Antimicrobial and Antioxidant Activities, Total Phenolic and Flavonoid Contents of Bee Pollen Crude Extracts

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Abstract: Bee pollen is a natural product from honeybees which is rich in bioactive compounds. Bee pollen grain and powder were extracted using conventional and sonication extraction methods. All bee pollen crude extracts could not inhibit growth of test pathogenic bacteria. The antioxidant activity was analyzed via DPPH free radical scavenging activity assay and found that the ethanolic extract of bee pollen grain obtained by conventional method had the highest antioxidant activities (p<0.05), 40.69±3.01 mg GAE/g extract. When phenolic content was evaluated, the aqueous extract of bee pollen grain by conventional method showed the highest total phenolic content (103.06±1.96 mg GAE/g extract) while ethanolic extract of bee pollen powder by conventional method exhibited the highest flavonoid content, 56.40±4.85 mg QE/g extract. The data obtained provides the guideline for insightful evaluation of bee pollen biological activities for designation of novel supplementary food products in the future.

Key words: Bee pollen, antioxidant, bioactive compound, sonication, honey.

1. Introduction

Bee pollen is known for its therapeutic properties attributed to several compounds such as minerals, vitamins, essential amino acids, phenolic acids, flavonoids, enzymes and co-enzymes [1]-[3].

Bee pollen is produced by mixing flower pollen with both the nectar and the bee salivary gland secretion. Afterwards, the bee is amassing at the tibia of their hind legs. Later, bee pollens are transported to the bee hive [4], [5]. Bee pollen is a food source of the bees. They contain concentrations of nutrients and rich in secondary metabolites [6]. The main biological components of bee pollen are phenolic acid derivatives and flavonoids. The flavonoids are so called secondary plant compounds which have different important physiological and pharmacological activities. They possess diverse biological properties such as antioxidant, anti-aging, anti-carcinogenic and anti-inflammatory [7].

Bee pollen is one of the most important sources of proteins, lipids, minerals and vitamins for bee survival. Recently, increasing evidence suggests its potential therapeutic benefits, including antioxidant properties and bioactive properties, as functional dietary food supplement. The previous studies have found that bee pollen consist of polysaccharides, pollen, moisture, proteins, lipids, ash, carotenoids and vitamins [8].

Additionally, the chemical composition of bee pollen also depends on the plant source, climatic conditions, soil type and bee's activities [9]. Bee pollen color has various shades ranging from bright yellow to black [10].

Bee pollen can inhibit growth of some pathogenic bacteria [11]. Some reports demonstrated that bee pollen extracts obtained from plants in the family Papaveraceae, Brassicaceae and Asteraceae could inhibit growth of *Bacillus subtilis, Escherichia coli*, *Klebsiella* spp., *Listeria monocytogenes*, *Pseudomonas aeruginosa and Staphylococcus aureu*s [12]. However, any bee pollen extracts could not show inhibitory effect on fungi (*Saccharomyces cerevisiae*, *Candida rugosa*, *Aspergillus niger* and *Rhizopus oryzae*) [13].

The aim of this study is to determine the biological activities of bee pollen. Accordingly, grain and powder of bee pollen are extracted by water and ethanol using conventional and sonication extraction methods.

2. Materials & Methods

Bee pollen obtained from Bee Products Industry Co., Ltd. was kept at -20°C prior to use. Different extraction methods (conventional and sonication) were performed using distilled water and ethanol as solvents.

2.1. Bee Pollen Extraction

For conventional method, each grain and powder bee pollen was extracted using two solvents, distilled water and ethanol, 95% (v/v). For aqueous extraction, the mixture of bee pollen and solvent, 1:10 ratio, was kept at 45˚C for 3 hours prior to filtration. Subsequently, the retentate was repeatedly extracted twice. The filtrates were combined together prior to solvent evaporation using a rotary evaporator (Heidolph, Germany) and lyophilization (FTS systems, USA). Meanwhile, the ethanolic extraction was similarly done but at room temperature for 24 hours. The lyophilized crude extracts were kept at -20˚C until used. When used, the crude extracts were reconstituted in the corresponding solvent and adjusted to the desired concentration.

