 2016; DOS SANTOS et al., 2017a; FERREIRA et al., 2017), leishmanicida (DUTRA et al., 2019), antiviral (COELHO et al., 2015), gastroprotetora (RIBEIRO-JUNIOR et al., 2015), antimicrobiana (LIBERIO et al., 2011; ARAÚJO, 2013; DA CUNHA et al., 2013; DE SOUSA et al., 2015, SILVA et al., 2016; DOS SANTOS et al., 2017a; SOUZA JÚNIOR et al., 2019), anti-inflamatória (DOS SANTOS et al., 2017b) e antitumoral (CINEGAGLIA et al., 2013; DA CUNHA et al.,

2016; BARTOLOMEU et al., 2016; CUNHA 2017; PEREIRA, 2021; BARBOZA et al., 2020). Estudos de Barboza et al. (2019) indicaram baixa toxicidade da geoprópolis.

2.2 Composição química da geoprópolis

A composição química da geoprópolis é complexa, pois é dependente da visita das abelhas nas resinas das espécies vegetais (VILLA-BÔAS, 2018). As principais classes de compostos identificadas por pesquisadores são fenólicos, ácidos graxos, açúcares, álcoois e terpenos. Diferentes técnicas analíticas são empregadas para identificação dos compostos químicos, como cromatografia líquida de alta eficiência acoplada a espectrometria de massas (CLAE/EM), cromatografia gasosa acoplada a espectrometria de massas (CG/EM) e ressonância magnética nuclear de hidrogênio e carbono 13 (RMN ^{1}H e ^{13}C) (Tabela 2).

A partir das análises químicas realizadas por Bankova et al. (1998) da geoprópolis das espécies *Melipona quadrifasciata anthidioides* e *Melipona fasciculata,* foram identificados ácidos graxos de cadeia longa (esteárico, palmítico, mirístico e oleico), ácido cinâmico, diterpenos, ácidos láctico e fosfórico

Dutra et al. (2008) submeteram amostras da geoprópolis coletadas nos municípios de Arari, São João Batista e São Bento, da Baixada Maranhense, a cromatografia em camada delgada, após revelar no UV e borrifar com $AlCl_3$ 1%, constataram a presença de substâncias flavonoídicas.

Da Cunha et al. (2013) identificaram no extrato etanólico da geoprópolis de *Melipona scutellaris* e sua respectiva fração hexânica por CG/EM, a presença de benzofenonas, como compostos majoritários e a ausência de flavonoides.

O fracionamento biomonitorado do extrato etanólico da geoprópolis de *Melipona scutellaris* utilizando Sephadex LH-20 e separação por CLAE de fase normal, levaram ao isolamento de dois novos ésteres de ácido cinâmico, cumarinas do tipo mameia (5,7-dihidroxi-6-(3-metil-2-butenil)-8- (4-cinamoil-3-metil-1-oxobutil)-4-propil-cumarina e 5,7-di-hidroxi-6-(4-cinamoil-3-metil-1-oxobutil)-4-fenilcumarina), assim como cinco cumarinas conhecidas (mammeigina, hidroximammeigina, mammeisina, cinamoiloxi-mammeisina e mammeina) e a benzofenona prenilada (ent-nemorosona) (DA CUNHA et al., 2016).

Na investigação química do extrato metanólico da geoprópolis *de Melipona interrupta* por Da Silva et al. (2013) e suas respectivas frações clorofórmica e acetato

de etila, as quais foram submetidas a cromatografia em coluna e em cromatografia em camada delgada preparativa, levaram ao isolamento dos flavonoides 5,7,4'-trihidroxiflavonona, 3,5,6,7,4'-pentahidroxiflavonol, miricetina-3-O-β-D-glucopiranosídeo e naringenina-4'-O-β-D-glucopiranosídeo.

De Souza et al. (2013) realizaram separação cromatográfica utilizando Sephadex LH-20 da fração de acetato de etila da geoprópolis de *Melipona subnitida*, isolando nove compostos fenólicos, os quais foram identificados por CLAE, sendo dois fenilpropanóides (6-O- *p*-coumaroil-D-galactopiranose e 6-O-cinamoil-1-O-*p*-coumaroil-*β*-D-glucopiranose) e sete flavonóides (7-O-metil-naringenina, 7-O-metil aromadendrina, 7,4'-di-O-metil-aromadendrina, 4'-O-metil-campferol, 3-O-metil-quercetina, 5-O-metil-aromadendrina e 5-O-metil kaempferol).

De Souza et al. (2018) identificaram por cromatografia líquida acoplada a espectrometria de massas (CL/EM), 51 compostos fenólicos da geoprópolis de *Melipona subnitida* Ducke, sendo quatro glicosídeos de galoil, onze acil-(cinamoil/coumaroil)-hexosídeos, vinte e três acil-(cinamoíl/coumaroil)-galoil-, hexosídeos, doze flavonoides (agliconas e O-glicosídeos acilados) e o ácido elágico.

Dos Santos et al. (2017b) demonstraram que a composição química do extrato hidroetanólico da geoprópolis de *Melipona orbignyi* possui substâncias fenólicas, como coumaroil-galoil-hexosídeo, digaloil-coumaroil-hexosídeo, cinamoil-galoil-hexosídeo, digaloil-cinamoil-hexosídeo, dicoumaroil-hexosídeo, dicoumaroil-galoil-hexosídeo, cinamoil-coumaroil-hexosídeo, cinamoil-coumaroil-galoil-hexosídeo, aromadendrina, metil-aromadendrina e naringenina, além de terpenos (sesquiterpenos, diterpenos e triterpenos).

Compostos voláteis foram identificados no extrato da geoprópolis de *Melipona beecheii* por Torres-González et al. (2016), como β-fencheno (14,53–15,45%), estireno (8,72–9,98%) e benzaldeído (7,44–7,82%). Outra classe relevante de voláteis identificados foram os monoterpenos, como (Z)–ocimenona (5,33–5,67%), *α*-pineno (3,98–4,66%), *m*-cimeno (3,90–4,58%), *trans*-isocarveol (2,71–2,89%), limoneno (2,66–2,90%), verbenona (2,46–2,72%), β-pineno (2,15–2,57%), acanfolenal (2,09–2,35%), *m*-cimeneno (2,05–2,33%), *trans*-pinocanfona (2,05–2,19%) e *trans*-pulegol (1,89-2,23%).

Batista et al. (2016) identificaram na geoprópolis de *Melipona fasciculata por* CL/EM, terpenos, incluindo cicloartano, cicloartenol, oleanano, β-amirina, cicloursano e ácido-urs-12-en-24-óico, bem como esteroides, fenólicos, ácidos graxos e açúcares.

Compostos fenólicos pertencentes as classes de ácidos fenólicos e taninos hidrolisáveis (galotaninos e elagitaninos) foram identificados na geoprópolis de *Melipona fasciculata* por CL/EM. O estudo correlacionou o alto teor dos compostos fenólicos com a ação antioxidante da geoprópolis produzida pela espécie (Dutra et al., 2014).

Barboza et al. (2020) identificaram flavonóides glicosilados, taninos hidrolisáveis, antraquinonas, catequinas, triterpenos e saponinas triterpênicas nos extratos da geoprópolis de *Melipona fasciculata*, oriundos dos municípios de Viana e Pinheiro, no estado do Maranhão.

A composição química dos extratos da geoprópolis de espécies de *Melipona* estão descritas na tabela 2.

Tabela 2. Dados da literatura da composição química da geoprópolis do gênero *Melipona*

Espécie de abelha	Local de Origem	Classe de compostos	Compostos químicos	Método Analítico	Referências
Melipona scutellaris	Bahia, Brasil	Fenólicos	5,7-dihidroxi-6-(3-metil-2-butenil)-8-(4-cinamoil-3-metil-1-oxobutil)-4-propil-cumarina, 5,7-dihidroxi-6-(4-cinamoil-3-metil-1-oxobutil)-4-fenillcumarina, mammeigina, hydroximammeigina, mammeisina, cinnamoiloxi-mammeisina, mammeina, *ent*-nemorosona, 2-propensaeure 3-fenil-trimetilsililéster, ácido 1,2-benzenodicarboxílico	CLAE/EM; CG/EM	Da Cunha et al., 2013; 2016
Melipona interrupta	Amazonas, Brasil	Fenólicos	naringenina, aromadendrina, naringenina-4'-O-β-glicopiranosídeo, miricetina-3-O-β-glicopiranosídeo	CLAE/EM; RMN	Da Silva et al., 2013
Melipona subnitida	Paraíba, Brasil	Fenólicos	6-O-p-coumaroil-D-galactopiranose, 6-O-cinamoil-1-O-p-coumaroil-D-glucopiranose, 7-O-metil-naringenina, 7-O-metil aromadendrina, 7,4'-Di-O-metil aromadendrina, 4'-O-metil kaempferol, 3-O-metil quercetina, 5-O-metil aromadendrina, 5-O-metil kaempferol, 3-O-metil quercetina, tri-hidroxiflavanona-O-coumaroil-hexosídeo, naringenina, di-hidroxiflavanona-O-coumaroil-hexosídeo, di-hidroxi-metoxi-flavanona-O-coumaroil-hexosídeo, dihidroxi-metoxi-flavanona-O-coumaroil-hexosídeo, 7-O-metil naringenina, aromadendrina, 7-O-metil-aromadendrina, di-O-galoil-glicose, tetra-O-galoil-hexosídeo, ácido elágico, 6-O-*p*-coumaroil-α-D-galactose, coumaroil-hexosídeo, 6-O-*p*-coumaroil-β-D-galactose, 6-O-*p*-coumaroil-α-D-galactose, coumaroil-hexosídeo, di-O-coumaroil-hexosídeo, 1,6-di-O-(E)-coumaroil glicopiranosídeo,	CLAE/EM; RMN	De Souza et al., 2013; 2018

CLAE/EM= Cromatografia líquida de alta eficiência acoplada a espectrometria de massas; CG/EM= Cromatografia gasosa acoplada a espectrometria de massas; RMN= Ressonância Magnética Nuclear

Tabela 2. Continuação.

Espécie de abelha	Local de Origem	Classe de compostos	Compostos químicos	Método analítico	Referências
Melipona subnitida	Paraíba, Brasil	Fenólicos	di-O-coumaroil-hexosídeo, 6-O-cinamoil-1-O-*p*-coumaroil-β-D-glicopiranose, coumaroil-O-cinamoil-hexosídeo, coumaroil-O-galoil-hexosídeo, coumaroil-di-O-galoil-hexosídeo, cinamoil-O-galoil-hexosídeo, cinamoil-di-O-galoil-hexosídeo, di-O-coumaroil-O-galoil-hexosídeo, 1,6-di-O-(E)-coumaroil-2-O-galoil-β-D-glicopiranosídeo, 1-O-(E)-coumaroil-6-O-(E)-cinamoil-2-O-galoil-β-D-glicopiranosídeo, coumaroil-O-cinamoil-O-galoil-hexosídeo	CLAE/EM; RMN	De Souza et al., 2013; 2018
Melipona quadrifasciata anthidioides	Mato Grosso do Sul, Brasil	Fenólicos	ácido benzóico, álcool *p*-cumárico, ácido vanilínico, ácido cinâmico, ácido diidroferúlico, vanilina, coniferilaldeído, coumaroil-galoil-hexosídeo, digaloil-coumaroil-hexosídeo, cinamoil-galoil-hexosídeo, digaloil-cinamoil-hexosídeo, dicoumaroil-hexosídeo, dicoumaroil-galoil-hexosídeo, cinamoil-coumaroil-hexosídeo, cinamoil-coumaroil-galoil-hexosídeo, aromadendrina, metil-aromadendrina, naringenina, ácido gálico, ácido elágico, ácido *p*-cumárico, O-galoil hexosídeo, metil-narigenina, luteolina-metil-éster, quercetina-3-metil-éster	CLAE/EM; GC/EM	Bankova et al., 1998; Rubinho et al., 2020
	Paraná, Brasil	Ácidos graxos	ácido láurico, ácido mirístico, ácido pentadecanóico, ácido palmítico, ácido palmitoléico, ácido margarínico, ácido esteárico, ácido oleico		
		Ácido orgânico	ácido láctico		
		Terpenos	ácido diterpênico		

CLAE/EM= Cromatografia líquida de alta eficiência acoplada a espectrometria de massas; CG/EM= Cromatografia gasosa acoplada a espectrometria de massas; RMN= Ressonância Magnética Nuclear

Tabela 2. Continuação.

Espécie de abelha	Local de Origem	Classe de compostos	Compostos químicos	Método analítico	Referências
Melipona beecheii	Veracruz, México	Fenólicos	β-fencheno, estireno, benzaldeído, (Z)-ocimenona, *α*-pineno, *m*-cimeno, *trans*-isocarveol, limoneno, verbenona, β-pineno, α -canfolenal, *m*-cimeneno, *trans*-pinocanfona e *trans*-pulegol	CG/EM	Torres-González et al., 2016
Melipona orbignyi	Mato Grosso do Sul, Brasil	Fenólicos	coumaroil-galoil-hexosídeo, digaloil-coumaroil-hexosídeo, cinamoil-galoil-hexosídeo, digaloil-cinamoil-hexosídeo, dicoumaroil-hexosídeo, dicoumaroil-galoil-hexosídeo, cinamoil-coumaroil-hexosídeo, cinamoil-coumaroil-galoil-hexosídeo, aromadendrina, metil-aromadendrina e naringenina	CLAE/EM	Dos Santos et al., 2017b
		Terpenos	sesquiterpenos, diterpenos e triterpenos		
Melipona fasciculata	Maranhão/Piauí, Brasil	Fenólicos	ácido gálico, HHDP-galoilglicose, HHDP-glicose, di-HHDP-glicose, trigaloil glicose, HHDP-digaloilglicose isômero, ácido vanolêico dilactona, trisgaloil-HHDP-glicose isomêro, di-HHDP-galoilglicose, trigaloil-HHDP-glicose, ácido elágico, ácido protocatecuico, corilagina, miricetina-3-O-arabinopiranosídeo, purina, taxifolina 7-O-ramnosídeo, isoschaftosídeo, tifaneosídeo, dihidroquercetina-C-glicosídeo, narigenina-C-glicosídeo, vitexina-O-galato, pinobanksina glicosilada, dihidroquercetina-3-O-ramnosídeo, galocatequina-xilose, alquilresorcinol, ácido anacárdico, ácido heptedecenil salicílico, ácido nonadecenil salicílico, ácido pentadecenil salicílico	CLAE/EM; CG/EM	Bankova et al., 1998 Batista et al., 2016; Araújo et al., 2015; Dutra et al., 2014; 2019; Barboza et al., 2020

CLAE/EM= Cromatografia líquida de alta eficiência acoplada a espectrometria de massas; CG/EM= Cromatografia gasosa acoplada a espectrometria de massas; RMN= Ressonância Magnética Nuclear.

Tabela 2. Continuação.

Espécie de abelha	Local de Origem	Classe de compostos	Compostos químicos	Método analítico	Referências
Melipona fasciculata	Maranhão/Piauí, Brasil	Fenólicos	heptadecilresorcinol, nonadecilresorcinol, pentadecilresorcinol, heptadecadienilresorcinol, ácido *p*-hidroxibenzoico, ácido cinâmico, ácido *cis*-*p*-cumárico, ácido *trans*-*p*-cumárico, álcool benzílico, hidroquinona, 3- (2-hidroxifenil) -propanol, 3- (4-hidroxifenil) -propanol, *p*-hidroxibenzaldeído, δP-hidroxiacetofenona pinobanksina, dihidroximetoxiflavanona	CLAE/EM; CG/EM	Bankova et al., 1998 Batista et al., 2016; Araújo et al., 2015; Dutra et al., 2014; 2019; Barboza et al., 2020
		Terpenos	ácido 3-oxo-urs-12-en-24-oic, cicloursano,cicloartenol, ácido oleanólico, lupenona, taraxerona, dipterocarpol, marsformosanona, β-amirina, 3-[Xyl]-28-Glc-fitolacagenina, lupeol, *α*-amirenona, *β*-amirenona, cetona triterpênica, ácido desidroabiético, ácido diterpênico, friedooleanano-3-on		
		Ácido orgânico	ácido glicônico, ácido glicólico, ácido quínico, ácido glicurônico, ácido láctico e ácido metilmalônico		
		Antraquinona	ácido xantolacaico A		
		Ácido inorgânico	ácido fosfórico		
		Ácidos graxos	ácido palmítico, ácido esteárico, ácido linoleico, ácido melíssico, ácido octenoico, ácido oleico, ácido araquídico, ácido beénico, ácido lignocérico, ácido láurico, ácido pentadecanóico e ácido palmitoléico		

CLAE/EM= Cromatografia líquida de alta eficiência acoplada a espectrometria de massas; CG/EM= Cromatografia gasosa acoplada a espectrometria de massas; RMN= Ressonância Magnética Nuclear.

Tabela 2. Continuação.

