UNIVERSIDADE VILA VELHA - ES

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCAS FARMACÊUTICAS

ESTUDO DA COMPOSIÇÃO QUÍMICA E DO POTENCIAL BIOATIVO DE GEOPRÓPOLIS E DA PRÓPOLIS DE ABELHAS SEM FERRÃO NATIVAS DO ESPÍRITO SANTO

ARIANE PINHEIRO CRUZ BERGAMINI

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Tese apresentada à Universidade Vila Velha, como pré-requisito do Programa de Pós-graduação em Ciências Farmacêuticas, para a obtenção do grau de Doutora em Ciências Farmacêuticas.

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ARIANE PINHEIRO CRUZ BERGAMINI

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Aprovada em 29 de junho de 2023,

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“Esforce-se para não ser um sucesso,
mas sim para ser valioso” (Albert Einstein).

SUMÁRIO

LISTA DE ABREVIATURAS E SIGLAS

AAPH - 2,2'-azobis(2-amidinopropano)dihidroclorido

ABTS+ - 2,2-azinobis-(3-etilbenzotiazol-6-sulfonato)

AME-ES – Associação dos Meliponopultures do Espírito Santo

ANOVA – Análise de Variância

ANVISA – Agência Nacional de Vigilância Sanitária

ASF – abelha sem ferrão

BHT - 2,6-tert-butil-1-hidroxi-tolueno

DMSO - Dimetilsulfóxido

DPPH - 2,2-difenil-1-picrilhidrazil

ELISA - Ensaio Imunoenzimatico

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária

ESI-FT-ICR-MS – *Fourier transform ion cyclotron resonance mass spectrometry combined with a direct infusion electrospray ionization* - espectrometria de massas por ressonância ciclotrômica de íons com transformada de Fourrier acoplada à ionização por eletrospray.

FRAP - poder antioxidante de redução férrica

GAE – *gallic acid equivalent* – equivalente de ácido gálico

IC_{50} - concentração do extrato requerida para reduzir a quantidade de radicais livres por 50%

IDAF - Instituto de defesa agropecuária e florestal do Espírito Santo

LBA – líquido bronqueoalveolar

LPS – Lipopolissacarídeo

MB - *Melipona bicolor*

MC - *Melipona capixaba*

MDA – Malondealdeído

MFC – *minimal fungicidal concentration* – concentração mínima fungicida

MIC – *minimal inhibitory concentration* – concentração inibitória mínima

MM - *Melipona mondury*

MQ - *Melipona quadrifasciata*

MTT - (brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio)

QE – *quercetin equivalent* – equivalente de quercetina

TC – *Tetragona clavipes*

TNFα – *tumor necrosis fator* - fator de necrose tumoral

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RESUMO

Bergamini, Ariane Pinheiro Cruz, Dr., Universidade Vila Velha – ES, junho de 2023. **Estudo da composição química e do potencial bioativo de geoprópolis e da própolis de abelha sem ferrão nativas do Espírito Santo.** Orientador: Prof. Dr. Marcio Fronza.

A própolis e geoprópolis são produtos derivados da resina vegetal e secreção salivar de abelhas sem ferrão. São usados na medicina popular devido aos seus componentes bioativos. No entanto, registros científicos que comprovem tais atividades de espécies ocorrentes no Espírito Santo – Brasil ainda são escassos. O objetivo deste estudo foi avaliar a composição química e as atividades biológicas da geoprópolis e própolis de cinco espécies de abelhas sem ferrão durante as estações seca e chuvosa. A caracterização físico-química foi realizada por análise de polifenóis totais, flavonoides, taninos e por espectrometria de massas por ressonância ciclotron de íons com transformada de Fourrier acoplada à ionização por eletrospray. A atividade antioxidante foi investigada pelos métodos de sequestro dos radicais DPPH e ABTS. Testes *in vitro* foram realizados para avaliar a produção de óxido nítrico, superóxido, citocina TNFα e determinar as atividades fungistatica, fungicida e citotóxica. Os resultados obtidos revelaram altos teores de fenólicos totais e flavonóides, uma promissora atividade antioxidante, anti-inflamatória e antifúngica. Os extratos apresentaram diferenças, quanto a composição e atividade biológica, entre as espécies e sazonalidade. Particularmente, o extrato de geoprópolis de *M. bicolor* e *M. capixaba,* coletados durante a estação seca, exibiram as maiores atividades antioxidantes e anti-inflamatórias, respectivamente. Especificamente, o extrato de própolis de *T. clavipes* coletado na estação chuvosa apresentou melhor atividade antifúngica quando comparado as demais espécies e estação. Os resultados sugerem que as própolis e geoprópolis de abelhas sem ferrão do Espírito Santo possuem potencial terapêutico devido à sua composição química e atividades biológicas. No entanto, são necessários mais estudos para aprofundar o conhecimento sobre esses produtos naturais e seu uso na medicina popular. A sazonalidade e a variação entre as espécies evidenciam a importância de considerar fatores ambientais e genéticos na obtenção desses produtos de qualidade. Essas descobertas contribuem para a valorização e conservação das abelhas sem ferrão, bem como para a busca de novas terapias baseadas em produtos naturais.

Palavras-chaves: abelha sem ferrão, própolis, geoprópolis, antioxidante, anti-inflamatório, antifúngico.

ABSTRACT

Bergamini, Ariane Pinheiro Cruz, Dr., Vila Velha University – ES, June 2023. **Chemical characterization and bioactive potential study of stingless bees geopropolis and propolis from Espírito Santo.** Advisor: Prof. Dr. Marcio Fronza.

Propolis and geopropolis are products derived from plant resin and salivary secretion of stingless bees. They are used in folk medicine due to their bioactive components. However, scientific records confirming such activities of species occurring in Espírito Santo, Brazil, are still scarce. The aim of this study was to evaluate the chemical composition and biological activities of geopropolis and propolis from five species of stingless bees during the dry and rainy seasons. Physicochemical characterization was performed by analysis of total polyphenols, flavonoids, tannins, and Fourier transform ion cyclotron resonance mass spectrometry coupled with electrospray ionization. Antioxidant activity was investigated using DPPH and ABTS radical scavenging methods. In vitro tests were conducted to evaluate nitric oxide production, superoxide production, TNF-α cytokine, and determine fungistatic, fungicidal, and cytotoxic activities. The results revealed high levels of total phenolics and flavonoids, promising antioxidant, anti-inflammatory, and antifungal activities. The extracts showed differences in composition and biological activity among the species and seasons. Particularly, geopropolis extract from M. bicolor and M. capixaba, collected during the dry season, exhibited the highest antioxidant and anti-inflammatory activities, respectively. Specifically, propolis extract from T. clavipes collected during the rainy season showed the best antifungal activity compared to the other species and season. These findings suggest that propolis and geopropolis from stingless bees in Espírito Santo have therapeutic potential due to their chemical composition and biological activities. However, further studies are needed to deepen the knowledge about these natural products and their use in folk medicine. Seasonality and variation among species highlight the importance of considering environmental and genetic factors in obtaining high-quality products. These discoveries contribute to the valorization and conservation of stingless bees, as well as the search for new therapies based on natural products.

Keywords: stingless bee, propolis, geopropolis, antioxidant, anti-inflammatory, antifungical

INTRODUÇÃO GERAL

INTRODUÇÃO GERAL

As abelhas sem ferrão (ASF) e seus produtos, tais como própolis e geoprópolis têm despertado interesse a nível nacional e internacional devido ao valor ambiental, sócio econômico e terapêutico (POTTS et al., 2016).

Esses insetos são agentes polinizadores fundamentais para a conservação dos ecossistemas e da biodiversidade das espécies vegetais (IMPERATRIZ-FONSECA et al., 2012). Atuam como bioindicadores da qualidade ambiental por serem sensíveis a agrotóxicos e são utilizadas como ferramentas em ações de educação ambiental (BRASIL, 2017).

No mundo há uma tendência para o consumo de produtos naturais e os governantes tentam alcançar os Objetivos de Desenvolvimento Sustentável (ODS), que incluem uma vida saudável e ambiente ecológico (UN, 2023). No Brasil, o Ministério da Saúde incluiu a apiterapia como nova prática integrativa no Sistema Único de Saúde (BRASIL, 2018). Esta prática utiliza produtos produzidos pelas abelhas nas colmeias como a apitoxina, geléia real, pólen, própolis, mel e outros. Especificamente, a própolis tem sido consumida como agente promotora da saúde devido a suas propriedades antiviral, antibacteriana, antifúngicas e antiparasitárias (ZULHENDRI et al., 2021). Desta forma, o uso de produtos apícolas sem ferrão como a própolis contribui para os ODS e pode revolucionar as indústrias alimentícias, farmacêuticas e cosméticas, pois suas propriedades terapêuticas já são reconhecidas (LAVINAS et al., 2018; ROZMAN et al., 2022).

Para que o setor industrial tenha mais confiança para investir nos projetos com produtos das ASF, existe a necessidade de padronização dessa matéria-prima e comprovações científicas de seu potencial, porém estudos sobre estes temas ainda são escassos (ROZMAN et al., 2022).

Mesmo com todos seus benefícios, há relatos que as ASF se encontram ameaçadas de extinção devido a inúmeros fatores, tais como: desmatamentos, mudanças climáticas, pesticidas, culturas geneticamente modificadas, patógenos e espécies exóticas invasoras (BROWN et al., 2014; LOPES et al., 2005; POTTS et al., 2016). Por isso, normativas de manejo destas abelhas, tais como Lei nº 11077 de 27/11/2019 – DOE/ES estabelece que a criação de ASF seja restrita à região geográfica de ocorrência natural. Isso tem restringido os projetos científicos, pois apenas instituições locais de pesquisa acabam acessando as criações da região (IDAF-ES, 2019).

Não existem estudos sobre a composição química e propriedades bioativas da geoprópolis e própolis de ASF coletadas no Espírito Santo (ES). Trabalhos científicos demonstram que essas composição e propriedades são influenciadas pela biodiversidade da flora, área geográfica, sazonalidade e espécies de abelha amostradas em outros estados brasileiro (CAMPOS et al., 2015; DUTRA et. al, 2014). Desta forma, a hipótese deste estudo é que a espécie de abelha e a sazonalidade alteram a composição físico-química da geoprópolis e própolis coletadas no ES, bem como modifica suas propriedades antioxidantes, anti-inflamatórias e antifúngicas.

Neste contexto, este trabalho objetivou a investigação da composição físico-química e a avaliação do potencial bioativo da própolis de abelhas sem ferrão nativas do Espírito Santo.

Considerando os resultados obtidos por meio das metodologias propostas, o trabalho foi dividido em dois capítulos. O primeiro capítulo apresenta os principais metabólitos secundários e as propriedades antioxidante, anti-inflamatória e antifúngica da própolis das espécies *Melipona quadrifasciata*, *Melipona mondury*, *Melipona capixaba* e *Melipona bicolor.* E o segundo capítulo descreve a composição físico-química da própolis da espécie *Tetragona clavipes* e suas propriedades antioxidante e antifúngica, cuja atividade foi diferenciada e pesquisada melhor considerando o tempo de morte e sinergismo.

FUNDAMENTAÇÃO TEÓRICA

FUNDAMENTAÇÃO TEÓRICA

Abelhas sem ferrão e seus produtos

Os insetos da ordem *Hymenoptera*, família *Apidae*, tribo *Meliponini* são conhecidos como meliponíneos ou abelhas sem ferrão (ASF) por possuírem ferrão atrofiado, sendo incapazes de ferroar, o que facilita seu manejo (VILLAS-BÔAS, 2012). Mais de 500 espécies foram descritas e encontradas em áreas tropicais e subtropicais: América do Sul, América Central, sul da América do Norte, África, Sudeste da Ásia e Norte da Oceania (HRNCIR et al., 2016). Segundo levantamentos realizados, 244 espécies estão distribuídas em todo o Brasil, sendo encontradas 39 espécies no Espírito Santo, nativas da Mata Atlântica. A lista detalhada das espécies de ASF de ocorrência no estado do Espírito Santo está descrita na Tabela 1 (DE MENEZES, 2014; IDAF-ES, 2019).

As cinco espécies estudas neste trabalho são cultivadas na meliponicultura, sendo conhecidas popularmente como: Mandaçaia (*Melipona quadrifasciata*), Uruçu amarela (*Melipona mondury*), Uruçu capixaba (*Melipona capixaba*), Guaraipo (*Melipona bicolor*) e Borá (*Tetragona clavipes*) (Figura 1).

A distribuição geográfica dessas espécies é diversificada no Brasil: *Melipona quadrifasciata, Melipona mondury, Melipona bicolor* e *Tetragona clavipes* são encontradas nas regiões sudeste, sul, nordeste e norte; e a espécie *Melipona capixaba* é encontrada somente na Mata Atlântica do Espírito Santo, principalmente em locais com altitude entre 700 m e 1.000 m (RESENDE et al., 2008).

A *Melipona capixaba* é categorizada como vulnerável e em perigo, nas listas capixaba e nacional de espécies ameaçadas de extinção, respectivamente (INMA, 2019; ICMBIO, 2018).

Visando a proteção e a utilização desses polinizadores, no Brasil são implementadas regulamentações (BRASIL, 2004; BRASIL, 2017). Há alguns anos, institutos e empresas (ex. EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária) publicam diretrizes/manuais para otimizar a meliponocultura e auxiliar a transformar a atividade em uma ferramenta de desenvolvimento sustentável (JAFFÉ et al., 2015; VILLAS-BÔAS, 2012). Em março de 2018, o Ministério da Saúde incluiu a apiterapia (prática que utiliza produtos produzidos pelas abelhas nas colmeias como a apitoxina, geléia real, pólen, própolis, mel e outros) como nova prática integrativa no Sistema Único de Saúde (BRASIL, 2018). E mais recentemente, em abril de 2019, o Instituto de Defesa Agropecuária e Florestal do Espírito Santo (IDAF) regulamentou a

identificação e os requisitos de qualidade que deve apresentar o mel de abelhas sem ferrão (IDAF-ES, 2019). Esses esforços confirmam a importância da regulamentação e padronização de práticas e produtos relacionados a abelha sem ferrão.

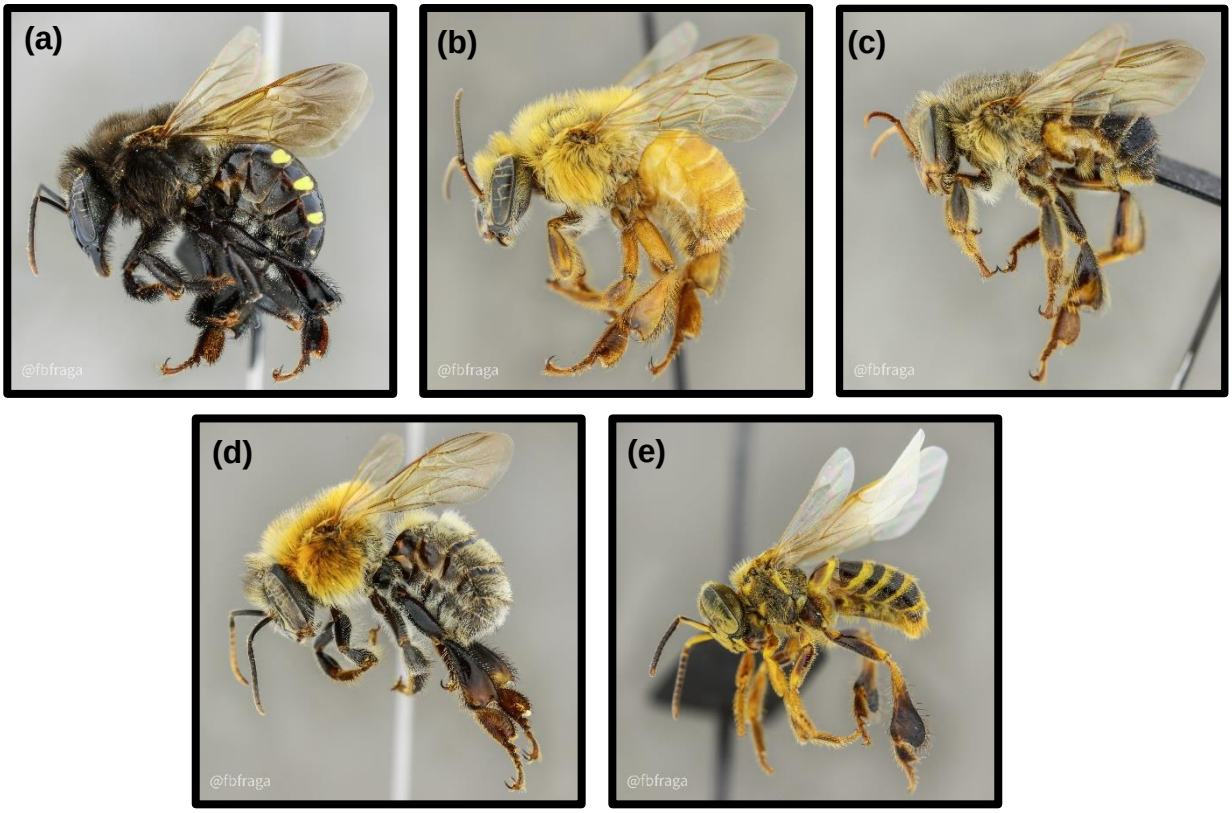

Figura 1 - Fotos das abelhas (a) *Melipona quadrifasciata*, (b) *Melipona mondury*, (c) *Melipona capixaba,* (d) *Melipona bicolor* e (e) *Tetragona clavipes* (Figura 1). Fonte: portfólio do biólogo entomólogo Felipe Bertholdi Fraga elaborado com amostras deste trabalho.

Tabela 1 - Lista de espécies de abelhas sem ferrão de ocorrência no estado do Espírito Santo.

Nome científico (descrita por)	Nome popular
Cephalotrigona capitata (Smith, 1854)	Mombução, papa-terra, abelha-papaterra, currunchos, guare negro, mombuca, eiruzú, negrito, eirusú, eirusú-grande, mumbuca, bombuca, jiu-butu.
Friesella schrottkyi (Friese, 1900)	Mirim preguiça, mosquito-remela.
Frieseomelitta dispar (Moure, 1950)	-
*Frieseomelitta meadewaldoi (Cockerell, 1915)**	Moça-branca, caveca, perna-longa
Frieseomelitta varia (Lepeletier, 1836)	Moça branca, mané-deabreu, manvel-d'abreu, mehnodjành.
Lestrimelitta ehrhardti (Friese, 1931)	-
Lestrimelitta rufipes (Friese, 1903)	Iraxim, limão
Leurotrigona muelleri (Friese, 1900)	Lambe olhos, mirim
Melipona (Eomelipona) bicolor (Lepeletier, 1836)	Guaraipo, guarubú, pé-de-pau e urusû-pé-de-pau
Melipona (Michmelia) capixaba (Moure & Camargo, 1994)	Uruçu-preta, uruçu-negra, uruçu-dasterras-frias, uruçu-capixaba.
Melipona (Michmelia) fuliginosa (Lepeletier, 1836)	Uruçu-boi, uruçu-preto,mel-de-anta, tapiiei, tapiieira, tapieira, uruçu, mandury-preto, turuçu, nara-bunábisuki.
Melipona (Eomelipona) marginata (Lepeletier, 1836)	Mandurium, monduri, taipeira, urussú-mirim, guarupú do mecudo, manduri, manduri menor, minduri, gurupu do miúdo ou taipeira.
Melipona (Michmelia) mondury (Smith, 1863)	Mondury, tuiuva, tujuva, tujuba, monduri, mondiri, uruçu amarela.
Melipona (Melikerria) quinquefasciata (Lepeletier, 1836)	Uruçu do chão, mandaçaia do chão, tumbihkihrasd-ivihgwih, urusû, urusû-do-chão, mandasaia-do-chão, mandassaia-do-chão, mandury, erereúamarilla-de-tierra
Melipona (Melipona) quadrifasciata (Lepeletier, 1836)	Mandaçaia
Nannotrigona testaceicornis (Lepeletier, 1836)	Iraí
Oxytrigona flaveola (Friese, 1900)	-
Oxytrigona tataira (Smith, 1863)	Caga-fogo, tataira, cagafogo, barra-fogo, botafogo, eitátá, ei-tata, eirátatá, atura, kangàràkrá-kamrek.
Paratrigona subnuda (Moure, 1947)	Jatahy-da-terra, mirim-sem-brilho, mirins-da-terra.
Partamona criptica (Pedro & Camargo, 2003)	-
Partamona helleri (Friese, 1900)	-
Partamona sooretamae (Pedro & Camargo, 2003)	-
Plebeia droryana (Friese, 1900)	Mirim droryana Inhati, jatahymosquito, miri-guazú, mosquitinho.
Plebeia lucii (Moure, 2004)	Mirim
Plebeia meridionalis (Ducke, 1916)	-
Plebeia phrynostoma (Moure, 2004)	Boca de sapo
Plebeia poecilochroa (Moure & Camargo, 1993)	-
Plebeia remota (Holmberg, 1903)	Mirim guaçú, abelha-preguiçosa, preguiçosa, mirim pintada, mirim preguiça, mirim rendeiro, tujuvinha.
Scaptotrigona tubiba (Smith, 1863)	Tubiba, tubíba, tubi, tapissuá, tubi-bravo, bocca-raza, tuibá.
Scaptotrigona xanthotricha (Moure, 1950)	Mandaguari amarela, trompeta, tujumirim, mandagoari, abelhafedente, abelha canudo, jandaíra pequena, jandaíra boca-de-cera.
Scaura atlantica (Melo, 2004)	-
Schwarziana quadripunctata (Lepeletier, 1836)	Guiruçu, iruçu da terra, abelha-mulata, guiruçu, mulatinha, abelhado-chão, papaterra, irussú-mineiro, irussú-do-chão, eiraihvihgwih, doncellita, señorita, mombucamirim, mombuquinha.
Schwarzula timida (Silvestre, 1902)	Lambe-olhos, lambiolhos, frecheira, mosquito-do-ouvido.
Tetragona clavipes (Fabricius, 1804)	Borá, vorá, jataí gigante, vamos-embora, i-kàikà.
Tetragonisca angustula (Latreille, 1811)	Maria-seca, virginitas, virgencitas, angelitas, abelhas-ouro, mariita, mariola, jatai-verdadeira, española, ingleses, mosquitinha-verdadeira, my-krwàt, jimerito, ramichiamarilla, moça-branca, jatahy-amarello, trez-portas, jatihy, jatai-piqueno, jatay, jaty, jatahy, mosquito-amarelo.
Trigona braueri (Friese, 1900)	Mel-de-cachorro, vaca, abelha-de-cachorro, abelha-cachorro.
Trigona hyalinata (Lepeletier, 1836)	Xupé, irapuã, abelha brava, guaxupé, arapuá, timba-preta.
Trigona spinipes (Fabricius, 1793)	Karavosá, eira-apuá, arapuá, abelha-decachorro, urapuca, irapuà, carabozà, irapoan, ira-puam,eirapuã, irapuãn, mbápý, carabozá, eiráapuá, xupé-pequeno, mehñykamrek.
Trigonisca intermedia (Moure, 1900)	-

Adaptado de: DE MENEZES, 2014; IDAF-ES, 2019 *Sinônimos para: *Frieseomelitta francoi* (Moure, 1946); *Frieseomelitta freiremaiai* (Moure, 1963).

Própolis e geoprópolis

Própolis é constituída por uma mistura de resina vegetal coletadas pelas abelhas na flora local e secreções salivares destes insetos (BANKOVA et al., 2000). Geoprópolis é uma mistura de barro com solo, lodo e/ou partículas de areia produzida por algumas espécies de meliponíneos (SHANAHAN e SPIVAK, 2021). Estes materiais são usados na construção, vedação, manutenção e assepsia da colmeia (SIMONE-FINSTROM e SPIVAK, 2010)

As fontes de resinas são descobertas pelas abelhas usando pistas visuais e olfativas que concentram combinações de mono e sesquiterpenos voláteis (LEONHARDT et al., 2011). Leonhardt et al. (2011) também sugeriram que as resinas coletadas do ambiente são modificadas dentro da colmeia e que cada espécie pode possuir diferentes enzimas ou agentes microbianos associados que alteram a resina (LEONHARDT et al., 2011). Neste contexto, o estudo da própolis de diferentes espécies é interessante para desvendar estas possíveis modificações das resinas.

No Brasil, própolis é definida como produto de características físicas resinosas e composição variável, coletada a partir de várias espécies vegetais e que sofre adição de secreções da abelha. Quando possui marcadores químicos definidos, diferenciados qualitativa e quantitativamente, conforme a região geográfica de origem, o produto é definido como própolis específica (BRASIL, 2011). Os extratos desses produtos podem ser registrados como suplementos alimentares ricos em compostos fenólicos (BRASIL, 2018). E, aos seus constituintes, aplicam-se as especificações estabelecidas na Instrução Normativa nº 3, de 19 de janeiro de 2001, que aprova os regulamentos técnicos de identidade e qualidade de produtos de abelha. Como essas normas foram elaboradas com base nas características dos produtos das abelhas com ferrão, *Apis mellifera*, alguns estudos têm verificado especificações fora da qualidade determinada pela IN 3/2001 (BRASIL, 2001). A exemplo, Cardozo et. al. (2015) obtiveram teores de flavonoides baixos para seus extratos (CARDOZO et al., 2015). Diferenças das amostras de própolis e geoprópolis também foram verificadas no estudo de Barth e da Luz 2003, cujos dados demonstram que a geoprópolis possui conteúdo mineral e de solo e ausência de tricomas vegetais (que podem alterar os resultados obtidos nas análises de cinzas e microscopia propostas pela IN 3/2001) (BARTH e DA LUZ, 2003; BRASIL, 2001). Isso demonstra a necessidade de adequações da regulamentação atual para o estabelecimento da qualidade de própolis de abelhas sem ferrão.

