**IBJ**
*Indonesian Biodiversity Journal*
*http://ejurnal-mapalus-unima.ac.id/index.php/ibj*

***indonesian. biodivers.j.***
**Vol. 2, No. 1: 18-29**
**ISSN : 2722-2659**

# THE BIOACTIVE CONTENT AND ANTIOXIDANT ACTIVITY OF HONEY BEEHIVES (*Apis dorsata* BINGHAMI) FROM THE BOGANI NANI WARTABONE BOLAANG MONGONDOW NATIONAL PLANT

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*Received: February 9, 2021* *Accepted: April 20, 2021*

## Abstract

Bee Honey (*Apis dorsata* Binghami) Is a subspecies of *A. dorsata* which is only found in sulawesidan island and surrounding islands and until now has not been successfully cultivated. Various studies have been conducted species of trees used for nesting places, nesting behavior, biotic conditions, physical surrounding nesting trees and nesting characteristics. This study aims to obtain secondary metabolite components and scientific data of antioxidant activity of honeybee extract (*Apis dorsata* Binghami) derived from Bogani Nani Wartabone Bolaang Mongondow National Park, North Sulawesi Province. The method of extraction in this research was conducted using maceration with methanol solvent, phytochemical test and antioxidant activity analysis using method (1,1-definil-2-picrilhidrazil) by measuring absorbance absorption at maximum wavelength using UV-Vis spectrophotometer. The results of the phytochemical test show that the honeycomb (*Apis dorsata* Binghami) contains alkaloids, flavonoids, saponins and tannins. From the analysis results showed that with the increasing concentration of extracts, the greater the antioxidant activity is marked by decreasing the value of IC50. The honeycomb (*Apis dorsata* Binghami) has IC50 of 212.42 ppm in comparison with vitamin C IC50 of 59.34 ppm.

**Keywords:** Honey Bee's Nest (*Apis dorsata* Binghami), Secondary Metabolite, Antioxidant

## INTRODUCTION

The forest honey bee, *Apis dorsata* Fabricius is the main producer of honey in Indonesia which supports the national economic sector and contributes to the process of forest regeneration through pollination services (Starr *et al*., 1987; Appanah 1993; Momose *et al*., 1998; Itioka *et al*. 2001). ). The bee *A. dorsata* Binghami is a subspecies of *A. dorsata* that is only found in Sulawesi and the surrounding islands and has not yet been successfully cultivated (Mokosuli *et al*., 2017; Hadisoesilo 2001: Lo *et al*., 2010).

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The nests of *A. dorsata* usually hang on the branches of large trees and cluster on one tree with the number reaching tens to hundreds of nests (Kahono *et al*., 1999; Hadisoesilo 2001; Hepburn and Radloff 2011; Mead 2013). In general, *A. dorsata* tends to like the habitus of tall trees with relatively open branches and not too dense canopy as nesting sites (Starr *et al*., 1987; Mokosuli, *et al*., 2013), although it has been found that there is only one colony in one area. tree. Colonies of *A. dorsata* of the genus Apis are honey-producing insects that are useful for human life. Other products from *A. dorsata* that are popular for consumption or use besides honey include wax, propolis, pollen and royal jelly. Beeswax or bee wax is the best result from combing *A. dorsata* beehives. The results of these candles are closely related to the size of the nests of *A. dorsata* which have a length of up to two meters and a width of one meter and can be even larger (Hadisosilo, 2001).

Propolis is one of the natural products produced by honey bees, and has been widely used as a medicine or supplement, mouthwash, anti-inflammatory, disease therapy, accelerate wound healing, and others. In addition, propolis has many benefits and special potential, because it has properties as anti-bacterial, anti-viral, and can inhibit cancer growth. Seeing the potential of propolis, it is necessary to scientifically prove the potential and use of propolis, especially as an antibacterial (Salatino *et al*., 2005).

Bioactive compounds are compounds contained in the bodies of animals and plants. This compound has various benefits for human life, including being used as a source of antioxidants, antibacterial, anti-inflammatory, and anticancer (Prabowo *et al*., 2014) stating that various studies on bioactive compounds have been carried out for human health purposes, ranging from being used as supplements to medicine for humans. There are bioactive compounds that can function as antibacterial, anticancer, anti-inflammatory and antioxidant (Bintang *et al*., 2007).

