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The Flavonoid Content of Bajakah Honey in Peatlands and Uplands in Kalimantan, Indonesia

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Abstract: The bajakah plant is a type of plant used by the Dayak ethnic community in Central Kalimantan. This plant is used to treat various diseases, especially cancer, and degenerative and supplements to increase stamina. The results of previous research found that bajakah contains phenolics, flavonoids, tannins, and saponins. The research also showed that red bajakah (Uncaria acida) revealed high flavonoid content in its stems, leaves, and twigs. Trigona honey (Heterotrigona itama) also contains high levels of flavonoids. This study aimed to determine whether a mixture of Bajakah stem extract and trigona honey from peatlands and uplands could increase the flavonoid content. The results showed that the flavonoid content increased with increasing red bajakah stem extract concentration with trigona honey from the peatlands and uplands. The highest results were obtained with a mixture of 0.225 mg of bajakah extract and 15 ml of trigona honey from peatlands with a flavonoid content of 29.580 mg/ml and a concentration of 0.150 mg/ml of bajakah extract and 15 ml of trigona upland honey, producing flavonoid content of 32.580 mg/ml.

Keywords: bajakah, trigona honey, peatland, upland, flavonoid.

印度尼西亞加里曼丹泥炭地和高地巴賈卡蜂蜜的類黃酮含量

摘要：海盜植物是加里曼丹中部達雅族社區使用的一種植物。這種植物用於治療各種疾病，尤其是癌症，以及退行性疾病和增強體力的補充劑。之前的研究結果發現，海盜含有酚類物質、黃酮類物質、單寧酸和皂甙。研究還表明，紅色海盜(鉤藤酸)在其莖、葉和細枝中顯示出高類黃酮含量。特里戈納蜂蜜(異三角藻)還含有高含量的類黃酮。本研究旨在確定海盜莖提取物與來自泥炭地和高地的特里戈納蜂蜜的混合物是否可以增加類黃酮含量。結果表明，泥炭地和高地的三葉草蜂蜜中的黃酮類化合物含量隨著紅色海盜莖提取物濃度的增加而增加。使用0.225毫克海盜提取物和15毫升來自泥炭地的特里戈納蜂蜜的混合物獲得了最高的結果，其中類黃酮含量為29.580毫克/毫升，濃度為0.150毫克/毫升的海盜提取物和15毫升特里戈納陸地蜂蜜，產生的類黃酮含量為 32.580毫克/毫升。

关键词：海盜，三角洲蜂蜜、泥炭地、高地、類黃酮。

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1. Introduction

Indonesia has various types of plants and is one of the mega-biodiversity countries in the world. Indonesia also has the second largest tropical forest in the world and has more than 20,000 species of medicinal plants, but until now, only 1,000 species have been recorded and 300 species are used for traditional medicine [1]. Generally, people in Indonesia have a habit of using traditional medicine as an alternative medicine to treat various diseases. These plants contain chemical compounds known as secondary metabolites.

Bajakah plants are a type of plants that are used by the Dayak people who live around the forest, as medicinal plants for various diseases and supplements to restore stamina. Based on the test conducted by [2] that Bajakah kampala contains phenolics, flavonoids, tannins and saponins. According to [3], in general, red and yellow bajakah on stems, leaves, and twigs contain high levels of flavonoids. This shows that in general, bajakah can be effective for treating various diseases, especially cancer. [4] also stated that bajakah kampala can treat cancer. [5] stated that the extract of Bajakah kampala stem contained phenolic content of 12.33 mg GAE/g. Bajakah kampala has also been shown to accelerate wound healing [6]. Bajakah belongs to the category of the genus Spatholobus and includes hairy plants on woody trees and the Phaseoleae tribe.