Considerando que as adequações citadas anteriormente devem ser baseadas em comprovações científicas, verificamos a escassez de estudos relacionados aos produtos de abelha sem ferrão nativas do Espírito Santo. Por exemplo, a *Melipona bicolor* e a *Melipona capixaba* (abelha de ocorrência exclusiva capixaba) foram estudadas quanto a genética, morfologia e características comportamentais, não sendo observados estudos sobre a composição química e a atividade biológica da própolis nessas espécies (ABDALLA et al., 2003; ABDALLA e CRUZ-LANDIM, 2004; HILÁRIO et al., 2000; HILÁRIO e IMPERATRIZ-FONSECA, 2009; MOURE e CAMARGO, 1994; RESENDE et al., 2008; ROCHA at al., 2002; SERRA et al., 2012)

No levantamento bibliográfico acerca de estudos com produtos de abelhas sem ferrão aplicáveis a saúde (com restrição temporal de janeiro/2000 a junho/2023 e estratégia de busca: *stingless bee and propolis*), verificou-se que nenhuma amostra de própolis foi coletada no estado do Espírito Santo (Lista detalhada do levantamento bibliográfico das ASF na Tabela 2). Sabendo que a flora local influencia a composição da própolis, o estudo com as ASF nativas da Mata Atlântica capixaba é de grande valia (CARDOZO et al., 2015). A exemplo, Sawaya e colaboradores em 2006 verificaram que o extrato da planta *Schinus terebenthifolius* (conhecida no Brasil como "aroeira vermelha") apresentou compostos em comum com a própolis da abelha *T. angustula* (SAWAYA et al., 2006). Outro estudo realizado por Sawaya et al. (2007) confirmaram a *S. terebenthifolius* como fonte vegetal importante para a geoprópolis das abelhas *T. angustula, M. quadrifasciata, Tetragona clavipes* e *Melipona scuttelaris* (SAWAYA et al., 2007).

Composição e propriedades da própolis

Comercialmente no Brasil, medicamentos à base de própolis de uso tópico, na cavidade bucal, com as indicações de uso como anti-inflamatório, anti-séptico e cicatrizante, são registrados junto a ANVISA com isenção da comprovação de eficácia e segurança (BRASIL, 2016). Esses medicamentos são isentos pois sua segurança e eficácia são constatadas pelo uso tradicional e dados científicos. Numa pesquisa ao banco de dados *Pub Med*, com restrição temporal de janeiro/2000 a junho/2023 e estratégia de busca *propolis,* foram encontrados mais de 4000 trabalhos sobre própolis de abelha, sendo que o número reduz para 88 quando a pesquisa é restringida ao produto de ASF (com estratégia de busca *propolis and stingless bee*) (PUB MED, 2023). Estas informações sugerem que a base de dados é maior para o

produto apícola da espécie *Apis mellifera*, havendo a necessidade de estudos complementares para as ASF.

Pesquisadores têm adotadas diversas técnicas analíticas para a avaliação da presença e a quantificação dos principais metabólitos secundários da própolis, com foco nos métodos cromatográficos e espectrofotométricos, sendo às vezes finalizado com técnicas de elucidação estrutural, tal como ressonância magnética nuclear – NMR (tabela 2) (ABDULLAH et al., 2020; DE SOUZA et al., 2013; ARAÚJO et al., 2016; ARAÚJO et al., 2011; BADIAZAMAN et al., 2018; BONAMIGO et al., 2017; BUTELLI-FIANCO et al., 2013; CAMPOS et al., 2014; CAMPOS et al., 2015; CAMPOS et al., 2011; CHOUDHARI et al., 2012; CINEGAGLIA et al., 2013; CISILOTTO et al., 2018; DA CUNHA et al., 2013; DE SOUZA et al., 2018; DOS SANTOS et al., 2017a; DOS SANTOS et al., 2017b; DOS SANTOS et al., 2017c; DUTRA el al., 2019; DUTRA et al., 2014; FARNESI et al., 2009; FRANCHIN et al., 2012; JUNIOR et al., 2019; KUSTIAWAN et al., 2017, 2015, 2014; LIBÉRIO et al., 2011; MOHAMED et al., 2020; MIORIN et al., 2003; PAZIN et al., 2017; SAWAYA et al., 2009; TORRES et al.,2018; UMTHONG, S et al., 2009; UTISPAN et al., 2017; VONGSAK et al., 2015). Podemos observar também que o etanol foi o principal solvente extrativo utilizado para o desenvolvimento estas investigações fitoquímicas, uma vez que, a maioria dos compostos biotivos da própolis possuem caracter polar.

Adicionalmente, os dados da tabela 2 monstram a diversidade química da própolis de abelhas sem ferrão constituída de fenilpropanóides, flavonóides, compostos fenólicos, taninos, terpenos, saponinas, alcaloides, óleos essenciais, ácidos graxos e açúcares (LAVINAS et al., 2018; SANCHES et al., 2017). Amostras advindas da mesma espécie e de diferentes localidades apresentam perfis químicos distintos, a exemplo, própolis da *Melipona scutellaris* do Tocantins continha flavonoides e catequinas (ARAÚJO et al., 2016), enquanto a própolis da mesma espécie da Bahia demonstrou a presença de cumarinas e benzofenona (DA CUNHA et al., 2013). Ao contrário, em amostras de diferentes espécies de abelhas de diversas regiões do Brasil foi identificado o flavonoide quercetina como um dos constituintes da própolis de *M. fasciculata* (Mato Grosso do Sul), *M. quadrifasciata* (Santa Catarina), *M. subnitida* (Paraíba) e *T. angustula* (Santa Catariana) (BONAMIGO et al., 2017; DOS SANTOS et al., 2017b; JUNIOR et al., 2019; TORRES et al., 2018).

Essa composição química constituída de flavonoides e compostos fenólicos é correlacinada as atividades farmacológicas, cujos relatos científicos descrevem as propriedades anti-inflamatória, antioxidante e antimicrobiana da

própolis/geoprópolis produzida por diferentes espécies de abelhas sem ferrão (LAVINAS et al., 2018; SANCHES et al., 2017).

Tabela 2 - Lista de espécie de abelhas sem ferrão com dados de estudos tais como: local de retirada da amostra (estado/país), tipo, extrato, componentes, polifenóis e flavonóides.

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Frieseomelitta longipes	Pará/ Brasil	Própolis	Hidrodestilação - Clevenger Extrato etanólico	**Óleos essenciais, monoterpenos e sesqueterpenos:** β-Copaene, β-trans-Bergamotene, α-Guaiene, Aromadendrene, α-Himachalene, α-Humulene, E-β-Farnesene, 9-epi-(E)-Caryophyllene, allo-Aromadendrene, cis Muurola-4(14), 5-diene, trans Cadina-1(6), 4-diene, g-Gurjunene, g-muurolene, Germacrene D, β-Chamigrene, α-Curcumene, Unknown 2, β-Selinene, Unknown 3, cis β-Guaiene cis, γ-Amorphene, Valencene, Bicyclogermacrene, α-Muurolene, β-Bisabolene, γ-Cadinene, δ-Cadinene, (E)-γ-Bisabolene, trans Cadina-1(2),4-diene, α-Cadinene, Germacrene B, Caryophyllene oxide, 1,10-di-epi-cubenol, 7- *epi* -Nemorosona, Xantocimol, Guttiferone C ou D, Gambogenona, Aristofenona A, Benzofenona poliprenilada, (1R, 5R, 7R) -3-Benzoil-7 - [(2E) -3,7-dimetil-2,6-octadieno-1-il] -4-hidroxi-8,8-dimetil-5- (3-metil-2-buten-1-il)biciclo [3.3.1] não-3-eno-2,9-dione	GC-MS LC-ESI-MS / MS	NE	NE	(DE SOUZA et al., 2018)
Frieseomelitta varia	Minas Gerais/ Brasil	propolis	Solução clorofórmica	ácido 3,5-diprenil-4-hidroxicinâmico (artepelin C)	LC/MS NMR	NE	NE	(CAMPOS et al., 2011)
Geniotrigona thoracica	Malásia	Propólis	Extrato metanólico	Terpenóides, cumarinas, flavonóides, óleos essencias, compostos aromáticos insaturados.	TLC	(mg GAE/g) 15,27 ± 0,12 (DGN) 13,07 ± 0,62 (BST) 9,23 ± 0,37 (LDG) 17,96 ± 0,64 (TM) 23,43 ± 0,50 (GM)	(mg QE/g) 9,52 ± 0,54 (DGN) 17,22 ± 0,16 (BST) 13,34 ± 0,20 (LDG) 11,33 ± 0,03 (TM) 14,06 ± 0,36 (GM)	BADIAZAMAN et al., 2019)
Geniotrigona thoracica (G.thoracica)	Sudeste Ásiatico	Própolis	Extrato etanólico	álcoois, fenóis, ácidos carboxílicos, aminas, alcenos e ésteres	FTIR	(mg/g) 299.4 ± 2.6	(mg/g) 2192.7 ± 12.3	(ABDULLAH et al., 2020)
Geniotrigona thoracica	Malásia	Propólis	Extrato etanólico	Compostos fenólicos, terpenóides, esteroides, álccol de açúcar, ácidos graxos (30 compostos)	GC-MS	NE	NE	(NAZIR et al., 2018)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Heterotrigona itama	Sudeste Ásiatico	georópolis	Extrato etanólico	álcoois, fenóis, ácidos carboxílicos, aminas, alcenos e ésteres	FTIR	(mg/g) 275.2 ± 3.5	(mg/g) 2391.0 ± 16.1	(ABDULLAH et al., 2020)
Heterotrigona itama	Malásia	georópolis	Extrato etanólico	Ácidos fenólicos, flavonas, triterpenos e fitosterol (28 compostos)	UHPLC-QTOF/MS	NE	NE	(ZHAO et al., 2017)
Heterotrigona itama	Nalásia	georópolis	Extrato hidroalcoolico	Flavonoides, taninos e fenólicos totais	UHPLC-MS/MS TGA-FTIR	NE	NE	(LIM et al., 2023)
Lepidotrigona terminata Smith	Chanthaburi/ Tailândia	Própolis	Extrato Etanólico	Compostos fenólicos (gama-mangostin e alfa-mangostin)	UV	(g GAE/100g) 2.16 ± 0.10	NE	(VONGSAK et al., 2015
Lepidotrigona ventralis Smith	Chanthaburi/ Tailândia	Própolis	Extrato Etanólico	Compostos fenólicos (gama-mangostin e alfa-mangostin)	UV	(g GAE/100g) 3.15 ± 0.25	NE	(VONGSAK et al., 2015
Melipona fasciculata	Tocantins/ Brasil	Própolis	Extrato Etanólico	ácido gálico , catequina, galocatequina e hesperidina.	TLC, HPLC	(mg GAE/g) 631.29 ± 4.22	NE	(ARAÚJO et al., 2016)
Melipona fasciculata	Maranhão/ Brasil	Geoprópolis	Extrato hidroalcoólico (HAE) e frações hexano e clorofórmio, gel a base de geoprópolis com Natrosol ® e propilenoglicol	Fenólicos e Flavonóides (HAE-1, HAE-2 e HAE-3) triterpenos (HAE-1, HAE-2)	UV	(% de GAE) 67,4 ± 2,0 - HAE-1 14,6 ± 2,3 - HAE-2 51,2 ± 3,9 - HAE-3	(% de QE) 1,07 (0,04) - HAE-1 2,91 (0,22) - HAE-2 1,11 (0,01) - HAE-3	(LIBÉRIO et al., 2011)
Melipona fasciculata	Maranhão/ Brasil	Geoprópolis	Extrato etanólico	Hexoses, glucitol, ácido glucuronílico, inositol e triterpenos.	GC-MS	NE	NE	(ARAÚJO et al., 2016)
Melipona fasciculata anthidioides	Mato Grosso do Sul/ Brasil	Própolis	Extrato etanólico	Fração Hexano Estigmasterol, *β* - Sitosterol, *β*-Amyrin, Taraxasterol, *α*- Amyrin, *β*-Amirina Acetato, Tocopherol. Fração Água Ácido vanílico, Ácido cafeico, Vanilina, Ácido p-cumarico, Ácido ferúlico, Ácido benzóico,Quercetina, Luteolina, Ácido cinâmico, Apigenina.	GC-MS (fração em hexano) HPLC (fração em água)	NE	NE	(BONAMIGO et al., 2017)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Melipona fasciculata	Maranhão/ Brasil	Geoprópolis	extrato hidroalcoólico (HEG), frações hexano (HF), clorofórmio (CF), acetato de etila (EAF)	HEG (Ácido palmítico, Ácido esteárico, Ácido quínico, Ácido, Ácido gálico, Ácido elágico, Ácido protocatecuico, frutose, glicose, manose, xilose, glicerol, xilitol, inositol). HF (Ácido palmítico, Ácido esteárico, Ácido quínico, Ácido oleico, Ácido araquídico, Ácido beénico, Ácido lignocérico, -Amirin, Lupenona, Ácido oleanólico, Campesterol, Estigmasterol, -Sitosterol) CF (Ácido palmítico, Ácido esteárico, Ácido gálico, Ácido elágico, Ácido protocatecuico, Ácido oleanólico, -Sitosterol) EAF (Ácido gálico, Ácido elágico, Glicose) HAF (Ácido palmítico, Ácido esteárico, Ácido gálico, Ácido elágico, Ácido quínico, Glicose, Manose, Xilose, Glicerol, Xilitol, Inositol)	GC / MS HPLC / UV	NE	NE	(DUTRA et al., 2019)
Melipona fasciculata Smith	Maranhão/ Brasil	Geoprópolis	Extrato etanólico	pentoses, hexoses, álcoois de açúcar, ácidos urônicos, dissacarídeos, alquilresorcinóis e triterpenos,	GC-MS	NE	NE	(CINEGAGLIA et al, 2013)
Melipona fasciculata Smith	Maranhão/ Brasil	Geoprópolis	Extrato hidroalcoólico (HEG) Fração hidroalcoólica (HAF), hexano (HF), clorofórmio (CF), acetato de etila (EAF)	HHDP-galloylglucose (corilagina), HHDP-glicose, di-HHDP-glicose (pedunculagina / casuarina), glicose trigaloil, Isômero HHDP-digaloilglucose (telimagrandina I), ácido valoneico dilactona, isômero c de trisgaloil-HHDP-glicose, di-HHDP-galloylglucose (potentilina / casuarictina)., trigaloil-HHDP-glicose (telimagrandina II), Ácido elágico, Ácido gálico.	HPLC / UV HPLC-DAD-ESI-MS	GAE (%) (HEG) 47,78 ± 0,04 (HAF) 36,0 ± 0,04 (HF) 6,32 ± 0,02 (CF) 20,53 ± 0,01 (EAF) 57,90 ± 0,02	NE	(DUTRA et al., 2014)
Melipona mondury	Bahia/ Brasil	Geoprópolis	Extrato Etanólico (EEGP) e frações butanol (BFGP), hexano (HFGP), acetato de etila (EAFGP)	Compostos Fenólicos	(ESI (-)-EM) UHPLC-MS UHPLC-MS / MS UV	(µg GAE/mg) EEGP - 144,4 ± 0,01 HFGP - 40,12 ± 0,4 EAFGP - 140,9 ± 0,28 BFGP - 303,1 ± 0,14	NE	(DOS SANTOS et al., 2017c)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Melipona orbignyi	Mato Grosso do Sul/ Brasil	Geoprópolis	Extrato hidroalcoólico	Aromadendrina, naringenina, metil-aromadendrina e metil-naringenina e derivados de ácidos fenólicos glicosilados (goloil, cinamoil e coumaroil)	HPLC-DAD-MS UV	(mg GAE/g) 121 ± 0,6	(mg QE/g) 19,9 ± 1,1	(DOS SANTOS et al., 2017a)
Melipona orbignyi	Mato Grosso do Sul/ Brasil	Geoprópolis	Extrato etanólico	Ácidos benzóicos, Ácidos dihidrocinâmicos, Ácidos cinâmico, Fenil, benzil, cafeatos de cadeia longa, Ácidos cumárinicos C prenilados, Ácidos diterpenicos, Álcoois triterpênicos e Açúcares e outros.	GC-MS CGL	(mg GAE / 100 g) 211 ± 7,5	(mg QE / 100 g) 23 ± 1,0	(CAMPOS et al., 2014)
Melipona quadrifasciata	Santa Catarina/ Brasil	Própolis	Extrato etanólico	terpenóides e flavonóides	UPLC-QTOF / MS	NE	NE	(CISILOTTO et al., 2018)
Melipona quadrifasciata	São Paulo/ Brasil	Própolis	Extrato etanólico	NE	NE	NE	NE	(FARNESI et al., 2009)
Melipona quadrifasciata	Santa Catarina/ Brasil	Própolis	Extrato hidroalcoolico	Catequina, epicatequina, aromadendrina, narigenina, pinocembrina e ácido p-coumarico	HPLC-ÉSI-MS / MS	(mg GAE/g) 4.87 ± 0,2 a 64.93 ± 0.6	(mg QE/g) 0.78 ± 0,2 a 8.48 ± 0.3	(HOCHEIM et al., 2019)
Melipona quadrifasciata anthidioides	São Paulo / Brasil	Própolis	Extrato Etanólico *Frações* Hexano Diclorometano Acetato de etila Água	EE - ácido p-cumárico FH - Ácido cinâmico, Artepillin, FDM - Ácido p-cumárico, Ácido cinâmico. FAC - Ácido p-cumárico, Ácido cinâmico FA - Ácido cafeico, Ácido p-cumárico	HPLC	(mg/g) EE - 70.5 ± 0.8 FH - 8.0 ± 0.2 FDM - 61.5 ± 1.1 FAC - 93.0 ± 1.9 FA - 91.0 ± 2.3	(mg/g) EE - 10.0 ± 0.1 FH - 2.6 ± 0.1 FDM - 14.2 ± 0.2 FAC - 14.7 ± 0.1 FA - 9.1 ± 0.1	(PAZIN et al., 2017)
Melipona quadrifasciata anthidioides	Mato Grosso do Sul / Brasil	Própolis	Extrato Etanólico	Ácido gálico, o-coumaroyl O-galloyl hexoside, Di-O-galloyl O-cinnamoyl hexoside, p-coumaric hexoside acid, O-cinnamoyl O-galloyl hexoside, Aromadendrin, p-coumaric acid, Ellagic acid, O-galloyl hexoside, O-cinnamoyl O-coumaroyl hexoside, Naringenin, Methyl-aromadendrin, Methyl-naringenin, Luteolin-methyl-ether, Quercetin-3-methyl-ether	LC-ESI-QTOF-MS	NE	NE	(RUBINHO et al., 2020)
Melipona quadrifasciata anthidioides	Mato Grosso do Sul / Brasil	Própolis	Extrato Etanólico	Ácido gálico, ácido elágico, flavonoides, diterpenos e triterpenos	HPLC-ÉSI / MS	NE	NE	(CAMPOS et al., 2023)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Melipona quadrifasciata quadrifasciata	Santa Catarina/ Brasil	Própolis	Extrato Etanólico	Ácido gálico, vanilina, ácido r-cumárico e quercetina	HPLC-DAD e UPLC-Q / TOF-MS / MS	(mg GAE/g) 3,87 ± 0,32	(mg QE/g) 0,14 ± 0,03	(TORRES et al., 2018)
Melipona quadrifasciata quadrifasciata	Paraná/ Brasil	Própolis	Extrato Etanólico	Ácidos cinâmicos, ácidos fenólicos, flavonoides, ácido graxos, diterpenos, triterpenos, polifenois e lipídeos fenólicos	HPLC	NE	NE	(SUREK et al., 2021)
Melipona quadrifasciata quadrifasciata	Argentina	Própolis	Extrato dietil éter	Monoterpenos, sesquiterpenos, álcool e ácidos alifáticos, ésteres e aromáticos.	GC-MS	NE	NE	(ISIDOROV et al., 2022)
Melipona scutellaris	Bahia/ Brasil	Geoprópolis	Extrato etanólico (EEGP) frações hexano (HF), clorofórmio , acetato de etila e aquosa (AF)	Compostos Fenólicos	NE	(mg GAE / g) 127 ± 1,9 - EEGP 38 ± 0,7 - HF 138 ± 0,6 - AF	NE	(FRANCHIN et al., 2012)
Melipona scutellaris	Tocantins/ Brasil	Geoprópolis	Extrato etanólico	ácido elágico, ácido gálico , catequina, galocatequina, hesperidina, kaempferol,, morine rutina.	TLC, HPLC	(mg GAE/g) 620,01 ± 6,45	NE	(ARAÚJO et al., 2016)
Melipona scutellaris	Bahia/ Brasil	Geoprópolis	Extrato etanólico, Fração hexano (HF), clorofórmio (CF) e acetato de etila (EAF)	2-propensaeure 3-fenil-trimetilsililéster Ácido 1,2-benzenodicarboxílico outros compostos apresentaram similaridade com a classe: benzofenonas poliprenileadas	RP-HPLC GC-MS	NE	NE	(DA CUNHA et al., 2013)
Melipona subnitida	Paraíba/ Brasil	Geoprópolis	Extrato etanólico e frações hexano, acetato de etila e metanol	Fenilpropanóides (6- *Op* -coumaroil- *D*- galactopiranose e 6- *O* -cinamoil-1- *Op*- coumaroil- *β* - *D*- glucopiranose) Flavonoides (7- *O* -metil-naringenina, 7- *O*- metil-aromadendrina, 7,4′-di- *O*- metil-aromadendrina, 4 ′ - *O*- metilcaempferol, 3-*O*- metil quercetina, 5- *O*- metil-aromadendrina e 5- *O*- metilcaempferol).	IV, LC-ESI-MS, NMR e RMN 2D	(mg GAE/g) EtOH 63.9 ± 8.6 Hexano 25.6 ± 0.5 EtOAc 115.8 ± 0.8 MeOH : H2O 40.0 ± 7.8	—	(DE SOUZA et al., 2013)

