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Macro and Micronutrient Content of Raw Propolis Collected from Different Regions in Indonesia

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ABSTRACT

The aim of this study was to determine the nutrients content of raw propolis from three regions in Indonesia including Bintan, Lampung, and South Sulawesi. Carbohydrates, protein, fat, ash, moisture, and fibers were determined using AOAC method, alongside B-complex vitamins through High-Performance Liquid Chromatography (HPLC) and several minerals with the use of inductively coupled plasma-optical emission spectrometry (ICP-OES). Additionally, the differences of nutrients content were analyzed using ANOVA followed by Duncan's post-hoc test. The result showed that Indonesian raw propolis mostly contained crude fat (38.67–61.64%), dietary fibre (45.02–58.72%), and carbohydrate (25.80–64.91%). Propolis from South Sulawesi had significantly higher protein and crude fat content than other samples ($p<0.05$). A significant amount of sodium, potassium, copper, zinc, iron, and calcium content was found in propolis from Bintan ($p<0.05$). Potassium, phosphor, manganese, magnesium, pyridoxine and folic acid were significantly found in propolis from Lampung ($p<0.05$). Our study shows that Indonesian raw propolis could be utilized in developing functional foods. However, the contents may differ between the regions.

Keywords: Indonesian propolis, macronutrient, mineral, raw propolis, vitamin

INTRODUCTION

Propolis is a resin collected by bees from the leaves and buds of diverse plant species in varying geographical locations to maintain the environmental integrity of hives (Iio *et al.* 2012; Woźniak *et al.* 2019). It has been popularly used as traditional cures for rheumatism, sprains, diabetes, anemia, alongside for dental treatments from time immemorial (Ramnath & Venkataramegowda 2016). Also, these substances have exhibited biological activities, including antioxidant, anti-inflammatory, antimicrobial, antiviral, and anti-cancerous propensities. Moreover, these components have been utilized for the generation of functional foods and beverages for the enhancement of human health (Pasupuleti *et al.* 2017; Woźniak *et al.* 2019).

Previous studies have reported that the chemical compounds of raw propolis were dependent on several factors including bee species, tree, herbaceous plant types, botanical origins, geographical location, and extraction method, as well as collecting time (Woźniak *et al.* 2019). Moreover, these dynamics were observed to affect the color, odor, and therapeutic effects of propolis. Phenols (phenolic acids, flavonoids, and their esters), carbohydrates, hydrocarbons, vitamins, amino acids and carboxylic acids were discovered to be the major bioactive compounds in propolis responsible for the medicinal qualities, along with the ability to scavenge reactive oxygen species (Ramnath *et al.* 2015; Nunes *et al.* 2018).

Several researchers had investigated the chemical constituents of propolis from Indonesia (Hasan *et al.* 2014; Fikri *et al.* 2019), India (Ramnath *et al.* 2015), Europe, Poland (Woźniak *et al.* 2019), Canada (Cottica *et al.* 2015), Mexico (Hernandez Zerate *et al.* 2018),

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and Brazil (Mello & Hubinger 2012). The results demonstrated the tremendous dependence of these compounds on the origin. Meanwhile, data regarding the nutrient content of Indonesian propolis remain limited. Besides, propolis from Bintan, Lampung and South Sulawesi are well-known propolis in Indonesia procured from the cultivation of stingless bees (*Trigona spp*) with a forest area of 200 million ha, and potential yields of 2,243 tons per 4 months or 6,729 tons per year (Mahani *et al.* 2011). In addition, research on Indonesian stingless bee propolis is still inadequate (Fikri *et al.* 2020). Therefore, the research aimed to evaluate the macro and micronutrient contents of raw propolis from these three distinctive parts in Indonesia.

METHODS

Design, location, and time

The study adopted an experimental design to evaluate the nutrient contents of raw propolis (*Trigona sp)* collected from three different regions in Indonesia. This research was conducted at Saraswanti Indo Genetech Laboratory and Chemical Laboratory of Pakuan University from March to October 2019. The samples were obtained in March 2019 and stored in the freezer at a temperature of -25^{o}C before the samples were analyzed in the laboratory. According to Bankova *et al.* (2016), raw propolis has the ability to remain frozen in an air-tight container for several years.

Materials and tools

Crude propolis was obtained from Bintan (Riau Islands), Lampung, and Luwu (South Sulawesi). The samples were packed with a sealed plastic bag and transported in a cool box with ice pack from its origin to our laboratory. The materials and tools for analysis consisted of H_2SO_4, selenium, NaOH, H_3BO_3, HCl, phenol phtalyne (PP) dan bromcresol green- methyl red (BCG-MM), thiamin, riboflavin, nicotinamide, niacin, pyridoxine, folic acid, HNO_3, internal standard yttrium 100 mg/l, phosphoric acid, and acetonitrile.