For sonication extraction using a sonication bath (Crest, USA), grain and powder bee pollen were sonicated twice, 30 minutes at 50 Hz each, prior to filtration [14]. The filtrate obtained was subsequently evaporated and lyophilized as mentioned above. When used, the crude extracts were dissolved in sterile distilled water to the desired concentration.

2.2. Antibacterial Activity Determination

Pathogenic bacteria including *B. cereus* TISTR 687, *E. coli* ATCC 25922, *E. coli* O157: H7 DMST 12743, methicillin resistant *S. aureus* DMST 20625, *Salmonella* Typhi DMST 22842, *Shigella dysenteriae* DMST 1511, *S. aureus* ATCC 25923 and *Vibrio cholerae* DMST 2873 were used as test bacteria. All of them were grown in Mueller-Hinton broth (MHB) (Merck, Germany) at 37°C for 24 hours. Each culture broth was adjusted its turbidity equivalent to a No. 0.5 McFarland standard prior to swab onto Mueller Hinton Agar (MHA) (Merck, Germany).

Antibacterial activity was determined by an agar disc diffusion method [2], [15]. A 6-mm sterile paper disc soaked with 500 mg/ml of each crude extract was placed onto the prepared MHA mentioned above. Gentamicin, 0.1 mg/ml, and sterile distilled water were used as positive and negative controls, respectively. The test plates were incubated at 37◦C for 24 hours. The diameters of inhibitory clear zone were observed and measured.

2.3. Antioxidant Properties by Using Free Radical Scavenging Assay

The antioxidant activity of the bee pollen crude extract was determined by the free radical scavenging ability using 2,2-diphenyl-a-picrylhydrazyl (DPPH) radical scavenging assay [16], [17], as described earlier

with some modifications. Briefly, 50 µL of each crude extract was mixed with 0.1 mM DPPH solution, 150 µL, and incubated in the dark at room temperature for 20 minutes. The absorbance of the mixture was measured at 517 nm. Gallic acid was used as a positive control. The ability of the sample to scavenge DPPH radical was determined according to the equation below:

$$\text{DPPH inhibition (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is absorbance of methanol, a negative control, and A_{sample} is absorbance of sample.

2.4. Total Phenolic Content by the Folin–Ciocalteu Method

By modified method of Babaa and Malik [18], 250 µL of crude extract were made up to 1.25 mL with deionized water (DI water), mixed with 125 µL of 50% Folin–Ciocalteu reagent, followed by the addition of 250 µL of absolute ethanol for 5 minutes, after that, mixed with 250 µL of 5% (w/v) sodium carbonate. Then, the mixture was incubated at room temperature in the dark for 1 hour, and absorbance was measured at 725 nm. The total phenolic content was calculated from the calibration curve. The results were expressed as mg of gallic acid equivalent per g extract.

2.5. Total Flavonoid Content by the Aluminium Chloride Colorimetric Method

By modified method of Babaa and Malik [18], 0.5 mL of each crude extract were mixed with 100 µL of 10% aluminium chloride, added with 1.5 mL of methanol and, mixed with 100 µL of 1M potassium acetate solution. Next, the mixture was made up to 2.80 mL with DI water. The mixture was allowed to stand for 30 minutes in the dark. The absorbance was measured at 415 nm. The total flavonoid content was calculated from a calibration curve and was expressed as mg Quercetin equivalent per g extract.

3. Results and Discussion

All bee pollen crude extracts could not inhibit growth of test pathogenic bacteria. For the bee pollen grain, the ethanolic extract had the highest antioxidant activities (p<0.05), 40.7 ± 3.0 mg GAE/g extract when tested by the DPPH assay (Fig. 1) while the aqueous extract showed the highest amount of total phenolic acid, 103.06±10.9 mg GAE/g extract (p<0.05) (Fig. 2). Meanwhile, the ethanolic extract of bee pollen powder presented the highest value of total flavonoid content, 56.4±4.8 mg QE/g extract (p<0.05) (Figure 3).

