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Chemical constituents and antioxidant activity of *Borussus flabellifer*, *Elaeis guineensis*, *Mimosa diplotricha* and *Mimosa pigra*

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Abstract

Four pollen types were found in bee pollen in Palmyra Palm (*Borussus flabellifer* L.), Oil palm (*Elaeis guineensis* Jacq.), Sensitive plant (*Mimosa diplotricha* C. Wright ex Suavale) and Giant sensitive tree (*Mimosa pigra* L.). Lipids, proteins, fiber, ash and humidity and DPPH scavenging activity of 4 pollen species were evaluated by AOAC standard methods. Result showed that the highest protein and lipids contents were found in *M. diplotricha* pollen (38.85±0.53%) and *B. flabellifer* bee pollen (1.84±0.1%), respecively. IC_{50} (143.9 µg/mL) of *M. pigra* pollen displayed the best antioxidant activity. However, it is less when compared to BHT (IC_{50} 5.0 µg/mL) and gallic acid (IC_{50} 0.32 µg/mL).

Keywords: Bee pollen; chemical content; antioxidant

Introduction

Bee pollen is collected by honey bee *Apis mellifer*. The bees are extremely discriminate to select the best pollen from millions of presented grains by using their accumulated forelegs. Then, pollen grains are mixed with 10% nectar sticky substance for packing together with α,β-galactosidase which secreted from their hypopharyngeal gland of stomachs. Allowing the packed pollens are adhered to rear legs in "pollen baskets" in order to safely transport it to their hives. (Carpes et al., 2009). Bees used pollens to provide for larvae and worker and stored pollen pellets in hive. When pollen pellets were kept for a long time, they will be produced lactic acid formation (Herbert et al., 1978). Honey bee seeks various flowering plants to make bee pollen, so the products from bee pollen were not uniform of nutritional value depend on botanical sources. Bee pollen was claimed as supplementary food and given some medicinal properties. Bee pollen rich of essential nutrition composed of protein (up to 35%), lipid, reducing sugars, non-reducing sugars, crude fiber, pectin, mineral salts, and amino acid (Herbert et al., 1978; Campos et al., 1996). The different botanical origins of bee

pollen have affected to quality and quantity of nutritional value. Bee pollen contains many varieties of pigments, of which only a small number have been isolated. Certain pigments are water-soluble, while others are fat-soluble. This accounts for the many varied colors of honey (including the ambers and greens), and the yellow of beeswax is a fat-soluble pigment. Amount of chemical contents can access original sources of plants. It is known that bee pollen contains lipids, sugars, proteins, amino acids, vitamins, carotenoids, polyphenolics such as flavonoids and carbohydrates. Especially the carbohydrates are derived from the nectar with which the flower pollen has been mixed in the flowers. The phenolic composition of pollen principally consists of flavonol glycosides and of hydroxycinnamic acids. This composition tends to be species-specific and has been related to the therapeutic properties (antibiotic, antineoplasic, antidiarrhoeic and antioxidant) of pollen.

The antioxidant property of bee pollen also praised them to be superb dietary food. There are several diseases being caused by free radical substances such as cardiovascular disease, some types of cancer (Rice-Evans et al., 1996). Free radical molecules are very harmful to human, for example, if they attract to one of paired electron from nucleic acid that will be factor to malformation of cell. There are different antioxidant activity rely on botanical sources as the same as chemical contents; in addition, bee pollen also found the phenolic compounds that are mostly found bioflavonoid (Almeida-muradian et al., 2005) involved with flavonol concentration (Almaraz-Abarca et al., 2007).

The aims of this study to investigate pollen species of each sample, and to determine both of chemical contents and antioxidant activity of bee pollen samples. Three samples obtained from Southern region and another one from Central region of Thailand.

Materials and Methods

Bee pollen samples

Three samples were collected from Southern region; two from Krasaesin district and one from Thepa district, and the another one bought from Central region of Thailand during 2010-2012. All of samples were conducted to laboratory and dehydrated at 60ºC for 2 days then kept all sample at -4°C in freezer for further analysis.

Identification of pollen

Botanical sources of each pollen sample were identified under light microscope by comparing with pollen of plant grown around the study sites. We divided some pollen sample contaminated with other species and a minor accepted percentage was <10%.

Determination of chemical contents

Total proteins were determined by the Kjeldahl method from 2 g of bee pollen samples and used 6.25 factor as conversion to calculate. Total lipids were extracted by using Soxhlet extractor with petroleum ether as solvent. The amounts of sample were used 2 g. Moisture contents were determined at 100°C in oven and weight by using gravimetry until constant weight. Ash contents were determined by incineration in furnace at 550°C for 2

hours by using 2 g of samples. Fiber contents were determined by AOAC method. All of samples were made in triplicate.