Antioxidants are substances that can delay, slow down and prevent the oxidation process (Mokosuli, *et al*., 2008). Antioxidants are very beneficial for health and play an important role in maintaining the quality of food products. The benefits of antioxidants for health and beauty, for example to prevent cancer and tumors, constriction of blood vessels, premature aging, and others. Antioxidants in food products can be used to prevent oxidation processes that can cause damage, such as rancidity, changes in color and aroma, and other physical damage (Tamat *et al*., 2007).

## MATERIALS AND METHODS

The research materials used were vitamin C (-tocopherol) phosphate buffer 0.1 M Ph 7.0, linolenic acid, 98% methanol, thiobarbituric acid (TBA). The tools used in this study were test tubes, pipettes, ependorf, incubator, votex, autoclave, spectrophotometer, aluminum foil, petri dish, tweezers, paper labe, threaded dark bottle. Sampling site in Bogani Nani Wartabone National Park. The analysis was carried out at the molecular laboratory, Faculty of Mathematics and Natural Sciences, Manado State University.

### Research procedure

The nests used are from *Apis dorsata* honey bees that live in Bogani Nani Wartabone National Park, Bolaang Mongondow. nests that will be used only good nests and are not contaminated with

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anything. Sampling was done by separating the honeycomb from the species. The weight of the honeycomb sample to be taken is approximately 1 kg. nests that have been taken, then extracted using 98% methanol and macerated for 48 hours. From the maceration process it produces extract which will be filtered using filter paper, from the filtering results it produces filtrate which will be evaporated with a rotary evaporator and produces a crude extract of *Apis dorsata* Binghami honey bee nest. The tests carried out included phytochemical testing including alkaloids, flavonoid saponins, tannins, triterpenoids and steroids (Harbourne Method, 1996) DPPH method (Mokosuli, *et al*., 2008).

**a. Determination of Percent Inhibition**

The percentage of inhibition is the percentage that shows the activity of the radical. The percentage of inhibition of DPPH radicals from each concentration of sample solution can be calculated by the formula:

$$\% \text{ Inhibition} = \frac{A_{blangko} - A_{sample}}{A_{blangko}} \times 100\ \%$$

Where Ablangko is the absorbance value of the DPPH solution without extract (blank DPPH solution), Asampel is the absorbance value of the tested sample (Brand And William *et al*., 1995)

**b. Determination of IC50 Value (Ihibition Concentration)**

After the percentage of inhibition was obtained for each concentration, the sample concentration and the percentage of inhibition obtained were plotted on the x and y axes, respectively, in the linear regression equation y = a ± bx. The equation is used to determine the IC50 value of each sample expressed by a y value of 50 and the x value to be obtained as IC50. The IC50 value is the concentration of the sample that can reduce DPPH radicals as much as 50% of the initial concentration (Molyneux, 2004).

**Data analysis**

Determination of IC50 of honeycomb extract was carried out using IBM SPSS regression analysis. The data processing method was carried out by comparing the test results with positive controls, then explained with qualitative results as reinforcement to support quantitative data.

## RESULTS AND DISCUSSION

**Sample Extraction Results**

The honey bee nest sample extraction process was carried out by the maceration method. This method was chosen because the process is easy and the equipment used is quite simple. The principle of maceration is that the solvent used in the maceration process will enter the sample cell through the cell wall, so that the contents of the cell will dissolve due to the difference in concentration between the solution inside and outside. Cells go through a diffusion process so that there will be an equilibrium between the solution inside the cell and the solution outside the cell (Ulfah, 2015: 27) Maceration in this study used methanol because it was able to attract secondary metabolites as a whole compared to other solvents and could penetrate the cell wall of the sample. so that it is able to dissolve the honeycomb and minimize the dissolved wax which is a nuisance in the extraction process. The honeycomb sample that has been

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divided into several parts and cut into small pieces with the aim of expanding the surface of the contact area between the solvent and simplicia so that the maceration process can take place effectively, then the sample is immersed in methanol solvent and stirred so that the solvent diffuses into the cell. to dissolve the compounds contained in the honeycomb sample and will mix with the surrounding liquid so that an equilibrium occurs. This happens because the concentration of the environment outside the cell is higher than the concentration inside the cell so that the contents of the cell including the active substances will be pulled out and dissolved in the solvent (Yuliana, *et al*., 2015: 68).

