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PÓLEN DE *Melipona fasciculata* Smith: valor nutricional e atividades biológicas

JOSÉ WILSON CARVALHO DE MESQUITA

São Luís
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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Maranhão, como requisito parcial para obtenção do título de Doutor em Ciências da Saúde.

Orientadora: Profª Drª Maria Nilce de Sousa Ribeiro

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RESUMO

A pesquisa de ingredientes bioativos para consumo humano tem aumentado em função da crescente consciência do consumidor da relação entre esses produtos e benefícios associados relacionados a saúde e bem-estar. Produtos de abelhas, como mel, própolis, geoprópolis, cera, geleia real e pólen são usados desde a antiguidade por suas ações terapêuticas e potencial nutracêutico. Considerando a cadeia produtiva, a bioatividade e a composição química de produtos de *Melipona fasciculata*, a Tiúba-do-Maranhão, este estudo propôs realizar a caracterização polínica, físico-química, química, nutricional e avaliar as propriedades antioxidante e anti-inflamatória do pólen de *M. fasciculata* cultivada no Maranhão. Foram coletadas 17 amostras de pólen de *M. fasciculata* entre abril de 2015 e abril de 2017, em 5 municípios do estado do Maranhão, cobrindo os biomas com maior área no estado em alguns de seus perfis fitogeográficos. A análise palinológica das amostras coletadas mostrou os tipos polínicos dominantes em Santa Luzia do Paruá como *Borreria verticillata* (Rubiaceae), *Hyptis* sp. (Lamiaceae), *Protium* sp. (Burseraceae), *Senna* sp. (Fabaceae) e *Mauritia* sp. (Arecaceae), em Palmeirândia, Viana e Anajatuba foram *Pontederia parviflora* (Pontederiaceae) e *Myrcia* sp. (Myrtaceae) e nas amostras de Chapadinha, *Mimosa* sp. (Mimosaceae) e *Eucalyptus* sp. (Myrtaceae). A análise microbiológica mostrou todas as amostras com baixíssima presença de contaminantes microbiológicos. A composição nutricional indicou que as amostras apresentam similaridade e que estão dentro dos parâmetros recomendados pelo Ministério da Agricultura para o pólen apícola, com exceção da umidade para duas amostras e teor de cinzas para uma. As amostras foram agrupadas segundo sua localidade de coleta e sazonalidade climática (estação seca e estação chuvosa), em seguida foram submetidas à extração por maceração por 72 horas com etanol 70% e hidromódulo 1:8, totalizando 10 extratos de pólen. O rendimento de extração variou entre 53,8 e 64,8%. Os extratos obtidos foram submetidos aos doseamentos de flavonoides totais e compostos fenólicos. Os extratos do pólen de Santa Luzia do Paruá (estação chuvosa), Palmeirândia (estação seca), Viana (estação seca), Anajatuba (estação chuvosa) e Chapadinha (estação seca) foram submetidos à análise por cromatografia líquida de alta eficiência com detector UV-Vis. Com o auxílio de padrões comerciais e utilizando o método da coeluição foi possível identificar a presença de β-sitosterol, ácido elágico, ácido cumárico, isoquercitrina, quercitrina, miricetina, naringenina, canferol, catequina, β-amirina, ácido cumárico, rutina, quercetina, ácido gálico e apigenina. Os extratos obtidos a partir das amostras de pólen de *M. fasciculata* coletados em Santa Luzia do Paruá (ESLPs e ESLPc), Palmeirândia (EPALs e EPALc) e Chapadinha (ECHAs), foram fracionados e cada uma de suas frações foi submetida à avaliação da atividade antioxidante pelo ensaio do DPPH. O resultado da avaliação da atividade antioxidante expresso em CE_{50} variou entre 156,24 e 1764,67 µg/ml. As frações clorofórmicas de todas as amostras apresentaram melhor atividade antioxidante. Os extratos obtidos à partir das amostras de pólen de *M. fasciculata* coletados em Santa Luzia do Paruá na estação chuvosa (ESLPc), Palmeirândia na estação chuvosa (EPALc) e Chapadinha na estação seca (ECHAs) foram utilizados para avaliação da atividade anti-inflamatória *in vitro* através do teste de inibição das enzimas cicloxigenase 1 e 2. A amostra de Chapadinha apresentou as maiores porcentagens de inibição enzimática nas concentrações de 10 e 50 µg/mL, chegando a inibir 61,7 e 58,6% de COX1 e COX2, respectivamente.

Palavras-chave: Abelha; Pólen; Qualidade; Composição; *Melipona fasciculata*.

ABSTRACT

Research into bioactive ingredients for human use has increased as result of greater consumer awareness of the relationship between these products and associated benefits related to health and well-being. Bee products such as honey, propolis, geopropolis, wax, royal jelly and pollen have been used since antiquity for their therapeutic properties and nutraceutical potential. Considering the production chain, bioactivity and chemical composition of *Melipona fasciculata* products this study proposed the palynological characterization, physicochemical, chemical, nutritional profile, antioxidant and anti-inflammatory properties of *M. fasciculata* pollen extracts cultivated in Maranhão. A total of 17 pollen samples of *M. fasciculata* were collected between April 2015 and April 2017 in 5 municipalities in the state of Maranhão, covering the biomes with the largest area in the state in some of their phytogeographic profiles. The palynological analysis of the collected samples showed the dominant pollen types in Santa Luzia do Paruá as *Borreria verticillata* (Rubiaceae), *Hyptis sp*. (Lamiaceae), *Protium sp*. (Burseraceae), *Senna sp*. (Fabaceae) and *Mauritia sp*. (Arecaceae), Palmeirândia, Viana and Anajatuba were *Pontederia parviflora* (Pontederiaceae) and *Myrcia sp*. (Myrtaceae) and those of Chapadinha, *Mimosa sp*. (Mimosaceae) and *Eucalyptus sp*. (Myrtaceae). The microbiological analysis showed all the samples with very low presence of microbiological contaminants. The nutritional composition indicated that the samples presented similarity and that they are within the parameters recommended by the Ministry of Agriculture, except for humidity for two samples and ash content for one. The samples were grouped according to their locality of collection and climatic seasonality (dry season and rainy season), after which they were extracted by maceration for 72 hours with 70% ethanol and 1: 8 hydromodule, totalizing 10 pollen extracts. The extraction yield varied between 53.8 and 64.8%. The extracts obtained were submitted to the total flavonoid and total phenolic compounds assays. The extracts of pollen from Santa Luzia do Paruá (rainy season), Palmeirândia (dry season), Viana (dry season), Anajatuba (rainy season) and Chapadinha (dry season) were submitted to high efficiency liquid chromatography with UV detector -Vis. The presence of β-sitosterol, ellagic acid, coumaric acid, isoquercitrin, quercitrin, myricetin, naringenin, canferol, catechin, β-amirine, coumaric acid, rutin, quercetin, gallic acid and apigenin. The extracts obtained from the pollen samples of M. fasciculata collected in Santa Luzia do Paruá (ESLPs and ESLPc), Palmeirândia (EPALs and EPALc) and Chapadinha (ECHAs) were fractionated and each of their fractions was submitted to the evaluation of the antioxidant activity by the DPPH assay. The result of the evaluation of the antioxidant activity expressed in EC50 varied between 156.24 and 1764.67 μg/ml. The chloroform fractions of all the samples presented better antioxidant activity. The extracts obtained from pollen samples of M. fasciculata collected in Santa Luzia do Paruá in the rainy season (ESLPc), Palmeirândia in the rainy season (EPALc) and Chapadinha in the dry season (ECHAs) were used to evaluate the anti-inflammatory activity in vitro by the inhibition test of cyclooxygenases 1 and 2. The Chapadinha sample presented the highest percentages of enzymatic inhibition in the concentrations of 10 and 50 μg/mL, reaching to inhibit 61.7 and 58.6% of COX1 and COX2, respectively.

Key-words: Bee; Pollen; Quality; Composition; *Melipona fasciculata*.

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1 INTRODUÇÃO

A pesquisa de ingredientes bioativos para consumo humano tem aumentado em função da crescente consciência do consumidor da relação entre o uso desses produtos e benefícios associados com a manutenção da saúde e bem-estar. Porém, as inter-relações entre dieta e saúde são complexas e exigem dados de composição de alimentos representativos e precisos (PAZ et al., 2015).

Na busca por uma dieta saudável verifica-se tendência na pesquisa de alimentos funcionais, definidos como "alimentos que comprovadamente afetam beneficamente uma ou mais funções orgânicas, além dos efeitos nutricionais adequados, de uma forma relevante para melhorar a saúde e prevenir e/ou reduzir o risco de doença" (STRINGHETA et al., 2007).

Evidências crescentes apoiam a hipótese de que alimentos funcionais que contenham componentes fisiologicamente ativos podem melhorar a saúde. Entretanto investigações adicionais são necessárias para fundamentar o benefício potencial de alimentos em que a relação dieta-saúde não esteja cientificamente validada em uma extensão suficiente (ASSMANN et al., 2014).

No Brasil, alegações de propriedades funcionais ou de saúde devem ser embasadas por estudos científicos que sejam adequados para comprovar o efeito alegado. Os alimentos de competência da fiscalização pela Agência Nacional de Vigilância Sanitária (ANVISA) que veiculem essas alegações devem ser enquadrados e registrados na categoria de alimentos com alegações de propriedades funcionais ou de saúde (BRASIL, 1999) ou na categoria de substâncias bioativas e probióticos isolados (BRASIL, 2002). Sendo que estudos em modelos animais, *ex vivo* ou *in vitro*, podem ser apresentados como informações de suporte para a relação proposta.

Produtos de abelhas, como mel, própolis, geoprópolis, cera, geleia real e pólen são usados desde a antiguidade por suas ações terapêuticas e potencial nutracêutico (RAO et al., 2016). Os produtos de *Apis mellifera* (apícolas) com maior importância comercial são o mel, devido ao seu valor energético, e o pólen, por apresentar valor nutricional. O consumo de pólen com finalidade de suplementação alimentar tem se destacado nos últimos anos, uma vez que contém grandes quantidades de ácidos graxos, carboidratos e aminoácidos, além de possuir dispositivos legais que estabelecem parâmetros de qualidade viabilizando sua comercialização em vários países (YANG et al., 2013).

O pólen apícola é o resultado da aglutinação de diferentes grãos de pólen colhidos pelas abelhas, misturados com néctar e substâncias salivares, sendo sua composição química variável de acordo com a espécie vegetal, condições ambientais, idade e estado nutricional da planta de onde os grãos de pólen foram coletados (MORAIS et al., 2011). Diversas ações biológicas do pólen apícola já foram demonstradas: prevenção de distúrbios da próstata (SHOSKES et al., 2003), imunomoduladora, antimutagênica, anti-inflamatória e antioxidante (PASCOAL et al., 2014).

No Brasil, há outras abelhas que produzem pólen, como os meliponíneos, conhecidos como "abelhas sem ferrão" por apresentarem o ferrão atrofiado. Apesar de não ser tão difundido como a apicultura, o cultivo dos meliponíneos (meliponicultura) vem sendo praticado há bastante tempo (NOGUEIRA-NETO, 1997).

A meliponicultura desenvolvida no Maranhão destaca-se principalmente pelo cultivo de *Melipona (Melikerria) fasciculata* Smith, 1854 (Hymenoptera, superfamília Apoidea, família Apidae, subfamília Meliponinae, tribo Meliponini) conhecida como "tiúba" ou "tiúba do Maranhão", a abelha sem ferrão mais comum no estado e uma das principais fontes de renda para várias famílias indígenas e rurais que a cultivam para produção de mel, cera, geoprópolis e pólen (ALBUQUERQUE et al., 2013).

O Maranhão, por ser uma região de transição, possui um grande número de ecossistemas, o que fornece a matéria prima de suporte para o trabalho das abelhas, que se origina na abundante biodiversidade. Além disso, a ação polinizadora dessas abelhas pode ainda aumentar a produção agrícola e promover a regeneração da vegetação natural (ALBUQUERQUE et al., 2013).

Os meliponíneos, em busca de recursos florais, visitam um espectro diversificado de flores, transportando involuntariamente grãos de pólen. Dessa forma, o conhecimento da flora meliponícola é essencial para o estabelecimento de programas de conservação desses animais (CARVALHO; MARCHINI, 1999) e para implementação de uma meliponicultura produtiva baseada na exploração racional destes organismos.

Estudos com a geoprópolis produzida por *M. fasciculata* cultivada no Maranhão demonstraram diversas ações biológicas como antimicrobiana, imunomodulatória e antioxidante (LIBERIO et al., 2011; DUTRA et al., 2014; BATISTA et al., 2016). Entretanto, há poucos estudos sobre a composição química e as atividades biológicas do pólen de *M. fasciculata*.

Estes dados evidenciam o grande potencial de mercado que a espécie *M. fasciculata* possui pela peculiaridade dos produtos oferecidos, fácil manejo e por

necessitar de pouco investimento, assim como a necessidade de estudos que indiquem os benefícios do seu consumo.

Considerando a bioatividade e composição química de produtos de *M. fasciculata* este estudo propõe realizar a caracterização polínica, físico-química, química, nutricional e avaliar as propriedades antioxidante e anti-inflamatória do pólen de *M. fasciculata* cultivada no Maranhão, visando demonstrar o potencial alimentício e/ou terapêutico deste produto, e, consequentemente favorecer o desenvolvimento sustentável da meliponicultura no estado.

2 REFERENCIAL TEÓRICO

2.1 Meliponíneos

Os meliponíneos ou abelhas sem ferrão, são insetos eussociais, um grupo ecologicamente importante, que atuam na manutenção da diversidade das plantas nativas e cultivadas como efetivos polinizadores. São encontrados em grande parte das regiões de clima tropical e em algumas regiões de clima temperado e subtropical. Por possuírem o ferrão atrofiado, são incapazes de ferroar, e consequentemente fáceis de manejar (NOGUEIRA-NETO, 1997). Além da ausência de ferrão, estas abelhas diferem das demais por construírem ninhos em troncos de árvores, fendas em pedras ou solo, ou pendurados em galhos; os favos são sobrepostos horizontalmente, depositam pólen misturado ao mel, e expulsam ou inutilizam os machos após a fecundação da rainha (FABICHAK, 1989; VELTHUIS et al., 2003).

No Brasil foram identificadas 244 espécies de abelhas sem ferrão, principalmente nas regiões Norte e Nordeste, correspondendo a cerca de 20% de todas as espécies neotropicais de abelhas sem ferrão (PEDRO, 2014). Elas são responsáveis por 40 a 90% da polinização da flora nativa, auxiliando no reflorestamento e identificação das espécies vegetais (KERR, 1987).

No estado do Maranhão, *Melipona fasciculata*, popularmente conhecida como tiúba, ou tiúba-do-maranhão, é a espécie de abelha sem ferrão mais frequentemente cultivada, especialmente em áreas de cerrado e campos alagados, devido ao seu alto valor de mercado e a produção concomitante de cera, pólen e geoprópolis (BATISTA et al., 2016). A sua criação é feita principalmente em caixas rústicas sem padronização (RÊGO; ALBUQUERQUE, 2010; MARTINS et al., 2011).

O pólen acumulado nas colônias é a principal fonte de proteínas das abelhas e suas larvas (SILVA; PAZ, 2012). Consiste na mistura de saliva com grãos de pólen coletados pelas abelhas na flora disponível na região. Para manutenção de suas colônias com centenas de milhares de indivíduos e altas taxas de reprodução, as abelhas precisam forragear grande quantidade de alimento de um grande número de plantas a partir de diversas famílias (RAMALHO et al., 2007). A presença de numerosos tipos de pólen indica que as abelhas procuram alimentos em um vasto número de espécies vegetais, a distâncias substanciais (BARROS et al., 2013).

O armazenamento do alimento é feito em células de cera que são preenchidas com mel e pólen (FREITAS, 2003). Embora existam variações, o ninho da maioria dos meliponíneos é formado por uma área de cria, constituída por favos sobrepostos na

horizontal, onde existem diversas células de crias; e potes de cera, para estocagem de pólen e mel (NOGUEIRA-NETO, 1997). Nos potes de estocagem de pólen estão a massa de pólen e sucos digestivos que serão posteriormente fechados para que ocorra a fermentação, a massa fermentada apresenta cor marrom levemente amarelada, odor característico, pH ácido, com baixo número de micro-organismos, e pronto para ser consumido pelas abelhas (MACHADO, 1971; SILVA; ZUCOLOTO, 1994). Os grãos de pólen manipulados pelos meliponíneos recebem o nome de samora nas regiões Centro-Oeste e Sudeste, e de saburá ou samburá na região Amazônica e no Nordeste.

2.1.1 Composição química e atividades biológicas do pólen de meliponíneos

A composição química do pólen pode variar de acordo com a espécie vegetal, condições ambientais, idade e estado nutricional das plantas que as abelhas visitaram (FUNARI et al., 2003; BARRETO et al., 2006). Poucos são os estudos sobre a composição química de pólen de abelhas sem ferrão, predominando estudos com pólen apícola. Em geral a composição química do pólen contém carboidratos, aminoácidos, proteínas, lipídios, vitaminas, minerais, ácidos fenólicos, flavonoides e triterpenos (CAMPOS et al., 1997; VILLANUEVA et al., 2002; SILVA et al., 2006; CARPES, 2008).

O pólen coletado por *Melipona compressipes manoensis*, *Melipona seminigra seminigra*, *Melipona seminigra merrilae*, *Frieseomelitta* sp. e *Scaptotrigona* sp. apresentou alto teor de açucares solúveis totais, açucares redutores e amido (MARQUES-SOUZA et al., 2002). Amostras de pólen acumuladas por espécies de *Melipona* cultivadas no estado do Amazonas (*M. compressipes manoensis*, *M. rufiventris paraensis*, *M. seminigra merrilae*) demonstraram teor de 15,7% a 23,8% de proteínas, 1,9 a 9,3% de lipídios e 26,4 a 57,4% de glicídios (SOUZA et al., 2004). Em pólen acumulado por *Melipona marginata* e *Melipona scutellaris*, cultivadas em meliponários no estado de São Paulo foi observada a presença de esteroides (0,33 a 53,16%) (FERREIRA-CALIMAN et al., 2012).

O pólen acumulado por *Melipona subnitida* Ducke, é constituído por flavonoides (naringenina, triacetina, isoramnetina e 8-metoxiherbacetina), aminoácidos, açúcares e *β*-sitosterol (SILVA et al., 2006; SILVA et al., 2014). Composto essencialmente de 98% de pólen de *Mimosa gemmulata* (Mimosoideae) (pólen de cor amarela) e de 89,4% de pólen de plantas da família Fabaceae (pólen de cor marrom).

Silva et al. (2009) demonstraram que o pólen acumulado por *Melipona rufiventris* possui ação antioxidante e é composto por ácido p-hidroxicinâmico e pelos flavonoides dihidroxiquercetina, isoramnetina, isoramnetina-3-*O-6''-O-E-p*-coumaroil-*β*-D-glicopiranosídeo, luteolina e quercetina.

Os flavonoides miricetina, dihidromiricetina, quercetina e isoramnetina foram identificados no pólen acumulado por *Scaptotrigona bipunctata* cultivada em Fortaleza, Ceará (LINS et al., 2003).

O extrato metanólico de pólen da abelha sem ferrão malaia *Lepidotrigona terminata* apresentou atividade antioxidante pelo ensaio de inibição do radical 1,1-difenil-2-picrilidrazina (DPPH) (CE_{50} de 0,5 mg/mL), atividade antiproliferativa contra células MCF-7 (CI_{50} de 15 mg/mL) e efeito quimiopreventivo, ao ser combinado com medicamentos usados na quimioterapia, como a cisplatina. A média de inibição das células tumorais foi potencializada pelo extrato de pólen em cerca de 50% (OMAR et al., 2016). Efeito antiproliferativo similar foi encontrado em estudo de avaliação de extrato de pólen de quatro espécies de abelhas sem ferrão nativas da Indonésia, *Trigona incisa*, *Timia apicalis*, *Trigona fusco-balteata* e *Trigona fuscibasis* (KUSTIAWAN et al., 2014).

