

The Chemical Composition of Trigona Honey in Bone, South Sulawesi

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Abstract

Introduction Trigona honey (*Tetragonula biroi*) is a type of honey produced by stingless bees that is one of the local products in South Sulawesi with the *Tetragonula* species widely known to the public. **Objective** This study was to determine the nutritional content of trigona honey and components of its secondary metabolite compounds as herbal therapy for the disease. **Method** This study aims to analyze the content of bioactive compounds of trigona honey is using phytochemical test and proximate test method. **Result** Phytochemical test show trigona honey had bioactive compounds flavonoid, alkaloid and triterpenoid. Proximate test using thin-layer chromatography (TLC) showed that methanol extract of honey trigona (*tetragonula biroi*) contains vitamin C 0.15%, flavonoids 159.62 ppm, polyphenols 133.52 ppm, and there are magnesium, calcium, zinc and beta carotene. **Conclusion** Trigona honey contains metabolite compounds such as polyphenols, flavonoids, alkaloids, and triterpenoids.

Keywords : Trigona honey, Nutritional, bioactive compounds

Introduction

There is a new interest in the treatment of different diseases using herbal products as they are normally safe and recommended by WHO when there is a lack of modern medicines¹. Indonesia as a developing country with abundant natural resources encourages its citizens to use natural ingredients or herbal products as a therapy for various diseases. More than one-tenth of plant species, approximately 50,000 species are used for pharmaceutical products².

Several herbal products can be used as therapy for infectious and non- infectious diseases, like *Curcuma xanthorrhiza* *Sygium aromaticum*³, *Musa L.paradisiaca*

*L (MPL)*⁴, Red fruit (*Pandanus conoideus*)⁵, *Curcuma longa*⁶, Miana Leaves (*Coleus scutellarioides (L)*)⁷, Andaliman (*Zantoxylum acanthopodium DC*)⁸, Curcumin⁹, Sapodilla fruit¹⁰, Ajwa dates¹¹ and *Thalassia hemprichi* grass¹².

Trigona honey is produced by stingless bees¹³, and has unique characteristics in terms of color, taste, viscosity, water content, sugar, calcium, protein, fat, and carbohydrates¹⁴. Honey produced by bees without sting has a different taste and aroma, more liquid texture, a darker color, and slightly sour taste and undergoes slow crystallization¹⁵. The taste comes from the resin of the plant where the bees build hives and pots. It varies depending on vegetation including flowers and trees that bees visit¹⁶.

Trigona honey contains vitamins that function as antibiotics, antitoxins, and antioxidants and increase the immune system. It also (*Teragonula Biroi*) contains protein, fat, carbohydrates, sugar, energy, vitamin C, beta carotene, calcium, magnesium, zinc, flavonoids,

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and polyphenols because trigona bees can collect nectars from the deepest part of flowers. It results in the high nutritional values it contains¹⁷

According¹⁸, honey contains more than 150 polyphenol compounds including flavonoids, phenolic acids, catechins, and derivatives cinnamic acid which function as antioxidants. The flavonoid compound is the largest group in phenolic compounds.

Material and Methods

Sample Preparation

1 bee boxes of the fresh sample was taken from a beehive, after that, the clean sample was put in bottle and stored in the refrigerator during the analysis. Honey sampling was repeated three times. The livestock was obtained from the beekeeping in Bontocane Village, Kahu Subdistrict, Bone Regency of South Sulawesi.

The Nutrition Test (Proximate)

The solution was made by mixing 10 mL of honey with 1 Liter of water, then was homogenized by stirring, aerated. The honey sample was analyzed at the Food Chemistry Laboratory, Faculty of Animal Husbandry, Hasanuddin University, Makassar.

Phytochemical Test

0.1 gram extract was added with 10 mL chloroform and a few drops of ammonia. The chloroform fraction was separated and acidified with a few drops of concentrated H₂SO₄. The acidic fraction was taken and divided into 3 tubes, then was added with *dragendorf*, *Meyer*, and *Wagner* reagents. The presence of alkaloids was characterized by the formation of white deposits in *Meyer's* reagents, red deposits in *dragendorf's* reagents, and brown deposits in *Wagner's* reagents^{19,20}.

