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ANTIOXIDANT AND PHYSICO-CHEMICAL PROPERTIES AND BOTANICAL ORIGIN OF POLLEN COLLECTED BY *Apis cerana* Fabricius FROM SELECTED APIARIES IN LAGUNA AND BATANGAS PROVINCES (PHILIPPINES)

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ABSTRACT

The physico-chemical and antioxidant properties of the ethanolic extracts of pollen collected by the Asian honey bee, *Apis cerana* Fabricius, from Laguna and Batangas Provinces (Luzon I., Philippines) were determined. The botanical origin of the bee pollen samples was also identified. The percent moisture ranged from 19.50 ± 0.17 to 22.44 ± 0.13; % ash, from 2.89 ± 0.02 to 3.58 ± 0.01; % crude fat, from 0.75 ± 0.12 to 2.70 ± 0.09; % crude fiber, from 1.07 ± 0.16 to 2.76 ± 0.14; % crude protein, from 15.15 ± 0.02 to 20.49 ± 0.95; % total carbohydrates (nitrogen-free extract), from 52.67 ± 0.90 to 56.03 ± 0.09; % sugar, i.e., total sugars, reducing sugar and apparent sucrose, from 9.63 ± 1.35 to 17.30 ± 2.14; from 7.28 ± 3.09 to 14.68 ± 3.50; and from 2.24 ± 2.20 to 2.62 ± 1.54, respectively; phosphorus content, from 1839.7 ± 43.7 to 2062.9 ± 7.5 mg P/kg; and sodium content, from 14.13 ± 0.14 to 33.57 ± 0.91 mg Na/kg. *In vitro* determination of antioxidant properties of the bee pollen samples includes 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Capacity with EC_{50} values of 7.86 ± 0.11 mg/mL to 8.02 ± 0.08, Hydrogen Peroxide Scavenging Capacity with EC_{50} of 30.86 ± 0.07 to 31.87 ± 0.09 µg/mL and Ferric Ion Reducing Antioxidant Power (FRAP) values of 2.69 ± 0.37 to 3.01 ± 0.49 mmol Fe^{2+}/g extract. All bee pollen samples were multifloral with 19 different pollen types. Variations on the values obtained were attributed to the variation of geographical location and floral sources of the pollen in the samples.

Key words: antioxidant properties, *Apis cerana*, Asian honey bee, bee pollen, physico-chemical properties

INTRODUCTION

The Asian honey bee, *Apis cerana* Fabricius, is one of the bee species commonly reared in apiaries in the Philippines. While bees obtain energy from honey in the form of simple sugars, pollen is their major source of proteins, minerals, fats and other substances. Bee pollen is a fine powder-like material produced by honey bees by mixing pollen gathered from flowering plants with nectar and bee secretions (Hassan, 2011) and used as the primary food source for the hive.

For humans, bee pollen is considered an important food in traditional medicine and as supplemental and alternative nourishment because of proven health-enhancing effects. Worldwide, pollen preparations are distributed as dietary supplements (Kroyer & Hegedus, 2001). Bee pollen is promoted as a health food with a wide range of nutritional and therapeutic properties. Honey bee-collected pollen is an apicultural product composed of nutritionally valuable substances which include proteins, lipids, sugars, fibers, minerals, amino acids and vitamins (Carpes et al., 2009; Freire et al., 2012). A high concentration of reducing sugars, essential amino acids, unsaturated and saturated fatty acids, the presence of Zn, Cu, Fe, and high K/Na ratio make honey bee pollen a potentially valuable food (Campos et al., 2008).

Bee-collected pollens around the globe have also been found to contain polyphenolic compounds and may act as potent antioxidant (Rebiai & Lanez, 2012; Kroyer & Hegedus, 2001; Graikou et al., 2011; Freire et al., 2012). However, studies on pollen collected by *A. cerana* are very limited. Evaluation of the physico-chemical and antioxidant properties of this particular bee pollen is necessary to distinguish its nutritive value and potential health benefits. Understanding properties of our bee pollen may lead to discovering other potential applications in the market and may contribute to the standardization of the physico-chemical and antioxidant properties of bee pollen in the Philippines. Since the exact chemical composition depends on the plants from which the worker bees gather the pollen, knowing the botanical origin of bee pollen is also necessary to understand the variation in its chemical composition. Thus, this study aimed to determine and compare the physico-chemical and antioxidant properties and botanical origin of pollens collected by Asian honey bees in selected apiaries in Laguna and Batangas provinces, located in southern Luzon.

MATERIALS AND METHODS

Sample Collection

Pollen samples of *A. cerana* were collected from selected apiaries of Alaminos and Los Baños in Laguna and Balete in Batangas. Samples were composite from different colonies of the said honeybee to represent each town.

Samples were collected through the use of forceps, by manually separating the pollen from the bee combs. These were ground as fine as possible using mortar and pestle. Each sample was then placed in a clean container, properly labeled and stored in the refrigerator until the analysis. All analyses were performed in three trials.