Espécie	Localização (estado/país)	Próprolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Melipona subnitida	Paraíba / Brasil	Geoprópolis	Extrato etanólico	Tetrahidroxi-flavanona, Tetrahidroxiflavona, 3-O-metil-quercetina, Éter dimetílico de miricetina, Naringenin, Triidroxiflavona, Tetrahidroxiflavona, Triidroxi-metoxi-flavona, Triidroxi-metoxi-flavona, Tetrahidroxi-metoxi-flavona, Éter dimetílico de quercetina, Éter dimetílico de quercetina, Hidroxi-metoxi-chalcona, Trihidroxi-metoxi-flavanona, Hidroxi-metoxi-flavanona, Diidroxi-dimetoxi-flavona, Tetrahidroxi-metoxi-flavona, Pentahidroxiflavona Diidroxitrimetoxiflavona, 7-O-metil naringenina, Diidroximetoxiflavona, Diidroxi-dimetoxi-flavona, Diidroxi-dimetoxi-flavona, Diidroxitrimetoxiflavona, Diidroxi-metoxi-chalcona.	UHPLC-PDA-QTOF-MS / MS	NE	NE	(JUNIOR et al., 2019)
Melipona subnitida	Rio Grande do Norte / Brasil	Geoprópolis	Extrato hidroetanólico	Flavonol, flavamonol, flavonona, flavona, chalcona, fenilpropanoides e taninos	UHPLC-QTOF-MS / MS	(mg GAE/g) 42.2 ± 2.8 a 116.7 ± 8.7	NE	(SOUSA-FONTOURA et al., 2020)
Meliponula ferruginea	Tanzânia	própolis	Extrato etanólico	Açúcar e derivados, ácidos aromáticos, ácidos graxos, diterpenos, cardanol, resorcinol, ácido amacardico, ácido químico, ácido caffeoilquínico, triterpenos	GC-MS	NE	NE	(POPOVA et al., 2021)
Plebeia remota	Paraná/ Brasil	Própolis	Extrato Etanólico	Ácidos cinâmicos, ácidos fenólicos, flavonoides, ácido graxos, diterpenos, triterpenos, polifenois e lipídeos fenólicos	HPLC	NE	NE	(SUREK et al., 2021)
Scaptotrigona aff.postica	Argentina	Própolis	Extrato dietil éter	Monoterpenos, sesquiterpenos, álcool e ácidos alifáticos, ésteres e aromáticos.	GC-MS	NE	NE	(ISIDOROV et al., 2022)
Scaptotrigona aff.postica	Maranhão/ Brasil	Própolis	Extrato hidroalcoólico	Fenol Total, Flavonóides, Ácidos Fenólicos e Ésteres	UV	11.95 ± 0.80%	0.55 ± 0.07%	(ARAÚJO et al., 2011)
Scaptotrigona bipunctata	Porto Alegre/ Brasil	Própolis	Extrato etanólico	Polifenóis totais e Flavonóides	UV TLC	GAE 6,06%	NE	BUTELLI-FIANCO et al., 2013)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Scaptotrigona bipunctata	Paraná/ Brasil	Própolis	Extrato Etanólico	Ácidos cinâmicos, ácidos fenólicos, flavonoides, ácido graxos, diterpenos, triterpenos, polifenois e lipídeos fenólicos	HPLC	NE	NE	(SUREK et al., 2021)
Scaptotrigona bipunctata	Santa Catarina/ Brasil	Própolis	Extrato etanólico	8 alcalóides piperidínicos e 3 flavonas	UPLC-QTOF / MS	NE	NE	(CISILOTTO et al., 2018)
Scaptotrigona aff.postica	Maranhão/ Brasil	Própolis	Extrato hidroalcoólico	Terpenos (di- e triterpenos) e Cumarinas	Prospecção química	NE	NE	(ARAÚJO et al., 2010)
Scaptotrigona bipunctata	São Paulo/ Brasil	Própolis	Extrato Etanólico	NE	ESI (-) – MS	NE	NE	(SAWAYA et al., 2009)
Scaptotrigona depilis	São Paulo/ Brasil	Própolis	Extrato Etanólico	NE	ESI (-) – MS	NE	NE	(SAWAYA et al., 2009)
Scaptotrigona depilis	Mato Grosso do Sul/ Brasil	Própolis	Extrato etanólico	Fração Hexano *β* - Sitosterol, *β*- Amyrinl, *α*- Amyrin, *β*- Amirina Acetato, Tocopherol. Fração Água Vanilina, Ácido p-cumarico, Ácido ferúlico, Ácido benzóico, Ácido cinâmico.	GC-MS (fração em hexano) HPLC (fração em água	NE	NE	(BONAMIGO et al., 2017)
Scaptotrigona depilis	Mato Grosso do Sul/ Brasil	Própolis	Extrato etanólico	triterpenos	HPLC-ESI-MS	NE	NE	(CAMPOS et al., 2023)
Scaptotrigona spp.	Maranhão / Brasil	Própolis	Extrato Etanólico *Frações* Hexano Diclorometano Acetato de etila Água	EE - nd FH - nd FDM - Ácido cinâmico FAC - nd FA - nd	HPLC	(mg/g) EE - 10.0 ± 0.3 FH - 5.5 ± 0.1 FDM - 17.5 ± 1.1 FAC - 41.5 ± 1.6 FA – 19.5 ± 0.8	(mg/g) EE - 4.3 ± 0.1 FH - 0.51 ± 0.01 FDM - 2.6 ± 0.1 FAC - 12.5 ± 0.4 FA - 10.9 ± 0.4	(PAZIN et al., 2017)
Tetragona clavipes	São Paulo / Brasil	Própolis	Extrato Etanólico *Frações* Hexano Diclorometano Acetato de etila Água	EE - nd Não houve análise nas demais frações	HPLC	(mg/g) EE - 12.5 ± 0.3	(mg/g) EE - 1.8 ± 0.1	(PAZIN et al., 2017)
Tetragona clavipes	Argentina	Própolis	Extrato dietil éter	Monoterpenos, sesquiterpenos, álcool e ácidos alifáticos, ésteres e aromáticos.	GC-MS	NE	NE	(ISIDOROV et al., 2022)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Tetragonisca fiebrigi	Mato Grosso do Sul/ Brasil	Própolis	Extrato etanólico	Ácido benzóico, Caffeato de cinnamyl, Café de benzila, Ácido hidrocinâmico, Ácido cinâmico, Éster etílico de ácido hidrocinâmico, Ácido p-cumarico, Ácido 3-fenil-p- cumárico, Frutose, Glicose, Ácido Caurenóico, Ácido 4-metoxibenzoico, retinol, colesterol, tocoferol.	GC-MS	NE	NE	(CAMPOS et al., 2015)
Tetragonisca fiebrigi	Argentina	Própolis	Extrato dietil éter	Monoterpenos, sesquiterpenos, álcool e ácidos alifáticos, ésteres e aromáticos.	GC-MS	NE	NE	(ISIDOROV et al., 2022)
Tetragonisca Quadrifasciata	Santa Catarina/ Brasil	Própolis	Extrato aquoso (AE) e hidroalcoólico (HE)	AE (rutina, ácido gálico, gallo-catequina, galato de epicatequina e ácido siríngico). HE (quercetina, epi-galocatequina, ácido *p* -OH-benzóico, epigalocatequina galato e ácido cumarico)	HPLC-UV	(mg GAE/g) (AE) - 189,5 ± 3,9 (EH) - 82,4 ± 3,8	(mg QE/g) (AE) - 0,74 ± 0,11 (HE) - 0,79 ± 0,04	(DOS SANTOS et al., 2017b)
Tetragonisca angustula	Minas Gerais/ Brasil	Própolis	Extrato etanólico	Ácido 3,5-diprenil-4-hidroxicinâmico Ácido 3-prenil-4-hidroxicinâmico,, Ácido 2,2-dimetil-8-prenil-2H-1-benzopiran- 6-propenóico, Derivado de ácido cinâmico 1.	HPLC	NE	NE	(MIORIN, P.L. et al., 2003)
Tetragonisca angustula	Minas Gerais/ Brasil	Própolis	Extrato etanólico	Ácido 3,5-diprenil-4-hidroxicinâmico (D), Ácido 3-prenil-4-hidroxicinâmico (B), Ácido 2,2-dimetil-8-prenil-2H-1-benzopiran- 6-propenóico (F),Derivado de ácido cinâmico 1 (E)	HPLC	NE	NE	(MIORIN et al., 2003)
Tetragonisca angustula	Santa Catarina/ Brasil	Própolis	Extrato etanólico	Ácido gálico	HPLC-DAD e UPLC-Q / TOF-MS / MS	(mg GAE/g) 1,26 ± 0,17	(mg QE/g) 0,15 ± 0,02	(TORRES et al., 2018)
Tetragonisca angustula	Porto Alegre/ Brasil	Própolis	Extrato etanólico	Polifenóis totais e Flavonóides	UV TLC	GAE 5,00%	NE	BUTELLI-FIANCO et al., 2013)
Tetragonisca angustula	Santa Catarina/ Brasil	Própolis	Extrato aquoso (AE) e hidroalcoólico (HE)	AE - (quercetina, ácido gálico e galocatequina). HE - (quercetina, ácido *p* -OH-benzóico, caféico, e ácidos cumaricos)	UV e NMR HPLC-UV	(mg GAE/g) (AE) - 42,0 ± 1,8 (HE) - 34,9 ± 1,1	(mg QE/g) (AE) - 0,93 ± 0,06 (HE) - 0,82 ± 0,03	(DOS SANTOS et al., 2017b)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Tetragonula pagdeni	Chanthaburi/ Tailândia	Própolis	Extrato Etanólico	Gama-mangostina e Alfa-mangostina	UV HPLC	(g GAE/100g) 12.83 ± 0.72	NE	(VONGSAK et al., 2015
Tetrigona apicalis	Malásia peninsulares	Própolis	Extrato etanólico	Undecane, Cyclohexane, Cubebene, Copaene, Methanoazulene, Cycloprop[e]azulene, Caryophyllene, Naphthalene, Cyclodecadiene, Bicyclogermacrene, Benzodioxole, Naphthalene, Tricycloundecene, Cycloprop[e]azulenol, Cadinol, Aristolene epoxide, Amyrin	GC-MS	NE	NE	(MOHAMED et al., 2020)
Tetrigona apicalis	Thailândia	Própolis	Extrato Hidroalcoolico	Ácido gálico, eriodictiol, isoquercetina, quercetina, hidroquinona, catequina, ácido tânico	HPLC	(mg PGE/g) 14.74	NE	(KRAIKONGJIT et al., 2018)
Tetrigona binghami (T. binghami)	Sudeste Ásiatico	Própolis	Extrato Etanólico	álcoois, fenóis, ácidos carboxílicos, aminas, alcenos e ésteres	FTIR	(mg/g) 275.9 ± 2.1	(mg/g) 2151.9 ± 12.1	(ABDULLAH et al., 2020)
Trigona sp	Maharashtra/ India	Própolis	Extrato etanólico	Nitrometano, 3,3-dimetilpentano, 2,5-dihirotiofeno, Ácido 3-hidroxibutanoico, Etil diazoacetato, Ácido acrílico de 2-(metilenecicloprópil) etil éster, 5-fenoximeti-1,3,4-tiadiazol-2-aminaacetato, 3-ciclohexen-1-ol, Boro (metanamina)tris(triflurometil), Etilvinilacetileno, 2-metil, 1-penten-3-ino, 2-(3-bromo-3 buten-1-il) -1,3-dioxolano, (SS)-ou(RR) - 2,3 hexanodiol, 1,2,3,5-tetraquis-O-(trimetilsilil), arabinofuranose, 1,3,4,5,6-pentakis-O-(trimetilsiil)-, O-metiloxima, D-frutose, 1,2,3,4,5-tetraquis-O-(trimetilsilil, ribitol, 3,3-difenil-1-trimetilsilil-ciclopropeno, Ácido alfa- [p-bromofenil}-O-amino cinâmico, Ácido hidroxisterico fenacil éster, 4-etilformanilida, ácido-1,2-diidro-4,6-dimetil-2-oxo-nicotínico, Ácido 1,2,-benzenodicarboxílico, 11-dien-2-ona, 4,beta.H,5.alfa.- ermofila-1 (10), (RR) - (+), 3,2,4-trimetil-4-p-tolil-cilcopentanol.	GC-MS	NE	NE	(CHOUDHARI et al., 2012)

Espécie	Localização (estado/país)	Própolis/ geoprópolis	Extrato	Componentes propostos	Método analítico	Polifenóis (unidade)	Flavonóides (unidade)	Referência
Trigona incisa, Trigona apicalis, Trigona fuscobalteata e Trigona fuscibisca	Samarinda/ Indonésia	Própolis	Extrato metanólico, Frações n-hexano, acetato de etila e metanol	NE	NE	NE	NE	(KUSTIAWAN et al., 2014)
Trigona incisa	Samarinda/ Indonésia	Própolis	Extrato metanólico e Frações n-hexano, acetato de etila e metanol	Cardol (5-pentadecil resorcinol)	1D-TLC, NMR	NE	NE	(KUSTIAWAN et al., 2015; 2017)
Trigona laeviceps	Samut Songkhram/ Tailândia	Própolis	Extrato metanólico e água	NE	NE	NE	NE	(UMTHONG et al., 2009)
Trigona sirindhornae	Chantaburi/ Tailândia	Própolis	Extrato etanólico, frações metanólica e acetato de etila	Flavonóides e derivados do ácido cinâmico	RP-HPLC	NE	NE	(UTISPAN et al., 2017)

AM, extrato de própolis de *Apis Mellifera* (amostra comparativa); EA, extrato aquoso (amostra); EE, extrato etanólico (amostra); EM, extrato metanólico (amostra); ESI–MS Mass spectrometry with electrospray ionization; FAE, fração acetato de etila (amostra); FB, fração butanol (amostra); FC, fração clorofórmio (amostra); FH, fração hexano (amostra); FHA, fração hidroálcoolica (amostra); FTIR – Fourier transform infrared; GAE – Gallic acid equivalente; GC–MS Gas chromatography–mass spectrometry; HPLC - High-performance liquid chromatography; LC–MS Liquid chromatography–mass spectrometry; ND, não detectado; NE, não estudado; NIR - Near-infrared; NMR - Nuclear magnetic resonance; OE, óleo essencial (amostra); PGE – Pirogalol equivalente; QE – quercetin equivalente; TGA - thermogravimetric analysis; TLC - Thin layer chromatography; UPLC–MS Ultra-performance liquid chromatography–mass spectrometry; UV Ultraviolet spectroscopy

Atividade antioxidante

A capacidade da própolis de diminuir ou neutralizar radicais livres, os responsáveis por danos oxidativos nos tecidos, também tem sido investigada. Essa atividade é relacionada a presença de flavonoides e compostos fenólicos, cujas estruturas químicas conferem estabilidade oxidativa ao organismo diminuindo os danos associados aos radicais livres (LAVINAS et al., 2018; REIS et al., 2019). Torres et al. (2018) investigaram as propriedades antioxidantes do extrato etanólico da própolis de ASF e verificaram que a atividade está relacionada diretamente com a concentração de fenóis e flavonoides do extrato, reforçando a afirmativa anterior (TORRES et al., 2018).

Os estudos da atividade antioxidante de própolis produzido por diferentes espécies de ASF estão resumidos na tabela 3. Os resultados são significativos e variam conforme o método utilizado na avaliação da atividade biológica, tipo de extrato, período de coleta das amostras e espécie de abelha estudada.

As principais metodologias aplicadas foram: captura de radicais livres DPPH (2,2-difenil-1-picrilhidrazil) e ABTS+ (2,2-azinobis-(3-etilbenzotiazol-6-sulfonato); método FRAP (poder antioxidante de redução do ferro); ensaio de inibição da hemólise oxidativa; avaliação da inibição de peroxidação lipídica em eritrócitos humanos e análise usando β-caroteno /ácido linoleico. A maioria dos autores utilizaram mais de um método a fim de avaliar comparativamente a atividade biológica. Nos estudos que propunham os métodos DPPH, ABTS+ e FRAP, extrato de própolis de ASF demonstrou poder de inibição de radicais livres similares aos padrões positivos, em alguns casos. Em outras análises, a própolis demonstrou a capacidade de proteção contra hemólise oxidativa e habilidade de reduzir os níveis de malondialdeido (MDA), que é um produto da peroxidação lipídica advindo do estresse oxidativo. Porém, na avaliação de Bonamigo et al. (2017), o extrato de própolis da espécie *Plebeia droryana* não foi capaz de inibir o conteúdo de MDA gerado pela ação do agente oxidante 2,2'-azobis(2-amidinopropano)dihidroclorido (AAPH) (BONAMIGO et al., 2017).

Em alguns trabalhos o extrato de própolis de ASF demonstrou um maior poder antioxidante quando comparado ao da espécie com ferrão (*Apis Mellifera*), em outros foi observado uma menor atividade (DE SOUZA et al., 2018).

O extrato etanólico (EE) demonstrou uma maior atividade antioxidante do que o extrato aquoso (EA) (FIKRI et al., 2019).

Sawaya et al. (2009) observaram que o período de coleta de amostras de cada espécie altera a atividade antioxidante, que se apresentou aumentada (resultados baixos de ED_{50}) na primavera. Com isso, os autores concluíram que a estação do ano afeta a composição e propriedade da própolis (SAWAYA et al., 2009).

Os estudos mostram que a atividade antioxidante varia com a espécie de abelhas, pois estas possuem preferências por plantas específicas na produção de própolis (BONAMIGO et al., 2017).

De modo geral, os autores concluem que a própolis de ASF possui potencial terapêutico para o tratamento e/ou prevenção de doenças relacionadas ao estresse oxidativo.

Tabela 3 - Atividade antioxidante de própolis produzida por diferentes espécies de abelhas sem ferrão.

Espécie	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (mmol FeSO4/g extrato)	Ensaio inibitório de a-glucosidase IC50 (µg/mL)	Ensaio de inibição de hemólise oxidativa (efeito protetor)	Avaliação da inibição da peroxidação lipídica em eritrócito humano usando MDA	Análise da inibição da peroxidação lipídica usando B-caroteno/ácido linoléico (%)	Referência
Frieseomelitta longipes	OE-1= 8,47 ± 0,00040 OE-2= 8,64 ± 0,00001 OE-3= 8,81 ± 0,00002 AM= 3,74 ± 0,00020 BHT (C+) = 6,00 ± 0,00020	NE	NE	NE	NE	NE	OE-1= 75,6 ± 0,7 OE-2= 75,1 ± 1,1 OE-3= 73,1 ± 1,1 AM= 75,5 ± 1,1 BHT (C+) = 86,4 ± 0,7	(DE SOUZA et al., 2018)
Geniotrigona Thoracica	EM= Variou de 53µg (BST) a 190 µg (DGN), algumas estavam inativas	NE	NE	NE	NE	NE	NE	(BADIAZAMAN et al., 2019)
Lepidotrigona ventralis	EE= 428.61 ± 26.24 AA= 7.33 ± 0.85	EE= 576.40 ± 48.17 AA= 6.07 ± 1.69	EE= 97.42 ± 24.78 AA= 7698.02 ± 698.03	EE= 387.97 ± 38.71 AC= 155.82 ± 6.69	NE	NE	NE	(VONGSAK et al., 2015)
Lepidotrigona terminata	EE= 1228.89 ± 67.29 AA= 7.33 ± 0.85	EE= 605.39 ± 56.32 AA= 6.07 ± 1.69	EE= 61.14 ± 5.86 AA= 7698.02 ± 698.03	EE= 469.66 ± 76.55 AC= 155.82 ± 6.69	NE	NE	NE	(VONGSAK et al., 2015)
Melipona Fasciculata Smith	HEG= 5,24 ± 0,12 CF= 17,86 ± 0,05 EAF= 3,75 ± 0,10 HAF= 8,81 ± 0,15 FH= nd AG= 1,83 ± 0,03 AE= 1,20 ± 0,01 Trolox= 5,11 ± 0,04	HEG= 2,56 ± 0,17 CF= 16,29 ± 0,02 EAF= 1,44 ± 0,03 HAF= 4,41 ± 0,04 FH= nd AG= 0,73 ± 0,04 AE= 1,20 ± 0,08 Trolox= 2,36 ± 0,04	HEG= 14,70 ± 0,43 CF= 2,52 ± 0,01 EAF= 17,87 ± 0,28 HAF= 15,06 ± 0,98 FH= nd AG= 25,87 ± 0,28 AE= 51,32 ± 1,98 Trolox= 9,09 ± 0,10	NE	NE	NE	NE	(DUTRA et al., 2014)
Melipona Fasciculata Smith	EE= 29,81 ± 2,49 AM= 260,34 ± 12,27 RT= 53,29 ± 4,10 AA= 19,20 ± 2,53	NE	NE	NE	NE	NE	NE	(ARAÚJO et al., 2016)
Melipona Mondury	EE= 6,91 ± 017 HF= 20,22 ± 0,2 EAF= 6,58 ± 0,04 BF= 2,23 ± 0,05 AG= 1,45 ± 0,01 Trolox = 1,54 ± 0,01	EE= 5,96 ± 0,08 HF= 20,51 ± 0,15 EAF= 5,5 ± 0,028 BF= 0,87 ± 0,003 AG= 1,08 ± 0,04 Trolox = 1,20 ± 0,035	NE	NE	NE	NE	EE= 27,99 ± 0,2 HF= 4,7 ± 0,001 EAF= 26,45 ± 0,4 BF= 78,57 ± 0,3 AG= 54,7 ± 0,4 Trolox = 83,69 ± 0,7	(DOS SANTOS et al., 2017c)

Espécie	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (mmol FeSO4/g extrato)	Ensaio inibitório de a-glucosidase IC50 (µg/mL)	Ensaio de inibição de hemólise oxidativa (efeito protetor)	Avaliação da inibição da peroxidação lipídica em eritrócito humano usando MDA	Análise da inibição da peroxidação lipídica usando B-caroteno/ácido linoléico (%)	Referência
Melipona Orbignyi	EE= 40 ± 4,8 AA= 3 ± 0,4	NE	NE	NE	Protege os eritrócitos contra a hemólise induzida pelo agente (50mM) durante os primeiros 120 min de incubação.	Reduz os níveis de MDA de todas as concentrações testadas (50-125 µg / mL) quando os eritrócitos foram incubados com o agente AAPH (50mM)	NE	(CAMPOS et al., 2014)
Melipona Orbignyi	EH= 18,3 ± 2,8 AA= 3,2 ± 0,9 BHT= 20,3 ± 5,6	EH= 10,3 ± 0,5 AA= 1,8 ± 0,05 BHT= 8,1 ± 0,7	NE	NE	Protege os eritrócitos contra a hemólise induzida pelo AAPH (50mM) durante os primeiros 240 min de incubação (50-125 µg/mL). Após 240 min as concentrações de 25 e 50 µg/mL reduziu a hemólise em 40,9% ± 8,0% e 93,2% ± 0,8%, respectivamente, quando comparado ao controle de AAPH. Apresentou maior atividade do que o controle AA.	Reduz os níveis de MDA em 89,75% ± 2,1% na concentração de 50 µg / mL quando os eritrócitos são incubados com o agente AAPH (50mM) por 240 min, e apresentou maior atividade do que o controle AA.	NE	(DOS SANTOS et al., 2017a)
Melipona subnitida	85 a 951 ± 1	NE	NE	NE	NE	NE	NE	(SOUSA-FONTOURA et al., 2020)
Tetragonisca Quadrifasciata	AE = Atividade aumentada HE= 117,5 AAAE= 141,1 ± 3,7 AAEH= 657,5 ± 0	NE	NE	NE	NE	NE	AE= 7,9 ± 3,7 HE= 2,8 ± 1,3	(DOS SANTOS et al., 2017b)

Espécie	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (mmol FeSO4/g extrato)	Ensaio inibitório de a-glucosidase IC50 (µg/mL)	Ensaio de inibição de hemólise oxidativa (efeito protetor)	Avaliação da inibição da peroxidação lipídica em eritrócito humano usando MDA	Análise da inibição da peroxidação lipídica usando B-caroteno/ácido linoléico (%)	Referência
Melipona quadrifasciata anthidioides	mg/g EE= 60,91 ± 2,01 BHT= 22,84 ± 7,87 AA= 3,32 ± 0,65	mg/g EE= 13,45 ± 1,81 BHT= 20,46 ± 2,78 AA= 2,50 ± 0,48	NE	NE	Protege os eritrócitos contra a hemólise induzida pelo agente AAPH (50mM) durante os primeiros 240 min de incubação atingindo 37,7 ± 10,4% para uma concentração de 125 µg / mL.	Reduz níveis de MDA de todas as concentrações testadas (50-125 µg / mL) quando os eritrócitos foram incubados com o agente AAPH (50mM) durante 240 min atingindo 74,4 ± 6,1 em uma concentração de 125 µg / mL	NE	(BONAMIGO et al., 2017)
Melipona quadrifasciata	151,37 ± 7,9 a > 1000	NE	NE	NE	NE	9,85 ± 1,0 A 38,48 ± 0,6 %	NE	(HOCHEIM et al., 2019)
Melipona quadrifasciata	mg/mL EE= 241,8	NE	NE	NE	NE	NE	NE	(TORRES et al., 2018)
T. angustula	mg/mL EE= 2433,0	NE	NE	NE	NE	NE	NE	(TORRES et al., 2018)
Melipona scutellaris	µg/mL EE=50,23 ± 1,60 AM= 845,38 ± 31,60 RT= 53,29 ± 4,10 AA= 19,20 ± 2,53	NE	NE	NE	NE	NE	NE	(ARAÚJO et al., 2016)
Melipona subnitida	EtOH= 27 ± 0.4 Hexano= 239,3 ± 1,1 EtOAc= 10,1 ± ,.0 MeOH:H_2O= 38,5 ± 0,5 AA= 2,1 ± 0,0	EtOH= 12,2 ± 0,1 Hexano= 32,3 ± 0,3 EtOAc= 4,3 ± 0,0 MeOH:H_2O= 26,2 ± 0,3 Trolox= 3,0 ± 0,1	NE	NE	NE	NE	EtOH= 35,4 ± 4,0 Hexano= 26,3 ± 3,5 EtOAc= 55,1 ± 1,9 MeOH:H_2O= 20,7 ± 1,6 Trolox= 81,3 ± 0,3	(DE SOUZA et al., 2013)
Scaptotrigona bipunctata	µg/mL Média EE= 183 Novembro EE= 43	NE	NE	NE	NE	NE	NE	(SAWAYA et al., 2009)

Espécie	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (mmol FeSO4/g extrato)	Ensaio inibitório de a-glucosidase IC50 (µg/mL)	Ensaio de inibição de hemólise oxidativa (efeito protetor)	Avaliação da inibição da peroxidação lipídica em eritrócito humano usando MDA	Análise da inibição da peroxidação lipídica usando B-caroteno/ácido linoléico (%)	Referência
Scaptotrigona depilis	EE= nd BHT= 22,84 ± 7,87 AA= 3,32 ± 0,65	EE= 80,04 ± 0,31 BHT= 20,46 ± 2,78 AA= 2,50 ± 0,48	NE	NE	Protege os eritrócitos contra a hemólise induzida pelo AAPH (50mM) durante os primeiros 120 min de incubação atingindo 63,5 ± 10,7% para uma concentração de 125 µg / mL.	Reduz níveis de MDA de todas as concentrações testadas (50-125 µg/mL) quando os eritrócitos foram incubados com o agente AAPH (50mM) durante 180 min.	NE	(BONAMIGO et al., 2017)
Melipona quadrifasciata anthidioides	µg / mL EE= 60,91 ± 2,01 BHT= 22,84 ± 7,87 AA= 3,32 ± 0,65	µg / mL EE= 13,45 ± 1,81 BHT= 20,46 ± 2,78 AA= 2,50 ± 0,48	NE	NE	Protege os eritrócitos contra a hemólise induzida pelo oxidante AAPH(50mM) por até 240 min, com inibição da hemólise atingindo 37,7 ± 10,4% para uma concentração de 125 µg / mL.	Reduz níveis de MDA de 74,4 ± 6,1%, durante 240 minutos a uma concentração de 125 µg / mL	NE	(BONAMIGO et al., 2017)
Scaptotrigona depilis	µg/mL Média EE= 593 Outubro EE= 113	NE	NE	NE	NE	NE	NE	(SAWAYA et al., 2009)
Scaptotrigona ssp	µg/mL Média EE= 310 Novembro EE= 77	NE	NE	NE	NE	NE	NE	(SAWAYA et al., 2009)
Tetragonisca angustula	µg/mL AE= > 1000 HE= > 1000 AA= 0	NE	NE	NE	NE	NE	AE= 7,2 ± 1,0 HE= 7,6 ± 0,9	(DOS SANTOS et al., 2017b)

Espécie	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (mmol FeSO4/g extrato)	Ensaio inibitório de a-glucosidase IC50 (µg/mL)	Ensaio de inibição de hemólise oxidativa (efeito protetor)	Avaliação da inibição da peroxidação lipídica em eritrócito humano usando MDA	Análise da inibição da peroxidação lipídica usando B-caroteno/ácido linoléico (%)	Referência
Tetragonisca fiebrigi	NE	µg / mL EE= 119,6 ± 20,5 BHT= 22,8 ± 4,2 AA= 1,3 ± 0,2	NE	NE	Protege os eritrócitos contra a hemólise induzida pelo oxidante AAPH (50mM) por até 240 min na maior concentração (125 µg/mL) 46 ± 3,6 %	Reduz níveis de MDA quando os eritrócitos foram incubados com o agente AAPH (50mM) durante 240 min na maior concentração (125 µg/mL) 39,5 ± 2,4 %	NE	(CAMPOS et al., 2015)
Tetragonula pagdeni	µg / mL EE= 122.71 ± 11.76 AA= 7.33 ± 0.85	µg / mL EE= 59.52 ± 10.76 AA= 6.07 ± 1.69	(mmol Fe 2+ / g) EE= 279.70 ± 20.55 AA= 7698.02 ± 698.03	EE=70.79 ± 6.44 AC= 155.82 ± 6.69	NE	NE	NE	(VONGSAK et al., 2015)
Trigona sp	µg/mL EE= 153 Trolox= 460 ± 0,64 mg/mg de EE	NE	NE	NE	NE	NE	NE	(CHOUDHARI et al., 2013)
Melipona quadrifasciata anthidioides	ug/mL EE Abs. Óptica 11.05 ± 0.6 ESR 8.4 ± 0.3	NE	NE	NE	NE	NE	NE	(PAZIN et al., 2017)
Tetragona clavipes	ug/mL EE Abs. Óptica 374.0 ± 4.0 ESR 218.0 ± 3.0	NE	NE	NE	NE	NE	NE	(PAZIN et al., 2017)
Scaptotrigona spp	ug/mL EE Abs. Óptica 512.0 ± 10.0 ESR 180.0 ± 3.0	NE	NE	NE	NE	NE	NE	(PAZIN et al., 2017)
Tetrigona Apicalis	NE —	EEP 1.68 mg/mL trolox 0.31 mg/mL	NE	NE	NE	NE	NE	(MOHAMED et al., 2020)

Espécie	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP (mmol FeSO4/g extrato)	Ensaio inibitório de a-glucosidase IC50 (µg/mL)	Ensaio de inibição de hemólise oxidativa (efeito protetor)	Avaliação da inibição da peroxidação lipídica em eritrócito humano usando MDA	Análise da inibição da peroxidação lipídica usando B-caroteno/ácido linoléico (%)	Referência
Geniotrigona thoracica (G. thoracica) *Heterotrigona itama (H. itama)* *Tetrigona binghami (T. binghami)*	mg/L *G. thoracica* 570.2 ± 6.1 *H. itama* 76.5 ± 1.3 *T. binghami* 1975 ± 22.5 *TAC (capacidade total antioxiddante) mg AAE/g* *G. thoracica* 42.5 ± 0.5 *H. itama* 317.6 ± 5.4 *T. binghami* 12.3 ± 0.3 AA 24.3 mg/ L	NE	NE	NE	NE	NE	NE	(ABDULLAH et al., 2020)

NE – Não estudado

Atividade anti-inflamatória

O processo inflamatório leva a liberação de citocinas pro-inflamatórias como TNF-α, IL-1β e IL-6, que na infecção estimulam a migração de células de defesa. Adicionalmente, as espécies reativas de oxigênio, como óxido nítrico e superóxido, são moléculas que causam peroxidação lipídica e estresse oxidativo numa resposta inflamatória. O efeito exarcerbado desta resposta pode ter efeito deletério nos tecidos e contribuir para a patogênese de doenças, como artrite reumatoide, asma, arterosclerose, sepse, entre outras (TU et al., 2022).