Moreover, the conventional extraction method provided the higher antioxidant activities than that of the sonication extraction significantly (p<0.05) (Table 1).

Table 1. Antioxidant Activity, Total Phenolic and Total Flavonoid Content of Bee Pollen Crude Extracts

Crude extract	Bee Pollen	DPPH (mg GAE/g extract)	Phenolic (mg GAE/g extract)	Flavonoid (mg QE/g extract)
Conventional extraction				
Ethanolic	Grain	40.69± 3.01 ^{a}	66.53± 10.90 $^{b, c}$	36.60± 0.20 ^{b}
	Powder	0.52± 0.15 ^{d}	60.66± 3.40 $^{c, d}$	56.40± 4.85 ^{a}
Aqueous	Grain	21.27± 2.63 ^{b}	103.06± 1.96 ^{a}	12.20± 1.73 ^{c}
	Powder	11.20± 3.89 ^{c}	54.46± 2.38 ^{c}	7.53± 0.30 ^{d}
Sonication extraction	Grain	0.03± 0.02 ^{d}	58.80± 0.40 $^{c, d}$	9.53± 0.30 $^{c, d}$
	Powder	12.60± 0.81 ^{c}	70.93± 2.66 ^{b}	12.00± 1.58 ^{c}

Statistically significant difference (p<0.05) are indicated by different low case letters (a-d) within sample for each methodology (Data were expressed as mean ± standard deviation (n=3))

