

## EVALUATION ANTIOXIDANT ACTIVITY, PHENOLIC CONTENT AND COLOUR OF INDONESIAN STINGLESS BEE HONEY AND STING BEE HONEY CULTIVATED IN INDONESIA

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Received: 8 August 2022, Revised and Accepted: 18 October 2022

## ABSTRACT

**Objective:** This study's objective was to evaluate the antioxidant activity, phenolic content, and color of Indonesian stingless and sting bee honey and to assess the correlation between antioxidant, phenolic content, and color.

**Methods:** The Indonesian bee honey sample's antioxidant activity was measured using d 2,2-diphenyl-1-picrylhydrazyl (DPPH). Folin-Ciocalteu was used to determine the phenolic content. The color was determined using a colorimeter.

**Results:** The values of antioxidant activity (IC<sub>50</sub>) ranged between 3.58±0.03 µg/ml and 64.27±0.13 µg/ml. *Heterotrigona itama* sample from South Sumatra has the highest antioxidant activity, followed by *Apis dorsata* from Bangka Belitung, *A. cerana* from North Sumatra, *Tetragonula fuscobalteata* from West Nusa Tenggara, and *Tetragonula biroi* South Sulawesi. The total phenolic content (TPC) of samples ranged from 0.0543±0.003 to 0.1760±0.002 mg GAE/g of honey. The samples *A. cerana* from North Sumatra, *T. biroi* from South Sulawesi, *A. dorsata* from Bangka Belitung, and *T. fuscobalteata* from West Nusa Tenggara presented the highest quantities of TPCs. The L\*, a\*, and b\* values ranged (3.08±0.1–56.19±1.2, 0.845±0.03–28.57±0.42, and 1.19±0.22–56.51±0.9), respectively significant correlation between antioxidant activity, color, and phenolic content, and of Indonesian bee honey.

**Conclusion:** Indonesian bee honey has a different value of antioxidant activity and phenolic content. The differences characteristic between Indonesian bee honey samples influenced by bee type (species) and its region. The dark honey has higher antioxidant activity than light honey.

**Keywords:** Antioxidant, Stingless bee honey, Sting bee honey, Phenolic, Colour.

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## INTRODUCTION

The honeybees are divided into two tribes; the first is Meliponini; the other tribe, Trigonini, is a species found throughout the tropics. Various genera represent this tribe. Honey produced by stingless bees and traditional *Apis mellifera* is the two varieties of honey now produced and marketed worldwide [9]. Honey is made by hydrolyzing sugars in plant nectar into monosaccharides glucose and fructose with invertase enzymes secreted by bees' hypopharyngeal glands [28]. Honey is a concentrated aqueous solution containing various carbohydrates such as fructose, glucose, maltose, sucrose, and other oligo- and polysaccharides [18]. Phenol compounds, amino acids, vitamins, minerals, enzymes, and organic acids are among the more than 180 chemicals in honey [2]. Honey is recognized as a food source and a medicinal by current and historical generations, customs, and civilizations. Islam recommended honey and was given its chapter in the holy book, the Holy Quran [8]. It is well known that honey benefits the human body due to its high concentration of bioactive chemicals [14]. Antioxidant substances such as flavonoids and phenolic, organic acids, carotenoids, proteins, amino acids, and enzymes are responsible for honey's health benefits [10]. Honey is a well-known functional food with medical implications. Antioxidant capacity is primarily responsible for its functional qualities [29]. Honey's main therapeutic benefit is its antioxidant activity [1]. Honey is used as a natural food supplement; honey has a variety of medical and health benefits. It has been proven to be a promising therapeutic antioxidant agent for various biodiverse diseases [3]. Natural honey's scavenging and redox capabilities have been linked to its abundant bioactive components such as phenolic acids, flavonoids, vitamins, and enzymes. However, the biological content of honey and the total antioxidant capacity is determined by the geographical and botanical areas and the type of bees [26]. Honey's composition and bioactive compounds largely depend on floral source, geographical location, and seasonal and environmental conditions [20].

