Vol. 18(4), pp. 43-49, May, 2024
DOI: 10.5897/JMPR2023.7343
Article Number: 0EF6F3B72130
ISSN 1996-0875

http://www.academicjournals.org/JMPR

ACADEMIC JOURNALS
expand your knowledge

Journal of Medicinal Plants Research

Full Length Research Paper

Pollen extracts from the Soconusco region: Chemical profile and effect against methicillin-resistant *Staphylococcus aureus*

Eduardo Lozano Guzmán[1]*, Luis Ángel Morán Núñez[1], Maribel Cervantes Flores[1], Adolfo Padilla Mendiola[1], María Guadalupe Nieto Pescador[1], Gerardo Alfonso Anguiano Vega[1], Víctor Jesús Albores Flores[2], Alfonso López García[2] and Julieta Grajales Conesa[2]

[1]Faculty of Chemical Sciences, Juárez University of Durango State, Durango, Mexico.
[2]Biosciences Institute, Autonomous University of Chiapas, Chiapas, Mexico.

Received 6 April, 2024; Accepted 10 May, 2024

Pollen has been widely known to have beneficial properties for humans, including fighting harmful microorganisms. On the other hand, bacterial resistance is an obvious global problem that has increased over the years. For this reason, the study of the antimicrobial properties of substances such as pollen could play an important role in this area. The present work focused on evaluating the antimocrobial effect of pollen collected by five bee species (*Melipona becheii, Melipona solani, Scaptotrigona mexicana, Scaptotrigona pectoralis* and *Tetragonisca angustula*) from the Soconusco (in Chiapas, Mexico) against methicillin-resistant *Staphylococcus aureus*. Four types of solvents were tested (isopropanol, acetonitrile, methanol, and water). The compounds were separated and identified by High Performance Liquid Chromatography. The inhibition study was done by microdilution and MCI was determined. The pollen structures were analyzed by microscopy. Among the compounds detected with greatest abundance are: chrysin, riboflavin, myricetin and nordihydroguayaretic acid stand out. The structure of pollen was studied by microscopy. The results show little antimicrobial effectiveness in all extracts. However, a first approximation to the chemical profile of pollen from Soconusco is achieved, which is important since there are not many reports in this regard.

Key words: Pollen, microbial inhibition, Soconusco, chemical profile, native bee, methicillin-resistant *Staphylococcus aureus* (MRSA).

INTRODUCTION

The term pollen comes from the Greek “paluno” which means to *distribute* or *disperse*. The term passed into Latin as “pollen”, meaning *flour* or *dust*. Carl Von Linné defined it as the dust spread by the male organs of flowers and whose function is fertilization (Arrazola Bonilla, 2020). Although the botanical and geographical origin of pollen can vary considerably, its chemical composition, in general, its chemical composition generally includes

*Corresponding author. E-mail: elozano@ujed.mx.

nutritional compounds such as carbohydrates, proteins, vitamins, and minerals, along with polyphenols, primarily flavonoids (Graikou et al., 2011). Other compounds have been found in bee pollen from different botanical origins, such as tannins, anthocyanins, stilbenes, coumaric acid, cinnamic acid and caffeic acid (Kaškonienė et al., 2020). In any case, the compounds present may vary according to the collection region, vegetation, species of forager bee, and time of year of collection (Amal et al., 2019).

Regarding their structure, pollen grains have two walls that protect them: the intina, which is in contact with the gamete, and the exine, a resistant covering located on the outside. The exine can be smooth, with engravings, pores, grooves, areolas, warts, or spines, which provide it with a very varied ornamentation and favor its adherence to the stigmas during pollination (Fontes et al., 2020).