Os extratos acetato de etila, ricos em flavonoides, dos polens amarelo e marrom da abelha *M. subnitida* demonstrou atividade antioxidante utilizando o ensaio de DPPH, com CE_{50} de 41,9 e 43,7 mg/mL, respectivamente (SILVA et al., 2006).

De acordo com a literatura os compostos fenólicos e flavonoides são extremamente benéficos à saúde humana, pois diminuem o risco de doenças degenerativas, reduzindo o estresse oxidativo (SILVA et al., 2006; FEÁS et al., 2012; KOMOSINSKA-VASSEV et al., 2015).

Tendo em vista a escassez de trabalhos sobre as propriedades biológicas e perfil químico do pólen de *M. fasciculata*, a elucidação dessas características seria de fundamental importância para validar o uso potencial deste produto ainda pouco investigado como medicamento ou alimento funcional.

2.1.2 Espectro polínico de produtos de meliponíneos

A análise palinológica combinada com a análise físico-química é útil na caracterização de produtos de meliponíneos, como mel, geoprópolis e pólen, produzidos regionalmente com características diferentes (BARROS et al., 2013). Oferecendo

resultados que podem ser utilizados na análise e rotulagem de amostras que beneficiam as atividades econômicas destes produtos (BARTH, 1998; BARTH; LUZ, 2003).

Em amostras de mel de *M. fasciculata* oriunda do município de Palmeirândia, Maranhão, espécies das famílias Pontederiaceae e Mimosaceae foram as mais representativas na análise polínica (MARTINS et al., 2011). Albuquerque et al. (2013) demonstraram as espécies botânicas utilizadas como recursos florais para *M. fasciculata* nessa região, visando o manejo dessas abelhas.

Barros et al. (2013) analisaram o pólen de 16 amostras de geoprópolis de *M. fasciculata* em municípios de três diferentes regiões geográficas do Maranhão (Palmeirândia, Barreirinhas e Belágua) e constataram que em Palmeirândia os tipos de pólen mais comum foram em ordem de importância: *Mimosa pudica*, *Mimosa caesalpiniifolia*, *Eucalyptus* e Euphorbiaceae; em Barreirinhas: *Mimosa pudica*, *Mimosa caesalpiniifolia*, *Chamaecrista*, *Bauhinia*, *Erechtites*, *Eucalyptus* e Euphorbiaceae; e em Belágua: *Solanum*, *Chamaecrista*, *Psidium*, Arecaceae, *Desmodium* e Meliaceae. Os mesmos autores demonstraram os principais tipos polínicos de geoprópolis de *M. fasciculata* em áreas alagadas e de cerrado no estado do Maranhão (RIBEIRO et al., 2016). Em trabalho realizado pelo mesmo grupo foi determinada a composição florística usada por *M. fasciculata* em áreas de Floresta Amazônica no estado do Maranhão (CARVALHO et al., 2016).

A abelha *M. subnitida,* cultivada em área de restinga no Maranhão, acumulou 58 tipos de pólen, pertencentes à família Fabaceae, Melastomaceae, Myrtaceae e Dilleniaceae (PINTO et al., 2014).

No espectro polínico em mel de *Melipona mandacaia* Smith, cultivada na região semiárida do estado da Bahia, foi constatado como predominante o pólen de *Piptadenia rigida* (Mimosaceae) (ALVES et al., 2006).

Diversos estudos apontam que as abelhas do gênero *Melipona*, apesar de consideradas generalistas, possuem preferência por coletar pólen de espécies vegetais com anteras poricidas (ANTONINI et al., 2006; RAMALHO et al., 2007; CARVALHO et al., 2016). Em várias oportunidades, pode-se perceber que a abundância do recurso polínico não alterou a frequência de coleta por *M. fasciculata*, demonstrando uma fidelidade à fonte vegetal (RAMALHO et al., 2007).

As informações sobre o espectro polínico ajudam a compreender as interações biológicas entre as plantas e seus polinizadores, assim como o comportamento de

competição entre as espécies por alimentos em diferentes ecossistemas (BARTH, 1998). Esse conhecimento contribui para o fortalecimento da meliponicultura.

3 OBJETIVOS

3.1 Objetivo geral

Realizar a caracterização polínica, físico-química, química, nutricional e avaliar as atividades antioxidante e anti-inflamatória do pólen de *Melipona fasciculata* Smith (tiúba).

3.2 Objetivos específicos

a) Analisar os parâmetros físico-químicos e nutricionais do pólen de *Melipona fasciculata*;
b) Analisar o espectro polínico do pólen de *M. fasciculata*;
c) Quantificar polifenois e flavonoides totais dos extratos de pólen de *M. fasciculata*;
d) Identificar a composição química dos extratos de pólen de *M. fasciculata*;
e) Investigar a ação antioxidante *in vitro* de extratos do pólen de *M. fasciculata;*
f) Investigar a ação anti-inflamatória *in vitro* de extratos do pólen de *M. fasciculata;*
g) Obter perfil químico-nutricional do pólen *in natura* e do extrato de pólen.

4 RESULTADOS

4.1 Capítulo I

A timeline of the biological activity assessment of stingless bee products

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A timeline of the biological activity assessment of stingless bee products

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Abstract

Stingless bee produce honey, propolis, geopropolis, pollen and cerumen. These products are used in popular practice as therapeutic agents. Thus, this review summarizes the studies of biological activity evaluation of stingless bee products samples worldwide. In last years, the publications have presented data on antimicrobial, anticancer, antioxidant and anti-inflammatory action along with chemical characterization. Despite growing and widespreding around the world, studies on biological activities are still scarce for many types of stingless bees and their products. It is clear that there is vast future research to validate the therapeutic potential of stingless bee products.

Keywords: stingless bee; antimicrobial; cytotoxic; antioxidant; anti-inflammatory.

1. Introduction

Stingless bees (Hymenoptera, Apidae, Meliponini) form the largest group of eusocial bees in the world, comprising more than 500 species (Michener, 2013). They are called stingless bees because they have no sting or have stunted sting and are therefore unable to sting. They present pantropical distribution, being found in countries of South and Central America, Africa, Southeast Asia and Australia. In Brazil there are more than 200 registered species, belonging to 29 genera (Pedro, 2014), such as *Melipona*, *Trigona*, *Scaptotrigona* and *Plebeia* (Figure 1).

Stingless bees play an important role in pollinating native vegetation and in maintenance of plant diversity, making them essential for the conservation of diverse ecosystems (Michener, 2007; Camargo & Pedro, 2013). In addition to the ecological function, stingless bees produce honey, propolis, geopropolis, wax and accumulate pollen, products that are fundamental for their food, defense and survival (Bankova, 2005; Kerr, 1996), and are highly valued and consumed by humans (Schwarz, 1948; Dos Santos & Antonini, 2008).

From the perspective of ethnobiology, there are reports of the use as food, in the treatment of diseases, production of handicrafts and activities related to religious rites in different communities: Uwa de Colombia (Falchetti & Nates-Parra 2002), Kayapó

(Posey, 1983; 1984), Pankarare (Costa-Neto, 1998), Enanene-Nawe (Dos Santos & Antonini, 2008), Mby'a in Brazil (Rodrigues, 2006), Mby'a and Ava Guarani in Paraguay (Zamudio et al., 2010), multicultural groups between Brazil and Argentina (Zamudio & Hilgert, 2012).

Beekeeping of stingless bees, known as meliponiculture, is a rapidly expanding activity with social, environmental and economic relevance (Cortopassi-Laurino et al., 2006; Salatino et al., 2019), however, neither all products made by the bees are being properly used, possibly due to ignorance on the part of meliponiculturists and consumers. Adopting some management practices, such as inspecting and feeding the colonies, can help optimize meliponiculture and make the activity a powerful tool for sustainable development (Jaffé et al., 2015). In addition, chemical and biological characterization can contribute to the valorization of bees and their products.

Regarding the evaluation of the chemical and pharmacological composition of bee products, studies have focused mainly on material from *Apis mellifera* bees. A contrary scenario exists for stingless bee products, because although they are popularly used as therapeutic resources to treat influenza, asthma, cough, wounds, eye problems and weakness (Alves & Alves, 2011), they do not yet have specific legislation to regulate the use and marketing of their products.

Evidence of the therapeutic properties of stingless bee products from different parts of the world will allow in the future the use of these natural resources as an alternative in the treatment of various diseases. Thus, the aim of this review is to present a historical narrative of research on the biological activities of stingless bee products: honey, pollen, propolis, geopropolis and cerumen.

2. Methodology

This is historical narrative review which the literature survey was performed between 2000 and 2020, using PubMed, Web of Science and Scopus databases and the following terms in association: “stingless bee” and “biological activity”.

3. Honey

Honey is a food substance produced by honey bees from the nectar of flowers (floral honey) or secretions from live parts of plants or excretions of plant-sucking insects (honeydew honey) that bees collect, transform, combine with their own specific substances, store and they let to mature in the honeycomb. It serves as food for bees in

the winter season and is the most consumed bee product by humans (Brazil, 2000; Costa & Oliveira, 2005; Ximenes et al., 2011).

Floral honey can be classified as unifloral or monofloral, when the collected nectar is originated from flowers of the same family, genus or species, or multifloral and polyphloral, when nectar is collected from different floral origins (Brazil, 2000; León-Ruiz et al., 2011). Monofloral honeys are rarer, more difficult to produce and have characteristic aromas, indicating the origin of nectar, consequently they are more valued commercially (León-Ruiz, 2011; Vanhanen et al., 2011). In Brazil, the main honeys of Meliponines are from *Acacia polyphyla*, *Anadenanthera macrocarpa*, *Citrus*, *Eucalyptus*, *Mimosa caesalpinifolia*, *Myrcia*, *Piptadenia rigida*, *Pontederia parviflora*, *Schinus*, *Solanum* and *Vernonia polyanthes* (Bazlen, 2000; Barth, 2004; Alves et al., 2006; Almeida, 2007; Martins et al., 2011).

Meliponines such as *Melipona* spp., *Scaptotrigona* spp. and *Trigona* spp., elaborate differentiated honeys, with particular sensory, physical and chemical characteristics and higher commercial value when compared to *A. mellifera* honey (Garedew et al., 2004; Torres et al., 2004; Almeida-Muradian et al., 2007; Guerrini et al., 2009). The most relevant differences are higher values of water, free acidity, electrical conductivity, maltose and nitrogen, and lower values of diastase (Vit et al., 1994).

3.1 Chemical composition

They contain simple sugars in their composition, as well as organic acids, amino acids, enzymes, minerals and vitamins (De Camargo et al., 2006; Bogdanov et al., 2008). The composition and quality of each honey depends on factors such as floral and geographical origin, climatic conditions, storage and bee species (Bertoncelj et al., 2007; Biluca et al., 2016; De Sousa et al., 2016). Therefore, studies with stingless bees must present entomological data, with the popular and scientific names of bees (Souza et al., 2006).

Recently, it was suggested that the classification of stingless bee honeys by origin of bee species can be determined using the Nuclear Magnetic Resonance (NMR) and Liquid chromatography-mass spectrometry (LC-MS) (Razali et al., 2018). Among the various classes of bioactive substances that occur in honey, we highlight the phenolic compounds, which in addition to the numerous benefits to human health, the total phenolic content represents an important tool in determining the floral origin of honey (Andrade et al., 1997; Iurlina et al., 2009; Bertoncelj et al., 2011).

The interest in stingless bee honey led researchers to develop quality standard parameters, showing rich composition and functional variability due to various causes. Vit et al. (2004) proposed quality criteria based on water content, reducing sugars, sucrose, acidity, ash, hydroxymethylfurfural and diastatic activity for honeys of *Melipona*, *Scaptotrigona* and *Trigona*. However, the moisture variation makes quality assessment difficult, because moisture depends on stingless bee species and the area where it is produced (Bijlsma et al., 2006).

3.2 Biological activities

Among the studies with honey, the antimicrobial and antioxidant activities were the most investigated (Table 1).

In 2004, Torres et al. published what would become the first attempt to validate the popular use of stingless bee honey by assessing biological activity and analyzing its chemical composition. The antimicrobial activity of *Tetragonisca angustula* honey collected in Colombia was evaluated by microcalorimetric experiments and agar diffusion bioassays against strains of the filamentous fungi *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride*, against gram-positive bacteria *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus megaterium* and *Bacillus brevis*, as well as two species of gram-negative bacteria, *Escherichia coli* and *Pseudomonas syringae*. In this study, none of the fungi was sensitive to the dilutions tested. Both gram-positive and gram-negative bacteria reacted differently. Diluted honey samples resulted in dense growth of all strains tested, because the enrichment of the medium was superior to the impact of hydrogen peroxide (substance identified as responsible for the activity in this study) on inoculated bacteria (Hormese).

In the evaluation of honey samples from *Melipona* sp. native to Ecuador, Guerrini et al. (2009) found in general a similar chemical composition to that previously obtained for *Apis mellifera* honey in other South American countries (Souza et al., 2006; Almeida-Muradian et al, 2007), with differences in glucose/fructose ratio and in high content of β-tocopherol for fresh samples, in addition, it was found a characteristic coumarin profile in extracts obtained using Amberlite-XAD resin. The antioxidant activity evaluated by DPPH radical scavenging method and β-carotene bleaching assay (88.1 ± 11.1 DPPH inhibition%; 70.8 ± 8.90 β-carotene inhibition%), as well as the evaluation of growth inhibition of *Enterococcus faecalis*, *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* were superior to those obtained with *Apis* monofloral honeys. Honey extracts

also showed antimutagenic activity in the *Saccharomyces cerevisiae* strain D7 assay, demonstrating the ability of stingless honey to protect against DNA damage.

Samples of honey from *Trigona carbonaria*, an Australian stingless bee, were evaluated for antimicrobial activity through a variety of methodologies such as agar diffusion, agar dilution, broth microdilution and time-kill assay. As previously shown, honey displayed a broad spectrum of action against bacteria but not against fungi (Boorn et al., 2010).

In a review study conducted on honey from *Melipona beecheii*, from the Yucatan Peninsula (Mexico), it was concluded that honey of this species has bioactive characteristics that allow it to be considered a natural source of food with antioxidant properties and has nutritional importance by protein richness (Cauich et al., 2015).

Illustrating the evolution of the techniques employed, the antimicrobial activity of Brazilian honey samples of *Scaptotrigona bipunctata* and *S. postica* were evaluated against *Staphylococcus aureus* (ATCC25923 and ATCC29213), *Staphylococcus epidermidis* (ATCC12228), *Enterococcus faecalis* (ATCC29212), *Enterococcus faecium* (ATCC6569), *Streptococcus mutans* (UA159), *Streptococcus pyogenes* (ATCC19615), *Escherichia coli* (ATCC25922 and ATCC8739), *Salmonella enterica* serovar *Enteritidis* (ATCC13076), *Klebsiella pneumoniae* (ATCC700603) and *Pseudomonas aeruginosa* (ATCC27853 and ATCC9027) by agar well diffusion assay (AWD), definition of minimum inhibitory concentration (MIC) by microdilution assays, checkerboard assay, time-kill assay, non-peroxide antibacterial activity (NPA) and scanning electron microscope (SEM). The values for minimum inhibitory concentration ranged from 0.62 to 10% (v/v) and were potentiated 4 to 32 times when the honeys were combined.

The images obtained by SEM showed inhibition of division and cell rupture for *Scaptotrigona bipunctata* and *S. postica* samples, respectively. In the combined samples, scanning showed both situations at the same time (Nishio et al., 2016). In the same direction, Zamora et al. (2017) have shown that the protein fraction of *Tetragonisca angustula* honey samples from Costa Rica has antimicrobial effect and is a promising candidate for new therapeutic agents, but honey samples from *Melipona beecheii* did not show relevant activity.

The literature is still scarce about native stingless bee species from Africa, and they have only recently begun to have their potential prospected. Nweze et al. (2017) tested the antioxidant activity of honey samples from *Hypotrigona* sp. and *Melipona* sp. of Nigeria by antioxidant equivalent—ascorbic acid assay and ferric reducing power

(FRAP). Honey samples from *Hypotrigona* sp. showed higher results (AAE 342.33 ± 0.78 mg/kg; FRAP 666.88 ± 1.73 μM Fe (II)/100 g) than *Apis mellifera* (AAE 298.13 ± 7.38 mg/kg; FRAP 511.40 ± 14.31 μM Fe (II)/100 g).

Regarding the evaluation of the cytotoxic activity, honey from four stingless bee species (*Trigona incisa*, *Trigona apicalis*, *Trigona fuscibalteata*, *Trigona fuscibisca*) native to Indonesia were tested against five cancer cell lines (HepG2, SW620, ChaGo-I, KATO-III e BT474), however only HepG2 (human liver carcinoma cells) was broadly sensitive. The authors believe that cytotoxic activity is slightly higher than *Apis* honey due to stingless bee honey be stocked in pots made of propolis, unlike the waxed honeybee pot (Kustiawan et al., 2014). This is the only work reporting the anticancer activity of stingless bee honey, so further investigation is necessary.

4. Propolis and geopropolis

Among the stingless bee products, the most prominent are propolis and geopropolis, as they are used by bees, especially to protect the hive against microbial infections and to seal holes and crevices, it is also used in folk medicine for its therapeutic properties (Marcucci, 1995).

Propolis is a resinous material produced by bees, which mix resins collected from different parts of plants with salivary secretions and enzymes. The coloration varies from yellowish green to dark brown tones depending on the origin and age (Ghisalberti, 1979; Marcucci, 1995).

Geopropolis is the result of a mixture of resinous material from plants, wax, salivary secretions and earth or clay, which is used by bees to protect them against insects and microorganisms, using it to repair cracks or damage to the hive (thermal isolation and against enemies), covering the beehive wall, reinforcing the combs, preparing aseptic sites for the queen bee laying and mummifying invading insects. While to the propolis is not added to earth or clay (Kerr, 1987; Marcucci, 1995).

Propolis research has focused on *A. mellifera*, which has several biological activities. However, the therapeutic potential of propolis and geopropolis of stingless bees has been shown.

4.1 Chemical composition

Propolis is rich in phenolic compounds, of which flavonoids are the most abundant, as well as terpenoids, diterpenic acids, phenolic acids and coumarins. The

composition depends on the season, bee species, vegetation and collection area, and consequently can influence biological activity. Thus, various chemical types of propolis have been described according to the plant visited (Velikova et al., 2000; Massaro et al., 2014).

The choice of plant by the bees depends on the region. In southern Brazil, stingless bees collected Araucaria resins (Sawaya et al., 2007). However, *Tetragonisca angustula* bees in all regions of Brazil preferred *Schinus terebenthifolius* as a source of propolis plants (Sawaya et al., 2006). On the other hand, it has been reported that neither bee species nor geographic location determines the chemical composition of Meliponine propolis and the choice of its plant source, respectively. Bees use the first plant exudate they encounter during their flights (Velikova et al. 2000).

Geopropolis presents mainly polyphenolic compounds: phenolic acids (Abreu et al., 2006; Cunha et al., 2009; Cardozo et al., 2015; Araújo et al., 2016; Batista et al., 2016) tannins (Dutra et al. ., 2014), flavonoids (Silva et al., 2013; De Souza et al., 2013), prenylated coumarins and benzophenones (Da Cunha, 2016), terpenes: monoterpenes, sesquiterpenes, diterpenes and triterpenes, fatty acids, steroids and saponins (Bankova & Popova, 2007; Dutra et al., 2008; Araújo et al., 2015; Batista et al., 2016), volatile compounds (Torres-González et al., 2016). As in propolis, the chemical composition of geopropolis also varies according to the flora visited by the bees, the region and the time of collection (Bankova, 2009).

