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Physicochemical and Nutritional Composition of Pot-pollen of Stingless bee *Melipona beecheii* in Yucatan, Mexico

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Abstract

The stingless bee *Melipona beecheii*, known as "Xunan Kab" in the Mayan language, is the only species that has been domesticated by the Mayans since immemorial time. Pot-pollen is collected, processed, and stored by stingless bees, in cerumen containers, which is used for its nutritional value in the larval and human diets. It is made up of natural floral pollen mixed with nectar and bee secretions, and fermented by microbes associated with stingless bees. The proximal analysis of the pot-pollen on a dry basis showed moisture, protein, fat, and ash content of 35.59 %, 36.9 %, 12.65 %, and 1.87 % respectively. The aminoacids analysis of pot-pollen proteins showed a high content of essential aminoacids such as leucine, methionine, threonine, valine among others. The methanolic extract of pot-pollen as well as its corresponding hexane and chloroform fractions presented antibacterial activity against *Listeria monocytogenes* and *Pseudomonas aeruginosa*. Additionally, eleven metabolites were identified from the hexane and chloroform fraction of pot-pollen, palmitic acid (1), γ-sitosterol or β-sitosterol (2), myristic acid (3), 5-octadecene (4), 9-octadecene (5), pentadecanoic acid, 14-methyl, methyl ester (6), palmitic acid, 1,1-dimethylethyl ester (7), dotriacontane (8), heptadecane (9), stearic acid butyl ester (10), and 2-methyltetracosane (11). Pot-pollen from *Melipona beecheii* is a superfood with a high nutritional value due to its high content of protein with a good percentage of essential aminoacids, fatty acids composition and metabolites with antimicrobial activity.

Keywords: antimicrobial activity, essential aminoacids, fatty acids, *Melipona beecheii*, pot-pollen protein, γ-sitosterol

1. Introduction

Natural products, including bee products, are particularly appreciated by consumers and used for therapeutic purposes as alternative drugs (Denisow & Denisow-Pietrzyk., 2016 pp 4303-4309). Natural bee products such as honey, propolis, geopropolis, wax, royal jelly, and pollen have been extensively employed since ancient times due to their wide pharmacological and nutritional content (Rao *et al.*, 2016 pp 657-664; Vit *et al*., 2024a pp 1-6; Vit *et al*., 2024b p 3879; Araque & Vit., 2024 pp 1-8).

Pollen is the male reproductive cell produced in the spermatophytes of anthers, and plays an essential role in the life cycle of flowering plants. Pollen grains are excellent sources of carbohydrates, proteins, lipids, vitamins, and minerals necessary for plant growth, development, and fusion with a female gamete (Denisow & Denisow-Pietrzyk., 2016 pp 4303-4309; Fatrcová-Šramková *et al*., 2016 pp 176-181; Kyselka *et al*., 2018 pp 11018-11026; Yang *et al*., 2013 pp 708-718). Several studies have shown that the pollen collected by honey bees is very selective and the bulk of pollen comes from a few plants only. Bee pollen is one of the richest natural foods and has nutritional and medicinal value. These key properties of bee pollen are due to the medicinally important bioactive compounds it contains and explain its use in traditional medicine for the treatment of various

diseases (Denisow & Denisow-Pietrzyk., 2016 pp 4303-4309; Jannesar *et al*., 2017 pp171-182; Kaur *et al*., 2013 pp 65-68; Vit *et al*., 2024a pp 1-6; Vit *et al*., 2024b p 3879; Araque & Vit., 2024 pp 1-8).

Stingless bees (Hymenopea; Apidae; Meliponini) comprise a diverse group of eusocial bees, especially diversified in the tropics. They are considered one of the most important pollinators and also the most significant ecosystem service providers in several tropical ecosystems (Gaona *et al*., 2019 p 22; Quezada-Euán., 2018 pp167-192; Slaa *et al*., 2006 pp 293-315). Stingless bees are an important component in the complex pollinator networks of most tropical forest ecosystems. Additionally, stingless bees are considered polyletic because of their ability to collect pollen and nectar from a variety of non-related plants (Biesmeijer *et* al., 2005 pp 444-450; Eltz *et al*., 2001 pp 273-279; Gaona *et al*., 2019 p 22). To know how these bees forage in tropical forests is a priority if we are to guarantee their conservation and the services these insects provide (Gaona *et al*., 2019 p 22; Schleuning *et al*., 2012 pp 1925-1931).

The Meliponini tribe is a group of stingless bee species found around the world which are characterized as having social organization and bearing an atrophied and non-functional sting, which gives rise to their popular name of stingless bees. The Meliponini tribe belongs to the Apinae subfamily of the Apidae family. *Trigona* and *Melipona* are the most well-known of these tropical bees (Ferreira *et al*., 2017 p 298; Heard, 1999 pp 183-206; Silva *et al.*, 2009 pp173-178; Yam-Puc *et al*., 2019 pp 358-363). The *Melipona beecheii* species has been of great importance and widely exploited in America since pre-Columbian times, in particular by Mesoamerican cultures such as the Maya. *M. beecheii* was practically the only bee species domesticated by the Maya due to its nest size, to the tameness of the bees, and to the excellent flavor, therapeutic properties and attractive golden appearance of its honey (Quezada-Euán *et* al., 2001 pp 160-167; Yam-Puc *et al*., 2019 pp 358-363).