Bee pollen has been widely known to have beneficial properties for humans, among the most important of which are antibacterial, antifungal, anticarcinogenic, antioxidant, and anti-inflammatory properties, among others. There are also records that it has been used successfully as a treatment for benign prostatitis (Morais et al., 2011). In pollen from different regions of Chile, a considerable antimicrobial effect was observed against *Streptococcus pyogenes*. Inhibition against other pathogens such as *Pseudomonas aureginosa*, *Staphylococcus aureus* and *Escherichia coli* was also evaluated, but these pollens did not present zones of inhibition. In pollen from stingless bees from the Amazon, Brazil, on the other hand, antibacterial activity was reported against *P. aeruginosa*, as well as *Candida albicans* (Carneiro et al., 2019). Another marked difference between different regions is the phenolic content, for example, in samples collected in parks in Portugal, the phenolic content varied between 10.5 and 16.8 mg of gallic acid equivalents per gram (GAE/g) (Morais et al., 2011), in regions of Vienna and New Zealand the content was lower, however, in pollens of Brazilian origin the phenol content is the highest reported, with values between 19.28 and 48.90 mg GAE/g (Pascoal et al., 2014). On the other hand, when we talk about antioxidant activity, Greek pollen has been shown to be at least 10 times more active than pollen from Brazilian regions, but it also turns out to have less antioxidant activity than pollen from certain regions of Arizona (Graikou et al., 2011).

For all this, it is considered that pollens from other poorly studied regions, such as the Soconusco region, Chiapas, in Mexico, deserve an adequate study approach. Soconusco is a jungle region in southern Mexico rich in tropical vegetation and abundant water. At least five species of native stingless bees occur in their habitat. In the present study, two types of pollen extracts collected by these species were made, which were tested against methicillin-resistant *S. aureus* (MRSA) and an approach to the chemical profile was made using high performance liquid chromatography coupled to a diode array detector (HPLC-DAD).

MATERIALS AND METHODS

Pollen samples

The collection of pollen samples was done in the Soconusco (which is a region south of the state of Chiapas, in Mexico), during the summer-autumn of 2021. The samples were taken directly from the hives. Table 1 shows the sampling site, its geolocation, the main crops in that region and the species of bee from which the samples were taken.

Obtaining extracts

The extracts were obtained by sonication with ultrasound based on what was described by Amal et al. (2019) and Vit and Santiago (2008). Four extracts were obtained by using four different solvents, which were applied from lowest to highest polarity: Isopropanol, Acetonitrile, Methanol and Water. In all cases, a 200 mg pollen sample was taken and crushed in a mortar until a fine powder was obtained. Each sample in each solvent was subjected to sonication at 14 kHz at 45°C in two sessions of 30 min each, with an interval of 15 min between them, and then they were kept at rest for 1 h protected from light. The tubes were centrifuged at 6,500 rpm for 10 min. The supernatant was taken from each of the tubes and filtered on a 0.45 µm pore nitrocellulose filter. Then, an aliquot of each extract, in its corresponding solvent, was placed in a vial for analysis in high-performance liquid chromatography.

The rest of the supernatant was evaporated in a stream of nitrogen and a water bath at 50°C until dry, then they were reconstituted in water and kept refrigerated until the microbial inhibition assay. The yield of the extracts was also determined by dry weight of a 1 mm aliquot of each extract subjected to evaporation in a stream of nitrogen to dryness. The results are expressed in mg/mL of dry solids. Additionally, the pollens from each extract of all solvents were analyzed with a VanGuard model 1486FLi trinocular fluorescence phase electron microscope, provided with a 100W HBO lighting system with a mercury light source to compare the state of the pollen grains before and after the extraction process.

Microbial inhibition assay

The microbial inhibition tests were carried out according to the methodology described by Taroco et al. (2006) with some modifications. The MRSA strains used were obtained from healthy carriers in the community, identified by biochemical means, and preserved in skim milk medium at -20°C. A suspension of MRSA cells was prepared in Muller Hinton broth and the extract reconstituted in water was applied to them at dilutions of 50, 25, 12, 6, 3, 1.5 and 0.75% v/v. A 10 µL aliquot of each mixture was plated with Muller Hinton agar and incubated at 37°C for 24 h. After the incubation time, the colonies were counted and the Minimum Inhibitory Concentration (MIC) was determined, expressed in mg/ml of dry solids. The MIC is established as that concentration at which there is no longer appreciable growth during the incubation time. All inhibition assays were performed in triplicate.

