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REGULAR ARTICLE

Chemical and bioactive characterization of pot-pollen produced by *Melipona* and *Scaptotrigona* stingless bees from Paria Grande, Amazonas State, Venezuela

Vit Patricia[1,2,3]*, Santiago Bertha[1], Pedro Silvia R.M[4], Javier Ruíz[1], Favian Maza[2], Peña-Vera María[5], Pérez-Pérez Elizabeth[5]

[1] Food Science Departament, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida 5101, Venezuela, [2]Currently, Prometeo Researcher, Faculty of Agricultural and Livestock Sciences, Universidad Técnica de Machala, Machala, El Oro, Ecuador, [3]Honorary Associate Biomedical Sciences, School of Medical Sciences, The University of Sydney, 75 East Street, Lidcombe, NSW 1825, Australia, [4]Biology Department, Faculty of Philosophy, Science and Letters, Universidade de São Paulo, Ribeirão Preto, SP, Brazil. [5]Laboratory of Molecular Biology, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida 5101, Venezuela

ABSTRACT

The chemical composition and bioactivity indicators (flavonoids, polyphenols, and antioxidant activity) of bee pollen produced by two Meliponini bee genera (*Melipona* and *Scaptotrigona*) in Paria Grande, Amazonas state, Venezuela, were compared. Proximal analyses were performed in triplicate following standard methods. Moisture, ash, and crude fat were done by volumetric methods, proteins by the microKjeldahl method (digestion with sulfuric acid and catalysts, distillation of ammonia by steam with sodium hydroxide and sodium thiosulphate, collection in boric acid and titration with hydrochloric acid), and carbohydrates by difference. Bioactivity was measured by spectrophotometry: The antioxidant activity by the ABTS method, flavonoids with a modified aluminum chloride method, and polyphenols with Folin-Ciocalteu. The following compositional and bioactivity variations were observed for the contents of moisture (43.49- 48.54) g/100g pollen, ash (1.94-2.33) g/100g pollen, fat (3.19-6.72) g/100g pollen, proteins (16.80-18.32) g/100g pollen, carbohydrates (27.62-31.03) g/100g pollen, antioxidant activity (373.5 – 493.6) μg TEAC/100g pollen, flavonoids (1,110.7-1,644.9) mg QE/100 g pollen, and polyphenols (1,576.9-3,905.6) mg GAE/100 g pollen. This study will benefit further nutrional proposals on this proteinaceous food of bee origin, and supports Venezuelan meliponiculture in the Amazone.

Keywords: Antioxidant activity; Flavonoids; Meliponini; Proximal analysis; Pot pollen; Polyphenols

INTRODUCTION

Bee pollen –as well as honey and propolis, has been widely studied for *Apis mellifera*, as recently reviewed (Vit, 1999). In Venezuela the botanical origin (Barth et al., 2011) and the chemical composition of *Apis mellifera* pollen loads from Los Andes in Cacute (Vit et al., 2008) and Misintá (Vit and Santiago, 2008), Mérida state. Also the antioxidant activity of aqueous, ethanolic and methanolic extracts of yellow, ochre, orange and brown pollen loads was compared (Pérez-Pérez et al., 2012).

The Warime Cooperative of Paria Grande Meliponicultors in the Amazonas state, Venezuela operates since year 2005. These Piaroa or Huottuja meliponicultors –also named stingless bee keepers, harvest honey and pollen produced in cerumen pots by stingless bees (Meliponini) (Pérez and Salas, 2008) and currently is expanded to 120 producers as informed by the president Mr. Alfonso Pérez in 2013. Studies on chemical composition of pot-pollen produced by stingless bees from the genus *Melipona*, will support the inclusion in sanitary procedures required for controlled marketing, to support this initiative of ancestral knowledge and recovery of native Indian traditions and environmental protection. This pollen is very sour and creamy compared with dried *Apis mellifera* pollen loads, because Meliponini ferment their pollen inside the nest; possibly it is richer in lactic acid as we found in stingless bee pot-honey (Vit et al., 2011). Menezes et al. (2012) proposed method to collect unfermented Meliponini pollen loads to increase acceptance, because in Brazil consumers prefer unfermented pot-pollen of *Scaptotrigona depilis*. However,

***Corresponding author:**
Vit Patricia, Food Science Department, Faculty of Pharmacy and Bioanalysis, Universidad de Los Andes, Mérida 5101, Venezuela.
E-mail: vitolivier@gmail.com

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in Venezuela sour taste of pot-pollen is highly appreciated because it reminds lemon, indeed when mixed with water it is named "pollen-lemonade".