Previous chemical studies in an ethanol-soluble fraction of sunflower bee pollen have resulted in the identification of a number of polyamides of hydroxycinnamic acids. The main metabolites found were tri-p-coumaroylspermidines together with other amides of hydroxycinnamic acids of spermidine and putrescine (Kyselka *et al*., 2018 pp 11018-11026). Similarly, in the ethyl acetate extract of pot-pollen from *Melipona rufiventris*, the metabolites p-hydroxycinnamic acid, dihydroquercetin, isorhamnetin, isorhamnetin-3-O-(6’’-O-E-p-coumaroyl)-β-D-glucopyranoside, luteolin, and quercetin were isolated and identified (Silva *et al.*, 2009 pp 173-178). Additionally, chemical studies in a hydroethanolic extract of pot-pollen from *Melipona fasciculata* identified many metabolites, including gluconic acid, gluconic acid derivative, kaempferol, kaempferol derivative, 6-hydroxykaempferol-3,6-digluoside-7-glucoronide, ellagic acid, ellagic acid dimer, quercetin-3,4’-diglucoside, linoleic acid and linolenic acid (Lopes *et al*., 2019 pp 4512).

Various studies carried out on different bee pollens have demonstrated that its chemical composition and biological activity depend on the plant species and nectar sources that the pollinizing bees visit (Denisow *et al*., 2016 pp 4303-4309). Recent studies of the ethanolic extracts of pot-pollen from *Melipona beecheii* have reported its important antibacterial activity against *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Listeria monocytogenes*. Likewise, the ethanolic extracts of pot-pollen from *M. beecheii* presented antioxidant activity. The main components found by GC-MS in the ethanolic extracts of pot-pollen from *M. beecheii* were palmitic acid, linoleic acid and linolenic acid, with their respective ethyl esters (Calderón-Martínez et al., 2024 p e202401355).

In our continuing search of compounds with nutritional potential and biological properties from bee products, and with the aim of contributing to the available information on the stingless bee *M. beecheii*, the main objective of this research was to evaluate the metabolites and antibacterial properties present in the pot-pollen of this species.

2. Methods

2.1 Entomological Identification

Collection of stingless bee specimens, deposited in stingless bee collection at Universidad Intercultural Maya de Quintana Roo identified as *Melipona beecheii* (Bennett, 1831) by Dr. Ricardo Ayala.

2.2 Pot-pollen from M. beecheii

Pot-pollen samples collected and processed by *M. beecheii* were taken directly from pollen pots of their nests at the “Flor de Mayo" meliponary in Mani, Yucatan, Mexico (20.3931° N, 89.3918° W). After collection, pot-pollen samples were placed in a sterile container and kept in refrigeration at 4 °C until use (Calderon *et al*., 2024 p e202401355).

2.3 Physicochemical Assay

The characterization of the pollen was carried out through different proximal analysis techniques, according to Bujang *et al*., 2021 p e0247327). The percentage of moisture was carried out by the oven drying method at 105 °C until constant weight. The percentage of moisture was calculated with the following formula: % Moisture = ((Pi−Pf)/Pi)*100. Where Pi: Initial weight of the sample (g) (wet) and Pf: Final weight of the sample (g) (Dry). In the case of ashes, the previously dehydrated pot-pollen samples were calcined in a muffle at 550°C for 4 hours. The percentage of ashes was obtained using the following formula: % Ashes= (P2−P0) * (100/P1−P0). Where: P0: Weight of crucible (g), P1: Weight of the crucible containing the sample (g), P2: Weight of the crucible and the residue after incineration (g). For the fat percentage, cellulose cartridges of constant weight were used, to which 2 g of dehydrated pot-pollen were placed, and they were inserted into the 83 Soxhlet extractor. The formula to obtain the percentage of fat is as follows: % Fat= (B−A/PM) *100. Where: B: Weight of the cellulose cartridge with sample (g) (before introducing into the equipment), A: Weight of the cellulose cartridge with sample (g) (after introducing into the equipment), PM: Weight of the dehydrated sample (g). The protein percentage was carried out by the Kjeldahl method. 250 mg of fresh pot-pollen was used, which was digested with 10 mL of sulfuric acid as described in the test. The protein percentage was calculated with the following formula: % Protein = (volume (mL) x normality (HCl) * 1.4 * 6.25) / sample weight (g).

2.4 Determination of Aminoacids Profile by HPLC

Samples of dry and wet pot-pollen were used for determining aminoacid profile using high-performance liquid chromatography-phase reverse with diode array detector, according to the methodology of Alaiz et al., 1992 p 181-186 without modifications; in a Dionex UltiMate 3000 Thermo scientific UHPLC using a C-18 Grace column of 300 mm x 3.9 mm, 125 A, 10 µm.

Forty mg of each pot-pollen sample were processed using acid and alkaline hydrolysis, the standards of aminoacids used as reference were AA-S-18 and L-Tryptophan HPLC grade of Sigma- Aldrich. The aminoacids content was calculated using an aminoacid standard curve with a correlation coefficient of 0.997 and the detection limits were 2.5 to 200 pmol aa/µL.