Proximate Chemical Analyses

Proximate analyses were performed to determine the physico-chemical properties of the pollen samples based on the following methods. Moisture content was determined through oven-drying method as described by AOAC (2000). For ash content, the bee pollen samples from moisture determination were ignited in a muffle furnace following the methods by AOAC (2000). Extractable fat was determined through the Goldfisch method while the crude fiber content was analyzed through the Weende method, both described also by AOAC (2000). Total nitrogen was determined using Kjeldahl method and was used for the calculation of the crude protein content. Total carbohydrates (nitrogen-free extract, NFE) was calculated from the results of the proximate analysis.

Analyses for specific carbohydrates were done using the methods described by Madamba (2000). Total sugars of pollen samples were determined through the phenol-sulfuric acid method, while analysis of reducing sugars was performed using the 3,5-dinitrosalicylic acid (DNS) method. The percentage of apparent sucrose for the bee pollen samples was calculated by deducting the % reducing sugar from the % total sugar.

Mineral determination was used to quantify specific minerals present in the sample. The analyses were based on the method described by AOAC (2000). Dry ashing was used as the sample preparation for elemental analysis of sodium and phosphorus in the bee pollen samples. Total phosphorus was determined using the ascorbic acid method, while sodium content was determined through atomic emission spectroscopy (AES) at 589 nm.

Determination of the Antioxidant Properties

Four grams of each bee pollen sample was extracted using 20 mL 70% ethanol at 70 °C for 2h in amber bottles. The reaction mixture was left overnight in the dark to maximize the yield. The supernatant was decanted and the residue was then re-extracted in the same manner. The ethanol extracts were finally combined and the solvent was evaporated to dryness using the rotary evaporator. The extracts were weighed and then redissolved in 25 mL 70% ethanol and stored in the dark until analysis.

Antioxidant properties of the bee pollen samples were determined using *in vitro* analysis. For the DPPH radical scavenging activity of the pollen extracts, the method described by Silva et al. (2005) was used. The H_2O_2

scavenging activity of the pollen extracts were determined using the method described by Escudero et al. (2008). Ferric reducing antioxidant power (FRAP) of the pollen extracts were analyzed also using the methods by Escudero et al. (2008).

Identification of botanical origin of the bee pollen samples

From the bee pollen samples, 0.5 g was dissolved in five mL distilled water and centrifuged for two min. The supernatant was decanted and the recovered residue was treated with four mL glacial acetic acid and centrifuged again for two min. The resulting residue was added with acetolysis solution (9:1 acetic anhydride: sulfuric acid) and placed in a hot water bath with occasional stirring for two min and was centrifuged for another two min. Four mL glacial acetic acid were again added to the residue and centrifuged for two min and about four mL warm water were also placed in the residue and centrifuged for two min. Four milliliters of 95% ethanol were added to the residue and stirred and centrifuged for two min. Four milliliters of glycerin were added to the residue and stirred. Pollen slides were prepared from each sample with 9x9 grid marks. The recovered pollen types were identified from the UPLB Bee Program Library. Pollen grains were identified and counted (for frequency) under a microscope. As far as possible, pollen types were identified to species but in some cases, only family identification was possible. The following terms was used for pollen frequency classes: predominant pollen (more than 45% of the pollen counted), secondary pollen (16–45%), important minor pollen (3-15%), and minor pollen (>3%). Then, bee pollen samples were classified as monofloral (if >65% of one species is present) or multifloral (Louveaux et al., 1978).

Statistical Analyses

The data were subjected to one-way analysis of variance (ANOVA) to test if there was an existing significant difference between the calculated values of the bee pollen samples resulting from different geographical origin. This was followed by the Duncan's Multiple Range Test (DMRT, post hoc analysis) to compare all pairs of mean with the difference at $p< 0.05$ considered as significant.

RESULTS AND DISCUSSION

Proximate Chemical Analysis

The proximate analyses (Figure 1) revealed the physico-chemical characteristics of the pollen types collected by *A. cerana* reared in selected apiaries in Laguna and Batangas. Moisture content of the fresh bee pollen samples ranged from 19.50% to 22.44%. Statistical analysis showed significant differences on the mean % moisture of the pollen samples from different

locations. The highest moisture content was measured from bee pollen from Balete, followed by Los Baños and Alaminos. Data obtained in this study agreed with those obtained by Bertoncelj et al. (2018) for Slovenian bee pollen, with a mean moisture content of 24.0% and those reported by Sattler et al. (2015) for fresh bee pollen from southern Brazil with mean moisture content of 16.6%. Bee pollen, being highly hygroscopic is affected by environmental conditions (Carpes et al., 2009) such as high humidity which may account for the high moisture content of the samples. Moisture content also varies depending on the composition of floral origin of bee pollen samples. Ideally, water content of fresh bee pollen is between 20-30% and the results obtained were within the range of the set standard by Campos et al. (2008).