Honey's antioxidant properties protect the body from the damaging effects of free radicals [6]. They serve as scavengers of free radicals, peroxy radicals, and metal chelators [11]. Honey's antioxidants are flavonoids, mono phenolics, polyphenolics, and Vitamin C. Honey includes aqueous and lipophilic antioxidants, allowing it to function as an optimal natural antioxidant at several cellular levels. By protecting antioxidant enzymes and reducing oxidative stress, this activity reduces cellular damage caused by free radicals; as a result, the inflammatory process is reduced. Honey can boost antioxidant levels in the plasma when taken orally [31]. Hydrogen peroxide, superoxide anion, singlet oxygen, and hydroxyl radicals are reactive oxygen species and contain pro-oxidant properties that harm cellular lipids, proteins, and DNA. Antioxidants decrease the adverse effects of free radicals by inhibiting their creation or eliminating them [21]. Antioxidants are helpful for more than just scavenging free radicals. They have the potential to alter signal transduction pathways that are influenced by free radicals through oxidative stress and are involved in cellular responses (e.g., inflammation, survival, proliferation, and death) in various diseases [4].

There are five species of native honey bees and at least 46 species of stingless bees in Indonesia, with honey bees and stingless bees being the most diverse. The stingless bee's range is relatively extensive, encompassing all of Indonesia. The country's vast geographic extent, diversified topological and environmental terrain, and complex geological history may all contribute to Indonesia's high species richness and unique distribution of bees [17]. Honey is a chemically varied substance whose composition is heavily influenced by the kind and species of plant from which the bees collect nectar [32]. Honey's content varies widely and is mainly determined by the floral source; however, seasonal and environmental conditions can also influence its composition and biological effects [5]. The color of bee honey varies from colony to colony and depends on the plants from which they collect nectar [7]. The biological content of honey and the total

antioxidant capacity is determined by the geographical and botanical areas and the type of bees [26]. This finding emphasizes the significance of understanding the biological properties of Indonesian stingless bee honey varieties from various states to understand the differences in phytochemical components, particularly antioxidant activity. The variance in these compounds may influence the choice of honey for various medical uses [12]. This study's objective was to evaluate the color, phenolic content, and antioxidant activity of Indonesian stingless and sting bee honey and to assess the correlation between antioxidant, phenolic content, and color.

**MATERIALS AND METHODS**

**Materials**

*Sample collection*

In this study, eighteen Indonesian stingless and sting honey samples were collected from beekeepers from different geographic regions in Indonesia (Table 1 and Fig. 1). Honey samples were obtained over 1 month, from December 2021 until January 2022. The honey samples were preserved in bottles and tightly closed, then kept in the dark at

room temperature (27±2°C) before analyses were performed. All honey samples were analyzed in duplicate.

**Methods**

*Determination of the antioxidant activity*

DPPH radical scavenging assay measures the antioxidant scavenging ability of 2,2-diphenyl-1-picrylhydrazyl [15]. 1 g of honey was dissolved in methanol added up to 25 ml, and 0.5 mL of a DPPH solution (160 PPM) was combined with 0.125–2 mL of this solution; for 30 min, the sample solution and a DPPH solution (blank) were kept in the dark at room temperature. A spectrophotometer was used to measure the absorbance of the solutions at 517 nm. The sample concentration providing 50% of the half maximal inhibitory concentration (IC50) was calculated by interpolation from the graph of radical scavenging activity percentage against sample concentration.

$$RSA = \frac{Absorbance\ DPPH - Absorbance\ sample}{Absorbance\ DPPH} \times 100$$

*Total Phenolic Content (TPC)*

Folin-Ciocalteu (FC) was used to determine the phenols in the samples [22]. As a stock solution, 5 mg of gallic acid (200 PPM) reagent was produced in distilled water. The calibration curve was performed by taking 0, 0.2, 0.4, 0.6, 0.8, 1, and 1.2 ml of gallic acid solution dissolved in 0.5 FC reagent and 2.5 ml of Na2CO3, then completing the volume to 25 ml of distilled water as a standard reference. Incubated in darkness at ambient temperature to 40 min, then read absorbance. Honey samples were diluted in methanol, then combined with 0.5 mL FC reagent and 2.5 mL Na2CO3 solution, completing the volume to 25 ml with distilled water. A spectrophotometer measured the absorbance at 725 nm after 40 min at room temperature in the dark. Gallic acid was used for a calibration curve (Fig. 2) and the results will be presented as mg GAE/100 g honey.

*Color*

The color was determined using a colorimeter; the system the International Commission on Illumination\*a\*b\* and D 65 illuminate (L\*- lightness, a\*- psychometric tone indicating red, and b\*- psychometric chroma indicating yellow) [27]. The measurement was conducted through the measuring pan. 5 g of each bee honey sample is placed in the supplied space in the glass cell, and corresponding values are read directly on display. Duplicates were obtained and the average was reported.