Steroid / Triterpenoid test

A total of 1 g of sample was dissolved with 25 mL of hot ethanol (50°C), then filtered into a porcelain dish and evaporated until dry. The residue was dissolved with ether and transferred into a test tube, then added with 3 drops of anhydrous acetic acid and 1 drop of concentrated H₂SO₄ (*Test Lieberman Burchard*). Red or purple color indicates the presence of triterpenoids while green or blue indicates that there are steroids there.

Flavonoid Test

The sample was added with 0.1 mg magnesium powder and 0.4 mL of amyl alcohol (a mixture of 37% hydrochloric acid and 95% ethanol with the same volume) and 4 mL of alcohol then the mixture was shaken. The formation of red, yellow, or orange in the amyl alcohol layer shows the existence of flavonoids.

Alkaloid Test

The sample was weighted and extracted with ammoniacal chloroform. It was then filtered with cotton and transferred to tubes A and B. *Dragendorff's* and reagents *Wagner's* were added into tubes A and B. Sample positively contained alkaloids if there was reddish deposit formed in tube A and brownish deposit in tube B.

Triterpenoid Test

The sample was weighted and extracted with ethanol. After that, it was strained using cotton and heated to dry. It was extracted again with chloroform and water (1: 1). 2 drops of chloroform extract was dried. 1 drop of concentrated sulfuric acid and anhydrous acetic acid were added. The sample positively contains triterpenoids if it experiences red or brown discoloration but positively contains steroids if it changes in blue, purple, or green.

Measurement of total phenols / polyphenols

The blank used was a mixture of 0.5 mL methanol, 2.5 mL of Folin Ciocalteu reagent dissolved in water, and 2.5 mL of NaHCO₃ 7.5%. The samples were then incubated at 45°C for 45 minutes. Repetition was carried out three times and the absorbance was measured at a wavelength of 765 nm.

Measurement of Total Flavonoids

A total of 10 mg of extract was dissolved in 10 mL of distilled water, then 5 mL of Honey solution was taken and added with 0.3 mL of NaNO₂ 5%. In the next step, the Honey mixture was added with 0.3 mL AlCl₃ 10% which was dissolved with methanol and incubated at room temperature for 5 minutes. After incubation, 2 mL of 1 M NaOH and distilled water, were added until the volume reached 10 mL²¹.

Results and Discussion

Phytochemical Compounds of Trigona Honey

Table : 1 Results of Phytochemical Tests of Trigona Honey (*Tetragonula Biroi*)

No	Phytochemical test	Result	Conclusion
1.	Flavonoid	With mayer reagents there was yellow deposits formed	Positive
2.	Saponin	Brownish ring was not formed	Negative
3.	Alkaloid	With Dragendorff reagents, there was orange deposits formed	Positive
4.	Tannin	Foam formed as high as 1,5 cm for 30 second, there was not greenish black	Negative
5.	Triterpenoid	With LB, Intensive yellow fluorescence there was formed	Positive

From the table 1, Phytochemical test results of Trigona Honey indicate that there are bioactive compounds in Trigona Honey which are flavonoid, alkaloid, and triterpenoid compounds .

Table 2 : Composition of Trigona Honey (*Tetragonula Biroi*), Bone, South Sulawesi

Parameters	Trigona honey
Vitamin C	0.15 %
Protein	1.32 %
Fat	0,23 %
Carbohydrate	64.12 %
Flavonoid	159.62 ppm
Beta carotene	10.61 ppm
Polyphenol	133.52 ppm
Calcium	273.23 ppm
Magnesium	338.94 ppm
Zinc	12.49 ppm
pH	5.76

The table 2 show that of Trigona Honey, show that there was 0.15% vitamin C, 1.32% Protein, 0.23% Fat, 64.12% carbohydrates, 10.61 ppm beta carotene, 273, 23 calcium, 338.94 magnesium, and 12.49 ppm zinc and the pH was 5.76. The results of measurements on

total phenol generally show that trigona honey contains more phenols. The polyphenol value of trigona honey was 133.52 ppm while the total flavonoid of trigona honey (ethanol test of 96%) was 159.62 ppm. Stingless bee honey has a different taste and aroma, a more liquid

texture and undergoes slow crystallization.