Protein content of the bee pollen samples ranged from 15.15% to 20.49% (Figure 1). From the reference standard proposed by Campos et al. (2008) for crude protein of dried pollen at 10-40%, data obtained in this study were within the set range. Similar data ranges were also obtained in the studies of bee pollen from southern Brazil (Carpes et al., 2009; Sattler et al., 2015) and southeast Australia (Somerville & Nichol, 2006). Mean protein content of the bee pollen samples among the locations showed significant differences at 5% level of significance. Furthermore, post hoc analysis (DMRT) suggested that mean crude protein differed among all locations. Values obtained showed that pollen from Alaminos had the highest crude protein content while that from Balete had the least. According to Sczcesna (2006), crude protein varies greatly depending on the variation of its botanical origin. In addition, Nogueira et al. (2012) also reported differences in crude protein under different environmental conditions. The bulk of the nitrogen content of bee pollen is in the protein fraction, being the second most abundant macromolecule in pollen after carbohydrates.

Significant differences were also found in the mean crude fat of bee pollen from different locations (one-way ANOVA at 5% significance level and DMRT). Bee pollen samples from Los Baños had the lowest crude fat content (0.75%). The percent crude fat of bee pollen samples from Alaminos and Balete were 2.70% and 2.36%, respectively. Results for crude fat analysis were within the 1-13% range set by Campos et al. (2008) as reference standard and the 1-10% range of quality criteria set by the Swiss Food Manual (Bogdanov, 2004). Moreover, crude fat content of the samples corroborates the data presented by Somerville (2005) for bee pollen from southeast Australia with mean value of 2.52%. High protein and low fat content in bee pollen make it a suitable food supplement (Carpes et al., 2009; Sattler et al., 2015).

Significant differences were found for the data of the mean ash content of bee pollen samples from different locations, which ranged from 2.89% to 3.58%. Bee pollen from Alaminos had the highest average percent ash while that from Balete had the lowest. Values of the ash content of bee pollen samples were within the range of the set standard of 2-6% by the Swiss regulation (Campos et al., 2008) and below the maximum value of 4% set by Brazil standard (Sattler et al., 2015). Data obtained in this study were similar to those reported by Martins et al. (2011) which ranged from 1.33% to 4.13% for Brazilian bee pollen.

Mean crude fiber content of bee pollen samples from Los Baños (2.76%) was higher and significantly different from that of Alaminos and Balete, while values were not significantly different between Alaminos and Balete at 1.07% and 1.13%, respectively. Nevertheless, the values obtained were within the standard range of 0.3-20% (Campos et al., 2008; Bogdanov, 2004). Values obtained were slightly lower than the data reported for bee pollen from Southern Brazil with mean crude fiber of 3.4% (Carpes et al., 2009). Like crude fat and ash, considerable differences in the values of crude fiber can be attributed to the botanical origin of the bee pollen samples.