Chemical profile

The chemical profile of each extract was determined using an Agilent 1260 Infinity HPLC equipped with an S2150UV diode array detector (DAD). Twenty µL of each extract were injected into the equipment. Separation of compounds was carried out on a Phenomenex C8, 4.6 × 250 mm reversed phase column with a particle size of 5 µm. Elution was performed at a flow rate of 0.2

Table 1. Sample collection.

Code	Sampling site	Main crops in the area	Predominant bee species
P1	Cacahoatan Lat.14°59´19.16´´N ; Lon.92°09´54.42´´W	Rambutan and coffe	*S. mexicana*
P2	Tuxtla chico Lat.14°56'37.2"N; Lon.92°10'37.0"W	Rambutan, coconut, cashew, and cocoa	*M. beecheii, M. solani,* and *S. mexicana*
P3	Tapachula Lat.14°53´22.59´´N; Lon.92°12´59.62´´W	Mango	*M. beecheii* and *S. mexicana*
P4	Mazatán Lat.14°51'39.5"N; Lon.92°28'25.7"W	Rambutan and banana	*S. mexicana*
P5	Cantón La Pita rancho agroecológico “Ayol” Lat.14°49'46.0"N; Lon.92°17'42.0"W	Avocado, sapote, papaya, and banana	*S. pectoralis*
P6	San Juan, Tapachula Lat.14°54'00"N; Lon.92°19'01"W	Coffee, mango, rambutan, cashew cocoa, and pine nut	*T. angustula*

Table 2. Gradient used in compound separation.

Time (min)	Water (%)	ACN (%)	Isopropanol (%)	Flow (mL/min)
0	100	0	0	0.2
7	70	30	0	0.2
15	50	50	0	0.2
25	20	20	60	0.2
35	10	10	80	0.2
45	0	10	90	0.2

mL/min in a gradient mode. The total running time was set at 45 min. Table 2 shows the gradient process used. The solvents used were water, acetonitrile (ACN), and isopropanol.

The compounds were identified by comparison of their UV/vis spectra with those reported in the literature (PubChem) (Vargas-Fajard, 2020). Quantification was not performed due to the complexity in acquiring standards; however, the concentration ratio is reported as peak area.

RESULTS

Extracts

Apparently, the sonication process of the various pollens facilitates the solvation of compounds adhered to the granules, but is not strong enough to fracture the structures. Figure 1 shows the photographs obtained before and after sonication of the aqueous extract. Panel A1 represents the grains magnified at 40x and the scale (5 µm) shown at the bottom. Panel A2 shows the grains at the same magnification but after sonication. Similarly, panels B1 and B2 show pollen grains before and after sonication but magnified at 100x. There are no relevant observable changes in the grain structures. However, as described subsequently, various compounds were found in the extracts. This suggests that these compounds are not precisely a constituent part of these grains, but come from substances that adhere to them and are part of the flowers from which they were collected by bees.

As can also be seen, after sonication a slight gray coloration was identified around the grain, while this coloration is more intense or black in the grains before the sonication process, which suggests that this part is being diluted in the medium and corresponds to the identified compounds. The same was identified in the isopropanol, acetonitrile and methanol extracts.