A modulação da própolis e geopóprolis de ASF na inflamação é reportada em estudos *in vivo* e tem sido atribuída aos compostos fenólicos, cuja estrutura química age no organismo prevenindo danos oxidativos e diminuindo a produção de citocinas. Franchin et al. (2012) induziram o processo inflamatório em camundongos e verificaram que estes animais tratados com geoprópolis de *Melipona scutellaris* apresentaram menores concentrações de IL-1β, e TNF-α e menor nocicepção, demonstrando a ação anti-inflamatória deste produto de abelha sem ferrão (FRANCHIN et al., 2012).

De Farias et al. (2014) investigaram o papel da própolis de *Scaptotrigona aff. postiça* em doenças inflamatórias como a asma (DE FARIAS et al., 2014). Os pesquisadores verificaram nos camundongos que após o tratamento com própolis o número de células inflamatórias (leucócitos) diminuiu no líquido broncoalveolar, a inflamação peribroncovascular com infiltrado de células polimorfonucleares reduziu e a concentração de interferon-γ (IFN-γ) no soro diminuiu. Com isso, os autores sugeriram que a própolis pode ser benéfica no tratamento de asma alérgica por inibir a migração de células inflamatórias para o espaço alveolar e a progressão sistêmica da inflamação (DE FARIAS et al., 2014).

Ação antimicrobiana

As própolis e geoprópolis de ASF possuem importante função antimicrobiana natural, pois as abelhas as produzem como mecanismo de defesa contra agentes invasores, vedação e assepicia das colmeias (SIMONE-FINSTROM e SPIVAK, 2010). Esta ação antimicrobiana tem sido demonstrada para bactérias gram-positivas, gram-negativas, fungos e parasitas que possuem relevância clínica por afetarem a saúde humana e/ou veterinária (Tabela 4) (ARAÚJO et al., 2016; BUTELLI-FIANCO et al., 2013; CAMPOS et al., 2011, 2014, 2015, 2023; CHOUDHARI et al., 2012; DA CUNHA et al., 2013; DE SOUZA et al., 2018; DOS SANTOS et al., 2017a, 2017b, 2017c; DUTRA et al., 2019; FARNESI et al., 2009; FERNANDES et al., 2001; ISODOROV et al., 2022; JÚNIOR et al., 2019; KRAIKONGJIT et al., 2018; LIBERIO et al., 2011; MIORIN et al., 2003; POPOVA et al., 2021; SUREK et al., 2021; TORRES et al., 2018).

Campos et al. (2015) verificaram que as gram-positivas demonstram ser mais sensíveis à ação da própolis do que as gram-negativas, atribuindo este efeito a diferença estrutural das paredes celulares destas bactérias (CAMPOS et al., 2015). E quando comparada a antibióticos de referência, tal como ampicilina, a atividade farmacológica da própolis demonstra ser semelhante (DE SOUZA et al., 2018).

A atividade antifúngica da própolis da ASF *Frieseomelitta longipes* foi observada na concentração de 250 µg/mL contra *Candida albicans* e *Candida tropicalis* (DE SOUZA et al., 2018). Com concentrações maiores do extrato da própolis (> 3mg/mL) das espécies *Tetragonisca fiebrigi* e *Melipona orbignyi*, Campos et al (2014 e 2015) observaram propriedades antifúngicas contra *Candida glabrata* e *Candida albicans* (CAMPOS et al., 2014; 2015).

Diferentes estudos associam os compostos fenólicos e terpenos com a atividade antimicrobiana da própolis de ASF (SALOMÃO et al., 2008; VELIKOVA et al., 2000). Esses compostos danificam a membrana celular e, inibem a síntese de ácido nucleico e metabolismo energético dos microorganismos. Adicionalmente, essas substâncias interferem nos fatores de virulência, incluindo enzimas, toxinas e outras moléculas sinalizadoras (CUSHNIE e LAMB, 2011).

Poucos estudos abordam a influência do conteúdo inorgânico (minerais, partículas de solo / argila) ou mesmo material orgânico associado à geoprópolis, como a microbiota nativa ou organismos em decomposição (LAVINAS et al., 2018). Menegatti et al. (2018) isolaram do alimento larval de abelhas *Melipona scutellaris* a bactéria *Paenibacillus polymyxa* ALLI-03-01, que produz (L)-(−)-3-phenyllactic acid e

fusaricidinos (compostos ativos contra fungos entomopatogênicos e *Paenibacillus larvae* que são patógenos das colmeias de abelha sem ferrão) (MENEGATTI et al., 2018).

Zulhendri et al (2021) relataram que uma das atuações da própolis em relação as propriedades antimicrobianas e parasitárias é diretamente no patógeno, inibindo suas enzimas, proteínas e metabólitos necessários para a invasão no hospedeiro, replicação de seu material genético ou produção de energia para sua sobrevivência. A outra ação está relacionada ao hospedeiro, pois a própolis ajuda a manter o status antioxidante celular do hospedeiro ao longo da infecção (ZULHENDRI et al., 2021). Diante disso, própolis e geoprópolis são produtos promissores na busca de novos compostos para terapias que requerem a associação suas propriedades.

Tabela 4 - Atividade antimicrobiana de própolis produzida por diferentes espécies de abelhas sem ferrão.

Espécie	Microorganismos testados (concentração de inibição µg/mL)	Método	Referência
Frieseomelitta longipes	*Bacillus cereus (7.8–15.6)* *Staphylococcus aureus (125)* *Pseudomonas aeruginosa (31.3–62.5)* *Escherichia coli (125)* *Candida albicans (2.5–250)* *Candida tropicalis (250)*	MIC	(DE SOUZA et al., 2018)
Frieseomelitta varia	*Bacillus subtilis e Staphylococcus aureus (62,5 e 250)*	MIC, MBC	(CAMPOS et al., 2011)
Melipona fasciculata	*S. mutans* *C. albicans*	Disco de difusão, CIM, CBM, viabilidade	(LIBERIO et al., 2011)
Melipona fasciculata	*Leishmania amazonenses*		(DUTRA et al., 2019)
Melipona fasciculata	*P. insidiosum*	MIC	(ARAÚJO et al., 2016)
Melipona mandaçaia	*Staphylococcus aureus (160)*	CIM	(FERNANDES JR et al., 2001)
Melipona mondury	*Staphylococcus aureus* *Pseudomonas aeruginosa*	MIC, MBC	(DOS SANTOS et al., 2017c)
Melipona orbignyi	*Staphylococcus aureus (3100)* *Escherichia coli (na)* *Candida albicans (3100)*	MIC, MBC, MFC	(CAMPOS et al., 2014)
Melipona orbignyi	*S. aureus > E. faecalis > E. coli >* *P. aeruginosa > C. neoformans > C. albicans.*	MIC, MBC	(DOS SANTOS et al., 2017a)
Melipona quadrifasciata	*S. aureus (250)* *P. aeruginosa (500)* *E. coli (>1000)* *U. urealiticum (150)* *M. mycoides (250)* *M. genitalium (250)* *M. capricolum (500)* *M. pneumoniae FH (250)* *M. pneumoniae 129 (125)* *M. haminis (500)*	MIC, MBC	(DOS SANTOS et al., 2017b)

Espécie	Microorganismos testados (concentração de inibição µg/mL)	Método	Referência
Melipona quadrifasciata	*Staphylococcus aureus* *P. aeruginosa*	Disco de difusão	(FARNESI et al., 2009)
Melipona quadrifasciata	*Staphylococcus aureus ATCC® 6538™ (3000)* *Methicillin-resistant S.aureus ESA 175 (3580)* *Methicillin-resistant S.aureus ESA 159 (3920)* *Enterococcus faecalis ATCC® 43300™ (4750)* *Vancomycin-resistant E.faecalis ESA 201 (5330)* *Vancomycin-resistant E.faecalis ESA 361 (5830)* *Escherichia coli ATCC® 29998™ (6000)* *Cephalosporin-resistant E.coli ESA 37 (7250)* *Cephalosporins-resistant E.coli ESA 54 (7750)* *P. aeruginosa ATCC® 15442™ (8420)* *Imipenem-resistant P.aeruginosa ESA 22 (9330)* *Imipenem-resistant P.aeruginosa ESA 23 (9920)*	MIC	(CAMPOS et al., 2023
Melipona quadrifasciata quadrifasciata	*S. aureus* (ATCC 6538) (333 ± 118) *S. aureus* (ATCC 33591) (250) *E. faecalis* (ATCC 29212) (333 ± 118) *E. faecalis* (ATCC 51299) (500)	MIC	(SUREK et al., 2021)
Melipona quadrifasciata quadrifasciata	*Staphylococcus aureus (2000–7000)* *MRSA* *Enterococcus faecalis* *Escherichia coli* *K. pneumoniae*	MIC	(TORRES et al., 2018)
Melipona quadrifasciata quadrifasciata	*P. larvae (125)* *S. aureus (31.25)* *B. cereus (0.12)* *B. subtilis (0.12)* *E. coli (125)* *P. aeruginosa (500)* *C. albicans (31.25)*	MIC	(ISIDOROV et al., 2022)
Melipona scutellaris	*Streptococcus mutans* *Staphylococcus aureus* *MSRA* *Enterococcus faecalis* *Actinomyces naeslundii* *Pseudomonas aeruginosa*	MIC, MBC	(DA CUNHA et al., 2013)

Espécie	Microorganismos testados (concentração de inibição µg/mL)	Método	Referência
Melipona scutellaris	*Staphylococcus aureus (230)*	MIC	(FERNANDES JR et al., 2001)
Melipona sp.	*Staphylococcus aureus (170)*	MIC	(FERNANDES JR et al., 2001)
Melipona subnitida	*Candida albicans* *Candida krusei* *Candida glabrata* *Candida tropicalis* *Candida guillermondii* *Candida parapsilosis*	MIC	(JÚNIOR et al., 2019)
Meliponula ferruginea	*S. aureus (ATCC 25923) (625)* *E. faecalis (ATCC 29212) (312.5)* *L. monocytogenes (ATCC 7644) (156.3)* *C. albicans (ATCC 10239) (625)* *E. coli (ATCC 25922) (625)* *P. aeruginosa (ATCC 27853) (312.5)* *S. typhi (ATCC 14028) (2500)*	MIC	(POPOVA et al., 2021)
Nonnotrigona testaceicornis;	*Staphylococcus aureus (9900)*	MIC	(FERNANDES JR et al., 2001)
Partamona sp.	*Staphylococcus aureus (140)*	MIC	(FERNANDES JR et al., 2001)
Plebeia remota	*S. aureus* (ATCC 6538) (417 ± 118) *S. aureus* (ATCC 33591) (250) *E. faecalis* (ATCC 29212) (250) *E. faecalis* (ATCC 51299) (>1000)	MIC	(SUREK et al., 2021)
Scaptotrigona bipunctata	*Staphylococcus aureus* *Escherichia coli*	MIC, MBC	(BUTELLI-FIANCO et al., 2013)
Scaptotrigona bipunctata	*S. aureus* (ATCC 6538) (>1000) *S. aureus* (ATCC 33591) (>1000) *E. faecalis* (ATCC 29212) (>1000) *E. faecalis* (ATCC 51299) (>1000)	MIC	(SUREK et al., 2021)

Espécie	Microorganismos testados (concentração de inibição µg/mL)	Método	Referência
Scaptotrigona depilis	*Staphylococcus aureus ATCC® 6538™ (1670)* *Methicillin-resistant S.aureus ESA 175 (2000)* *Methicillin-resistant S.aureus ESA 159 (2670)* *Enterococcus faecalis ATCC® 43300™ (3000)* *Vancomycin-resistant E.faecalis ESA 201 (3500)* *Vancomycin-resistant E.faecalis ESA 361 (4670)* *Escherichia coli ATCC® 29998™ (3500)* *Cephalosporin-resistant E.coli ESA 37 (5750)* *Cephalosporins-resistant E.coli ESA 54 (6500)* *P. aeruginosa ATCC® 15442™ (6830)* *Imipenem-resistant P.aeruginosa ESA 22 (8250)* *Imipenem-resistant P.aeruginosa ESA 23 (8750)*	MIC	(CAMPOS et al., 2023
S. postica	*P. larvae (31.25)* *S. aureus (125)* *B. cereus (1.95)* *B. subtilis (7.81)* *E. coli (500)* *P. aeruginosa (500)* *C. albicans (125)*	MIC	(ISIDOROV et al., 2022)
Scaptotrigona sp	*Staphylococcus aureus* *E. coli*	Disco de difusão	(FARNESI et al., 2009)
Scaptotrigona sp	*Staphylococcus aureus (8230)*	MIC	(FERNANDES JR et al., 2001)
Tetragonisca angustula	*Staphylococcus aureus* *MRSA* *Enterococcus faecalis* *Escherichia coli* *K. pneumoniae*	MIC	(TORRES et al., 2018)
Tetragonisca angustula	*Staphylococcus aureus*	MIC	(MIORIN et al., 2003)
Tetragonisca angustula	*Staphylococcus aureus*	MIC	(FERNANDES JR et al., 2001)

Espécie	Microorganismos testados (concentração de inibição µg/mL)	Método	Referência
Tetragona clavipes	*P. larvae (125)* *S. aureus (31.25)* *B. cereus (0.12)* *B. subtilis (0.12)* *E. coli (125)* *P. aeruginosa (500)* *C. albicans (31.25)*	MIC	(ISIDOROV et al., 2022)
Tetragonisca fiebrigi	*P. larvae (31.25)* *S. aureus (31.25)* *B. cereus (0.49)* *B. subtilis (0.49)* *E. coli (500)* *P. aeruginosa (500)* *C. albicans (31.25)*	MIC	(ISIDOROV et al., 2022)
Tetragonisca fiebrigi	*Staphylococcus aureus* *Staphylococcus epidermidis* *Enterococcus faecalis* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Proteus mirabilis* *Candida glabrata* *Candida albicans*	MIC, MBC, MFC	(CAMPOS et al., 2015)
Tetrigona apicalis	*S. aureus ATCC25923 (6000)* *E. coli ATCC 25922 (12000)* *P. aeruginosa ATCC27853 (24000)*	MIC	(KRAKORGJIT et al., 2017)

Espécie	Microorganismos testados (concentração de inibição µg/mL)	Método	Referência
Trigona sp	*Escherichia coli ATCC 117* *Salmonella typhimurium ATCC 23564* *Bacillus subtilis ATCC 6633* *Staphylococcus aureus ATCC 6538* *Salmonella abony NCTC 6017* *Streptococcus pyogenes* *Staphylococcus epidermidis ATCC 1228* *Staphylococcus schleiferi* *Staphylococcus aureus* *Streptococcus mutans* *Klebsiella pneumoniae* *Acinetobacter baumannii* *Candida glabrata NCIM 3236*		(CHOUDHARI et al., 2012)
Trigona spinipes	*Staphylococcus aureus (9470)*	MIC	(FERNANDES JR et al., 2001)

MRSA, *Staphylococcus aureus* resistente a Meticilina; na (nenhuma atividade)

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CAPÍTULO 1

Chemical characterization, antioxidant, anti-inflammatory and antifungal activities of geopropolis from four *Meliponini* stingless bee species

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ABSTRACT

Geopropolis are products derived from plant resin and stingless bees salivary secretion and are used in folk medicine for their therapeutic properties. However, scientific records evidencing such properties from species occurring in Atlantic Forest – Brazil are still scarce. The aim of this study is investigate the chemical composition and biological features of geopropolis gathered from four species of stingless bees during different seasons. The chemical composition of the hydroalcoholic extract of geopropolis was determined by the total polyphenols, tannins, and flavonoid content, and by ESI-FT-ICR MS analysis. The antioxidant activity was investigated by DPPH and $ABTS^{+}$ scavenging tests. *In vitro* assays were used to evaluate the production of nitric oxide, superoxide, and cytokines and to determine the fungistatic and fungicidal activity. The data indicated that geopropolis present polyphenol, flavonoid, and tannin contents. A total of 56 compounds were proposed using ESI-FT-ICR MS. Additionally, geopropolis extract act on fungi that causes dermatophytosis and acts on oxidative stress arising from the inflammatory process, displaying differences between species and seasonality. Our results contribute to a better understanding of the chemical and biological of geopropolis being considered a promising natural product in the pharmaceutical industry.

Keywords: *stingless bee; Melipona; antioxidants; anti-inflammatory; antifungal; geopropolis*

1. INTRODUCTION

Brazilian biodiversity is known worldwide for being one of the most exuberant in the world and for having a significant number of species that are objects of scientific study, attracting the interest of the most avid researchers (Valli et al., 2018). Inserted in this context, stingless bees have concentrated much of the attention due to their ability to produce compounds with promising bioactive potential (Ferreira et al., 2020; Lavinas et al., 2018; Popova et al., 2021). In particular, stingless bees of the Order *Hymenoptera*, family *Apidae*, and tribe *Meliponini* are found in tropical and subtropical areas: South, Central, and North America, Africa, Asia, and Oceania (Hrncir et al., 2016). In Brazil, 244 species are found, and 39 species are described in the Atlantic Forest in the Espírito Santo state (de Menezes, 2014; Lavinas et al., 2018). Among these species, *Melipona (Eomelipona) bicolor* (Lepeletier, 1836), *Melipona (Michmelia) capixaba* (Moure & Camargo, 1994), *Melipona (Michmelia) mondury* (Smith, 1863) and *Melipona (Melipona) quadrifasciata* (Lepeletier, 1836) that produce geopropolis.

Geopropolis are products of stingless bees that help protect the box hives' structural and antiseptic protection against external factors. While geopropolis is a mixture of salivary gland secretions from bees, wax, plant resins and soil, silt, and/or sand particles (Shanahan and Spivak, 2021). Its chemical diversity is influenced by flora biodiversity, geographic area, seasonality, collection time, and bee species (Campos et al., 2015; Dutra et al., 2014). So far, there are no studies on the chemical composition and biological activities of many stingless bees native to the Atlantic Forest, including *Melipona bicolor* and *Melipona capixaba*. This specie, *M capixaba*, is a stingless bee exclusively occurring in Espirito Santo, Brazil. Both species, *M. capixaba* and *M. bicolor* have been only studied regarding genetics, morphology, and behavioral characteristics (Abdalla and Cruz-Landim, 2004; Hilário et al., 2000; Hilário and Imperatriz-Fonseca, 2009; Moure and Camargo, 1994; Resende et al., 2008; Rocha et al., 2002; Serra et al., 2012).

Studies have demonstrated the chemical diversity of stingless bee geopropolis consisting of phenylpropanoids, flavonoids, phenolic compounds, tannins, terpenes, saponins, alkaloids, essential oils, fatty acids, and sugars (Lavinas et al., 2018; Sanches et al., 2017). The presence of phenolic compounds, such as flavonoids (quercetin and kaempferol) is usually correlated to the antioxidant activity of geopropolis. The chemical structures of these compounds are characterized by having

at least one hydroxyl group, which confers oxidative stability to the organism by decreasing the damage associated with free radicals, the ones responsible for oxidative damage in tissues (Lavinas et al., 2018; Reis et al., 2019). Torres et al. (2018) investigated the antioxidant properties of the ethanolic extract of stingless bee propolis and found that the activity is directly correlated with the concentration of phenols and flavonoids in the extract, reinforcing the previous statement (Torres et al., 2018).

Recent reviews concerning the biological activity of *Meliponini* geopropolis reported a broad range of bioactivity. The activity varies based on the method used to evaluate the biological activity, type of extract, period of collection of samples, and bee species studied (Lavinas et al., 2018; Popova et al., 2021; Sanches et al., 2017). The primary methodologies applied were the free radical capture DPPH (2,2-diphenyl-1-picrylhydrazyl) and $ABTS^{+}$ (2,2-azinobis-(3-ethyl benzothiazole-6-sulfonate); FRAP (iron reducing antioxidant power) method; oxidative hemolysis inhibition assay; assessment of lipid peroxidation inhibition in human erythrocytes and analysis using β-carotene/linoleic acid. Using DPPH, $ABTS^{+}$, and FRAP methods, the stingless bee propolis extracts have demonstrated free radical inhibition power similar to chemical compounds widely known for their antioxidant action, employed as the positive standards (e.g., gallic acid, ellagic acid, ascorbic acid, BHT) (de Souza et al., 2018; dos Santos et al., 2017a; dos Santos et al., 2017b). Stingless bee geopropolis also demonstrated the ability to protect against oxidative hemolysis and reduce malondialdehyde (MDA) levels, a product of lipid peroxidation arising from oxidative stress. However, in the evaluation of Bonamigo et al. (2017), the extract of propolis from the species *Plebeia droryana* was not able to inhibit the content of MDA generated by the action of the oxidizing agent 2,2'-azobis (2-amidinopropane) dihydrochloride (Bonamigo et al., 2017). Overall, the authors conclude that stingless bee geopropolis has therapeutic potential for the treatment and/or prevention of oxidative stress-related diseases.

Other authors have reported a significant antimicrobial capacity of geopropolis (Farnesi et al., 2009; Velikova et al., 2000), acting mainly in two ways, one that acts directly on the pathogen, making it difficult to invade the host by interfering in crucial steps of its metabolism, and another that acts on the host itself, improving the immune capacity to face potential invading microorganisms and the possible damage caused by them (Zulhendri et al., 2021).

In this context, this study aimed to investigate the chemical composition and biological properties of geopropolis from four stingless bees meliponas from the Espírito Santo state collected in different seasons of the year.

2. MATERIAL AND METHODS

2.1 Sample collection

The geopropolis samples from the four selected stingless bee species of the *Meliponini* tribe - *Melipona quadrifasciata* (MQ), *Melipona mondury* (MM), *Melipona capixaba* (MC), *Melipona bicolor* (MB) - were collected in the city of Santa Maria Jetibá (20º05'52"S, 40º36'56"W), Espírito Santo, Brazil, in the dry season of 2019 (September) and in the rainy season of 2020 (February). Geopropolis samples were taken directly from the hives by spatulation, packaged, and correctly identified in plastic bags, and stored under protection from light and at a temperature of 0ºC.

2.2 Preparation of the extracts

The stingless bee geopropolis samples collected were first subjected to mechanical disintegration in a ball mill and then to extraction by ultrasound-assisted maceration using 70% ethanol according to the method described by (Dutra et al., 2014) with modifications. After extraction, the obtained material was filtered, rotary-evaporated, lyophilized, and stored at -20°C until analysis.

2.3 Determination of flavonoids, total phenolics, and tannins

The spectrophotometric method determined the total flavonoid content after the reaction with aluminum chloride (10% w/v), according to (Asem et al., 2020). Quantification was done by constructing a standard curve of quercetin (100 - 700 µg/mL) and determined by reading the absorbances in a spectrophotometer (Molecular Devices Spectra MAX 190) at 415 nm. The total flavonoid content of the geopropolis extracts was expressed as mg quercetin equivalent (QE)/g dry extract.

The Folin-Ciocalteau method described by Krepsky (Krepsky et al., 2012) performed quantification of total phenolics and tannins. An analytical curve was prepared with gallic acid (10 - 50 µg/mL) for this determination. The contents were determined by reading the absorbances performed in a spectrophotometer (Molecular Devices Spectra MAX 190) at 715 nm. The tannin content was estimated by the difference between the total phenolic and non-phenolic content in the extracts, based

on polyvinylpolypyrrolidone (PVPP) precipitation. The results were expressed as mg gallic acid equivalents (GAE)/g dry extract.

The experiments were performed in triplicate on different days.

2.4 Mass Spectrometry (ESI-FT-ICR MS)

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) combined with a direct infusion electrospray ionization (ESI) provided a detailed molecular view of geopropolis extracts. The samples were dissolved in 1 mL of methanol and analyzed by high-resolution mass spectrometry (HRMS). The mass spectrometer was the 9.4 T Solarix (Bruker Daltonics, Bremen, Germany). The analyzes were performed in the electrospray source and the negative ion mode, ESI(-), over a mass range of m/z 150–1500. The ESI source conditions were as follows: a nebulizer gas pressure of 1.5 bar, a capillary voltage of 4.0 – 4.4 kV, and a capillary transfer temperature of 200 C°. Ion time accumulation was 0.005 – 0.030 s. The time of flight was 0.950 sec. ESI(-)FT-ICR mass spectra were acquired by accumulating 16 scans of time-domain transient signals in 4 mega-point time-domain data sets. All mass spectra were externally calibrated using Arginine (m/z from 150 to 1500). A mass accuracy of <10 ppm provided the unambiguous molecular formula assignments for singly charged molecular ions. Mass spectra were processed using Data Analysis software (Bruker Dantonics, Bremen, Germany). Elemental compositions of the compounds were determined by measuring the mass-to-charge ratio (m/z), considering the error and double bond equivalents (DBE) values (Ribeiro et al., 2021).

The structural formulas of the compounds were obtained by ChemSpider software and compared with compounds frequently described in the scientific literature for bee products.

2.5 Antioxidant activity

The antioxidant activity of the geopropolis extracts was determined using radical scavenging methods, in particular, ABTS+ 2,2-azinobis (3-ethyl benzo ethybenzothiazole) and DPPH 2,2-diphenyl-1-picrylhydrazyl (Re et al., 1999). The results were expressed as IC_{50} (µg/mL) and compared with quercetin. The experiments were performed in triplicate on different days.

2.6 Cell viability

Cell viability was assessed using the colorimetric MTT test (Mosmann, 1983). Briefly, RAW 264.7 macrophages and fibroblast L929 were seeded in 96-well plates at 8 x 104 cells/well. After overnight, cells were treated with different geopropolis extract concentrations (0.1 - 150 µg/mL) for 24 h at 37 ºC in 5% CO_2. Then, the MTT solution was added to each well and the formazan crystals were dissolved with dimethyl sulfoxide (DMSO). The optical density of each well was measured at 595 nm wavelength by the microplate reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices, USA). The results were reported as a percentage of viable cells.

2.7 Nitric oxide analysis

RAW 264.7 macrophages were plated at a concentration of 8 x 10^4 cells mL-1 in 96-well plates and incubated overnight (37ºC at 5% CO_2). Geopropolis extract was added at concentrations of 0.1 to 20 µg/mL. LPS (1 µg/mL) was added at all wells after 1h, except for the negative control. L-NIL (30 µM) was used as a positive control. After 20 h of incubation, 100 µL of the supernatant was transferred to another plate and the nitrite quantification was performed by adding 100 µL of the Griess solution (Green et al., 1982). Results expressed as mean ± SD of the nitrite concentration (µM) calculated by regression analysis of a standard curve of sodium nitrite.