4.2 Biological activities

Because bees use these products as protection against infections, it has led to work that has evaluated their antibacterial and antifungal actions. In the early 2000s the analysis of propolis samples from 12 Brazilian stingless bees showed significant activity against *Staphylococcus aureus*, but weak activity against *Candida albicans* and weak or no action against *Escherichia coli*. In this study, a relationship between antibacterial activity and the percentage of diterpenic acids or the combination of gallic acid and diterpenic acids was observed (Velikova et al., 2000).

Similar results were found with *Trigona laeviceps* propolis from Thailand, where the aqueous extract and the methanolic extract inhibited in dose-dependent manner microbial growth in the following order of sensitivity: *S. aureus*> *E. coli*> *C. albicans*. However, neither one showed activity against *Aspergillus niger*. The antimicrobial activity of the aqueous extract was higher than that of methanolic, suggesting that the

antimicrobial action is due to the presence of polar substances (Umthong et al., 2009). Still in Thailand, Sanpa et al. (2015) also investigated the antibacterial activity of propolis collected by *T. laeviceps* and another stingless bee, *Tetrigona melanoleuca*. The ethanolic extract and the isolated compounds (six xanthones, one triterpene and one lignan) of *T. laeviceps* propolis showed higher antibacterial activity than *T. melanoleuca* propolis, which presented triterpenes as main constituents.

The evaluation of antimicrobial activity of *Melipona scutellaris* geopropolis of Brazil showed similar results. The antimicrobial activity was specially found against *S. aureus* and methicillin resistant *S. aureus* (MRSA) strains, as well as against *Streptococcus mutans*, with minimum inhibitory concentration (MIC) values below 50 μg/mL, and poor growth inhibition of gram-negative bacteria. However, the authors identified mainly nonpolar compounds, and benzophenones as the main active compounds (Da Cunha et al., 2013).

Melipona orbignyi propolis, collected in Mato Grosso do Sul, Brazil, showed activity against *S. aureus* (MIC = 3.1 mg/mL) and *C. albicans* (MIC = 3.1 mg/mL), and was not effective against *E. coli*. In the chemical composition predominated diterpenic acids and aromatic acids (Campos et al., 2014). Santos et al. (2017) using *M. orbignyi* geopropolis from the same locality obtained slightly different results. They demonstrated antimicrobial activity against gram-positive and gram-negative bacteria, and fungi, in the following sensitivity sequence: *S. aureus* (MIC = 6.1 mg/mL)> *E. faecalis*> *E. coli* (MIC = 10.5 mg/mL)> *P. aeruginosa*> *C. neoformans*> *C. albicans* (MIC = 23.0 mg/mL). Flavonoids, derived from glycosylated phenolic acids and terpenes (sesquiterpenes, diterpenes and triterpenes) were identified. The difference found in the antimicrobial activity of the last two mentioned studies may be related to the chemical composition, which may have been influenced by the way the extract was obtained, since Santos et al. (2017) made cold extraction with ethanol 70% and Campos et al. (2014) employed ethanol 80% in a hot extraction.

The antimicrobial action was demonstrated in other studies performed by the same group with stingless bees found in Brazil, *Tetragonisca fiebrigi* and *Melipona quadrifasciata anthidioides*. The ethanolic extract of *T. fiebrigi* propolis, containing phenolic compounds, alcohols, aromatic acids and terpenes, was active against gram-positive, gram-negative bacteria and fungi. However, activity was higher against gram-positive bacteria *S. aureus* (MIC = 0.55 mg/mL) (Campos et al., 2015). Similarly, the hydroethanolic extract of *M. q. anthidioides*, which showed di- and trigaloyl and

phenylpropanyl heterosidic derivatives, flavanones, diterpenes and triterpenes, induced cell death in all evaluated gram-positive, gram-negative and yeast bacteria, including clinical isolates resistant to antimicrobial drugs (Santos et al., 2017). Growth inhibition of *S. aureus* was also observed for propolis extracts of stingless bees from Australia (*Tetragonula carbonaria*), which presented flavanones in their composition (Massaro et al., 2014).

More recently, Georgieva et al. (2019) evaluated the antimicrobial and antioxidant activity of propolis collected from *Lisotrigona cacciae* bee hives in Vietnam. Compounds isolated from the extract inhibited *S. aureus*, *E. coli* and *C. albicans*, while the crude extract was inactive against *C. albicans*. For the isolated xanthone, α-mangostin, significant activity was observed against *S. aureus* with MIC and MBC of 0.31 μg/mL. However, for antioxidant action the extract and all tested compounds were inactive (IC_{50}> 1000 μg/mL), except for the 10,11-dihydroxydracaenone C compound which displayed good antioxidant capacity with IC_{50} of 116.0 μg/mL.

The results of these studies prove the antimicrobial potential of propolis and geopropolis of stingless bee, especially against *S. aureus*. Concerning constituents responsible for antibacterial action, phenolic compounds exert an important contribution, they inhibit the growth of bacteria, promoting damage to the cell membrane and inhibiting the synthesis of nucleic acids and energy metabolism. Flavonoids, for example, form a complex with cell wall components and therefore inhibit other adhesions. In addition, these compounds interfere with virulence factors such as enzymes, adhesins, and inflammatory cytokine production (Cushnie & Lamb, 2011; Farhadi et al., 2019).

Scaptotrigona postica geopropolis showed potential inhibition of herpes simplex virus type 1 (HSV-1) with effect on all stages of virus replication, also inhibiting virus entry into cells, which could be attributed to the high content of C-glycosylflavones (Coelho et al., 2015).

The biological properties of propolis and geopropolis are diverse and more recently studies have shown that these products from stingless bees from different parts of the world have anti-proliferative and cytotoxic action.

Stingless bee *Trigona laeviceps* is native to Thailand where it can be commercially cultivated to produce propolis in quantities comparable to *A. mellifera*. Propolis produced by *T. laeviceps* was extracted with water and methanol separately and tested for antiproliferative and cytotoxic activity on colorectal cancer cells (SW620). The results showed higher antiproliferative activity for the aqueous extract. Treatment of cells with

aqueous extract at a concentration of 200μg/mL caused changes in cell morphology and DNA fragmentation (Umthong et al., 2009). Then, justified by the fact that the use or consumption of propolis in the form of crude extract is not recommended, the same group decided to purify the ethanolic crude extract of *T. laeviceps* propolis using a bioassay-guided isolation procedure and test the *in vitro* antiproliferative activity in five cancer cell lines (Chago, KATO-III, SW620, BT474 and Hep-G2) compared to two normal cell lines (CH-liver) and fibroblasts (HS-27). The enrichment produced a fraction, 30DCM-F3, which provided the highest cytotoxicity in cancer cell lines but the lowest cytotoxicity in normal cells. The authors noted that the perform of more purification steps led to lower IC_{50} values (μg/mL) and the activity is derived from a combination of compounds (Umthong et al., 2011). Additionally, dichloromethane extracts from propolis collected from *Trigona sirindhornae*, Thailand's stingless bee, produced cytotoxic effects against metastatic squamous cells of head and neck carcinoma, with IC_{50} values of 199.8 ± 1.05 μg/mL (DMEP-B) and 76.33 ± 1.24 μg/mL (DMEP-C) (Utispan et al., 2017).

Stingless bee propolis of Indian origin showed potent antineoplastic activity in the MCF-7 (human breast cancer), HT-29 (human colon adenocarcinoma), Caco-2 (human epithelial colorectal adenocarcinoma), and B16F1 (murine melanoma) cell lines, with IC_{50} value of 250 μg/mL for all cell lines tested. The proposed mechanism of action is that propolis extract induces apoptosis due to DNA fragmentation, as evidenced by the TUNEL test (Choudari et al., 2013).

Kustiawan and colleagues published a sequence of studies evaluating the cytotoxic activity of Indonesian native stingless bee propolis. Initially, they did a screening with propolis of *Trigona* spp. against five cancer cell lines (HepG2, SW620, ChaGo-I, KATO-III and BT474), and observed that *T. incisa* propolis extract showed the highest *in vitro* cytotoxicity in SW620 cells (6% survival at 20 μg/mL) (Kustiawan et al., 2014). Then, the extract of this propolis was fractionated and tested against the same cell lines previously evaluated. The main active compound of the *T. incisa* propolis fraction (F45) was found to be a cardol isomer able to induce early apoptosis (programmed cell death) of SW620 cells at IC_{50} (4.51 μg/mL) and IC_{80} (6.84 μg/mL), respectively, within 2-6 h of incubation, and cell cycle arrest at the G1 subphase (Kustiawan et al., 2015). Finally, they demonstrated that cardol induces SW620 cell death through increased oxidative stress and mitochondrial apoptotic pathway (Kustiawan et al., 2017).

In Brazil, a study was conducted with two native bee species, *Scaptotrigona depilis* and *Melipona q. anthidioides*. In both, DPPH and ABTS assay, the ethanol extract

of propolis of *M. q. anthidioides* (EEP-M) showed better antioxidant activity (DPPH IC_{50} 60.91 μg/mL; ABTS IC_{50} 13.45 μg/mL) compared with ethanol extract of propolis of *S. depilis* (EEP-S) (DPPH IC_{50} not calculated; IC_{50} ABTS 80.04 μg/mL). The extracts showed concentration-dependent cytotoxicity. At the highest concentration tested (500 μg/mL), the cell growth of erythroleukemic cells (K562) were 32.6 ± 3.2% (EEP-S) and 21.2 ± 4.1% (EEP-M). The extract promoted death by necrosis and late apoptosis. However, no toxic or lethal effects were observed against the nematode *Caenorhabditis elegans*. The activities exerted by the extracts are attributed to their chemical composition, which includes phytosterols, terpenes, phenolic compounds, and tocopherol, and possibly to the synergy between different compounds present in propolis (Bonamigo et al., 2017).

With respect to the geopropolis of *M. q. anthidioides*, dos Santos et al. (2017) evaluated its chemical composition and therapeutic properties, demonstrating antioxidant, antimutagenic, anti-inflammatory and antimicrobial activities. The total concentrations of phenolic compounds and flavonoids present in hydroethanolic extract were 118.7 ± 2.8mg GAE/g extract and 25.4 ± 2.8mg QE/g extract, respectively. Di- and trigaloyl and phenylpropanyl heteroside derivatives, flavanones, diterpenes, and triterpenes were identified in the extract.

Propolis and geopropolis are products of stingless bee with richness of active compounds, known to possess interesting antiproliferative and cytotoxic properties. They showed activity in several human cancer cells like colorectal cancer cells. The mode of action involves induction of apoptosis mediates by the mitochondrial apoptotic pathway.

5. Pollen

Bees use plant species as trophic resources, which may be nectariferous plants, polliniferous and those that have both resources, i.e., polliniferous-nectariferous plants (Villanueva, 2002; Barth, 2004). Poliniferous plants are almost exclusively pollen suppliers for bees (Barth, 2004). According to Nogueira-Neto (1997) while collecting pollen from various flowers, the bees transport and transfer the pollen from one flower to another, performing entomophile pollination. They also carry pollen when sucking the nectar from the flowers. *Melipona* bees, especially *Melipona fasciculata*, collect botanical pollen from anthers of various plant species and mix with nectar and salivary secretions and put in pollen basket located on their tibias, forming pollen pellets (Nogueira-Neto, 1997; Castaldo & Capasso, 2002).

These bees collect and transport pollen to the hive, where it is stored, undergoing physicochemical changes due to fermentative processes (Penedo et al., 1976). These processes differ according to the bee group and allow better assimilation of nutrients and preservation of stored food (Machado, 1971). Food is stored in wax cells that look like small round pots when they are with honey and small tubes when they are with pollen (Freitas, 2003).

Although there are variations, the nest of most meliponines, of the genus *Melipona* and other genera, is formed by a brood area, consisting of horizontally overlapping combs, where there are several brood cells; and cerumen pots for storing pollen and honey (Nogueira-Neto, 1997).

5.1 Chemical composition

Regarding the chemical composition of stingless bee pollen, there are few studies when compared to those with pollen from *A. mellifera*. Bee pollen is the result of agglutination of different pollen grains harvested by bees, mixed with nectar and its salivary substances (Silva et al., 2009; Morais et al., 2011), which stands out for its therapeutic properties, as well as food supplement, due to the presence of nutritive components such as proteins, essential amino acids, vitamins, lipids, and minerals (Villanueva et al., 2002; Marchini et al., 2006; Feás et al., 2012; Komosinska-Vassev et al., 2015).

Pollen contains phenolic acids, flavonoids and triterpenes (Campos et al., 1997; Villanueva et al., 2002; Silva et al., 2006). Phenolics and flavonoids are extremely beneficial to human health because they reduce the risk of degenerative diseases, reducing oxidative stress (Silva et al., 2006; Feás et al., 2012; Komosinska-Vassev et al., 2015). Chemical composition may vary according to plant species, environmental conditions, age and plant nutritional status (Funari et al., 2003; Barreto et al., 2006).

5.2 Biological activities

In popular practice, bee pollen has been used to relieve colds, flu, ulcers and early aging (Lyngheim & Scagnetti, 1979; Hanssen, 1979), as well as nutritional supplementation, as it contains nutritionally essential substances (Fernandes-da-Silva & Serrão, 2000), being considered a super food that can improve the functions of the brain, heart, liver and prostate (Fratellone et al., 2016).

Lopes et al. (2019) demonstrated higher anti-inflammatory and antinociceptive effects for the first time from the pollen collected by *M. fasciculata* compared to products to others bees, including Apis mellifera, evidencing the potential use of stingless bee products in the development for new therapeutic agents.

The product of stingless bees with less scientific production evaluating therapeutic potential is pollen (Figure 1). In 2014, Kustiawan et al. evaluated samples from four species from Indonesia: *Trigona incisa*, *Trigona fusco-balteata*, *Trigona fuscibasis* and *Timia apicalis*. Overall, it was observed that pollen extracts had lower cytotoxic activity than propolis and honey.

6. Cerumen

Meliponines produce the cerumen by mixing plant resin with wax. According to the type and quantity of resin the color can be from very light yellow to dark coloured. Cerumen is a pliable and soft material used to construct brood cells, storage pots, involucrum, as well as nest entrance, which serves in a defense mechanism against undesirable visitors (Jalil, 2014).

The cerumen is very little investigated and was first evaluated less than a decade ago by Massaro et al. (2011), which samples from colonies of *Tetragonula carbonaria*, an Australian bee, were analyzed for chemical composition definition using mass spectrometry techniques, such as gas chromatography-mass spectrometry (GC-MS), and for evaluation of the *in vitro* inhibition capacity of lipoxygenase enzyme activity. A rich composition of diterpenes and phenolic acids was observed, and the inhibition of 5-lipoxygenase was similar to Trolox. Hamilton et al. (2017) confirmed radical scavenging activity and interference with 5-LOX-LTB4 signaling of *T. carbonaria* cerumen extracts. However, they pointed out that further investigation would be needed to determine if the extracts will provide therapeutic benefits for medical conditions in which oxidative stress and inflammation are implicated.

Tetragonula laeviceps colony cerumen from Thailand was purified to obtain the bioactive compound α-mangostin. The pure compound displayed cytotoxic activity in five cell lines. The mechanism of action involves caspase-independent apoptosis. In addition, *in vivo* cytotoxicity and angiogenesis were evaluated in zebrafish embryos with monitoring of the expression of genes involved in angiogenesis (*vegfaa* and *vegfr2*) by real time PCR. All tests have proven the potential of α-Mangostin, isolated from *T.*

laeviceps cerumen, as a compound with high anticancer potential (Nugitrangson et al., 2016).

This study identified 104 biological evaluations carried out in studies with the various stingless bee products from 2000 to 2020. The review shows the growth of knowledge produced in this area of science over the years. The most analyzed product was propolis, and the least evaluated were cerumen and pollen (Figure 1). There was a predominance of studies assessing cytotoxic and antimicrobial activities. Antioxidant action was reported only 11 times and anti-inflammatory action in a much smaller amount only 4 times (Figure 2). Despite the number of biological studies, in this review was not found studies about the therapeutic potential of stingless bee products in diseases such as diabetes, immunological disorders, parasitic and cardiovascular diseases. More importantly, studies on stingless bee products that have not been studied within the wide diversity of *Meliponini* that exist are lacking.

Interestingly, many researches on biological activity have performed the chemical characterization of the products. Some studies have used bioassay-guided isolation procedures. From crude extracts to isolated molecules were tested. The detailed data on chemical composition helps to understand if the compounds act synergistically to achieve the effect or if the action is due to a specific compound. These data may guide the technological use of the total extracts or purified fractions, or even isolated substances into medicines, healthy foods and food supplements, and cosmetics.

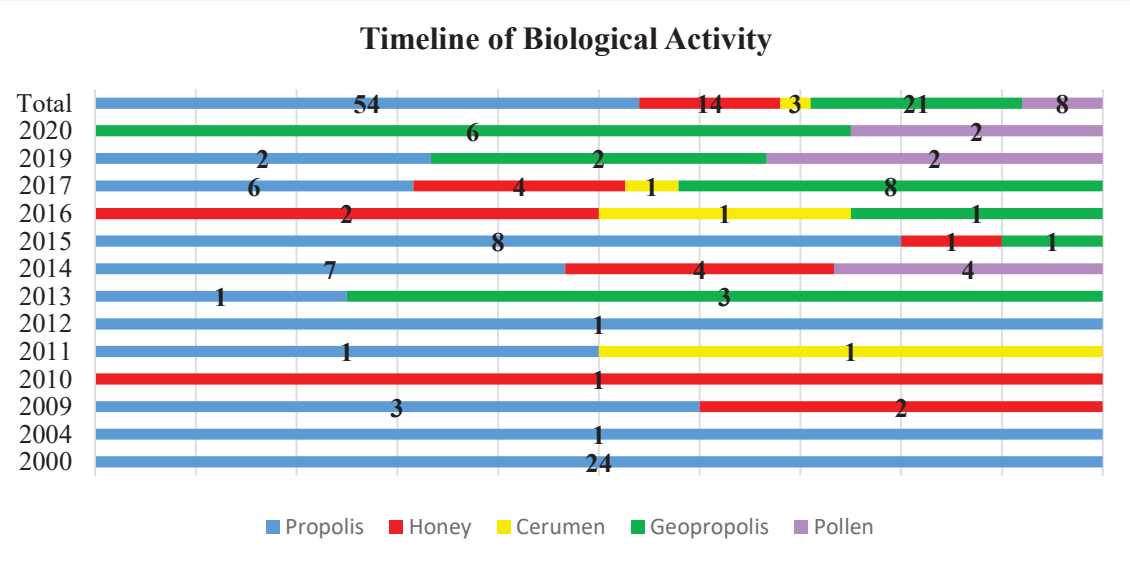

Figure 1. Timeline of studies with products from stingless bee.

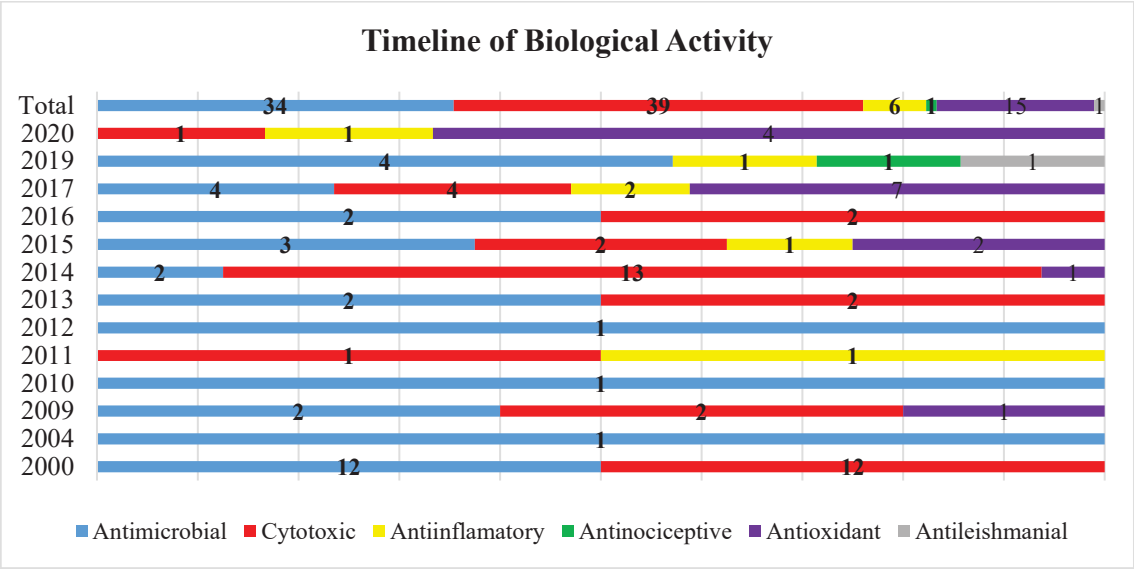

Figure 2. Timeline of biological activities evaluated with products from stingless bee.