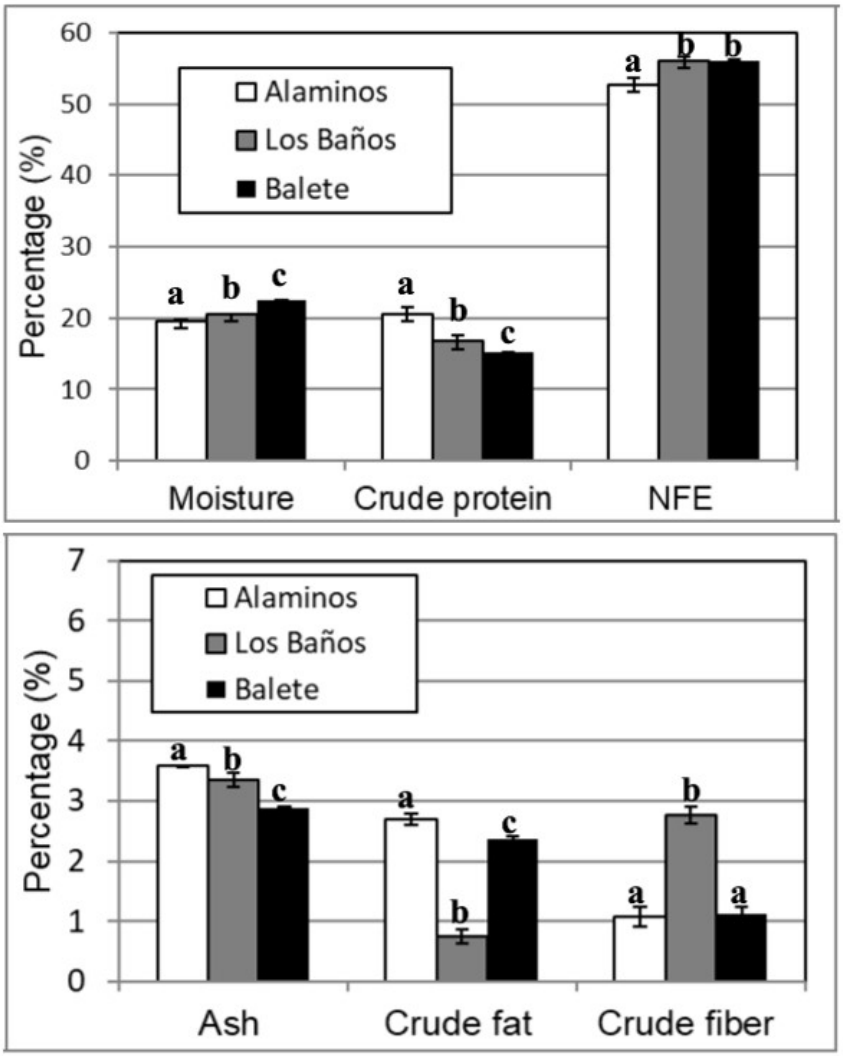

Figure 1. Proximate analysis of pollen collected by the Asian honey bee, *Apis cerana*, from selected apiaries in Laguna (Alaminos and Los Baños) and Batangas (Balete). Values are means of 3 trials expressed as mean ± standard deviation; the same letter above the bars shows no significant difference at 5% level of significance.

Carbohydrates as nitrogen-free extract (NFE) of the bee pollen samples ranged from 52.67 to 56.03%. Data for bee pollen from Alaminos (52.67%) were within the standard range of 13-55% (Campos et al., 2008) and significantly different from that of Los Baños and Balete. No significant difference was found between Los Baños and Balete samples and their values were a bit higher compared to the standard range. However, since total carbohydrates as NFE was calculated by subtracting the sum of all the percent constituents from 100%, results may not be very accurate because of possible errors accumulated from all of the analyses performed.

Carbohydrate Analysis

Figure 2 shows that total sugars of the pollen samples ranged from 9.63% to 17.30%. That of the pollen sample from Balete was significantly different from those of samples from Alaminos and Los Baños, while data for Alaminos and Los Baños samples were not significantly different. Data obtained in this study were lower that those resulting from liquid chromatography-mass spectrometer from Slovenian bee pollen with data ranging from 24.71% to 55.35% (Bertoncelj et al., 2018) and that of bee pollen from Southern Brazil with mean total sugar of 52.1%, analyzed spectrometrically (Carpes et al., 2009). Total sugars of bee pollen was relatively low compared to the sugar content of honey which constitutes about 95% of honey's dry matter (Bogdanov, 2011; De Melo et al., 2017). It should be noted that honey also contributed to the sugar content of bee pollen since honey and other secretions are used by bees to hold pollen together in their hives (Campos et al., 2008).

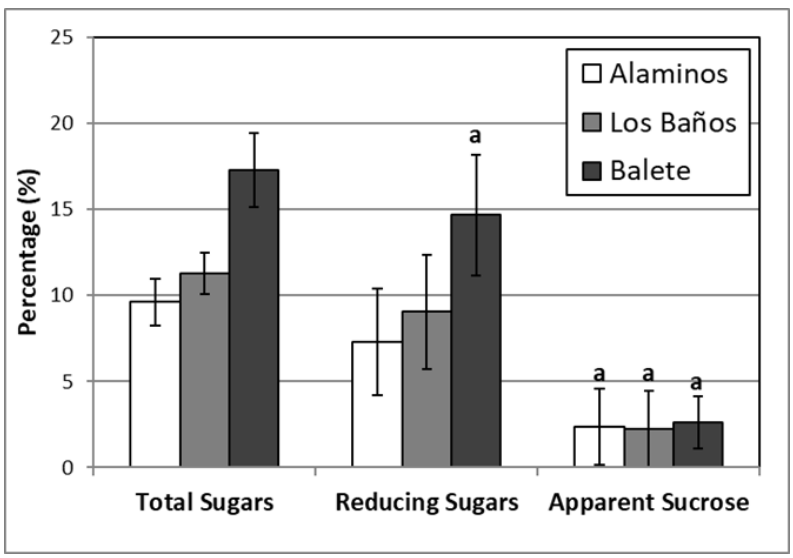

Figure 2. Carbohydrate analysis of pollens collected by the Asian honey bee, *Apis cerana,* from selected apiaries in Laguna and Batangas. (Values are means of 3 trials expressed as mean ± standard deviation, same letter above the bars shows no significant difference at 5% level of significance).