2.5 Chromatographic Analysis

Vacuum Liquid Chromatography (VLC) and column chromatography purifications were performed using E.M. Merck TLC-grade silica gel 60GF and E.M. Merck silica gel (70-230 mesh), respectively. Analytical TLC experiments were carried out using aluminum-backed silica gel (60F254) plates (E.M. Merck, 0.2 mm thickness). The various components in the chromatograms were visualized by dipping the plates in a solution of phosphomolybdic acid (20 g) and ceric sulfate (2.5 g) in 500 ml of sulfuric acid (5%), followed by drying and gentle heating. GC-MS analyses were run on a Hewlett Packard 5890 gas chromatograph connected to a mass selective detector (MSD) (model 5975) [GC conditions: Split injection of 1 ml of sample; Ultra 1 column (25 m × 0.2 mm i.d.), flow rate 1.0 ml/min (Nitrogen); oven temperature program T1= 100° C (3 min), T2= 280° C (30 min), gradient 10° C/min, injector 300° and detector (FID) 300° C]. The components of each fraction were identified by comparing the MS spectra with those previously reported, by matching fragmentation patterns with those in the NIST05 library.

2.6 Extraction and Isolation

The pot-pollen samples (159.35 g) were combined and extracted three times by maceration with methanol for 24 h. The resulting product from the three extractions was combined, filtered and concentrated under reduced pressure at 40 °C to produce 50.72 g (31.83 %) of crude extract. The crude extract was suspended in a methanol:water mixture (7:3, v/v) and the resulting aqueous suspension was fractioned with n-hexane (three times, 2:1, v/v) and then with chloroform (three times, 2:1, v/v) yielding two fractions (3.59 and 1.95 g) of increasing polarity. The hexane fraction (3.59 g) was subjected to VLC purification, eluting with increasing amounts of heptane in chloroform and chloroform in methanol to produce five main fractions (A-E). Fraction D was successively purified by gravity column chromatography eluting with hexane/acetone (9:1) and hexane/ethyl acetate/methanol (8:1:1), by means of which it was possible to obtain two semi-pure fractions, I (25.3 mg) and II (6.2 mg). The chloroform fraction was successively purified by gravity column chromatography eluting with ethyl ether/chloroform/methanol (5:4:1) and hexane/acetone (8:2) to obtain one semi-pure fraction, III (3.7 mg).

2.7 Antibacterial Activity

The antibacterial activity assays were carried out with the strains *Pseudomonas aeruginosa* (ATCC 27853) and *Listeria monocytogenes* (ATCC 15313). Bacterial cultures were carried out in Petri dishes with Müeller Hilton agar at 37°C for 24 h. Bacterial inoculums were prepared by diluting cell biomass in 0.85% NaCl, adjusting to

0.5 on the McFarland scale. The bacterial suspension was diluted approximately to a concentration of 1 x 106 colony forming units per milliliter (CFU/mL). For antibacterial activity, disk diffusion and microdilution methods were used (Ramón-Sierra *et al.*, 2021 p 100177). The crude extract and fraction I (dissolved in DMSO) were evaluated. In the first method, 6mm diameter filter paper discs were used with the agents to be evaluated, in Petri dishes with Müeller Hilton agar, these were incubated at 37°C for 72 h. In the second method, bacterial growth was evaluated by optical density at 600 nm in the presence of different concentrations of the agents in 96-well microplates with Müeller Hilton medium and incubated at 37°C for 72 h at 250 rpm. Both assays were done in triplicate. The positive control was amikacin (10 µg/mL) and the negative control was DMSO (10 %).

3. Results

3.1 Physicochemical Composition of M. beecheii Pot-pollen

Table 1 shows the results of the proximal analyses carried out on the pot-pollen collected and processed by *M. beecheii. M. beecheii* pot-pollen had high protein content. This high concentration of protein makes *M. beecheii* pot-pollen a food with high nutritional value.

Table 1. Proximal analyses of pot-pollen from *M. beecheii*

Parameters	*M. beecheii* pollen (%)
Moisture	35.59±0.16
Ash	1.87±0.18
Fat	12.65±1.4
Protein	36.9±0.51

3.2 Amino Acid Profile of M. beecheii Pot-pollen

Table 2 shows the amino acid composition of *M. beecheii* pot-pollen, it was observed that it contains a percentage greater than 50 % of essential and semiessential amino acids compared to not essential aminoacids. All essential and semiessential amino acids are present in the pot-pollen, arginine, methionine/cystine, and threonine had high content. In the case of not essential aminoacids, aspartic acid/asparagine, and glutamic acid/glutamine presented the highest content.

Table 2. Composition of Amino Acids (g/100g of protein) in Pot-pollen of *Melipona beecheii*

Amino acid name	**Amino acid content**
Arginine	13.31 ± 0.04
Histidine *	1.60 ± 0.19
Isoleucine *	3.96 ± 0.17
Leucine *	9.33 ± 0.00
Lysine *	3.29 ± 1.01
Methionine */Cysteine	7.57 ± 0.27
Phenylalanine *	4.40 ± 0.27
Threonine *	5.36 ± 0.38
Tryptophan *	2.62 ± 0.35
Valine *	4.94 ± 0.08
Aspartic acid/Asparagine	10.61 ± 1.18
Glutamic acid/Glutamine	15.41 ± 0.21
Alanine	3.47 ± 0.02
Glycine	4.53 ± 0.05
Proline	2.09 ± 0.49
Serine	6.12 ± 0.22
Tyrosine	2.57 ± 0.39

*Essential aminoacids

3.3 Chromatographic Analysis of Crude Extract and Fraction of M. beecheii Pot-pollen

Successive chromatographic purifications of the hexane fraction of *M. beecheii* pot-pollen, using a combination of VLC and gravity column chromatography, resulted in the isolation of two semi-pure fractions, I and II. Additionally, successive chromatographic purifications of the chloroform fraction using a gravity column chromatography gave semi-pure fraction III.