DPPH radical scavenging method

The antioxidant activity was accessed by DPPH radical scavenging assay. We used 3 mL test tube; 1 mL of extract from bee pollen that was varied concentration to measure and 0.5 mL DPPH. The extraction applied for 70% ethanol as solvent according to Carpes (2008). DPPH have changed from deep purple to bright yellow, therefore level of color depend on a large of quantity of antioxidant activity by using colorimetric principle. Absorbance value was monitored by using spectrophotometer at 520 nm after set at room temperature for 30 minutes (Kim et al., 2006). Antioxidant activity was displayed in term of IC_{50} (half maximal inhibitory concentration) and calculated by mean of linear regression between concentration and in $ug.mL^{-1}$ and mean percentage of antioxidant activity. BHT and gallic acid were monitored as standard to compare the antioxidant capacity. 1 mL of 70 % ethanol and 0.5 mL of DPPH were use as sample blank. The formula clarifies as equation 1. All analysis made triplicate and Ducan test were applied between the mean at a level of $p<0.05$.

$$\% \text{ inhibition} = \frac{(\text{OD control} - \text{OD sample}) \times 100}{\text{OD control}} \qquad (1)$$

Where, OD control is the absorbance of the blank sample, and OD sample is the absorbance of samples or standard sample.

Result and Discussion

Pollen species of bee pollen were investigated form samples in different botanical resource; Both Palm (*Borussus flabellifer* L.) and Giant sensitive plant (*Mimosa diplotric-ha* C. Wright ex Suavale) from Amphoe Krasaesin, Oil palm (*Elaeis guineensis* Jacq.) from Amphoe Thapa, and Giant sensitive tree (*Mimosa pigra* L.) from Lopburee province. Mutiflora pollen bees from Amphoe Krasaesin were showed in both Palm and Giant sensitive (ration 1:20) as same as sample from Lopburee province (> 80% was Giant sensitive tree pollen bee). Oil palm from Amphoe Thapa was only monoflora pollen bee. Sizes and shapes under light microscope of pollen bees from *M. diplotricha*, *B. flabellifer*, *E. guineensis* and *M. pigra* were showed in Figure 1. The chemical contents composed of protein, lipid, fiber, moisture and ash. Bee pollen of *M. diplotricha* showed the highest protein content Brazil (Carpes et al., 2009) and bee-collected pollen in Poland (Szczêsna, 2002; 1998). Not only it it has a great deal of protein composition but it also had low lipid compositions (1.77±0.28%)

Table 1 The chemical contents of bee pollen samples.

Samples	Total protein (%)	Total Lipids (%)	Crude fiber (%)	Moisture (%)	Ash (%)
M. diplotricha	38.61±0.47	1.77±0.28	0.08±0.12	*	3.14±0.08
B.flabellifer	24.07±1.42	5.22±0.42	4.73±0.77	14.23±0.54	3.05±0.36
E.guineensis	21.86±0.45	0.92±0.22	0.91±0.48	10.84±0.24	3.96±0.08
M. pigra	29.51±0.27	1.45±0.11	0.36±0.30	10.43±0.54	5.67±0.13

* Not shown; All values are statistically different (n = 3, $p<0.05$); Data are expressed as means±SD.

Table 2 The standard antioxidant compared with all bee pollen samples.

Samples	Total protein (%)
Standard Gallic acid	0.324
Standard Butylated hydroxytoluene (BHT)	5.047
Mimosa pigra	143.861
Mimosa diplotricha	354.651
Elaeis guineensis	2,176.14
Borassus flabellifer	2,215.24

All values are statistically different (n = 3, $p<0.05$). Data are expressed as means ± SD.