Espécie de abelha	Local de Origem	Classe de compostos	Compostos químicos	Método analítico	Referências
Melipona fasciculata	Maranhão/Piauí, Brasil	Açúcares	glicose, frutose, manose, arabinose, galactose, fucose, sorbose, xilose, ribose, aucubina	CLAE/EM; CG/EM	Bankova et al., 1998 Batista et al., 2016; Araújo et al., 2015; Dutra et al., 2014; 2019; Barboza et al., 2020
		Álcools	eritritol, arabitol, sorbitol, glicerol, xilitol, inositol,		
		Esteróides	lanosterol, acetato lanosterol, *β*-sitosterol, estigmasterol, campesterol		

CLAE/EM= Cromatografia líquida de alta eficiência acoplada a espectrometria de massas; CG/EM= Cromatografia gasosa acoplada a espectrometria de massas; RMN= Ressonância Magnética Nuclear.

2.3 Câncer

O câncer é uma das doenças que mais tem causado mortalidade no mundo, com maior incidência de câncer de mama (2,3 milhão), pulmão (2,2 milhão), colorretal (1,9 milhão), próstata (1,4 milhão) e estômago (1,1 milhão) (SUNG et al., 2021). Para o Brasil, a estimativa para cada ano do triênio 2020 - 2022 aponta que ocorrerão 625 mil casos novos de câncer (450 mil, excluindo os casos de câncer de pele não melanoma). O câncer de pele não melanoma será o mais incidente (177 mil), seguido pelos cânceres de mama e próstata (66 mil cada), cólon e reto (41 mil), pulmão (30 mil) e estômago (21 mil) (INCA, 2020),

O câncer surge a partir de uma mutação genética, ou seja, de uma alteração no DNA da célula, que passa a receber instruções erradas para as suas atividades. As alterações podem ocorrer em genes especiais, denominados proto-oncogenes, que a princípio são inativos em células normais. Quando ativados, os proto-oncogenes tornam-se oncogenes, responsáveis por transformar as células normais em células cancerosas. Dividindo-se rapidamente, estas células tendem a ser muito agressivas e incontroláveis, determinando a formação de tumores, que podem espalhar-se para outras regiões do corpo (RANG et al., 2016; INCA, 2020).

O processo de desenvolvimento do câncer, denomina-se carcinogênese, a qual possui alterações moleculares e celulares que envolvem 03 (três) estágios distintos: iniciação, promoção e progressão (PAN et al., 2011; INCA, 2020).

Na fase de iniciação, os genes sofrem ação dos agentes cancerígenos, que provocam alterações em alguns de seus genes. Apesar das alterações, ainda não é possível se detectar um tumor clinicamente. As células alteradas se encontram predispostas para a ação de um segundo grupo de agentes que atuará no próximo estágio (INCA, 2020).

Na fase de promoção do tumor, a célula iniciada é transformada em célula maligna, de forma lenta e gradual. A estimulação do promotor de tumor deve continuar por um longo período (semanas, meses ou anos) nos tecidos-alvo. Os efeitos promocionais são reversíveis. Quando o tumor desaparece, ocorre regressão do tumor, possivelmente através de mecanismos de apoptose. Alguns componentes da alimentação e a exposição excessiva e prolongada a hormônios são exemplos de fatores que promovem a transformação de células iniciadas em malignas (TANAKA et al., 2013; INCA, 2020).

A progressão envolve a conversão de células tumorais em células invasoras, de forma lenta e gradual. Nesta etapa, as células alteradas se multiplicam descontroladamente, independente da presença ou ausência de estímulos carcinogênicos. Esta fase se caracteriza pela irreversibilidade, instabilidade genética, produção de fator de crescimento, invasão, metástases e alterações no metabolismo e morfologia das células afetadas. Neste momento, o câncer já está instalado, evoluindo até o surgimento das primeiras manifestações clínicas da doença (PAN et al., 2011; TANAKA et al., 2013; INCA, 2020).

2.3.1 Câncer de pulmão

São estimados para cada ano do triênio 2020-2022, 17.760 novos casos de câncer de pulmão em homens e 12.440 em mulheres no Brasil. Esses valores correspondem a um risco estimado de 16,99 casos novos a cada 100 mil homens e 11,56 para cada 100 mil mulheres. No país, o câncer de pulmão configura-se entre os principais em incidência, ocupando a terceira posição entre os homens e quarta posição entre as mulheres (Figura 4) (INCA, 2020).

Localização Primária	Casos	%
Próstata	65.840	29,2%
Cólon e reto	20.520	9,1%
Traqueia, brônquio e pulmão	17.760	7,9%
Estômago	13.360	5,9%
Cavidade oral	11.180	5,0%
Esôfago	8.690	3,9%
Bexiga	7.590	3,4%
Linfoma não Hodgkin	6.580	2,9%
Laringe	6.470	2,9%
Leucemias	5.920	2,6%

Localização Primária	Casos	%
Mama feminina	66.280	29,7%
Cólon e reto	20.470	9,2%
Colo do útero	16.590	7,4%
Traqueia,brônquio e pulmão	12.440	5,6%
Glândula tireoide	11.950	5,4%
Estômago	7.870	3,5%
Ovário	6.650	3,0%
Corpo do útero	6.540	2,9%
Linfoma não Hodgkin	5.450	2,4%
Sistema nervoso central	5.220	2,3%

*Números arredondados para múltiplos de 10.

Figura 4 - Distribuição proporcional dos dez tipos de câncer mais incidentes estimados para 2020-2022 por sexo, exceto pele não melanoma, no Brasil.

Fonte: INCA (2020)

Os padrões geográficos globais nas mortes relacionadas ao câncer de pulmão seguem de perto aqueles em incidência devido à baixa sobrevida e à alta taxa de letalidade desta doença. Em todo o mundo, o câncer de pulmão é a principal causa de morte relacionada ao câncer em homens e a segunda causa em mulheres. Em 2018, cerca de 1,8 milhão de mortes ocorreram (1,2 milhão em homens e 576.100 em mulheres), sendo responsáveis por 1 em 5 mortes relacionadas ao câncer em todo o mundo (AMERICAN CANCER SOCIETY, 2018; BRAY et al., 2018).

O tabagismo é considerado como principal fator de risco, o qual é responsável por 80% a 90% de todos os diagnósticos de câncer de pulmão, entretanto, existem vários outros fatores de risco identificados como casualmente associados à etiologia do câncer de pulmão, como por exemplo, exposição ao gás radioativo invisível, rádon, que é encontrado no solo, exposições ocupacionais, incluindo arsênio, berílio, cádmio, cromo e escapamento de diesel, histórico de doenças respiratórias não infecciosas, como DPOC (Doença Pulmonar Obstrutiva Crônica), enfisema ou bronquite crônica, infecções causadas por *Chlamydia pneumoniae*, *Mycobacterium tuberculosis* e HIV e anormalidades genéticas, como a superexpressão do receptor do fator de crescimento epidérmico (EGFR) (HOY et al., 2019; SCHABATH; COTE, 2019).

Cerca de 25% dos diagnósticos de câncer de pulmão são entre os não fumantes, e aproximadamente 60% a 80% das mulheres com diagnóstico de NSCLC (câncer de pulmão de células não pequenas) nunca foram fumantes. As exposições associadas ao risco de câncer de pulmão são também consideradas fatores para os não fumantes, como por exemplo, o fumo passivo, fumaça de cozinha, radiação ionizante, gás radônio, suscetibilidade genética herdada, exposições ocupacionais, doenças pulmonares preexistentes e vírus oncogênicos (SCHABATH; COTE, 2019).

Os tumores de câncer de pulmão são classificados em duas grandes categorias histológicas: câncer de pulmão de células não pequenas (NSCLC), que é a causa de cerca de 85% dos casos, e de câncer de pulmão de células pequenas (CPCP), que responde pelos 15% restantes. NSCLC inclui carcinoma escamoso de pulmão (LUSC), adenocarcinoma pulmonar (LUAD) e subtipos de carcinoma de células grandes (DURUISSEAUX; ESTELLER, 2018).

Apesar da hemoptise seja considerada como principal sintoma primário do câncer de pulmão, ela ocorre apenas em 7% a 10% dos pacientes. Os sintomas mais prevalentes no estágio inicial do câncer de pulmão são: tosse, dispneia, dor, fadiga, sintomas depressivos e náuseas ou vômitos. Além disso, outros sintomas podem ser

incluídos, como, rouquidão, perda de peso, anorexia, derrame pleural, disfagia, linfadenopatia e síndromes paraneoplásicas (HOY et al., 2019).

2.3.2 Resistência à terapia antineoplásica

Segundo Iqbal et al. (2018), o aparecimento tardio do câncer de pulmão, a heterogeneidade do subtipo histológico do tumor, a compreensão restrita da biologia do tumor e o desenvolvimento de resistência aos medicamentos são as principais causas do mau prognóstico desta doença.

O aparecimento invariável de resistência adquirida aos medicamentos dificulta a duração da resposta do tumor e também representa a principal barreira para um aumento na sobrevida dos pacientes. As células tumorais podem desenvolver resistência aos medicamentos durante tanto na fase inicial quanto na fase tardia da terapia antineoplásica. Existem dois tipos de resistência a medicamentos: resistência intrínseca ou primária e resistência adquirida ou secundária (LIM; MA, 2019).

A resistência intrínseca acontece quando inicialmente o tumor não diminui de tamanho após a terapia antineoplásica. Isto pode ser explicado pela heterogeneidade do tumor ou entre diferentes locais do tumor dentro de um hospedeiro. Em contrapartida, a pesquisa na compreensão da resistência adquirida aos medicamentos tem se concentrado amplamente em decifrar o mecanismo de resistência molecular em tecidos tumorais que surgiram como doenças mensuráveis de progressão clínica. Neste caso, estão incluídos os mecanismos dependentes do alvo como mutações secundárias ou variação do número de cópias dentro do alvo original e mecanismos não-dependentes do alvo que levam à ativação de vias de sinalização alternativas ou fenotípicas/histológicas (SINI, 2018; LIM; MA, 2019).

Especificamente, em câncer de pulmão, a resistência pode ser detectada em pacientes que fazem uso dos inibidores da tirosinase (TKIs) e apresentam mutações ativadoras do receptor EGF (EGFR) e rearranjos dos genes ALK. Em pacientes com NSCLC mutante EGFR, os TKIs foram introduzidos na terapêutica depois da terapia padrão da cisplatina, melhorando a taxa de resposta geral (ORR), entretanto, uma pequena parte desses pacientes não conseguem se beneficiar desta terapia, devido as mutações herdadas específicas (resistência primária) e ainda assim, inicialmente, quando respondem a essas drogas podem desenvolver uma resistência secundária, geralmente dentro de um ano a partir do início do tratamento. Outras formas de

resistência adquirida podem ser identificadas, como, alteração na histologia do tumor, aumento na produção do fator de crescimento e expressão da bomba de efluxo da droga (SINI et al., 2018).

Além disso, casos de resistência secundária parecem ser causados pela amplificação do oncogene MET em 5% a 20% dos pacientes durante o tratamento com erlotinibe ou gefitinibe (inibidores da tirosinase) e potencialmente tão alto quanto 30% em pacientes com resistência adquirida ao osimertinibe. Mutações em PIK3CA e amplificações do receptor tipo 2 do fator de crescimento epidérmico humano (HER-2) também tem sido considerados mecanismos de resistência adquirida em câncer de pulmão (DUMA et al., 2019).

2.3.3 Linhagens celulares de câncer de pulmão de células não pequenas (NSCLC)

A linhagem celular de adenocarcinoma humano A549 é classificada como carcinoma de células não pequenas (CPNPC). Foi descrita, em 1972, por D.J. Giard, et al., através de uma cultura de explante de tecido pulmonar adenocarcinômico de um homem caucasiano de 58 anos, expressa algumas características das células ATII (alvéolo pulmonar humano primário tipo II), incluindo a síntese de fosfolipídios, corpos lamelares citoplasmáticos e microvilosidades apicais (GIARD et al., 1973; LIEBER et al., 1976).

A linhagem celular A549 também pode ser classificada como hipotriploide, apresentando número cromossômico modal de 66, que ocorre em 24% das células. Os números modais de 64 e 67 são relativamente comuns, com ploidias mais altas ocorrendo em uma taxa infrequente (0,4%). Estas foram bem caracterizadas ao longo dos anos e são valiosas para pesquisadores que as utilizam rotineiramente como modelos i*n vitro* e i*n vivo.* Essas células são de natureza escamosa e são responsáveis pela difusão de substâncias como água e eletrólitos através dos alvéolos dos pulmões. As células A549 são células epiteliais basais alveolares humanas que crescem aderentemente como uma monocamada *in vitro* e servem como hospedeiros de transfecção adequados (LIEBER et al., 1976).

A linhagem NCI-H460 é um tipo de adenocarcinoma pulmonar humano, o qual foi derivado do líquido pleural de um paciente com câncer de pulmão de grandes células por A.F. Gazdar e associados, em 1982. As células NCI-H460 expressam

mRNA p53 facilmente detectável em níveis comparáveis ao tecido pulmonar normal e não exibem anormalidades estruturais de DNA grosseiras. As células NCI-H460 coram positivamente para queratina e vimentina, mas são negativas para proteína tripleto de neurofilamentos (AMOÊDO et al., 2011).

2.4 Atividade antitumoral da geoprópolis

Cinegaglia et al. (2013) demonstraram efeito citotóxico do extrato etanólico da geoprópolis de *Melipona fasciculata* sobre as células de osteosarcoma canina, de maneira dose e tempo dependente (24, 48 e 72h). A ação foi evidenciada pela análise morfológica, mostrando a sensibilidade dessas células ao extrato da geoprópolis, cuja composição química obtida por CG/EM identificou alquilresorcinóis, triterpenos, pentoses, hexoses, dissacarídeos e ácidos urônicos, os quais podem estar relacionados à ação citotóxica.

Da Cunha et al. (2013) apontaram a atividade antiproliferativa de extrato etanólico da geoprópolis de *Melipona scutellaris* contra linhagens tumorais de glioma (U251), melanoma (UACC-62), mama (MCF-7), ovário resistente a múltiplas drogas (NCI/ADR-RES), rim (786-0), pulmão (NCI-H460), próstata (PC-3) e ovário (OVCAR-3). O crescimento total destas linhagens foi inibido em baixas concentrações do extrato comparado às linhagens normais (queratinócitos e fibroblasto murino normal), demonstrando dessa maneira, a seletividade do extrato da geoprópolis para as células tumorais.

A partir do extrato hidroetanólico da geoprópolis de *Melipona scutellaris,* Da Cunha et al. (2016) isolaram oito compostos, os quais foram testados sobre duas linhagens de câncer de cólon (COLO205 e KM12). Dentre as substâncias isoladas, as cumarinas mammeisina e mammeina apresentaram uma porcentagem alta de inibição do crescimento de 56 e 83%, respectivamente.

Bartolomeu et al. (2016) demonstraram que o extrato hidroetanólico da geoprópolis de *Melipona fasciculata* apresentou atividade citotóxica sobre o crescimento de células HEp-2 (carcinoma epidermóide de laringe), reduzindo significativamente a migração celular após 24h. No mesmo estudo, a combinação do extrato da geoprópolis com a doxorrubicina afetou significativamente a sensibilidade das células HEp-2 após 72 h, além disso, a combinação promoveu a apoptose dessas

linhagens tumorais, apresentando alterações morfológicas, como fragmentações de membrana citoplasmática (corpos apoptóticos) e perda da integridade de membrana.

Dos Santos et al. (2017a) testaram a atividade antiproliferativa de extrato etanólico da geoprópolis de *Melipona mondury*, coletada em Entre Rios, Bahia, contra carcinoma hepatocelular humano (HepG2), leucemia promielocítica humana (HL-60) e leucemia mielocítica crônica humana (K562), comprovando que os valores de CI_{50} dessas linhagens tumorais foram inferiores as células normais (linfoblastos humanos).

Oliveira et al. (2016; 2019) evidenciaram que a combinação do extrato hidroetanólico da geoprópolis de *Melipona fasciculata* Smith em combinação com a doxorrubicina aumentou a expressão de CD80, favorecendo a ativação de linfócitos T e estimulou a produção de TNF-α (atua na via da caspase-8 nas células tumorais) e IL-10 em monócitos humanos. Além disso, esta combinação inibiu a produção de IL-6 pelos monócitos, o que pode ser explicado pelo aumento da produção de IL-10. Quando IL-6 está regulada positivamente está associada ao crescimento do tumor e resistência à quimioterapia.

Barboza et al. (2020) demonstraram a citotoxicidade da geoprópolis em linhagens de câncer de pulmão e ovário, e, em especial na linhagem A2780, e foram observadas várias alterações nessas células quando tratadas com o extrato, especificamente na concentração mais alta exposta (62,5 µg/mL). As células tornaram-se arredondadas e encolhidas, exibindo densidade diminuída e destacando-se do substrato, sendo características sugestivas de morte celular induzida pelo extrato da geoprópolis mediada por apoptose. Tal fato foi confirmado com o aumento da expressão da caspase-3 clivada e do PARP clivado.