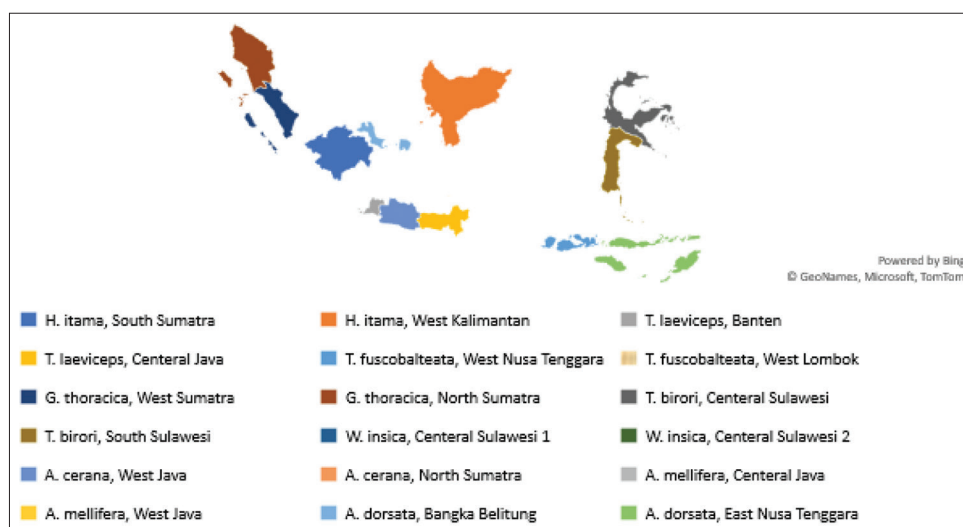
**Statistical analysis**

Average and standard deviation of data have been calculated. One-way ANOVA with Duncan's MRT new Multiple Range Test and LSD

**Table 1: Sources of bee honey samples collected from regions in Indonesia**

Type	Code	Species, Region
Stingless bee honey	H1	<i>H. itama</i> South Sumatra
	H2	<i>H. itama</i> , West Kalimantan
	H3	<i>T. laeviceps</i> , Banten
	H4	<i>T. laeviceps</i> , Central Java
	H5	<i>T. fuscobalteata</i> , West Nusa Tenggara
	H6	<i>T. fuscobalteata</i> , West Lobak
	H7	<i>G. thoracica</i> , West Sumatra
	H8	<i>G. thoracica</i> , North Sumatra
	H9	<i>T. biror</i> ), Central Sulawesi
	H10	<i>T. biror</i> ), South Sulawesi
Sting bee honey	H11	<i>W. insica</i> , Central Sulawesi 1
	H12	<i>W. insica</i> , Central Sulawesi 2
	H13	<i>A. cerana</i> , West Java
	H14	<i>A. cerana</i> , North Sumarta
	H15	<i>A. mellifera</i> , Central Java
	H16	<i>A. mellifera</i> , West Java
	H17	<i>A. dorsata</i> , Bangka Belitung
	H18	<i>A. dorsata</i> , East Nusa Tenggara

*Heterotrigona itama*: *H. itama*, *Tetragonula Moure laeviceps*: *T. laeviceps*: *Tetragonula Moure fuscobalteata*: *T. fuscobalteata*, *Geniotrigona thoracica*: *G. thoracica*, *Tetragonula Moure biro*: *T. biror*, *Wallacetrigona insica*: *W. insica*, *Apis cerana*: *A. cerana*, *Apis mellifera*: *A. mellifera*, *Apis dorsata*: *A. dorsata*



**Fig. 1: Location of Indonesian bee honey**

Least Significant Difference, Pearson's correlation coefficient (r) at a significant p=0.05 was used to evaluate the correlation between antioxidant activity, phenolic, and color of honey was calculated using Microsoft Office Excel and SPSS.

**RESULTS AND DISCUSSION**

**Antioxidant activity**

The results of the antioxidant activity analysis of stingless bee honey and sting honey were obtained using a spectrophotometer and different values of (IC50) were obtained in Table 2 and Fig. 3. The composition of honey changes depending on the source of the raw material (nectar or honeydew), the bee species, the edaphoclimatic conditions, the available floral supply, and the storage circumstances [13]. The antioxidant activity increases when the value of IC50 decreases but decreases when the value of IC50 increases.