The GC-MS analysis of the three semi-pure fractions permitted detection of eleven metabolites (two fatty acids, three ester fatty acid derivatives, two alkenes, three alkanes and, one sterol). The metabolites were identified based on the molecular weight and by comparing their fragmentation pattern in mass spectrometry with the compounds described in the NIST05 library and the literature data.

The chromatographic profile in the CG-MS analysis of fraction I (Fig. 1A) showed a number of peaks and metabolites 1 (t_R 14.00 min) and 2 (t_R 26.60 min) were identified. The mass spectrum of metabolite 1 showed a parent ion peak at *m/z* 256, suggesting the molecular formula $C_{16}H_{32}O_2$, which was identified as palmitic acid. Additionally, the mass spectrum of metabolite 2 showed a parent ion molecular at *m/z* 414 ($C_{29}H_{50}O$), which was identified as a β-sitosterol (2a) or γ-sitosterol (2b), a steroidal molecule (Fig. 2). It is not possible to distinguish the epimeric forms β-sitosterol and γ-sitosterol for metabolite 2 by mass spectrometry. β-sitosterol is one important kind of phytosterol, that is a Δ5 4-desmethyl sterol with an additional ethyl substituent in the side chain at C-24. This 24-ethyl substituent has α chirality which was already known. The only difference between these two epimers, β-sitosterol and γ-sitosterol, is the 24-ethyl substituent. The 24-ethyl in the side chain of γ-sitos-terol has beta chirality. It must be emphasized most strongly that the α-, β- assignments for side chain stereochemistry bear no relation to the use of α- and β- to define substituents attached to the sterol rings. The two systems of α/β assignments are quite unrelated (Liao *et al.*, 2018 pp 10748-10759; Sheng &Chen., 2009 pp 203-206).

On the other hand, the GC-MS analysis of fraction II (Fig. 1B) permitted identification of two metabolites (1 and 3). Metabolite 3 (t_R 11.93 min) presented a parent ion peak at *m/z* 228 ($C_{14}H_{28}O_2$) and from its fragmentation patterns was identified as myristic acid (3) (Dayhuff & Wells., 2005 pp 144-149).

The GC-MS analysis of fraction III (Fig. 1C) resulted in the identification of eight metabolites (4-11). Metabolites 4 (t_R 10.02 min) and 5 (t_R 12.29 min) showed the same parent ion peak at *m/z* 252 ($C_{18}H_{36}$) and from their fragmentation patterns were identified as two alkenes, 5-octadecene (4) and 9-octadecene (5), respectively (Adeosun *et al.*, 2013 pp138-141). The mass spectrum of metabolites 6 (t_R 13.66 min), 7 (t_R 16.10 min) and 10 (t_R 17.80 min) showed the parent ion peaks at *m/z* 270 ($C_{17}H_{34}O_2$), *m/z* 312 ($C_{20}H_{40}O_2$) and *m/z* 340 ($C_{22}H_{44}O_2$), and they were identified based on their fragmentation pattern as three fatty acid ester derivatives: pentadecanoic acid, 14-methyl, methyl ester (6) (Salem *et al*., 2011 pp 95-98; Williams., 1993 pp 159-164); palmitic acid, 1,1-dimethylethyl ester (7) (Radulović *et al.*, 2012 pp 2165-2185); and stearic acid butyl ester (10) (Gołębiowski *et al*., 2015 pp 213-222).

Finally, metabolites 8 (t_R 16.21 min), 9 (t_R 17.52 min) and 11 (t_R 17.85 min) presented parent ion peaks at *m/z* 450 ($C_{32}H_{66}$), *m/z* 240 ($C_{17}H_{36}$) and *m/z* 352 ($C_{25}H_{52}$) respectively, and from their fragmentation patterns were identified as the alkanes: dotriacontane (8) (Dauda *et al.*, 2022 pp 3215-3227), heptadecane (9) (Tsuchiya & Matsumoto *et al*., 1988 pp 149-155), and 2-methyltetracosane (11) (Muhammad *et al.*, 2015 pp 219-227).