Solvent replacement is carried out every 24 hours and the remaceration process is needed to replace the saturated solution with a new solvent so that all chemical compounds contained in the sample can be extracted optimally (Huliselan, *et al*., 2015: 159). Furthermore, filtering with cotton and calico cloth aims to separate the filtrate from the residue, the filtered filtrate is then evaporated at a temperature below 60$^{O}$C so that a thick extract is obtained and the solvent will evaporate. Evaporation results obtained honey bee nest extract with a weight of 83.4229 g.

**Analisis Fitokimia**

Phytochemical analysis is one way to determine the content of secondary metabolites in a sample. In this study, the phytochemical analysis used the Harborne (1996) procedure. The compounds analyzed included alkaloids, saponins, flavonoids, tannins, steroids and triterpenoids. Based on the phytochemical screening test of honey bee hive extract with 98% methanol as solvent, it showed positive results on the test of alkaloids, flavonoids, saponins and tannins, negative results on tests of tritepenoid and steroid compounds are shown in Table 1.

Table 1. Results of Phytochemical Analysis of Honeycomb (*Apis dorsata* Binghami)

| Group of compounds | Test results SLM |
|---|---|
| Alkaloids | + |
| Flavanoids | + |
| Saponins | + |
| Tannins | + |
| Triterpenoids | - |
| Steroids | - |

Description: (+) identified; (-) not identified

The group of alkaloid compounds in the phytochemical screening test showed positive results, which was indicated by the formation of a white precipitate in Meyer's reagent and brown in Wegner's reagent. Alkaloids are semi-polar compounds in this sample, it can be seen the presence of these compounds. The alkaloid test will show positive results if it is marked by the appearance of a precipitate

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and a brown color change after being reacted with Mayer's reagent. Mayer's reagent contains metal Hg and KI which will form a brownish yellow precipitate complex with alkaloid compounds. Marliana *et al*. (2005). In the alkaloid test with Meyer's reagent, it is estimated that nitrogen in the alkaloid will react with metal ions K+ from potassium tetraidormercurate (II) to form a precipitated potassium alkaloid complex (Svehla, 1990).

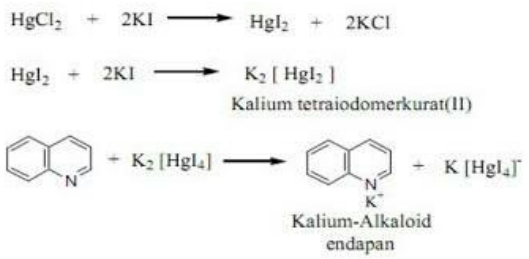


Figure 1. Estimated Reaction of Alkaloid Compounds With Meyer's Reagents (Marlina, 2005).

Positive results of alkaloids in the Wagner test with the formation of a brown precipitate. It is estimated that the precipitate is potassium-alkaloid. In the manufacture of Wagner's reagent, iodine reacts with I ions from potassium iodine to produce I3 ions which are brown in color. In the Wagner test, metal ions K+ will form covalent bonds with nitrogen in the alkaloids to form a precipitated potassium-alkaloid complex (Svhla, 1990).

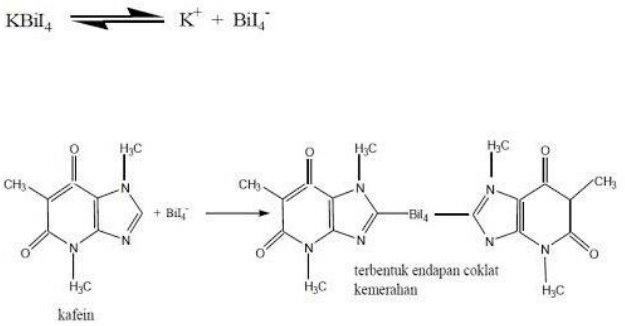


Figure 2. Reaction for the formation of a brown precipitate by the reagent (Marlina, 2005).