Regarding the place of origin of the product used in the studies, samples of stingless bees found in South and Central America, including Mexico, Africa, Asia and Australia (Figure 3) were used, where bees are native (Kerr & Maule, 1964; Rasmussen & Cameron, 2010).

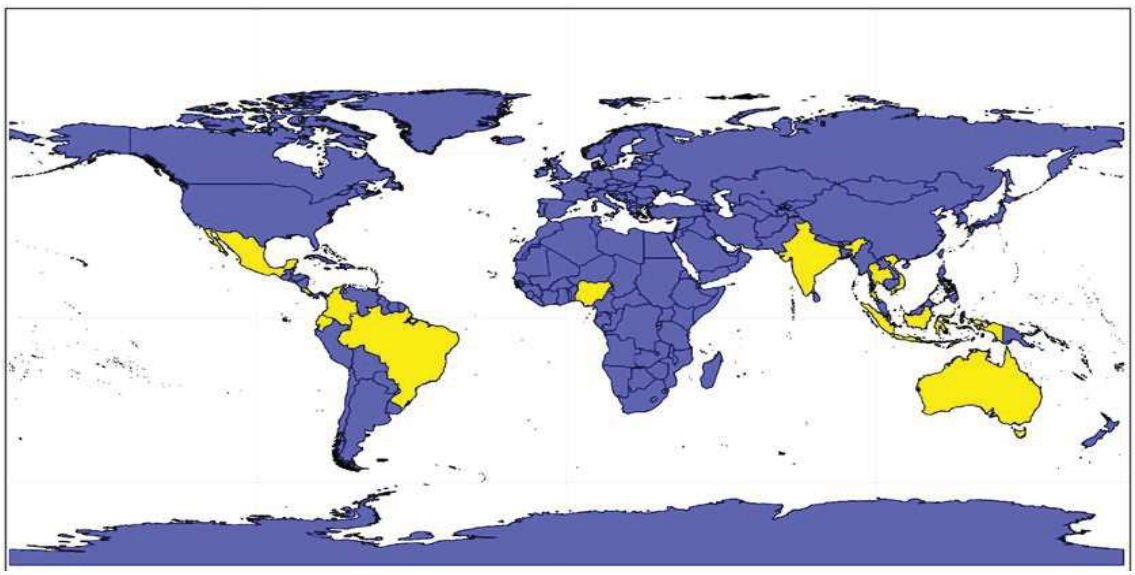

Figure 3. Distribution (in yellow) of the origin of stingless bee products evaluated in published studies on biological activity.

Table 1
Biological activity studies of products from stingless bee, distributed by author and year, country where the sample was collected, type of product, bee species, biological activity, and chemical analysis.

Author	Year	Country	Product	Species	Biological Activity	Chemical Analysis
Velikova et al.	2000	Brazil	Propolis	*Melipona quadrifasciata*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Melipona quadrifasciata*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Melipona favosa*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Melipona favosa*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Melipona scutellaris*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Melipona scutellaris*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Melipona marginata*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Melipona marginata*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Scaptotrigona bipunctata*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Scaptotrigona bipunctata*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Plebeia remota*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Plebeia remota*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Plebeia spp*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Plebeia spp*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Plebeia droryana*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Plebeia droryana*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Tetragonisca angustula*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Tetragonisca angustula*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Nanotrigona testacularis*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Nanotrigona testacularis*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Lestrimellata spp*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Lestrimellata spp*	Cytotoxic	Yes
Velikova et al.	2000	Brazil	Propolis	*Tetragona clavipes*	Antimicrobial	Yes
Velikova et al.	2000	Brazil	Propolis	*Tetragona clavipes*	Cytotoxic	Yes
Torres et al.	2004	Colombia	Honey	*Tetragonisca angustula*	Antimicrobial	Yes
Guerrini et al.	2009	Ecuador	Honey	*Melipona sp*	Antioxidant	Yes
Guerrini et al.	2009	Ecuador	Honey	*Melipona sp*	Antimicrobial	Yes
Umthong et al.	2009	Thailand	Propolis	*Trigona laeviceps*	Antimicrobial	No
Umthong et al.	2009	Thailand	Propolis	*Trigona laeviceps*	Antiproliferative	No
Umthong et al.	2009	Thailand	Propolis	*Trigona laeviceps*	Cytotoxic	No
Boorn et al.	2010	Australia	Honey	*Trigona carbonaria*	Antimicrobial	No
Massaro et al.	2011	Australia	Cerumen	*Tetragonula carbonaria*	Antiinflamatory	Yes
Umthong et al.	2011	Thailand	Propolis	*Trigona laeviceps*	Antiproliferative	No
Choudhari et al.	2012	India	Propolis	*Trigona sp*	Antimicrobial	Yes
Choudhari et al.	2013	India	Propolis	*Trigona sp*	Anticancer	No
Choudhari et al.	2013	India	Propolis	*Trigona sp*	Antioxidant	No
Da Cunha et al.	2013	Brazil	Geopropolis	*Melipona scutellaris*	Antimicrobial	Yes
Da Cunha et al.	2013	Brazil	Geopropolis	*Melipona scutellaris*	Antiproliferative	Yes
Da Cunha et al.	2013	Brazil	Geopropolis	*Melipona scutellaris*	Antibiofilm	Yes
Campos et al.	2014	Brazil	Propolis	*Melipona orbignyi*	Antimicrobial	Yes
Campos et al.	2014	Brazil	Propolis	*Melipona orbignyi*	Antioxidant	Yes
Campos et al.	2014	Brazil	Propolis	*Melipona orbignyi*	Cytotoxic	Yes
Kustiawan et al.	2014	Indonesia	Propolis	*Trigona incisa*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Honey	*Trigona incisa*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Pollen	*Trigona incisa*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Propolis	*Trigona apicalis*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Honey	*Trigona apicalis*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Pollen	*Trigona apicalis*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Propolis	*Trigona fuscobalteata*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Honey	*Trigona fuscobalteata*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Pollen	*Trigona fuscobalteata*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Propolis	*Trigona fuscibisca*	Anticancer	No
Kustiawan et al.	2014	Indonesia	Honey	*Trigona fuscibisca*	Anticancer	No

Table 1 (continued)

Author	Year	Country	Product	Species	Biological Activity	Chemical Analysis
Kustiawan et al.	2014	Indonesia	Pollen	*Trigona fuscibisca*	Anticancer	No
Massaro et al.	2014	Australia	Propolis	*Tetragonula carbonaria*	Antimicrobial	Yes
Campos et al.	2015	Brazil	Propolis	*Tetragonisca fiebrigi*	Antimicrobial	Yes
Campos et al.	2015	Brazil	Propolis	*Tetragonisca fiebrigi*	Antioxidant	Yes
Campos et al.	2015	Brazil	Propolis	*Tetragonisca fiebrigi*	Antiinflamatory	Yes
Campos et al.	2015	Brazil	Propolis	*Tetragonisca fiebrigi*	Cytotoxic	Yes
Coelho et al.	2015	Brazil	Geopropolis	*Scaptotrigona postica*	Antiviral	Yes
Cauich et al.	2015	Mexico	Honey	*Melipona beecheii*	Antioxidant	Yes
Kustiawan et al.	2015	Indonesia	Propolis	*Trigona incisa*	Cytotoxic	Yes
Kustiawan et al.	2015	Indonesia	Propolis	*Trigona incisa*	Anticancer	Yes
Sanpa et al.	2015	Thailand	Propolis	*Tetragonula laeviceps*	Antimicrobial	Yes
Sanpa et al.	2015	Thailand	Propolis	*Tetrigona melanoleuca*	Antimicrobial	Yes
Bartolomeu et al.	2016	Brazil	Geopropolis	*Melípona fasciculata*	Anticancer	Yes
Nishio et al.	2016	Brazil	Honey	*Scaptotrigona bipunctata*	Antimicrobial	No
Nishio et al.	2016	Brazil	Honey	*Scaptotrigona postica*	Antimicrobial	No
Nugitrangson et al.	2016	Thailand	Cerumen	*Tetragonula laeviceps*	Anticancer	Yes
Bonamigo et al.	2017	Brazil	Propolis	*Melipona quadrifasciata*	Antioxidant	Yes
Bonamigo et al.	2017	Brazil	Propolis	*Melipona quadrifasciata*	Cytotoxic	Yes
Bonamigo et al.	2017	Brazil	Propolis	*Melipona quadrifasciata*	Toxic	Yes
Bonamigo et al.	2017	Brazil	Propolis	*Scaptotrigona depilis*	Antioxidant	Yes
Bonamigo et al.	2017	Brazil	Propolis	*Scaptotrigona depilis*	Cytotoxic	Yes
Bonamigo et al.	2017	Brazil	Propolis	*Scaptotrigona depilis*	Toxic	Yes
Dos Santos et al.	2017	Brazil	Geopropolis	*Melipona quadrifasciata*	Antimutagenic	Yes
Dos Santos et al.	2017	Brazil	Geopropolis	*Melipona quadrifasciata*	Antiinflamatory	Yes
Dos Santos et al.	2017	Brazil	Geopropolis	*Melipona quadrifasciata*	Antimicrobial	Yes
Dos Santos et al.	2017	Brazil	Geopropolis	*Melipona quadrifasciata*	Antioxidant	Yes
Hamilton et al.	2017	Australia	Cerumen	*Tetragonula carbonaria*	Antioxidant	Yes
Kustiawan et al.	2017	Indonesia	Propolis	*Trigona incisa*	Anticancer	No
Nweze et al.	2017	Nigeria	Honey	*Hypotrigona sp*	Antioxidant	Yes
Nweze et al.	2017	Nigeria	Honey	*Melipona sp*	Antioxidant	Yes
Santos et al.	2017	Brazil	Geopropolis	*Melipona orbignyi*	Antioxidant	Yes
Santos et al.	2017	Brazil	Geopropolis	*Melipona orbignyi*	Antiinflamatory	Yes
Santos et al.	2017	Brazil	Geopropolis	*Melipona orbignyi*	Antimutagenic	Yes
Santos et al.	2017	Brazil	Geopropolis	*Melipona orbignyi*	Antimicrobial	Yes
Utispan et al.	2017	Thailand	Propolis	*Trigona sirindhornae*	Anticancer	Yes
Zamora et al.	2017	Costa Rica	Honey	*Tetragonisca angustula*	Antibiofilm	No
Zamora et al.	2017	Costa Rica	Honey	*Melipona beecheii*	Antibiofilm	No
Cambronero-Heinrichs et al.	2019	Costa Rica	"Microbiota"	*Tetragonisca angustula*	Antibiotic	No
Dutra et al.	2019	Brazil	Geopropolis	*Melipona fasciculata*	Antileishmanial	Yes
Georgieva et al.	2019	Vietnã	Propolis	*Lisotrigona cacciae*	Antimicrobial	Yes
Lopes et al.	2019	Brazil	Pollen	*Melipona fasciculata*	Antiinflamatory	Yes
Lopes et al.	2019	Brazil	Pollen	*Melipona fasciculata*	Antinociceptive	Yes
Ramón-Sierra et al.	2019	Mexico	Propolis	*Melipona beecheii*	Antimicrobial	Yes
Souza Júnior et al.	2019	Brazil	Geopropolis	*Melipona subnitida*	Antimicrobial	Yes
Lopes et al.	2020	Brazil	Pollen	*Scaptotrigona affinis postica*	Antiinflamatory	Yes
Lopes et al.	2020	Brazil	Pollen	*Scaptotrigona affinis postica*	Antioxidant	Yes
Silva et al.	2020	Brazil	Geopropolis	*Melipona mandacaia*	Antioxidant	Yes
Sousa-Fontoura et al.	2020	Brazil	Geopropolis	*Melipona subnitida*	Wound healing	Yes
Barboza et al.	2020	Brazil	Geopropolis	*Melipona fasciculata*	Cytotoxic	Yes
Barboza et al.	2020	Brazil	Geopropolis	*Melipona fasciculata*	Antiinflamatory	Yes
Barboza et al.	2020	Brazil	Geopropolis	*Melipona fasciculata*	Antioxidant	Yes

7. Conclusion

The studies realized along the time evidenced important biological activities of meliponine products, specially of propolis, including antimicrobial, cytotoxic, antioxidant, anti-inflammatory, antinociceptive and antileishmanial. However, the results should stimulate several options for future research into the therapeutic properties of all these stingless bee products. Finally, we hope that their beneficial effects will be properly used.

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4.2 Capítulo II

Melipona fasciculata Smith's pollen: nutrient composition and nutraceutical potential

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Melipona fasciculata Smith's pollen: nutrient composition and nutraceutical potential

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Highlights

- Comprehensive nutrient profiles, including Fatty Acids, for *Melipona fasciculata*'s pollen are presented;
- Principal component analysis for nutritional parameters showed 3 main components;
- Palynological study identified main types of pollen used at each region and a dendrogram graphic explain its similarity;
- Pollen Extracts were evaluated for Flavonoid and Polyphenol contents.
- Cloroformic fraction showed antioxidant and antiinflamatory potential.

Abstract

Research into bioactive ingredients for human use has increased as result of greater consumer awareness of the relationship between these products and associated benefits related to health and well-being. Bee products such as honey, propolis, geopropolis, wax, royal jelly and pollen have been used since antiquity for their therapeutic properties and nutraceutical potential. Considering the production chain, bioactivity and chemical composition of *Melipona fasciculata* products this study proposed the palynological characterization, physicochemical, chemical, nutritional profile, antioxidant and anti-inflammatory properties of *M. fasciculata* pollen extracts cultivated in Maranhão. A total of 17 pollen samples of *M. fasciculata* were collected between April 2015 and April 2017 in 5 municipalities in the state of Maranhão, covering the biomes with the largest area in the state in some of their phytogeographic profiles. The palynological analysis of the collected samples showed the dominant pollen types in Santa Luzia do Paruá as *Borreria verticillata* (Rubiaceae), *Hyptis sp*. (Lamiaceae), *Protium sp*. (Burseraceae), *Senna sp*. (Fabaceae) and *Mauritia sp*. (Arecaceae), Palmeirândia, Viana and Anajatuba were *Pontederia parviflora* (Pontederiaceae) and *Myrcia sp*. (Myrtaceae) and those of Chapadinha, *Mimosa sp*. (Mimosaceae) and *Eucalyptus sp*. (Myrtaceae). The microbiological analysis showed all the samples with very low presence of microbiological contaminants. The nutritional composition indicated that the samples presented similarity and that they are within the parameters recommended by the Ministry of Agriculture, except for humidity for two samples and ash content for one. The samples were grouped according to their locality of collection and climatic seasonality (dry season and rainy season), after which they were extracted by maceration for 72 hours with 70% ethanol and 1: 8 hydromodule, totalizing 10 pollen extracts. The extraction yield varied between 53.8 and 64.8%. The extracts obtained were submitted to the total flavonoid and total phenolic compounds assays. The extracts of pollen from Santa Luzia do Paruá (rainy

season), Palmeirândia (dry season), Viana (dry season), Anajatuba (rainy season) and Chapadinha (dry season) were submitted to high efficiency liquid chromatography with UV detector -Vis. The presence of β-sitosterol, ellagic acid, coumaric acid, isoquercitrin, quercitrin, myricetin, naringenin, canferol, catechin, β-amirine, coumaric acid, rutin, quercetin, gallic acid and apigenin. The extracts obtained from the pollen samples of *M. fasciculata* collected in Santa Luzia do Paruá (ESLPs and ESLPc), Palmeirândia (EPALs and EPALc) and Chapadinha (ECHAs) were fractionated and each of their fractions was submitted to the evaluation of the antioxidant activity by the DPPH assay. The result of the evaluation of the antioxidant activity expressed in EC_{50} varied between 156.24 and 1764.67 μg/mL. The chloroform fractions of all the samples presented better antioxidant activity. The extracts obtained from pollen samples of *M. fasciculata* collected in Santa Luzia do Paruá in the rainy season (ESLPc), Palmeirândia in the rainy season (EPALc) and Chapadinha in the dry season (ECHAs) were used to evaluate the anti-inflammatory activity *in vitro* by the inhibition test of cyclooxygenases 1 and 2. The Chapadinha sample presented the highest percentages of enzymatic inhibition in the concentrations of 10 and 50 μg/mL, reaching to inhibit 61.7 and 58.6% of COX1 and COX2, respectively

Keywords: Stingless bee; Meliponiculture; Nutrient composition; Fatty acids; Biodiversity; Food analysis; Food composition; Antiinflamatory; Antioxidant.

1. Introduction

Research on bioactive ingredients for human consumption has developed as a result of increased consumer awareness of the relationship between the use of bioactive ingredients and the benefits associated with maintaining health and well-being. However, the interrelationships between diet and health are complex and require representative and accurate food composition data (Paz et al., 2015).

In the quest for a healthy diet there is a tendency in the search for functional foods, defined by Stringheta et al. (2007) as "foods that have proven to beneficially affect one or more organic functions, in addition to the appropriate nutritional effects, in a way that is relevant to improving health and preventing and/or reducing the risk of disease".

Growing evidence supports the hypothesis that functional foods containing physiologically active components can improve health. However further investigations are needed to corroborate the potential benefit of foods in which the diet-health relationship is not scientifically validated to a sufficient extent (Assmann et al., 2014).

Bee products such as honey, propolis, geopropolis, wax, royal jelly and pollen have been used since ancient times for their therapeutic actions and nutraceutical potential (Rao et al., 2016).

Bee pollen (*Apis mellifera*) nutritional products of major commercial importance are honey, due to its energy value, and pollen, because its nutritional value. The consumption of pollen for the purpose of food supplementation has been outstanding in

recent years, since it contains large amounts of fatty acids, carbohydrates and amino acids, besides having legal devices that establish parameters of quality, making it possible to commercialize them in several countries (Yang et al., 2013).

Bee pollen is the result of the agglutination of different pollen grains harvested by bees, mixed with nectar and salivary substances, and their chemical composition varies according to plant species, environmental conditions, age and nutritional status of the plant from which the grains of pollen were collected (Campos et al., 2008; Morais et al., 2011). Several biological actions of bee pollen have been demonstrated: prevention of prostate disorders (Shoskes & Manickam, 2003), immunomodulatory, antimutagenic, anti-inflammatory and antioxidant (Pascoal et al., 2014).

There are other bees that accumulate pollen known as stingless bees (Hymenoptera: Apidae: Meliponini) because they have the atrophied stinger. The cultivation of these bees, Meliponiculture, has been practiced mainly by traditional people from tropical regions (Nogueira-Neto, 1997).

Meliponiculture is sustained by cultivation of many bee species in Brazil based on relative abundance in each region. At Maranhão state it stands out mainly for the cultivation of *Melipona (Melikerria) fasciculata* (tiúba*)*. This activity is one of the main sources of income for several indigenous and rural families that cultivate it for the production of honey, wax, and pollen (Albuquerque et al., 2013).

However, there are few studies on nutritional profile, chemical composition and biological activities of *Melipona fasciculata* pollen.