Bee pollen is used for its nutritional value in the human diet (Ioirish, 1985; Krell, 1996). It is made up of natural flower pollen mixed with nectar and bee secretions, compacted in the corbiculae (Michener, 1999). Honey and other bee products, such as royal jelly, pollen and propolis may be used as functional foods because of their naturally high antioxidant potential (Viuda-Martos et al., 2008). Silva et al. (2006) studied the composition and free radical scavenging activities of two samples of yellow and brown pollen loads of *Melipona subnitida* from the Brazilian Northerneast. These authors isolated naringenin, isorhamnetin, and D-mannitol from the *Mimosa gemmulata* yellow pollen, and β-sitosterol, tricetin, selagin, and 8-methoxiherbacetin from the Fabaceae brown pollen. The EC_{50} of ethanolic extracts in yellow and brown pollen was 104.5 ± 0.5, and 106.1 ± 1.3 μg/mL. Later, Silva et al. (2009) evaluated free radical scavenging activity of ethanolic extracts of pollen loads from stingless bee *Melipona rufiventris* "uruçu amarela", finding a concentration of a scavenger of active oxygen species of 104.1 ± 1.2 μg/mL. In recent studies, Tohamy et al. (2014) investigated the anti-mutagenic, anti-histopathologic and antioxidant effects of water extracts of Egyptian bee pollen (WEBP) in induced hepatic, renal, testicular and genotoxicity in male albino mice (*Mus muscullus*), finding that bee pollen is potent in exerting an ameliorative effect.

In Venezuela and Ecuador, bee pollen is not regulated, only honey norms for *Apis mellifera* are available from the Venezuelan Commission of Norms (COVENIN 1984 a,b) and the Ecuadorian Technical Norm from the Ecuadorian Institute of Normalization (NTE INEN, 1988). Besides the well known commercial pollen loads of *Apis mellifera*, there is another type of pollen produced in cerumen pots by stingless bees (Meliponini), this pot-pollen is produced by the Warime Cooperative of Meliponicultors in Paria Grande, and needs to be characterized. The objective of this work is to analyze the proximal content (moisture, ash, proteins and fat), and bioactivity indicators (total antioxidant activity TAA, flavonoid and polyphenol contents) of pot-pollen collected, processed and stored in cerumen pots by *Melipona* (Michmelia) sp. *aff. eburnea* and *Scaptotrigona* cf. *ochrotricha* stingless bees with a similar palynological origin in Paria Grande, Amazonas state, Venezuela.

MATERIALS AND METHODS

Pot-pollen samples: Approximately 100 g of pot-pollen produced by two stingless bee species were collected from three nests in Paria Grande, Amazonas state, Venezuela, and were kept frozen at -20°C until analysis.

Botanical origin: Natural pollen analysis was done following the Louveaux et al. (Louveaux et al., 1978) method, and the frequency classification: dominant (> 45%), accessory (15% to 45%), important isolated (3% to 14%) and rare (< 3%). Pollen plates were used for identification (Barth, 1970; Vit, 2005).

Entomological origin: The two stingless bee species known with ethnic names "tobillo morrocoy" and "sonquette", were kept in isopropyl alcohol for further entomological identification at Universidade de São Paulo, Ribeirão Preto, Brazil, as *Melipona* (Michmelia) sp. aff. *eburnea* Friese, 1900 and *Scaptotrigona* cf. *ochrotricha* (Buysson, 1892), respectively.

Proximal analyses: Proximal analyses were done in triplicate following oficial analytical methods (AOAC, 1999). Moisture, ash and fat were done by gravimetric methods; proteins were measured by the microKjeldhal method following sulfuric acid (Merck, Darmstadt, Germany) digestion, ammonia distillation by vapor flow with sodium hydroxide (Sigma Aldrich, USA) and sodium thyosulphate (Merck, Darmstadt, Germany), collection in boric acid (Sigma Aldrich, USA) and titration with hydrochloric acid); carbohidrates were calculated by difference.

Ethanolic homogenate preparations: A weight of 100 ± 10 mg of each pot-pollen type was placed on a glass homogenizer (Thomas No. A3528, USA), and 5 mL of ethanol 95% (v/v) (Riedel de Haën, Europe) were added, and homogenized on an ice bath. Homogenates were centrifuged in a BHG Optima II (USA) centrifuge at 3,000 rpm for 10 min, and supernatants were used for biochemical analysis (Pérez-Pérez et al., 2012).