Honey is one of the non-timber forest products whose benefits are countless, including as a health supplement and stamina. One type of honey that is widely cultivated by the community is the trigona bee (*Heterotrigona itama*) or kelulut bee. This type of bee is also classified as a stingless bee. Honey also contains micronutrients [7], so that honey is becoming popular as a supplement for maintaining health and stamina. Several studies have shown that honey from stingless bees has higher antioxidant activity than honey from *Apis* sp. [8, 9], so it has hepatoprotective and cardioprotective effects [10]. Honey is more trigona also has high antioxidant content because it has a high total phenolic [11].

Cultivation of trigona honey can be done in wetlands, especially in the peatland areas and in the highlands. The difference in the location of trigona bee cultivation will certainly result in different types of plants as feed for bees and result in differences in the content of secondary metabolites or phytochemicals. The mineral content, pollen, and phenolic content of honey influence the colour and taste of trigona honey [12]. Additionally, geographical origin and types of nectar-producing plants around beekeeping influence the colour. The results of the study show that darker colors have higher phenolic and flavonoid content than lighter coloured honeys [13-15].

In this study, we wanted to determine how the flavonoid content by mixing red bajakah extracts with various concentrations with trigona or kelulut honey, so that this combination resulted in the highest flavonoid content. Additionally, it will also be known that trigona honey cultivated in the peatlands or uplands has the highest flavonoid content when combined with red bajakah (*Uncaria acida*).

2. Materials and Methods

2.1. Field of Research and Sample Collections

Bajakah generally lived in primary and secondary forests with a very variable microclimate, with 29-31°C, the humidity of 75-81%, and light intensity of 20% [3]. The red Bajakah samples in this study were obtained from the peat-swamp forest area in Pulang Pisau Regency, Central Kalimantan. The Dayak ethnic community used this type of bajakah to treat various degenerative diseases and cancer, as well as to maintain body stamina.

The trigona honey used was taken from the results of trigona bee cultivation from the wetlands area in Barito Kuala Regency and the highlands in the Tanjung Regency area. Both regions are in South Kalimantan, Indonesia.

The location where the plant is collected is shown in Fig. 1.

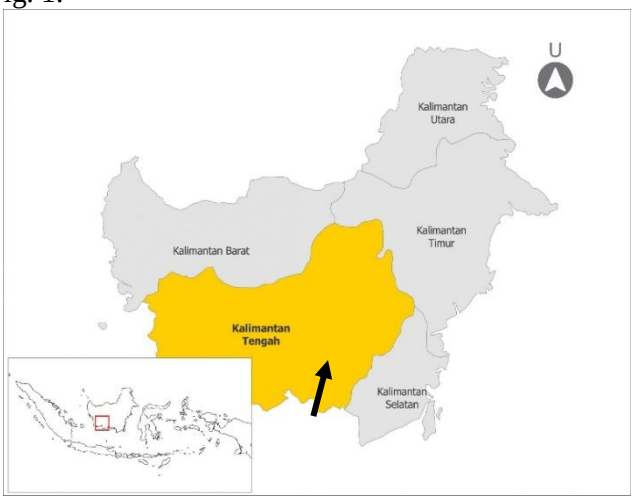

Fig. 1 Bajakah plant sampling locations

2.2. Preparation of Extracts and Flavonoids

The material used was the stem and bark of a red bajakah. It was cleaned with water (preferably with water flow) and then dried. The dried materials were put in a plastic bag so that the sample is maintained fresh. The materials were cut to become small, and it was made powder using a commercial blender, after that it was done extract. Red pirated extracts of various concentrations, namely, 0 g, 0.075 g, 0.150 g, 0.225 g, and 0.300 g, were mixed with 15 ml of trigona honey obtained from the peatlands and uplands.

Quantitative phytochemical testing was performed using Gravimetry. The working procedure for determining each parameter is as follows. In a quantitative flavonoid test, the sample was macerated for 24 h with 80% methanol. 80% methanol is used for maceration because 80% methanol is the best and most commonly used solvent for flavonoid extraction. The

extract was filtered, and the solvent was evaporated to dryness, so a dry solid of flavonoids was obtained. The flavonoid solids were weighed.