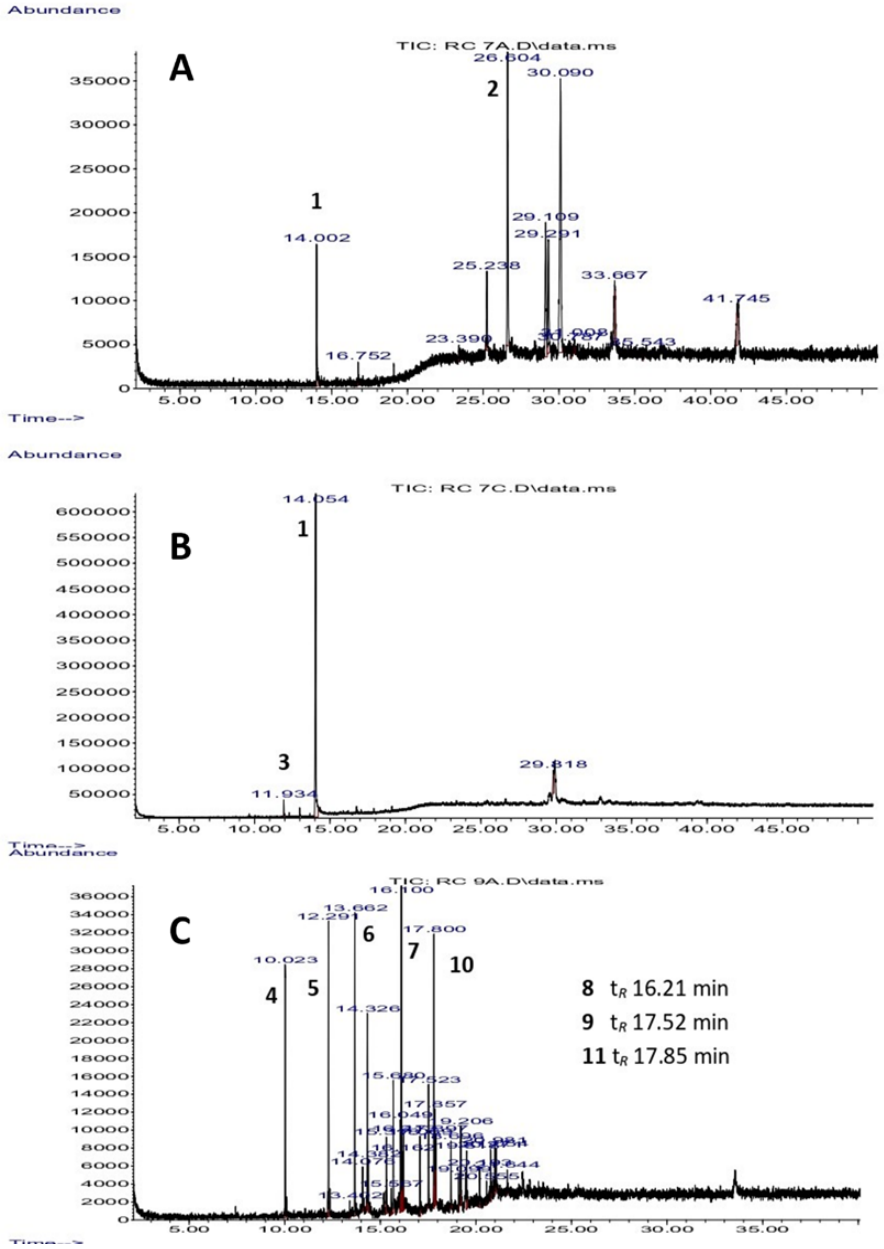


Figure 1. GC profile of fractions I-III of pot-pollen from *Melipona beecheii*. A. Fraction I. B. Fraction II and C. Fraction III

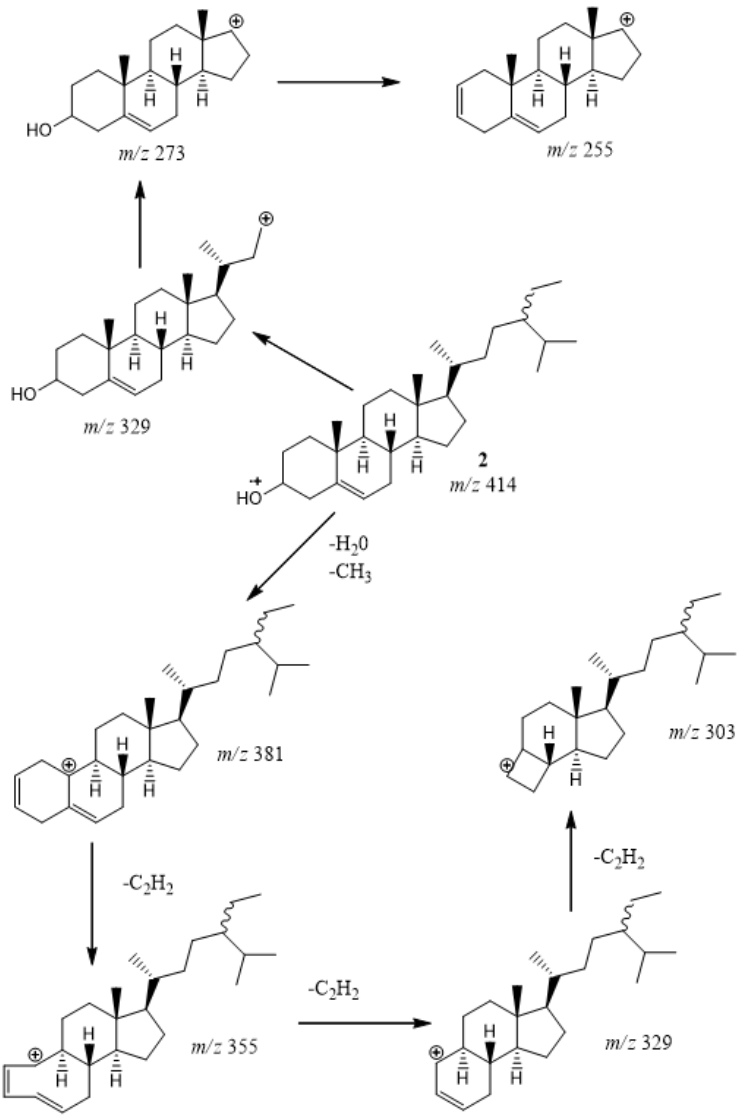


Figure 2. Fragmentation by mass spectrometry of metabolite 2, β-sitosterol (2a) or γ-sitosterol (2b)