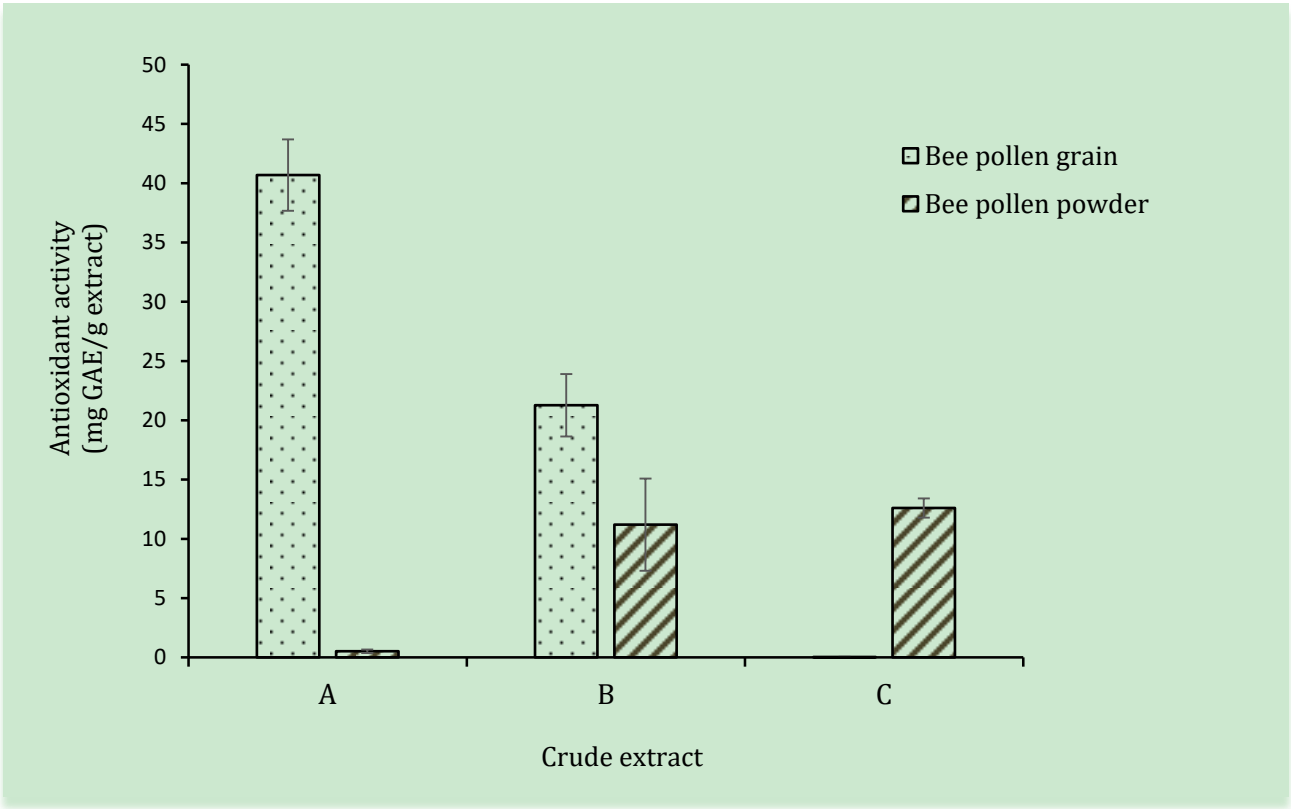

Fig. 1. Antioxidant activity of bee pollen crude extracts. The ethanolic extract (A) and the aqueous extract (B)obtained by the conventional method, and the aqueous extract obtained by the sonication method (C).

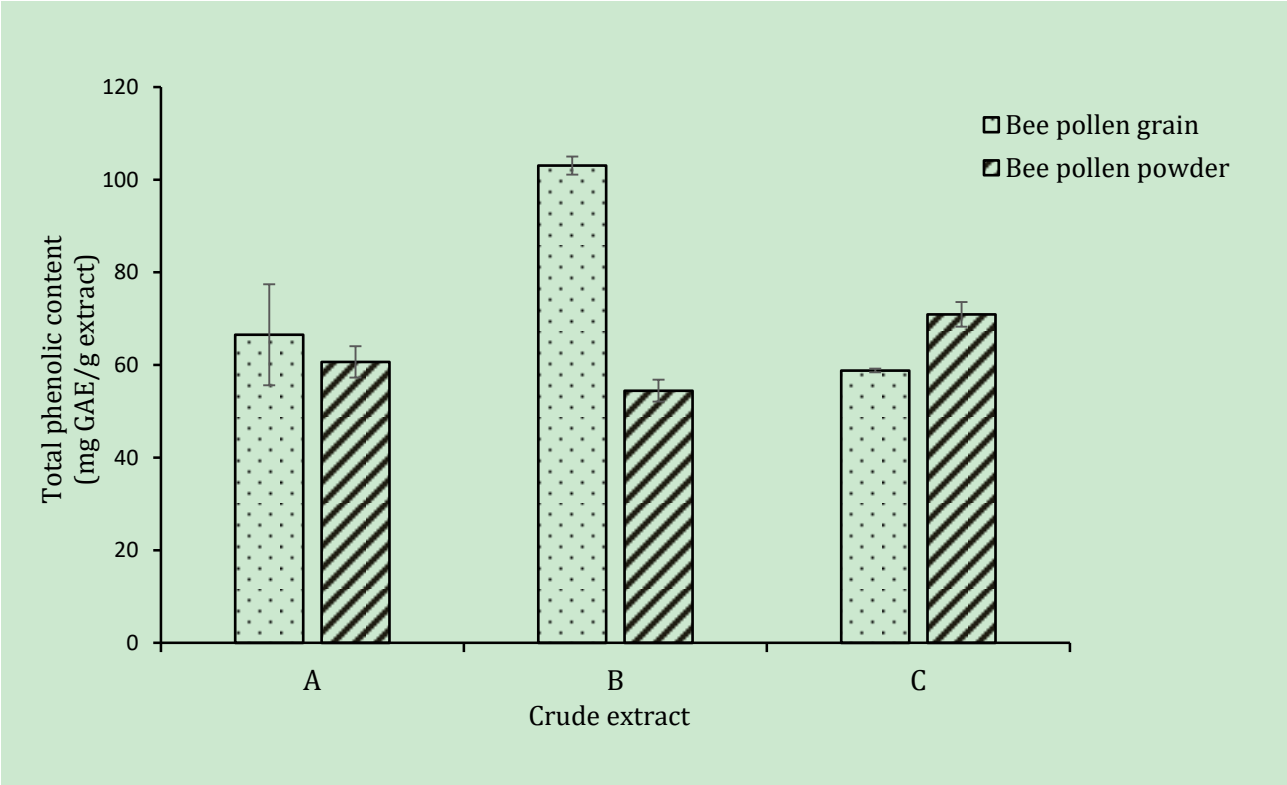

Fig. 2. Total phenolic content of grain and powder bee pollen extract. The ethanolic extract (A) and the aqueous extract (B) obtained by the conventional method, and the aqueous extract obtained by the sonication method (C).