Microbial inhibition

Six different pollens were used (Table 1) and four solvents (water, methanol, acetonitrile and isopropanol). In none of them was there an inhibitory effect. Figure 2 shows the growth of the various pollens in aqueous extraction. Box 1 corresponds to the uninoculated medium to check the sterility of the medium. Box 2

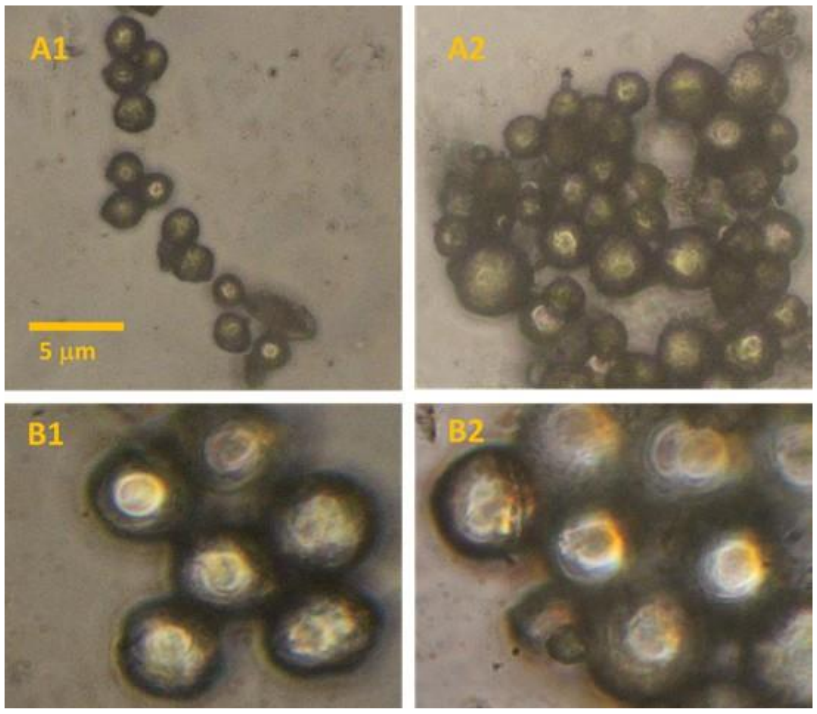

Figure 1. Microscope photographs of pollen grains before and after sonication magnified at 40x (A) and 100x (B).

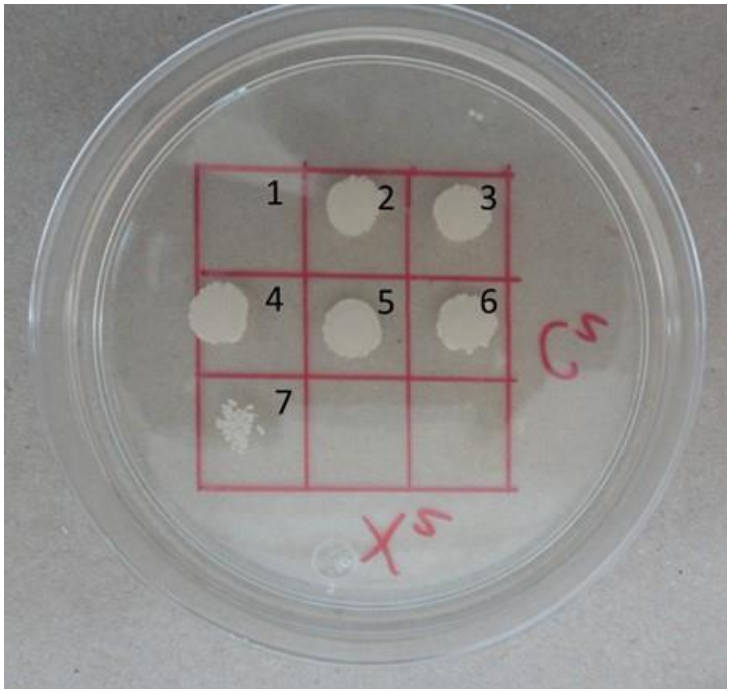

Figure 2. Growth of MRSA in aqueous extract.

corresponds to the aqueous extract P1, box 3 corresponds to the aqueous extract P2, etc.