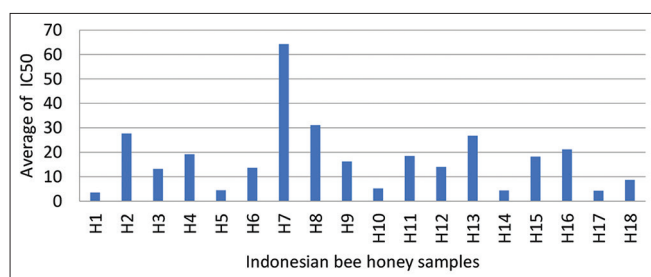


Fig. 2: Standard curve phenolic

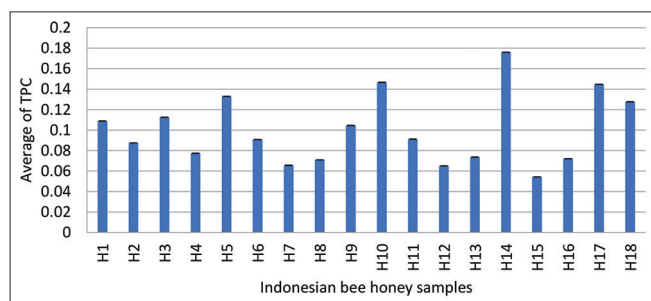


Fig. 3: Average antioxidant activity

Table 2 shows statistically significant differences in antioxidants. The values of antioxidant activity (IC50) ranged between 3.58±0.03 µg/ml and 64.27±0.13 µg/ml. *H. itama* sample from South Sumatra has the highest antioxidant activity (3.5888±0.03µg/ml), followed by *Apis dorsata* from Bangka Belitung (4.3179±0.04), *A. cerana* from North Sumatra (4.4349±0.21), *Tetragonula fuscobalteata* from West Nusa Tenggara (4.5493±0.07), and *Tetragonula biroi* from South Sulawesi (5.2248±0.005). Honey species derived from various floral sources offer potent antioxidative properties and function as scavengers for active oxygen species [23].

**TPC**

The results of the total phenolic analysis of stingless and sting bee honey were obtained using a spectrophotometer with different results shown in Table 2 and Fig. 4. The TPC of samples ranged from 0.0543±0.003 to 0.1760±0.002 mg GAE/g of honey. These figures are related to the botanical origin and the species of the honey-producing bees. The honey color is influenced by bee subspecies, nectar source, hive quality, honey age, and storage [25]. The samples H5, H10, H14, H17, and H18 presented the highest quantities of TPCs and the lowest TPCs were observed for the honey H7, H12, and H15; these results showed statistically different values. A study by Scripcă et al. [27] showed that the total polyphenols content of honey from Romania was less significant for acacia (0.08 mg/100 g) and rape honey (0.14 mg/100 g). In another study by da Silva et al., the TPC of the honey samples from Brazil ranged from 17 to 66 mg GAE/g. A study by Idris et al. [16] showed that the TPC of Sudanese honey samples ranged from 4.44 to 201.08 mg GAE/100 g. The results showed significant differences in TPC between Indonesian honey samples in both type and region.

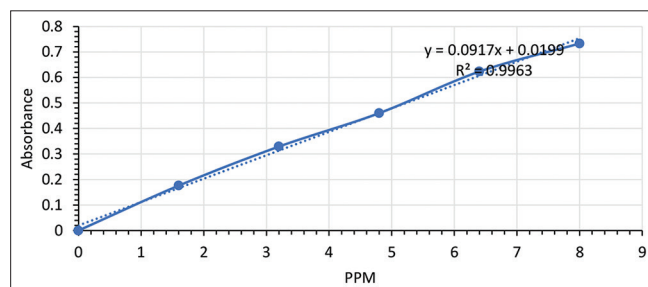


Fig. 4: Average of TPC

Table 2: Average antioxidant activity, total phenolic content, and color of Indonesian bee