On the other hand, reducing sugar content of the bee pollen samples among the different locations showed no significant differences (Figure 2). Data obtained in the present study were also lower compared to data obtained by Carpes et al. (2009) but agree with those of Sattler et al. (2015) for Brazilian and Kalaycıoğlu et al. (2017) for Turkish bee pollens. Mean apparent sucrose of samples from different locations showed no significant differences and were close to one another, with a tight range from 2.24% to 2.62% (Figure 2). Comparable sucrose values were reported by Szczesna (2007) for honey bee pollen from Poland, South Korea and China with data ranging from 1.33% to 4.13%. On the other hand, slightly lower sucrose contents (range, 0.05–0.28) were analyzed by Bertoncelj et al. (2018) for Slovenian bee pollen. Low sucrose can be explained by the enzyme invertase in bees that degrades sucrose to its monosaccharide units--glucose and fructose (Somerville, 2000) resulting to low apparent sucrose of the samples.

Mineral Determination

Elemental analyses of phosphorus and sodium of the bee pollen samples (Figure 3) indicate that Alaminos and Los Baños had similar mean total phosphorus content of 2043.1 and 2062.9 mg P/kg, respectively. These differed significantly from that of Balete which showed a lower amount of phosphorus at 1839.7 mg P/kg. Nevertheless, data obtained were within the standard range of 800-6000 mg P/kg set by Campos et al. (2008). A similar range was also reported by Altunatmaz et al. (2017) for Turkish bee pollen (mean = 2659.73 mg P/kg). Conversely, higher P contents were reported for honey bee pollen from New South Wales (Australia) with mean = 4600 mg P/kg (Somerville & Nicol, 2002) and for Brazilian bee pollen samples (mean = 6923.6 mg P/kg, by Carpes et al., 2009; = 4828 mg P/kg by, Morgano et al. 2012). Factors such as flower varieties from which the bee pollen was collected, climatic conditions, geography, other environmental conditions, genetic composition of the plant species, agricultural procedures (fertilization and agricultural spraying) and apicultural processes can contribute to these differences or similarities in mineral levels (Altunatmaz et al., 2017).

Mean sodium content showed statistically significant differences among the different locations. Sodium content of bee pollen samples was highest in Alaminos followed by Los Baños and lastly, Balete with mean values of 33.57, 19.50, and 14.13 mg/kg, respectively. Sodium levels in this study tended to be lower than those reported for Turkish bee pollen (range of 5497 to 6223 mg/kg) (Kalaycıoğlu et al., 2017) and Chinese pollen (range of 1072 to 2447 mg/kg) (Szczesna, 2007) but agree with some bee pollen samples from Brazil (range of 12.9 to 636 mg/kg) (Carpes et al. 2009) and from Australia (range of 16 to 480 mg/kg) (Somerville and Nicol 2002). Several studies have shown that the main minerals present in bee pollen are K, Mg, Na, Ca, and P (Campos et al., 2008: Morgano et al., 2012; Altunatmaz et al., 2017).

***In vitro* Determination of Antioxidant Activity**

Antioxidants of various origins are considered to be multifunctional and their activity depends on various parameters such as reaction mechanism, experimental conditions, and heterogeneity of the matrix. Thus, the antioxidant properties of the pollen extracts cannot be evaluated using only one method due to the complexity of their constituents (Morais et al., 2011).