As can be seen, only the P6 extract presents a small inhibitory effect but it is not really significant. In the other

Table 3. List of areas of compounds detected in pollens based on collecting species (uA × 10E8).

Compound	Collector bee species				
	M. beecheii	***M. solani***	***S. mexicana***	***S. pectoralis***	***T. angustula***
Baicaleina	34.9	-	-	-	-
Myricetin	157.5	428.94	153.9	1.96	164
Apigenin	10.68	31.5	-	3.88	48.7
Chrysine	189	1.74	6.39	277.66	6.08
Rivoflavin	-	276	4	1.41	3.03
Kaemferol	-	12	0.07	-	-
Nordihidroguayaretic acid	-	22.5	2.56	-	3.32
Calic acid	-	143	-	-	-
Naringenin	-	-	-	-	18.9
Total	4	7	5	4	6

Table 4. List of areas of compounds detected based on the solvents used (uA × 10E8).

Compound	Solvents			
	Isopropanol	**Acetonitrile**	**Methanol**	**Water**
Baicaleina	34.9			
Miricetin	362.4	1.29	514.54	26.74
Apigenin	3.88	31.5	51.3	8.08
Chrisine	1.69	195.4	272	11.74
Rivoflavin	-	-	283.03	1.41
Kaemferol	-	-	-	12.07
Nordihidroguayaretic acid	24.59	424.23	2.56	-
Galic acid	143	-	-	-
Naringenin	-	-	-	18.9

extracts with the other solvents there was no significant inhibition either.

Chemical profile

In a first approximation to the chemical profile of the extracts, various spectra were observed, which were compared with what was reported in the literature and, doing a broad search in public access databases, nine compounds of interest were detected, which appear in Table 3 and are reported in relation to the forager bee species. An approximation to the concentrations of each compound present in the samples, without having a reference standard and drawing calibration curves, is the evaluation of the areas of each chromatographic peak, since the area is directly proportional to the concentration of the substance detected. The areas, therefore, can give us an idea of the abundance of each compound, although this is not properly quantified.

Both, Tables 3 and 4, show the areas of the identified compounds. Empty boxes indicate that the compound was not found using the indicated solvent or species. The most abundant compounds were Myricetin, Chrysin and Gallic acid, with Kaepferol being the least abundant. The bee species in which the greatest number of compounds were detected was *Melipona solani* followed by *Tetragonisca angustula*.

On the other hand, it is expected that the various solvents used also present differences in the extracted compounds due to their polarity. Table 4 shows the area ratio of chromatographic peaks taking into account the solvent used without making a difference in the harvester species.

DISCUSSION

First of all, it must be highlighted that the importance of this work is that there are not many works on the chemical characterization of propolis from stingless bees from the Soconusco region. Grajales et al. (2021) reported in a study group of compounds, that propolis were detected from the Soconusco region, including hydroxycinnamic acids, flavanones, flavonoids and their glycosylated derivatives. However, there is no more in-

depth report. The same work reports that an antimicrobial effect was found in the combination of garlic (*Allium sativum*) with propolis obtained from two bee species (*M. solani* and *Scaptotrigona mexiana*), results that contrast with our work. It could be that propolis alone has little effectiveness but when combined with other substances, such as in this case, garlic, the combination becomes synergistic. It is feasible to ask ourselves if the same behavior could occur in the case of pollens, this leads us to propose a study where these combinations are tested.