Assim, os estudos de investigação do potencial antitumoral já desenvolvidos com geoprópolis de espécies de *Melipona*, com ênfase aos trabalhos com *Melipona fasciculata* estimulam a continuidade das pesquisas nesse segmento, na perspectiva real de Pesquisa & Desenvolvimento (P&D) de busca de novas opções no combate ao câncer.

3 OBJETIVOS

3.1 Objetivo geral

Realizar estudo de bioprospecção da geoprópolis de *Melipona fasciculata* Smith, coletadas no estado do Maranhão, em modelo de avaliação *in vitro* de atividade antitumoral em células de câncer de pulmão e identificação da composição química.

3.2 Objetivos específicos

- Realizar revisão bibliográfica de estudos pré-clínicos de própolis e geoprópolis de espécies de abelhas sem ferrão na área de câncer;
- Avaliar atividade antitumoral *in vitro* das frações apolares e polares do extrato hidroetanólico da geoprópolis de *Melipona fasciculata* em linhagens de câncer de pulmão e em células normais;
- Identificar as substâncias químicas das frações bioativas do extrato hidroetanólico da geoprópolis de *Melipona fasciculata* Smith.

4 RESULTADOS

Os resultados do presente trabalho estão organizados em dois capítulos:

- Capítulo I: Trata de uma revisão de literatura de estudos pré-clínicos anticânceres de própolis e geoprópolis de espécies de abelhas sem ferrão, que foi publicado na revista **Pharmaceuticals,** 14, 1161, 2021; https://doi.org/10.3390/ph14111161.
- Capitulo II: Apresenta resultados da citotoxicidade de frações separadas do extrato hidroetanólico da geoprópolis de *Melipona fasciculata* Smith sobre linhagens de câncer de pulmão e células normais, assim como a identificação química da fração bioativa.

  O artigo será submetido à revista **Toxicology *in vitro***.

4.1 Capítulo I – ARTIGO

Use of stingless bee propolis and geopropolis against cancer—a literature review of preclinical studies

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Review

Use of Stingless Bee Propolis and Geopropolis against Cancer—A Literature Review of Preclinical Studies

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Abstract: Cancer is one of the major maladies affecting humankind and remains one of the leading causes of death worldwide. The investigation of the biological activities of stingless bee products, especially propolis and geopropolis, has revealed promising therapeutic properties, especially in the research on new antineoplastic agents. This literature review of preclinical trials, involving biological assays of antitumor activity and identification of the chemical composition of propolis and geopropolis of stingless bee species, describes the cytotoxicity in tumor lineages (breast, lung, ovarian, liver, mouth, pharynx, larynx, colon, stomach, colorectal, cervix, kidney, prostate, melanoma, human glioblastoma, canine osteosarcoma, erythroleukemia, human chronic myelocytic leukemia, and human promyelocytic leukemia) of propolis and geopropolis of 33 species of stingless bees. The chemical composition of propolis and geopropolis was identified, indicating that these belong to the chemical classes of phenolic acids, flavonoids, coumarins, benzophenones, anthraquinones, alkaloids, terpenes, steroids, saponins, fatty acids, and carbohydrates and are possibly responsible for the cytotoxicity in tumor cells. Apoptosis was one of the main mechanisms of cytotoxicity of extracts and substances isolated from stingless bee products. Although the results found are encouraging, other preclinical studies and clinical trials are essential for the discovery of new anticancer agents.

Keywords: stingless bee products; new anticancer agents; propolis; geopropolis

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1. Introduction

Stingless bees, also known as meliponines, live in colonies and are characterized by having atrophied stingers [1]. They are social insects of great diversity and wide geographic distribution, occupying almost all of Latin America and Africa, besides southeast Asia and northern Australia [2–4]. There are more than 600 described species, and they are spread across all tropical and subtropical areas of the globe [5]. Among the genera with the largest number of known species are *Plebeia*, *Trigona*, *Melipona*, *Scaptotrigona*, and *Trigonisca* [6].

Meliponines make great contribution to environmental conservation, as they perform pollination of native plant species and contribute to a reduction in deforestation and environmental damage [4,7]. In addition, they are commercially known for their role in the production of natural products, such as honey, wax, royal jelly, propolis, and geopropolis and accumulation of pollen [3,4,8,9].

Propolis is a mixture of salivary secretions and plant resins collected by bees and is produced to seal the hive and prevent the entry of air and invading insects, besides having antimicrobial activity, protecting the colony from diseases [10,11].

Some meliponin species mix propolis with clay or soil. The result of this mixture is a resinous material more rigid than propolis. Despite the differences in the formation of both products, geopropolis has similar functions to propolis regarding the protection of the hive [12].

Different biological activities of propolis and geopropolis have been investigated worldwide, including antioxidant [13–16], antimicrobial [15,17], antileishmanial [18], antiviral [19], anti-inflammatory [20], healing [21], and antitumor [8,22–25] action.

The evaluation of the antitumor activity of propolis and geopropolis has been the object of research in several study groups. These stingless bee products have already been tested in many tumor models of head and neck, lung, liver, pancreas, kidney, prostate, skin, breast, gastric, and colon cancer, the results of which suggest the potential use of these natural compounds as part of complementary medical treatment of human tumors [17,24,26,27].

Considering the importance of natural products for the development of antineoplastic drugs, the present study conducted a literature review of studies of the effect of propolis and geopropolis produced by species of stingless bees against different tumor lineages and the identification of their chemical compounds responsible for the biological activity.

2. Results and Discussion

The selection of articles by primary search identified 2080 articles, of which 1622 were in ScienceDirect, 126 were in PubMed, 310 were in Scopus, and 22 were in Scielo. Articles indexed in two or more databases were considered only once. After the initial screening of titles, abstracts, and keywords, 31 articles were selected, as the others did not meet the inclusion criteria.

A total of 33 species of stingless bees producing propolis and geopropolis with antitumor potential were identified, of which 20 species (*Scaptotrigona affinis postica*, *Scaptotrigona bipunctata*, *Scaptotrigona depilis*, *Scaptotrigona* sp., *Melipona quadrifasciata quadrifasciata*, *Melipona quadrifasciata anthidioides*, *Melipona orbignyi*, *Trigona* spp., *Trigona incisa*, *Trigona apicalis*, *Trigona fuscobalteata*, *Trigona fuscibisca*, *Trigona laeviceps*, *Trigona sirindhornae*, *Tetragonisca fiebrigi*, *Tetrigona apicalis*, *Tetragonula pagdeni*, *Tetragonula biroi*, *Heterotrigona itama*, *Heterotrigona bakeri*, *Homotrigona fimbriata*, *Tetragonula testaceitarsis*, *Tetragonula sarawakensis*, *Tetragonula fuscobalteata*, *Tetragonula laeviceps*, *Lepidotrigona terminata*, *Lepidotrigona ventralis*, *Geniotrigona thoracica*, *Lisotrigona furva*, and *Plebeia remota*) are producers of propolis and three species (*Melipona fasciculata*, *Melipona mondury*, and *Melipona scutellaris*) are producers of geopropolis (Table 1).

Only two studies on the propolis cytotoxicity of stingless bees (*Scaptotrigona aff. postica* and *Tetragonula biroi*) were found in animal models [28,29] with the remaining studies being in vitro tests. These stingless bee products have already been tested in vitro on tumor cell lines, such as breast (MDA-MB-231, MCF-7, and BT-474), lung (A549, H460, SK-LU-1, and ChaGo-1), ovarian (ES2, A2780, NCI-ADR/RES, and OVCAR-03) cancer, liver (HepG2), mouth (KB), pharynx (HN30 and HN31), larynx (HEp-2), colon (CaCo-2, COLO205, SW620, and KM12), stomach (KATO-III, AGS, MKN-45, NUGC-4, and MKN-74), colorectal (HRT-18), cervix (HeLa), kidney (786-0), prostate (PC-3), melanoma (UACC-62, SK-MEL-28, and B16-F10), human glioblastoma (U251 and U343), canine osteosarcoma (OSA), and leukemia (K562 and HL-60) (Table 1).

Stingless bee products with antitumor potential come from seven countries (Brazil, India, Indonesia, Thailand, Malaysia, Philippine, and Vietnam) (Figure 1).

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Table 1. Propolis and geopropolis extracts of stingless bee species with anticancer activity in tumor cell lines.

Bee Species	Place of Origin	Product	Type of Preparation	Tumor Cells	Result	Type of Test	Chemical Identification	Ref.
Melipona fasciculata (Smith 1854)	Maranhão, Brazil	Geopropolis	Hydroethanolic extract	Canine osteosarcoma (OSA)	Dose- and time-dependent cytotoxicity	In vitro	No	[23]
				Human epidermoid laryngeal carcinoma (HEp-2)	Decrease in cell viability from 25 to 100 µg/mL		Yes [a]	[30]
				Human epidermoid laryngeal carcinoma (HEp-2)	Inhibition of cell proliferation and migration		No	[25]
				Lung cancer (A549 and H460) and ovarian cancer (ES2 and A2780)	Dose- and time-dependent cytotoxicity		Yes [a]	[8]
Melipona scutellaris (Latreille 1811)	Bahia, Brazil		Ethanolic extract	Glioma (U251), melanoma (UACC-62), breast (MCF-7), multidrug-resistant ovarian (NCI-ADR/RES), kidney (786-0), lung (NCI-H460), prostate (PC-3), and ovary (OVCAR-03)	Anti-proliferative activity		Yes [a]	[24]
Melipona mondury (Smith 1863)	Bahia, Brazil		Hydroethanolic extract	B16-F10 (melanoma murine), HepG2 (human hepatocellular carcinoma), K562 (human chronic myeloid leukemia), and HL-60 (human promyelocytic leukemia)	IC_{50} 24.2 to 46.6 µg/mL		Partially	[15]
Melipona quadrifasciata quadrifasciata (Lepeletier 1836)	Paraná, Brazil	Propolis	Ethanolic extract	MDA-MB-231 (triple-negative human breast adenocarcinoma), MCF-7 (human breast adenocarcinoma), HeLa (human cervical adenocarcinoma), HepG2 (human hepatocellular carcinoma), HRT-18 (human colorectal adenocarcinoma)	IC_{50} 97.53 to 155.1 µg/mL	In vitro	Yes [a]	[17]

Table 1. *Cont.*

Bee Species	Place of Origin	Product	Type of Preparation	Tumor Cells	Result	Type of Test	Chemical Identification	Ref.
Melipona quadrifasciata anthidioides (Lepeletier 1836)	Mato Grosso do Sul, Brazil	Propolis	Ethanolic extract	Erythroleukemia cell line (K562)	Decrease in cell growth to 21.2% ± 4.1% at 500 µg/mL	In vitro	Yes [a]	[31]
	Santa Catarina, Brazil		Ethanolic extract	Human melanoma (SK-MEL-28)	Decreased migration and invasion of melanoma cells		Yes [a]	[32]
Melipona orbignyi (Guérin-Méneville 1844)	Mato Grosso do Sul, Brazil		Ethanolic extract	Erythroleukemia cell line (K562)	Decrease in cell viability to less than 25% at 500 µg/mL		Yes [b]	[33]
Trigona spp.	Maharashtra, India		Hydroethanolic extract	Human breast adenocarcinoma (MCF-7), human colon adenocarcinoma (HT-29), human epithelial colorectal adenocarcinoma (CaCo-2), and murine melanoma cell lines (B16F1).	Time- and dose-dependent cytotoxicity IC_{50} 250 µg/mL		No	[26]
	Indonesia		Hydroethanolic extract	Breast (MCF-7)	Decrease in cell growth to 47.71%		Partially	[34,35]
Trigona sirindhornae (Michener and Boongird 2004)	Chantaburi, Thailand		Dichloromethane extract	Primary lesions of the pharynx (HN30) and lymph node metastases (HN31)	Dose-dependent cytotoxicity		No	[36]
Tetragonula pagdeni (Schwarz 1939)	Chanthaburi, Thailand	Propolis	Methanolic extract	Squamous cell carcinoma of the mouth (KB), hepatocellular carcinoma (HepG2), colon adenocarcinoma (CaCo-2), and melanoma (SK-MEL-28)	Cytotoxicity IC_{50} 33.38 to 80.81 µg/mL	In vitro	Yes [b]	[27]

Table 1. *Cont.*

Bee Species	Place of Origin	Product	Type of Preparation	Tumor Cells	Result	Type of Test	Chemical Identification	Ref.
Tetragonula testaceitarsis (Cameron 1901)	Kalimantan, Indonesia	Propolis	Ethanolic extract	Human breast cancer (MCF-7), human cervical adenocarcinoma (HeLa), and human colon cancer (CaCo-2)	Moderate decrease in cell viability to 75 µg/mL	In vitro	No	[37]
Tetragonula sarawakensis (Schwarz 1939)							No	
Tetragonula fuscobalteata (Cameron 1908)							No	
Tetragonula laeviceps (Smith 1857)							No	
Tetragonisca fiebrigi (Schwarz 1938)	Mato Grosso do Sul, Brazil	Propolis	Ethanolic extract	Erythroleukemia cell line (K562)	Dose-dependent cytotoxicity		Yes [a]	[20]
Trigona incisa (Sakagami and Inoue 1989)	Kalimantan, Indonesia	Propolis	Methanolic extract	Colon (SW620), liver (HepG2), stomach (KATO-III), lung (ChaGo-1), and breast (BT-474)	Anti-proliferative activity	In vitro	Yes [b]	[38–40]
Trigona apicalis (Smith 1857)							No	[38]
Trigona fuscobalteata (Cameron 1908)							No	
Trigona fuscibisca (Friese 1900)								
Heterotrigona itama (Cockerell 1918)			Ethanolic extract	Human breast cancer (MCF-7), human cervical adenocarcinoma (HeLa), and human colon cancer (CaCo-2)	Moderate decrease in cell viability to 75 µg/mL	In vitro	No	[37]
Heterotrigona bakeri (Cockerell 1919)							No	
Homotrigona fimbriata (Smith 1857)							Yes [b]	

Table 1. *Cont.*

Bee Species	Place of Origin	Product	Type of Preparation	Tumor Cells	Result	Type of Test	Chemical Identification	Ref.
Lepidotrigona terminata (Smith 1878)	Chanthaburi, Thailand	Propolis	Methanolic extract	Squamous cell carcinoma of the mouth (KB), hepatocellular carcinoma (HepG2), colon adenocarcinoma (CaCo-2), and melanoma (SK-MEL-28)	Cytotoxicity IC_{50} 74.30 to 264.78 µg/mL	In vitro	No	[27]
Trigona laeviceps (Smith 1857)	Samut Songkram, Thailand		Aqueous extract	Colon (SW620)	Decrease of cell viability to 23%		No	[41]
			Ethanolic extract	Colon (SW620), breast (BT-474), liver (HepG2), lung (ChaGo), and stomach (KATO-III)	Anti-proliferative activity IC_{50} 19.9 to 36.19 µg/mL		No	[42]
Lepidotrigona ventralis (Smith 1857)	Chanthaburi, Thailand		Methanolic extract	Squamous cell carcinoma of the mouth (KB), hepatocellular carcinoma (HepG2), colon adenocarcinoma (CaCo-2), and melanoma (SK-MEL-28).	Cytotoxicity IC_{50} 96.58 to 565.19 µg/mL		No	[27]
Geniotrigona thoracica (Smith 1857)	Perak, Malaysia		Ethanolic extract	Human breast adenocarcinoma (MCF-7)	Growth inhibition IC_{50} 38.9 µg/mL		No	[43]
Plebeia remota (Holmberg 1903)	Paraná, Brazil		Ethanolic extract	MDA-MB-231 (triple-negative human breast adenocarcinoma), MCF-7 (human breast adenocarcinoma), HeLa (human cervical adenocarcinoma), HepG2 (human hepatocellular carcinoma), and HRT-18 (human colorectal adenocarcinoma)	IC_{50} 41.76 to 76.1 µg/mL		Yes [a]	[17]
Tetragonula biroi (Friese 1898)	Lagunas, Philippines		Ethanolic extract	Gastric cancer cell lines (AGS, MKN-45, NUGC-4, and MKN-74)	Regression of macroscopic and histological lesions	In vitro and in vivo	Yes [a]	[29]

Table 1. *Cont.*

Bee Species	Place of Origin	Product	Type of Preparation	Tumor Cells	Result	Type of Test	Chemical Identification	Ref.
Scaptotrigona aff. postica (Latreille 1807)	Maranhão, Brazil	Propolis	Hydroethanolic extract	Ehrlich solid tumor	Inhibition of tumor progression	In vivo	Partially [a]	[28]
Scaptotrigona bipunctata (Lepeletier 1836)	Paraná, Brazil		Ethanolic extract	MDA-MB-231 (triple-negative human breast adenocarcinoma), MCF-7 (human breast adenocarcinoma), HeLa (human cervical adenocarcinoma), HepG2 (human hepatocellular carcinoma), and HRT-18 (human colorectal adenocarcinoma)	Cytotoxicity IC_{50} 54.89 to 112.23 μg/mL	In vitro	Yes [a]	[17]
Scaptotrigona bipunctata (Lepeletier 1836)	Santa Catarina, Brazil		Ethanolic extract	Human melanoma (SK-MEL-28)	Decreased migration and invasion of melanoma cells		Yes [a]	[32]
Scaptotrigona depilis (Moure 1942)	Mato Grosso do Sul, Brazil		Ethanolic extract	Human erythroleukemia cell line (K562)	Decrease in cell growth to 32.6 ± 3.2% at 500 μg/mL		Yes [a]	[31]
Scaptotrigona sp.	Maranhão, Brazil		Ethanolic extract	Human glioblastoma (U251 and U343)	Anti-proliferative activity		No	[22]
Tetrigona apicalis (Smith 1857)	Perak, Malaysia		Ethanolic extract	Human breast adenocarcinoma (MCF-7)	Proliferation inhibition IC_{50} 32.70 μg/mL		Yes [a]	[44]

a = chemical composition detailed in Table 2. b = compound isolation shown in Figure 2.