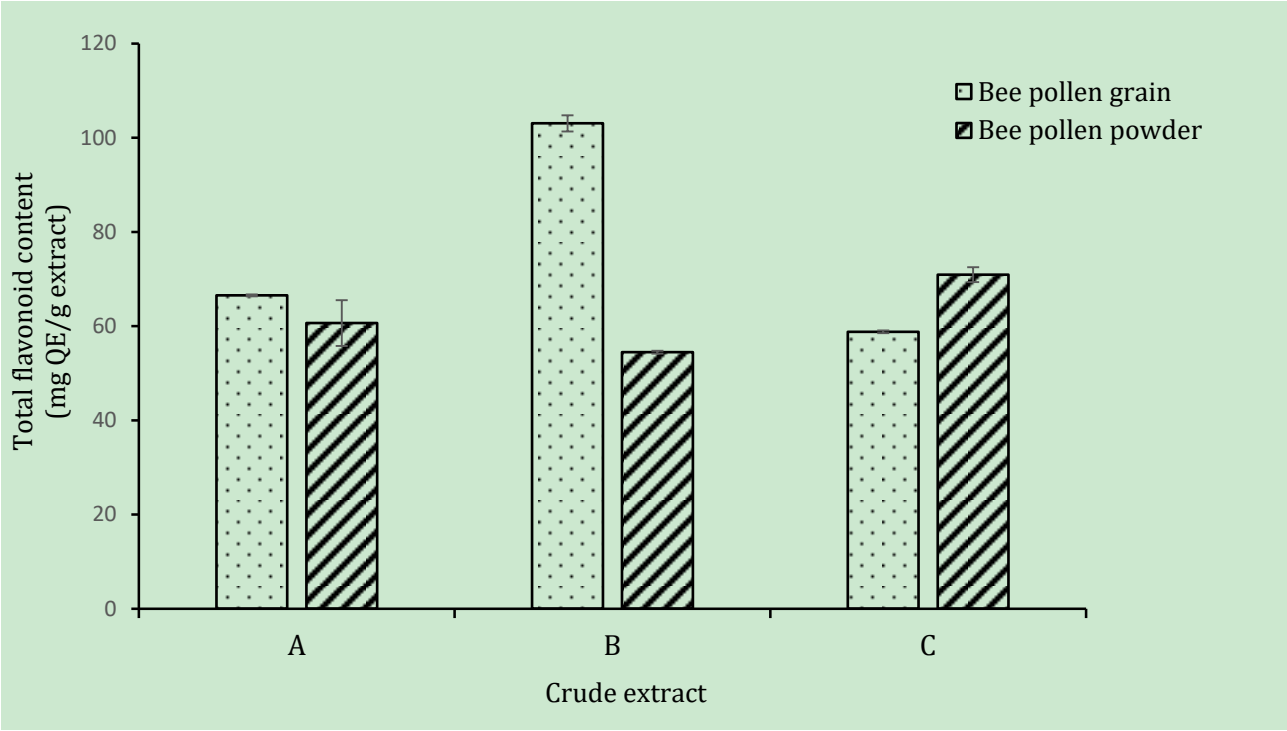

Fig. 3. Total flavonoid content of grain and powder bee pollen extract. The ethanolic extract (A) and the aqueous extract (B) obtained by the conventional method, and the aqueous extract obtained by the sonication method (C).

In this study, the antioxidant activities of bee pollen extracts are associated with the content of phenolic compounds which is similar to the previous reports [19], [20], [21]. On the other hands, this does not appear to be true for other biological activities [22], [23].