The group of flavonoid compounds in the phytochemical screening test showed positive results with the formation of a yellow color on the amyl alcohol layer. The addition of HCL in the flavonoid test was carried out to hydrolyze flavonoids into their aglycones by hydrolyzing O-glycosyl. Glycosyl will be replaced by H+ from acid because of its electrophilic nature. Glucosides in the form of sugars that are commonly encountered are glucose, galactose and riminose. Reduction with HCL produces complex compounds that are red, yellow or orange (Robinson 1985). According to Robinson (1995) the red color produced indicates the presence of flavonoids by concentrated magnesium hydrochloric acid.

Flavonoids are often good reducing compounds that inhibit oxidation reactions, both enzymatically and non-enzymatically, so that flavonoids are antioxidants that play a role in inhibiting the growth of cancer

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cells (Lisdawati, 2002). detected on the chromatogram or in solution (Harborne 1996). The red color in the flavonoid test is due to the formation of flavilium salts (Achmad, 1986) according to the following reaction.

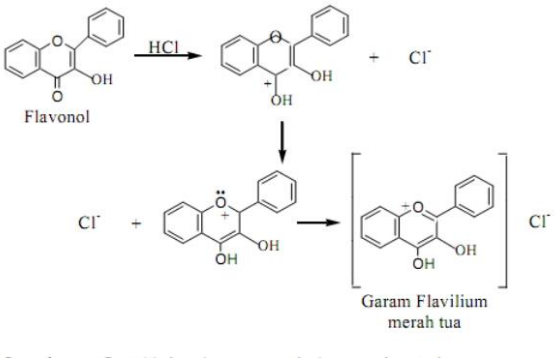


Figure 3. Reaction of Formation of Garum Flavilium (Achmad, 1986).

The group of saponin compounds in the phytochemical screening test showed positive results, indicated by the formation of stable foam. According to Robinson (1995) compounds that have popar and nonpolar groups are surface active so that when saponins are shaken with water they will form micelles. In the micellar structure, the polar groups are facing outward while the nonpolar groups are facing inward, this condition looks like foam. Saponins are given this name because of their soap-like properties (the Latin word sapo means soap). The foam caused by saponins is due to the structure of the constituent compounds, namely non-polar sepogenin chains and water-soluble polar side chains (Kristianingsi, 2002). nonpolar solvent (hydrophilic) surfactant that can lower the surface tension.

The group of tannin compounds in the phytochemical screening test showed positive results with the formation of a greenish-blue color. In the identification of tannins, the color change was caused by the addition of FeCI3 with one of the hydroxyl groups present in the tannin compound. The addition of FeCI3 produces a blackish green color which indicates the presence of condensed tannins (Sangi *et al*., 2008). The formation of blackish green or blue ink in the extract after adding FeCI3 because tannins will form complex compounds with FeCI3 (Halimah, 2010)

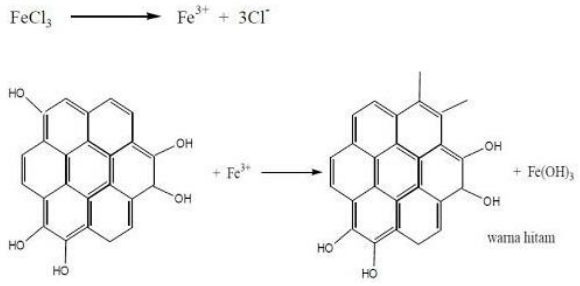


Figure 4. Reaction for the Formation of Complex Compounds with F3CI3 (Halima, 20010)

The triterpenoid and steroid compounds in the phytochemical screening test showed negative

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results, the red and green colors indicated the presence of triterpenoids and the green color indicated the presence of steroids. The results of the treterpenoid and steroid tests did not show this color. According to Harborne (1987), triterpenoid compounds are fat-soluble compounds. So based on the level of solubility, in the test the triterpenoid compound group was pulled with ether. However, in this study, the extraction of triterpenoid compounds was carried out using methanol as a solvent. This is because methanol is a universal solvent so that it can dissolve polar and non-polar analytes. Methanol can attract alkaloids, steroids, saponins, and flavonoids (Thompson, 1985).