The tools used for this analysis were analytical balance, knife, board, porcelain cup, desiccator, oven, furnace, Bunsen burner, Soxhlet, boiling flask, Kjeldahl flask, volumetric flask, magnetic stirrer, microwave digestion, ICP-OES Agilent Technologies 700 Series system, HPLC Shimadzu Prominence-iLC-2030C.

Procedures

Sample preparation was done by cutting raw propolis into small fractions to analyze their macro and micronutrient contents. Macronutrient analysis was conducted independently according to the official AOAC method (AOAC 2019): moisture content by method No. 934.01; ash content by method No. 923.03; protein by Micro-Kjeldahl method No. 960.52; crude fat by Soxhlet extraction method No. 963.15; and total dietary fiber by method No. 985.29. Carbohydrate content was calculated by difference.

Determination of minerals content. The mineral content was analyzed using ICP-OES (AOAC official method 2011.14). These samples were prepared by diluting 0.5–1.0 g sample in a vessel, subsequently mixed with 10 ml of concentrated HNO_3 for 15 minutes. Therefore, the mixture was subjected to destruction in microwave digestion at 150^{o}C for 10 and 15 minutes and then cooled. Afterward, the solution was poured into a 50 ml volumetric flask and measured using ICP-OES Agilent Technologies 700 Series system.

Determination of vitamins content. Analysis of vitamins was conducted using AOAC official method: thiamin by AOAC 968.27; riboflavin by AOAC 970.65; nicotinamide and niacin by AOAC 981.16; pyridoxine by AOAC 2004.07; and folic acid AOAC 992.05. The analysis was performed using the HPLC Shimadzu Prominence-iLC-2030C equipped with C18 column using phosphoric acid-acetonitrile (99:1) as the mobile phase at a flow rate of 0.3 ml/min, and Photodiode Array (PDA) and detector at 200–300 nm wavelength.

Data analysis

Data collection was conducted with 3 replications for each sample. The data were then analyzed using Microsoft Excel 2013 and SPSS for Windows Version 16. Furthermore, the significant differences in macro and micronutrient content were determined using analysis of variance followed by Duncan's post-hoc test with confidence interval of 95% ($p<0.05$).

RESULTS AND DISCUSSION

Proximate composition

Table 1 indicates the proximate composition of raw Indonesian propolis. The results show that propolis from South Sulawesi had the highest protein and crude fat content, while the lowest dietary fibre was found in propolis from Bintan ($p<0.05$). Moreover, propolis from Lampung had the highest ash and carbohydrate content ($p<0.05$). Meanwhile, moisture content did not differ between the samples ($p>0.05$). The variation is probably attributed to the environmental conditions, geographical location and herbaceous plant species around the beehives (Hasan *et al.* 2014). In addition, harvesting method may result in carbohydrate content variation due to sugar residue from honey (Fikri *et al.* 2020).

The macronutrient content analysis showed that Indonesian propolis generally contained high amount of crude fat (38.67–61.64%), dietary fibre (45.02–58.72%), and carbohydrate (25.80–64.91%). The samples contained relatively low water (7.44–8.76%) and protein content (2.02–3.05%). In addition, the mineral quantity was possibly high because the analyses of ash content showed more than 1%. Those macronutrients can be utilized as an ingredient in developing functional food and beverages. Powdered propolis has been frequently used in making either drug development or food component (Bankova *et al.* 2016).

Mineral content

Mineral is an essential micronutrient in human metabolism, primarily as a cofactor for enzyme activity. The measurement of mineral content was performed to identify the potential of propolis as a source of minerals. Table 2 shows a great variation in the minerals content of Indonesian propolis. Sodium, potassium, copper, zinc, iron, and calcium content were significantly higher in propolis from Bintan than other samples ($p<0.05$). Moreover, potassium, phosphor, manganese and magnesium were remarkably higher in propolis from Lampung ($p<0.05$). The present results are comparable with those of previous research on nutrients content of Indonesian propolis from East Java conducted by Halim *et al.* (2012).

The information on high mineral content in raw propolis is the basis for utilization in various applications. Developing propolis in the forms of powders or pills may help to meet human body requirements. Indeed, each mineral has a special benefit in the metabolism due to its role as an enzyme cofactor in our body (Berger *et al.* 2018).