Code	Antioxidant Activity (IC50 µg/ml)	TPC mg GAE/g of honey	Colour			Visual Color
			L*(D65)	b*(D65)	a*(D65)	
H1	3.5888±0.03 <sup>a</sup>	0.1089±0.003 <sup>def</sup>	10.59±1.53 <sup>b</sup>	14.935±1.63 <sup>c</sup>	10.84±1.64 <sup>b</sup>	
H2	27.7556±0.17 <sup>i</sup>	0.0874±0.003 <sup>bcd</sup>	40.26±2.0 <sup>i</sup>	16.765±1.93 <sup>c</sup>	46.39±4.9 <sup>shi</sup>	
H3	13.2600±0.99 <sup>d</sup>	0.1125±0.006 <sup>efg</sup>	31.605±2.65 <sup>fg</sup>	19.54±0.15 <sup>de</sup>	43.2±5.1 <sup>figh</sup>	
H4	19.1967±0.06 <sup>f</sup>	0.0775±0.006 <sup>abc</sup>	24.275±3.33 <sup>de</sup>	21.825±0.71 <sup>efg</sup>	33.15±4.9 <sup>de</sup>	
H5	4.5493±0.07 <sup>b</sup>	0.1330±0.006 <sup>gh</sup>	28.97±3.45 <sup>ef</sup>	22.72±0.13 <sup>fg</sup>	36.48±6.68 <sup>ef</sup>	
H6	13.6930±0.21 <sup>d</sup>	0.0908±0.001 <sup>cde</sup>	38.92±2.46 <sup>hi</sup>	21.06±1.82 <sup>ef</sup>	54.575±5.01 <sup>ij</sup>	
H7	64.2793±0.12 <sup>k</sup>	0.0655±0.003 <sup>ab</sup>	56.19±1.2 <sup>j</sup>	1.775±0.64 <sup>a</sup>	56.51±0.9 <sup>j</sup>	
H8	31.1717±1.03 <sup>j</sup>	0.0709±0.001 <sup>abc</sup>	11.105±3.1 <sup>b</sup>	17.255±1.8 <sup>cd</sup>	10.695±3.0 <sup>b</sup>	
H9	16.2469±0.37 <sup>e</sup>	0.1045±0.003 <sup>def</sup>	20.165±3.0 <sup>cd</sup>	24.05±2.4 <sup>g</sup>	21.495±5.1 <sup>c</sup>	
H10	5.2248±0.005 <sup>b</sup>	0.1466±0.00 <sup>h</sup>	17.87±0.45 <sup>c</sup>	11.335±0.07 <sup>b</sup>	20.555±0.9 <sup>c</sup>	
H11	18.5968±0.03 <sup>f</sup>	0.0911±0.001 <sup>cde</sup>	28.925±2.34 <sup>ef</sup>	20.76±0.25 <sup>ef</sup>	38.4±3.5 <sup>ef</sup>	
H12	14.0380±0.13 <sup>d</sup>	0.0650±0.003 <sup>ab</sup>	28.85±2.35 <sup>ef</sup>	21.52±0.25 <sup>efg</sup>	37.945±2.9 <sup>efg</sup>	
H13	26.8146±0.39 <sup>h</sup>	0.0736±0.00 <sup>abc</sup>	33.135±3.0 <sup>fg</sup>	21.945±1.23 <sup>efg</sup>	41.775±2.2 <sup>figh</sup>	
H14	4.4349±0.21 <sup>ab</sup>	0.1760±0.002 <sup>i</sup>	34.86±1.8 <sup>gh</sup>	17.165±0.01 <sup>cd</sup>	45.245±2.2 <sup>gh</sup>	
H15	18.3232±0.62 <sup>f</sup>	0.0543±0.003 <sup>a</sup>	31.75±1.6 <sup>fg</sup>	28.485±1.83 <sup>h</sup>	48.55±2.4 <sup>hij</sup>	
H16	21.2301±0.08 <sup>g</sup>	0.0721±0.00 <sup>abc</sup>	34.97±1.1 <sup>gh</sup>	20.56±0.51 <sup>ef</sup>	48.96±1.7 <sup>hij</sup>	
H17	4.3179±0.04 <sup>ab</sup>	0.1446±0.04 <sup>h</sup>	3.08±0.1 <sup>a</sup>	0.845±0.03 <sup>a</sup>	1.19±0.22 <sup>a</sup>	
H18	8.7509±0.19 <sup>c</sup>	0.1276±0.00 <sup>figh</sup>	20.165±3.1 <sup>cd</sup>	28.57±0.42 <sup>h</sup>	27.67±3.7 <sup>cd</sup>	
Total	17.52±14.27	0.1001±0.03	27.54±12.44	18.4±7.45	34.65±15.99	

They were not significantly different (p>0.05) among honey samples with the same letters, and very significant difference (p<0.001) among honey samples with different letters