2.8 Superoxide anion assay

The superoxide assay was used to determine the inhibitory effect of geopropolis extract on the production of the superoxide radical ($O_2^{\bullet -}$) in LPS-stimulated RAW 264.7 macrophages, as previously described by Soares et al (2021). Tempol (4-hidroxi-2,2,6,6-tetrametilpiperidin-1-oxil) (10 µM) was used as a positive control. The results were expressed as mean ± SD of superoxide anion production (%).

2.9 Measurement of cytokine

The supernatant of LPS-stimulated macrophages cell culture exposed to different concentrations of the geopropolis extracts (0.1 - 20 µg/mL) was used to quantify tumor necrosis factor-alpha (TNF-α) by enzyme-linked immunosorbent assay (ELISA), according to the manufacturer's instructions (eBioscience, San Diego, California, USA).

2.10 Antifungal activity

In the study reference strains were used. The fungi were *Aspergillus fumigatus* ATCC 16913, *Trichophyton rubrum* CBS 392.58, e *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90029 e *Candida parapisilosis* ATCC 22019. Sensitivity tests were performed using the broth microdilution method in 96-well microplates to determine the minimum inhibitory concentration (MIC). Per the standards for susceptibility testing set forth by reference documents M27-A3 and M38-A (CLSI 2008, 2010) of the Institute for Clinical Laboratory Standards. The antifungal solutions were prepared in dimethylsulfoxide (DMSO) and subsequently diluted in RPMI-1640 (Roswell Park Memorial Institute) buffered with MOPS (3-Nmorpholine-propane sulfonic acid) without glutamine, to obtain final concentrations in the range of 8 to 1024 µg/mL for the extracts and from 0.03 to 16 µg/mL for the conventional antifungals Fluconazole and Amphotericin B. The minimum inhibitory concentration was obtained through the concentration where no visual growth was observed after 24h for *Candida strains*, 48h for strains of *Aspergillus fumigatus*, and 96h for strains of *Trychophyton rubrum*. A strain of *Aspergillus flavus* ATCC 204304 was used as a reference to guarantee the quality of the test against drug dilution.

The CFM determination test was performed, adapting the protocol by Espinel-Ingroff et al. 2002. Transferring 20 µL of the microdilution wells in broth, referencing the MIC, 2xMIC and 4xMIC values, to the culture plate containing saubourad agar (ASD) and incubated at 35ºC for 24h for *Candida*, 48h for *Aspergillus*, and 96 h for *T. rubrum*. The CFM was defined as the lowest concentration that did not show any visual growth after incubation (Colony Forming Units), where it is considered that approximately 99% to 99.5% of the fungal pathogens tested died (Van et al., 2018)

2.6 Statistical analysis

The experiments were performed using an entirely randomized design. Data were analyzed with analysis of variance (ANOVA) ($p < 0.05$) followed by Tukey's test (Sokal and Rohlf, 1995) in the R program (R Foundation for Statistical Computing, version 3.1.1, 2014, Vienna, Austria). Statistical variations among species and within species were determined using double factorial analysis.

3. RESULTS

The highest yields were attributed to the geopropolis extracts collected in the dry season from *M. quadrifasciata*, *M. mondury, M. capixaba* and *M. bicolor* bees (yields 27.3, 27.4, 24.7 and 24.2% w/w, respectively). The yields of the rainy season were 16.6, 24.9, 21.6 and 12.3% w/w from MQ, MM, MC and MB bees, respectively.

Considering total polyphenols and geopropolis samples collected in the rainy season, *M. bicolor* stood out with 160.68 ± 5.70 mg of GAE/g of geopropolis extract, followed by *M. mondury* with 93.10 ± 11.50 mg of GAE/g of geopropolis extract. *M. capixaba* concentrated 80.67 ± 5.00 mg of GAE/g of geopropolis extract, while *M. quadrifasciata* exhibited only 18.03 ± 1.80 mg of GAE/g of geopropolis extract (Figure 1A).

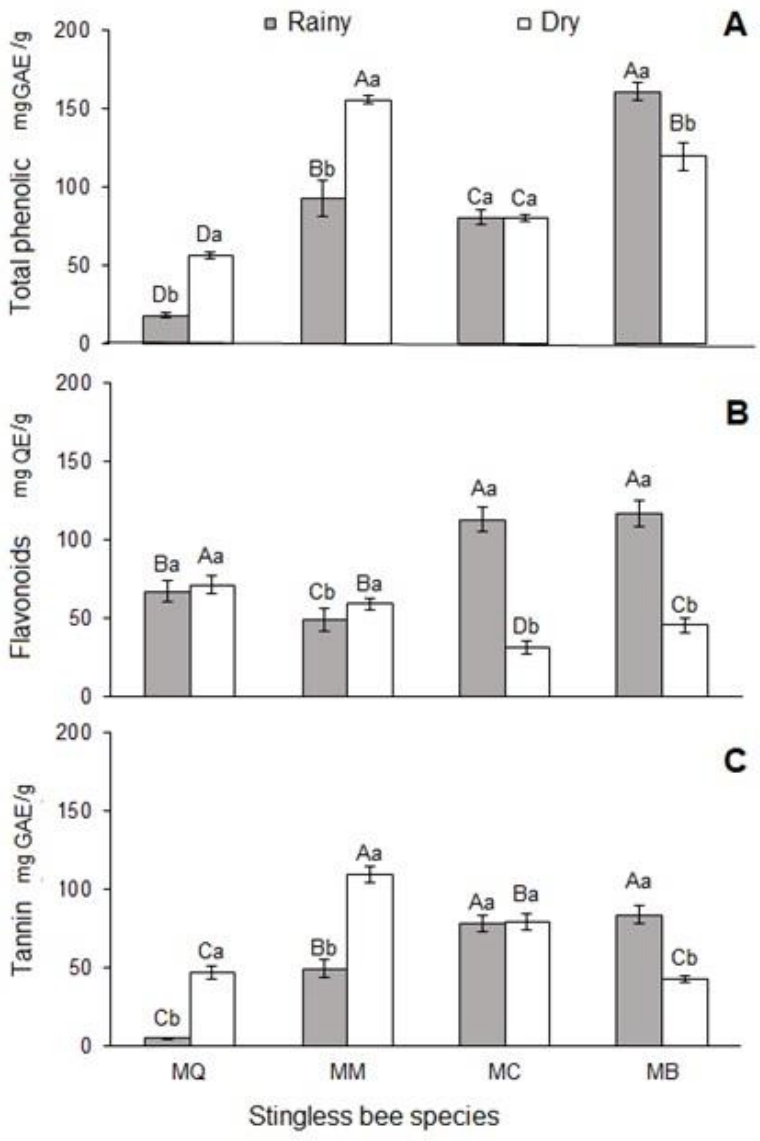

Figure 1 - Total phenolics (A), flavonoids (B), and tannins (C) contents of geopropolis extracts from *Melipona quadrifasciata* (MQ), *Melipona mondury* (MM), *Melipona*

capixaba (MC), and *Melipona bicolor* (MB) stingless bee obtained in the dry and rainy seasons. Values are represented as mean ± standard deviation of three independent triplicates. Values within each column followed by a different superscript letter(s) are significantly different at $P < 0.05$. *Upper case letters compare bee species in the same seasons. *Lower case letters compare the same bee in different seasons.

In the dry season, the total phenolic content found was higher in *M. mondury* with 155.68 ± 2.80 mg of GAE/g of geopropolis extract, similar to a previous study with the same species, which showed 144.4 ± 0.01 mg of GAE/g of extract (dos Santos et al., 2017c). *M. bicolor* exhibited 119.37 ± 8.70 mg of GAE/g, similar to *M. orbignyi* analyzed by Santos et al (2017a) which, 121.0 ± 0.6 mg of GAE/g in the propolis extract. Interestingly, the concentrations of polyphenols in the geopropolis of *M. bicolor* were higher than in the geopropolis of *M. mondury* in the rainy season (dos Santos et al., 2017a). *M. capixaba* presented 79.92 ± 1.90 mg of GAE/g of geopropolis extract, the only species that did not exhibit different seasons. *M. quadrifasciata* exhibited the lowest total phenolic content with 56.03 ± 2.30 mg of GAE/g of geopropolis extract, similar to 57.90 ± 0.02 mg of GAE/g reported by Dutra et al. (2017) in the ethyl acetate fraction the hydroalcoholic extract of geopropolis (Dutra et al., 2017).

The concentration of total flavonoid content (Figure 1 B) in the geopropolis obtained in the rainy season was higher for *M. bicolor* (116.82 ± 8.10 mg of QE/g). However, it differed statistically from *M. capixaba* (112.98 ± 7.60 mg of QE/g). In the geopropolis extract of *M. mondury*, the flavonoid content was 49.08 ± 7.20 mg of QE/g. *M. quadrifasciata* expressed 67.02 ± 6.80 mg of QE/g of geopropolis extract. The amount of flavonoids in the dry season decreased compared to the rainy season. However, about to the dry season species stood out, such as *M. quadrifasciata*, with 71.12 ± 5.80 mg of QE/g of ethanolic extract, a result that was higher than the one described by Pazin et al (2017) in the ethanolic extract of *Melipona quadrifasciata anthidioides* that presented 10.0 ± 0.1 mg of QE/g of ethanolic extract (Pazin et al., 2017). In this analysis, *M. quadrifasciata* was the only species that did not show significant variation in flavonoid concentration regardless of the season of geopropolis collection. No studies were found to corroborate the flavonoid analysis for the other species.

The content of tannins in the geopropolis extracts (Figure 1C) from the rainy season was higher in *M. bicolor* (83.85 ± 5.50 mg of GAE/g), and in *M. capixaba* (78.02 ± 5.30 mg of GAE/g). *M. mondury* presented a value of 49.32 ± 5.60 mg of GAE/g of

geopropolis extract. As the concentration of polyphenols in *M. quadrifasciata* was lower than other species in the same period, this characteristic was also observed for tannins, which concentrated only 4.90 ± 0.30 mg of GAE/g of ethanolic extract concerning the geopropolis of the same season. In the dry season, the predominance of tannins was 42.68 ± 2.10 mg of GAE/g of geopropolis extract of *M. mondury*. *M. capixaba* presented 79.50 ± 5.00 mg of GAE/g of extract; no variation of tannins was noted when comparing the geopropolis of the same species in the two seasons. Moreover, the representation of tannin concentration in *M. capixaba* was close to the concentration of polyphenols in this species. *M. quadrifasciata* presented 46.90 ± 4.20 mg of GAE/g of geopropolis extract and stood out compared to the concentration found for the rainy season. *M. bicolor* concentrated 42.68 ± 2.10 mg of GAE/g of geopropolis extract, making it the species with the lowest amount of tannins according to the comparison made. No data on quantifying tannins in geopropolis from *Meliponini* were found in the literature.

In Mass Spectrometry, 56 compounds were proposed considering the high resolution and accuracy of ESI(-) FT-ICR MS, as well as the low mass error (≤10 ppm), m/z (mass-to-charge ratio) values, and double bond equivalents (DBEs). The spectral profiles and presence of the proposed compounds are similar among the samples, with variations in relative intensities (Figure 1S and Table 1S).

As phenols, we propose the benzoic acid derivatives (compound 3 ion $[C_7H_{11}O_6]^-$ of m/z 191 identified as Quinic acid, compound 6 ion $[C_9H_9O_5]^-$ of m/z 197 identified as Syringic acid, compound 34 ion $[C_{13}H_{15}O_{10}]^-$ of m/z 331 identified as Monogalloylglycoside, compound 48 ion $[C_{21}H_{17}O_{13}]^-$ of m/z 477 identified as Digalloilshikimic acid), benzoaldehydes (compound 2 ion $[C_9H_9O_4]^-$ of m/z 181 identified as Syringaldehyde) and simple phenols (compound 8 ion $[C_{11}H_{11}O_4]^-$ of m/z 207 identified as Sinapaldehide, compound 40 ion $[C_{29}H_{49}O]^-$ of m/z 413 identified as Sitosterol, compound 27 ion $[C_{14}H_5O_8]^-$ of m/z 300 identified as Ellagic acid and its dimer, compound 53 ion $[C_{28}H_{11}O1_6]^-$ of m/z 603 identified as Ellagic acid dimer). These compounds have been described by other authors (Araújo et al., 2016; Bonamigo et al., 2017; dos Santos et al., 2017b; Dutra et al., 2019; Ferreira et al., 2020; Lopes et al., 2019, 2020; Silva et al., 2006).

The flavonoids proposed in this study were: compound 12 ion $[C_{15}H_9O_4]^-$ of m/z 253 identified as Chrysin, compound 15 ion $[C_{15}H_9O_5]^-$ of m/z 269 identified as Apigenin / Galangin, compound 16 ion $[C_{16}H_{13}O_4]^-$ of m/z 269 identified as Medicarpin, compound 17 ion $[C_{15}H_{11}O_5]^-$ of m/z 271 identified as Naringenin, compound 23 ion

$[C_{15}H_9O_6]^-$ of m/z 285 identified as Kaempferol / Luteolin, compound 24 ion $[C_{15}H_{11}O_6]^-$ of m/z 287 identified as Plathymenin / Eriodictyol / Aromadendrin, compound 25 ion $[C_{15}H_{13}O_6]^-$ of m/z 289 identified as Catechin / Epicatechin, compound 26 ion $[C_{16}H_{11}O_6]^-$ of m/z 299 identified as Hispidulin, compound 28 ion $[C_{15}H_9O_7]^-$ of m/z 301 identified as Quercetin / Tricetin, compound 29 ion $[C_{16}H_{13}O_6]^-$ of m/z 301 identified as Isoferreirin / Alnustinol / Ferreirin / Hesperetin, compound 30 ion $[C_{15}H_{11}O_7]^-$ of m/z 303 identified as Taxifolin / Dihydroquercetin, compound 31 ion $[C_{16}H_{11}O_7]^-$ of m/z 315 identified as Isorhamnetin / Selagin / Methoxyherbacetin, compound 32 ion $[C_{15}H_9O_8]^-$ of m/z 317 identified as Myricetin, compound 37 ion $[C_{25}H_{25}O_5]^-$ of m/z 405 identified as Diprenylgenistein, compound 38 ion $[C_{22}H_{31}O_{21}]^-$ - of m/z 407 identified as Galloyl pinocembrin, compound 41 ion $[C_{25}H_{25}O_6]^-$ of m/z 421 identified as Diprenylkaempferol / Cajanone, compound 43 ion $[C_{22}H_{15}O_9]^-$ of m/z 423 identified as Galloyl naringenin, compound 44 ion $[C_{15}H_{19}O_{10}]^-$ of m/z 431 identified as apigenin-glucoside, compound 45 ion $[C_{22}H_{15}O_{10}]^-$ of m/z 439 identified as Galloyl eriodictyol, compound 46 ion $[C_{21}H_{19}O_{11}]^-$ of m/z 447 identified as quercitrin, compound 47 ion $[C_{21}H_{19}O_{12}]^-$ of m/z 463 identified as Isoquercetin, compound 51 ion $[C_{26}H_{27}O_{15}]^-$ of m/z 579 identified as Carlinoside, compound 52 ion $[C2_7H_{31}O_{14}]^-$ of m/z 579 identified as Naringin, compound 54 ion $[C_{30}H_{25}O_{14}]^-$ of m/z 609 identified as prodelphinidin B4, compound 55 ion $[C_{27}H_{29}O_{16}]^-$ of m/z 609 identified as Rutin, and compound 56 ion $[C_{27}H_{29}O_{17}]^-$ - of m/z 625 identified as quercetin-Diglucoside. Other authors found these substances in stingless bee products such as geopropolis and pollen (Araújo et al., 2016; Belina-Aldemita et al., 2020; Bonamigo et al., 2017; Chong and Chua, 2020; Dembogurski, 2018; de Oliveira et al., 2019; de Souza et al., 2013, 2018; dos Santos et al., 2017a, 2017b; Ferreira et al., 2020; Funakoshi-Tago et al., 2016; Lopes et al., 2019, 2020; Othman et al., 2020; Silva et al., 2006).

In the phenylpropanoids class, the following derivatives of cinnamic acid have been proposed: compound 4 ion $[C_{10}H_9O_4]^-$ of m/z 193 identified as Ferulic acid, compound 9 ion $[C_{11}H_{11}O_5]^-$ of m/z 223 identified as Sinapic ac, compound 10 ion $[C_{14}H_{15}O_3]^-$ of m/z 231 identified as Drupanin / hydroxycinnamic acid, compound 11 ion $[C_{14}H_{15}O_4]^-$ of m/z 247 identified as Prenyl caffeate / Prenylated caffeic acid, compound 35 ion $[C_{16}H_{17}O_9]^-$ of m/z 353 identified as Chlorogenic acid / caffeoylquinic acid, compound 36 ion $[C_{16}H_{15}O_8]^-$ of m/z 359 identified as Rosmarinic acid, compound 50 ion $[C_{25}H_{23}O_{12}]^-$ of m/z 515 identified as Caffeoylquinic acid. These compounds have been described by other authors (Bonamigo et al., 2017; Dembogurski, 2018, de Oliveira et al., 2019; Ferreira et al., 2020; Othman et al., 2020).

The proposed major terpenic compounds were divided into sesquiterpene hydrocarbons, such as compound 7 ion $[C_5H_{23}]^-$ of m/z 203 identified as Copaene / Bergamotene / Guaiene / Aromadendrene / Himachalene / Humulene / Farnesene / Caryophyllene / muurolene / Germacrene / Chamigrene / Selinene / Valencene / Bisabolene / Cadinene, and oxygenated diterpenoid such as compound 33 ion $[C_{20}H_{25}O_4]^-$ of m/z 329 identified as Carnosol. Previous literature has reported these elements for their antioxidant and antimicrobial activity for propolis from the species *Friesomelitta longipes* (de Souza et al., 2018; Ferreira et al., 2020).

Short-chain fatty acids (which are carboxylic acids with a number of carbon atoms equal to or less than 6) were proposed, such as compound 5 ion $[C_6H_{11}O_7]^-$ of m/z 195 identified as Gluconic acid, and long-chain fatty acids, such as compound 13 ion $[C_{16}H_{31}O_2]^-$of m/z 255 identified as Acid palmitique, compound 14 ion $[C_{15}H_{19}O_4]^-$ of m/z 263 identified as Abscisic acid, compound 18 ion $[C_{18}H_{29}O_2]^-$- of m/z 277 identified as Linolenic acid, compound 19 ion $[C_{18}H_{31}O_2]^-$ of m/z 279 identified as Linoleic acid, compound 20 ion $[C_{18}H_{33}O_2]^-$ of m/z 281 identified as Oleic acid, and compound 21 ion $[C_{18}H_{35}O_2]^-$ of m/z 283 identified as Stearic acid. These compounds have been described by other authors (Dutra et al., 2019; de Oliveira et al., 2019; Ferreira et al., 2020; Lopes et al., 2020, 2019).

Xanthones with Mangostin (compound 39 ion $[C_{24}H_{25}O_6]^-$ of m/z 409) and Mangiferin (compound 42 ion $[C_{19}H_{17}O_{11}]^-$ of m/z 421) were proposed in this study and were also detected by Chong and Chua (2020). Other proposed compounds were sugar, alcohol, and benzaldehyde, such as Glucose (compound 1 ion $[C_6H_{11}O_6]^-$ of m/z 179), Vitamin A / Retinol (compound 22 ion $[C_{20}H_{29}O]^-$ of m/z 285), and Nemorosone / Propolone A (compound 49 ion $[C_{33}H_{41}O_4]^-$ of m/z 501), respectively. These compounds have been described by other authors (Campos et al., 2015; de Souza et al., 2018; Dutra et al., 2019).

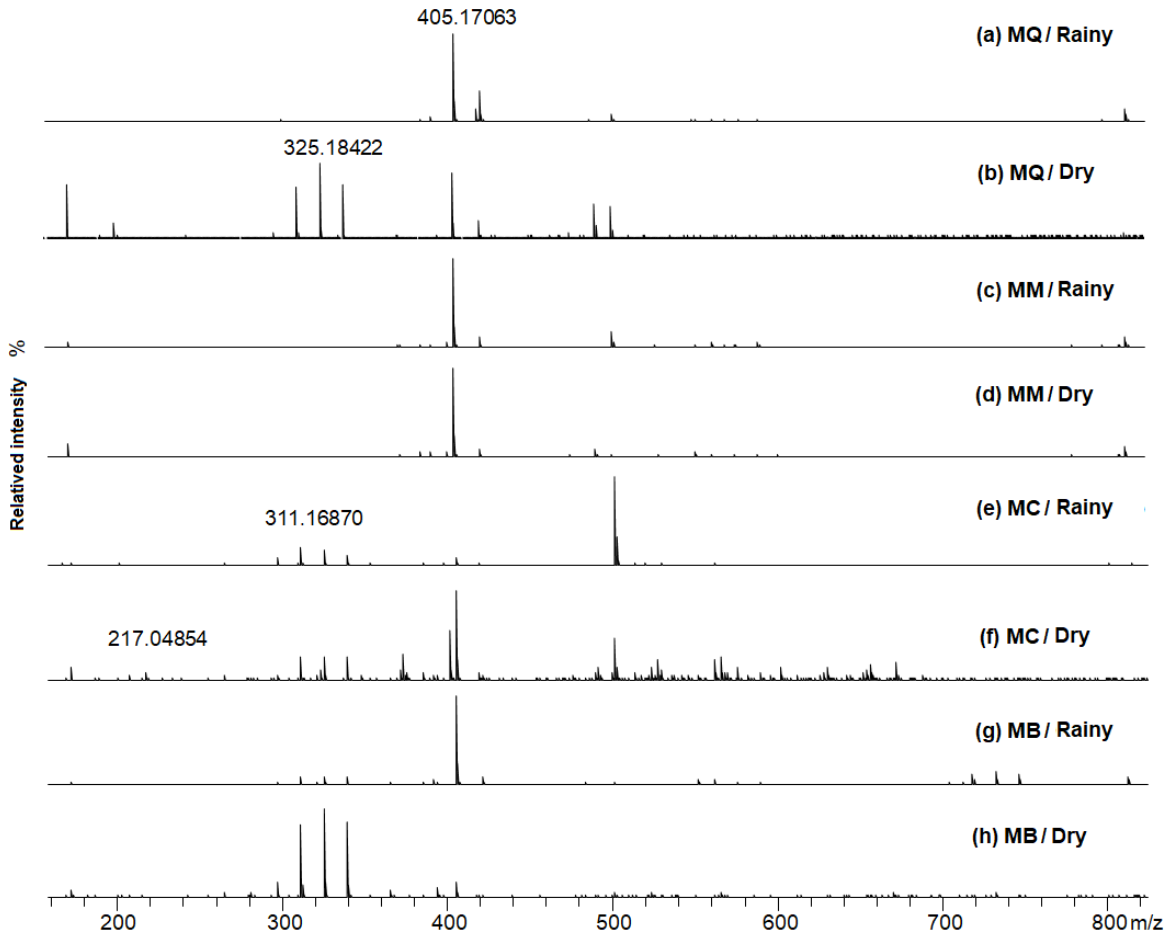

Figure 1S - ESI(-) FT-ICR MS spectral profiles of propolis extracts from *Melipona quadrifasciata* (MQ), *Melipona mondury* (MM), *Melipona capixaba* (MC), and *Melipona bicolor* (MB) stingless bee species and seasonal seasons (Dry and Rainy).

Table 1S - Chemistries species proposed for ESI(-)FT-ICR MS from propolis extracts from *Melipona quadrifasciata* (MQ), *Melipona mondury* (MM), *Melipona capixaba* (MC), and *Melipona bicolor* (MB) stingless bee species and seasonal seasons (Dry and Rainy).