3. Results

Different sources of nectar will make honey to have a different composition, taste, aroma, and physical appearance [7]. Additionally, external factors such as geographic location, plant vegetation, climate, air temperature and humidity, topography, and bee food sources (from nectar) also affect the characteristics of honey [16, 17]. Likewise, what happens due to the difference in growing places between peatlands and uplands, where in these two growing places will cause different types of plants that feed trigona bees.

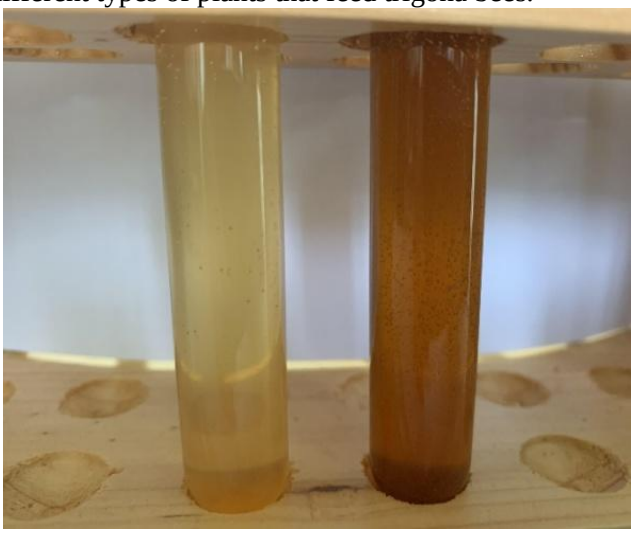

Fig. 2 Honey trigona samples from peatlands (left) and uplands (right)

The flavonoid content in trigona honey is quite high, namely, 10 mg/ml for the peatland areas and 20 mg/ml for the upland areas. The tendency of this difference is due to the different types of bee feed between the two growing places. The geographical origin and feed of the trigona honey bee influence the colour of honey. Trigona honey produces nectar around the farm area and until now there is no standard for its colour [18]. Some consumers prefer the colour of trigona honey, which is dark in colour. Consumers choose honey based on the knowledge that is generally applicable in the community, not based on scientific studies.

The results showed that darker honey had higher phenolic and flavonoid content than lighter coloured honey [13, 14, and 15]. But the results are still on the type of honey from stinging bees. The acidity of honey from the stingless bee (*Geniotrigona thoracica*) in Malaysia was 281.98 meq/kg [19]. The source of bee feed affects the colour, acidity, and sugar content of honey [20]. Honey with a light colour has a higher acidity [18].

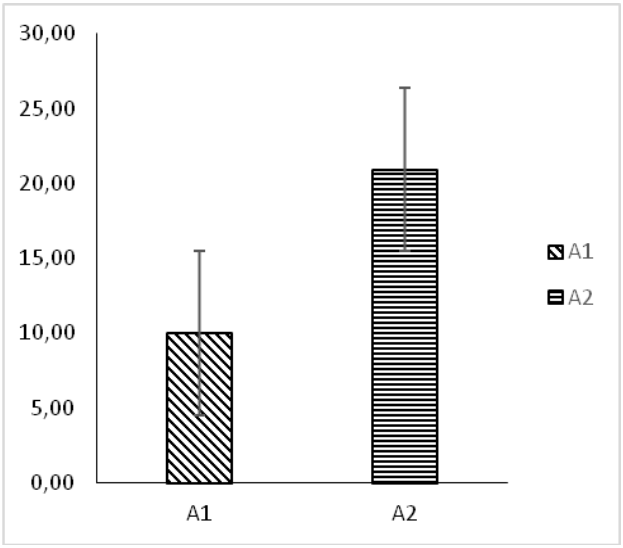

Fig. 3 Differences in flavonoid content in wetlands and uplands
Notes: A1 - trigona honey from peatlands; A2 - trigona honey from uplands

According to [21], the total flavonoid content is between 5.12–19.40 mg QE/100 g. [22] also reported the total flavonoid content of four types of Malaysian honey with a value of 14.20–156.82 mg CE/kg. This means that the levels of flavonoids in calliandra honey, rubber honey and randu honey in research in Malaysia are much higher than the flavonoid levels in honey in various countries including Indonesia. The feed factor of the bees greatly affects the flavonoid content produced.