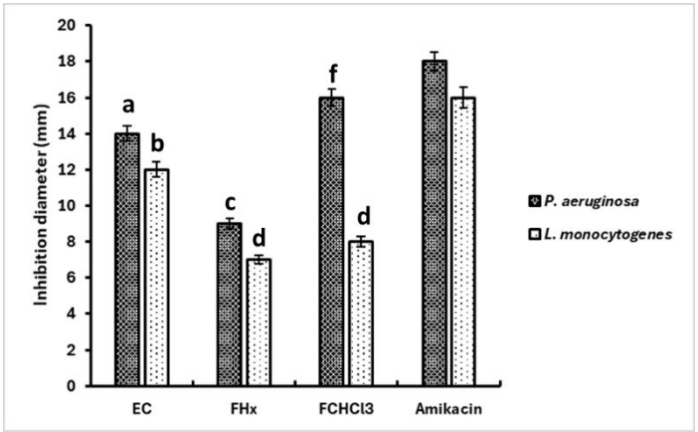
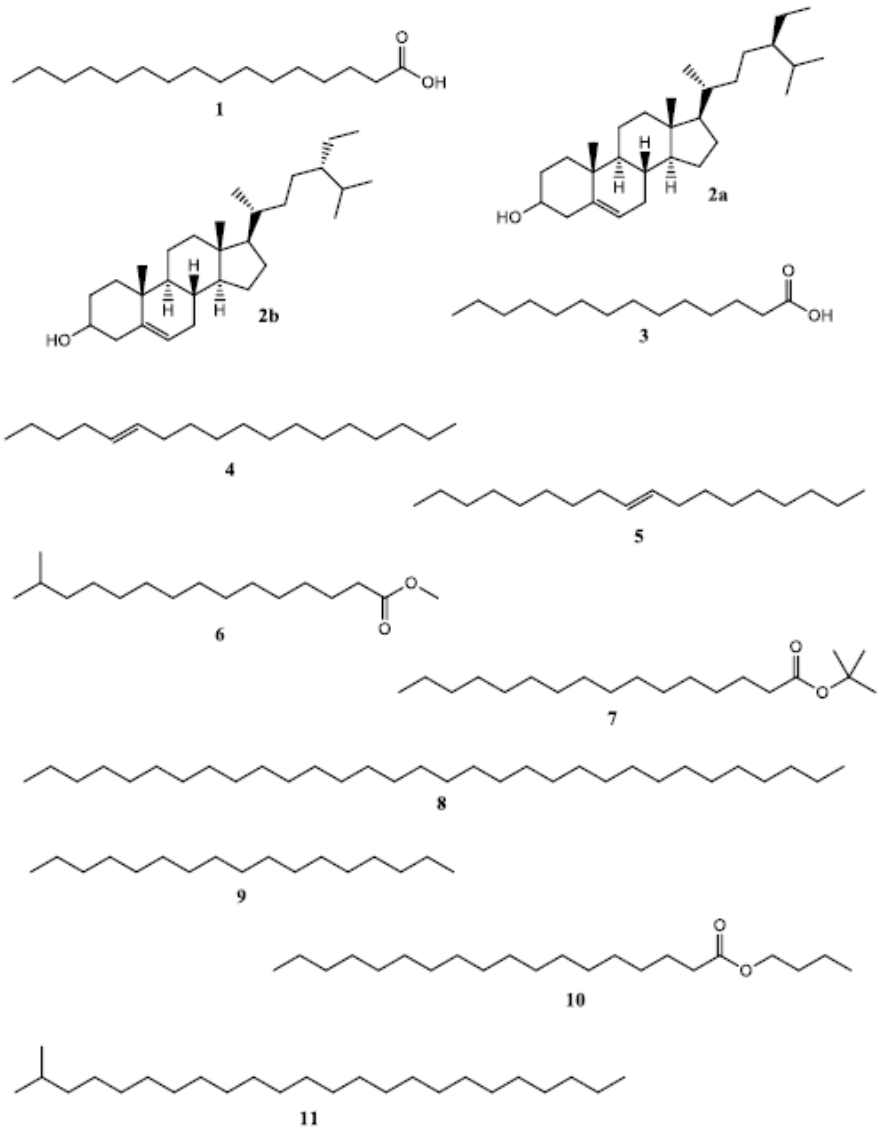


Figure 3. Antimicrobial activity of the crude extract (EC), fraction I hexane (FHx) and chloroform ($FCHCl_3$), and the antibiotic Amikacin against *P. aeruginosa* ATCC 27853 and *L. monocytogenes* ATCC 15313

Note: Different letters (significant difference). Equal letters (no significant difference). P-value < 0·05 was considered statistically significant.

Table 3. Metabolites identified in pot-pollen from *M. beecheii*

Number	t_R (min.)	Metabolite	Molecular weight	Molecular formula
1	14.00	Palmitic acid	256	$C_{16}H_{32}O_2$
2	26.60	β-sitosterol / γ-sitosterol	414	$C_{29}H_{50}O$
3	11.93.	Myristic acid	228	$C_{14}H_{28}O_2$
4	10.02	5-Octadecene	252	$C_{18}H_{36}$
5	12.29	9-Octadecene	252	$C_{18}H_{36}$
6	13.66	Pentadecanoic acid, 14-methyl, methyl ester	270	$C_{17}H_{34}O_2$
7	16.10	Palmitic acid, 1,1-dimethylethyl ester	312	$C_{20}H_{40}O_2$
8	16.21	Dotriacontane	450	$C_{32}H_{66}$
9	17.52	Heptadecane	240	$C_{17}H_{36}$
10	17.80	Stearic acid buthyl ester	340	$C_{22}H_{44}O_2$
11	17.85	2-methyltetracosane	352	$C_{25}H_{52}$



3.4 Antibacterial Activity of Crude Extract and Fraction of M. beecheii Pot-pollen

Figure 3 shows that the pollen metabolites of *M. beecheii* were able to inhibit the growth of both pathogens evaluated, with *P. aeruginosa* ATCC 27853 being the most susceptible to these metabolites, showing larger

inhibition zones for both the CE and Fraction I, while for *L. monocytogenes* ATCC 15313, smaller inhibition zones were obtained.