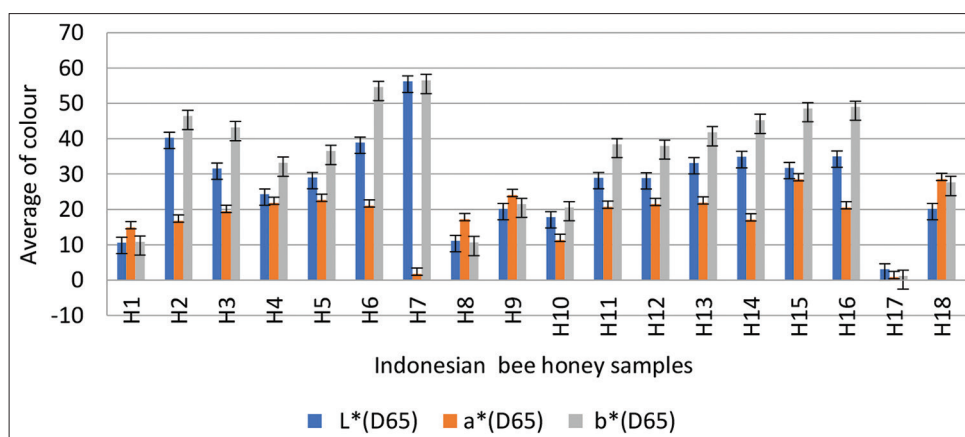


Fig. 5: Average of color

Table 3: The correlation between antioxidant activity, phenolic content, and color of Indonesian bee honey samples

Parameter	IC50	TPC	L*(D65)	b*(D65)	a*(D65)
IC50	1	-0.631**	0.611**	0.422**	-0.293*
TPC	-0.631**	1	-0.344*	-0.349*	-0.222
L*(D65)	0.611**	-0.344*	1	0.948**	0.045
b*(D65)	0.422**	-0.349*	0.948**	1	0.257
a*(D65)	-0.293*	-0.222	0.045	0.257	1

\*\*The correlation is significant at the 0.01 level

### Color

The results of color analysis of Indonesian bee honey were obtained using a colorimeter, and different values of L\*, a\*, and b\* were obtained. The average value of the analysis results is shown in Table 2 and Fig. 5. The L\*, a\*, and b\* values ranged (3.08±0.1–56.19±1.2, 0.845±0.03–28.57±0.42, and 1.19±0.22–56.51±0.9), respectively. Honey samples are divided into two categories based on their lightness value: Light honey with L\* > 50 and dark honey with L\* ≤ 50 [24]. The sample *G. thoracica*, West Sumatra, has the highest average L\* value (56.19±1.2; by visual comparison, it was also discovered to be the lightest, but the L\* value decreased further in *A. dorsata*, Bangka Belitung honey samples (3.08±0.1). *H. itama* honey sample from West Kalimantan has a high L\* value (40.26±2.0) compared with the *H. itama* honey sample from South Sumatra (10.59±1.53). The sample *A. dorsata* from Bangka Belitung has L\* value less than *A. dorsata* from East Nusa Tenggara. Considering this, the color of honey samples varies depending on the origin and species. A study by Wilczyńska and Ruskowska [30] showed that the L\*, a\*, and b\* values in samples of honey ranged from 20, 41–29, 22, 1, 67–5, 67 to 2, 43–16, 26, respectively.

The relationship is significant at level 0.05.

Table 3 shows the correlation between antioxidant activity, TPC, and the color of Indonesian bee honey. IC50 showed a strong positive correlation with L\* (r=0.611), which can be explained that a lower L\* will lead to lower IC50 in honey. A low value of IC50 means higher antioxidant activity; it can be explained that dark honey has higher antioxidant activity than light honey. Moderately positive correlation with b\* (r=0.422), strong negative correlation with TPC (r=-0.611). It can be explained that samples of Indonesian bee honey with higher TPC will lead to lower IC50 in honey, and a lower value of IC50 means high antioxidant activity. L\* showed a strong positive correlation with b (r=0.948).

### CONCLUSION

Based on the results, Indonesian bee honey has different antioxidant activity and phenolic content values. The differences characteristic between Indonesian bee honey samples influenced by bee type (species) and its region. Sting bee honey has antioxidant activity and

TPC higher than stingless bee honey. Significant correlation between antioxidant activity, phenolic content, and color of Indonesian bee honey that dark honey has higher antioxidant activity than light honey. Indonesian bee honey samples with higher TPC have a lower value of IC50 which means high antioxidant activity. Samples H14, H10, H17, H5, and H1, which contained the highest phenolics, are the best-value antioxidants and should be more widely consumed.

### ACKNOWLEDGMENTS

The authors thank the Ministry of Research and Technology and the Universitas Padjadjaran for their support in this publication.

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