A high water content and acidity are characteristics of trigona honey [23]. Improving the quality of trigona honey so that it meets SNI standards and suitable for marketing by reducing the water content, one of which is using a *dehumidifier* [24]. The water content of trigona honey can be reduced by up to 21% [25].

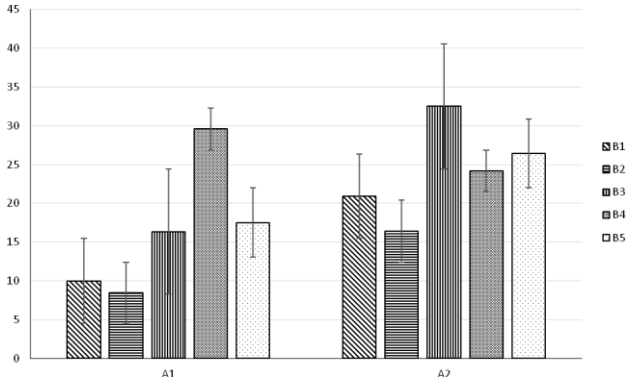

Fig. 4 Differences in flavonoid content in bajakah honey
Notes: A1 - trigona honey from wetlands; A2 - trigona honey from uplands; B1 - extracts of bajakah stem of 0 g; B2 - extracts of bajakah stem of 0.075 g; B3 - extracts of bajakah stem of 0.150 g; B4 - extracts of bajakah stem of 0.225 g; B5 - extracts of bajakah stem of 0.300 g

The test results on a mixture of bajakah honey and trigona honey collected in different places, namely, wetlands and uplands and mixed with different concentrations of bajakah extract (0 g, 0.075 g, 0.150 g, 0.225 g, and 0.300 g) showed differences in the content

of flavonoids. A tendency to increase the content of flavonoids with increasing concentrations of bajakah exists, but the highest content was found in a mixture of 15 ml and 0.225 mg trigona wetland honey at 29.580 mg/ml and trigona upland honey 15 ml and 0.150 mg at 32.580 mg/ml. However, at concentrations of Bajakah extract of more than 0.225 mg in trigona honey, there was a decrease in the content of flavonoids, as well as a decrease in trigona upland honey mixture with more than 0.150 mg.

Phenolic compounds and flavonoids are compounds are generally known as antioxidant compounds. According to [26], in honey, more than 150 polyphenolic compounds contain flavonoids, phenolic acids, catechins, and cinnamic acid derivatives, which are compounds that function as antioxidants. Flavonoid compounds are the largest group in phenolic compounds. [27] reported that the antioxidant activity in honey was mainly due to these two compounds because there was a strong correlation between the antioxidant activity with phenolic and flavonoid compounds.

Table 1 Results of the analysis of variance analysis of trigona honey mixture and the concentration of bajakah

sd	df	ns	ms	F (count)	F Table	
					5%	1%
Treatment	9	1721.00	191.22	2.85	2.39*	3.46
A	1	446.60	446.60	6.65	4.35*	8.1
B	4	890.93	222.73	3.32	2.87*	4.43
AB	4	383.47	95.87	1.43	2.87	4.43
Error	20	1343.04	67.15			
Total	29	3064.04	105.66			

A significant difference at the 95% level in trigona peatlands honey and uplands trigona honey, as well as at different concentrations of bajakah (0 g, 0.075 g, 0.150 g, 0.225 g, and 0.300 g) exists. However, there was no significant difference when trigona honey was mixed with various concentrations of bajakah.