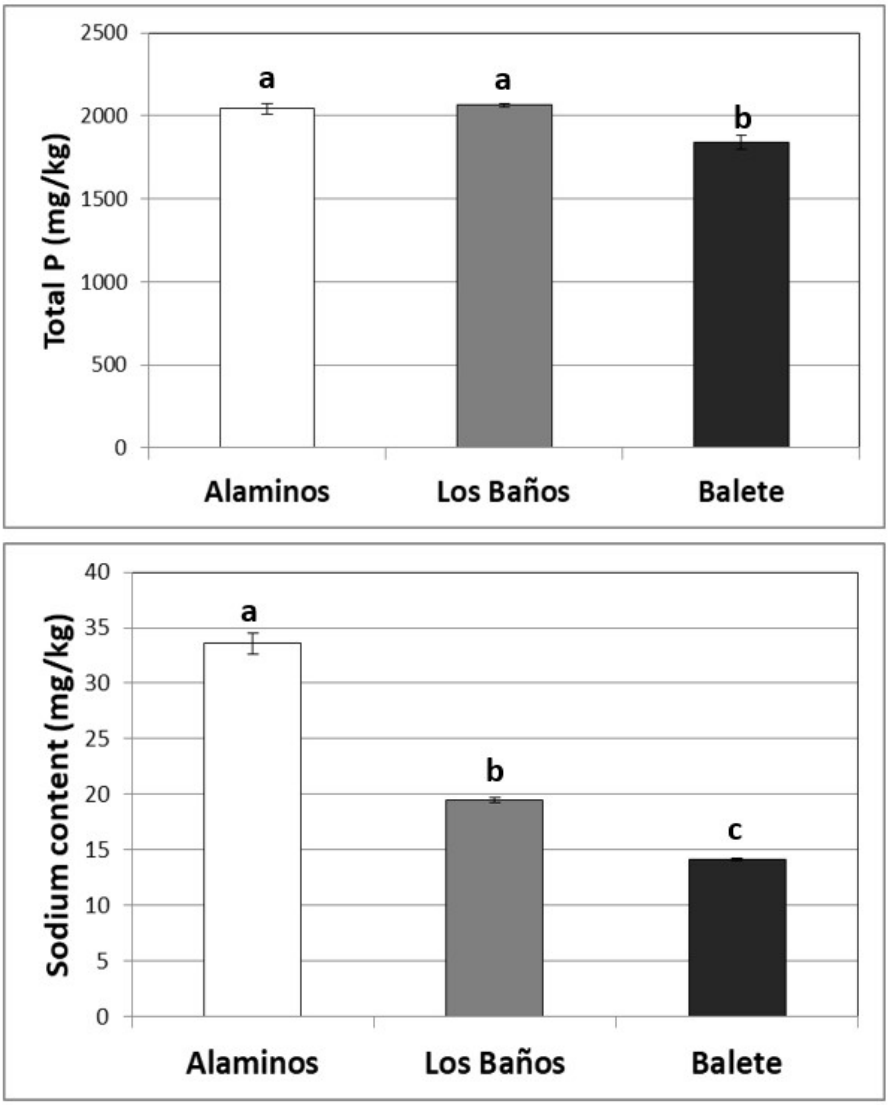

Figure 3. Total phosphorus and sodium content of pollen collected by the Asian honey bee, *Apis cerana,* from selected apiaries in Laguna and Batangas. (Values are means of 3 trials expressed as mean ± standard deviation, same letter above the bars shows no significant difference at 5% level of significance)

Results of the DPPH radical scavenging assay (Table 1) were reported as the EC_{50}, that is, the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%. Lower values indicate better antioxidant capacity of the bee pollen extracts (Carpes et al., 2009). Thus, the EC_{50} values were inversely proportional to the antioxidant activity of the samples (Sandagon, 2013). There were no significant differences among EC_{50} values of pollen samples from different locations. Ascorbic acid (positive control) has the highest effectiveness with EC_{50} of 7.40 since it is a well-known antioxidant. The EC_{50} of

the pollen samples and that of ascorbic acid were significantly different but the values obtained were not so far from each other. Carpes et al. (2009) reported lower EC_{50} values for DPPH radical scavenging activity of Brazilian bee pollen with a range of 0.81 to 4.69 mg/mL. Even much lower EC_{50} values were reported by LeBlanc et al. (2009) for the Sonoran Desert bee pollen with a range of 0.015 to 9.15 mg/mL and by Graikou et al. (2011) for the Greek bee pollen with mean EC_{50} value of 0.1814 mg/mL. Nevertheless, the results of antioxidant activities in the present study agree with those found by Meda et al. (2005), who analyzed 27 samples of different geographic origins from Burkina Faso and found a mean EC_{50} value of 10.60 mg/mL. In addition, the reduction of DPPH radical concentration together with the increase of pollen extract concentration was observed for all samples, and it was verified that the DPPH scavenging activity has a linear relationship with the concentration (Figure 4).

Table 1. Antioxidant activity of bee pollen samples from selected apiaries in Alaminos and Los Baños (Laguna) and Balete (Batangas).

Place of Origin	***In vitro* Assay**		
	DPPH Radical Scavenging EC_{50} (mg/mL)	H_2O_2 Scavenging EC_{50} (µg/mL)	FRAP values (mmol Fe^{2+}/g extract)
Alaminos	7.86 ± 0.11[a]	31.27 ± 0.09[a]	3.01 ± 0.49[a]
Los Baños	7.95 ± 0.21[a]	30.86 ± 0.07[b]	2.69 ± 1.04[a]
Balete	8.02 ± 0.08[a]	31.87 ± 0.09[c]	2.69 ± 0.37[a]
Ascorbic Acid	7.40 ± 0.04[b]	27.71 ± 0.02[d]	8.92 ± 0.49[b]

Values are means of 3 replicates expressed as mean ± standard deviation. Means in a column followed by the same letter are not significantly different at 5% level of significance.