Bee pollen from honey bee contains different amounts of total phenolic and flavonoid content. This may be linked to the type of floral and different diets of the bees [12], [14], [24]. Furthermore, the total phenolic acid and flavonoid contents of bee pollen extracts also depend on the polarity of solvent used in extraction. High solubility of phenolic and flavonoid compounds in polar solvents provides high concentration of these compounds obtained in the extraction [25]. The aqueous extraction of bee pollen grain in this study gave the highest total phenolic content. By the way, the high solubility of antioxidant in non-polar solvent provide the high concentration.

Freire [26] studied about the values of total phenolic and flavonoid contents compared with other studies which used ethanol, methanol and water extraction. They found that the difference type of extraction solvent had the difference effect on concentration of bioactive compound in the extracts.

4. Conclusions

Bee pollen extracts cannot inhibit growth of test enteric pathogenic bacteria. Free radical scavenging activity, amount of total phenolic acid and flavonoid contents depend on the method of extraction and polarity of solvent. Moreover, the conventional extraction had antioxidant activities higher than sonication extraction.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

WF extracted bee pollen, determined biological activity and analyzed the data. NT proved and edited manuscript.

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References

[1] Human, H., & Nicolson, S. W. (2006). Nutritional content of fresh, bee-collected and stored pollen of Aloe greatheadii var. davyana (Asphodelaceae). *Phytochemistry*, *67*, 1486-1492.

[2] Abouda, Z., Zerdani, I., Kalalou, I., Faid, M., & Ahami, M. T. (2011). The antibacterial activity of Maroccan bee bread and bee-pollen (fresh and dried) against pathogenic bacteria. *Research Journal of Microbiology*, *6(4)*, 376-384.

[3] Roulston, T. H., & Cane, J. H. (2000). Pollen nutritional content and digestibility for animals. *Plant Systematics and Evolution*, *222*, 187-209.

[4] Pereira, F. M., Freitas, B. M., Vieira, N. J. M., Lopes, M. T. R., Barbosa, A. L., & Camargo, R. C. R. (2006). Development of bee colonies with different protein foods. *Pesquisa Agropecúaria Brasileira*, *41(1)*, 1–7.

[5] Campos, M. R. G., Bogdanov, S., Almeida-Muradian, L. M. B., Szczesna, T., Mancebo, Y., Frigerio, C., & Ferreira, F. (2008). Pollen composition and standardisation of analytical methods. *Journal of Apicultural Research*, *47*, 156–163.

[6] Graikou, K., Kapeta, S., Aligiannis, N., Sotiroudis, G., Chondrogianni, N., Gonos, E., & Chinou, I. (2011). Chemical analysis of Greek pollen - Antioxidant, antimicrobial and proteasome activation. *Chemistry Central Journal*, *5*, 33.

[7] Yerlikaya, O. (2014). Effect of bee pollen supplement on antimicrobial, chemical, rheological, sensorial properties and probiotic viability of fermented milk beverages. *Fermented Milk Beverages, Mljekarstvo, 64(4)*, 268-279.

Roldán, A., Muiswinkel, G. C. J., Lasanta, C., Palacios, V., & Caro, I. (2011). Influence of pollen addition on mead elaboration: Physicochemical and sensory characteristics. *Food Chemistry*, *126*, 574-582.

[8] Silva, I. L., Pagliarini, M. A., Chagas, T.,E., Frühauf, M., Kempka, A. P., & Dornelles, R. C. P. (2017). Effect of supplementation with pollen and brewer's yeast in the fermentation and in the physicochemical properties of honey spirits. *International Food Research Journal*, *24(3)*, 1124-1134.

[9] Dubtsova, E. A. (2009). Structure, biological properties of honey, pollen and royal jelly and their possible use in nutrition therapy. *Experimental and Clinical Gastroenterology*, *3*, 36–41.

[10] Fatrcova-Šramkova, K., Nožkova, J., Kăcaniova, M., Mariassyova, M., Rovna, K., & Strĭcik, M. (2013). Antioxidant and antimicrobial properties of monofloral bee pollen. *Journal of Environmental Science and Health*, *48*, 133–138.