Various interesting physiological activities are exhibited by some triterpenoids and these compounds are active components in medicinal plants that have been used to treat diseases including diabetes, liver damage, and malaria. Some compounds may have ecological value for the plants that contain them because these compounds can work as insecticides or atifungals (Robinson, 1995). ), Steroids have a tendency as a source of antibacterial. Bangham and Horne (2006) stated that steroids can interact with cell membrane phospholipids which are impermeable to lipophilic compounds, causing decreased membrane integrity, changing cell membrane morphology, and ultimately causing cell membranes to become brittle and lysis.

**Antioxidant Activity Test**

Analysis of Determination of % Inhibition

Antioxidant activity test was carried out using a UV-Vis spectrophotometer. The results of the absorbance measurement from the blank DPPH showed that the optimum wavelength of DPPH was at 516 nm and further measurements using the DPPH radical attenuation method were carried out at that wavelength. The optimum wavelength (λ) of DPPH was obtained by measuring the absorbance of the blank DPPH solution at various wavelengths of 400-700 nm. The optimum wavelength of the blank DPPH solution can be shown from the absorbance curve (A) vs. wavelength (λ) as shown in Figure 5, which is the wavelength at the optimum absorbance. From the results of the measurement of maximum absorption, the maximum of DPPH is 516 nm, with research conducted by Erawati, 2012.

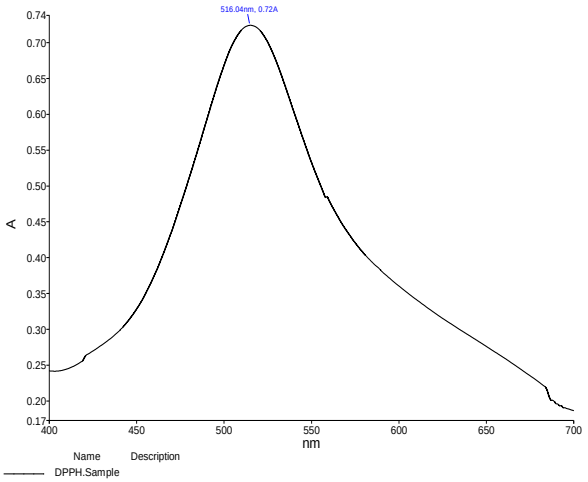


Figure 5. DPPH Absorbance Curve at Various Wavelengths.

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Measurement of the absorption of the extract mixed with DPPH was carried out after incubation for 30 minutes so that a reaction occurred between DPPH as a free radical and the sample being tested. Likewise, the measurement of the absorption of vitamin C mixed with DPPH was carried out after incubation for 30 minutes so that a reaction occurred between DPPH as a free radical and vitamin C. This test was carried out to determine the absorbance of DPPH remaining after adding the test material. If a compound has antioxidant activity, there will be a decrease in the absorbance value of DPPH. Measurement of DPPH absorbance was also carried out on DPPH in methanol p.a without the test material. The decrease in DPPH absorbance was indicated by a change in the color of the DPPH from purple to yellow.

Table 2. Presentation of DPPH Radical Inhibition of Honeycomb Extract (*Apis dorsata* Binghami) and Vitamin C.

| Concentration ppm | Absorbance(A) Honeycomb | Inhibis% |
|---|---|---|
| 10 | 0.758 | 29,87 |
| 50 | 0,552 | 48,93 |
| 100 | 0,411 | 61,97 |
| 200 | 0,382 | 64,66 |
| 250 | 0,424 | 60,77 |

| Concentration ppm | Absorbance(A) Vitamin C | Inhibisi% |
|---|---|---|
| 50 | 0,454 | 57,81 |
| 100 | 0,355 | 59,19 |
| 200 | 0,160 | 85,19 |

The percentage of free radical inhibition of the honeycomb extract shows that the higher the concentration of the extract, the lower the absorbance of the beehive, meaning that the honeycomb can ward off or reduce DPPH free radicals. Likewise with Vitamin C, the higher the concentration of vitamin C, the lower the absorbance. The difference in each value of % inhibition between beehive extract and vitamin C showed that at all concentrations of vitamin C the value of % inhibition was higher than that of beehive extract.