In another study, reported by Lidia Rosa et al. (2023), it is indicated that some honeys such as that of *Melipona bechei* are of monofloral origin. This could also be related to the antimicrobial effects of beehive products, since the amount of compounds present in them are not as varied as those of other species and therefore they are not usually very potent. The fact that some species are monofloral and others are not is also described by Cenet (2019) who reports a study of 6 pollens, six of which were monofloral. However, he reports that the extracts have great antimicrobial effectiveness against *Bacillus megaterium* and *S. aureus*. López et al. (2020) carried out the collection of honey and pollen for two years in the Soconusco region, indicating that only two species (*M. solani* and *Scaptotrigona mexicana*) are usually monofloral and that the others present a floral variation of 45%. In our case, the predominant crops from which the samples were taken are Rambutan, which is a permanent flowering tree with small flowers and whose fruit requires pollination. Of the regions shown, four have this crop. The second largest crop in our samples is coffee, whose floral components, especially caffeine, induce bees to return again and again to the flower. Therefore, we could classify our extracts as monofloral, which would explain the low antimicrobial effectiveness. It is necessary to compare with pollen that comes from areas whose fauna is more varied.

On the other hand, Lidia Rosa et al. (2023) report that *M. bechei* pollen from Cuba was effective in inhibiting *E. coli* ATCC 25922. However, it should be noted that this is a reference strain and does not present resistance. Therefore, it would be necessary to compare these pollens with *E. coli* isolates from various environments and verify if the effectiveness is the same.

Regarding the compounds detected in each bee species, the one that presented the greatest amount of compounds was *M. solani*. It is necessary to clarify that the compounds reported here are the ones detected but it does not mean that other compounds that are in lower abundance are not present. All species present myricetin and chrysin. Apigenin and riboflavin are found in four of the five species studied. Some of these compounds coincide with what has been reported in pollen from other regions such as Greece (Atsalakis et al., 2017) or Saudi Arabia (Amany et al., 2013).

Myricetin was detected in *M. bechei, M. solani* with the highest abundance, and in *S. mexicana* and *T. angustula* with a lower abundance. In this sense, de Sousa et al. (2020) report that extracts rich in myricetin were highly effective in inhibiting the growth of *S. aureus*, which we could not detect in our work. Another compound detected with certain abundance in our work was Chrysin, however, seems that this compound does not have reports as an antimicrobial, but there are many reports of properties as anticancer.

On the other hand, compounds that have been reported as potent antimicrobials were not found in great abundance in our extracts, such is the case of Nordihydroguayretic acid, which has been reported as a good antimicrobial and anticancer agent (Manda et al., 2020; Bridi et al., 2019). Although this compound was detected in three species (*M. solani*, *S. mexicana* and *T. angustula*), the abundance was very low.

Regarding the solvents used, it seems to be consistent with the solubility of each compound. By using isopropanol, which is a slightly polar solvent, the largest amount of myricetin was obtained, which is practically insoluble in water. However, methanol, which is more polar, also extracted large amounts of this compound, but water was not able to extract a considerable amount of myricetin. Nordihydroguayarectic acid was easily extracted with acetonitrile, this compound is also characterized by being poorly soluble in water. Methanol and water were not able to achieve an extraction, which is perfectly explainable by the polarity of the compound and solvents. More polar compounds such as methanol easily extracted chrysin and riboflavin.

Conclusions

The work provides a first approximation to the compounds abundant in pollens from the Soconusco region, but a more detailed analysis is needed to quantify these compounds. The extracts tested did not show effectiveness MRSA. It seems that sonication alone is not enough to extract all compounds due to the structure of the pollen itself, so extraction methods must be tested or complemented to fracture these structures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Amal D, Drira M, Sana B, Najla H, Kais M, Adel K, Ne´ji G (2019). Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. Arabian Journal of Chemistry 12(8):3075-3086.

Arrazola Bonilla JC (2014). Descripción morfológica del polen de 13 especies de plantas de importancia alergénica presentes en la zona urbana de la ciudad de puebla.