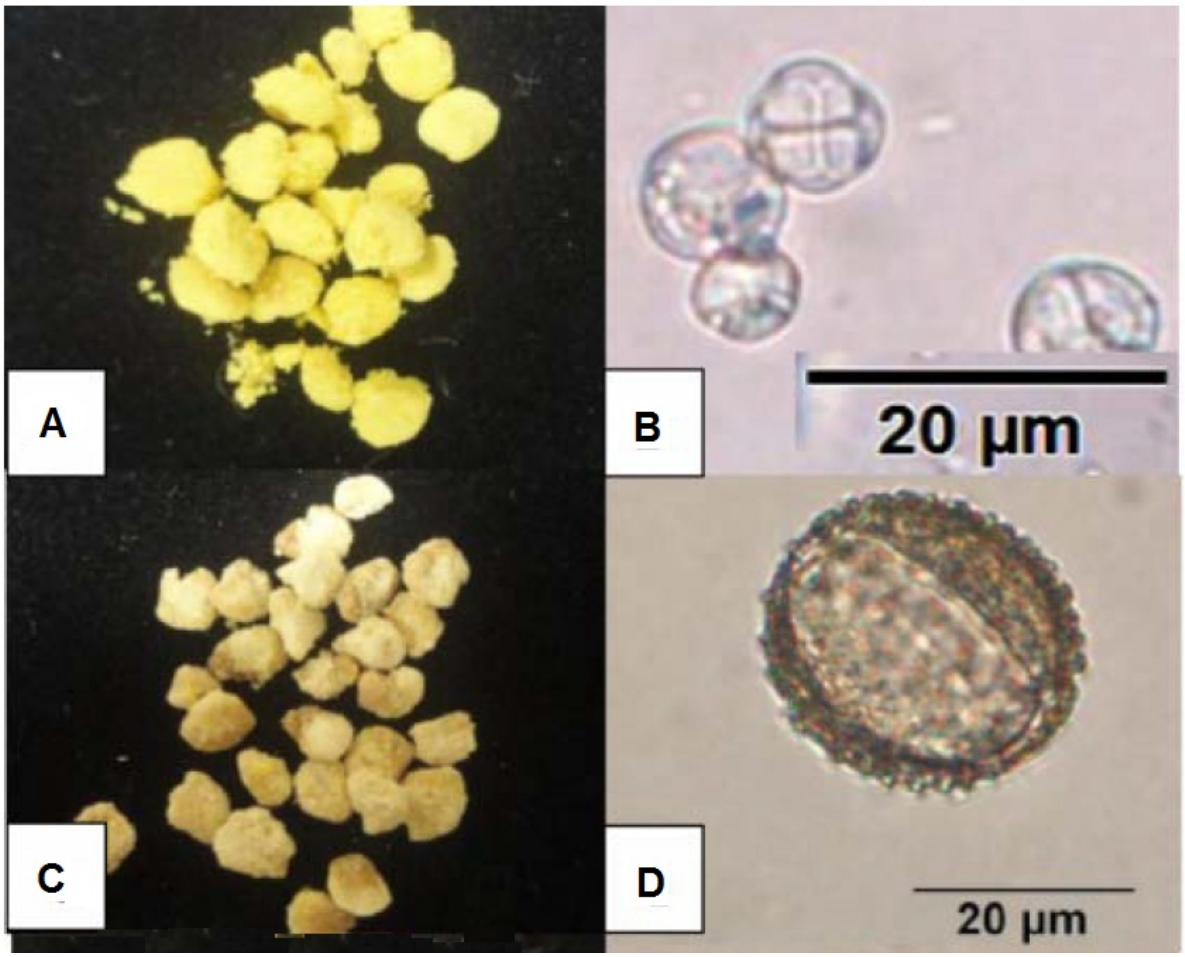

Figure 1. Morphology of four bee pollen samples. A-B: *M. diplotricha* (size 7.5x8 µM) and C-D: *B. flabellifer* (size 26.5x30.3 µM).

which raised it up to the supreme food supplement. The highest lipid contents found in *B. flabellifer* (5.22±0.42%) (Table 1); besides, we could notice unique a fat drop in a mass of bee pollen when dissolved in water that show tend to high fat product. Both of *B. flabellifer* (5.22±0.42%) and *M. diplotricha* (1.77±0.28%) displayed lipid contents accorded with the pollen standard requirement of Brazil (Campos et al., 2008). Bee pollen from *B. flabellifer* showed the highest quantity of lipid contents, crude fiber and moisture which amount of those contents exceed the pollen standard requirement of Brazil (Campos et al., 2008), especially the moisture contents were beyond the standard of bee pollen product (not more than