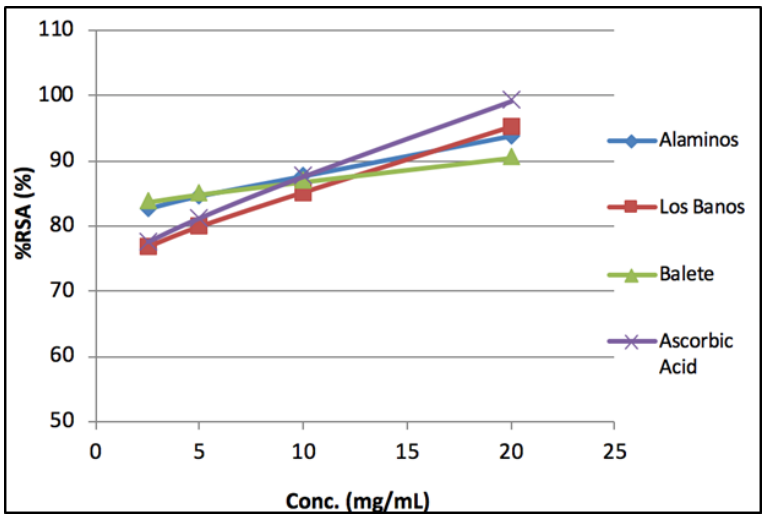

Figure 4. DPPH radical scavenging activity of ascorbic acid and extracts from pollen collected by the Asian honey bee, *Apis cerana,* from selected apiaries in Laguna and Batangas. (Values are means of 3 trials expressed as mean ± standard deviation, same letter above the bars shows no significant difference at 5% level of significance)

Values obtained from hydrogen peroxide assay were expressed in EC_{50} (µg/mL) (Table 1). For H_2O_2 scavenging activity, there were significant differences among samples from different locations and ascorbic acid. Mean H_2O_2 scavenging activity for the pollen samples was 31.33 µg/mL. Being the positive control, ascorbic acid gave the highest H_2O_2 scavenging activity at EC_{50} of 27.71 µg/mL. In their study of antioxidant properties of linden bee pollen, Jin et al. (2018), reported an O_2 scavenging activity of IC_{50} of 2290 µg/mL for the bee pollen samples and 6 µg/mL for the ascorbic acid (positive control). Similar to DPPH scavenging activity, ethanolic extracts of bee pollen from different apiaries showed a similar trend of increasing % scavenging activity as the concentration of the extract increases (Figure 5). The same trends were also reported for the antioxidant activity studies of other bee pollens (Graikou et al., 2011; Jin et al., 2018)

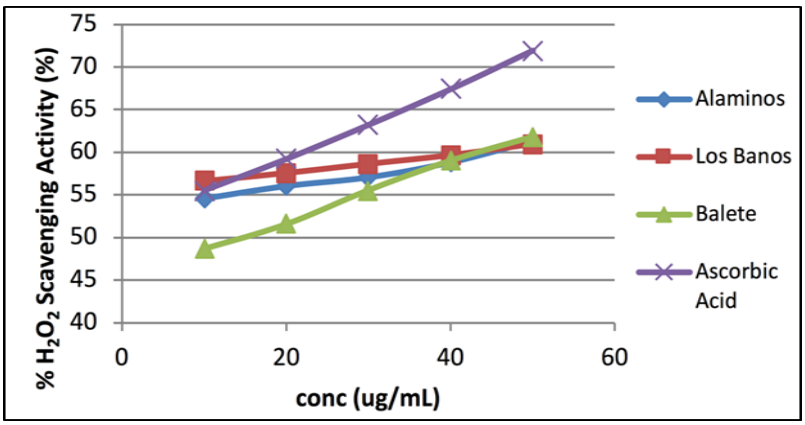

Figure 5. Hydrogen peroxide scavenging activity of ascorbic acid and extracts from pollen collected by the Asian honey bee, *Apis cerana,* from selected apiaries in Laguna and Batangas. (Values are means of 3 trials expressed as mean ± standard deviation, same letter above the bars shows no significant difference at 5% level of significance)

Total antioxidant potential of the bee pollen samples can be evaluated using the ferric reducing antioxidant power (FRAP) assay. As a measure of antioxidant power, FRAP is a simple, direct test of antioxidant capacity (Prior et al., 2005). FRAP values were expressed in mmol Fe^{2+}/g extract (See Table 1). No statistically significant differences were found among the FRAP values of the pollen extracts from different locations, while there were significant differences between the positive control and the bee pollen samples. Ascorbic acid was used as the positive control just like in the two previous assays, which showed the highest average FRAP value of 8.92 mmol Fe^{2+}/g extract while the bee pollen samples showed a mean value of 2.80 mmol Fe^{2+}/g. Higher FRAP values mean higher antioxidant capacity. A similar range of FRAP values were reported by LeBlanc et al. (2009) for the Sonoran Desert bee pollen with mean of 1.53 mM Fe^{2+} for the ethanolic extracts of the bee pollen.

A lot of studies have pointed out that the antioxidant activity of pollen is largely a result of the phenolic compounds and flavonoids that have free radical scavenging activity (Ares et al., 2018; Jin et al., 2018; LeBlanc et al., 2009),

although other constituents such as proteins and vitamins may also contribute to this property (Campos et al., 2008; Graikou et al., 2011). In addition, collection place, weather conditions, soil characteristics, time of the year when the collection was performed, and farming conditions were also factors tp be considered for antioxidant activity (Escudero et al., 2008).

Botanical Origin of Bee Pollen Samples

Melissopalynology is an important tool in determining the floral sources upon which the bees foraged. Each flower species has a unique pollen grain, which may be studied, using proper techniques, to determine the geographical origin and major floral sources of the honey bees (Nair et al., 2013). Physico-chemical characteristics and antioxidant properties of the bee pollen samples vary according to the botanical origin of the pollen composition in the samples.