The objective of this study was to determine the nutrient composition and its nutraceutical potential of pollen accumulated by the stingless bee *Melipona fasciculata* from four different phytogeographic regions in the state of Maranhão, Brazil, and to examine its antioxidants activities.

2. Materials and methods

2.1. Sampling protocol

In this study, 16 samples of bee pollen were collected from beehives of *Melipona fasciculata* (Tiúba) in 4 regions: 4 samples from Santa Luzia do Paruá (SLP: S 02º 52’349” W 45º 98’302”), 4 samples from Palmeirândia (PAL: S 02º 42’121” W 44º 53’452”), 4 samples from Viana (VIA: S 03º 10’131” W 44º 53’464”) and 4 samples from Chapadinha (CHA: S 03º 73’445” W 43º 31’908”), four municipalities belonging to the

state of Maranhão in the Northeast region of Brazil (Figure 1). These regions have differences in their diversity of flora. Environmental conditions found in Santa Luzia do Paruá allow higher humidity (Amazonia), Palmeirândia and Viana belongs to flooded fields, Chapadinha comprises more savanna like flora and has a drier season. These differences in floristic composition between the four regions can influence the physical and chemical composition of pollen stored by *Melipona fasciculata* bee. Each sample was identified using uppercase - SLP. PAL, VIA or CHA, representing the geographic origin, lowercase - d and r, representing dry or rain season and numbers (Table 1).

Figure 1. Location of collected samples.

The samples from each municipality were grouped according to the season of the year in which they were collected (Table 1). The in natura pollen were submitted to drying with air circulation at 38ºC, extracted by maceration with 70% ethanol/water (70:30, v/v) for 72h, with hydromodule of 1:8. The extractive solutions was filtered through Whatman no. 1 filter paper (Whatman, Durham, UK) in a Buchner funnel and concentrated to a small volume at 40 °C in a rotary evaporator under vacuum, obtaining the hydroalcoholic extract of pollen.

Table 1

Pollen extracts codification.

Pollen Samples	**Extract**
SLPd1 + SLPd2	SLPd
SLPr1+ SLPr2	SLPr
PALd1 + PALd2	PALd
PALr1 + PALr2	PALr
VIAd1 + VIAd2	VIAd
VIAr1 + VIAr2	VIAr
CHAd1 + CHAd2	CHAd
CHAr1 + CHAr2	CHAr

2.2 Palynological identification

Pollen samples were collected directly from the pollen pots in the *M. fasciculata* hives. Flowering plant species were also collected (about 1000m radius of the site) and sent for identification at the Maranhão Herbarium (MAR) of the Federal University of Maranhão (UFMA). The anthers of the plant species and the pollen of the collected meliponaries were prepared according to the methodology of Almeida-Muradian et al. (2005).

Slides were then prepared using glycerin jelly for optical microscopy analysis. The pollen grains were separated into pollen types according to their morphology. The identification of pollen types was based on the reference collection of plant pollen slides from the reference of the UFMA Bee Studies Laboratory. The classification system adopted for the level of family was the APG III (2009).

In total, 500 pollen grains from each pollen sample were counted. The means of three pollen samples were calculated. The quantitative results were classified as

frequency classes (Louveaux et al., 1978): predominant pollen ‘dp’ (more than 45% of the grains counted), secondary pollen ‘sp’ (15% to 45%), important minor pollen ‘imp’ (3% to 15%) and minor pollen ‘mp’ (less than 3%).

Minitab® 15 software was used to generate the dendrograms of percentage similarity of the types of pots analyzed.

2.3 Microbiological Determinations

In order to evaluate the microbial quality of the *Melipona fasciculata* bee pollen samples, they were analyzed for the presence of mesophilic microorganisms, yeasts and moulds, *Escherichia coli* and *Salmonella* (APHA, 2001).

2.3.1 Sample Preparation

A mass of 25 g of bee pollen were aseptically taken and homogenized for 3 min with 225 mL of pre-chilled (4 ± 0.5 °C) sterile peptone-physiological saline solution (0.1% neutral peptone + 0.85% NaCl in sterile deionized L1O, pH = 7.0 ± 0.05). Decimal serial dilutions were prepared from this homogenate in the same chilled sterile diluents (1:10 v/v). (APHA, 2001)

2.3.2 Enumeration of the Total Mesophilic Microorganisms

The total quantification of aerobic mesophilic microorganisms was done with sowing by incorporation of 1 mL for each decimal dilution to Petri plates culture medium containing Plate Count Agar (PCA - Himedia, Mumbai, India). The colony counting was made after incubation plates at 30 °C for 72 h and the results were expressed as colony forming units per gram of bee pollen ($cfu \cdot g^{-1}$) (APHA, 2001).

2.3.3 Enumeration of Yeast and Moulds

Moulds and yeasts enumeration was made on DG18 (dichloran-glycerol agar) and incubated at 25 °C for 5 days. Microbial counts were expressed as $cfu \cdot g^{-1}$.

2.3.4 Detection of *Escherichia coli*

For the quantification of total coliforms and *Escherichia coli*, SimPlate kit system from Bio Control (Merck, Darmstadt, Germany), was used. The supplied culture medium was pre-hydrated in 9 mL of distilled water, inoculated with 1 mL of the stock suspension and homogenized by vortex, following the manufacturer’s recommendations. The

contents were poured onto plates containing 84 wells. The liquid was evenly spread across the wells in a circular motion at low speed and the excess was removed. The plates were incubated at 35 °C for 24 to 48 h. The quantification of total coliforms was performed by counting the number of wells in which the color change occurred in the culture medium. The quantification of *E. coli* was performed by counting the number of wells in which the fluorescence was observed after exposure to a plate of ultraviolet light (UV) at 365 nm.

2.3.5 Detection of *Salmonella sp.*

The detection of *Salmonella* sp. was carried out using the immunodiffusion 1–2 test, as described in the AOAC method (2012). The results were obtained 16–20 h after pre-enrichment in buffered peptone water and the results were interpreted visually, by monitoring the development of an immunoband, which is a characteristic immobilization pattern of cells.

2.4 Physicochemical Analyses

2.4.1 Moisture Content

Approximately 2 g of each sample of bee pollen was dried at a temperature of 105 °C, until constant weight (AOAC, 2012).

2.4.2 Ash Content

The ash content was determined after ignition at 550 ± 15 °C by gravimetry. This determination was carried out according to AOAC (2012).

2.4.3 pH Values

pH was measured in the aqueous phase obtained after mixing 10 g of pollen in 75 mL of distilled water by a pH combined electrode connected to a pH 211 Microprocessor pH Meter (Hanna Instruments, Limena, Italia).

2.4.4 Total Acidity

Regarding total acidity, 2.5 g of bee pollen were mixed with CO2-free water and the titration was started with NaOH 0.05 N in a 5 mL per minute flow, until reaching the pH 8.5. This determination was carried out according to AOAC (2012).

2.4.5 Protein Content

Nitrogen content was determined using the Kjeldahl method (Quimis, Brazil). The crude protein (CP) content was calculated using the conversion factor of 6.25 (N × 6.25) (AOAC, 2012).

2.4.6 Fiber Content

The fiber content was determined according to the method of Van Soest et al. (1991) as adapted for Komarec (1993) and Senger et al. (2008), using neutral detergent and digestion by autoclaving.

2.4.7 Total Phenolic Content

The total polyphenol content in the hydroalcoholic extract of pollen was performed using Folin-Ciocalteau reagent and 20% sodium carbonate (NaCO3), like was described in Batista et al (2016). The TPC was calculated using a gallic acid calibration curve (2.5–40.0 µg/mL) and expressed as gallic acid equivalent (%). Analyses were performed in triplicate and the mean value was calculated for each sample (Dutra et al., 2014).

2.4.8 Flavonoid Content

For total flavonoid concentration we used photocolorimetric method with 5% methanolic aluminum chloride solution ($AlCl_3$), like was described in Lopes et al (2020). The concentration was calculated from the calibration curve constructed with standard quercetin solution (Merck, Germany) (1–30.00 µg/mL) and expressed as quercetin equivalent (%). The analyses were performed in triplicate (Dutra et al., 2008)

2.4.9 Reducing Sugars

Reducing sugars were determined using the DNS (dinitrosalicylic acid) method (AOAC, 2012).

2.5 Chemical Identification

Chemical identifications will be performed by gas chromatography coupled to mass spectrometry (GC-MS) and by high performance liquid chromatography with ultraviolet detector (HPLC-UV).

2.5.1 Determination of the Fatty-Acid Profile

The crude fat (CF) was extracted with petroleum ether using an automatic Soxtec device (FOSS, Soxtec™ 2050, Höganäs, Sweden) (AOAC, 2012). Fatty acid methyl esters were prepared from the extracted CF fraction by transesterification using MeOH in the presence of H2SO4. A sample containing 20 ± 0.5 mg of lipids was redissolved in 0.75 mL n-hexane; then 0.1 mL of 2 N KOH in MeOH was added and the solution was mixed in a vortex mixer, for 2 min (Quimis, Brazil) and dried over anhydrous Na_2SO_4. After phase separation, the superior layer of n-hexane containing the fatty acid esters was removed and injected on a gas-chromatograph model GC (Shimadzu, Japan) equipment, equipped with a split/spitless injector, a flame ionization detector (FID) and a column (30 m × 0.32 mm ID × 0.25 μm: stationary phase of 50% cyanopropylmethyl-50% phenylmethylpolysiloxane). The analysis of the methyl esters was performed using H2 as carrier gas at a pressure of 0.6 bar and at split ratio was of 1:40. The flow rate of the carrier gas was set at 4.0 mL·min−1. A thermal gradient from 170 to 240 °C at 3.5 °C·min−1 was used with the injector and detector temperatures at 240 °C. The injection volume was 1 μL. The Fatty acid methyl esters were identified by comparing the retention times of the peaks obtained in the bee pollen sample with those of known reference esters. The fatty acid composition was expressed as percent of major fatty acid methyl esters from the peak areas.

2.5.2 Determination of Phenolics

Pollen extracts were analyzed in a high-performance liquid chromatograph (Thermo Finnigan Surveyor Plus) coupled to an ultraviolet detector. An ACE 5 C-18 reverse phase analytical column (250 x 4.60 mm, 5 μm) was used. The sample compounds were separated at room temperature using a 0.6 mL/min flow gradient elution. The mobile phase consisted of purified water containing 0.1% acetic acid (A) and methanol (B). The gradient used was: 0-2 min, 5% B; 2-50 min, 5-80% B. The injection volume in the system was 10 μL and detection was performed at 254 nm. Identification was performed based on co-injection with standards.

2.6 Biological activity

2.6.1 Determination of *in vitro* antioxidant activity of pollen extracts

The antioxidant activity of hydroalcoholic pollens extracts and fractions was evaluated by using the DPPH free radical scavenging assay as described by Brand-Willians (1995) with modifications as described by our research group in Dutra et al. (2014). The samples of pollens extracts were solubilized on methanol at concentrations (30-480 µg/mL) different concentrations and added to a methanol solution of DPPH in methanol (40.0 µg/mL). After 30 minutes of reaction at room temperature in the dark, the absorbance of each solution was read at 517 nm in a Lambda 35 UV-Vis spectrophotometer (PerkinElmer, Inc., Waltham, MA, USA). Methanol ACS was used as a control and DPPH solution was used as blank. Trolox® (positive control) standards were treated under the same conditions as the samples. The percentage of antioxidant activity (% AA) was calculated by the equation:

$$\% \text{ AA} = (\%) = 100 - [(A_{\text{sample}} - A_{\text{blank}}) \times 100/A_{\text{control}}],$$

where A_{control} is the absorbance of the control (solution with DPPH radical and methanol) and A_{sample} is the absorbance of the radical in the presence of the samples.

The percentage of antioxidant activity was related to the sample concentration to obtain the efficient concentration (EC50), defined as the sample concentration required to cause a 50% inhibition of the initial DPPH concentration. All experiments were performed in triplicate.

2.6.2 *In vitro* anti-inflammatory activity evaluation of pollen extracts

The assay was performed according to the manufacturer's recommendations (Cox colorimetric inhibitor screening - Cayman Chemical®) in 96-well plates. Using the reagents: 0.1M tris HCl buffer pH 8, heme solution diluted with tris-HCl buffer; Cox-1 and Cox-2 enzymes; arachidonic acid; Potassium hydroxide; deionized water; colorimetric substrate, strontium ranelate and 70% ethanol. The 96-well plate was initially identified and inhibition tests were performed in triplicate for each pollen extract concentration tested (2, 10 and 50 µg/mL) and for the reaction controls: BW - control without enzyme; A- with enzyme only, without inhibitor. The 3 “BW” wells received 160 µL Tris-HCl buffer, 10 µL heme and 10 µL solvent - 70% ethanol, used to dilute pollen samples. The 3 wells “A” received 150 µL Tris-HCl buffer, 10 µL heme, 10 µL enzyme (Cox-1 or Cox-2) and 10 µL solvent - 70% ethanol. And the wells with pollen extract samples, potential Cox inhibitor, at concentrations (2, 10 and 50 µg/mL), received 150

µL of Tris-HCl buffer, 10 µL of heme, 10 µL of enzyme (Cox-1 or Cox-2) and 10 µL of the 70% ethanol-diluted solvent-pollen extract samples at their respective test concentration. After mixing all these reagents in each well, the plate was gently shaken for a few seconds and incubated for 5 minutes. Subsequently, 20 µL of the colorimetric substrate solution was added to each well of the plate and then 20 µL was added to each well of arachidonic acid, substrate of the COX-catalyzed enzyme reaction. The plate was again carefully shaken and incubated for exactly 2 minutes at 25 ° C. Being read at 590 nm. Absorbance readings were verified and the triplicate averaged. Subsequently, the mean BW value was subtracted from the absorbance averages of A (A - BW) and the mean absorbance of each pollen sample at each concentration tested (pollen sample mean [x] - BW). Then, the inhibition percentage itself was determined by subtracting and dividing the mean value of each sample (pollen sample at each concentration tested, already subtracted the BW value) from the mean absorbance value of A (already subtracted BW), and multiplying by 100.

2.7 Statistical Analysis

All the experiments were conducted in a fully randomized manner. In a first approach, it was performed an overall descriptive analysis, by calculating means, standard deviations and relative standard deviation percentages (RSD (%). To assess the linear dependence between pairs of variables, Pearson's correlation coefficients were calculated and presented in the form of correlation matrix. Shapiro-Wilk's test was used to verify whether data follow normal distributions for each category of the independent variable and normal QQ plot was used to confirm if the variables lacking normality could have an approximately normal distribution. ANOVA one-way was applied when variables had groups with normality and homogeneity of variances and Kruskal-Wallis test. To study the overall data's variability, principal component analysis (PCA), an unsupervised multivariate technic, was used to generate a new set of orthogonal variables (linear combinations of the original variables), principal components (PCs), which represents a new subspace where the main variation present in the original data is mainly represented by the first components. It was used for visualization of hidden groups, possible outliers and, considering the projections of the original variables' loadings, the variables' influence in samples distribution. All statistical studies were performed using Stata 12 software and adopting the 5% ($p < 0.05$) significance level.

3. Results and Discussion

Pollen samples were collected from meliponaries from four geographic regions in the state of Maranhão. These regions have different floristic diversity. These differences in floristic composition between the four regions can influence the physical and chemical composition of pollen stored by *Melipona fasciculata* bee. At state Maranhão, Brazil as an ecological transition region, has a large number of ecosystems, which provides the raw material to support the work of bees, which originates in abundant biodiversity. In addition, the pollinating action of these bees may also increase agricultural production and promote the regeneration of natural vegetation (Albuquerque et al., 2013).

Twenty-five pollen types belonging to 12 families were identified among the plants used by *Melipona fasciculata* in the study (Table 3). The diversity of the pollen used in each period by the bees presented little variation in terms of the number of species visited between the dry and rainy seasons, with the workers choosing alternatives accompanying the nearest and abundant flowering of the studied nests. This type of finding comprises the discussion about floral preference, the displacement of trophic exploration to one or a few sources that is much discussed in the investigation of the ecological habits of stingless bees. With varying conclusions about the existence or not of the direction of the habit of foraging. Although some authors conclude that stingless bees are not, in general, specialized visitors to a few plant species (Velthuis, 1997), with several studies pointing to generalist habits and the exploitation of a variety of trophic resources (Barth, 2004), regionalized approaches have shown that Meliponini bees often concentrate foraging on a few pollen sources (Ramalho et al., 1989; Ramalho et al., 1990).

The relative abundance and proximity of the resource directs the use of certain types and pollen, as observed in samples of flooded fields Palmeirândia (PAL) and Viana (VIA), with concentration of *Pontederia parviflora* in the rainy months and *Myrcia* sp in the dry months. Bees may present flower preferences to optimize the costs and benefits of foraging (Ramalho et al., 2007). The preference of one source over another may be due to the ease of collection, quantity or quality of resources, or as a means to avoid interactions with competitors (Cortopassi-Laurino & Ramalho, 1988). The preference of one source over another may be due to the ease of collection, quantity or quality of resources, or as a means to avoid interactions with competitors (Cortopassi-Laurino & Ramalho, 1988). However, with regard to the degree of anthropization of the study areas,

it was observed in *both Melipona scutellaris* (Ramalho et al., 2007) and *Melipona capixaba* (Luz et al., 2011).

From 12 plant families used by *M. fasciculata*, Fabaceae with 5 species stood out, followed by Myrtaceae (4 species), Mimosaceae and Arecaceae (3 species each). The most important species per sampled in a single per period were *Borreria verticillata*, *Protium* sp., *Pontederia parviflora*, *Myrcia* sp., *Eucalyptus* sp. and *Mimosa pudica* (Table 3).

Borreria verticillata are commonly visited by several species of native bees (Souza et al., 2015), and the high percentage of this pollen type in the analyzed samples demonstrates its trophic importance for *M. fasciculata* in the study area. The Pontederiaceae family was represented by *Pontederia parviflora* and *Eichornia* sp. types. Ribeiro et al. (2016) identified the Pontederia type in samples of geopropolis from Palmeirândia, Maranhão. Martins et al. (2011) analyzed the honey of 'tiúba' and also found that this type of pollen is significant. *Pontederia parviflora* Alexander ("little onion") is a typical species of flooded areas. Species of Pontederia, including *P. parviflora,* are important pollen sources for *Apis mellifera* in the other flooded fields in Brazil (‘Pantanal’) (Salis et al., 2009).

Species of the family Myrtaceae were important sources of pollen for the tiúba, as verified in the samples of savanna (CHAd and CHAr) and flooded fields (PALd, PALr, VIAd and VIAr), being also identified as accessory pollen in a vegetation sample of dense ombrophylous Forest/Secondary vegetation. The Myrtaceae family is one of the most important in several Brazilian vegetable formations, its flowers are commonly visited by meliponíneos (Gressler et al., 2006). Barros et al. (2013) in pollen analysis of *M. fasciculata* geoprópolis, observed among the most representative pollen types of Cerrado vegetation samples in Maranhão, Eucalyptus (Myrtaceae). Similar to the pollen type of savanna samples analyzed in this study. In the state of Maranhão, there are extensive eucalyptus plantations, mainly in the savanna areas.

The genus *Mimosa* provides a large amount of pollen to bees (Ramalho et al., 1990; Barth, 1998) in dry areas. Pollen grains of this genus are commonly found in samples of Melipona propolis in the Brazilian northeast (Luz et al., 2009). In this study it was identified as dominant pollen in samples collected in savanna area and as accessory pollen in samples of flooded fields, in the dry season.

The total number of pollen types exploited by *Melipona fasciculata* varied significantly between the four locations (Table 3). And variation from one sampling to

the next was relatively small in a same location, forming clusters of similarity despite the floristic temporal dynamics, as can be inferred from the analysis of similarity performed, samples from Chapadinha (Savana) closer related than the others (Amazonic areas) (Figure 2, Table 2).