Fraction, I (FHx) inhibited the growth of *P. aeruginosa* by 99% with concentrations of 6 mg/mL, while for *L. monocytogenes* it required twice as much to inhibit its growth. In the case of CE, the same minimum inhibitory concentration was required to inhibit both microorganisms. In the case of the chloroform fraction, a concentration of 7 mg/mL was required to eliminate 99.9% of the growth of *P. aeruginosa*, while in the case of *L. monocytogenes*, the fraction $FCHCl_3$ required 14 mg/mL. For the present study, 1 mg/mL of amikacin was used. All samples showed stability of their antimicrobial activity for 72 hours.

Table 4. Minimum Inhibitory Concentration (MIC) of Crude extract and its Fraction I of pot-pollen from *M. beecheii*

		Bacterial Strains	
Pot-pollen Crude Extract and Fraction I		*P. aeruginosa* ATCC 27853	*L. monocytogenes* ATCC 15313
Crude Extract (CE)	MIC (mg/mL)	8 ± 0.5	8 ± 1.0
FHx	MIC (mg/mL)	6 ± 0.5	12 ± 2.0
$FCHCl_3$	MIC (mg/mL)	7 ± 1.0	14 ± 1.0

4. Discussion

Pot-pollen is a product of high commercial value due to its beneficial nutritional contents of carbohydrates, lipids, proteins, amino acids, vitamins, minerals, sterols, carotenoids and polyphenols. Its quality depends on geographical, climatic factors, hive management and floral diversity (Yang *et al.*, 2013 pp 708-718).

In pot-pollen, moisture is an important quality parameter, since it is useful to avoid deterioration and proliferation due to the growth of detrimental bacteria, molds and insect larvae, particularly *Achroia grisella* or the moth that attacks the pot-pollen (Bogdanov, 2004 pp 334-341), and it is needed for fermentation by microbes associated with *M. beecheii* (Calderón-Martínez et al., 2024 p e202401355). The moisture content of pot-pollen from *M. beecheii* value was lower than that reported in pot-pollen from other stingless bees such as *Scaptotrigona* sp. and *Melipona scutellaris*, with 43.49±0.95% and 52.89±1.90% respectively (Alves *et al*., 2018 pp 349-360, Vit *et al*., 2016 pp 78-84), which could be due to climatic conditions, soil, and flowering. Pot-pollen contains essential elements for the development of stingless bees such as calcium, magnesium, sodium, potassium, iron, copper, zinc, manganese, silicon and selenium (Vit et al., 2018 pp 318-323; Vit et al., 2024b p 13). The percentage of ash in this research was 1.87 ± 0.18 %, obtaining similar results in the stingless bee *Scaptotrigona* sp. with 1.94 ± 0.35 % reported by Vit *et al.* (2016 pp 78-8416), similarly, in other stingless bee species such as *Tetragonisca angustula* and *Melipona scutellaris*, showed data greater than 2.06 ± 0.13 % and 4.72 ± 0.61 % respectively. In the case of fats, the pot-pollen of *M. beecheii* showed lipids of 12.65 ± 1.4 %. A background of this work showed a fat content of 9.47 ± 0.49 %, in pot-pollen of *M. beecheii* from Maní Yucatán. This variation may be due to the composition of the floral pollen depending on the region, the season of the year, the climate, and the type of flowering, since in *A. mellifera* pollen, it has shown a content of 10.79±7.16%, similar results to the previous ones (Yang *et al.*, 2013 pp 708-718). It has been reported that in bee pollen we can find essential fatty acids such as linoleic, γ-linoleic and archaic acids (Komosinska-Vassev *et al.*, 2015 pp 1-7). The pot-pollen of *M. beecheii* showed 24.00 ± 0.31 %, a value similar to 20.09 ± 2. 02%, corresponding to the pot-pollen of *M. beecheii* from Mani, Yucatan. The protein content is similar to that of the pot-pollen of other meliponines such as *Tetragonisca angustula* with 22.97±3.57% (Vit *et al*., 2016 pp78-84). The high values of this macronutrient in the pollen collected and processed by the bee *M. beecheii* means that this food reserve in the nest can be considered a superfood with high nutritional value.