**IC50 analysis**

The results of the sample absorbance measurements for each concentration can be seen in (figure 6). The antioxidant activity of the DPPH method is expressed by 50% Inhibition Concentration or IC50, which is the concentration of the sample that can inhibit DPPH activity by 50%. Based on the activity test data, the IC50 value of the honeycomb honeycomb methanol extract (*Apis dorsata* Binghami) was obtained from the results of linear regression calculations, where y = a ± bx. Variable y in this equation is

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as IC50, variables a and b are constants, where the value of x obtained is the amount of concentration required to reduce 50% of DPPH radicals. The value of r = 0.9219 illustrates that with increasing concentration of the extract, the greater the antioxidant activity. From the extract concentration vs. absorbance curve using the Excel 2010 program, the linear regression equation in this study was y = 10,659x + 7,1171 and r = 0,9219. This can be seen from the relationship curve of the concentration of the honeycomb honeycomb (*Apis dorsata* Binghami) methanol extract to the percent inhibition in (figure 6).

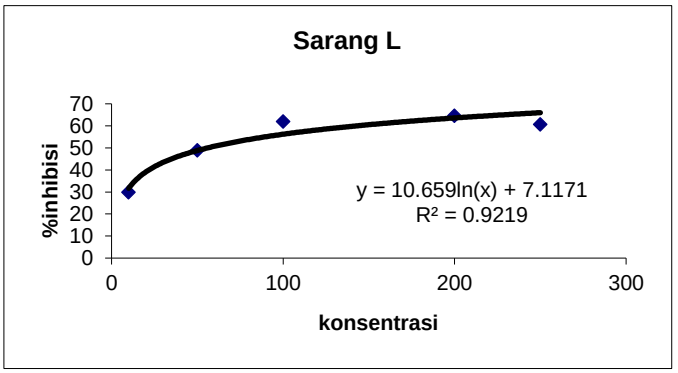


Figure 6. Linear Regression Curve of Honeycomb Honey Extract (*Apis dorsata* Binghami).

The IC50 value of the honeycomb honeycomb (*Apis dorsata* Binghami) methanol extract based on the calculation results obtained was 212.42 ppm. According to Molyneux (2004), if the IC50 value obtained ranges from 200-1000 ppm, then the substance is less active but still has potential as an antioxidant. According to Thamrin, *et al*. (2016: 59), a compound is said to be a very strong antioxidant if the IC50 value is 50 ppm, the compound is said to be a strong antioxidant if the IC50 value is between 50-100 ppm, the compound is said to be a moderate antioxidant if the IC50 value is between 101-150 and the compound is said to be weak antioxidant if the IC50 value is more than 151 ppm. The IC50 value of the honeycomb honeycomb (*Apis dorsata* Binghami) methanol extract when seen in the curve (figure 8) above, the concentration of the 100 ppm extract was able to inhibit 61.97% of DPPH radicals, the IC50 value of the extract required to inhibit 50% of DPPH radicals was based on the calculation is 212.42 ppm and the antioxidant activity index is less active but still has potential as an antioxidant.

The positive control used in this study was vitamin C. Vitamin C is a water-soluble antioxidant (Davies, et.al., 1991). The use of positive control in testing this antioxidant activity is to determine how strong the antioxidant potential is in the methanol extract of Sarang Lebah Madu (*Apis dorsata* Binghami) when compared to vitamin C. If the IC50 value of the sample is the same or close to the IC50 value of the positive control, it can be said that the sample potential as an alternative antioxidant. On (figure 7).

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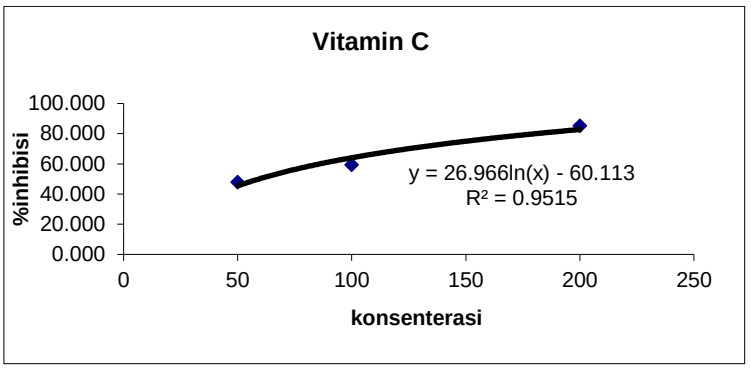


Figure 7. Comparative Linear Regression Curve for Vitamin C.