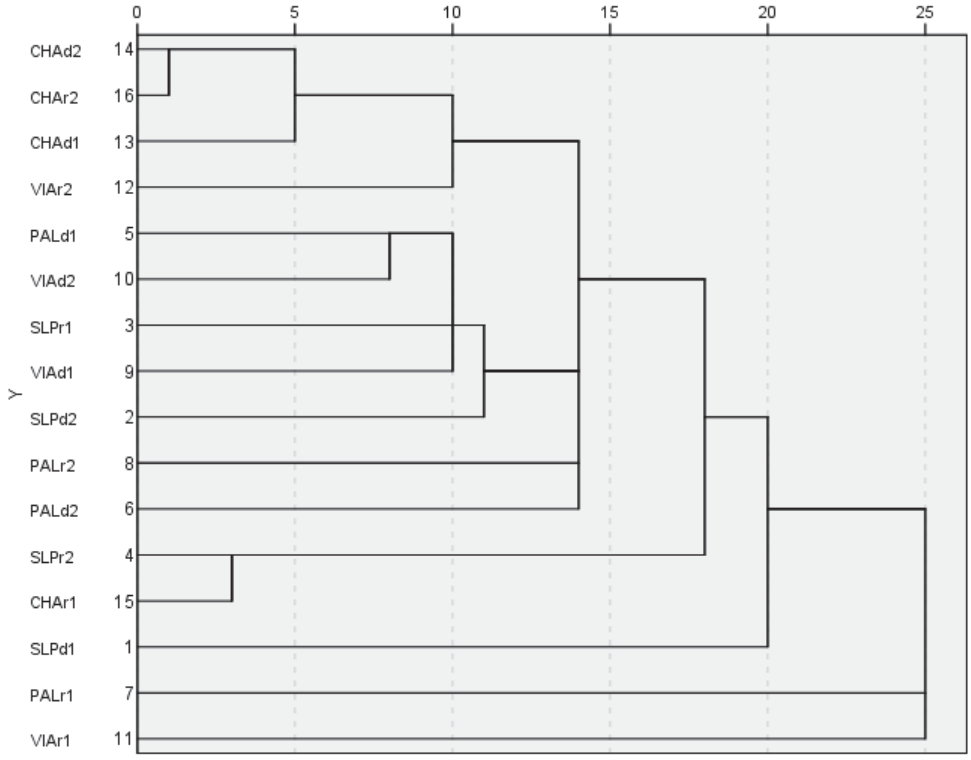

Figure 2. Dendrograms of similarity of the types of pollen analyzed.

Table 2
Similarity Matrix between pollen samples.

Case	Euclidian Quadratic Distance															
	1:SLPd1	2:SLPd2	3:SLPr1	4:SLPr2	5:PALd1	6:PALd2	7:PALr1	8:PALr2	9:VIAd1	10:VIAd2	11:VIAr1	12:VIAr2	13:CHAd1	14:CHAd2	15:CHAr1	16:CHAr2
1:SLPd1	0,000															
2:SLPd2	19,656	0,000														
3:SLPr1	33,206	16,812	0,000													
4:SLPr2	17,435	28,711	20,615	0,000												
5:PALd1	19,591	13,769	10,703	21,642	0,000											
6:PALd2	14,010	10,669	17,008	13,044	18,133	0,000										
7:PALr1	13,646	22,267	18,816	28,368	20,939	17,766	0,000									
8:PALr2	33,319	25,172	9,994	30,631	7,547	33,742	24,384	0,000								
9:VIAd1	26,795	12,923	5,962	20,876	17,614	7,695	17,062	24,926	0,000							
10:VIAd2	20,211	6,405	5,892	25,306	5,234	11,456	13,819	11,277	6,190	0,000						
11:VIAr1	14,651	18,041	25,200	33,555	19,643	18,379	12,499	20,425	22,842	13,249	0,000					
12:VIAr2	20,907	20,020	10,588	13,425	13,011	8,791	16,081	16,353	12,761	11,484	12,699	0,000				
13:CHAd1	10,110	16,074	17,700	9,282	9,031	7,702	16,097	22,752	18,736	13,524	20,309	7,891	0,000			
14:CHAd2	14,017	23,476	20,120	13,994	7,903	15,260	19,781	15,750	25,821	15,745	15,693	6,036	3,715	0,000		
15:CHAr1	18,667	29,850	16,807	2,881	21,046	16,395	26,040	24,268	17,307	22,020	28,975	14,093	15,278	17,282	0,000	
16:CHAr2	18,204	22,922	21,332	17,143	8,206	21,088	23,896	13,549	31,731	18,046	19,500	9,829	5,660	1,909	21,626	0,000

Table 3
Pollen spectra, percentages and frequency classes of the pollen types in bee bread accumulated by *Melipona (Merrichelia) fasciculata* (Apidae, Meliponini) from 4 areas in Maranhão State, northeastern Brazil.

Plant Family	Pollen Type	Pollen Samples															
		SLPd1	SLPd2	SLPr1	SLPr2	PALd1	PALd2	PALr1	PALr2	VIAd1	VIAd2	VIAr1	VIAr2	CHAd1	CHAd2	CHAr1	CHAr2
Rubiaceae	*Borreria verticillata*	39% (sp)		51% (dp)	34% (sp)												
Burseraceae	*Protium* sp.	14% (imp)	46% (dp)	33% (sp)	12% (imp)												
Solanaceae	*Solanum* sp.			7% (imp)	19% (sp)									11% (imp)	16% (sp)		
Lamiaceae	*Hyptis* sp.	14% (imp)		5% (imp)	27% (sp)												
Fabaceae	*Senna* sp.	26% (sp)	1% (mp)														
Myrtaceae	*Psydium* sp.		14% (imp)														
Arecaceae	*Mauritia* sp.		24% (sp)					1% (mp)	2% (mp)			1% (mp)	5% (imp)				4% (imp)
Arecaceae	*Attalea speciosa*		13% (imp)					1% (mp)	3% (imp)			1% (mp)	2% (mp)	13% (imp)	19% (sp)	8% (imp)	7% (imp)
Pontederiaceae	*Pontederia parviflora*					71% (dp)	56% (dp)	10% (imp)	4% (imp)	77% (dp)	51% (dp)	10% (imp)	9% (imp)				
Pontederiaceae	*Eichornia* sp.					11% (imp)	11% (imp)	6% (imp)	22% (sp)	8% (imp)	22% (imp)	1% (mp)	2% (mp)				
Mimosaceae	*Mimosa caesalpiniifolia* .						10% (imp)		7% (imp)	1% (mp)	8% (imp)			19% (sp)	18% (sp)	16% (sp)	11% (imp)

dp: predominant pollen; sp: secondary pollen, imp: important minor pollen; mp: minor pollen.

Table 3 (continued)

Plant Family	Pollen Type	Pollen Samples															
		SLPd1	SLPd2	SLPr1	SLPr2	PALd1	PALd2	PALr1	PALr2	VIAd1	VIAd2	VIAr1	VIAr2	CHAd1	CHAd2	CHAr1	CHAr2
Mimosaceae	*Mimosa pudica*						12% (imp)		6% (imp)	2% (mp)	3%			11% (imp)	9% (imp)	31% (sp)	25% (sp)
Myrtaceae	*Eugenia* sp.							18% (sp)	20% (sp)			22% (sp)	19% (sp)				
Myrtaceae	*Myrcia* sp.							51% (dp)	32% (sp)			54% (dp)	44% (sp)				
Myrtaceae	*Eucalyptus* sp.													26% (sp)	22% (sp)	11% (imp)	16% (sp)
Bignoniaceae	*Tabebuia* sp.	3% (mp)															
Fabaceae	*Chamaecrista hispidula*					7% (imp)		1% (mp)		6% (imp)	3% (imp)	3% (imp)	4% (imp)	5% (imp)	2% (mp)		
Hypericaceae	*Vismia guianensis*					3% (imp)											
Fabaceae	*Machaerium*					4% (imp)	8% (imp)										
Hypericaceae	*Vismia* sp.													9% (imp)	6% (imp)	20% (sp)	13% (imp)
Arecaceae	*Astrocaryum* sp.													1% (mp)	2% (mp)	8% (imp)	9% (imp)
Melastomataceae	*Mouriri* sp.													2% (mp)	1% (mp)	3% (imp)	7% (imp)
Mimosaceae	*Acacia* sp.				1% (mp)										1% (mp)		3% (imp)

dp: predominant pollen; sp: secondary pollen, imp: important minor pollen; mp: minor pollen.

Table 3 (continued)

Plant Family	Pollen Type	Pollen Samples															
		SLPd1	SLPd2	SLPr1	SLPr2	PALd1	PALd2	PALr1	PALr2	VIAd1	VIAd2	VIAr1	VIAr2	CHAd1	CHAd2	CHAr1	CHAr2
Fabaceae	*Neptunia* sp.					2% (mp)	1% (mp)	6% (imp)	2% (mp)	1% (mp)	5% (imp)	1% (mp)	1% (mp)				
Fabaceae	*Machaerium* sp.										4% (imp)	2% (mp)	9% (imp)				
Less than 0,5%		2%	1%	2%	4%	2%	2%	4%	2%	4%	3%	3%	3%	2%	2%	2%	3%
Undertemined		2%	1%	2%	3%	2%	1%	2%		1%	1%	2%	2%	1%	2%	1%	2%
Total		100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%

dp: predominant pollen; sp: secondary pollen, imp: important minor pollen; mp: minor pollen.

Bee pollen is a food of rich nutritional value, having in its composition carbohydrates, lipids, proteins, fibers, vitamins and minerals. In Brazil there is still no official regulations determining identity and quality parameters for *M. fasciculata* pollen, only for bee pollen (from *Apis mellifera*). According to Normative Instruction (Brazil, 2001), which provides Technical Regulation for Identification and Quality of Beekeeping Pollen for market purposes, it must present the following characteristics: moisture maximum ash content of 4%, minimum lipid content of 1,8%, minimum protein content of 8%, total sugar content of 14,5 to 55,0%, minimum crude fiber content of 2%, pH between 4 and 6 and maximum free acidity of 300 mEq/kg.

The mean free acidity was 125.7 ± 18.34 mEq/kg. The mean pH values were 3.9 ± 0.30. The mean moisture content was 25.4 ± 1.76%. When comparing these data with the requirements for Apis mellifera pollen, it was verified that all the samples presented results within the specification for free acidity and humidity, and mean pH below the established range (pH 4 to 6), with some samples showing pH above 4, especially those collected in the municipalities of Palmeirândia and Viana. Souza et al. (2004) found higher moisture values in pollen samples from stingless bees (36.9 ± 11.1%). Reis (2001) found moisture values of 23.0% for Apis pollen (in natura).

Pollen stored by *M. fasciculata* is a product that undergoes fermentation process during its storage in the wax pots inside colonies. *Melipona* species add enzymatic secretions to the collected pollen, and this one acquires peculiar characteristics that make it a product different from the floral pollen (Kerr et al., 1996; Villanueva et al., 2002).

The free acidity index in correlation with the pH and humidity of the pollen samples are valuable indicators for establishing the state of conservation of this food. Moisture indices in the pollen *in natura* are variable and depend on the period of production. For commercial purposes, the pollen *in natura* would have to undergo a process of drying and stabilization, until reaching a maximum of 4% of humidity, to preserve its characteristics at the time of collection.

Average value for ash was 3.9 ± 0.6%, wich comprises with regulation for bee pollen. Ash values less than 4% was also found by Souza et al. (2004) in pollen of Meliponini in Amazon. Higher values for this test may be due to differences in floral pollen collection or contamination with inorganic material, even with geopropolis (Almeida-Muradian et al., 2012).

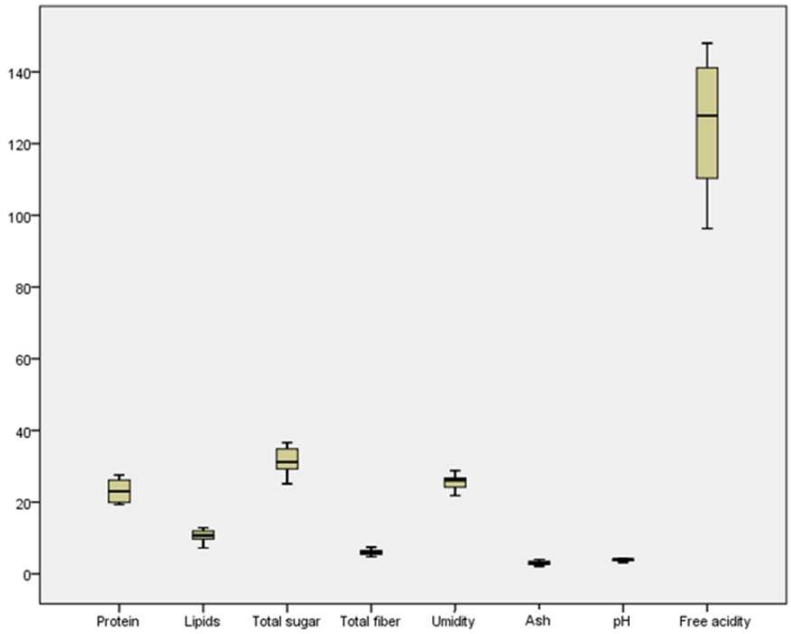

Figure 3. Physicochemical and nutritional composition of *in vitro* samples of *Melipona fasciculata* (Box plot, absolute values).

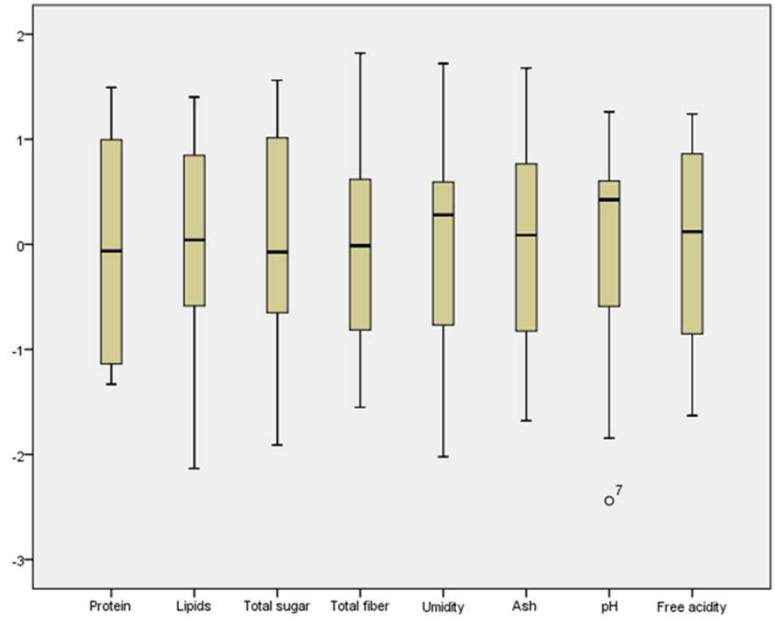

Figure 4. Physicochemical and nutritional composition of *in vitro* samples of *Melipona fasciculata* (Box plot, standardized values).

From Figure 3, the moisture and pH values presented negative asymmetry. The values for total sugars showed positive asymmetry. The other variables or parameters were symmetrical.

Table 4
Physicochemical and nutritional composition of *in vitro* samples of *Melipona fasciculata.*

Sample	Energy (Kcal/g)	Protein (%)	Fat (%)	Carbohydrates (%)	Fiber (%)	Moisture (%)	Ash (%)	Free Acidity (mEq)	pH
SLPd1	291,2 ± 5,0 [a]	27,6 ± 0,3 [a]	7,2 ± 0,1 [a]	29,0 ± 0,7	5,4 ± 0,3	27,1 ± 0,2	3,7 ± 0,3	138,0 ± 2,0	3,7 ± 0,06
SLPd2	292,6 ± 6,1 [a]	19,7 ± 0,2 [b]	8,0 ± 0,2 [b]	35,6 ± 1,0	7,5 ± 0,5	26,2 ± 0,6	3,1 ± 0,1	136,7 ± 0,6	4,0 ± 0,06
SLPr1	323,2 ± 4,0[b]	19,6 ± 0,3 [b]	12,8 ± 0,2 [c]	32,3 ± 0,3	6,7 ± 0,3	26,4 ± 0,4	2,1 ± 0,1	117,0 ± 1,7	3,7 ± 0,06
SLPr2	312,2 ± 9,8 [b]	26,4 ± 0,4 [c]	11,8 ± 0,3 [d]	25,1 ± 1,5	6,4 ± 0,3	26,9 ± 0,2	3,5 ± 0,3	97,3 ± 2,1	4,2 ± 0,06
PALd1	323,4 ± 5,0 [b]	22,3 ± 0,2 [d]	10,5 ± 0,2	35,0 ± 0,3	5,4 ± 0,3	24,5 ± 0,7	2,3 ± 0,2	127,3 ± 2,1	4,2 ± 0,06
PALd2	306,4 ± 3,2[c]	23,1 ± 0,4 [e]	10,9 ± 0,2	28,9 ± 0,8	7,3 ± 0,3	26,0 ± 0,3	3,8 ± 0,2	148,0 ± 3,6	4,0 ± 0,06
PALr1	305,8 ± 6,6 [c]	26,0 ± 0,4 [c]	9,2 ± 0,1	29,7 ± 1,3	5,9 ± 0,3	26,5 ± 0,4	2,7 ± 0,4	147,7 ± 2,1	3,2 ± 0,06
PALr2	328,5 ± 7,5 [b]	20,1 ± 0,3 [b]	11,3 ± 0,4 [d]	36,6 ± 1,2	4,8 ± 0,3	25,0 ± 0,3	2,2 ± 0,3	98,0 ± 2,6	3,7 ± 0,06
VIAd1	322,1 ± 9,5 [b]	19,3 ± 0,3 [b]	12,3 ± 0,5 [c]	33,4 ± 0,6	7,2 ± 0,3	24,9 ± 0,2	2,8 ± 0,2	146,6 ± 3,5	3,8 ± 0,06
VIAd2	311,9 ± 8,1 [b]	19,6 ± 0,3 [b]	10,5 ± 0,5	34,8 ± 0,7	6,2 ± 0,3	26,3 ± 0,4	2,6 ± 0,4	142,3 ± 1,2	3,9 ± 0,06
VIAr1	306,7 ± 8,2 [c]	21,5 ± 0,5 [f]	9,1 ± 0,2	34,8 ± 0,6	5,1 ± 0,3	25,5 ± 0,5	4,0 ± 0,1	140,0 ± 1,7	3,4 ± 0,06
VIAr2	330,5 ± 6,2[d]	22,9 ± 0,4 [d]	12,9 ± 0,2	30,7 ± 1,0	6,1 ± 0,3	23,8 ± 0,4	3,6 ± 0,2	123,7 ± 2,3	3,7 ± 0,06
CHAd1	318,9 ± 7,6 [b]	27,0 ± 0,3 [a]	10,3 ± 0,4	29,6 ± 1,0	6,3 ± 0,3	23,7 ± 0,3	3,1 ± 0,2	128,3 ± 5,7	4,1 ± 0,06
CHAd2	330,8 ± 8,5 [b, d]	26,1 ± 0,2 [c]	11,1 ± 0,1	31,7 ± 0,2	5,1 ± 0,3	22,6 ± 0,4	3,4 ± 0,2	120,3 ± 1,2	4,0 ± 0,06
CHAr1	309,4 ± 2,0 [c]	24,1 ± 0,1 [g]	12,2 ± 0,4 [c, d]	25,9 ± 0,5	5,7 ± 0,3	28,8 ± 0,4	3,4 ± 0,1	96,3 ± 4,6	4,0 ± 0,06
CHAr2	329,4 ± 5,7 [b]	26,1 ± 0,1 [c]	10,2 ± 0,2	33,4 ± 0,3	5,4 ± 0,3	21,8 ± 0,2	3,1 ± 0,2	103,7 ± 3,2	4,1 ± 0,06
Total	315,2 ± 12,63	23,2 ± 2,95	10,6 ± 1,63	31,7 ± 3,44	6,0 ± 0,85	25,4 ± 1,76	3,1 ± 0,6	125,7 ± 18,34	3,9 ± 0,30

Table 5 shows that there is a correlation between the study variables.