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Figure 1. Locations of stingless bee species producing propolis and geopropolis with antitumor potential worldwide.

2.1. Cytotoxicity Tests

2.1.1. Ehrlich Tumor

Ehrlich tumor is a model of mammary adenocarcinoma of female mice that is used in the evaluation of antitumor drugs [45]. According to Araújo et al. [28], animals inoculated by Ehrlich tumor cells in the paws treated with the hydroethanolic propolis extract produced by *Scaptotrigona aff. postica* at doses of 0.5 and 5 mg/kg showed significant inhibition in tumor development from the 6th day after inoculation. There was a significant increase in the number of cells in the spleen and bone marrow of animals treated with the extract in relation to the control, showing that these doses induce an increase in the production of peripheral immune cells and precursors.

2.1.2. Glioblastoma

Glioblastomas are the most frequent and aggressive primary brain tumors, classified, according to the World Health Organization (WHO), as grade IV due to their malignancy [46]. Borges et al. [22] showed an in vitro antiproliferative effect of the ethanolic propolis extract produced by *Scaptotrigona* sp. against human adult glioblastoma cell lines (U251 and U343), with a decrease in cell proliferation by 48% to 59% in 72 h at a dose of 2 mg/mL, as well as a reduction in colony formation. They also observed that the combination of the propolis extract (2 mg/mL) with temozolomide (50 μM) had a synergistic antiproliferative effect, reducing cell proliferation to less than 20%. This association showed superior results related to the extract and drug when evaluated separately.

2.1.3. Erythroleukemia

Erythroleukemia is a rare form of acute myeloid leukemia characterized by the proliferation of erythropoietic elements in the bone marrow; erythroblasts with foreign, lobulated nuclei; and pathological myeloblasts in the peripheral blood [47].

The mechanism of in vitro cytotoxicity of the ethanolic propolis extract produced by *Melipona orbignyi* against erythroleukemia cells (K562) was elucidated by Campos et al., [33], demonstrating that the cell viability decreased to less than 25% at the concentration of 500 μg/mL and that necrosis was the predominant form of cell death of cells treated with propolis. The data are interesting with regard to therapy against tumor cells resistant to cell death by apoptosis, which usually occurs with the use of conventional chemotherapy.

The in vitro cytotoxicity of the ethanolic propolis extract produced by *Tetragonisca fiebrigi* against erythroleukemia cells (K562) was higher with progressive increase in concentration. The most effective cytotoxic concentrations of the ethanolic extract propolis were 250 and 500 μg/mL, which promoted cell death by necrosis (23% ± 1.0% and 56% ± 1.4%) and secondary necrosis (10% ± 1.8% and 13% ± 0.8%), respectively. At the highest concentration evaluated (500 μg/mL), there was a 67% ± 2.5% reduction in viable cells [20].

Bonamigo et al. [31] observed the in vitro cytotoxic activity of ethanolic propolis extracts of *Scaptotrigona depilis* and *Melipona quadrifasciata anthidioides* against erythroleukemia cells (K562) as the concentration increased with cell growth of 32.6% ± 3.2% and 21.2% ± 4.1%, respectively. At the concentration of 500 μg/mL, by flow cytometry using the annexin and propidium iodide markers, after 24 h of treatment, the ethanolic propolis extract produced by *Scaptotrigona depilis* promoted death by necrosis in 52.9% ± 4.1% of the cells and death by late apoptosis in 12.1% ± 0.6% of the cells. The ethanolic propolis extract produced by *Melipona quadrifasciata anthidioides* promoted, after 24 h of treatment, death by necrosis in 57.5% ± 3.8% of the cells and death by late apoptosis in 19.4% ± 1.6% of the cells.

2.1.4. Melanoma

Melanoma skin cancer originates from normal pigment cells called melanocytes. These melanocytes produce melanin, the pigment responsible for giving color to the skin and which protects the body from damage by the sun's ultraviolet rays. Similar to other cells in the body, melanocytes can transform into cancer cells and when this transformation occurs, the result is the development of melanoma [48].

According to Cisilotto et al. [32], in vitro cytotoxicity of the hydroethanolic propolis extract produced by *Scaptotrigona bipunctata* against melanoma cells (SK-MEL-28) occurs by cell death by apoptosis, also evidenced in the accumulation of reactive oxygen species (ROS), reduction of mitochondrial membrane potential ($\Delta\psi m$), and induction of decreased levels of Bcl-2 proteins (antiapoptotic proteins) and AKT-3 (cell-growth-related protein). The extract also causes a decrease in migration and invasion of melanoma cells.

The combination of the extract (30 μg/mL) with the antineoplastic vemurafenib (15 μM) against melanoma cells demonstrated a synergistic effect, showing a cytotoxic effect, suggesting reduced resistance and increased cell death in cells with BRAF (proto-oncogene regulator of cell function) mutation.

2.1.5. Osteosarcoma Cells

Osteosarcoma is a primary malignant bone tumor that can occur in any age group but mainly affects children, adolescents, and young adults and can also occur in animals [49]. Cinegaglia et al. [23] demonstrated that the hydroethanolic geopropolis extract produced by *Melipona fasciculata* exerts an in vitro cytotoxic effect against canine osteosarcoma cells in a dose- and time-dependent manner (24, 48, and 72 h). This was also evidenced by morphological analysis, showing the sensitivity of these cells to the extract.

2.1.6. Laryngeal Carcinoma

Laryngeal carcinoma is among the most common head and neck cancers, accounting for about 2.4% of all newly diagnosed cases and 0.7% of all cancer-related deaths occurring worldwide/year [50].

Studies by Bartolomeu et al. [25] demonstrated in vitro cytotoxic activity of the hydroethanolic geopropolis extract produced by *Melipona fasciculata* against the growth of HEp-2 cells (larynx epidermoid carcinoma) and significant reduction in cell migration after 24 h of treatment with the extract. In the same study, the combination of the extract (25 mg/mL) with doxorubicin (1 mM) significantly affected the sensitivity of HEp-2 cells after 72 h, promoting apoptosis of this tumor lineage, presenting morphological changes, such as cytoplasmic membrane fragmentations (apoptotic bodies), loss of membrane, and integrity. Araújo et al. [30] also verified a significant decrease in cell viability observed after 6 h of incubation with 50 and 100 μg/mL of extract, and after 24, 48, and 72 h of incubation, there was a significant decrease in cell viability from 25 to 100 μg/mL.

2.1.7. Ovarian Adenocarcinoma

Ovarian cancer is the second-most-common gynecological neoplasm, second only to cervical cancer. Almost all ovarian neoplasms (95%) are derived from the epithelial cells lining the ovary [51].

Da Cunha et al. [24] demonstrated the in vitro antiproliferative activity of the ethanolic geopropolis extract produced by *Melipona scutellaris* against ovarian adenocarcinoma with a multidrug-resistant phenotype (NCI/ADR-RES) and ovarian adenocarcinoma (OVCAR-03), with an IC_{50} range from 11.93 to 23.92 μg/mL. The total growth of these strains was inhibited at low concentrations when compared to normal strains (keratinocytes and normal murine fibroblast with IC_{50} of 43.20 and 52.73 μg/mL, respectively), thus demonstrating the selectivity of the ethanolic extract geopropolis for tumor cells.

The in vitro cytotoxicity of the hydroethanolic geopropolis extract produced by *Melipona fasciculata* (specifically at the concentration of 62.5 μg/mL, the highest exposed) against ovarian cancer lineage (A2780) was demonstrated through the visualization of several alterations in the morphology of these cells, such as cell rounding and shrinkage, presenting decreased density, standing out from the substrate. These characteristics are suggestive of cell death induced by the hydroethanolic extract mediated by apoptosis. This fact was confirmed with the increased expression of cleaved caspase-3 and of PARP, which is poly(ADP-ribose) polymerase cleaved by the Western blotting technique [8].

2.1.8. Colorectal Adenocarcinoma

Colorectal cancer (CRC) accounts for 9% of all cancers worldwide, making it the second-most-common cancer in women and the third-most-common cancer in men. Adenocarcinoma arising from the rectal and colonic epithelium accounts for 90% of the CRC cases [52–54].

Umthong et al. [41] observed decreased cell viability for 23% of the colon cancer cells (SW620) treated with the aqueous propolis extract produced by *Trigona laeviceps*, and morphological changes were visualized in these cells, such as chromatin condensation, DNA fragmentation, internucleosomal DNA degradation, cell shrinkage, membrane blebbing, nuclear pyknosis, and apoptotic body formation, events typical of the apoptosis process.

Choudhari et al. [26] suggested that the hydroethanolic propolis extract produced by *Trigona* spp. exhibits in vitro cytotoxicity against human colon adenocarcinoma tumor cell lines (HT-29) as the incubation time and concentration were increased. The IC_{50} value found was 250 μg/mL. Morphological changes in cells demonstrated typical changes of apoptosis, i.e., apoptotic membrane blebbing and detachment of cells.

Kustiawan et al. [38] observed the in vitro cytotoxicity of methanolic, hexane, and ethyl acetate propolis extracts of *Trigona incisa*, *Trigona apicalis*, *Trigona fuscobalteata*, and *Trigona fuscibisca* species against human colon tumor cells (SW620), with IC_{50} ranging from 62 to 124 μg/mL.

2.1.9. Carcinoma of the Pharynx

Pharynx cancer represents about 25% of malignant tumors affecting this area and 2% of all malignant diseases. The most prevalent histological type, in more than 90% of the patients, is squamous cell carcinoma [51].

Utispan et al. [36] evaluated the in vitro cytotoxicity of three fractions derived from the dichloromethane propolis extract produced by *Trigona sirindhornae* against cell lines derived from primary pharyngeal lesions (HN30) and lymph node metastases (HN31), both from the same patient. The fractions significantly decreased the viability of both cell lines at concentrations of 50 to 200 μg/mL.

2.1.10. Gastric Adenocarcinoma

Stomach cancer is also called gastric cancer. The adenocarcinoma type accounts for about 95% of the stomach tumor cases and mostly affects men around 60–70 years of age. About 65% of the patients are over 50 years old [51].

Gastric cancer tumor lines (AGS, MKN-45, NUGC-4, and MKN-74) were treated in vitro with the ethanolic propolis extract produced by *Tetragonula biroi* by Desamero et al. [29], which revealed a proportional reduction in cancer cell proliferation as a function of higher concentration and longer incubation times, showing an IC_{50} range from 39 to 925 μg/mL after 72 h. Data confirmed in an in vivo assay using an animal model mimicking a gastric adenocarcinoma of a differentiated type indicated that after treatment with an ethanolic extract, there was a remarkable regression of macroscopic mucosal elevation, corresponding histologically to a substantial reduction in the pyloric mucosal thickness and infiltration of lymphocyte T.

In view of this, the evaluation of the antitumor activity of stingless bee products showed encouraging results. The predominance of in vitro studies to the detriment of animal assays was observed. In addition, the mechanism of cell death was not so explored and detailed in some studies. Sparse data from anticancer trials of stingless bees propolis and geopropolis demonstrate the need for these trials to prove the efficacy and safety of these products.

2.2. Chemical Identification of Antitumor Extracts from Propolis and Geopropolis

The chemical composition of propolis and geopropolis from stingless bees is shown in Table 2. The main classes of compounds identified are phenolics compounds (comprising phenolic acids, flavonoids, coumarins, and benzophenones) terpenes, steroids,

alkaloids, fatty acids, and sugars. Qualitative approaches were used to define the classes of compounds; and analytical techniques, such as high-performance liquid chromatography coupled to mass spectrometry (HPLC/MS), liquid chromatography coupled to mass spectrometry (LC/MS), and gas chromatography coupled to spectrometry (GC/MS), were employed to identify the compounds.

β-amyrin, a compound identified in samples from stingless bees *Tetrigona apicalis*, *Scaptotrigona bipunctata*, *Melipona quadrifasciata anthidioides*, and *Melipona fasciculata*, was the subject of a study by Wen et al. [55] that highlighted the significant cytotoxic activity of this substance against HepG2 (hepatocellular carcinoma) cells. The cytotoxic effects were justified by the induction of apoptosis and the arrest of the G2/M cycle in a dose-dependent manner.

Cinnamic acid, a chemical compound identified in *Melipona orbignyi* and *Tetragonisca fiebrigi* samples, was explored by [56], who observed a reduced cell proliferation rate and a significant change in nuclear cytoplasmic ratio of nasopharyngeal carcinoma (NPC) after treatment with cinnamic acid. In addition, the treatment partially restored normal cell morphology and drove cell differentiation toward a benign phenotype and revealed cell death by apoptosis.

Ma et al. [57] showed that the compound taraxerone (identified in *Tetrigona apicalis* and *Melipona fasciculata* samples) exerts potent antiproliferative effects against A-549 (lung adenocarcinoma) in a strong dose-dependent and time-dependent manner. Furthermore, fluorescence microscopy revealed that taraxerone is able to induce cell shrinkage and chromatin condensation, recorded features of apoptosis.

p-Coumaric acid, identified in samples of species *Scaptotrigona bipunctata*, *Melipona quadrifasciata anthidioides*, and *Tetragonisca fiebrigi*, was studied by Sharma et al. [58], who observed significant inhibition of the proliferation of A375 (human melanoma) and B16 (mouse melanoma) cells after treatment with p-coumaric acid, as well as morphological changes in these cells after 48 h of treatment with different concentrations of the compound. They found increased levels of cleaved caspase-3 and cleaved caspase-9 in A375 and B16 cells, indicating that apoptosis is regulated by the family of caspases.

Artepillin C, present in the ethanolic extract produced by *Scaptotrigona bipunctata*, exhibited dose- and time-dependent cytotoxic effects on prostate cancer (HSC-3) cell lines. Flow cytometry analysis showed that 22% of the HSC-3 cells untreated with the compound suffered spontaneous cell death, while 77.32% of the cells were killed in response to the highest dose of artepillin C at 72 h. The antitumor activity of artepillin C is mediated by one of the following mechanisms: induction of cell cycle arrest in cancer cells, inhibition of angiogenesis, and inhibition of the oncogenic PAK1 signaling cascade [59].

Gallic acid, identified in the hydroethanolic extract geopropolis of *Melipona mondury* and in the ethanolic extract propolis of *Melipona quadrifasciata quadrifasciata*, inhibited the progression of prostate cancer cells (PC-3), was a mitochondrial potential enhancer ($\Delta\Psi m$), and increased the number of apoptotic cells and DNA fragmentation. A Western blot analysis revealed negatively regulated expression of histone deacetylases (HDAC) 1 and 2, reported in various cancers, leading to the positive regulation of acetyl-p53 expression at the protein level, subsequent to the negative regulation of cell-cycle-related gene expression, i.e., proliferating cell nuclear antigen (PCNA) and cyclin D1 and E1; positively regulating the expression of the cell cycle arrest gene p21; and regulating the expression of genes related to the intrinsic apoptosis pathway, such as Bax, Bcl-2, cleaved caspase-3, and poly(ADP-ribose) polymerase [60].

Apigenin, presented in the ethanolic extract produced by *Melipona quadrifasciata anthidioides* propolis, inhibited proliferation, prevented cell cycle progression, and promoted apoptosis in both ovarian cancer cells (SKOV3) and cisplatin-resistant cells (SKOV3/DDP). In addition, apigenin reduced mitochondrial transmembrane potential and elevated caspase-3/cleaved caspase-3 and Bax/Bcl-2 ratios in both cell types. Quantitative reverse transcription PCR and Western blotting results demonstrated that apigenin significantly downregulates Mcl-1 transcription and translation levels in SKOV3 and SKOV3/DDP cells, which is responsible for its cytotoxic functions and chemosensitizing effects [61].

Table 2. Chemical composition of propolis and geopropolis extracts of stingless bee species.