Total antioxidant activity by the ABTS method: For the method of ethanolic decolorization of ABTS solution (Sigma, Canada), ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{+\bullet}$) was produced by reacting 7 mM stock solution with potassium persulfate (Merck, Darmstadt, Germany) to a final concentration of 2.45 mM (in water), in the dark at room temperature (RT) for 12-16 h before use (Re et al., 1999) [43]. For this pollen analysis, the $ABTS^{+\bullet}$ solution was diluted with 20% ethanol (v/v) up to 0.60-0.70 absorbance units at 735 nm, 100 μL of homogenates were diluted in ethanol, and mixed with 7.5 mL of $ABTS^{+\bullet}$ solution diluted in ethanol 20% (v/v). Absorbance values were measured 6 min after mixture. A solution of 8 mM Trolox (Sigma, Steinheim, Germany) was used as antioxidant standard. Trolox was diluted to obtain 1, 2, 4 and 8 μM in 5 mM PBS buffer (pH 7.4). Decolorization percentages at 734 nm after 6 min were

calculated and plotted as a function of different Trolox concentrations, and TAA was reported accordingly. TAA value for pollen is equivalent to Trolox concentration that produces the same decolorization percentage. Purified antioxidants like quercetin, melatonin and lipoic acid were used as controls of the antioxidant activity. Results are expressed as μg Trolox equivalents (TEAC) per 100 g honey.

Flavonoid content: Flavonoid content was measured using a modification of the aluminum chloride method (Woisky and Salatino, 1998). The flavonoid concentration was measured with a calibration curve using standard solutions of quercetin (Sigma, Steinheim, Germany) diluted up to 25, 50 and 100 μg/mL in ethanol 80% (v/v). Standard solutions (0.5 mL) were mixed with 1.5 mL 95% (v/v) ethanol, 0.1 mL of aluminum chloride (Fisher Scientific, New Jersey, USA) 10% (w/v), 0.1 mL of potassium acetate (Sigma Aldrich, USA) 1M and 2.8 mL of distilled water. After incubation at ambient temperature for 30 min, absorbance was recorded at 415 nm. In a similar way, 0.5 mL of ethanolic extracts of pollen homogenates reacted with aluminum chloride for flavonoid content determinations. Results are expressed as mg quercetin equivalents (QE) per 100 g honey.

Polyphenol content: Polyphenol content was analyzed by spectrometry at 765 nm using Folin-Ciocalteu (Sigma-Aldrich, St. Louis, USA) reagent (Singleton, 1999). One hundred microliters of pollen homogenates were mixed with 500 μL of Folin-Ciocalteu's reagent diluted 1/10 with water, to which 400 μL of sodium carbonate was added (Sigma, Steinheim, Germany) 7.5% (w/v). Absorbance at 765 nm was recorded after 10 min of reaction at 37°C, against a blank with MQ water instead of ethanolic extract. The polyphenol concentration was estimated with a calibration curve using a solution of 0.1 g/L of gallic acid (Sigma, Steinheim, Germany) as standard (0, 0.25, 0.05 and 0.1 g/L). Results are expressed as mg equivalents of gallic acid (GAE) per 100 g honey.

Statistical analysis

All experiments were done in triplicate. Data were analyzed by ANOVA ($P< 0.05$), with average comparison through post hoc Scheffé test using SPSS 12.0 software (SPSS, 2004) to compare variations of physicochemical composition and bioactivity in the two pollen types with different entomological origin (*Melipona* sp. and *Scaptotrigona* sp.). Media ± SD are given for each parameter.

RESULTS

The pot-pollen produced by two species of *Melipona* aff. *eburnea* and *Scaptotrigona* cf. *ochrotricha* had two dominant pollen types; one of the Fabaceae Papilionoideae family 46% in the Melipona, and 20% in the *Scaptotrigona* pot-pollen, and the other of the Malpighiaceae family that was found as less frequent accessory pollen 26% in *Melipona*, but dominant with 54% in *Scaptotrigona* pot-pollen. Other pollen types from Apocinaceae, Arecaceae, Bixaceae, Euphorbiaceae, Fabaceae Mimosoideae, Lamiaceae, Malvaceae, Myrtaceae, and Poaceae families were also detected with frequencies lower than 3%.

The chemical composition of pot-pollen produced by *Melipona* and *Scaptotrigona* bees from Paria Grande, were done by proximal analysis moisture, ash, fat, protein and carbohydrate contents is given in Table 1.