Table 2 Results of the DUNCAN difference test

Treatment	Middle Value	Different Value									
		A2B3	A1B4	A2B5	A2B4	A2B1	A1B5	A2B2	A1B3	A1B1	A1B2
A2B3	32.50										
A1B4	29.58	2.92									
A2B5	26.42	6.08	3.17								
A2B4	24.17	8.33	5.42	2.25							
A2B1	20.92	11.58	8.67	5.5	3.25						
A1B5	17.50	15*	12.08	8.92	6.67**	3.42*					
A2B2	16.42	16.08*	13.17	10	7.75**	4.5**	1.08				
A1B3	16.33	16.17*	13.25	10.08	7.83**	4.58**	1.17	0.08			
A1B1	10.00	22.5**	19.58*	16.42*	14.17**	10.92**	7.5**	6.42**	6.33**		
A1B2	8.42	24.08**	21.17**	18**	15.75**	12.5**	9.08**	8**	7.92**	1.58	

To get the honey with the highest flavonoid content, A2B3 honey obtained from cultivation in the upland area with a concentration of 0.150 mg piracy is 32,500 mg/ml. However, if using trigona honey from the peatlands, the concentration of bajakah used was 0.225 g with flavonoid content of 29.580 mg/ml.

Table 3 Results of the B (bajakah stem extract) factor test

Factor	Middle Value	Different Value				
		B4	B3	B5	B1	B2
B4	26.88					
B3	24.42	2.46				
B5	21.96	4.92	2.46			
B1	15.46	11.42*	8.96	6.5		
B2	12.42	14.46**	12*	9.54	3.04	

The highest levels of flavonoids were found at the concentration of B4 (0.225 g), but the results were not statistically significantly different from B3 and B5, but significantly different from B1 and very significant from B2. Therefore, it is recommended to production pirated honey with the highest flavonoid content, the composition of B4, B5, and B3 is chosen. Choose B3 if you want to save costs, because the extract content is the lowest, but the flavonoids are still equivalent to B5 and B4.

4. Discussion

The mixture of bajakah extract with trigona honey will increase the flavonoid content contained in the mixture. However, at a certain concentration of bajakah extract, will also result in decreased flavonoid content. The content of 0.150 g red bajakah extract mixed with 15 ml of trigona honey from the uplands resulted in the highest flavonoid content of 32.500 mg/ml, as well as 0.225 g of red bajakah extract and 15 ml of trigona honey tasted from peatlands. The flavonoid content in honey from the peatlands and uplands is different. The flavonoid content in the upland area tends to be higher than that in the peatlands; this difference is due to the source of food from trigona bees.

Differences in feed will also affect the colour of the honey produced and the content of flavonoids produced. The results of the study show that darker colors have higher phenolic and flavonoid content than lighter coloured honeys [13, 14, and 15]. Honey colour is correlated with phenolic and flavonoid content. Dark honey tends to have higher phenolic content [28], and vice versa in lighter honey [14, 15]. The dark colour of honey indicates the dominant anthocyanin content in honey [29].

The content of flavonoids in bajakah honey tends to

be influenced by the colour of the honey, where this colour is related to the content contained in the honey. Honey with a light/yellow colour contains several flavonoid substances such as anthocyanins. Honey contains about 200 compounds such as vitamins, enzymes, amino acids, and minerals, with the main ingredients consisting of water and sugar [30].

5. Conclusion

A mixture concentration of 0.150-g red bajakah honey extract and 15 ml of trigona honey from the uplands contained the highest flavonoids of 32.500 mg/ml. If you want to use trigona honey from peatlands, you need 0.225 g of red bajakah extract. The flavonoid content in bajakah honey is dependent on the flavonoid content contained in Trigona honey, which is a mixture of red bajakah stem extract. The flavonoid content in trigona honey in the uplands tends to be higher compared to peatlands, because the feed available in the uplands is more varied and the honey colour is darker.

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