Bee Species	Place of Origin	Product	Class of Compounds	Chemical Compounds	Method	Ref.
Scaptotrigona bipunctata (Latreille 1807)	Paraná/Santa Catarina, Brazil	Propolis	Alkaloids	Lelobanonoline, 2-[6-(2-hydroxy-propyl)-1-methyl-[2]piperidyl]-1-phenylethanone, norlobelanidine, norlobeline, lobeline, and lobelanidine	HPLC/MS	[17,32]
			Terpenes	α-Amyrin/β-amyrin and 4R,5R,9R,10R-13-hydroxypodocarp-8(14)-en-19-oic acid		
			Phenolic compounds (phenolic acids, flavonoids, coumarin, stilbenes, phenylpropanoids, and tannins)	Vicenin, liquiritigenin, formononetin, drupanin, p-coumaric acid, acid ferulic, biochanin A, kaempferol methyl ether, dihydrokaempferide, retusin 8-methyl ether, betuletol, artepillin C, 4-hydroxy-3(E)-(4-hydroxy-3-methyl-2-butenyl)-5-prenylcinnamic acid, 3-hydroxy-2,2-dimethyl-8-prenyl-2H-1-benzopyran-6-propenoic acid, artepillin C derivative, anacardic acid, dicaffeoylquinic, and (E)-3-{4-hydroxy-3-[(E)-4-(2,3-dihydrocinnamoyloxy)-3-methyl-2-butenyl]-5-prenylphenyl}-2-propenoic acid		
			Fatty acids	Palmitic acid, oleic acid, stearic acid, and eicosapentaenoic acid		
Melipona fasciculata (Smith 1854)	Maranhão, Brazil	Geopropolis	Phenolic compounds	Anacardic acid, heptedecenyl salicylic acid, nonadecenyl salicylic acid, pentadecenyl salicylic acid, heptadecadecylresorcinol, nonadecadecylresorcinol, pentadecadecadienylresorcinol, heptedecadienylresorcinol, taxifolin 7-*O*-rhamnoside, isoschaftoside, typhaneoside, dihydroquercetin-*C*-glycoside, narigenin-C-glycoside, vitexin-*O*-gallate, glycosylated pinobanksin, dihydroquercetin-3-O-rhamnoside, and gallocatechin-xylose	HPLC/MS	[8,30]
			Terpenes	Lupeol, α-amyrin, β-amyrin, α-amyrenone, β-amyrenone, triterpene ketone, taraxerone, dipterocarpol, marsformosanone, and 3-[Xyl]-28-Glc-phytophthalacagenin		
			Anthraquinone	Xantholaccaic acid A		
			Organic acids	Glycuronic acid, methylmalonic acid, and gluconic acid		
Melipona scutellaris (Latreille 1811)	Bahia, Brazil	Geopropolis	Benzofenones	Propensaeure 3-phenyl-trimethylsilylester and 1,2-benzenedicarboxylic acid	GC/MS	[24]

Table 2. *Cont.*

Bee Species	Place of Origin	Product	Class of Compounds	Chemical Compounds	Method	Ref.
Melipona quadrifasciata quadrifasciata (Lepetetier 1836))	Paraná, Brazil	Propolis	Phenolic compounds	p-Coumaric acid, ferulic acid, ellagic acid, gallic acid, naringenin, aromadendrin, isosakuranetin, dihydrokaempferide, aromadendrin methyl ether, cinnamoyl-galloyl-hexoside, anacardic acid, cinnamoyl-coumaroyl-hexoside, dicoumaroyl-hexoside, digalloyl-cinnamoyl-hexoside, digalloyl-coumaroyl-hexoside, cinnamoyl-coumaroyl-galloyl hexoside, and dicoumaroyl-galloyl-hexoside	HPLC/MS	[17]
			Terpenes	Sugiol, pimaric acid, isocupressic acid, cupressic acid, junicedric acid, mangiferonic acid, and isomangiferolic acid		
Melipona quadrifasciata anthidioides (Lepeletier 1836)	Mato Grosso do Sul, Brazil	Propolis	Phenolic compounds	p-Coumaric acid, vanilic acid, caffeic acid, vanillin, ferulic acid, benzoic acid, quercetin, luteolin, cinnamic acid, and apigenin	HPLC/MS; GC/MS	[31]
			Terpenes	Stigmasterol, β-sitosterol, β-amyrin, taraxasterol, α-amyrin, β-amyrin acetate, and pinusenocarp		
Melipona quadrifasciata anthidioides (Lepeletier 1836)	Santa Catarina, Brazil	Propolis	Phenolic compounds	7-*O*-methyl aromadendrin, 5-hydroxy-4′,7-dimethoxy flavone, 2′-hydroxynaringenin, narigenin, and p-coumaric	HPLC/MS	[32]
			Phenylpropanoids	4-*O*-(6″-*O*-p-coumaroyl-β-D-glucopyranosyl)- and 6-*O*-cinnamoyl-1-*O*-*p*-coumaroyl-β-D-glucopyranoside		
			Terpenes	Abieta-8,11,13,15-tetraen-18-oic acid, abietic acid, 7-hydroxydehydroabietic acid, and inumakiol		
Melipona orbignyi (Guérin-Méneville 1844)	Mato Grosso do Sul, Brazil	Propolis	Phenolic compounds	Dihydrocinnamic acids, cinnamic acids, benzoic acids, coumarin C-prenylated acids, and long-chain caffeates	GC/MS	[33]
Melipona mondury (Smith 1863)	Bahia, Brazil	Geopropolis	Phenolic compounds	Gallic acid	HPLC/MS	[15]
			Terpenes	Not specified		
Trigona spp.	Maharashtra, India	Propolis	Unidentified	Unidentified	-	[26]
	Indonesia	Propolis	Alkaloids, flavonoids, saponins, tannins, steroids, and triterpenes	Unidentified	Chemical approach	[34]

Table 2. *Cont.*

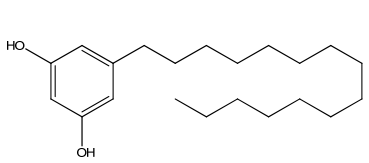
Bee Species	Place of Origin	Product	Class of Compounds	Chemical Compounds	Method	Ref.
Scaptotrigona aff. postica (Latreille 1807)	Maranhão, Brazil	Propolis	Terpenes and coumarins	Unidentified	Phytochemical approach	[28]
Scaptotrigona depilis (Moure 1942)	Mato Grosso do Sul, Brazil	Propolis	Terpenes	β-Sitosterol, β-amyrin, α-amyrin, and β-amyrin acetate	GC/MS; HPLC/MS	[31]
			Phenolic compounds	Vanillin, p-coumaric acid, ferulic acid, benzoic acid, and cinnamic acid		
Tetragonula biroi (Friese 1898)	Lagunas, Philippines	Propolis	Carbohydrates, steroids, alkaloids, anthraquinones, and phenols	Unidentified	Phytochemical approach	[29]
Tetragonisca fiebrigi (Schwartz 1938)	Mato Grosso do Sul, Brazil	Propolis	Phenolic acids	Benzoic acid, cinnamic acid, p-coumaric acid, 3-phenyl-p-coumaric acid, and benzyl caffeate	GC/MS	[20]
			Phenylpropanoids	Cinnamyl caffeate, hydrocinnamic acid, and hydrocinnamic acid ethyl ester		
			Terpene	Kaurenoic acid		
			Sugars	Fructose and glucose		
			Lipids	Tocopherol, cholesterol, and retinol		
Tetrigona apicalis (Smith 1857)	Perak, Malaysia	Propolis	Hydrocarbon	Undecane	GC/MS	[44]
			Phenolic compound	Myristicin		
			Terpenes	β-Elemene, α-cubebene, copaene, cyperene, α-gurjunene, caryophyllene, α-caryophyllene, γ -cadinene, germacrene D, bicyclogermacrene, δ-amorphene, β-selinene, aromadendr-1-ene, spathulenol, caryophyllene oxide, 1, 2-dimethyl-3, 5-bis(1-methylethenyl)-, humulene epoxide II, α-cadinol, aristolene epoxide, taraxerone, β-amyrin, and α-amyrin		
Plebeia remota (Holmberg 1903)	Paraná, Brazil	Propolis	Fatty acid	Arachidonic acid	HPLC/MS	[17]
			Terpenes	Sugiol, totarol, communic acid, agathic acid, isocupressic acid, cupressic acid, dihydroagathic acid, and 15-acetoxy-cupressic acid		

HPLC/MS = high-performance liquid chromatography coupled to mass spectrometry. GC/MS = gas chromatography coupled to mass spectrometry.

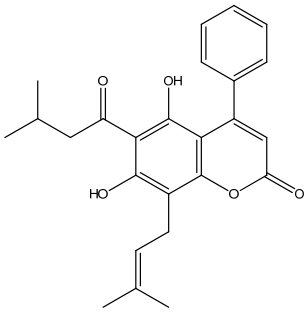
Thus, the compounds identified in propolis and geopropolis extracts have already been studied by different researchers, demonstrating their antitumor potential. Phenolic compounds and terpenes were the most present classes of compounds in stingless bee products.

2.3. Isolation of Compounds

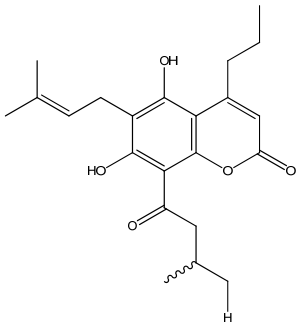
Compounds isolated from propolis and geopropolis of stingless bee species that were tested against tumor cell lines are shown in Figure 2.



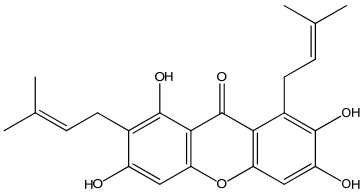
cardol (**1**)



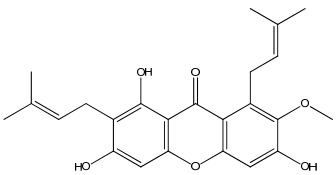
mammeisin (**2**)



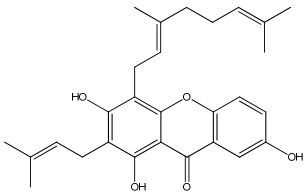
mammein (**3**)



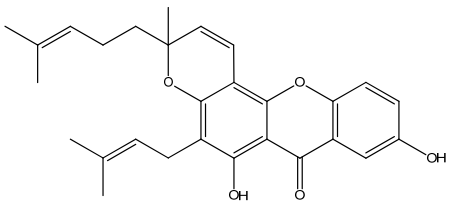
γ-mangostin (4)



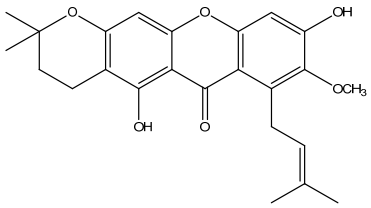
α-mangostin (**5**)



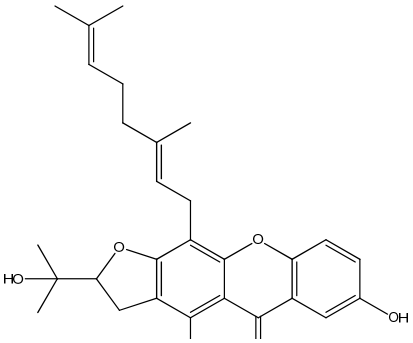
cochinchinone A **(6)**



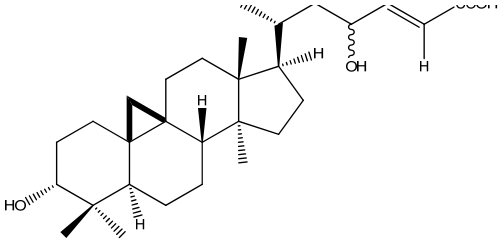
cochinchinone J (**7**)



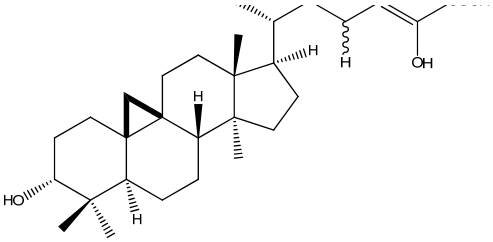
3-isomangostin (**8**)



cratoxylumxanthone B (**9**)

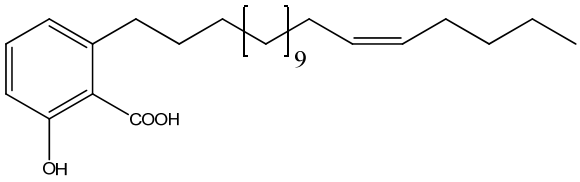


23-hydroxyisomangiferolic acid B (**10**)

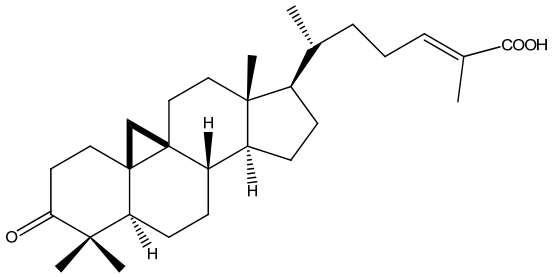


27-hydroxymangiferonic acid (**11**)

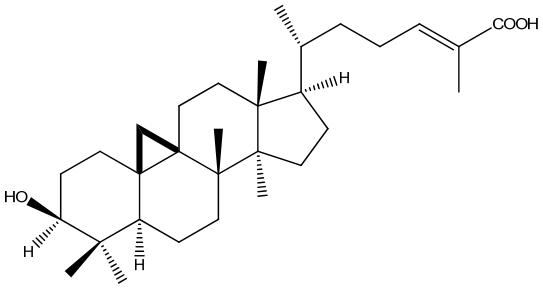
Figure 2. *Cont.*



2-hydroxyl-6-(14′ Z-nonadecenyl)benzoic acid (**12**)



Mangiferonic acid (**13**)



Mangiferolic acid (**14**)

Figure 2. Compound **1** was isolated from the methanolic extract propolis of *Trigona incisa*. Compounds **2** and **3** were isolated from the hydroethanolic extract geopropolis of *Melipona scutellaris*. Compounds **4** and **5** were isolated from the methanolic extract propolis of *Tetragonula pagdeni*. Compounds **4**, **6**, **7**, **8**, and **9** were isolated from the hydroethanolic extract propolis of *Lisotrigona furva*. Compounds **10**, **11**, and **12** were isolated from the ethanolic extract propolis of *Trigona* minor. Compound **13** was isolated from the ethanolic extract propolis of *Homotrigona fimbriata*. Compound **14** was isolated from the acetate extract propolis of *Lisotrigona furva*.

Kustiawan et al. [38–40] submitted the methanolic propolis extract produced by *Trigona incisa* to chromatographic fractionation, isolating, among other compounds, cardol (**1**), which was identified by NMR spectrometric analysis. Biological cytotoxicity tests indicated that compound **1** induces cell death by apoptosis in the initial incubation period (≤6 h) and modulates cell cycle arrest in the G1 subphase in SW620 cells (colon cancer cells). Kustiawan et al. [40] observed that compound **1** promotes changes in cell morphology in SW620 cells; a significant increase in caspase-3 and caspase-9 activities; and cleavage of pro-caspase-3, pro-caspase-9, and PARP.

Eight compounds were isolated from the hydroethanolic geopropolis extract produced by *Melipona scutellaris* by [62] and tested in vitro against two colon cancer cell lines (COLO205 and KM12). The coumarins mammeisin (**2**) and mammein (**3**) (Figure 2) showed a higher average percentage of growth inhibition, of 56% and 83%, respectively. The mechanism of cytotoxicity of the extract against tumor cells was not investigated by the authors.

Vongsak et al. [27] tested the in vitro cytotoxicity of the methanolic propolis extract produced by *Tetragonula pagdeni* against oral squamous cell carcinoma (KB), hepatocellular carcinoma (HepG2), colon adenocarcinoma (CaCo-2), and melanoma (SK-MEL-28) cell lines and observed variation in IC_{50} values from 33. 38 to 80.81 μg/mL. In contrast, the IC_{50} value of normal human fibroblast cells was 228.75 μg/mL, demonstrating greater selectivity of propolis metabolites toward tumor cells. From the said extract, γ-mangostin (**4**) and α-mangostin (**5**) were isolated by preparative thin layer chromatography and their structures identified by spectrometric methods (NMR ^{1}H and ^{13}C) (Figure 2). These substances expressed comparable cytotoxicity to the positive control, doxorubicin, against these tumor cell lines, with IC_{50} values from 2.84 to 15.12 μg/mL and 1.63 to 7.07 μg/mL for (**4**) and (**5**), respectively.

Twenty-three chemical compounds were isolated from the hydroethanolic propolis extract produced by *Lisotrigona furva* by Oanh et al. [63], among which, cochinchinone A (**6**), cochinchinone J (**7**), cratoxylumxanthone B (**8**), 3-isomangostin (**9**), and γ-mangostin (**4**), (Figure 2) were tested against mouth epidermoid carcinoma (KB), human hepatoma (HepG-2), human lung adenocarcinoma (SK-LU-1), and human breast adenocarcinoma (MCF-7). These compounds showed activity on the tested tumor cell lines, with compound (**4**) demonstrating greater activity compared to others, showing an IC_{50} value of 2.10 and 2.73 μg/mL, respectively, in human hepatoma and human lung adenocarcinoma.