N°	m/z (exper)	Molecular Formulas [M-H]-	DBE	error (ppm)	Structural Formulas	MQ Rainy	MQ Dry	MM Rainy	MM Dry	MC Rainy	MC Dry	MB Rainy	MB Dry	Reference
1	179.05636	$C_6H_{11}O_6$	1	-0.21	Glicose	ND	ND	ND	ND	D	ND	ND	ND	(Campos et al., 2015; Dutra et al., 2019)
2	181.05066	$C_9H_9O_4$	5	1.24	Syringaldehyde	ND	ND	ND	ND	D	ND	ND	ND	(Ferreira et al., 2020)
3	191.05618	$C_7H_{11}O_6$	2	7.76	Quinic acid	ND	ND	ND	ND	D	ND	ND	ND	(Dutra et al., 2019)
4	193.04973	$C_{10}H_9O_4$	6	4.83	Ferulic acid	ND	ND	ND	ND	D	D	ND	ND	(Bonamigo et al., 2017; de Oliveira et al., 2019; Othman et al., 2020)

N°	m/z (exper)	Molecular Formulas [M-H]-	DBE	error (ppm)	Structural Formulas	MQ Rainy	MQ Dry	MM Rainy	MM Dry	MC Rainy	MC Dry	MB Rainy	MB Dry	Reference
5	195.05105	$C_6H_{11}O_7$	1	2.43	Gluconic acid	ND	ND	ND	ND	D	D	D	D	(Lopes et al., 2020, 2019)
6	197.04563	$C_9H_9O_5$	5	8.21	Syringic acid	D	D	ND	ND	D	ND	D	ND	(dos Santos et al., 2017b; Ferreira et al., 2020)
7	203.17996	$C_{15}H_{23}$	4	-5.10	Copaene / Bergamotene / Guaiene / Aromadendrene / Himachalene / Humulene / Farnesene / Caryophyllene / muurolene / Germacrene / Chamigrene / Selinene / Valencene / Bisabolene / Cadinene	D	D	D	D	D	D	D	D	(de Souza et al., 2018)
8	207.06619	$C_{11}H_{11}O_4$	6	6.60	Sinapaldehyde	D	D	ND	ND	D	D	ND	D	(Ferreira et al., 2020)
9	223.05994	$C_{11}H_{11}O_5$	6	5.64	sinapic ac	ND	ND	D	D	ND	D	ND	D	(Ferreira et al., 2020)
10	231.10276	$C_{14}H_{15}O_3$	7	7.95	Drupanin / hydroxycinnamic acid	ND	ND	D	ND	ND	ND	ND	D	(Dembogurski, 2018)
11	247.09771	$C_{14}H_{15}O_4$	7	8.27	Prenyl caffeate / Prenylated caffeic acid	D	ND	D	ND	ND	ND	D	D	(Dembogurski, 2018)
12	253.04847	$C_{15}H_9O_4$	11	-9.99	Chrysin	D	ND	D	D	ND	D	D	D	(Funakoshi-Tago et al., 2016)
13	255.23296	$C_{16}H_{31}O_2$	1	-8.31	Acide palmitique	D	D	D	D	D	D	D	D	(Dutra et al., 2019; Ferreira et al., 2020)
14	263.1280	$C_{15}H_{19}O_4$	4	9.82	Abscisic acid	D	D	D	D	D	D	D	D	(de Oliveira et al., 2019)
15	269.0455	$C_{15}H_9O_5$	11	-9.08	Apigenin / Galangin	D	D	ND	ND	ND	D	D	D	(Bonamigo et al., 2017; de Oliveira et al., 2019; Ferreira et al., 2020; Othman et al., 2020)
16	269.080	$C_{16}H_{13}O_4$	8	8.26	Medicarpin	ND	ND	D	D	D	D	D	D	(Funakoshi-Tago et al., 2016)
17	271.0612	$C_{15}H_{11}O_5$	11	-10.01	naringenin	D	D	D	D	D	D	D	D	(Chong; Chua, 2020; dos Santos et al., 2017a; Ferreira et al., 2020; Silva et al., 2006)
18	277.21742	$C_{18}H_{29}O_2$	4	7.61	Linolenic acid	D	D	D	D	D	ND	ND	ND	(Lopes et al., 2019)

N°	m/z (exper)	Molecular Formulas [M-H]-	DBE	error (ppm)	Structural Formulas	MQ Rainy	MQ Dry	MM Rainy	MM Dry	MC Rainy	MC Dry	MB Rainy	MB Dry	Reference
19	279.23306	$C_{18}H_{31}O_2$	3	-9.27	Linoleic acid	D	D	D	D	ND	D	D	D	(Lopes et al., 2019; Isidorov et al, 2022)
20	281.24878	$C_{18}H_{33}O_2$	2	-0.62	Oleic acid	ND	ND	D	D	D	D	D	D	(Isidorov et al, 2022)
21	283.26442	$C_{18}H_{35}O_2$	1	-7.79	Stearic acid	D	D	D	D	ND	D	D	D	(Dutra et al., 2019)
22	285.2316	$C_{20}H_{29}O$	6	-9.92	Vitamin A / Retinol	D	D	ND	ND	ND	ND	D	ND	(Campos et al., 2015)
23	285.04051	$C_{15}H_9O_6$	11	-2.87	Kaempferol / Luteolin	ND	ND	D	D	D	D	D	ND	(Araújo et al., 2016; de Oliveira et al., 2019; Ferreira et al., 2020; Lopes et al., 2020, 2019; Othman et al., 2020)
24	287.05611	$C_{15}H_{11}O_6$	10	9.72	Plathymenin / Eriodictyol / Aromadendrin	D	D	D	D	D	D	D	D	(Funakoshi-Tago et al., 2016)
25	289.06978	$C_{15}H_{13}O_6$	9	9.35	Catechin / Epicatechin	D	D	D	D	D	D	D	D	(Araújo et al., 2016; dos Santos et al., 2017b; Ferreira et al., 2020)
26	299.05618	$C_{16}H_{11}O_6$	11	-5.71	Hispidulin	D	D	ND	ND	D	ND	ND	D	(Ferreira et al., 2020)
27	300.99926	$C_{14}H_5O_8$	12	-4.15	Ellagic acid	D	D	D	D	ND	D	D	ND	(Araújo et al., 2016; Dutra et al., 2019; Ferreira et al., 2020; Lopes et al., 2020, 2019)
28	301.03569	$C_{15}H_9O_7$	11	9.94	Quercetin / Tricetin	ND	ND	ND	ND	D	D	D	D	(de Souza et al., 2013; Belina-Aldemita et al., 2020; Bonamigo et al., 2017; dos Santos et al., 2017b; Ferreira et al., 2020)
29	301.07194	$C_{16}H_{13}O_6$	10	5.95	Isoferreirina / Alnustinol / Ferreirina / Hesperetin	D	ND	D	ND	D	ND	D	D	(Araújo et al., 2016)
30	303.05118	$C_{15}H_{11}O_7$	10	9.92	Taxifolin / Dihydroquercetin	D	D	ND	ND	D	ND	ND	ND	(Ferreira et al., 2020)
31	315.05126	$C_{16}H_{11}O_7$	11	7.73	Isorhamnetin / Selagin / Methoxyherbacetin	D	D	D	D	D	D	D	D	(Othman et al., 2020; Silva et al., 2006)
32	317.03032	$C_{15}H_9O_8$	11	-4.02	Myricetin	ND	ND	D	D	ND	ND	D	ND	(Ferreira et al., 2020)

N°	m/z (exper)	Molecular Formulas [M-H]-	DBE	error (ppm)	Structural Formulas	MQ Rainy	MQ Dry	MM Rainy	MM Dry	MC Rainy	MC Dry	MB Rainy	MB Dry	Reference
33	329.17604	$C_{20}H_{25}O_4$	8	9.94	carnosol	D	ND	D	ND	ND	D	D	ND	(Dembogurski, 2018; Ferreira et al., 2020)
34	331.06632	$C_{13}H_{15}O_{10}$	6	-7.49	monogalloylglucose	D	D	D	D	D	D	D	D	(Lopes et al., 2020)
35	353.08788	$C_{16}H_{17}O_9$	8	-8.00	Chlorogenic acid / caffeoylquinic acid	D	D	D	D	D	D	D	D	(Dembogurski, 2018; de Oliveira et al., 2019; Ferreira et al., 2020)
36	359.07581	$C_{18}H_{15}O_8$	11	9.47	Rosmarinic acid	D	D	ND	ND	D	D	ND	ND	(Ferreira et al., 2020)
37	405.17089	$C_{25}H_{25}O_5$	13	-0.35	Diprenilgenistein a	D	D	D	D	D	D	D	D	(de Souza, 2018)
38	407.20102	$C_{22}H_{31}O_{21}$	15	9.40	Galloyl pinocembrin	D	D	D	D	ND	ND	D	D	(Chong and Chua, 2020)
39	409.172943	$C_{24}H_{25}O_6$	11	-5.35	Mangostin	D	D	ND	ND	ND	D	ND	D	(Chong and Chua, 2020; Vongsak et al., 2015)
40	413.37882	$C_{29}H_{49}O$	5	-9.66	Sitosterol	D	D	D	D	D	D	D	ND	(Bonamigo et al., 2017; Silva et al., 2006)
41	421.16576	$C_{25}H_{25}O_6$	13	-0.75	Diprenilkaempferol / Cajanona	D	D	D	D	D	D	D	D	(de Souza, 2018)
42	421.084900	$C_{19}H_{17}O_{11}$	11	6.35	Mangiferin	D	D	D	D	D	D	D	D	(Chong and Chua, 2020)
43	423.07106	$C_{22}H_{15}O_9$	15	9.80	Galloyl naringenin	D	D	D	D	ND	D	D	ND	(Chong and Chua, 2020)
44	431.09859	$C_{21}H_{19}O_{10}$	12	6.91	apigenin-glucoside	D	D	D	D	D	D	D	ND	(Lopes et al., 2020)
45	439.0639	$C_{22}H_{15}O_{10}$	15	9.84	Galloyl eriodictyol	D	D	D	D	D	D	D	D	(Chong and Chua, 2020)
46	447.09331	$C_{21}H_{19}O_{11}$	12	-10.03	quercitrin	D	D	ND	ND	D	D	D	D	NR
47	463.08836	$C_{21}H_{19}O_{12}$	12	8.94	Isoquercetin	D	D	D	D	D	D	D	D	(Ferreira et al., 2020)
48	477.07226	$C_{21}H_{17}O_{13}$	13	0.66	digalloylshikimic acid	D	D	D	D	D	D	D	D	(Lopes et al., 2020)
49	501.30110	$C_{33}H_{41}O_4$	13	-0.14	Nemorosone / Propolone A	D	D	D	D	D	D	D	D	(de Souza, 2018)
50	515.11991	$C_{25}H_{23}O_{12}$	14	3.10	caffeoylquinic acid	ND	ND	ND	ND	D	ND	ND	ND	(Dembogurski, 2018)
51	579.13571	$C_{26}H_{27}O_{15}$	13	-5.74	Carlinoside	D	D	D	D	D	D	D	D	NR
52	579.16647	$C_{27}H_{31}O_{14}$	12	-4.33	Naringin	D	D	D	D	D	D	D	D	(de Oliveira et al., 2019; Ferreira et al., 2020)

N°	m/z (exper)	Molecular Formulas [M-H]-	DBE	error (ppm)	Structural Formulas	MQ Rainy	MQ Dry	MM Rainy	MM Dry	MC Rainy	MC Dry	MB Rainy	MB Dry	Reference
53	603.00535	$C_{28}H_{11}O_{16}$	23	0.78	ellagic acid dimer	D	D	D	D	ND	D	D	ND	(Lopes et al., 2019)
54	609.12554	$C_{30}H_{25}O_{14}$	18	8.75	prodelphinidin B4	ND	ND	ND	ND	D	D	ND	ND	NR
55	609.1478	$C_{27}H_{29}O_{16}$	13	-2.79	Rutin	D	D	D	D	D	D	D	D	(Araújo et al., 2016; Belina-Aldemita et al., 2020; de Oliveira et al., 2019; dos Santos et al., 2017b; Ferreira et al., 2020)
56	625.1412	$C_{27}H_{29}O_{17}$	13	-7.95	quercetin-diglucoside	D	D	D	D	D	D	D	D	(Belina-Aldemita et al., 2020; Lopes et al., 2020, 2019)

* M/Z (mass-to-charge ratio); DBE (Double Bound Equivalence); ppm (parts per million); ND (No Detected); D (Detected); NR (No referenced).

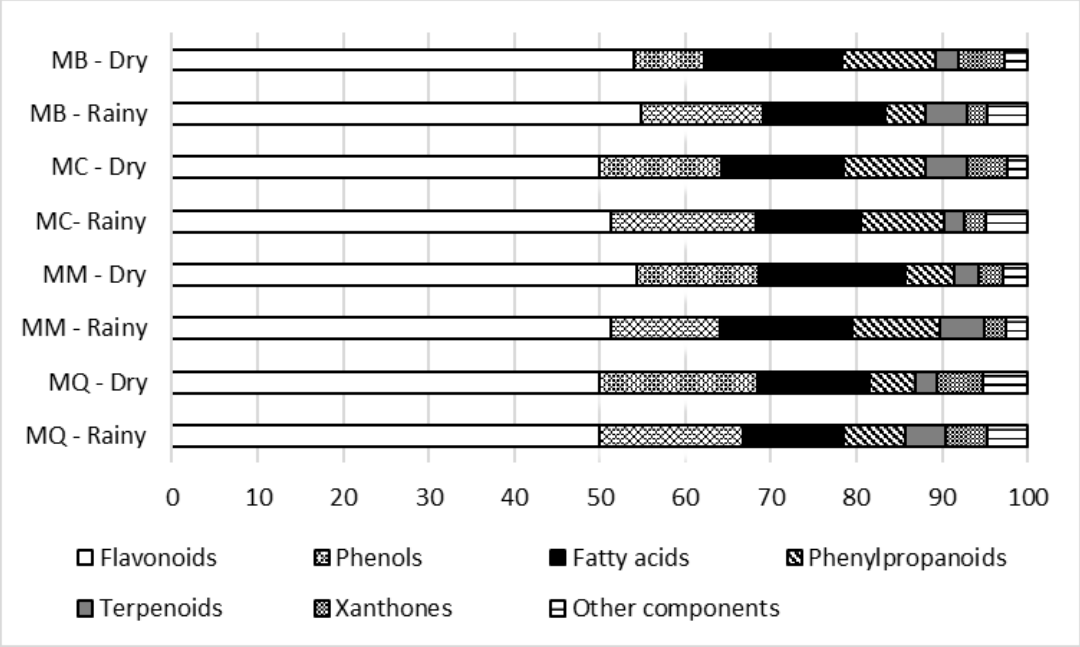

Figure 2S - Main groups of compounds proposed in the propolis extracts from *Melipona quadrifasciata* (MQ), *Melipona mondury* (MM), *Melipona capixaba* (MC), and *Melipona bicolor* (MB) stingless bee and seasonal seasons (Dry and Rainy).

Since our studies demonstrated that geopropolis from the samples contains compounds with antioxidant properties, we evaluated this property using ABTS and DPPH free radical scavenging chemical methods. Both tests observed the highest antioxidant activity with the *M. bicolor* sample. Geopropolis showed significant differences between the seasons in inhibiting DPPH and ABTS radicals (Figure 2).

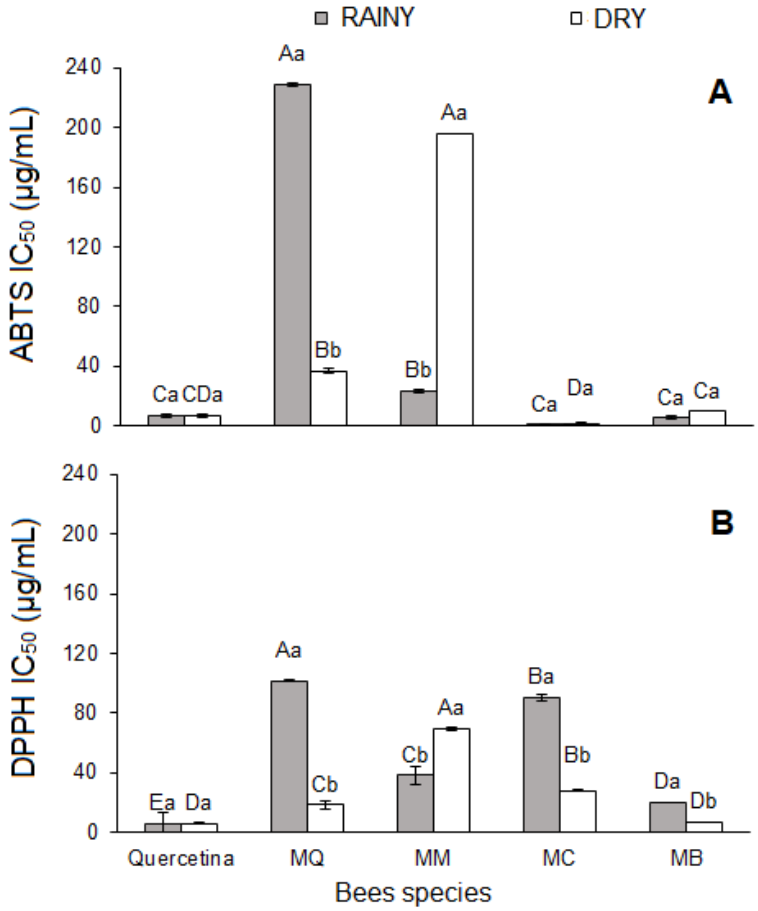

Figure 2 - Antioxidant activity of geopropolis extracts from *Melipona quadrifasciata* (MQ), *Melipona mondury* (MM), *Melipona capixaba* (MC), and *Melipona bicolor* (MB) stingless bee and seasonal seasons. Figures 2A and 2B determine the capacity to scavenge the organic radicals ABTS+ 2,2-azinobis (3-ethethyl benzothiazolesulfonate) and DPPH 2,2-diphenyl-1-picrylhydrazyl, respectively. Values are represented as mean ± standard deviation of three independent triplicates. Values within each column followed by a different superscript letter(s) are significantly different at $P < 0.05$. *Upper case letters compare bee species in the same seasons. *Lower case letters compare the same bee in different seasons.

M. bicolor extracts from rainy and dry seasons showed inhibitory activity against DPPH radical with IC_{50} of 20.03 ± 1.93 µg/mL and IC_{50} of 7.02 ± 0.21 µg/mL, respectively, and the property of geopropolis collected in the dry season was similar to that observed for standard positive quercetin.

Geopropolis extracts from the dry and rainy seasons of *M. bicolor* and *M. capixaba* also showed inhibitory activity against the ABTS radical similar to the standard positive quercetin, showing the antioxidant potential of geopropolis from these species, which had not yet been reported in the scientific literature (Figure 2A).

The effects of geopropolis extracts on cell viability were evaluated using the MTT colorimetric method in macrophage (RAW 264.7) and fibroblast (L929) strains. At concentrations from 0.1 to 20 µg/mL, no extract showed cytotoxicity for RAW 264.7 and L929 strains. Thus, subsequent anti-inflammatory activity experiments were also performed considering these concentrations.

The anti-inflammatory effect of geopropolis extracts was evaluated through the production of inflammation mediators, such as nitric oxide (NO) and superoxide (O2), and cytokine (TNF-α). The geopropolis extracts of the 4 species studied and at the 4 concentrations evaluated showed a significant reduction in the production of NO, produced by macrophages (RAW 267.2) stimulated by LPS, however, no dose-dependent effect was observed, as seen in Figure 3A.

In the intracellular analysis of the inhibition of superoxide anion production produced by macrophages (RAW 267.2) stimulated by LPS, after treatment with geopropolis extracts, it was observed that the samples had a reducing effect on the production of superoxide anion. The effect was dose-dependent when compared to the LPS control group. Significant reductions were observed at the 20 µg/mL concentration of the extracts, except for the MQ species. This reduction in superoxide anion production in the MC extract was similar to that observed for the Tempol positive control, which was not observed for the other extracts (Figure 3B).

After 24 h of LPS-stimulated macrophages, the release of TNF-α significantly increased in the cellular supernatant, indicating an inflammatory macrophage response. In contrast, the geopropolis extracts significantly reduces the production of TN F-α at the 20 µg/mL (Figure 3C)

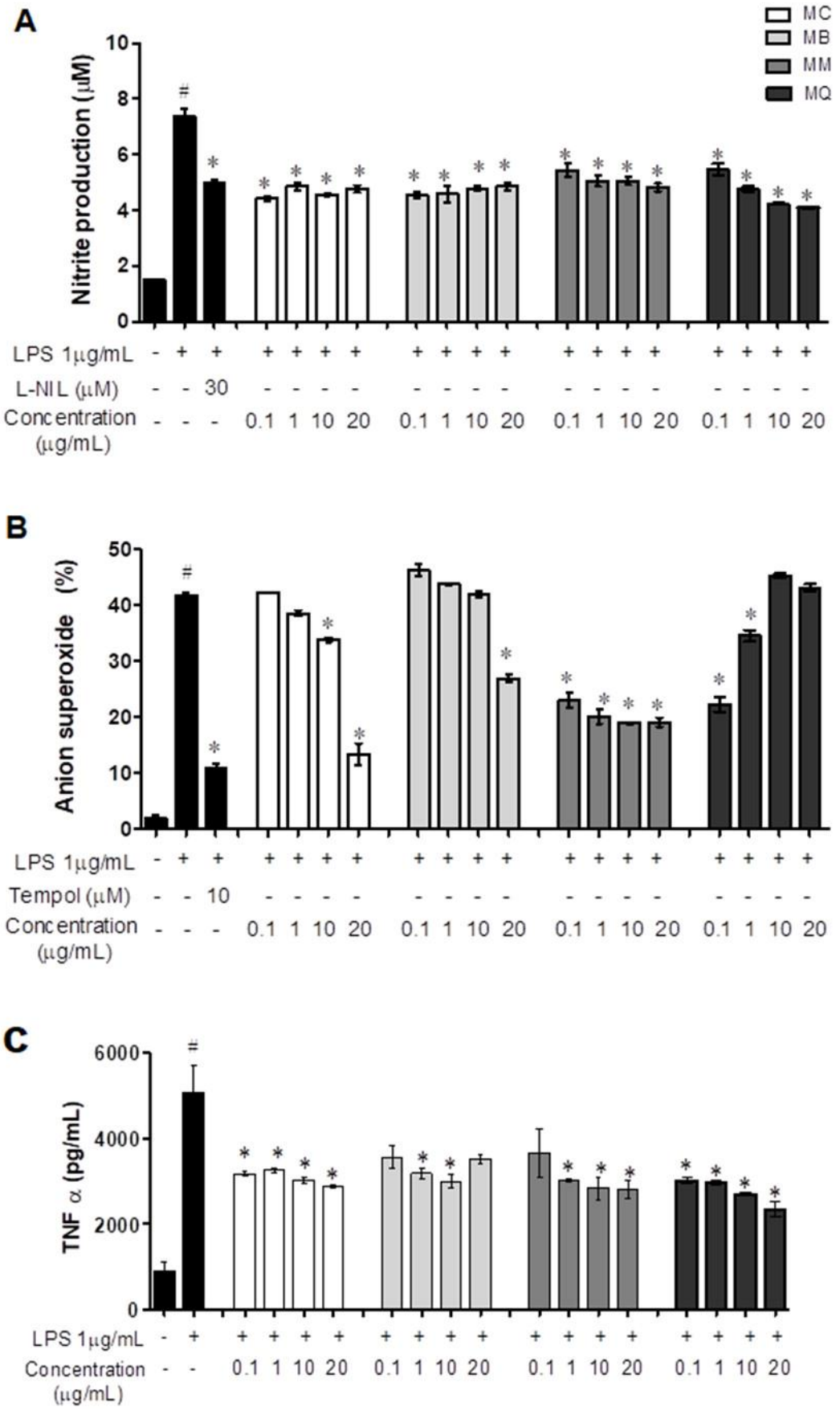

Figure 3 - Effect of *Melipona capixaba* (MC), *Melipona bicolor* (MB), *Melipona mondury* (MM), and *Melipona quadrifasciata* (MQ) geopropolis extracts on nitric oxide

(A) and anion superoxide (B) production *in vitro* in cultured LPS-stimulated macrophages, and effect of these geopropolis extracts on the concentration of pro-inflammatory cytokine TNF-α (C). RAW 264.7 macrophages were exposed to different geopropolis extract concentrations in the presence or absence of LPS. Results were expressed as mean ± SD. # Significant ($p < 0.05$) compared to the negative control. * Significant ($p < 0.05$) compared to the control + LPS group

In this study, the fungicidal and fungistatic activity of geopropolis (Table 1) using different species of fungi was verified. Up to the evaluated concentration (1024 µg/mL) the MQ and MB geopropolis extracts showed antifungal activity against *M. furfur* and *T. rubrum*, which was not observed for the other extracts. Specifically, dry season MQ geopropolis demonstrated fungicidal properties at a concentration of 256 µg/mL.

Table 1 - The activity geopropolis extracts from *Melipona quadrifasciata* (MQ), *Melipona mondury* (MM), *Melipona capixaba* (MC), and *Melipona bicolor* (MB) against fungal strains estimated by MIC – minimal inhibitory concentration, MFC – minimal fungicidal concentration

	Strain	*Malassezia furfur* CCT 1349		*Trichophyton rubrum* CCT 5506		*Candida albicans* ATCC 10231		*Candida albicans* ATCC 146		*Candida parapsilosis* ATCC 20019		*Aspergillus fumigatus* ATCC 16913	
		MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
MQ	dry	1024	>1024	256	256	>1024	>1024	>1024	>1024	>1024	>1024	1024	>1024
	rainy	>1024	>1024	512	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
MM	dry	>1024	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
	rainy	>1024	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
MC	dry	>1024	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
	rainy	>1024	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
MB	dry	512	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
	rainy	>1024	>1024	1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024	>1024
Fluconazole		2	>1024	>1024	16	0,5	4	0,5	4	2	4	128	>1024
Amphotericin B		-	-	0,06	0,06	0,06	0,06	0,25	0,25	0,25	0,25	-	-

(-) no tested. Results in µg/mL

4. DISCUSSION

Many factors can influence the extraction process, such as type and amount of solvent, size of the particles of the raw material, time, temperature, and extraction technique (Simões et al., 2017). Due to their efficiency in extracting polyphenols and flavonoids, ethanol and hydroethanolic mixtures are the most common solvents used to extract bioactive compounds from propolis (Martinello et al., 2021). Dos Santos et al. (2017b) obtained higher yields with hydroalcoholic extract of propolis from *M. quadrifasciata* and *T. angustula* bees (yields 24.4% w/w and 5.0% w/w, respectively) when compared to the aqueous extract of the same species (yields for *M. quadrifasciata* of 6.2% w/w and fo *angustula* 2.1% w/w) (dos Santos et al., 2017b). Using the same extraction procedure as this work, Dutra et al. (2014) reported an extractive yield of 8.80% for *M. fasciculata*. The ultrasound-assisted maceration with 70% ethanol for 30 min led to a significant reduction in extraction time compared to conventional methods. Moreover, the use of these extraction conditions has efficient (Cavalaro et al., 2019).

The chemical composition of stingless bee geopropolis can be influenced by the vegetation of the collection site and the species of stingless bees (Asem et al., 2020). In the different seasons of the year (including the dry and rainy seasons) the flowering period of different plant species occurs. We could observe that seasonality is one of the main factors that can interfere with the concentration profile of geopropolis phytochemicals, between species, as well as within the same species.

This variation in the concentration of compounds with bioactive potential found in geopropolis is due to plant biodiversity, and this factor must be considered in the standardization of this apicultural product. The regulatory agency in Brazil (IN/MAPA n° 3/2001) does not have specific requirements for stingless bee products. Its specification is based on data found for the species *Apis Mellifera* (sting bee) and requires minimum levels of 0.25% m/m (= 2.5 mg/g) and 0.50% m/m (= 5.0 mg/g) of flavonoids and phenolics, respectively. Considering that the results obtained in this study are above the values cited above, we suggest that the standard can be adopted as a reference for stingless bee geopropolis.

This is the first study to propose the chemical composition of the geopropolis of *M. mondury*, *M. capixaba*, *M. Bicolor* bees. Their compounds are potentially rich in antioxidant, anti-inflammatory, antimicrobial, and chemopreventive properties, which will be explained later (Lavinas et al., 2018; Popova et al, 2021; Sanches et al, 2017). However, other analytical techniques may be necessary for the structural elucidation

of these compounds. That is based on the configuration's lack of data, and the binding position of some functional groups cannot be distinguished by ESI(-)FT-ICR MS. We suggest using appropriate standards and high-performance methods (e.g., liquid chromatography-HPLC and nuclear magnetic resonance-NMR) as complementary techniques.

We suggest that these compounds may be naturally present in the resin collected by the bees, coming from the secondary metabolism of the plants. In addition, they may have been generated by the action of bees' salivary enzymes, which can modify the collected resin. Leonhardt et al. (2011) suggested that resins collected from the environment are modified within the hive and that each species may have different enzymes or associated microbial agents that alter the resin. In this context, our work showed that there were no significant modifications of the resins of different species relating to the groups of compounds, since similar percentages were observed among representatives of flavonoids (≅ 52 %), fatty acids (≅ 14 %), phenols (≅ 14 %), phenylpropanoids (≅ 8 %), terpenoids (≅ 4 %), xanthones (≅ 4 %) and other components (≅ 4 %) (Figure 2S).