In Table 2 is presented data on total antioxidant activity (TAA), flavonoid and polyphenol contents of ethanolic homogenates of *Melipona* and *Scaptotrigona* pot-pollen, besides the total antioxidant activity of purified antioxidants like quercetin, melatonin and lipoic acid used as controls of the antioxidant activity.

DISCUSSION

Stingless bee origin is estimated in the late Cretaceous (Engel and Michener, 2013), and since then also their interactions with plants (Lunau, 2004). Bees forage plants based on a cost-benefit balance. Besides floral attractiveness to a particular stingless bee species, other factors determine pollen choices and rate of visitation: 1. Presence of competitors, 2. Distance from the nest to the resources, 3. Bee communication strategy, 4. Number of bees in the nest, 5. Type of soil, 6. Temperature, 7. Light (Kajobe, 2007; Corbet et al., 2008).

The dominant pollen types were from the family Fabaceae Papilionoideae (46%) *Melipona*, and Malpighiaceae (54%) *Scaptotrigona* pot-pollen. The higher fat content of *Scaptotrigona* pollen could be explained by the oil glands of Malpighiaceae. Although most genera of Malpighiaceae are generally pollinated by oil collecting bees like the Centridini group (Rego et al., 2006). Novais and Absy (2013) studied pollen pots of Amazonian *Tetragonisca angustula* in Belterra and Santarém, Pará, Brazil found that *Byrsonima*, *Cecropia*, *Clidemia hirta*, *Davilla kunthii*, *Eriope*, *Myrcia* and *Vismia guianensis* were the most significant pollen types with frequencies above 70%. Absy et al. (1984) recommend that plants of identified pollen sources should be cultivated near to meliponaries to support stingless beekeeping as potentially sustainable economic activity. Particularly, planting native species of the genera *Astrocaryum*, *Bactris*, *Byrsonima*, *Citharexylum*, *Couma*, *Euterpe*, and *Vitex*, is adviced to facilitate beekeeping especially in seasons of pollen

Table 1: Proximal analysis of pot-pollen from *Melipona* sp. and *Scaptotrigona* sp.

Pollen type "ethnic name" *Bee species*	n	Moisture (g/100 g)	Ash (g/100 g)	Fat (g/100 g)	Proteins (g/100 g)	Carbohidrates (g/100 g)
"Tobillo morrocoy" *Melipona* sp. *aff. eburnea*	3	48.54±0.41[b]	2.33±0.10[b]	3.19±0.11[a]	18.32±0.10[b]	27.62±0.50[a]
"Sonquette" *Scaptotrigona* sp. *cf. ochrotricha*	3	43.49±0.95[a]	1.94±0.35[a]	6.72±0.58[b]	16.80±0.21[a]	31.03±1.08[b]

Table 2: Bioactivity properties of *Melipona* and *Scaptotrigona* pot-pollen ethanolic homogenates

Pollen type "ethnic name" *Bee species*	n	Total antioxidant activity (TEAC/100 g pollen)	Flavonoid content (mg of QE/100 g pollen)	Polyphenol content (mg GAE/100 g pollen)
"Tobillo morrocoy" *Melipona* sp. *aff. eburnea*	3	373.5±21.0[d]	1,576.9±35.8[a]	3,905.6±64.0[b]
"Sonquette" *Scaptotrigona* sp. *cf. ochrotricha*	3	493.6±2.0[e]	1,110.7±66.8[a]	1,644.9±16.0[b]
Quercetin		130.4±12.5[c]		
Melatonin		112.7±8.7[b]		
Lipoic acid		67.8±2.4[a]		

scarcity; and considering that with the pollination service of the bees, local food production may also increase (Rech and Absy, 2011). These authors reported *Scaptotrigona* sp. pot-pollen with three secondary pollen types from two species of the family Fabaceae Papilionoideae: *Aldina latifolia* (24.2%), *Swartzia dolichopoda* (30.8%), and *Vitex cymosa* Lamiaceae (34.5%)

The botanical origin of the genus *Byrsonima* (Malpighiaceae) found here as a less frequent source in the *Melipona* and *Scaptotrigona* pot-pollen was also reported for *Centris* (Ribeiro et al., 2008) and *Cephalotrigona femorata* with a secondary frequency of 33.4 % (Absy et al., 1984). The Malpighiaceae family offers oil from elaiophore calyx glands to pollinators (Pedro, 1994). However, pollination by pollen-collecting bees has probably shifted to remove their calyx glands in a group of the over 950 species of Malpighiaceae in the New World, after an almost obligate relationship of very specialized pollinators that explain the floral conservatism of Neotropical Malpighiaceae (Anderson, 1979).