The ethanolic propolis extract produced by *Trigona minor* was subjected to partionation with solvents of different polarities. The n-hexane extract showed the most potent preferential cytotoxicity against human pancreatic cancer cells (PANC-1), with an IC_{50} value of 3.6 μg/mL. Further separation and purification of this fraction led to the identification of 16 triterpenoids, most notably 23-hydroxyisomangiferolic acid B (**10**) and 27-hydroxyisomangiferolic acid (**11**), which showed stronger preferential cytotoxicity, with IC_{50} values of 4.3 and 3.7 μM, respectively. Subsequently, compound (**10**) was evaluated for its effect on the cell morphology of PANC-1 cells. When these cells were treated with 5 μM of compound (**10**) for 24 h, the PANC-1 cells changed morphologically and gave a unique red fluorescence, indicating the apoptosis process. Furthermore, in the colony formation assay in PANC-1 cells, compound (**10**) significantly inhibited colony formation in a concentration-dependent manner [64].

Three more substances were isolated from the ethanolic propolis extract produced by *Trigona minor*, with emphasis on 2 hydroxyl-6- (14′Z-nonadecenyl) benzoic acid (**12**), which showed preferential cytotoxicity against the human pancreatic cell line PANC-1, with an IC_{50} value of 2.4 μM. The cytotoxicity of this compound is related to the substituents on the alkenylphenol ring. The presence of the carboxylic acid group or one more hydroxyl group appears to increase the activity [65].

The ethanolic propolis extract produced by *Homotrigona fimbriata* was fractionated by silica gel column chromatography, leading to the isolation of mangiferonic acid (**13**), which showed moderate cytotoxicity, with IC_{50} = 96.76 mM in MCF-7 cells, IC_{50} > 110.04 mM in HeLa cells, and IC_{50} > 110.04 mM in CaCo-2 cells [37].

Chromatographic separation of the ethyl acetate propolis extract produced by *Lisotrigona furva* led to the isolation of five cycloartane-type triterpenes, which were tested on lung cancer cell lines (LU-1) and breast cancer cell lines (MCF-7), most notably mangiferolic acid (**14**), which showed an IC_{50} value of 13.33 and 62.85 μg/mL, respectively [66].

3. Materials and Methods

This review covered ScienceDirect, Scopus, Pubmed, and Scielo databases, as updated on October 2021. The references obtained in the review were consulted and analyzed in detail. The key words employed alone or in combination in the literature review were propolis, geopropolis, stingless bee, cancer, cytotoxicity, and antiproliferative. Articles on propolis from sting bee species (i.e., *Apis mellifera*) were excluded from the search.

4. Conclusions

The propolis and geopropolis extracts from stingless bees analyzed in this revision had diverse and complex chemical compositions, and their constituents belong to the chemical classes of phenolic acids, flavonoids, coumarins, benzophenones, terpenes, steroids, alkaloids, fatty acids, and sugars. The extracts and isolated substances showed selective cytotoxicity against different tumor cell lines, suggesting an antineoplastic potential and synergism with standard chemotherapeutics. Although the preliminary results of propolis and geopropolis from stingless bees are encouraging, further preclinical studies and clinical trials are essential to validate the safety, efficacy, and effectiveness of the products from these species in cancer therapy.

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4.2 CAPÍTULO II – ARTIGO

Phenolic compounds from the stingless bee *Melipona fasciculata* Smith inhibit lung cancer cell proliferation in vitro.

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Artigo será submetido a **Toxicology *in vitro***

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ABSTRACT

One of the main therapeutic problems of lung cancer is antineoplastic resistance. Natural products of animal origin are promising in the discovery of new antitumor molecules. Geopropolis is a natural product produced by *Melipona fasciculata* Smit, a stingless bee. The hydroethanolic extract of geopropolis of this species had a cytotoxic effect on lung and ovarian cancer cells, but there are no further studies with purified fractions of crude extract.This article aimed to evaluate the cytotoxicity of the subfractions of the ethyl acetate fraction obtained by partitioning the hydroethanolic extract of geopropolis from *Melipona fasciculata* Smith in lung cancer strains (A549 and H460), as well as the chemical identification of the bioactive subfractions. Geopropolis samples were collected in a meliponary in the city of Viana, Maranhão, Brazil, dried, triturated (powder) and extracted by maceration, the extractive solution filtered, concentrated in a rotary evaporator and lyophilized, obtaining the lyophilized hydroethanolic extract (EHGV). The EHGV was subjected to liquid-liquid partition, obtaining the hexane (HF), ethyl acetate (EAF) and hydromethanolic fractions (HMF). The EAF was subjected to biodirected fractionation through column chromatography, which resulted in 13 subfractions (FRGE-1 to FRGE-13). Bioactive subfractions were identified by LC and LC–ESI/IT–MS/MS. The in vitro cytotoxicity of fractions and subfractions were evaluated in lung cancer cell lines (H460 and A549) and normal cells (HUVEC) by the 3-4,5-dimethylthiazol-2,5-diphenyl tetrazolium bromide (MTT) assay and the selectivity indices were determined. HF, EAF and HMF inhibited cell proliferation of lung cancer cell lines (H460 and A549) within 48h. FRGE-9 and FRGE-11 are three times more active in tumor cells (A549) than in normal cells. Phenolic compounds were identified in the two bioactive subfractions FRGE-9 (dihydroxybenzoic acid, apigenin-6-C-glucoside and luteolin-5-O-beta-rutinoside) and FRGE-11 (diosmetin, and myricetin-3-galactoside). Diosmetin, apigenin-6-C-glucoside, luteolin-5-O-beta-rutinoside and myricetin-3-galactoside are being identified for the first time in the geopropolis from *M. fasciculata.* The results suggest that the antitumor actions of lung cancer (A549 and H460) of the FRGE-9 and FRGE-11 subfractions are related to their chemical composital.

Keywords: cytotoxicity; lung cancer, chemical composition, bee products.

1. Introduction

Lung cancer is one of the leading causes of death in men and women (American Cancer Society, 2018; Bray, 2018). Antineoplastic resistance and toxicity are the main obstacles to therapeutic success against this neoplasm (Lim; Ma, 2019).

Natural products, especially those of animal origin, such as the products of stingless bees, may be promising in the search for new molecules and/or alternative adjuvants in cancer therapy, since several studies have demonstrated antioxidant, anti-inflammatory and antitumor activity of propolis and geopropolis indicating potential

for cancer treatment (Lavinas et al., 2019; Al-Hatamleh et al., 2020; Nainu et al., 2021; Campos et al., 2021; Pereira et al., 2021).

The stingless bee, *Melipona fasciculata* Smith (Hymenoptera: Apidae: Meliponini), commonly known as tiúba or tiúba do Maranhão is cultivated for honey production, but accumulates pollen, produces wax and geopropolis, which is a mixture of plant resins, secretions, wax and earth/or clay, is used in the hive as aseptic material, in defense against invaders and in regulating the internal temperature (Nogueira-Neto, 1997).

Previous studies of the hydroethanolic extract of geopropolis of *M. fasciculata* have demonstrated antitumor activity against canine osteosarcoma tumor cell lines (Cinegaglia et al, 2013), cytostatic action against human laryngeal epidermoid carcinoma cells (Araújo et al. 2015), evaluation of the combination of *M. fasciculata* geopropolis extracts with the antitumour drugs carboplatin, methotrexate and doxorubicin in human laryngeal epidermoid carcinoma (Bartolomeu et al., 2016) and decreased cell viability in human monocytic leukaemia cell lines (Oliveira et al., 2016).

Our research group revealed the sensitivity of ovarian and lung cancer cells to the hydroethanolic extract *of Melipona fasiculata* geopropolis from Viana, Maranhão, as well as the identification of triterpenes with ursane, oleanane and damarene skeletons, glycosylated triterpenic saponin, flavonoids and aromatic organic acids (Barboza et al., 2020).

However, there are no antitumor tests related to the separation into apolar and polar fractions of the hydroethanolic extract of geopropolis of *M. fasciculata*, as well as chemical identification of these fractions.

Considering the incidence and mortality of lung cancer, the search for new sources of effective and safe antitumor drugs is necessary. In this perspective, this article aims to evaluate the cytotoxicity of subfractions of the ethyl acetate fraction obtained by the partition of the hydroethanolic extract of geopropolis of *M. fasciculata* Smith in lung cancer cell lines (A549 and H460) and normal cells (HUVEC), as well as the chemical identification of bioactive subfractions.

2. Materials and methods

2.1 Geopropolis samples

The samples of geopropolis of *Melipona fasciculata* Smith were collected from beehive in meliponary in the municipality of Viana (03'13'13 " S and 45º00'13 "83 W), in the state of Maranhão, Brazil, in 2018. Geopropolis was removed from hives with a spatula, placed in a sterile container and kept refrigerated at 4^0 C until use. This present research is registered on National System of Genetic Heritage Management and Associated Traditional Knowledge (SISGEN) under the code ABCEA59.

2.2 Extraction of samples

The geopropolis samples were triturated to until powder (200 g) and were extracted by maceration with 70% etanol/water (70:30, v/v) for 6 days at a solid: solvent ratio of 1 to 5 (w/v), with solvent renewal every 48 h. The resulting product from the all extractions was combined, filtered and concentrated in a rotary evaporator under vacuum at 40°C, and lyophilized (using 15 µM of Hg at 100ºC for 48 h) obtaining lyophilized hydroethanolic extract of geopropolis which were coded for EHGV and kept refrigerated until their use.

2.2.1 Liquid-liquid partition of the lyophilized hydroethanolic extract of geopropolis (EHGV)

The EHGV was dissolved in methanol:water (1:1, v/v) under mechanical stirring, the hidromethanolic solution obtained was successively partitioned with hexane and ethyl acetate. The solutions obtained were separately, filtered and dried (anhydrous Na_2SO_4) and concentrated in a rotary evaporator under reduced pressure at 40ºC, obtaining the hexanic fraction (HF), ethyl acetate (EAF) and hydromethanolic fraction (HMF). The fractions were subjected to yield determination.

2.4 Chromatographic fractionation of ethyl acetate fraction (EAF)

The EAF (1.2g) was subjected to chromatographic fractionation on a silica gel column (silica gel 60, 70-230 mesh, 0.063-0.200 mm) and eluted with hexane*,* hexane/ethyl acetate, ethyl acetate/metanol and metanol. Out of the resulting 84 fractions, the fractions were combined obtaining 13 sub-fractions encoded from FRGE-1 to FRGE-13.

2.5 LC and LC–ESI/IT–MS/MS Analysis

The FRGE-9 and FRGE-13 subfractions were analyzed by LC-ESI/IT–MS/MS (LC-20AD, Shimadzu Corp, Kyoto, Japan) and a Phenomenex Gemini C-18 (250 x 4.6mm, 5 µm) column at 25 ^{0}C, was used.The mobile phase used was Milli-Q water (Millipore) containing 0.1% formic acid (eluent A) and methanol (eluent B). Elution was performed on a linear gradient of: 0 min- 5% B; 1−45 min, 100% B, 60 min. The subfractions were diluted in methanol and 0.1% Milli-Q water of formic acid and filtered through a nylon filter (0.22 µm, Allcrom Sao Paulo, Sao Paulo, Brazil). The samples volume injected into the system were 10 µL, with a flow rate of 1 mL/min and UV–Vis detection at 254 nm. The LC was coupled to a mass spectrometer (Amazon Speed ETD, Bruker, MA, USA) equipped with electrospray ionization (ESI) and an ion trap (IT) type analyzer in negative mode under the following conditions: 5 kV capillary voltage, capillary temperature of 325 °C entrainment gas (N_2) flow 12 L/min, nitrogen nebulizer at a pressure of 10 psi. The acquisition range was *m*/*z* 100–1000, with two or more events. The compounds were identified on the basis of the molecular ion mass fragmentation.

3. Biological tests

3. 1 Cell Culture

For the experiments, lung tumour cell lines (NCI-H460 and A549) and normal cell lines (HUVEC) acquired from the American Type Culture Collection (ATCC, Manassas, VA, USA) were being used. The strains were maintained in RPMI medium (Sigma, USA) supplemented with 0.2% (w/v) sodium bicarbonate, 10% (v/v) fetal bovine serum (Gibco/Invitrogen, USA), 1% (v/v) stabilised solution of penicillin (100 units/mL) and streptomycin (100 µg/mL) (Gibco/Invitrogen, USA) in an incubator with controlled temperature conditions (37°C) with an atmosphere of 5% CO_2.

3.2 Cytotoxicity Activity

Fractions HF, EAF, HMF and subfractions FRGE-1 to FRGE-13 were evaluated in cytotoxicity assays using 3-4,5 dimethylthiazol-2,5 diphenyl tetrazolium bromide (MTT) according to Mosmann's methodology (1983). Tumor cell lines were plated (1 x 10^4 cells/well) in 96-well plates and, after 24h of culture, treated with the fractions at

concentrations of 15.62, 31.25, 62.5, 125, 250 µg/mL for 48h. Subsequently, the cells were incubated with 20 µL of MTT solution (5 mg/mL) for 4h. The plates were centrifuged at 450 g and, to allow the formazan crystals to be solubilized, 100µL of dimethylsulfoxide (DMSO) was added to each well and the spectrophotometric reading of the absorbance, at a wavelength of 538 nm, was performed in a reader of plates (MR-96 A, Bioclin, Brazil). As control of the experiments, untreated cells were used. The experiments were performed in triplicate.

3.3 Selectivity index

The selectivity index (SI) of fractions HF, EAF, HMF and subfractions FRGE-1 to FRGE-13 were performed according Hasoon & Kadhim (2021). The selectivity indices of the fractions and subfractions were obtained from the ratio between the IC_{50} values of non-tumor cells and IC_{50} of tumor cells expressed in the equation:

$$SI = \frac{IC_{50}\ (\text{non-tumor cells})}{IC_{50}\ (\text{tumor cells})}$$

Cell values greater than or equal to 2.0 were considered to indicate that the sample was twice as active in tumor cells as in normal cells.

4 Statistical analyses

Cytotoxicity data were initially normalized (mean ± standard deviation) transformed into Log (X) and analyzed by nonlinear regression to obtain IC_{50} for each fraction using GraphPad Prism 8.0 software (GraphPad Inc., San Diego, CA, USA). Data were subjected to statistical analysis; two-way ANOVA test followed by the Dunnet test, considering $p < 0.05$ as significant. All in vitro studies were performed in triplicate, represented by independent biological assessments.

4. Results

Liquid-liquid partition of the lyophilized hydroethanolic extract of geopropolis (EHGV)

The partition of the EHGV resulted in the fractions hexanic (HF), ethyl acetate (EAF) and hydromethanolic fraction (HMF). The HMF (30%) and EAF (26%) showed higher yield than the HF (4%).

Cytotoxicity activity

HF, EAF and HMF were investigated for the cytotoxic activity tested for cell growth inhibition for lung cancer cell lines (H460 and A549) and normal cells (HUVEC) All three fractions showed low toxicity to normal cells (IC_{50} > 250 µg/mL). HMF showed lower toxicity for lung cancer strains, presenting IC_{50} 196.50 µg/mL and 275.90 µg/mL. HF had better cytotoxicity with IC_{50} 40.99 µg/mL and 51.83 µg/mL (Table 1).

Table 1. Inhibitory concentration (IC_{50}) of the hexanic fraction (HF), ethyl acetate fraction (EAF) and hydroethanolic fraction (HMF) obtained of the lyophilized hydroethanolic extract geopropolis of *Melipona fasciculata* Smith (EHGV) for inhibition of cell proliferation in lung cancer cell lines (H460 and A549) and normal cells (HUVEC) in 48h.

Samples	Cell lines		
	H460	A549	HUVEC
HF	40.99 µg/mL	51.83 µg/mL	>250.00 µg/mL
EAF	118.70 µg/mL	142.10 µg/mL	>250.00 µg/mL
HMF	196.50 µg/mL	275.90 µg/mL	>250.00 µg/mL

HF= Hexanic fraction. EAF= Ethyl acetate fraction. HMF= Hydromethanolic fraction. IC_{50} values calculated by non-linear regression equation log (inhibitor) versus response—variable slope by the MTT assay. Concentration required to inhibit cell growth by 50% as determined by the dose response curve. Values are expressed as mean of cytotoxicity assays (n = 3).

HF was more cytotoxic, but of low yield, which would make the next step of the research difficult, therefore, EAF was chosen for fractionation in column chromatography, generating 84 subfractions, which were analyzed by thin layer chromatography (TLC) and pooled according to their similarity, resulting in 13 subfractions (FRGE-1 to FRGE-13).