B-complex vitamins profile of Indonesian propolis

B-complex vitamins is water-soluble vitamin which our body needs. Table 3 shows the B-complex vitamins profile of the three samples of Indonesian propolis. Thiamine and riboflavin were not detected in all samples. However, nicotinamide and pyridoxine were found in all samples. Pyridoxine content in propolis from Lampung was significantly detected (99.25 mg/100 g) ($p<0.05$), where consuming 1.31 g of raw propolis from Lampung could meet 100% RDA (RDA for pyridoxine is 1.3 mg). Moreover, propolis from Lampung contained higher folic acid (8533.13 µg/100 g) compared to the other samples ($p<0.05$). Folic acid has an important role

Table 1. The proximate composition of three Indonesian propolis

Parameters	Origin of propolis		
	Bintan	Lampung	South Sulawesi
Moisture (%)	8.76±0.113[a]	8.13±0.031[a]	7.44±0.016[a]
Ash (%)	1.31±0.006[c]	3.42±0.016[a]	2.07±0.026[b]
Protein (%)	2.04±0.033[b]	2.02±0.041[b]	3.05±0.005[a]
Crude fat (%)	38.67±0.337[b]	39.38±0.13[b]	61.64±0.047[a]
Dietary fibre (%)	45.02±0.653[b]	57.66±0.032[a]	58.72± 0.472[a]
Carbohydrate (%)	49.21±0.42[b]	64.91±0.49[a]	25.80±0.04[c]

[*] All of data were expressed as means±SD; values in the same row followed by different superscript are significant at $p<0.05$ by ANOVA followed by Duncan's multiple range test

Table 2. Mineral composition of three Indonesian propolis

Parameters (mg/100 kg)	Indonesian region		
	Bintan	Lampung	South Sulawesi
Sodium	73.915±1.039[a]	28.395±0.318[b]	15.53±0.014[c]
Potassium	237.11±3.309[b]	913.92±3.734[a]	118.23±1.252[c]
Phospor	305.52±4.165[b]	1146.4±13.371[a]	146.4±2.440[c]
Copper	13.725±0.290[a]	13.17±0.042[b]	12.875±0.049[b]
Zinc	1.565±0.035[a]	1.26±0.000[b]	0.805± 0.007[c]
Iron	4.195±0.120[a]	3.255±0.064[c]	3.89±0.014[b]
Manganese	9.81±0.240[b]	23.685±0.120[a]	8.545±0.078[c]
Calcium	173.1±3.642[a]	141.12±0.311[b]	99.335±0.106[c]
Magnesium	74.36±1.216[b]	98.4±0.636[a]	39.065±0.233[c]

[*]All of data were expressed as means±SD; values in the same row followed by different superscript are significant at $p<0.05$ by ANOVA followed by Duncan's multiple range test

in lowering risks of birth defects, cardiovascular disease, and cancer (Kennedy 2016). In addition, niacin content was only found in propolis from Bintan and South Sulawesi, where niacin content in propolis from South Sulawesi is significantly higher than other samples ($p<0.05$).

Our results confirm that propolis compounds are strongly affected by location, resin souces, and bee types (Woźniak *et al.* 2019). The bees play a vital role in plant source selection. This animal only collects important components from the environment for hive protection against fungal and bacterial infections. According to Huang *et al.* (2014), *Populus* was identified as the main resin source of propolis in subtropical regions. Meanwhile, bees in tropical regions use plentiful resin sources from *Baccharis dracunculifolia*, *Clusia*, *Macaranga*, *Pinus*, *Acacia paradoxa*, *Cupressus*, and *Dalbergia*. Even though the source of resin varies between the regions, the bees seem to have a preference. *Trigona incisa* produced East Kalimantan

Table 3. B-complex vitamins profile of three Indonesian propolis

Vitamin	Origin of propolis		
	Bintan	Lampung	South Sulawesi
B_1 (Thiamin)	ND	ND	ND
B_2 (Riboflavin)	ND	ND	ND
B_3 (Nicotinamide) (mg/100 g)	7.99±0.11[b]	5.88±0.1[1c]	14.29±0.36[a]
B_3 (Niacin) (mg/100 g)	2.71±0.08[b]	ND	12.10±0.31[a]
B_6 (Pyridoxine) (mg/100 g)	2.32±0.01[c]	99.26±0.33[a]	5.67±0.04[b]
B_9 (Folic acid) (ug/100 g)	ND	8533.13±76.16	ND

[*]All of data were expressed as means±SD; values in the same row followed by different superscript are significant at $p<0.05$ by ANOVA followed by Duncan's multiple range test; ND = Not Detected

propolis from mango trees (Kustiawan *et al.* 2014). Popova *et al.* (2012), Bankova *et al.* (2014), Sanpa *et al.* (2015), and Fikri *et al.* (2020) reported that the botanical origin of propolis from Fiji, Thailand, and Indonesia is also mango plant.

CONCLUSION

The nutritent content of Indonesian propolis from Bintan, Lampung and South Sulawesi remarkably varies. Propolis from South Sulawesi had significantly higher protein and crude fat content than other samples. The dietary fibre of all propolis samples was high. Propolis from Bintan had significantly higher sodium, potassium, copper, zinc, iron, and calcium content than other samples. Moreover, potassium, phosphor, manganese and magnesium were significantly higher in propolis from Lampung. In addition, propolis from lampung had a significant amount of pyridoxine and folic acid. These findings support that the environmental and geographical location are essential for quality control of propolis.

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AUTHOR DISCLOSURES

The authors have no conflict of interest.

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