[11] Carpes, S. T., Beginini, R., Alencar, S. M., & Masson, M. L. (2007). Study of preparation of bee pollen extracts, antioxidant and antibacterial activity. *Ciencia e Agrotecnologia*, *31*, 1818–1825.

[12] Salles, J., Cardinault, N., Patrac, V., Berry, A., Giraudet, C., Collin, M. L., Chanet, A., Tagliaferri, C., Denis, P., Pouyet, C., Boirie, Y., & Walrand, S. (2014). Bee pollen improves muscle protein and energy metabolism in malnourished old rats through interfering with the Mtor signaling pathway and mitochondrial activity. *Nutrients*, *6*, 5500–5516.

[13] Mărghitaş, L. A. L., Stanciu, O. G., Dezmirean, D. S., Bobiş, O., Popescu, O., Bogdanov, S., & Campos, M. G. (2009). In vitro antioxidant capacity of honeybee-collected pollen of selected floral origin harvested from Romania. *Food Chemistry*, *115*, 878-883.

[14] Collins, C. H., Lyne, P. M., & Grange, J. M. (1995). *Collins and lynes Microbiological Methods*, 7th Edn. Butterworth. Heinman. Oxford 493.

[15] Villaño, D., Fernández-Pachán, M. S., Moyá, M. L., Troncoso, A. M., & García-Parrilla, M. C. (2007). Radical scavenging ability of polyphe-nolic compounds towards DPPH free radical. *Talanta*, *71*, 230.

[16] Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry*, *113*, 1202–1205.

[17] Babaa, S. A., & Malik, S. A. (2015). Determination of total phenolic and flavonoid content, antimicrobialand antioxidant activity of a root extract of Arisaema jacquemontii Blume. *Journal of Taibah University for Science*, *9*, 449–454.

[18] Melo, A. A. M., Estevinho, M. L. M. F., Sattler, J. A. G., Souza, B. R., Freitas, A. S., & Barth, O. M., & Almeida-Muradian, L. B. (2016). Effect of processing conditions on characteristics of dehydrated bee-pollen and correlation between quality parameters. *Journal of Food Science and Technology*, *65*, 808–815.

[19] Rebiai, A., & Lanez, T. (2012). Chemical composition and antioxidante activity of Apis mellifera bee pollen from northwest Algeria. *Journal of Fundamental and Applied Sciences*, *4*, 155–163.

[20] Almaraz-Abarca, N., Campos, M. G., Ávila-Reyes, J. A., Naranjo-Jiménez, N., Herrera-Corral, J., & González-Valdez, L. S. (2004). Variability of antioxidant activity among honeybee-collected pollen of different botanical origin. *Interciencia Journal*, *29*, 574–578.

[21] Pascoal, A., Rodrigues, S., Teixeira, A., Féas, X., & Estevinho, L. M. (2014). Biological activities of commercial bee pollens: Antimicrobial, antimutagenic, antioxidant and anti-inflammatory. *Food and Chemical Toxicology*, *63*, 233–239.

[22] Leja, M., Mareczek, A., Wyzgolik, G., Klepacz-Baniak, J., & Czekonska, K. (2007). Antioxidative properties of bee pollen in selected plant species. *Food Chemistry*, *100*, 237–240.

[23] Nagamitsu, T., & Inoue, T. (2002). Foraging activity and pollen diets of subterranean stingless bee colonies in response to general flowering in Sarawak, Malaysia. *Pidologie*, *33*, 303–314.

[24] Stanković, M. S. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of Marrubium peregrinum L. extracts. *Kragujevac Journal of Science*, *33*, 63–72.

[25] Freire, K. R. L., Lins, A. C. S., Dórea, M. C., Santos, F. A. R., Camara, C. A., & Silva, M. S. (2012). Palynological origin, phenolic content and antioxidant properties of honey bee-collected pollen from Bahia, Brazil. *Molecules*, *17*, 1652–1664.

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