Table 5
Sample correlation matrix.

	Protein	**Lipids**	**Total sugar**	**Total fiber**	**Free acidity**	**Humidity**	**Ash**	**pH**
Protein	1,0000							
Lipids	-0,2600	1,0000						
Lipids	-0,2600	1,0000						
Total sugar	-0,5865	-0,2598	1,0000					
Total fiber	-0,3337	0,1340	-0,2347	1,0000				
Humidity	-0,2928	0,0389	-0,4130	0,3973	1,0000			
Ash	0,4551	-0,2655	-0,4343	0,0148	-0,0206	1,0000		
pH	0,2412	0,0946	-0,2443	0,1651	-0,1192	0,0871	1,0000	
Free acidity	-0,1883	-0,4326	0,1525	0,4126	0,1743	0,1425	-0,3712	1,0000

According to Table 6, there are only 3 components with variance values above 1. The first component (CP1) explains 27.06% of the sample variability. While the second (CP2) and the third (CP3) explain, respectively, 23.35% and 21.29%. Cumulatively these three components explain 71.70% of the total sample variation. That is, the 8 original variables were reduced by 3 components.

Table 6
Total variance

Component	Initial Values		
	Total	% de variance	% cumulative
1	2,16	27,06	27,06
2	1,87	23,35	50,41
3	1,70	21,29	71,70
4	0,95	11,83	83,53
5	0,64	8,06	91,58
6	0,47	5,93	97,52
7	0,20	2,48	100,00
8	0,00	0,00	100,00

According to Table 7, the higher the value of the component, the greater the representativeness of the variable. In PC1, the most representative variable was protein content. For PC2, total fibers and humidity. In relation to PC3 was the free acidity.

Table 7

Matrix of components and variables

Variables	Component		
	PC 1	PC 2	PC 3
Protein	0,59886	-0,07378	0,22453
Lipids	-0,00466	0,03143	-0,65428
Total sugar	-0,48377	-0,43671	0,15492
Total fiber	-0,16740	0,57337	-0,12181
Humidity	-0,12194	0,55661	-0,12683
Ash	0,40473	0,22379	0,36307
pH	0,33783	0,01662	-0,29092
Free acidity	-0,29411	0,33753	0,50012

Figure 5 shows high humidity values in components 1 and 2 in the SLPd sample. There is a relationship between high values of component 2 of total fiber and pH in the VIAr sample. There are also low concentrations of Total sugar values in components 1 and 2 in the PALr sample. Relationship of high protein component 1 values in the CHAd sample.

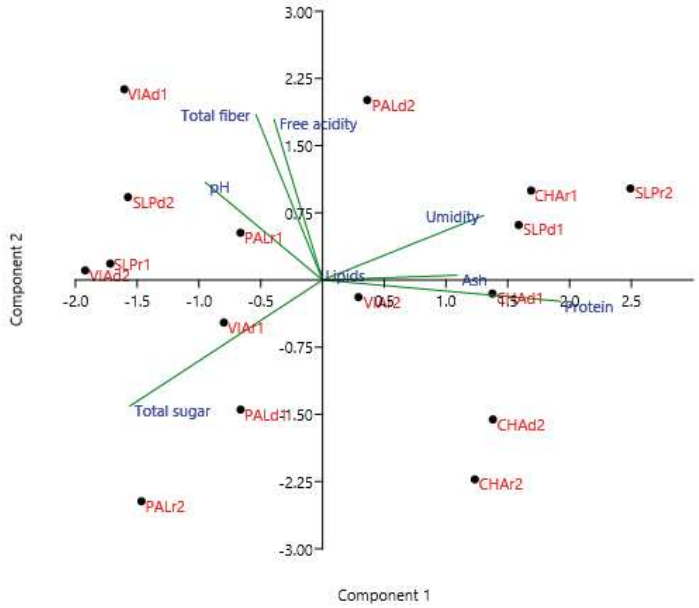

Figure 5. Representation of the values of components 1 and 2 for all samples.

As shown in Figure 6, there is a relationship between large values of components 1 and 3 with humidity and protein. In VIAr and PALd samples there is a concentration of high values of component 3 and low values of component 1 in the variables total sugar and pH. Trend of low values of components 1 and 3 for the total fiber and free acidity variables. In samples SLPd and CHAd there is a relationship of high component 1 values and low component 3 values for the ash variable.

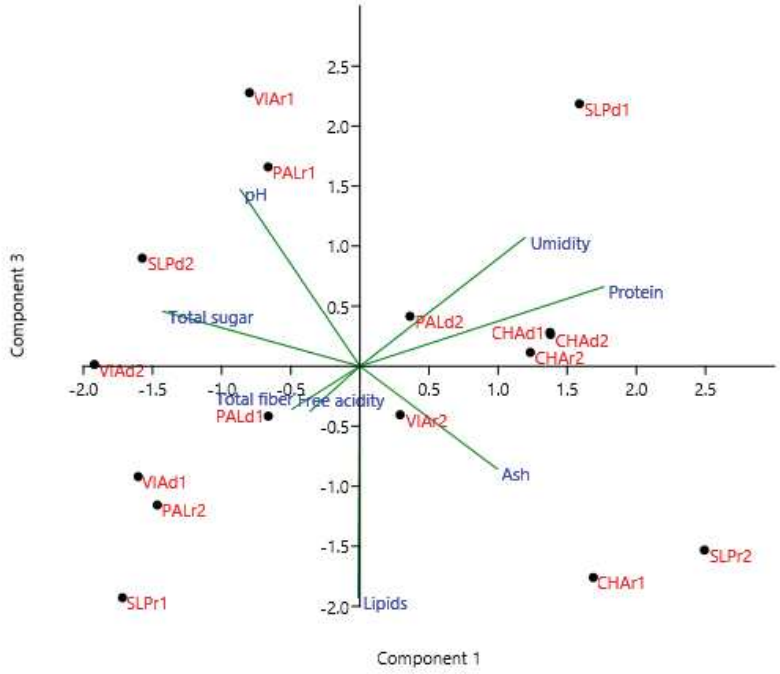

Figure 6. Representation of the values of components 1 and 3 for all samples.

According to Figure 7, in the first quadrant there is the relationship of high values of components 2 and 3 with the pH and moisture in sample VIAd. And also, the concentration of high component 3 values and low component 2 values for total sugar and protein variables in the PALd sample. Finally, the trend of high component 2 values and low component 3 values for the lipids, total fiber and free acidity variables in the SLPd sample.

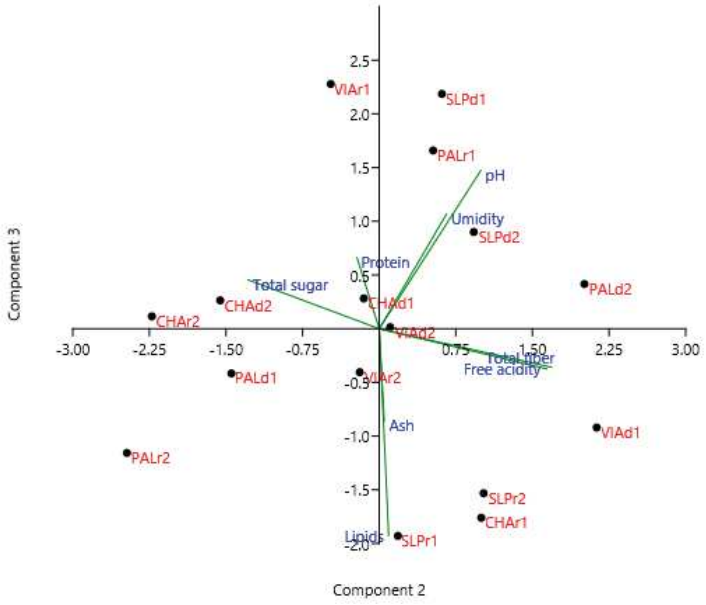

Figure 7. Representation of the values of components 2 and 3 for all samples.

Results of the microbiological quality evaluation of *M. fasciculata* pollen samples collected from municipalities of Maranhão are expressed in Table 8. In assessing the quality of a food and therapeutic product, one of the parameters to be assessed is the microbiological quality. As there are no specific parameters for meliponin pollen, the total aerobic and fungal quantification was analyzed, as well as the search for *Escherichia coli* and *Salmonella* sp., causative agents of gastroenteritis, which reflect hygiene and cleaning problems during handling. The results demonstrated that this product has an excellent degree of microbiological quality, with very low levels of contamination.

Table 8
Results of the microbiological quality evaluation of *M. fasciculata* pollen samples collected from April 2015 to April 2017 in municipalities of Maranhão.

Sample	**Total Aerobic Count (CFU/g)**	**Yeasts and Moulds Count (CFU/g)**	***Escherichia coli***	***Salmonella* sp.**
SLPd1	<10	<10	Absent/g	Absent/g
SLPd2	<10	$1x10^2$	Absent/g	Absent/g
SLPr1	<10	<10	Absent/g	Absent/g
SLPr2	<10	<10	Absent/g	Absent/g
PALd1	<10	$1x10^2$	Absent/g	Absent/g
PALd2	<10	<10	Absent/g	Absent/g
PALr1	<10	<10	Absent/g	Absent/g
PALr2	<10	<10	Absent/g	Absent/g

Table 8. (continued)

Sample	Total Aerobic Count (CFU/g)	Yeasts and Moulds Count (CFU/g)	*Escherichia coli*	*Salmonella* sp.
VIAd1	<10	<10	Absent/g	Absent/g
VIAd2	$1x10^2$	<10	Absent/g	Absent/g
VIAr1	<10	$1x10^2$	Absent/g	Absent/g
VIAr2	<10	<10	Absent/g	Absent/g
CHAd1	<10	<10	Absent/g	Absent/g
CHAd2	<10	$1x10^3$	Absent/g	Absent/g
CHAr1	<10	<10	Absent/g	Absent/g
CHAr2	<10	<10	Absent/g	Absent/g

All samples were analyzed further for composition of fatty acids and the results are shown in Table 9. Total polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA) and saturated fatty acid (SFA) contents ranged from 58.30 – 60.13 g/100 g (59.15± 0.76 g/100g), 2.70 – 4.06 g/100 g (3.35± 0.57 g/100g) and 36.40 – 38.11 g/100 g (37.52± 0.77 g/100g), respectively. Markiewicz-Żukowska et al. (2013) found in bee bread (accumulated pollen from Apis) predominant α-linoleic, linoleic, oleic and 11,14,17-eicosatrienoic acid species, otherwise, as in our study Mayda et al. (2020) found predominance of palmitic acid. Overall, literature confirms that accumulated pollen can be used as a source of fatty acids.

Table 9
Fatty acid composition.

Fatty Acid (%)	SLP	PAL	VIA	CHA	Average
Caprylic	0,06	0,11	0,03	0,12	0,08 ± 0,04
Capric	0,22	0,34	0,22	0,24	0,26 ± 0,06
Lauric	0,02	0,07	0,01	0,04	0,04 ± 0,03
Myristic	0,23	0,20	0,26	0,23	0,23 ± 0,02
Palmitic	33,00	31,08	31,60	30,79	31,92 ± 0,98
Stearic	1,85	2,71	2,51	1,84	2,23 ± 0,45
Oleic	1,44	1,77	2,33	2,48	2,01 ± 0,49
Linoleic	28,87	28,98	34,97	19,98	28,20 ± 6,18
Linolenic	30,34	29,96	25,16	38,32	30,95 ± 5,45
Arachidic	2,73	3,42	1,77	4,38	3,08 ± 1,10
Erucic	1,26	1,38	1,14	1,58	1,34 ± 0,19

Pollen samples were grouped according to collection location and climatic seasonality (dry season and rainy season), then submitted to extraction. The extractive process employed provided a good yield, which ranged from 55.0 to 64.8% (Table 10).

Table 10
Yield of hydroalcoholic pollen extracts of *Melipona fasciculata*.

Extract	Yield
SLPd	64,8%
SLPr	61,6%
PALd	59,3%
PALr	55,7%
VIAd	56,1%
VIAr	58,2%
CHAd	61,1%
CHAr	55,0%

The extracts obtained were submitted to the total flavonoid and phenolic compounds assays, the results are expressed in Table 11. The phenolic compounds content ranged from 11.90 to 29.94mg gallic acid/100g. They were significantly higher ($p < 0.05$) in EPALs and EVIAs extracts, with 29.94 and 29.62mg of gallic acid/100g, respectively. The pollen samples extracts collected during the dry season presented higher phenolic compounds content than the rainy season extracts.

Table 11
Total polyphenols and flavonoids content of hydroalcoholic pollen extracts of *Melipona fasciculata*.

Extract	Total polyphenols (mg gallic acid/100g)	Flavonoid content (mg quercetin/100g)
SLPd	$13,57 \pm 0,06^{a}$	$3,37 \pm 0,18^{a}$
SLPr	$11,90 \pm 0,10^{b}$	$4,18 \pm 0,13^{b}$
PALd	$29,94 \pm 0,05^{c}$	$17,20 \pm 0,20^{c}$
PALr	$26,39 \pm 0,44^{d}$	$13,92 \pm 0,06^{d}$
VIAd	$29,62 \pm 0,21^{c}$	$15,42 \pm 0,11^{e}$
VIAr	$24,57 \pm 0,41^{e}$	$11,33 \pm 0,07^{f}$
CHAd	$16,35 \pm 0,22$f	$12,90 \pm 0,06^{g}$
CHAr	$13,19 \pm 0,17^{a}$	$7,70 \pm 0,08^{h}$

Different letters mean statistical difference ($p<0,05$)

The pollen extracts from Santa Luzia do Paruá (rainy season), Palmeirândia (dry season), Viana (dry season) and Chapadinha (dry season) were subjected to analysis by high performance liquid chromatography with UV-Vis detector (Tables 12 to 15).

Table 12
Chemical identification by high performance liquid chromatography with UV-Vis detector of *M. fasciculata* pollen extract collected in Santa Luzia do Paruá (rainy season).

Peak	***rt* (min)**	**External Standard**
1	13,165	β-Sitosterol
2	13,530	Ellagic Acid
3	20,140	Coumaric Acid
4	21,973	Isoquercitrin
5	26,790	Quercitrin
6	27,773	Myricetin
7	34,067	Naringenin
8	38,018	Kaempferol

Table 13
Chemical identification by high performance liquid chromatography with UV-Vis detector of *M. fasciculata* pollen extract collected in Palmeirândia (dry season).

Peak	***rt* (min)**	**External Standard**
1	12,135	Catechin
2	13,060	*β*-Sitosterol
3	13,530	Ellagic Acid
4	14,053	*β*-Amirin
5	20,140	Coumaric Acid
6	21,973	Isoquercitrin
7	23,630	Rutin
8	26,790	Quercitrin
9	27,773	Myricetin
10	34,067	Naringenin
11	38,018	Kaempferol

Table 14
Chemical identification by high performance liquid chromatography with UV-Vis detector of *M. fasciculata* pollen extract collected in Viana (dry season).

Peak	*rt* (min)	External Standard
1	26,667	Quercitrin
2	31,972	Quercetin
3	34,000	Naringenin

Table 15
Chemical identification by high performance liquid chromatography with UV-Vis detector of *M. fasciculata* pollen extract collected in Chapadinha (dry season).

Peak	*rt* (min)	External Standard
1	9,690	Gallic Acid
2	21,973	Isoquercitrin
3	31,985	Quercetin
4	34,067	Naringenin

Several flavonoid types have been previously isolated and identified from pollen samples, the most common being flavonol-3-O-diglycoside. Silva et al. (2006) identified naringenin in a *Melipona subnitida* pollen. Among the flavonoids that occur in pollen bee (*Apis mellifera*) there is also canferol, quercetin and isoramnetin (Komosinska-Vassev et al., 2015). In our study we identified canferol in the M*elipona*. *fasciculata* pollen extract collected in Palmeirândia in the dry season and quercetin in the pollen extract collected in Viana in the dry season. Myricetin and quercetin flavonoids identified in the *M. fasciculata* pollen extracts were also identified in the pollen accumulated by stingless bee *Scaptotrigona bipunctata* (Lins et al., 2003).

This work presents for the first time the chemical characterization of the pollen accumulated by *M. fasciculata* in different phytogeographic domains, contributing to the elaboration of a chemical quality profile of pollen accumulated by this meliponin, and consequently helping in the direction of studies of evaluation of biological and biological activities on the economic value of this product.

The extracts obtained from the *M. fasciculata* pollen samples were fractionated and each one of its fractions was submitted to the evaluation of the antioxidant activity by the DPPH assay (Table 16).

Table 16
Antioxidant activity of *M. fasciculata* pollen extracts and their fractions by DPPH assay.

Sample	EC_{50} (µg/mL)				
	EB	**FH**	**FC**	**FAet**	**FB**
SLPd	572,47	852,17	167,63	524,30	501,70
SLPr	487,29	1764,67	177,32	210,73	541,36
PALd	496,37	709,14	156,24	517,30	481,20
PALr	427,31	628,63	198,00	886,00	1002,40
VIAd	623,94	696,56	202,13	771,51	981,49
VIAr	599,58	701,23	206,71	795,07	872,45
CHAd	557,66	716,57	162,39	540,27	576,22
CHAr	567,26	675,71	182,91	636,66	826,33

EB = raw extract; FH = hexanic; FC = cloroformic; FAet = ethyl acetate; FB = butanolic; EC_{50} = median effective concentration

The evaluation of antioxidant activity by the DPPH assay has been widely used to verify the DPPH radical sequestering capacity in bee products such as pollen (Graikou et al., 2011; Freire et al., 2012; Choi et al., 2016).

The result of the evaluation of the antioxidant activity expressed in EC50 ranged from 156.24 to 1764.67 µg/mL. The chloroform fractions of all samples showed better antioxidant activity. Phenolic extracts showed potent cuts of free radical activity in the DPPH radical compared to hexane and ethanolic extracts. The high polyphenol content may be responsible for the high antioxidant activity.

The extracts were also subjected to the *in vitro* anti-inflammatory activity evaluation by the type 1 cyclooxygenase (COX1) and type 2 cyclooxygenase (COX2) inhibition test (Figure 8). The choice of samples was guided by the antioxidant results, therefore, 3 samples (CHAd, PALd and SLPd) were tested.

The results obtained in the inhibition assay suggest an anti-inflammatory potential. Dry season pollen extracts from Chapadinha and Palmeirândia against COX 1 and Santa Luzia do Paruá against COX 2.