The following compositional variations were observed in Table 1 for the contents of moisture (43.49 – 48.54) g/100g pollen, ash (1.94-2.33) g/100g pollen, fat (3.19-6.72) g/100g pollen, proteins (16.80-18.32) g/100g pollen and carbohidrates (27.62-31.03) g/100g pollen. The pollen collected by *Scaptotrigona* was 5% drier and had almost double fat content than that of *Melipona*. These differences, besides the 1.5% higher protein content of *Melipona* pollen, caused its lower carbohydrate content, almost 4% compared with the *Scaptotrigona* pollen. Compared to *Apis mellifera* fresh pollen from Cacute (Vit et al., 2008) and Misintá (Vit and Santiago, 2008), Venezuela; pot-pollen from the two Meliponini species investigated here showed a higher moisture than 13.24-17.93 %, higher fat than (1.73-5.37%), lower proteins than (24.17-52.56%), and similar ash content (1.60-2.18%).

The TAA of ethanol extracts of pot-pollen from *Melipona* was lower than that of *Scaptotrigona* (Table 2). Similarly, lower values of polyphenol contents were also observed in the *Melipona* pot-pollen, compared to *Scaptotrigona* pot-pollen. There is a positive relation between polyphenol content and antioxidant activity (TAA values), but not with the flavonoid content, as previously informed in another research for unifloral honey (Vit et al., 2010). Freire et al. (2012) determined phenolic and flavonoid contents, and antioxidant properties of twenty-five bee pollen harvested during a nine-month period from the Canavieiras municipality (Northeastern Brazil). The total phenolic contents ranged from 41.5 to 213.2 mg GAE/g, and antioxidant activities based on the (ABTS) correlated with the total phenolic contents. Morais et al. (2011) determined phenolic content and antioxidant properties of pollen from five Portuguese Natural-Parks such as Parque Nacional Peneda Gerês (PNPG); Parque Natural do Montesinho (PNM); Parque Natural do Alvão (PNA); Parque Natural da Serra da Estrela (PNSE) and Parque Natural do Douro Internacional (PNDI). The phenolic contents, determined 10.5 and 16.8 mg GAE/g of extract, in bee pollen from PNM and PNDI, respectively. The free radical scavenging measured showed the highest effective extract - PNM with EC_{50} 2.16, followed by PND with 2.24 mg/mL.

Several methods for determining the antioxidant activity in honey have been used, and among them the ABTS assay is referred to by many authors. This assay assesses the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical cation ($ABTS^{\bullet+}$) (Suarez-Alvarez et al., 2009). Besides to the simplicity of this assay, there is little correlation between the TEAC value and the number of electrons an antioxidant can give away. In spite of this, the ABTS assay is considered an easy and accurate method for use in the antioxidant capacity studies in honey because it allows

to determine the radical scavenging ability present in the honey by the hydrogen-donation reaction. On the other side, honey phytochemicals are mainly represented by the extensive class of phenolic compounds, being the major class the flavonoids (flavonols, flavanols and flavones), followed by phenolic acids (benzoic acids, phenylacetic and hydroxycinnamic acids). In pollen samples, the highest quantity of flavonoids exists like glycosides, namely aglycones, being quercetin the majoritarian compound (Bogdanov, 2011).

It has been widely demonstrated that flavonoids are very effective as scavengers of reactive oxygen species (ROS): peroxide, hydroxyl and superoxide radicals, as well as against reactive nitrogen species (RNS) like nitric oxide and peroxyl nitrite (Yen and Lai, 2003). Moreover, flavonoids have high affinity for proteins and other biological macromolecules, such as hormones and nucleic acids, and for divalent ions of metals; and also have high catalytic ability for the electron transfer and act as radical scavengers. Flavonoids are capable as act like antioxidants because have substituents with a high capacity of donate hydrogen/electrons with adequate reduction potentials; and the ability to relocate the resulting radical (Rivas-Gonzalo and García-Alonso, 2002). It has been described some structural criteria necessary for effectiveness in capturing radicals of flavonoid molecules, through ABTS radical cation assay, among them: (i) the presence of the structure 3',4'-o-dihydroxy on B aromatic ring (catechol), which gives greater stability to the formed radical; (ii) the existence of hydroxyl groups on carbons 5 and 7 of A ring; (iii) the double bond located at position 2,3 in conjunction with the 4-oxo and 3-hydroxy on C ring, responsible for the relocation from the B ring (the peroxyl radicals produced are stabilized by the resonance effect of the aromatic nuclei); and (iv) the appearance of the hydroxyl groups in position 3 and 5 with 4-oxo function in rings A and C, and with 2,3 double bond between positions generate the maximum potential free radical scavenger (Burda and Oleszek, 2001; Heim et al., 2001; Rivas-Gonzalo and García-Alonso, 2002). Depending of its structure, the antioxidant activity of some flavonoids present in pollen samples cannot be determined by ABTS assay because the mechanism involved is not electron/hydrogen transfer, the principal mechanism evaluated in this method.