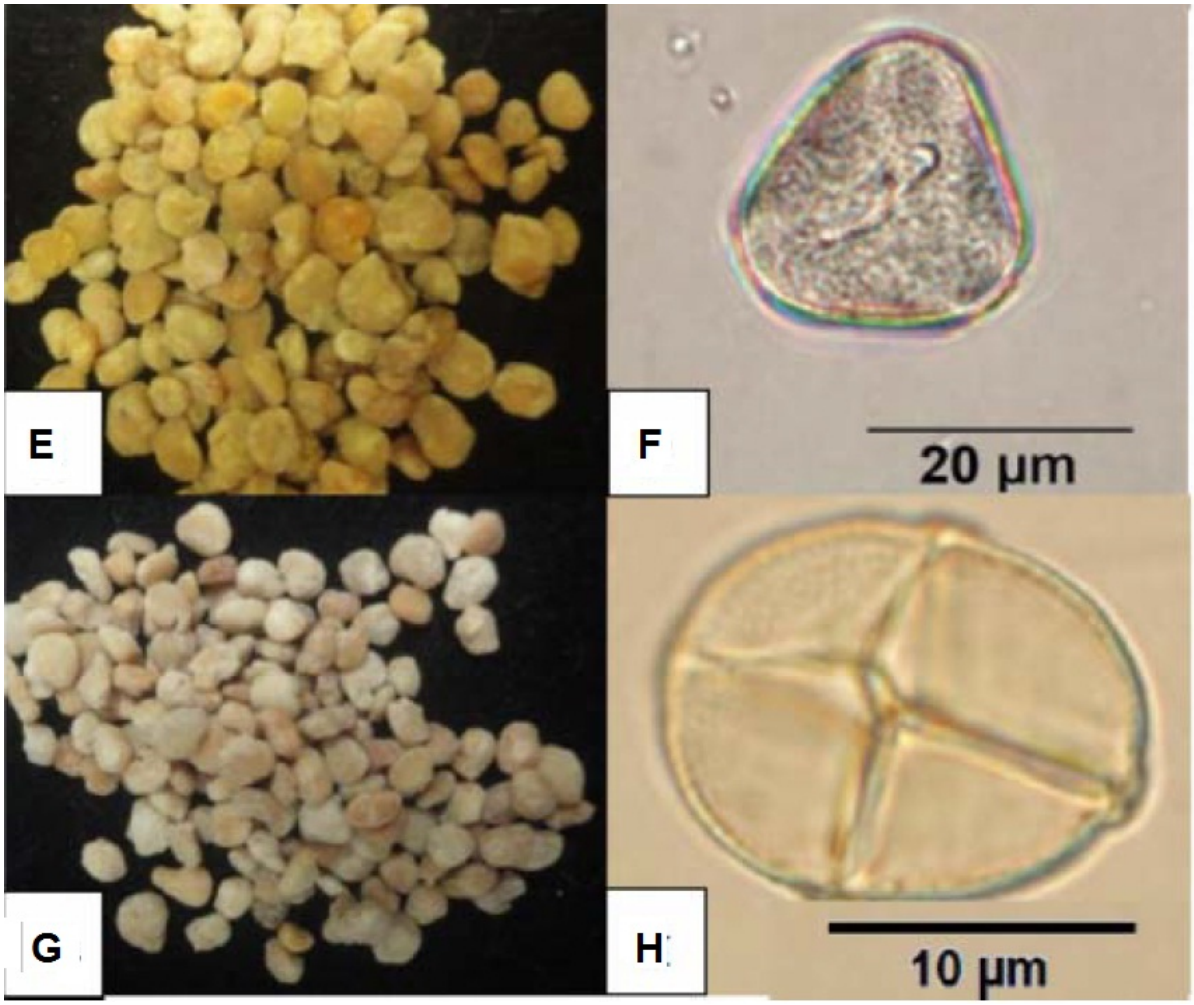

Figure 2. Morphology of four bee pollen samples. E-F: *E. guineensis* (size 20.8 x22.4 µM) and G-H: *M. pigra* (size 13.5x16.4 µM).

8%). The cause occurred in the tropical Oil Palm is economic plantation cultivated in Southern Thailand, so it was found monoflora pollen. Results of protein, lipid, crude fiber, moisture and ash contents were 21.86±0.45%, 0.92±0.22%, 0.91±0.48%, 10.84±0.24%, and 3.96±0.08 %, respectively. The work has two main objectives to determine the chemical co-mposition of pollen pellet samples of *Apis mellifera* L., including moisture, proteins, lipids, ash and to identify the floral origin of these pollen pellets in order to recognize a monofloral and mutifloral condition.

Antioxidant activity values of pollen bees were measuring by DPPH assay and showed in term of IC_{50}. We used two positive control which there were IC_{50} value 0.324 ug/mL in gallic acid that act as a natural antioxidant and 5.047 ug/mL in BHT. Scavenging activities of *M. pigra*, *M. diplotricha*, *E. guineensis* and *B. flabellifer* were 28.54-, 70.36-, 435.20- and 433-folds, respectively, compared with folds of BHT. Even though this study showed low values of beneficial natural antioxidant from sample plants, we could test the anti-radical activities with suitable methods, such as malondialdehyde (MDA) assay and thibarbituric acid-reactive-substances (TBARS) assay in further. From this study found all bee pollen sample is not suitable for using in anti-oxidant but it will be benefit to young larvae of

worker bee in high protein of *M. diplotricha*, *M. pigra* and *B.flabellifer*, which are the pre dominant species in the south of Thailand.

Conflicts of Interest

We declare that we have no conflict of interest.

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