According to Bravo (1998), among the most common and important low molecular weight, phenolic compounds found in plants are the simple phenolic derivatives and flavonoids. The simple phenols have an aromatic ring (C_6) and hydroxyl attached to their chemical structure with different methylation and hydroxylation of the aromatic ring (Bravo, 1998). In the flavonoids group were included the compounds consisting of the $C_6C_3C_6$ diphenylpropane structure with two aromatic rings linked through three carbons that usually form an oxygenated heterocycle and whose structure is conjugated with sugars (heterosides) or not (aglycone) (Simões et al., 2017). Phenylpropanoid derivatives (C_6 C_3) are also an important group of low molecular weight phenolics, the most important of which are the hydroxycinnamic acid derivatives (p-coumaric, caffeic, ferulic, sinapic acids) (Bravo, 1998). The chemical structures proposed in our study have been highlighted by other authors due to their anti-inflammatory, antioxidant, antimicrobial activities and the most frequently detected bioactive compounds were Kaempferol, quercetin, and rutin (Araújo et al., 2016; Belina-Aldemita et al., 2020, Bonamigo et al., 2017; Chong and Chua, 2020; Dembogurski, 2018, de Oliveira et al., 2019; de Souza et al., 2013, 2018; dos Santos et al., 2017a, 2017b; Ferreira et al., 2020; Funakoshi-Tago et al., 2016; Lopes et al., 2019, 2020; Othman et al., 2020; Silva et al., 2006).

Xanthones are plant phenolic compounds with a $C_6C_1C_6$ carbon skeletal structure, whose name originated from the Greek word "Xanthos," meaning yellow (Remali et al., 2022). Mangostin and Mangiferin proposed in this study, also detected by Chong and Chua 2020, who justified the finding due to the presence of an orchard near the *Heterotrigona itama* bee hive. These xanthones are known to possess antitumor, antioxidant, antidiabetic, antimicrobial, and anti-inflammatory properties (Aljunaid et al., 2020).

Sawaya et al (2009) observed that the sampling period of each species changes the antioxidant activity, which was increased (low ED50 results) in spring (Sawaya et al., 2009). With this, the authors concluded that the year's season affects propolis's composition and properties, corroborating with results obtained in our study.

The samples showed significant differences among the species for both DPPH and ABTS radical inhibition. The studies of Bonamigo and coworkers in 2017 also show that the antioxidant activity varies with the species of bees, as they have preferences for specific plants in the production of geopropolis (Bonamigo et al., 2017).

The negative effects on the production of nitric oxide, superoxide anions and inflammatory interleukin TNF-α indicate the modulation of the inflammatory signaling pathways, which contributes to the control of oxidative stress and consequent suppression of the inflammatory process. In this context, studies have shown that both NO, O_2 and TNF-α are involved in numerous pathophysiological processes and, therefore, are considered important therapeutic targets (Cinelli et al., 2019).

The stingless bee as a form of defense against invading agents and to preserve life in the hive, propolis has an important natural fungicidal function. The fungicidal and fungistatic activity observed in our study resembles that described by other authors. De Souza et al. (2018). verified the antifungal activity of geopropolis (250 µg/mL) from the stingless bee *Frieseomelitta longipes* against *Candida albicans* and *Candida tropicalis*. With higher concentrations (> 7 mg/mL and > 3 mg/mL) of geopropolis from the species *Tetragonisca fiebrigi* and *Melipona orbignyi*, Campos et al. (2015, 2014) observed antifungal properties against *Candida glabrata* and *Candida albicans*, respectively.

Zulhendri et al. (2021) reported that one of the actions of propolis concerning antimicrobial and parasitic properties is directly on the pathogen, inhibiting its enzymes, proteins and metabolites necessary for invasion in the host, replication of its genetic material or production of energy for its survival. The other action is related

to the host, as propolis helps maintain the host's cellular antioxidant status throughout the infection.

5. CONCLUSIONS

For the first time in the literature, stingless bee geopropolis samples from four Brazilian species exhibited high levels of flavonoids, phenolics, tannins, and additionally a proposed content of fatty acids, phenylpropanoids, terpenoids, xanthones, and other components that jointly showed excellent antioxidant activity, which can be attributed to the presence of such bioactive compounds. Notably, it was observed that seasonality affect the chemical composition of geopropolis and alter its antioxidant activity due to the synergistic effects of its compounds. Despite the evidence showing that geopropolis from *Melipona quadrifasciata, Melipona mondury, Melipona capixaba, Melipona bicolor* bees possess antioxidant, anti-inflammatory and antifungal potential, further studies on the structure-activity relationships, molecular mechanisms, and applications of geopropolis for the treatment and/or prevention of oxidative stress-related diseases are recommended.

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Conflict of interests

The authors declare no conflict of interest.

CRediT authorship contribution statement

Ariane Pinheiro Cruz Bergamini: Conceptualization, Formal analysis, Investigation, Data Curation, Writing – original draft. **Brendo Victor Siqueira de Almeida Bergamini, Flavia Vitorino de Araújo Porto, Iana Soares Pessoa, Mirilláiny Anacleto Virginio, Victor da Rocha Fonseca:** Investigation, Validation. **Victor Paulo Mesquita Aragão:** Formal analysis, Validation. **Wanderson Romão, Denise Coutinho Endringer, Rodrigo Scherer:** Methodology, Validation. **Marcio Fronza:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing,

Supervision, Project administration, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

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CAPÍTULO 2

Chemical profile, antioxidant, antifungal and cytotoxic activities of propolis from the stingless bee *Tetragona clavipes*

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ABSTRACT

Propolis, comprising a blend of resin and mud, is exclusively synthesized by stingless bees. Despite its popular recognition for its medicinal properties, a limited number of studies have evidenced its biological activities. In this context, the purpose of this study was to determine the chemical profile and the antioxidant, antifungal and cytotoxic activities of *Tetragona clavipes* propolis. The hydroalcoholic extract of propolis was prepared and its chemical profile determined by Fourier transform ion cyclotron resonance mass spectrometry combined with a direct infusion electrospray ionization. Total polyphenols and flavonoids content were determined by colorimetric methods. Antioxidant activity was determined by the capacity to scavenge the free radicals 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid). The antifungal activity was evaluated by determining the minimum inhibitory concentration and minimum fungicidal concentration against *Aspergillus fumigatus*, *Trichophyton rubrum*, *Candida albicans* and *Candida parapisilosis*. Cytotoxic activity was determined by the MTT method. The results obtained revealed high contents of total phenolics, a promising level of antioxidant and antifungical activities. *T. clavipes* propolis from the rainy season showed better antioxidant and antifungal activities and higher content of flavonoids, with less variability of chemical species. The extracts exhibited fungistatic activity for *C. albicans*, *C. parapisilosis* and *A. fumigatus*; and fungicide for *T. rubrum.* Additionally, sample collected during the rainy season demonstrated synergism with fluconazole and amphoterecin B for *T. rubrum* and additivity for *C. albicans*, *C. parapisilosis.* In summary, the results of *T. clavipes* propolis revealed high levels of total phenolics, indicating its potential as a rich source of bioactive compounds, significant antioxidant activity and a promising antifungal activity against common pathogenic fungi. These findings contribute to our understanding of the therapeutic potential of *T. clavipes* propolis and provide a basis for further research and development of natural products for various applications in medicine and healthcare.

***Palavras-chave**:*

Stingless bee, antifungal, antioxidant, propolis, Tetragona clavipes

1. INTRODUCTION

The global dissemination of a disease as we witnessed with COVID-19, as well as the evidence of pathogens resistant to conventional treatments, point to the need to find new pharmaceutical compounds and complementary therapies to the ailments that devastate humanity. As an example, we have the rise of fungal infections, that have become a global health issue due to the increase in the immunocompromised population (be it because of acquired immunodeficiency syndrome (AIDS), chemotherapy in cancer patients, receiving an organ transplant, serious illnesses, or post-operative procedures), as well as the pathogens' resistance to even the most modern antifungal medications such as Fluconazole (Friedman and Schwartz, 2019; Ozarowski and Karpinski, 2023; Sai and Azim, 2016).

Despite the available evidence, these infections continue to be neglected, resulting in a high mortality rate and causing the demise of approximately 1.6 million individuals annually. Notably, representatives of the *Candida* spp and *Aspergillus* spp genera demonstrate mortality rates exceeding 60% among the affected population (Bassetti et al., 2018; Bongomin et al., 2017; Janbon et al., 2019). The spectrum of these infections ranges from dermatophytosis, which typically presents as asymptomatic, to invasive and systemic infections that carry the potential for fatality. Dermatophytosis is confined to the outermost layer of the body, colonizing keratinized tissues. The predominant fungal species associated with dermatophytosis include representatives of the dermatophyte fungi, among which *Trichophyton rubrum* holds prominence. Meanwhile, systemic infections disseminate throughout the host's organism, being able to manifest itself in solid organs and/or bloodstreams, especially in immunosuppressed patients (Kim, 2016). *Candida albicans* and *Aspergillus fumigatus* are among the most virulent of this subgroup (Murray, 2017).

The therapeutic options for fungal infections are inherently limited due to the evolutionary similarities between fungi, being eukaryotic organisms, and their human hosts. Consequently, medications such as amphotericin B, although frequently employed in the treatment of certain ailments, are associated with numerous adverse effects including nephrotoxicity, hepatotoxicity, cardiotoxicity, phlebitis, as well as fever and chills (Zaitz, 2010). Consequently, the existing repertoire of antifungal medications can be categorized into merely four classes (polyenic, azoles, echinocandins, and allylamines), exhibiting a limited spectrum of selectivity. Furthermore, the emergence of antifungal resistance further exacerbates this situation, posing significant challenges in the realm of clinical management (Arastehfar et al., 2020; Martinez-Rossi, 2018).

Natural products and their derived compounds serve as a widely employed alternative for therapeutic and prophylactic purposes in the management of fungal infections. The abundant resources offered by nature possess remarkable efficacy rates in treating various diseases caused by fungal pathogens (Aldholmi et al., 2019; Broda, 2020; Scorzoni et al. 2016). Bee products, including the propolis produced by stingless bees, encompass a wide array of chemical compounds that play a pivotal role in determining various biological activities. These compounds contribute to the diverse and multifaceted nature of bee products, augmenting their potential for numerous biological functions (Kurek-Gorecka et al., 2020).

In March 2018, the Brazilian Health Ministry included apitherapy as a new integrative practice in the Unified Health System (Sistema Único de Saúde – SUS) (BRASIL, 2018). This practice uses bee products such as apitoxin, royal jelly, pollen, propolis, honey and others. The integration of this practice normally including the topical and oral uses of propolis from *Apis mellifera*. The choice of this stinging species is due to its standardization and regulation based on scientific studies.

On the other hand, stingless bee propolis has drawn attention as a potential source of antimicrobial agents due to its role in maintaining hive asepsis (Simone-Finstrom and Spivak, 2010). However, despite its promising attributes, scientific research concerning stingless bee propolis from *Tetragona clavipes* remains limited in scope and depth. Further investigations are warranted to unravel the full potential of this unique natural product. In this context, the purpose of this study was to evaluate the chemical profile, antioxidant, antifungal and cytotoxic activities of *Tetragona clavipes* propolis.

2. MATERIAL AND METHODS

2.1 Sample collection

Samples of propolis from the *Tetragona clavipes* stingless bee (Fabricius, 1804) was collected in Santa Maria Jetibá (20º05'52"S, 40º36'56"W), Espírito Santo, Brazil, in the dry season of 2019 (September) and in the rainy season of 2020 (February). Propolis samples were taken directly from the hives by spatulation, packaged, correctly identified in plastic bags, and stored at -20°C protected from light.

2.2 Preparation of the hydroalcoholic extract of propolis

Samples of propolis were subjected to mechanical disintegration in a ball mill and after then submitted to extraction procedure by ultrasound-assisted maceration using 70% ethanol (Dutra et al., 2014). Subsequently, the extract was filtered, concentrated in a rotary evaporator, lyophilized to obtain the dry extract, and stored at -20°C protected from light until analysis.

2.3 Determination of flavonoids and total phenolics

The total flavonoid content in the propolis extracts was determined using the spectrophotometric method, as described by Asem et al. (2020). The reaction with aluminum chloride (10% w/v) was employed, followed by quantification through the construction of a standard curve using quercetin (100 - 700 µg/mL) as the reference compound. Absorbances were measured at 415 nm using a Molecular Devices Spectra MAX 190 spectrophotometer. The total flavonoid content of the propolis extracts was expressed as milligrams of quercetin equivalent (QE) per gram of dry extract. The quantification of total phenolics was conducted using the Folin-Ciocalteu method, as described by Krepsky et al. (2012). To determine the phenolic contents, an analytical curve was prepared using gallic acid (10 - 50 µg/mL) as the reference compound. Absorbances were measured at 715 nm (Molecular Devices Spectra MAX 190). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry extract. The experiments were performed in triplicate on different days.

2.4 Mass Spectrometry (ESI-FT-ICR MS)

Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) combined with a direct infusion electrospray ionization (ESI) provided a detailed molecular view of propolis extracts. The samples were dissolved in 1 mL of methanol and analyzed by high-resolution mass spectrometry (HRMS). The mass spectrometer

was the 9.4 T Solarix (Bruker Daltonics, Bremen, Germany). The analyzes were performed in the electrospray source and the negative ion mode, ESI(-), over a mass range of m/z 150–1500. The ESI source conditions were as follows: a nebulizer gas pressure of 1.5 bar, a capillary voltage of 4.0 – 4.4 kV, and a capillary transfer temperature of 200 C°. Ion time accumulation was 0.005 – 0.030 s. The time of flight was 0.950 sec. ESI(-)FT-ICR mass spectra were acquired by accumulating 16 scans of time-domain transient signals in 4 mega-point time-domain data sets. All mass spectra were externally calibrated using Arginine (m/z from 150 to 1500). A mass accuracy of <10 ppm provided the unambiguous molecular formula assignments for singly charged molecular ions. Mass spectra were processed using Data Analysis software (Bruker Dantonics, Bremen, Germany). Elemental compositions of the compounds were determined by measuring the mass-to-charge ratio (m/z), considering the error and double bond equivalents (DBE) values (Ribeiro et al., 2021). The structural formulas of the compounds were acquired through the utilization of ChemSpider software. Subsequently, these structural formulas were subjected to a comparative analysis with compounds that have been extensively documented in scientific literature concerning bee products.

2.5 Antioxidant activity

The antioxidant activity of the propolis extracts was determined using radical scavenging methods, specifically, $ABTS^{+}$ (2,2-azinobis (3-ethyl benzo ethybenzothiazole)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) (Re et al., 1999). The results were expressed as IC_{50} (µg/mL) values and compared to the reference compound, quercetin. The experiments were conducted in triplicate on different days.

2.6 Antifungal activity

The study employed reference strains to ensure consistency and standardization in the experimental procedures. The fungi strains used were *Aspergillus fumigatus* ATCC 16913, *Trichophyton rubrum* CBS 392.58, *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90029 e *Candida parapisilosis* ATCC 22019. The determination of minimum inhibitory concentration (MIC) was conducted through sensitivity tests using the broth microdilution method in 96-well microplates. The susceptibility testing procedures was performed according to the established standards outlined in reference documents M27-A3 and M38-A (CLSI 2008, 2010) published by the Institute for Clinical Laboratory Standards. The antifungal solutions

were prepared in dimethylsulfoxide (DMSO) and subsequently diluted in RPMI-1640 (Roswell Park Memorial Institute) buffered with MOPS (3-Nmorpholine-propane sulfonic acid) without glutamine, to obtain final concentrations in the range of 8 to 1024 µg/mL for the extracts and from 0.03 to 16 µg/mL for the conventional antifungals Fluconazole and Amphotericin B. The minimum inhibitory concentration was obtained through the concentration where no visual growth was observed after 24h for *Candida strains*, 48h for strains of *Aspergillus fumigatus*, and 96h for strains of *Trychophyton rubrum*. A strain of *Aspergillus flavus* ATCC 204304 was used as a reference to guarantee the quality of the test against drug dilution.

The determination of the minimal fungicidal concentration (MFC) was conducted by adapting the protocol outlined by Espinel-Ingroff et al. (2002). The MFC test was performed by transferring 20 µL of the microdilution wells in broth, which corresponded to the MIC, 2x MIC and 4x MIC values, into the culture plate containing saubourad agar (ASD). Subsequently, the inoculated plates were incubated at 35ºC for 24 h in the case of *Candida*, 48 h for *Aspergillus*, and 96 h for *T. rubrum*. The MFC was defined as the lowest concentration that did not show any visual growth after incubation (Colony Forming Units), where it is considered that approximately 99% to 99.5% of the fungal pathogens tested died (Van et al., 2018).

2.7 Time-kill curve

The assay was performed using the adaptations of Ghannoum et al. 2013. to the method described by Klepser et al.1998. The suspensions of the test microorganisms (*Trychophyton rubrum*) were adjusted according to the CLSI 2008 protocol, to obtain suspensions of test organism of 1-3 x 103 CFU/mL in RPMI broth together with the propolis extract concentrations of 0.25x, 0.5x, 1x, 2x, 4x and 8x the MIC for each isolate. A positive control without the presence of drug was prepared. At predetermined times (0, 6, 12, 24, 36 and 48h), a 100 µL sample was removed from the test concentrations and diluted in 900 µL of saline solution. Then, a 30 µL aliquot of each dilution was seeded onto ABD plates. Tests were performed in duplicates. Colony counts were determined after 4 days (96 h) of incubation at 30°C. A time-kill curve was plotted for the log10 number of conidia/mL versus time. MIC, MIC ÷ 2, MIC ÷ 4 and MIC ÷ 8.

2.8 Synergism test

The synergism test was verified from the combination of two substances (propolis extratct and antifungical fluconazole or anfotericin B) was determined using the checkerboard technique to derive the fractional inhibitory concentration (FIC) index. A serial dilution was used. Then, 50 µL of each test substance was added horizontally (Containing the inhibitory and sub-innotory concentrations of the antifungal corresponding to the MIC to MIC ÷ 256 range) and vertically (Containing the inhibitory and sub-innotory concentrations of the extract corresponding to the range from MIC to MIC ÷ 64). Finally, 100µL of a solution containing fungal inoculum was added (104 CFU/mL) according to the standardization established by CLSI. Growth and sterility controls were also used. The assay was performed in duplicate and the microplates were incubated for 24h for *Candida* and 96h for *T. rubrum* under incubation at 35°C. The results were interpreted visually in a reading mirror, the dye risasurine was added to the plate to facilitate visual reading.

The FIC index was calculated as the sum of FICA + FICB, where A is propolis extrct and B is antifungical. FICA, in turn, is calculated using the MICA combined/MICA alone ratio, while FICB = the MICB combined/MICB alone ratio. This index was interpreted as follows: synergism (< 0.5), additivity (0.5 - 1.0) and antagonism (> 1.0) (Barchiesi et al., 1997; De Castro et al., 2015).

2.9 Cytotoxic activity

Cytotoxic activity was assessed using the colorimetric MTT test (Mosmann, 1983). Briefly, fibroblast (L929) and a human melanoma cell line (MV-3) were seeded in 96-well plates at 8 x 10^4 cells/well. After overnight, cells were treated with different propolis extract concentrations (0.1 - 150 µg/mL) for 24 h at 37 ºC in 5% CO_2. Then, the MTT solution was added to each well and the formazan crystals were dissolved with dimethyl sulfoxide (DMSO). The optical density of each well was measured at 595 nm wavelength by the microplate reader (Multi-Mode Microplate Reader, Filter Max F5, Molecular Devices, USA). The results were reported as IC_{50} (µg/mL).

2.10 Statistical analysis

The data are show as the mean ± standard deviation (SD) and were analyzed for statistically significant differences between the groups, using Student´s *t*-test and analysis of variance (ANOVA) followed by Tukey's test using the Prism 5 GraphPad Software. The results were considered significant when $p < 0.05$.

3. RESULTS

Significant variations were detected in both the quantitative and qualitative outcomes of the physical-chemical analyses. The propolis samples obtained during the rainy season exhibited a notably elevated concentration of phenolic compounds (76.68 ± 1.74 mg GAE/g of propolis) in contrast to the dry season samples, which recorded a lower content (26.11 ± 1.04 mg GAE/g of propolis) (Table 1S). Conversely, the levels of flavonoids remained comparable between the dry and rainy seasons, with values of 22.82 ± 4.33 mg QE/g of propolis and 23.97 ± 1.04 mg QE/g of propolis, respectively.

Table 1S - Total phenolic and flavonoids contents of *Tetragona clavipes* propolis extract.

Propolis	Total phenolic (mg GAE/g of propolis)	Total flavonoids (mg QE/g of propolis)
T. clavipes (dry)	26.11 ± 1.04[b]	22.82 ± 4.33[a]
T. clavipes (rainy)	76.68 ± 1.74[a]	23.97 ± 7.38[a]

*Significant differences ($p < 0.05$) between groups were identified by Student´s t-test, as indicated by different letters assigned to each group within the same column. The experiments were performed in triplicate (n = 3) and the results are presented as mean ± standard deviation (SD). GAE – Gallic acid equivalent. QE – Quercetin equivalent.

The utilization of high-resolution and accurate ESI(-) FT-ICR MS allowed for the identification of 28 compounds, among which approximately 82% were detected in the propolis samples collected during the dry season, while only 64% were found in the propolis samples from the rainy season. Consequently, the rainy season propolis samples exhibited a reduced diversity in terms of the proposed chemical species identified through ESI(-) FT-ICR MS (Table 2S).

Table 2S - Chemistries species proposed for ESI(-)FT-ICR MS from propolis extracts from *Tetragona clavipes* stingless bee (dry and rainy seasons).

N°	m/z (exper)	Molecular Formulas [M-H]-	DBE	error (ppm)	Structural Formulas	*T. clavipes* (dry)	*T. clavipes* (rainy)	Ref.
1	223.05994	$C_{11}H_{11}O_5$	6	-5.50	Sinapic acid	D	ND	(FERREIRA et al., 2020)
2	247.09771	$C_{14}H_{15}O_4$	7	-0.04	Prenyl caffeate / Prenylated caffeic acid	D	ND	(DEMBOGURSKI, 2018)
3	253.04847	$C_{15}H_9O_4$	11	5.30	Chrysin	D	ND	(FUNAKOSHI-TAGO et al., 2016)

N°	m/z (exper)	Molecular Formulas [M-H]-	DBE	error (ppm)	Structural Formulas	*T. clavipes* (dry)	*T. clavipes* (rainy)	Ref.
4	263.1280	$C_{15}H_{19}O_4$	4	-0.53	Abscisic acid	D	ND	(DE OLIVEIRA et al., 2019)
5	269.0455	$C_{15}H_9O_5$	11	-4.37	Apigenin / Galangin	D	D	(BONAMIGO et al., 2017b; DE OLIVEIRA et al., 2019; FERREIRA et al., 2020; OTHMAN et al., 2020)
6	285.2316	$C_{20}H_{29}O$	6	2.28	Vitamin A / Retinol	D	ND	(CAMPOS et al., 2015b)
7	285.04051	$C_{15}H_9O_6$	11	-0.53	Kaempferol / Luteolin	ND	D	(ARAÚJO et al., 2016; DE OLIVEIRA et al., 2019; FERREIRA et al., 2020; LOPES et al., 2019, 2020; OTHMAN et al., 2020)
8	299.05618	$C_{16}H_{11}O_6$	11	-5.81	Hispidulin	ND	D	(FERREIRA et al., 2020)
9	300.99926	$C_{14}H_5O_8$	12	9.45	Ellagic acid	D	ND	(ARAÚJO et al., 2016; DUTRA et al., 2019; FERREIRA et al., 2020; LOPES et al., 2019, 2020)
10	303.05118	$C_{15}H_{11}O_7$	10	6.66	Taxifolin / Dihydroquercetin	D	ND	(FERREIRA et al., 2020)
11	315.05126	$C_{16}H_{11}O_7$	11	6.44	Isorhamnetin / Selagin / Methoxyherbacetin	D	ND	(OTHMAN et al., 2020; SILVA et al., 2006)
12	329.17604	$C_{20}H_{25}O_4$	8	5.07	Carnosol	D	D	(DEMBOGURSKI, 2018) (FERREIRA et al., 2020)
13	331.06632	$C_{13}H_{15}O_{10}$	6	4.80	Monogalloylglucose	ND	D	(LOPES et al., 2020)
14	405.17089	$C_{25}H_{25}O_5$	13	0.07	Diprenilgenisteina	D	D	(DE SOUZA, 2014)
15	409.172943	$C_{24}H_{25}O_6$	11	-0.35	Mangostin	D	ND	(CHONG and CHUA, 2020; VONGSAK et al., 2015)
16	413.37882	$C_{29}H_{49}O$	5	8.90	Sitosterol	ND	D	(BONAMIGO et al., 2017; SILVA et al., 2006)
17	421.16576	$C_{25}H_{25}O_6$	13	-0.45	Diprenilkaempferol / Cajanona	D	D	(DE SOUZA, 2014)
18	421.084900	$C_{19}H_{17}O_{11}$	11	3.24	Mangiferin	D	D	(CHONG and CHUA, 2020)
19	423.07106	$C_{22}H_{15}O_9$	15	5.64	Galloyl naringenin	ND	D	(CHONG and CHUA, 2020)
20	439.0639	$C_{22}H_{15}O_{10}$	15	-9.75	Galloyl eriodictyol	D	D	(CHONG and CHUA, 2020)
21	447.09331	$C_{21}H_{19}O_{11}$	12	6.36	Quercitrin	D	D	NR
22	463.08836	$C_{21}H_{19}O_{12}$	12	-7.93	Isoquercetin	D	D	(FERREIRA et al., 2020)
23	477.07226	$C_{21}H_{17}O_{13}$	13	-4.85	Digalloylshikimic acid	D	D	(LOPES et al., 2020)
24	501.30110	$C_{33}H_{41}O_4$	13	-4.20	Nemorosone / Propolone A	D	D	(DE SOUZA, 2014)
25	579.13571	$C_{26}H_{27}O_{15}$	13	-6.78	Carlinoside	D	D	NR
26	579.16647	$C_{27}H_{31}O_{14}$	12	8.82	Naringin	D	ND	(DE OLIVEIRA et al., 2019; FERREIRA et al., 2020)
27	609.1478	$C_{27}H_{29}O_{16}$	13	6.94	Rutin	D	D	(ARAÚJO et al., 2016; BELINA-ALDEMITA et al., 2020; DE OLIVEIRA et al., 2019; DOS SANTOS et al., 2017b; FERREIRA et al., 2020)
28	625.1412	$C_{27}H_{29}O_{17}$	13	0.55	Quercetin-diglucoside	D	D	(BELINA-ALDEMITA et al., 2020; LOPES et al., 2019, 2020)

* M/Z (mass-to-charge ratio); DBE (Double Bound Equivalence); ppm (parts per million); ND (No Detected); D (Detected); NR (No referenced).