Discussion

Table 1 shows the data we obtained about bioactive compounds including flavonoids, alkaloids, and triterpenoids. Trigona honey with ethanol solvent showed a positive response to the Flavonoid test. This indicated that Trigona honey (*Tetragonula Biroi*) contains flavonoid bioactive compounds.

Analysis of the secondary metabolite compounds of trigona honey used two methods, namely phytochemical screening as a qualitative test and measurement of polyphenols and flavonoids as a quantitative test. Phytochemical screening aims to determine the chemical content in the form of secondary metabolites of a sample. Phytochemical test results showed that trigona honey contains chemical compounds, namely alkaloids, flavonoids, and triterpenoids, but does not contain tannins and saponins. The polyphenols in this study were regarded to be gallic acid because the measurement standard for total phenols used gallic acid.

Flavonoids have many good effects on the health of the human body including antioxidants that act as an anti-inflammatory by identifying the metabolic effects pathway of arachidonic acid, affecting prostaglandin production and histamine release, having antitumor activity by cutting tumor promoter activity, and antiviral agents that can break the synthesis of nucleic acids²². Flavonoids and various compounds *phenolic* in honey are the most important pharmacological compounds. These compounds have been proven to be able to ward off free radicals, protect lipids and other compounds (vitamin C) that are easily oxidized²³.

Flavonoids with free hydroxyl groups have a radical capture activity and the presence of more than one hydroxyl group, especially on ring B will increase their antioxidant activity. Also, the presence of ortho-catechol groups in the B flavonoid ring becomes a factor determining the high antioxidant capacity. The active ingredient of trigona honey (*Tetragonula biroi*) has antioxidant properties. The antioxidants substances can configure the important oxidative compounds to capture and prevent the establishment of chain reaction configuration and can donate hydrogen atoms that also affect the activity in reducing free radicals²⁴.

Alkaloids are compounds that have the ability to regenerate pancreatic β cells that have been damaged.

Alkaloids can reduce glucose levels by stimulating and inhibiting glucose synthesis by inhibiting the enzymes of glucose 6- phosphatase, fructose, 1, 6-biophosphatase, and can increase the oxidation of glucose glycogen through glucose 6- phosphate dehydrogenase. glucose 6- phosphatase and fructose 1,6-bisphosphatase are enzymes that can reduce the formation of glucose from the substrate²⁵.

Based on flavonoid data (Table 2), trigona honey has a high flavonoid content enough of 159.62 ppm. Therefore, it can be stated that the total flavonoids are more soluble in 96% ethanol solvent. In honey, there are more than 150 *polyphenolic* compounds including *flavonoids*, *phenolic acids*, *catechins*, and derivatives *cinnamic acid* which function as antioxidants²⁶. The *flavonoid* compounds are the largest group in phenolic compounds¹⁸.

Vitamin C is the main antioxidant in plasma against free radical (ROS) and also plays a role in cells. As a free radical scavenger, vitamin C can react directly with *superoxide* and *hydroxyl anions*, as well as various *hydroperoxides* fat²⁷. Beta carotene, lutein, and lycopene are included in this antioxidant. It was found that carotenoids reduce the risk of heart disease and some types of cancer, and strengthen the body's defense system. In addition to vitamins, minerals, and beta carotene, Trigona honey also contains polyphenols.

Polyphenols contain antioxidant compounds that are able to reduce oxidative stress by preventing the chain of changes of *superoxide* (O₂) to *hydrogen peroxide* (H₂O₂) through the reaction of *habe wells* and *fentors* will form *hydroxy radicals* (OH). Polyphenols donate hydrogen atoms from the aromatic hydroxyl (-OH) group to bind to free radicals and remove them from the body through the excretion system²⁴. In general, decreasing oxidative stress can reduce insulin resistance, delay, slow down, and prevent lipid oxidation processes that play a role in the process of MDA production, inhibit pancreatic β cell damage, nDNA and mtDNA²⁷. The health benefits of polyphenols are to prevent or treat chronic degenerative diseases such as diabetes, cancer, and blocked arteries²⁶.

Conclusion

Based on the result of this study, trigona honey (*Tetragonula Biroi*) contains bioactive compound in the form of flavonoid, alkaloid, triterpenoid and polyphenol which have the potential to be antyhyperglychemic.

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