Among the thirteen fractions tested, FRGE-9 and FRGE-11 had the best results, with cytotoxicity in cells line A549, with IC_{50}, respectively, 65.52 µg/mL and 63.87 µg/mL (Table 2).

Table 2 Inhibitory concentration (IC_{50}) of the subfractions FRGE-1, FRGE-2, FRGE-3, FRGE-4, FRGE-5, FRGE-6, FRGE-7, FRGE-8, FRGE-9, FRGE-10, FRGE-11, FRGE-12 and FRGE-13 for inhibition of cell proliferation in lung cancer cell lines (H460 and A549) and normal cells (HUVEC) in 48h.

Samples	Cell lines		
	H460	A549	HUVEC
FRGE-1	146.40 µg/mL	128.50 µg/mL	129.70 µg/mL
FRGE-2	133.60 µg/mL	125.80 µg/mL	58.82 µg/mL
FRGE-3	141.60 µg/mL	161.70 µg/mL	75.78 µg/mL
FRGE-4	79.20 µg/mL	138.70 µg/mL	115.20 µg/mL
FRGE-5	87.13 µg/mL	175.50 µg/mL	88.83 µg/mL
FRGE-6	86.50 µg/mL	>250.00 µg/mL	177.30 µg/mL
FRGE-7	88.58 µg/mL	251.10 µg/mL	>250.00 µg/mL
FRGE-8	112.60 µg/mL	172.50 µg/mL	>250.00 µg/mL
FRGE-9	>250.00 µg/mL	65.52 µg/mL	216.10 µg/mL
FRGE-10	178.80 µg/mL	>250.00 µg/mL	192.10 µg/mL
FRGE-11	>250.00 µg/mL	63.87 µg/mL	215.40 µg/mL
FRGE-12	>250.00 µg/mL	>250.00 µg/mL	120.30 µg/mL
FRGE-13	239.00 µg/mL	>250.00 µg/mL	168.50 µg/mL

FRGE-1, FRGE-2, FRGE-3, FRGE-4, FRGE-5, FRGE-6, FRGE-7, FRGE-8, FRGE-9, FRGE-10, FRGE-11, FRGE-12 and FRGE-13= subfractions obtained from the chromatographic fractionation of the ethyl acetate fraction (EAF) of the lyophilized hydroetanolic extract of geopropolis from *M. fasciculata* (EHGV). IC_{50} values calculated by non-linear regression equation log (inhibitor) versus response—variable slope by the MTT assay. Concentration required to inhibit cell growth by 50% as determined by the dose response curve. Values are expressed as mean of cytotoxicity assays (n = 3).

The results obtained through the MTT assay revealed that the fractions decreased the percentage of cell viability for lung cancer cells (H460 and A549) within 48h (Figure 1, 2 and 3).

At a concentration of 62.5 µg/mL, HF decreased less than 40% of the cell viability of A549 and H460 cells (Figure 1 and 2). At maximum concentration (250 µg/mL), FRGE-5 to FRGE-8 decreased cell growth from H460 to less than 20% (Figure 1). HF, EAF and HMF had no cytotoxicity for normal cells (HUVEC), unlike FRGE-1 to FRGE-4 which were cytotoxic for non-tumor cells at 250 µg/mL (Figure 3).

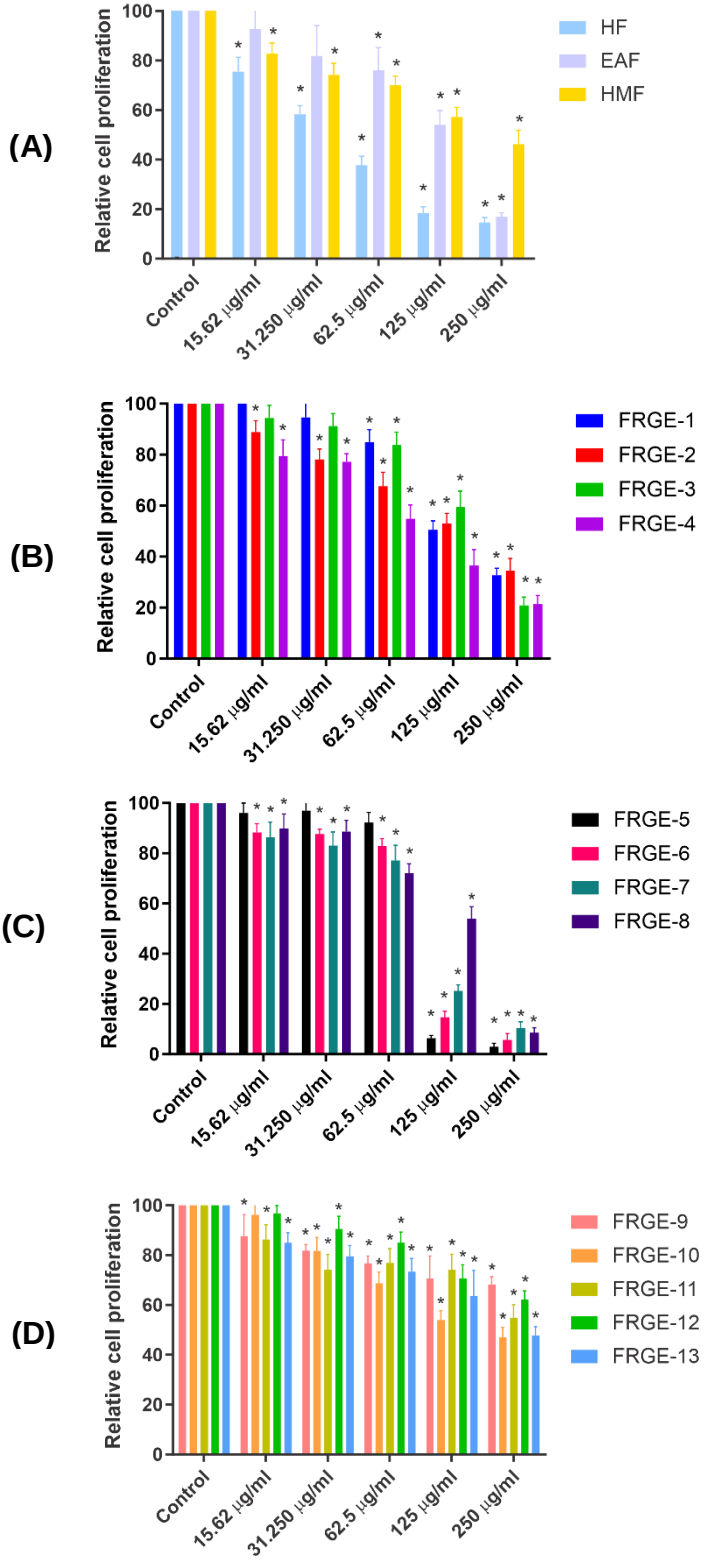


Figure 1 - Effects de HF, EAF, HMF **(A)**, FRGE-1 to FRGE-4 **(B)**, FRGE-5 to FRGE-8 **(C)**, FRGE-9 to FRGE-13 **(D)** in lung cancer cell line (H460) in 48h with statistical results. 2-way ANOVA with Dunnet test. (*indicates $p \leq 0.05$ as significant).

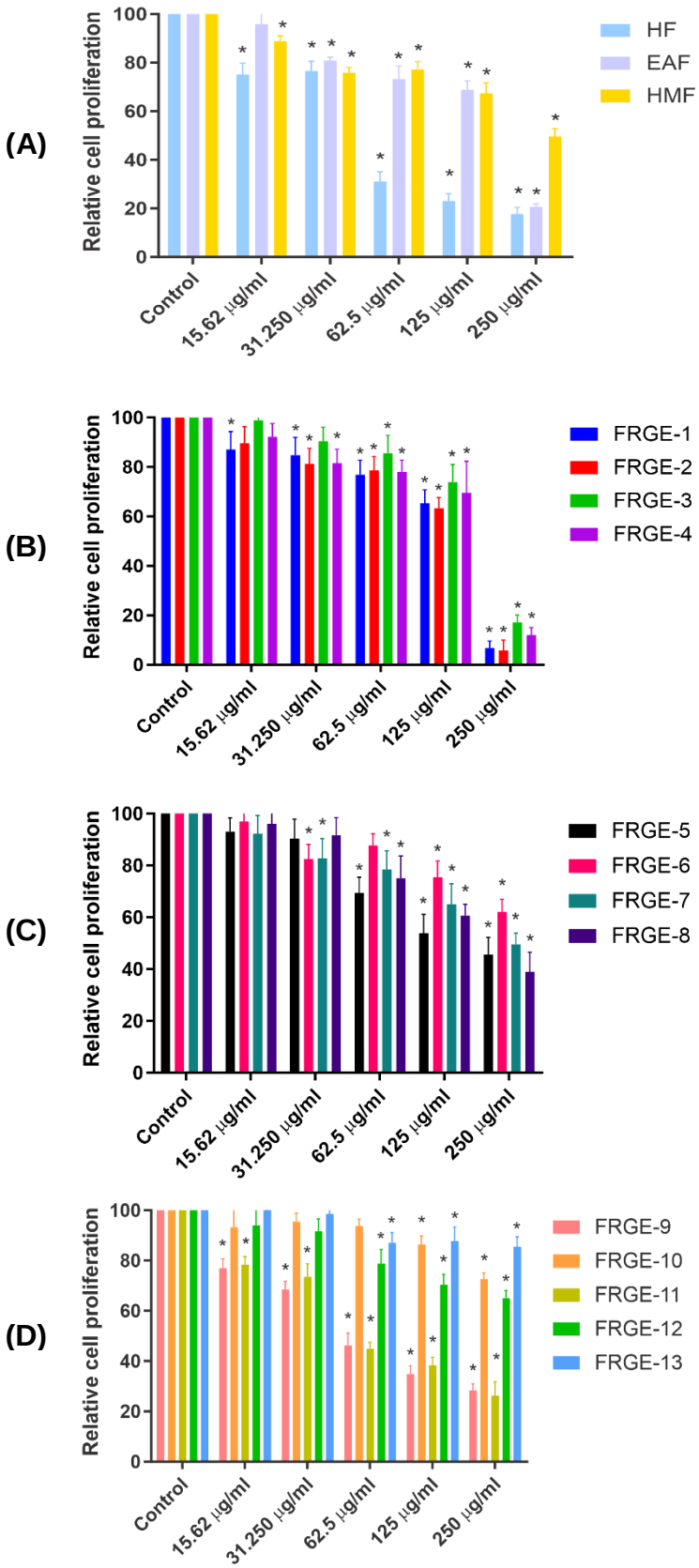


Figure 2 - Effects de HF, EAF, HMF **(A)**, FRGE-1 to FRGE-4 **(B)**, FRGE-5 to FRGE-8 **(C)**, FRGE-9 to FRGE-13 **(D)** in lung cancer cell line (A549) in 48h with statistical results. 2-way ANOVA with Dunnet test. (*indicates $p \leq 0.05$ as significant).

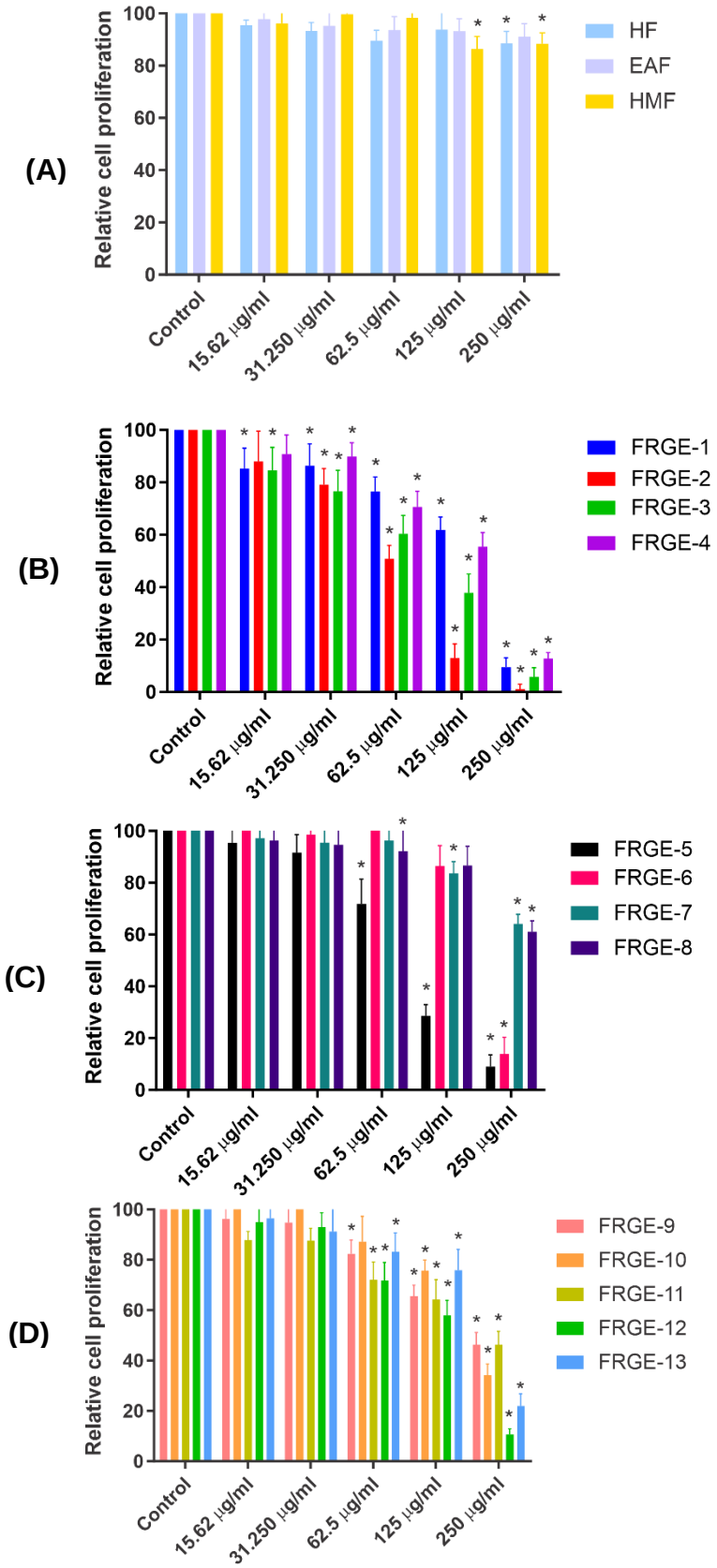


Figure 3 - Effects de HF, EAF, HMF **(A)**, FRGE-1 to FRGE-4 **(B)**, FRGE-5 to FRGE-8 **(C)**, FRGE-9 to FRGE-13 **(D)** in normal cell lines (HUVEC) in 48h with statistical results. 2-way ANOVA with Dunnet test. (*indicates $p \leq 0.05$ as significant).

Selectivity index (SI)

Analyzing data from Table 3, we observed that HF showed a significant selectivity index for the two lung cancer cell lines (H460 and A549). FRGE-6, FRGE-7 and FRGE-8 showed selectivity for H460, in contrast to FRGE-9 and FRGE-11 showed selectivity for A549.

Table 3. Selectivity index (SI) of fractions and sub-fractions of the lyophilized hydroetanolic extract of geopropolis from *Melipona fasciculata* Smith

Samples	Cells	
	H460	A549
HF	>6.09	>4.82
EAF	>2.10	>1.75
HMF	>1.27	>0.90
FRGE-1	0.88	1.00
FRGE-2	1.00	0.5
FRGE-3	0.43	0.46
FRGE-4	1.45	0.83
FRGE-5	1.01	0.50
FRGE-6	2.04	<0.70
FRGE-7	>2.82	>0.99
FRGE-8	>2.22	>1.44
FRGE-9	<0.86	3.29
FRGE-10	1.07	<0.76
FRGE-11	<0.86	3.37
FRGE-12	<0.48	<0.48
FRGE-13	0.70	<0.67

HF= Hexanic fraction. EAF= Ethyl acetate fraction. HMF= Hydromethanolic fraction. FRGE-1 to FRGE-13= Sub-fractions obtained from the chromatographic fractionation of the ethyl acetate fraction (EAF) of the lyophilized hydroetanolic extract of geopropolis (EHGV).

HPLC/UV and LC–ESI/IT–MS/MS Analysis

Considering that the FRGE-09 and FRGE-11 subfractions were the most active in the anticancer assays, their chemical compositions were analyzed by HPLC/UV−vis and LC–ESI/IT–MS/MS.

The HPLC chromatograms of the most bioactive subfractions are shown in Figures 4 and 5.