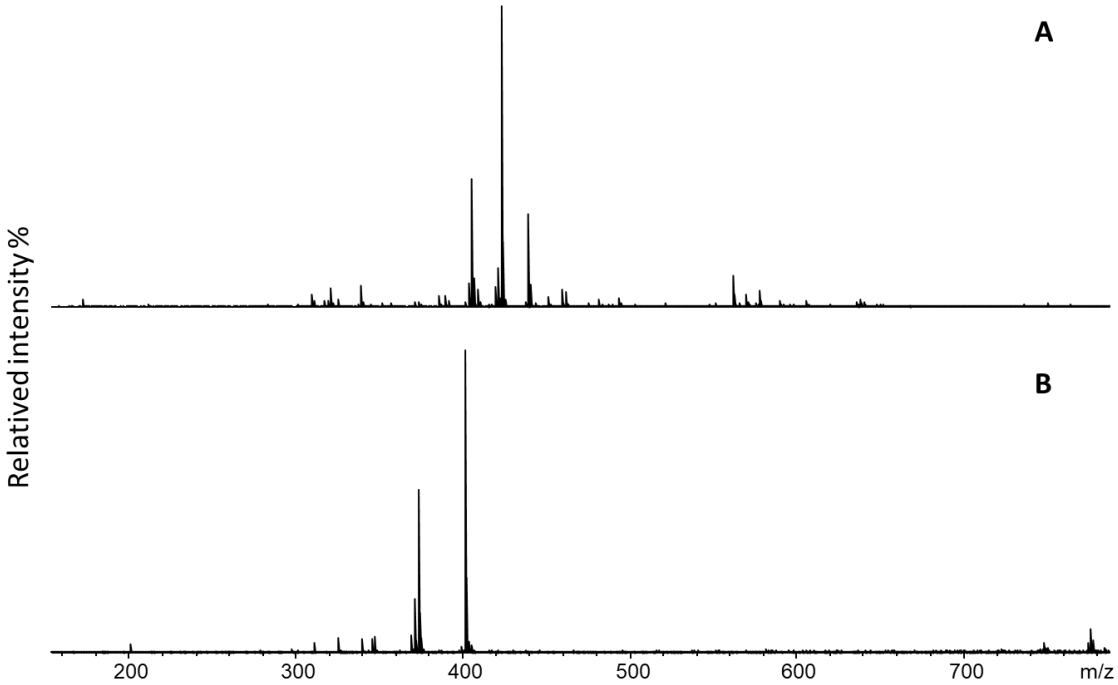

Figure 1S - ESI(-) FT-ICR MS spectral profiles of propolis extracts from *T. clavipes* stingless bee of the dry (A) and rainy (B) seasons.

Upon evaluating the spectral profiles and, the proposed compounds for each extract, it was observed that a significant proportion of the compounds were identified as flavonoids. Specifically, among the dry season samples, approximately 57% of the proposed compounds were categorized as flavonoids, while in the rainy season samples, this percentage increased to 67% (Figure 1S and Table 2S). The flavonoids proposed in this study are compound 3 $[C_{15}H_9O_4]^-$ ion at m/z 253 identified as Chrysin, compound 5 $[C_{15}H_9O_5]^-$ ion at m/z 269 identified as Apigenin/Galangin, compound 7 $[C_{15}H_9O_6]^-$ ion at m/z 285 identified as Kaempferol /Luteolin, compound 8 $[C_{16}H_{11}O_6]^-$ ion at m/z 299 identified as Hispidulin, compound 10 $[C_{15}H_{11}O_7]^-$ ion at m/z 303 identified as Taxifolin/Dihydroquercetin, compound 11 $[C_{16}H_{11}O_7]^-$ io at m/z 315 identified as Isorhamnetin/Selagin/Methoxyherbacetin, compound 14 $[C_{25}H_{25}O_5]^-$ ion at m/z 405 identified as Diprenylgenistein, compound 17 $[C_{25}H_{25}O_6]^-$ ion at m/z 421 identified as Diprenylkaempferol / Cajanone, compound 19 $[C_{22}H_{15}O_9]^-$ ion at m/z 423 Identified as Galloyl naringenin, composed 20 $[C_{22}H_{15}O_{10}]^-$ ion at m/z 439 identified as Galloyl Eriodictyol, composed 21 $[C_{21}H_{19}O_{11}]^-$ ion at m/z 447 identified as quercyitrin, compound 22 $[C_{21}H_{19}O_{12}]^-$ ion at m/z 463 identified as Isoquercetin, compound 25 ion $[C_{26}H_{27}O_{15}]^-$ ion at m/z 579 identified as Carlinoside, compound 26 $[C_{27}H_{31}O_{14}]^-$ ion at m/z 579 identified as Naringin, compound 27 $[C_{27}H_{29}O_{16}]^-$ ion at m/z 609 identified as Rutin and compound 28 $[C_{27}H_{29}O_{17}]^-$ ion at m/z 625 identified as Quercetin-

Diglucoside. All of these compounds have been previously identified by other studies (Funakoshi-tago et al., 2016; Araújo et al., 2016; Belina-Aldemita et al., 2020; Bonamigo et al., 2017; Chong and Chua, 2020; de Oliveira et al., 2019; de Souza et al., 2013; dos Santos et al., 2017b; Ferreira et al., 2020; Lopes et al., 2019, 2020; Othman et al., 2020; Silva et al., 2006).

The other compounds proposed by ESI(-) FT-ICR MS are non-flavonoid phenols such as fatty acids, phenylpropanoids and xanthones (Mangostin - compound 15 $[C_{24}H_{25}O_6]^-$ ion at m/z 409 and Mangiferin - compound 18 $[C_{19}H_{17}O_{11}]^-$ ion at m/z 421) and other non-phenolics such as terpenoids (compound 12 $[C_{20}H_{25}O_4]^-$ ion at m/z 329) (Table 2S).

The antioxidant activity of propolis, assessed by its ability to scavenge free radicals, was determined based on the concentrations needed to inhibit 50% of the radicals. The results demonstrated that the extract obtained during the rainy season (Table 1) exhibited lower values, indicating higher antioxidant potential. Moreover, the data obtained for the rainy season extract closely resembled the positive pattern observed with quercetin, further confirming the enhanced antioxidant activity of propolis collected during the rainy season.

Table 1 - Antioxidant activity of *Tetragona clavipes* propolis extract.

	Antioxidant activity (IC_{50} µg/mL)*	
	DPPH	**ABTS**
T. clavipes (dry)	143.03 ± 4.35^c	92.18 ± 6.05^c
T. clavipes (rainy)	15.88 ± 1.69^b	26.84 ± 1.75^b
Quercetin	5.89 ± 2.08^a	6.90 ± 1.16^a

*Significant differences ($p < 0.05$) between groups were identified by conducting a one-way ANOVA followed by Tukey's post hoc test, as indicated by different letters assigned to each group within the same column. The experiments were performed in triplicate (n = 3) and the results are presented as mean ± standard deviation (SD).

The fungicidal activity of the extracts was observed against *T. rubrum*, while fungistatic activity was noted against the other fungal species tested (Table 2). Geopropolis from the dry and rainy seasons exhibited fungicidal properties at concentrations of 256 µg/mL and 128 µg/mL, respectively. Notably, the propolis from the rainy season displayed remarkable inhibitory effects at lower concentrations, with a minimum inhibitory concentration (MIC) of 32 µg/mL for *C. albicans* and MIC of 8 µg/mL for *C. parapsilosis*. Consequently, further investigation into the rainy season

extract was conducted to examine the time of death and potential synergistic effects, thereby better understanding its antifungal potential.

Table 2 - The activity of propolis extracts against fungal strains estimated by the minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC).

Strain	***T. clavipes* (dry) (µg/mL)**		***T. clavipes* (rainy) (µg/mL)**		**Fluconazole (µg/mL)**		**Amphotericin B (µg/mL)**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
T. rubrum CCT 5506	256	256	128	128	4	16	0.06	0.06
C. albicans ATCC 10231	64	>1024	32	256	0.5	4	0.06	0.06
C. albicans ATCC 146	>1024	>1024	64	>1024	0.5	4	0.25	0.25
C. parapsilosis ATCC 22019	>1024	>1024	8	>1024	2	4	0.25	0.25
A. fumigatus ATCC 16913	1024	>1024	>1024	>1024	128	>128	0.125	0.125

The kill curves depicted in Figure 1 illustrated the time and concentration-dependent killing of *T. rubrum* by both propolis extract and fluconazole. The results indicated that propolis extract exhibited a more effective kill rate compared to fluconazole. Specifically, the propolis extract from the rainy season demonstrated a dose and time-dependent antifungal effect, achieving a killing effect on *T. rubrum* strains at a concentration of 2x MIC within 48 h. In contrast, fluconazole required a concentration of 4x MIC to achieve a similar killing effect.

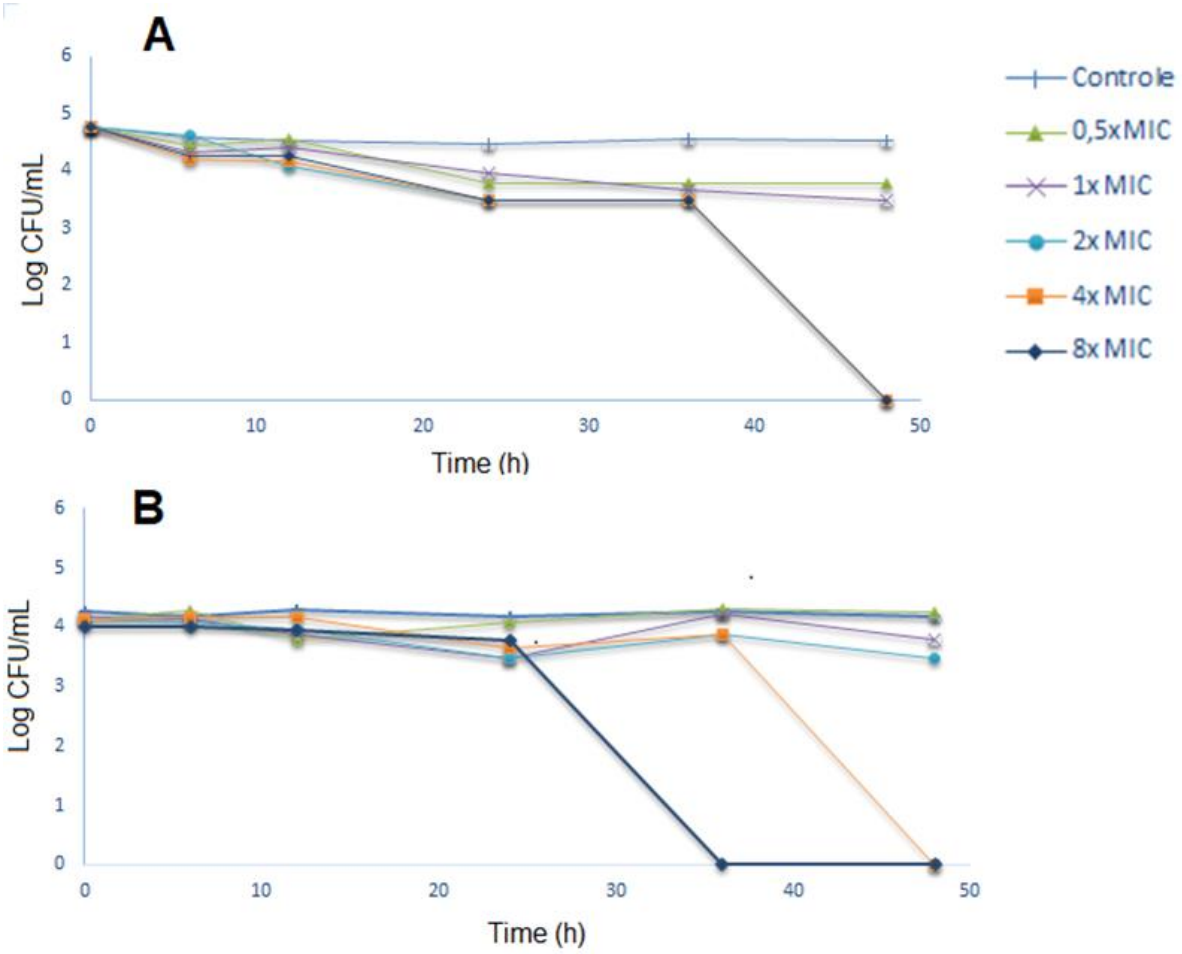

Figure 1 - Time Kill curve of propolis extracts of the rainy season (A) and fluconazole (B) against *Trichophyton rubrum.*

Table 3 presents the results demonstrating the synergistic effects of propolis extract when combined with fluconazole or amphotericin B against *T. rubrum*. The combination of propolis extract with amphotericin B resulted in a reduction of MIC values against *C. albicans*, indicating a synergistic effect with an FIC (Fractional Inhibitory Concentration) index value of 0.31. Additionally, the antifungal effects of propolis extract in combination with fluconazole or amphotericin B against *C. parapsilosis* were found to be additive. It is important to note that no antagonistic effects were observed in any of the combinations tested in Table 3.

Table 3 - The minimum inhibitory concentration (MIC) values (in µg/mL) and fractional inhibitory concentration index (FIC) values exhibited by propolis extract when combined with fluconazole and amphotericin B against specific strains using the synergy data method.

	Propolis extract / Fluconazole Combination				**Propolis extract / Amphotericin B Combination**			
Strain	FICA	FICB	FIC	Interp	FICA	FICB	FIC	Interp
C. albicans ATCC 10231	0.031	1	1.03	Add	0.06	0.25	0.31	Syn
C. albicans ATCC 146	0.015	0.5	0.51	Add	0.06	0.25	0.31	Syn
C. parapsilosis ATCC 22019	0.5	0.25	0.75	Add	0.25	0.5	0.75	Add
T. rubrum CCT 5506	0.007	0.25	0.26	Syn	0.25	0.25	0.5	Syn

Note: MIC = Minimum inhibitory concentration, FIC = Fractional inhibitory concentration index. The FIC index was calculated as the sum of FICA + FICB, where A is propolis extract and B is antifungical drug. This index was interpreted (Interp) as follows: If the total FIC index 0.5 it was evaluated as synergy (Syn), if 1 < FIC > 0.5 as additive (Add), if FIC > 1 as antagonist effect (Ant) (Barchiesi et al., 1997; de Castro et al., 2015).

The effects of propolis extracts on cell viability were evaluated using the MTT colorimetric method in melanoma (MV-3) and fibroblast (L929) cell line. The extracts showed cytotoxicity in both cell lines exhibiting IC_{50} bellow 100 µg/mL as observed in Table 3S.

Table 3S - Cytotoxic activity of the propolis extract in fibroblasts (L929) and melanoma (MV-3) cell line, IC_{50} values and 95% confidence intervals from three independent experiments are given.

Extract (Season)	**Cell line IC_{50} (µg/mL)**	
	L929	**MV-3**
T. clavipes (dry)	51.72 (45.27 to 59.10)	58.55 (49.60 to 94.76)
T. clavipes (rainy)	43.66 28.04 to 67.97)	52.54 (43.47 to 63.50)

4 DISCUSSION

The present study provides valuable insights into the chemical composition and biological activities of Tetragona clavipes propolis. These findings open avenues for discussion regarding its potential applications in medicine and healthcare. Studies conducted on propolis have indicated that its health benefits can be attributed due to the presence of phenolic compounds (Campos et al 2015, Dutra et al 2014). In a study conducted by Pazin et al. (2017) it was revealed that propolis obtained from the *T. clavipes* species collected in São Paulo had a polyphenols content of 12.5 ± 0.3 mg GAE/g and and a flavonoid content of 1.8 ± 0.1 mg QE/g (Pazin et al., 2017). The observed lower values of polyphenols and flavonoids in the propolis of the *T. clavipes* species collected in the study by Pazin et al. (2017) compared to our study may be attributed to the variations in the flowering period of plant species during different seasons. It is well-established that the chemical composition of stingless bee propolis can be influenced by the local vegetation, which may differ between geographical locations, seasons and bee species (Asem et al., 2020; Chong and Chua, 2020; Ferreira et al., 2020; Lopes et al., 2020).

The findings of this study indicate that the flavonoids, present in higher proportions in the samples, possess a diphenylpropane C6-C3-C6 structure consisting of aromatic and phenolic rings (Bravo, 1998). The distinctive structural characteristics of these compounds contribute to their antioxidant and antimicrobial properties (Bravo, 1998). Scientific studies have consistently identified Kaempferol and Rutin as the most frequently detected bioactive compounds in propolis as observed in this study (Araújo et al., 2019; Ferreira et al., 2020; Lopes et al., 2019, 2020; Othman et al., 2020; Belina-Aldemita et al., 2020; dos Santos et al., 2017b). It is indeed interesting to note that Kaempferol was not detected in the propolis extract from the dry season. This observation suggests that the presence of Kaempferol may contribute to the antioxidant activity of propolis. The absence of Kaempferol in the dry season extract implies that other compounds or factors may account for the antioxidant activity observed in that particular sample. Further investigation is warranted to explore the specific role of Kaempferol and its impact on the antioxidant properties of propolis. In contrast to the absence of Kaempferol in the dry season extract, the flavonoid was indeed present in the propolis extract from the rainy season. The presence of flavonoids in the rainy season extract suggests that these compounds may contribute to the observed antioxidant potential of the sample. However, to fully understand the antioxidant properties and potential benefits, further studies focusing on isolating and

characterizing these specific compounds are necessary. Such investigations would provide valuable insights into the individual contribution of the isolated compounds and their potential applications in various health-related areas. Previous study conducted by Pazin et al. in 2017 provided evidence of the antioxidant activity of *T. clavipes* propolis using the DPPH assay at a concentration of 218.0 ± 3.0 µg/mL. These findings align with our own results and support the notion that the biological activities of *T. clavipes* propolis cannot be attributed to a single compound but rather to the synergistic interactions among its components. This suggests that the combined effects of multiple compounds in propolis contribute to its overall biological activity, including antioxidant properties.

The terpenoid Carnosol observed in this study was reported in a previous investigation for its antioxidant and antimicrobial activity for propolis of the *Friesomelitta longipes* species (Ferreira et al., 2020). The two xanthones proposed in this study (Mangostin and Mangiferin) also have these properties and were detected by Chong et al (2020). These authors justified the finding due to the presence of an orchard near the *Heterotrigona itama* bee hive. Mangostin and Mangiferin are known to possess antitumor, antioxidant, antidiabetic, antimicrobial, and anti-inflammatory properties (Aljunaid et al., 2020).

Propolis helps maintain human cellular antioxidant status throughout infections, such as those caused by parasites and microorganisms (Zulhendri et al 2021). However, propolis acts directly on the pathogen, inhibiting its enzymes, proteins and metabolites necessary for host invasion, replication of its genetic material or energy production for its survival (Zulhendri et al 2021). In this context, our research was aimed at evaluating the activity of this raw material on different fungi that cause human health.

Propolis has an important natural fungicidal function, as it acts in the defense of the hive against pathogens. De Souza et al. (2018) demonstrated the antifungal activity of propolis from the stingless bee *Frieseomelitta longipes* against *Candida albicans* and *Candida tropicalis* within similar concentration (250 µg/mL) observed in this study. According to the literature, stingless bee propolis often exhibits antifungal activity at higher concentrations than those studied in our work. For example, propolis from *M. orbignyi* displayed antifungal potential by inhibiting the growth of *C. albicans* at a concentration of 3.1 mg/mL (MIC) and demonstrating fungicidal effects at 50 mg/mL (MFC) (Campos et al., 2014).

Different studies associate phenolic compounds and terpenes with the antimicrobial activity of stingless bee propolis (Salomão et al., 2008; Velikova et al., 2000). These compounds have been shown to exert their antimicrobial effects by damaging the cell membrane, inhibiting nucleic acid synthesis, and disrupting energy metabolism in microorganisms. Furthermore, they can interfere with various virulence factors, including enzymes, toxins, and signaling molecules, thereby contributing to their overall antimicrobial activity. (Cushnie, Lamb, 2011).

T. rubrum, *C. albicans* and *C. parapsilosis* are significant pathogens that play a crucial role in the development of various diseases (Bongomin et al., 2017; Janbon et al., 2019). The treatment of infections caused by these microorganisms has garnered significant attention due to the serious issues of antifungal resistance and drug toxicity. The emergence of antifungal resistance poses challenges in effectively treating these infections, while the potential toxicity of certain antifungal drugs further complicates therapeutic interventions. Therefore, there is a pressing need to address these challenges by exploring alternative treatment options, developing new antifungal agents, and promoting prudent use of existing antifungal medications to combat resistance and minimize drug toxicity. (Arastehfar et al., 2020; Kim, 2016; Martinez-Rossi et al. 2018). In this way, this study used two important commercial drugs that are routinely used for antifungal treatment. Fluoconazole has been reported due to its pathogens' resistance and Amphotericin B due to present many adverse effects such as nephrotoxicity, hepatotoxicity, cardiotoxicity, phlebitis, fever, and chills (Friedman and Schwartz, 2019; Murray, 2017; Sai and Azim, 2016; Zaitz, 2010). In the present study, we have made an innovated contribution by demonstrating that propolis derived from *T. clavipes* has the potential to serve as an alternative treatment option. This propolis extract offers advantages in addressing the challenges associated with fluconazole resistance and the limitations of toxicity associated with Amphotericin B. Our findings highlight that propolis exhibits significant antifungal activity and demonstrates synergistic and additive effects against fungi. This suggests that propolis holds promise as an effective antifungal agent and may represent a valuable therapeutic approach for managing fungal infections.

5 CONCLUSION

In conclusion, our findings offer significant insights into the potential medicinal applications of propolis derived from the stingless bee *Tetragona clavipes*. Particularly, propolis samples collected during the rainy season exhibit a diverse array of phytochemical compounds with notable antioxidant, fungicidal, and fungistatic activities. These properties are of pharmacological relevance and have the potential to be translated into human clinical trials. Moreover, given the variations in propolis composition based on geographic region, season, and bee species, our work contributes to the standardization of this valuable natural resource.

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Conflict of interests

The authors declare no conflict of interest.

CRediT authorship contribution statement

Ariane Pinheiro Cruz Bergamini: Conceptualization, Formal analysis, Investigation, Data Curation, Writing – original draft. **Brendo Victor Siqueira de Almeida Bergamini, Iana Soares Pessoa, Thiago Antônio de Sousa Cutrim, Tamires Cruz dos Santos, Matheus Campos dos Santos, Victor da Rocha Fonseca:** Investigation, Validation, Formal analysis. **Wanderson Romão, Denise Coutinho Endringer, Rodrigo Scherer:** Methodology, Validation. **Marcio Fronza:** Conceptualization, Methodology, Validation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

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CONSIDERAÇÕES FINAIS

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Os resultados e a revisão bibliográfica desta tese trazem uma compilação das características físico-químicas da própolis e geoprópolis de diferentes espécies de abelha sem ferrão encontradas no Brasil. Esta riqueza de dados deve ser utilizada para propor uma padronização desta matéria-prima.

A referência brasileira de padrão de qualidade de própolis (IN 3/2001) estabelece valores de no mínimo 0,5% e 5% m/m para flavonoides e compostos fenólicos, respectivamente. Além disso, classifica a própolis quanto ao teor de flavonoides em: baixo teor 1,0% m/m; médio teor > 1,0% - 2,0%; e alto teor > 2,0% m/m (BRASIL, 2001). Apesar desta normativa ser padronizada considerando a própolis de *Apis mellifera*, recomendamos que os parâmetros quantitativos sejam mantidos como referência para as ASF, visto que os valores para flavonóides (3,1% a 11,7% m/m) e fenólicos (5,6% a 16,1% m/m) encontram-se dentro do especificado.

Os demais parâmetros quantitativos de identidade e qualidade da própolis propostos pela IN 3/2001 devem ser melhor estudadas para o produto das ASF, pois estudos de perda por dessecação, cinzas, cera, atividade de oxidação, massa mecânica, solúveis em etanol e contaminantes são escassos.

Quanto as provas qualitativas, sugerimos que seja mantido o requerimento da norma regulamentar, que descreve que a própolis deve apresentar picos característicos das principais classes de flavonóides entre 200 e 400nm no espectro de absorção de radiações ultravioleta e visível. Uma vez que, os extratos etanólicos e hidroalcoolicos avaliados nas literaturas científicas e os estudados neste trabalho possuem flavonoides, sendo que os mais frequentemente observados são: kaempferol, quercetina, ácido elágico e rutina.

Destacamos que a rutina foi um composto proposto para todas as amostras de própolis e geoprópolis estudadas nesta tese, sendo um possível marcador químico nas análises de espectrometria ou cromatrografia numa rotina do controle de qualidade desta matéria-prima.

As estações chuvosa (setembro de 2019) e seca (fevereiro de 2020) foram determinadas considerando a precipitação total observada na região e no período da coleta das amostras (INCAPER, 2019, 2020). Como evidenciamos que a sazonalidade influencia nas características físico-químicas e propriedades biológicas da própolis e geoprópolis de ASF, recomendamos que os trabalhos realizados com o material descrevam as condições ambientais do local. Pois muitas publicações não apresentam esta informação, dificultando a padronização da qualidade do produto.

CONCLUSÕES

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Os resultados sugerem que as própolis e geoprópolis de abelhas sem ferrão do Espírito Santo possuem potencial terapêutico antioxidante, anti-inflamatório e antifúngico devido à sua composição química e atividades biológicas.

A sazonalidade e a variação entre as espécies evidenciam a importância de considerar fatores ambientais e genéticos na obtenção desses produtos de qualidade. O potencial bioativo da própolis e geoprópolis apresentou diferenças entre espécies e sazonalidade. Particularmente, o extrato de geoprópolis de *M. bicolor* e *M. capixaba* coletados durante a estação seca exibiram as maiores atividades antioxidantes e anti-inflamatórias, respectivamente. Especificamente, o extrato de própolis de *T. clavipes* coletado na estação chuvosa apresentou melhor atividade antifúngica quando comparado as demais espécies e estação.

O trabalho traz uma nova perspectiva no desenvolvimento de produtos cosméticos, alimentícios e farmacêuticos a partir de um produto natural. Apesar da complexidade química da própolis e geoprópolis de ASF e dificuldade de padronização, sua empregabilidade é promissora devido as atividades biológicas. Diante disto, são necessários mais estudos para aprofundar o conhecimento sobre esses produtos naturais e seu uso na medicina popular com a finalidade de elucidar os possíveis efeitos sinérgicos de seus componentes e os mecanismos celulares moleculares de ação da própolis e geoprópolis.

Conlui-se, enaltecendo que essas descobertas contribuem para a valorização e conservação das abelhas sem ferrão, bem como para a busca de novas terapias baseadas em produtos naturais.