There were a total of 19 different plant species (Figure 6) present in the bee pollen samples. Table 2 shows the floral sources and their average frequency in the pollen samples. The predominant pollen grains identified from Alaminos samples was *Mimosa pudica* L., with *Aeschynomene* sp. as secondary pollen source. *M. pudica* produces head-type of inflorescences abundantly with exposed stamens which allow easy access for foraging bees in collecting pollen grains (Cunanan-Deyto et al., 2012). Meanwhile, pollen samples from Los Baños had Fabaceae (=Leguminosae) and coconut, *Cocos nucifera* L., as the predominant and secondary sources, respectively. The Fabaceae is the third largest family of plants, with about 19,300 species that can be found almost entirely in the whole world (Estrella et al., 2006). Hence, its presence and relative abundance of flowers make it a preferred plant to be visited by the bees. Pollen samples from Balete consisted of Asteraceae (=Compositae) and Poaceae (=Graminae) as the predominant and secondary sources, respectively.

Note that coconut was present in all samples. Coconut produces pollen abundantly and bees are very much attracted to the taste and smell of its nectar (Cunanan-Deyto et al., 2012). Also, majority of the pollen types were found as important minor sources which only indicate diverse nectar sources in the locations of the apiaries. In fact, all bee pollen samples were classified as multifloral, which indicate that the study areas have diverse or wide range of vegetation. Moreover, being multifloral, contribution of other floral sources aside from the predominant and secondary sources would also affect the bee pollen properties.

SUMMARY AND CONCLUSION

Physico-chemical analyses of the *Apis cerana* pollen samples from bee colonies in Alaminos and Los Baños in Laguna Province, and Balete in Batangas Province were determined using proximate analysis, carbohydrate analysis, and mineral determination. In addition, antioxidant properties of the bee pollen

Figure 6. Identified botanical origin of bee pollen samples. (1) *Adenanthera* sp; (2) *Aeschynomene* sp; (3) *Albizia* sp; (4) *Alnus japonica* (Thunb.) Steud.; (5) Amaranthaceae; (6-7) Apocynaceae; (8) Arecaceae; (9) *Cassia* sp; (10) *Cocos nucifera* L.; (11) Asteraceae; (12) Poaceae; (13) *Heterospathe philippinensis* (Becc.) Becc.; (14) Fabaceae IV; (15) *Mangifera indica* L.; (16)*Mimosa pudica* L.; (17) *Pithecellobium dulce* (Roxb.) Benth.; (18) Poaceae; (19) Unidentified pollen.

Table 2. Identity and classification of botanical origin of the bee pollen samples.

Site	Botanical Origin	Average frequency	Classification	Monofloral/ Multifloral
Alaminos	*Aeschynomene* sp.	21.35	Secondary	Multifloral
	Apocynaceae	6.74	Important Minor	
	Cocos nucifera	1.87	Minor	
	Asteraceae	5.99	Important Minor	
	Poaceae	11.99	Important Minor	
	Heterospathe philippinensis	5.99	Important Minor	
	Mimosa pudica	46.07	Predominant	
Los Baños	*Adenanthera* sp.	1.16	Minor	Multifloral
	Albizia sp.	1.54	Minor	
	Alnus japonica	2.32	Minor	
	Amaranthaceae	0.77	Minor	
	Arecaceae	10.04	Important Minor	
	C. nucifera	16.22	Secondary	
	Asteraceae	8.11	Important Minor	
	Fabaceae IV	47.88	Predominant	
	Pithecellobium dulce	2.70	Minor	
	Poaceae	5.41	Important Minor	
	Unidentified pollen	3.86	Important Minor	
Balete	*Aeschynomene* sp.	4.76	Important Minor	Multifloral
	A. japonica	3.17	Important Minor	
	Apocynaceae	8.33	Important Minor	
	Cassia sp.	3.97	Important Minor	
	C. nucifera	2.78	Minor	
	Asteraceae	45.24	Predominant	
	Poaceae	18.65	Secondary	
	Mangifera indica	5.56	Important Minor	
	Unidentified pollen	7.54	Important Minor	

samples were evaluated using *in vitro* determination of antioxidant properties such as DPPH radical scavenging, hydrogen peroxide, and ferric reducing antioxidant power assays. Variations in the values obtained from the analyses can be attributed to different factors but generally, the composition of the pollen sources of the bee pollen samples greatly affects the properties obtained in the study as well the polyphenolic composition for its antioxidant activity. Identification of the botanical origin of bee pollen samples was also conducted. Pollen from 19 different plant species were present in the samples. All samples were classified as multifloral with *M. pudica*, other Fabaceae (=Leguminosae), and Asteraceae (=Compositae) as predominant sources for Alaminos, Los Baños, and Balete samples, respectively.

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