We can find that *Melipona beecheii* pot-pollen had proteins with high biological value such as ovalbumin and -lactoglobulin from egg and milk respectively, it contains comparable quantities of important human diet aminoacids as tryptophan, leucine and threonine are present in the aminoacid analyses. Several investigations have reported the high nutritional value of the pot-pollen such as *Tetragonula laeviceps* where essential amino acids: leucine and phenylalanine were highly present (Mohammad *et al*., 2021 p 957). Notably, tryptophan content in *M. beechei* pot-pollen is high, and according to daily aminoacids requirements for humans, 10 g of pot-pollen are enough to provide it. It was reported that the free aminoacids required by the human body are adequately provided even by 15 g of bee pollen (Thakur *et al*., 2020 pp 82-106). It has also been reported that asparagine glutamic acid and proline had the most content in bee pollen of *Apis mellifera* (Martín-Gómez *et al*., 2022 p 4013)

On the other hand, the main metabolites identified in the *M. beecheii* pot-pollen were fatty acids (1 and 3), a sterol (2), fatty acid ester derivatives (6, 7 and 10), and long-chain alkenes (4 and 5) and alkanes (8, 9 and 11). In the case of metabolites 1 and 3 identified in *M. beecheii* pot-pollen, they correspond to palmitic acid and myristic acid, while metabolites 6, 7 and 10 correspond to fatty acid ester derivatives from pentadecanoic acid, palmitic acid and stearic acid. Some lipids, including fats, are used to store energy, but most are used to form lipid / protein membranes (i.e., partitions that divide intracellular compartments and separate the cell from its environment) (Welte & Gould, 2017 pp1260-1272). It is important to mention, that in the present study were identified in the pot-pollen; saturated and unsaturated fatty acids and their derivatives; some of these have been previously reported in pot-pollen samples from *Tetragonula biroi* (Friese, 1898). Presence of saturated and unsaturated fatty acids and their derivatives confer to *Melipona beecheii* pot-pollen an important nutritional value; it has been reported that a balanced diet of saturated and unsaturated fatty acids, could prevent cardiovascular diseases (Belina-Aldemita *et al.*, 2019 p 103215). There are more than a hundred different types of fatty acids, although the most common in plants are oleic acid and palmitic acid (Cseke *et al.*, 2016 p 9). Moreover, metabolites 4, 5, 8, 9 and 11 in *M. beecheii* pot-pollen corresponded to long-chain alkanes (8, 9 and 11) and alkenes (4 and 5). Hydrocarbons correspond to a small group of less polar natural products that contain only hydrogen and carbon atoms. Aliphatic hydrocarbons are straight-chain hydrocarbons, which generally have an odd number of carbon atoms, as a result of decarboxylation of their fatty acid equivalents (Savage *et al*., 1997 pp 51-53). In plants, saturated hydrocarbons are universally distributed as waxy coatings (cuticle waxes) on leaves and as cuticle waxes on fruit surfaces. Typical examples include n-nonacosane (C29H60) and hentriacontane (C31H64) (Eglinton *et al*., 1962 pp89-102; Vrkoslav *et al*., 2010 pp220-231). Larger unsaturated hydrocarbons are also common as vegetable waxes. Exceptionally high amounts of alkenes have been detected in rye pollen (*Secale cereale*), rose petals (*Rosa* spp.) and sugar cane (*Saccharum* spp.). As the length of the chain and the degree of unsaturation increases, the hydrocarbons become waxy and then solid at room temperature. Waxes can be long chain hydrocarbons or fatty acid esters (Eglinton *et al* 1962 pp89-102; Savage *et al*., 1997 pp 51-53).

The search for metabolites from natural sources represents great potential for the development of products of biotechnological interest, focusing mainly on regions rich in biodiversity such as the Yucatan peninsula. Research into the chemical composition of the pot-pollen from stingless bee nests is of the utmost importance, since the metabolites present in this biological material represent a rich source of molecules with possible interesting biological properties that give the product added value. The current study of the metabolites present in the *M. beecheii* pot-pollen from the state of Yucatan was carried out with this in mind, and a total of eleven metabolites were identified. This work represents the first study of the metabolites present in the *M. beecheii* pot-pollen and this knowledge needs palynological analysis to reveal the variety of plants involved in the manufacture of said pot-pollen. Identification of the metabolites present in the *M. beecheii* pot-pollen will allow us to discover, in addition to their chemical structure, their probable potential for the development of new biotechnological products. Secondary and primary metabolites are of great importance to our health, nutrition, and economics. The importance of the study of metabolites (natural products) is due to their applications in biotechnology, for example we can mentioned a number of old and new peptidic drug entities, although formally synthetic in nature, are simply produced by synthetic methods rather than by the use of fermentation or extraction. Multibillion-dollar markets exist for the medically useful secondary metabolites, antibiotics and derivatives such as the β-lactam peptide antibiotics, glycopeptides, lipopeptides, polyketides, aminoglycosides, and others (Newman & Cragg., 2020 pp 770-803; Demain., 2007 pp 269-283).

5. Conclusions

Pot-pollen of *M. beecheii* is a food which contains a high percentage of good quality proteins, with a concentration of essential amino acids such as histidine, threonine and tryptophan, comparable with proteins considered of good nutritional quality such as albumin. Also, pot-pollen presents essential fatty acids important for human health. The present work represents the first study of metabolites found in the pollen collected and processed in cerumen pots by *M. beecheii* in the state of Yucatán, showing a variety of metabolites, highlighting the presence of essential amino acids and fatty acids, which are of great importance in larval and human nutrition. Finally, it is important to mention that the metabolites identified in the present work have other biological properties such as antimicrobial activity against *Pseudomonas aeruginosa* (ATCC 27853) and *Listeria monocytogenes* (ATCC 15313).

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Competing interests

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

Informed consent

Obtained.

Ethics approval

The Publication Ethics Committee of the Canadian Center of Science and Education.

The journal's policies adhere to the Core Practices established by the Committee on Publication Ethics (COPE).

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data sharing statement

No additional data are available.

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