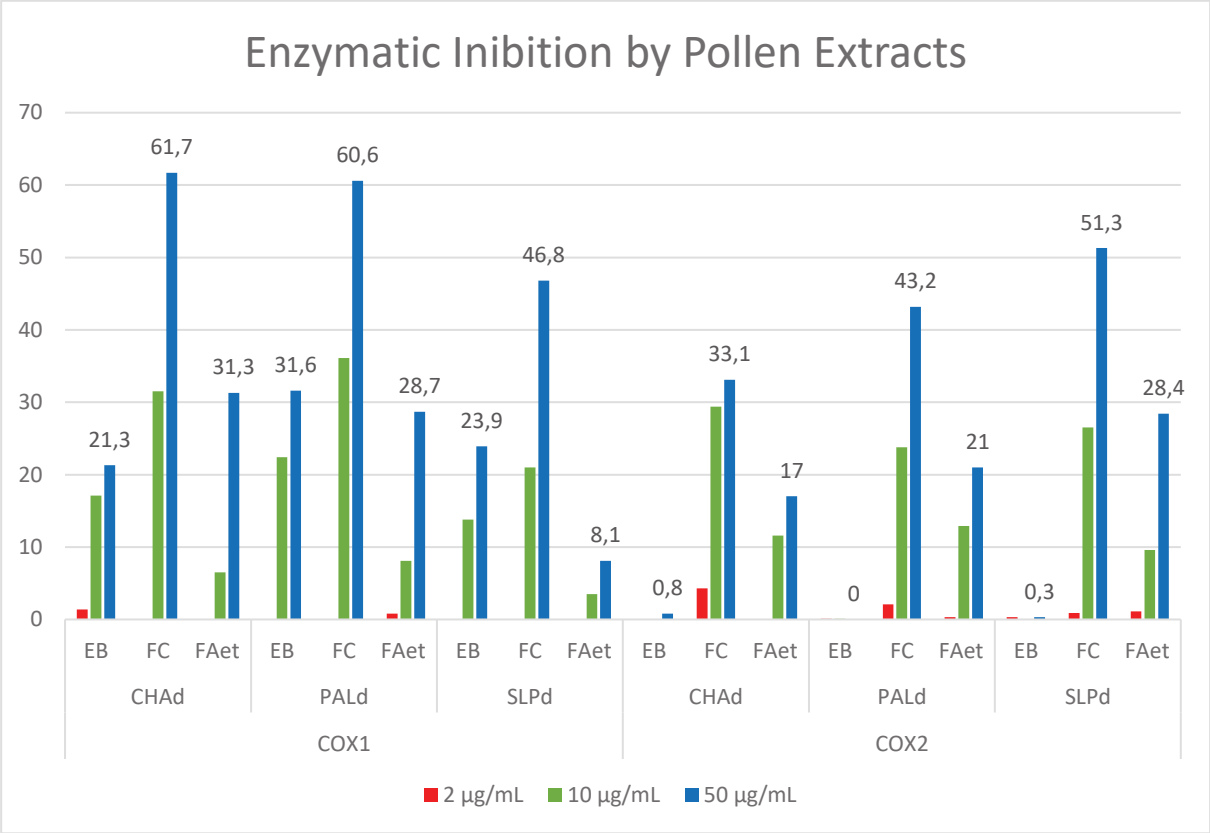

Figure 8. Percentage inhibition of COX1 and COX2 enzymes by *M. fasciculata* pollen extracts collected in Chapadinha in the dry season (CHAd): FC (blue), EB (gray) and FAet (orange).

Maruyama et al. (2010) found a potent anti-inflammatory action for ethanol pollen extract, inhibiting COX-2 production, and suggested that the flavonoids present in the extract may be responsible for the observed activity. Similar results were demonstrated in this study, where pollen extracts of *M. fasciculata* showed high content of phenolic compounds, evidenced by chemical analysis.

3.1 Limitations

Nutrient composition of foods is known to vary seasonally, depending on the stage of the life cycle, food availability, and changes in the wider environment. It was however, outside the scope of this study to attempt to sample to account for these variations. Furthermore, a relatively small size of pooled samples was used. In the local context and considering resource constraints, it was also not possible to obtain larger more representative sample sizes. Therefore, while it is recognized that these are limitations of the study, given the lack of existing data on nutrient composition of Meliponini pollen, the results are still of significant value, providing time and location specific estimates for comparison with future analyses.

4. Conclusions

The results obtained from the studied samples allowed to establish a nutritional profile of *Melipona fasciculata* pollen, including fatty acids profile. They also demonstrated that this product has an excellent degree of microbiological quality, with very low levels of contamination. Chemical analysis of pollen extracts of *M. fasciculata* revealed a composition rich in phenolic substances, of which compounds of the flavonoid class. Richness in phenolic and fatty acids imply in therapeutic potential for *M. fasciculata* pollen. Physicochemical and biological characterization studies are important for proper quality control and standardization of this material which may be beneficial as a dietary supplement as well as a functional food.

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5 CONSIDERAÇÕES FINAIS

Os dados da presente pesquisa estabelecem, pela primeira vez, os perfis químico e nutricional do pólen de *Melipona fasciculata* Smith. Definindo o grande valor nutricional deste produto do cultivo dessas abelhas nativas. Além disso, acrescentam a caracterização polínica sazonal e por região de coleta, incluindo avaliação das atividades antioxidante e anti-inflamatória dos extratos obtidos do pólen dessa espécie.

Tais dados continuam a expandir as possibilidades para o avanço de diversas pesquisas que possam continuar avaliando o potencial bioativo e nutricional do pólen de abelhas com ferrão atrofiado, promovendo ensaios de avaliação do impacto fisiológico de dietas ricas nesse bioproduto ou mesmo do isolamento de compostos ativos presentes ali.

O Maranhão é particularmente apto para o desenvolvimento da meliponicultura, uma atividade com baixo impacto ambiental e que pode prover produtos com elevado valor agregado e com grande potencial na biotecnologia e na nutrição humana.

Espera-se que os resultados aqui apresentados possam contribuir para a expansão dessa atividade e para a efetiva conservação dessas espécies de abelha.

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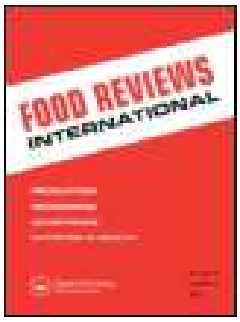
APÊNDICES



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Exploring the anticancer properties of essential oils from family Lamiaceae

Ludmilla Santos Silva de Mesquita, Tássio Rômulo Silva Araújo Luz, José Wilson Carvalho de Mesquita, Denise Fernandes Coutinho, Flavia Maria Mendonça do Amaral, Maria Nilce de Sousa Ribeiro & Sonia Malik

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Towards a better understanding of Artemisia vulgaris: Botany, phytochemistry, pharmacological and biotechnological potential

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Towards a Better Understanding of *Artemisia vulgaris:* Botany, Phytochemistry, Pharmacological and Biotechnological potential

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Article

Chemical Profile and Biological Activities of Essential Oil from *Artemisia vulgaris* L. Cultivated in Brazil

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Abstract: Essential oil from the leaves of *Artemisia vulgaris* L. (Compositae) cultivated in Brazil was investigated for its chemical composition and biological activities including antibacterial, antifungal, and anthelmintic. The constituents of essential oils isolated by hydro-distillation were examined by GC-MS and a total of 18 components were identified. The essential oil was dominated by oxygenated sesquiterpenes (44.4%), sesquiterpene hydrocarbons (33.3%), and oxygenated monoterpenes (16.6%). Caryophyllene (37.45%), germacrene D (16.17%), and humulene (13.66%) were the major components. The essential oils from *A. vulgaris* showed bactericidal and fungicidal properties against *Staphylococcus aureus* and *Candida albicans*, respectively. Anthelmintic activity against *Haemonchus contortus* was absent in this essential oil. Altogether above results indicate that essential oils from *A. vulgaris* can be used for various medicinal purposes.

Keywords: anthelmintic; antimicrobial; (compositae); caryophyllene; germacrene-D; humulene; GCMS-chromatography

1. Introduction

Increasing resistance and other side effects caused by the repeated use of similar antibiotics or anti-parasitics enforced the researchers to find suitable natural compounds as alternatives. A large number of plant derived compounds play an important role in the natural defence system against all living microorganisms [1]. In vitro investigations of antimicrobial and antifungal activities have been carried out for several medicinal crops including *Baccharis trimera* [2], *Zingiber officinale* [3,4], *Mikania glomerata* [5,6], *Mentha piperita* [3,7], *Syzygium aromaticum* [8], *Cymbopogon citratus* [9,10], *Allium sativum* [11,12], and *Psidium guajava* [6,11,13]. There is a huge interest in medicinal crops to extract oils and bioactive compounds to use them as food additives to delay or prevent growth and development of microorganisms [14–18].

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Antinociceptive Activity of *Borreria verticillata*: *In vivo* and *In silico* Studies

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Borreria verticillata (L.) G. Mey. known vassourinha has antibacterial, antimalarial, hepatoprotective, antioxidative, analgesic, and anti-inflammatory, however, its antinociceptive action requires further studies. Aim of the study evaluated the antinociceptive activity of *B. verticillata* hydroalcoholic extract (EHBv) and ethyl acetate fraction (FAc) by *in vivo* and *in silico* studies. *In vivo* assessment included the paw edema test, writhing test, formalin test and tail flick test. Wistar rats and Swiss mice were divided into 6 groups and given the following treatments oral: 0.9% NaCl control group (CTRL), 10 mg/kg memantine (MEM), 10 mg/kg indomethacin (INDO), 500 mg/kg EHBv (EHBv 500), 25 mg/kg FAc (FAc 25) and 50 mg/kg FAc (FAc 50). EHBv, FAc 25 and 50 treatments exhibited anti-edematous and peripheral antinociceptive effects. For *in silico* assessment, compounds identified in FAc were subjected to molecular docking with COX-2, GluN1a and GluN2B. Ursolic acid (UA) was the compound with best affinity parameters (binding energy and inhibition constant) for COX-2, GluN1a, GluN2B, and was selected for further analysis with molecular dynamics (MD) simulations. In MD simulations, UA exhibited highly frequent interactions with residues Arg120 and Glu524 in the COX-2 active site and NMDA, whereby it might prevent COX-2 and NMDA receptor activation. Treatment with UA 10 mg/Kg showed peripheral and central antinociceptive effect. The antinociceptive effect of *B. verticillata* might be predominantly attributed to peripheral actions, including the participation of anti-inflammatory components. Ursolic acid is the main active component and seems to be a promising source of COX-2 inhibitors and NMDA receptor antagonists.

Keywords: ***Borreria verticillata,*** **COX-2, NMDA receptor, molecular docking, molecular dynamics simulations**

INTRODUCTION

Pain is a warning system that informs the body about the occurrence of tissue damage (Nickel et al., 2012). In the pathophysiology of pain several biological actions are involved, including activation of cyclooxygenase 2 enzyme (COX-2) and N-methyl-D-aspartate (NMDA) receptor.

COX-2 is upregulated in the central nervous system in response to inflammatory factors. It is a rate-limiting enzyme for prostanoid production during inflammation

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Seasonal variation in the chemical composition and biological activity of the essential oil of *Mesosphaerum suaveolens* (L.) Kuntze

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Arboviruses

ABSTRACT

In tropical countries, arboviruses are public health threats, especially those transmitted by *Aedes aegypti* mosquitoes. The prevention of such diseases is based on vector control. However, the compounds currently used have disadvantages such toxicity to humans and environment, so larvicide plants is a promising alternative. The purpose of this study is to determine the best season to harvest aerial parts of *Mesosphaerum suaveolens* (L.) Kuntze to obtain its essential oil, and evaluate the effects of seasons on chemical composition, larvicidal activity against *A. aegypti*, and toxicology against *Artemia salina* and *Danio rerio* using such essential oils and its major compounds. The gas chromatography–mass spectrometry analysis of essential oil samples performed at different times during a year, showed qualitative and quantitative variation in the chemical composition. 1,8-Cineole is the major compound in all seasons. The dry season had a higher yield. The essential oils showed activity against *A. aegypti* larvae, with lethal concentrations varying from 90.8 to 135.2 µg/mL. Their major compounds present no activity up to the highest concentration tested (200 µg/mL). The toxicity for *A. salina* and *D. rerio* was lower than for *A. aegypti* larvae, ranging from 94.6 to 215.7 µg/mL. The results demonstrate potential of the essential oil of the aerial parts of *M. suaveolens* for the control of *A. aegypti* larvae, and evidence that the oil of the plant collected in the dry season is more promising for the control of the larvae and provides better yield.

1. Introduction

In tropical countries, arboviruses are important public health threats, since fast climatic changes, natural environment alterations and disordered occupation of urban areas with poor sanitary conditions favor viral amplification and transmission (Lopes et al., 2014).

Dengue (DENV 1-5), Chikungunya (CHIKV), Mayaro (MAYV) and Zika (ZIKV) are among the most widespread arboviruses in tropical countries. The high morbidity and mortality rates are high (Pereira Serra et al., 2016). *Aedes aegypti* is the most important vector of these arboviruses, it also transmits yellow fever in urban environments (Brazil, 2016).

The lack of vaccines against the main viruses transmitted by *A. aegypti* aggravates the situation and concentrates the control in the elimination of vectors, through the application of compounds to reduces or even eliminate the population of mosquitoes in areas where there is virus circulation. However, the compounds currently used as insecticides have disadvantages that limit their use such as high cost, selection of resistant mosquitoes, and toxicity to humans (Dias and Moraes, 2014; Rosa et al., 2016).

Safer alternatives have been researched aiming the control of this vector (Dias et al., 2015). The use of insecticidal and larvicide plants is a promising alternative since plant species produce bioactive compounds that usually act in synergism, are biodegradable, and present a low risk of developing vector resistance (Rosa et al., 2016; Martello et al., 2019).

Essentials oils are complex mixtures of several volatile secondary metabolites from plants, such as monoterpenes, sesquiterpenes, and phenylpropanoids (Bakkali et al., 2008; Dias et al., 2015; Pavela, 2015).

The chemical composition is genetically determined (intrinsic

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ORIGINAL ARTICLE

Pharmacognostic Evaluation of *Carapa guianensis* Aubl. Leaves: A Medicinal Plant Native from Brazilian Amazon

Tássio Rômulo Silva Araújo Luz[1], José Antonio Costa Leite[1], Samara Araújo Bezerra[1], Ludmilla Santos Silva de Mesquita[1], Edilene Carvalho Gomes Ribeiro[2], José Wilson Carvalho De Mesquita[1], Daniella Patrícia Brandão Silveira[1], Maria Cristiane Aranha Brito[2], Crisálida Machado Vilanova[3], Flavia Maria Mendonça do Amaral[1,3], Denise Fernandes Coutinho[1,2,3]

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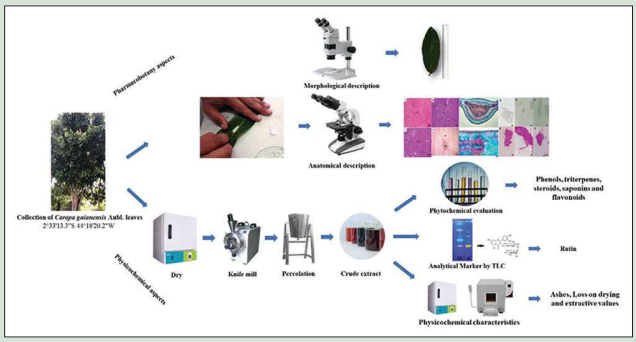
ABSTRACT

Background: *Carapa guianensis* Aubl., known as crabwood, has been used in folk medicine as anti-inflammatory, wound healing, and for the treatment of flu and colds. **Objective:** The present study aimed to establish the pharmacognostic features of *C. guianensis* leaves. **Materials and Methods:** The leaves were investigated according to the World Health Organization guideline on the pharmacognostic specification, which comprised macroscopic and microscopic assessment, phytochemical screening, and physicochemical characterization of the leaves, besides the microscopic analysis of the powder. **Results:** Leaves were characterized as a compound, coriaceous with elliptic shape, entire margin, acuminate apex, obtuse base, and opposite phyllotaxis. The epidermis has straight periclinal and anticlinal walls. Calcium oxalate crystals were observed in druses, anomocytic stomata just on a lower side (hypostomatic), and dorsiventral mesophyll. Phytochemical screening revealed the presence of flavonoids, saponins, triterpenes, and steroids in the crude extract. The values of the physicochemical parameters such as total ash, acid-insoluble ash, and loss on drying are 7.16%, 1.03% and 7.93%, respectively; the ethanol and water-soluble extractive values are 19.47% and 15.97%, respectively. **Conclusions:** The information obtained with botanical, physicochemical, and phytochemical studies could be used to identify *C. guianensis* and to certify the authenticity of commercial samples.

Key words: Andiroba, morphoanatomy, phytochemical screening, quality control, rutin

SUMMARY

- Rutin can be used as chemical marker for quality control of *C. guianensis*.
- The crude extract of the leaves has phenolic compounds, saponins, triterpenes and steroids.
- The leaves are characterized by the presence of cells with straight anticlinal walls and polyhedral shape, calcium oxalate crystals in the form of rosette in secondary ribs and anomocytic stomata.



Abbreviations Used: TLC: Thin-layer chromatography.

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INTRODUCTION

Medicinal plants are a rich source of secondary metabolites with interesting biological activities,[1] and since the beginning of civilization, plants have been used for man to heal, cure, or prevent diseases; they have great potential for producing new drugs of great benefit to humankind.[2,3]

Carapa guianensis Aubl. (*Meliaceae*), commonly known as andiroba and crabwood, is a tree distributed in the Amazon basin, Central America, and the Caribbean. In Brazil, it was included in a national list of medicinal plants with the potential to advance in the production chain and generate products of interest to the public health system.[4]

Ethnopharmacological studies have reported the therapeutic use of *C. guianensis*. Leaves and bark are used to prepare the tea for treating fever, worms, and skin illness.[5] The seed oil is largely utilized to treat diseases of the respiratory system, inflammation, and as an insect repellent.[6-9] Owing to its traditional use for therapeutic purposes for centuries, this species, mainly the oil has been investigated for its pharmacological properties. However, studies with the *C. guianensis* leaves are lacking. Therefore, it is required studies to validate the popular use of this plant species, in a real perspective of the development of products derived from *C. guianensis*.[10,11]

However, the legislation[12] emphasizes that for the development of herbal medicines, it is necessary to define parameters of efficacy, safety, and quality (authenticity, integrity, and purity) of both the raw material and the finished product, which also allows the inclusion in pharmacopoeias and official codes.

The integrity tests aimed at qualitative and quantitative evaluation of markers of plant material, assays based on characterization and chemical

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ANEXOS

JOURNAL OF FUNCTIONAL FOODS

AUTHOR INFORMATION PACK

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DESCRIPTION

Announcement: From January 2020, *Journal of Functional Foods* is an open access journal. Authors who publish in the Journal will be able to make their work immediately, permanently, and freely accessible.

Journal of Functional Foods continues with the same aims and scope, editorial team, submission system and rigorous peer review. We give authors the possibility to publish their top-quality papers in a well-established leading journal in the food and nutrition fields. The Journal will keep its rigorous criteria to screen high impact research addressing relevant scientific topics and performed by sound methodologies.

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Characterisation of healthy foods and functional constituents with reference to product development; preparation of natural and synthetic ingredients for use in foods, effects of processing (including packaging and storage) on functionality and improvement of product quality; verification, quality

control and traceability of natural and synthetic functional food ingredients and products will be considered.
The regulatory aspects of functional foods and related issues e.g. labelling, substantiation of health claims are also of interest together with those dealing with the value creation on the food chains based on the nutritional/healthy aspects.

The following papers are not within the scope of the Journal: Papers only dealing with food analysis and characterization of food structure and composition Papers focusing on the absorption kinetic of single bioactives Papers dealing with pure compounds having no connection with food

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FOOD CHEMISTRY

AUTHOR INFORMATION PACK

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DESCRIPTION

Food Chemistry has two open access companion journals *Food Chemistry: X* and Food Chemistry: Molecular Sciences.

The Aims and Scope of *Food Chemistry* are assessed and modified on an annual basis to reflect developments in the field. This means that research topics that have been deemed in scope previously may now fall outside of the scope of the journal as our scientific and technical understanding of the fields evolve and topics become less novel, original or relevant to *Food Chemistry.*

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– Chemistry relating to major and minor **components of food**, their nutritional, physiological, sensory, flavour and microbiological aspects;

– **Bioactive constituents** of foods, including antioxidants, phytochemicals, and botanicals. Data must accompany sufficient discussion to demonstrate their relevance to food and/or food chemistry;

– Chemical and biochemical composition and structure changes in molecules induced by processing, distribution and domestic conditions;

– **Effects of processing** on the composition, quality and safety of foods, other bio-based materials, by-products, and processing wastes;

–Chemistry of **food additives**, **contaminants**, and other agro-chemicals, together with their metabolism, toxicology and food fate.

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