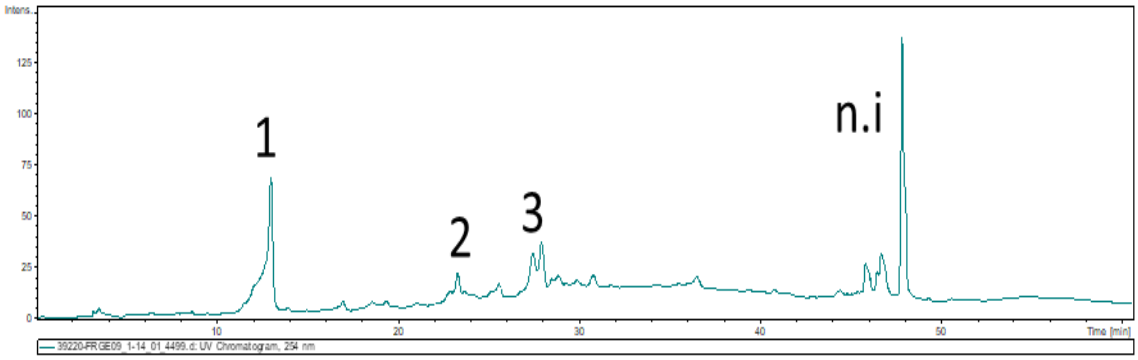


Figure 4. HPLC chromatogram of FRGE-09 detected at 254 nm. Peak number 1= dihydroxybenzoic acid; peak number 2= apigenin-6-C-glucoside; peak number 3=luteolin-5-O-beta-rutinoside; ni=not identified.

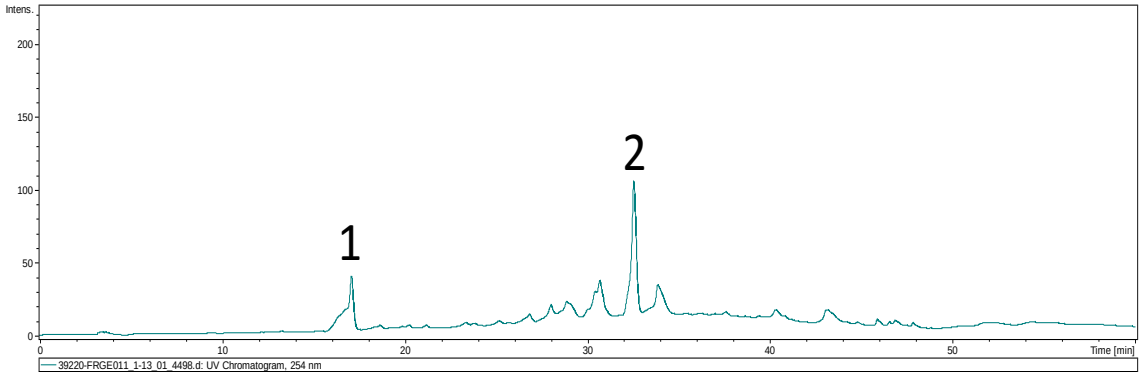
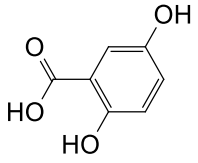
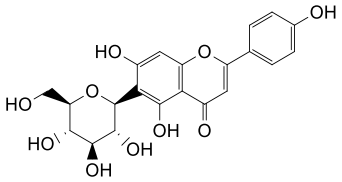
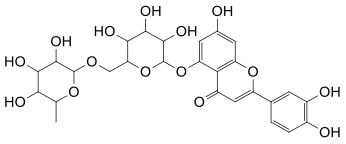


Figure 5. HPLC chromatogram of FRGE-11 detected at 254 nm. Peak number 1=diosmetin; peak number 2=myricetin-3-galactoside.

Chemical identifications of the FRGE-09 and FRGE-11 subfractions by LC–ESI/IT–MS/MS are described in tables 4 and 5.

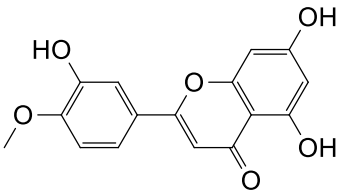
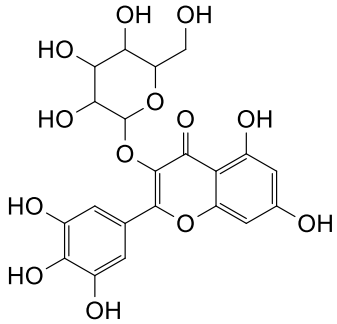
Table 4. Compounds identified in the FRGE-09, subfraction of obtained from the chromatographic fractionation of the ethyl acetate fraction (EAF) of the lyophilized hydroetanolic extract of geopropolis (EHGV) by LC-ESI/IT-MS/MS.

Peak	RT (min)	MW	$[M-H]^-$ m/z	MS/MS	Identification	Chemical structure
1	13.1	154	153	109	Dihydroxybenzoic acid	

Peak	RT (min)	MW	[M-H]- m/z	MS/MS	Identification	Chemical structure
2	23.2	432	431	311; 283	Apigenin-6-C-glucoside	
3	27.5	594	593	285	Luteolin-5-O-beta-rutinoside	
4	47.8	500	599	463	n.i	-

RT=retention time; MW=molecular weight; [M − H]⁻=molecular ion; MS=mass spectrometry; ni=not identified.

Table 5. Compounds identified in the FRGE-11, subfraction of obtained from the chromatographic fractionation of the ethyl acetate fraction (EAF) of the lyophilized hydroetanolic extract of geopropolis (EHGV) by LC-ESI/IT-MS/MS.

Peak	RT (min)	MW	[M-H]- m/z	MS/MS	Identification	Chemical structure
1	15.6	300	299	153	Diosmetin	
2	34.2	480	479	259; 237	Myricetin-3-galactoside	

RT=retention time; MW=molecular weight; [M − H]⁻=molecular ion; MS=mass spectrometry; ni=not identified.

5. Discussions

The present study demonstrates the cytotoxicity of FRGE-09 and FRGE-11 subfractions of the ethyl acetate fraction obtained by partitioning the hydroethanolic extract of geopropolis from *Melipona fasciculata* against lung cancer strains (A549 and H460), which are constituted by flavonoids, glycolyzed flavonoids and phenolic acid.

The hexane (HF), ethyl acetate (EAF) and hydromethanolic (HMF) fractions of the hydroethanolic extract of geopropolis *Melipona fasciculata* inhibited cell proliferation in lung cancer cell lines (H460 and A549) in 48h.

FH showed a decrease (40.99 µg/mL and 51.83 µg/mL) of cell viability in both tumor lines, unlike EAF and FHM, decreased cell viability from concentrations higher than 100 µg/mL

The hexane fraction of the ethanolic extract of geopropolis from *Melipona scutellaris* showed an IC_{50} of 9.55 µg/mL for the H460 strain (Da Cunha et al., 2013), unlike the IC_{50} of HF in this study (40.99 µg/mL).

The cytotoxic activity of the hexane (HFr) and ethyl acetate (AcEFr) fractions of the ethanolic extract of *Melipona mondury* geopropolis on B16-F10 (murine melanoma), HepG2 (human hepatocellular carcinoma), K562 (human chronic myeloid leukemia) and HL- 60 (human promyelocytic leukemia) after 72h showed a variation of IC_{50} for HFr and AcEFr, respectively, 24.24 to 46.62 µg/mL and 45.90 to 29.79 µg/mL (Dos Santos et, 2017). These IC_{50} results were close to the FH of geopropolis from *Melipona fasciculata* in lung cancer strains, while in the EAF and HMF fractions of this species, the IC_{50} were more distant. Subfractions of the ethyl acetate fraction (EAF) were also responsible for the cytotoxicity of the two pulmonary strains.

In the literature, we did not find reports of antitumor activity in lung cancer (H460 and A549) of subfractions resulting from the liquid-liquid partition of the hydroethanolic extract of geopropolis from *M. fasciculata*

Cinegaglia, et al. (2013) reported anticancer effects of the hydroethanolic extract of geopropolis from *M. fasciculata* on canine osteosarcoma cells. Bartolomeu et al. (2016) report the combinatorial effect of the hydroethanolic extract of geopropolis produced by *M. fasciculata* with anticancer drugs against human laryngeal epidermoid carcinoma (HEp-2) cells.

Araujo et al. (2015) indicate that hydroethanolic extract of geopropolis from *M. fasciculata* exhibited cytostatic action against human laryngeal epidermoid carcinoma cells.

Barboza et al. (2020) reported that the hydroethanolic extract of geopropolis *M. fasciculata* decreased by 50% the cell viability of lung cancer strains (A549 and H460) in 48h.

Analyzing data from Table 2, EAF, FRGE-9 and FRG-11 showed a significant selectivity index for the lung cancer cell line (A549).

According to Suffness & Pezzuto (1991), the selectivity index is a good parameter to assess whether there is a difference in toxicity between normal cell lines and tumor cells, suggesting a potential use of certain substances in future clinical trials. SI values greater than or equal to 2.0 were considered significant, indicating that the substance is more active in neoplastic cell lines than in normal cells.

The FRGE-6 and FRGE-8 subfractions were twice as selective for lung cancer tumor lineage (H460) than for normal cells (HUVEC). FRGE-9 and FRGE-11 were three times more selective for another lung cancer tumor cell line (A549) than for non-tumor cells.

The SI of the hexane fraction of the ethanolic extract of geopropolis from *Melipona scutellaris* was approximately 2 for the H460 strain (Da Cunha et al., 2013), a result lower than the selectivity indices of FH, that were >6.09.

Polyphenolic compounds were identified in the two bioactive subfractions FRGE-9 and FRGE-11 (Tables 3 and 4) being one flavonoid (diosmetin), three glycosylated flavonoids (apigenin-6-C-glycoside, luteolin-5-O-beta-rutinoside and myricetin -3-galactoside) and a phenolic acid (dihydroxybenzoic acid).

Dihydroxybenzoic acid has already been identified in the hydroethanolic extract of geopropolis from *M. fasciculata* Smith (Batista et al., 2016; Dutra et al., 2014; 2019).

Diosmetin, apigenin-6-C-glucoside, luteolin-5-rutinoside and myricetin-3-galactoside are being identified for the first time in the hydroethanolic extract of geopropolis from *M. fasciculata*.

Yin et al., (2009) demonstrated after lung cancer treatment (IMR-90) with dihydroxybenzoic acid, decreased lung cancer cell viability, DNA fragmentation, decreased mitochondrial membrane potential and increased caspase- 3 and caspase-8 in these cells. This substance also suppressed cell adhesion and production of VEGF

(vascular endothelial growth factor), IL-6, IL8 and ICAM-1 (Intercellular Adhesion Molecule 1) in cancer cells.

Flavonoids act on the complex molecular mechanisms of anti-cancer actions, such as the modulation of enzymes that metabolize carcinogens, specific arrest of the cell cycle and activation of programmed cell death, apoptosis, therefore, they are potential agents to act in preventive/therapeutic measures of cancer of lung. (Hou & Kumamoto, 2010; Khan & Mukhtar, 2015; Braicu et al., 2017).

Apigenin-6-C-glycoside inhibited the growth of large lung cancer cells (CORL-23) (Conforti et al., 2009). Furthermore, Cao et al., (2016) reported that this glycosylated flavonoid selectively suppressed the ability to form spheres tumors, as well as migration and invasion in lung cancer stem cells derived from NCI-H446 cells.

In vitro assays by Chen et al., (2019) showed that diosmetin induced selective apoptotic death in lung cancer cells (A549 and H1299), without causing significant toxicity to normal cells. In vivo studies, mice inoculated with A549 cells and then treated with diosmetin and paclitaxel demonstrated synergistic suppression of these cells. The absence of *in vitro* toxicity was confirmed after histological analysis of vital organs. The combination of diosmetin and paclitaxel provided greater inhibition of tumor growth compared to the two single treatments.

Luteolin is a promising substance capable of stimulating multiple targets in lung cancer. In human non-small cell lung cancer (NSCLC) cell line A549, it was shown to be effective against cancer cell proliferation, inducing cell death and suppressing cell migration. The induction of apoptosis is associated with the activation of caspases-3 and -9, altering the phosphorylation of MEK, the expression of Bcl-2 family proteins (Bax, Bcl-2) and its downstream kinase ERK, and the phosphorylation of Akt (Meng et al., 2016).

According to Suvarna et al., (2018), luteolin activates the intrinsic pathway of apoptosis by activating p53 and inducing DNA damage, and this occurs by inhibiting DNA topoisomerase enzymes. Furthermore, luteolin triggers sustained activation of JNK which can promote the apoptosis pathway, presumably through modulation of p53 or BAD and further suppresses cell survival pathways to lower the apoptosis threshold. This substance inhibits several survival pathways, such as PI3K/Akt, NF-κB, and MAPKs in cancer cells, which can mimic growth factor deprivation that blocks the signaling pathways triggered by growth factor.

A549 and H1299 cancer cells were exposed to X-rays with or without myricetin treatment. Comparison between groups showed that myricetin-treated groups significantly suppressed cell survival and proliferation, increased cell apoptosis and increased caspase-3 protein expression after in vitro exposure to X-rays. In in vivo assays, the rate of growth of tumor xenografts were significantly decreased in myricetin-treated irradiated mice (Zhang et al., 2014).

Myricetin exerted a dose-and time-dependent inhibitory effect on adhesion, invasion and migration of A549 cells in the absence of cytotoxicity. Gelatin or casein zymography assays showed that myricetin inhibited the metalloproteinase-2 (MMP-2) and urokinase-plasminogen activator (u-PA) activities of A549 cells. These two markers are fundamental for cell invasion and migration (Shih et al., 2009).

In the literature, antitumor activities of the flavonoids myricetin and luteolin are described in lung cancer strains, but for the glycosylated flavonoids (luteolin-5-rutinoside and myricetin-3-galactoside), so far there are no reports of this action.

The results suggest that the antitumor actions of fractions of geopropolis of *Melipona fasciculata* against lung cancer cells (A549 and H460) are related to the chemical constituents diosmetin, apigenin-6-C-glycoside, luteolin-5-O-beta-rutinoside and myricetin -3-galactoside and dihydroxybenzoic acid. Thus, the results obtained demonstrated the therapeutic value of the chemical constituents in the perspective of suggesting the future prototypes of lung cancer molecules.

6. Conclusion

The FRGE-9 and FRGE-11 subfractions obtained from the ethyl acetate fraction of the hydroethanolic extract of the geopropolis of *Melipona fasciculata* Smih showed relevant cytotoxicity for lung cancer strains (A549 and H460), which are constituted by dihydroxybenzoic acid, apigenin-6-C-glycoside, luteolin-5-O-beta-rutinoside (FRGE-9), diosmetin and myricetin-3-galactoside (FRGE-11) and are related to antitumor actions. Diosmetin, apigenin-6-C-glucoside, luteolin-5-rutinoside and myricetin-3-galactoside are being identified for the first time in the in the geopropolis of the species.

Thus, despite the promising results, cytotoxicity assays of diosmetin, luteolin-5-rutinoside and myricetin-3-galactoside, as well as the exploration of their mechanisms of action of antitumor activity, are necessary for the suggestion of future prototypes of molecules for the lung cancer treatment more effective and safe.

Declaration of interests

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

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5 CONSIDERAÇÕES FINAIS

A revisão de literatura de estudos pré-clínicos antitumorais de própolis e geoprópolis de espécies de abelhas sem ferrão demonstrou resultados de citotoxicidade desses produtos em diferentes linhagens tumorais e composição química complexa.

As frações FRGE-9 e FRGE-11, separadas da fração acetato de etila do extrato hidroetanólico da geoprópolis de *Melipona fasciculata* Smith, apresentaram citotoxicidade relevante para as duas linhagens de câncer de pulmão. A identificação dos constituintes químicos revelou a presença de quatro substâncias flavonóidicas, o flavonoide diosmentina e os flavonoides glicosilados, apigenin-6-C-glucoside, luteolina-5-rutinosídeo e miricetina-3-galactosídeo ainda não identificados no extrato hidroetanólico da geoprópolis *M. fasciculata* com destaque aos flavonóides glicosilados (luteolina-5-rutinosídeo e miricetina-3-galactosídeo) ainda não testados em células de câncer de pulmão.

Os resultados encontrados estimulam a necessidade de continuidade dos ensaios antitumorais dessas substâncias, bem como seus mecanismos de ação antitumoral, a fim de sugerir protótipos mais eficazes e seguros para o tratamento do câncer de pulmão.

As atividades antitumorais da geoprópolis de *Melipona fasciculata* poderão impulsionar a meliponicultura para o aproveitamento da geoprópolis e permitir sua exploração biotecnológica de forma sustentável, como materia prima para futuros produtos anticânceres de pulmão.

Ressalta-se que a meliponicultura é uma atividade de fácil manejo, baixo custo e o cultivo de *M. fasciculata* é utilizado para produção de mel no estado do Maranhão, e é uma das fontes de renda para várias famílias das regiões da Baixada e Cerrado maranhense e a geopropolis na maioria das vezes é descartado durante o processo de limpeza das colmeias de tiúba.

A exploração racional da geoprópolis produzida de *M. Fasciculata*, que é nativa no Maranhão, permitiria o fortalecimento da cadeia produtiva da meliponicultura (criação de abelhas sem ferrão), fomentaria a formação de recursos humanos e permitiria o desenvolvimento sustentável, pois a exploração para estudos químicos e biológicos não acarretaria impactos ambientais.

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