Amany MB, Arafat SM, Soliman HM (2013). Chemical analysis of olive

and palm pollen: Antioxidant and antimicrobial activation properties. Wudpecker Journal of Food Technology 1(2):14-21.
Atsalaki E, Chinou I, Makropoulou M, Karabournioti S, Graikou K (2017). Evaluation of phenolic compounds in *Cistus creticus* bee pollen from Greece. Antioxidant and antimicrobial properties. Natural Product Communications 12(11):1934578X1701201141.
Bridi R, Atala E, Pizarro PN, Montenegro G (2019). Honeybee pollen load: phenolic composition and antimicrobial activity and antioxidant capacity. Journal of natural products 82(3):559-565.
Carneiro ALB, Gomes AA, Alves da Silva L, Alves LB, Cardoso da Silva E, da Silva Pinto AC, Tadei WP, Pohlit AM, Simas Teixeira MF, Gomes CC, Naiff MDF (2019). Antimicrobial and Larvicidal activities of stingless bee pollen from Maues, Amazonas, Brazil. Bee World 96(4):98-103.
Cenet M (2019). Pollen analyses and antimicrobial properties of the natural honey from the east Mediterranean part of anatolia. Pakistan Journal of Zoology 51(02):541-548.
De Sousa MM, Araújo RDMP, Libório RC, Costa EC, de Araujo BN, dos Santos LM., ... & de Moraes Peixoto (2020). Antimicrobial potential of *Jurema preta* and *umburana*, native species of the Caatinga biome, on Staphylococcus isolated from small ruminants with mastitis. Semina: Ciências Agrárias 41(5supl1):2231-2244.
Fontes D, Jaramillo C, Moreno JE (2020). Pollen morphology of the Amacayacu Forest dynamics plot, western Amazon, Colombia. Palynology 44(1):32-79.
Graikou K, Suzanne K, Nektarios A, George S (2011). Chemical analysis of Greek pollen - Antioxidant, antimicrobial and proteasome activation properties. Chemistry Central Journal 5-33.
Grajales CJ, Elías CJ, Lozano GE, Moreno CF, Albores FV, López GA (2021). Actividad antimicrobiana de propóleos de abejas sin aguijón en combinación con ajo, *Allium sativum* (Amaryllidaceae). Revista de Biología Tropical 69(1):22-35.
Kaškonienė V, Adaškevičiūtė V, Kaškonas P, Mickienė R, Maruška A (2020). Antimicrobial and antioxidant activities of natural and fermented bee pollen. Food Bioscience 34:100532.
Lidia Rosa GP, Mademoiselle PP, Yudit AM, Yilieny MI, Yunialí FC (2023). Utilidad para la salud de productos de la colmena Melipona. InI jornada científica de profesores.
Manda G, Rojo AI, Martínez KE, Pedraza CJ, Cuadrado A (2020). Nordihydroguaiaretic acid: From herbal medicine to clinical development for cancer and chronic diseases. Frontiers in Pharmacology 11:500770.
Morais M, Moreira L, Feás X, Estevinho LM (2011). Honeybee-collected pollen from five Portuguese Natural Parks: Palynological origin, phenolic content, antioxidant properties and antimicrobial activity. Food and Chemical Toxicology 49(5):1096-1101.
Pascoal A, Rodrigues S, Teixeira A, Feás X, Estevinho LM (2014). Biological activities of commercial bee pollens: antimicrobial, antimutagenic, antioxidant and anti-inflammatory. Food and Chemical Toxicology 63:233-239.
Taroco R, Seija V, Vignoli R (2006). Métodos de estudio de la sensibilidad antibiótica. Temas de Bacteriología y Virología Médica, Oficina del libro FEFMUR, Uruguay 36(1):665-668.
Vargas Fajardo CJ (2020). Determinación y cuantificación de compuestos fenólicos en flores de Taraxacum officinale, mediante HPLC-DAD-MS y ensayos colorimétricos UV-VIS.
Vit P, Santiago B (2008). Composición química de polen apícola fresco recolectado en el páramo de Misintá de los andes venezolanos. Archivos Latinoamericanos de Nutrición 58(4):411-415.