More recently, Pascoal et al. (2014) evaluated the biological activities of eight commercial bee pollens purchased from the market. Phenolic contents varied from 32.15 to 18.55 GAE/g, flavonoids varied from 10.14 to 3.92 QE/g. All the samples exhibited antimutagenic and antimicrobial activity, being *Staphylococcus aureus* the most sensitive and *Candida glabrata* the most resistant of the microorganisms studied. These authors observed positive correlations between polyphenols, flavonoids, antioxidant activity and antimicrobial activity of *Apis mellifera* bee pollen. These are few evidences about the antioxidant activity of honey, pollen and propolis and its relationship with total polyphenol content, and especially flavonoid concentration.

There is no statistical data available for the bee industry in Venezuela, and this lack of organization is reflected in the low participation in international debates on biodiversity protection of feral bees endangered of extinction (Bouga, 2013), contamination of bee products with genetically modified organisma (GMO) and its residues (Haefeker, 2013), or the effects of pesticides in honeybee and authenticity of honey (Maxwell, 2013), as recently discussed in round tables of the APIMONDIA XXXXIII International Congress of Apiculture held in Kiev, Ucrania from the 29th September to the 4th. October 2013.

The fact that pollen regulations still need to be created in Venezuela, offers the advantage to suggest the inclusion of pot-pollen produced by Meliponini bees different from *Apis mellifera*, like *Melipona* and *Scaptotrigona*. Expanded quality criteria (microbiological, contaminants, trace elements, vitamins) recommended in the review for dried *Apis mellifera* pollen pellets (Campos et al., 2008) could also be adopted for fresh bee pollen; in their draft these authors suggested maximum moistures of 6-8 g/100, maximum ash of 6 g/100, minimum protein of 15 g/100, minimum fat of 1.5 g/100 g, and instead of carbohydrates measured by difference, a minimum of total sugars 40 g/100g.

CONCLUSIONS

In the current research moisture and ash contents measured with gravimetric methods, proteins (micro Kjeldahl), ether extract (Soxhlet), and bioactivity indicators measured by spectrophotometric methods to evaluate total antioxidant activity as free radical scavengers, flavonoid and polyphenol contents were compared in pot-pollen produced by *Melipona* (Michmelia) sp. aff. *eburnea* Friese, 1900 and *Scaptotrigona* cf. *ochrotricha* (Buysson, 1892) in Paria Grande, Amazonas state, Venezuela. The characterization is useful for a pot-pollen regulation database, and also as a basis for pharmaceutical studies.

The proximal composition and bioactivity of bee pollen produced by two species of stingless bees in Paria Grande, Amazonas state, Venezuela let the following observations: 1. Pot-pollen is a rich source of oligoelements in the diet given the high ash content measured in this study, 2. The pollen spectra varied according to the stingless bee species, 3. Fat content of pollen varied according to the stingless bee species and the plant they visited foraging pollen.

To our knowledge, this is the first report on the biological activities of Venezuelan stingless bee pot-pollen, and there is very small quantity of evidence of antioxidant activity on stingless bee pollen (Silva et al., 2006; Silva et al., 2009). Our data suggest that the ethanol extract of pot-pollen have a potent antioxidant activity, similar or higher than that found for purified antioxidants like quercetin, melatonin and lipoic acid, probably due to its polyphenol content and fermentive process. This is very important to support the use of pollen as a very energetic nutritional supplement since ancient times.

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Author contributions

V. P. wrote the project, collected and distributed the pot-pollen, S.B. and J.R. did the physicochemical analysis, P. S. R. M. did the entomological identifications, F.M. supervised the Prometeo Project, P.-P. E. supervised the antioxidant, flavonoid and polyphenol measurements of her student P.-V. M. Statistics were done by V. P. and P.-P. E. All authors wrote, read and approved the final manuscript.

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