The following is a linear regression curve of vitamin C measured using a UV-Vis spectrophotometer. Based on the activity test data, the IC50 value of vitamin C was obtained from the results of linear regression calculations, where y = 26.966x + 60.113 and r = 0.9515. the y variable in this equation is IC50, where the value of x obtained is the amount of concentration required to reduce 50% of DPPH radicals. The IC50 value of vitamin C can be seen in the curve (figure 9) above, the concentration of vitamin C at 100 ppm can inhibit 59.19% of DPPH radicals. The IC50 value of the extract required to inhibit 50% of DPPH radicals based on calculations is 59.34 ppm and is included in the strong category of antioxidant activity group.

## CONCLUSION

The results of the phytochemical screening test for Honey Beehive Extract (*Apis dorsata* Binghami) showed the presence of alkaloids, flavonoids, saponins, and tannins. Triterpenoid and steroid compounds were not identified. With the identification of four groups of compounds in the sample, it can be concluded that the honey bee nest sample (*Apis dorsata* Binghami) from Bogani Nani Wartabone Bolaang Mongondow National Park has the potential as a source of bioactive antioxidants.

The results of antioxidant analysis using the DPPH method of Honey Beehive Extract (*Apis dorsata* Binghami) showed an IC50 of 212.42 ppm compared to Vitamin C with an IC50 of 59.34 ppm. These results indicate that the honeycomb honeycomb (*Apis dorsata* Binghami) methanol extract has the potential to be developed as a source of bioactive antioxidants.

## REFFERENCES

Appanah S. 1993. Mass flowering of dipterocarp forests in the aseasonal tropics. *J Biosci*. 18(4): 457–474.

Achmad, S.A. 1986. *Kimia Organik Bahan Alam.* Jakarta: Karnunika.

Bintang L.A.K, Sinurat A.P , Purwadira 2007. Penambahan ampas mengkudu sebagai senyawa bioaktif terhadap performans ayam broiler . JITV 12(1) : 1-5.

Hadisoesilo S. 2001. Keanekaragaman spesies lebah madu asli Indonesia. Pusat Penelitian dan Pengembangan Hutan dan Konservasi Alam, Bogor. *Biodiversitas.*2:123-128.

Brand Williams, & W. Cuvelier, M.E. (1995) Use of free radical method to evaluate antioksidant activity. *Food science and technology,* 28(1), 25- 30.

Bangham AD, Horne RW. 2006. Action of Saponinsonbiologikal cell membranes. *Journal Nature* 196(1):952-953.

Erawati, 2012. Uji Aktivitas Antioksidan Ekstrak Daun Garciniada edalanthera Pierre Dengan metode DPPH (*1,1-diphenyl-2-picrylhidrazyl*) dan Identifikasi Golongan Senyawa Kimia Dari Fraksi Paling Aktif. [Skripsi]. Depok: Universitas Indonesia.

Hepburn R, Radloff SE. 2011. *Honeybees of Asia*. New York (US). Berlin Heidelberg.

Harborne JB. 1996. Metode Fitokimia, penentuan dan cara modern menganalisis Tumbuhan. Penerjemah : Patmawinata K dan Soediro I. Penerbit ITB Bandung.

Halima, 2010. Uji Fitokimia dan Uji Toksisitas Ekstrak Tanaman Anting Anting (*Achalypa Indica Linn*) Terhadap Larva Udang ( *Artemia Salina Leach*). Malang: Jurusan Kimia Universitas Islam Negri Malang.

Harborne, 1987 . Metode Fitokimia Penentuan Cara Modern Menganalisis Tumbuhan.

Huliselan, Yosina M., dkk. "Aktivitas Antioksidan Ekstrak Etanol, Etil Asetat dan n-Heksan dari Daun Sesewanua (*Clerodendron squamatum* Vahl.)". *Ilmiah Farmasi* 4, no. 3 (Agustus 2015): h. 155-163.

Itioka T, Inoue T, Kaliang H, Kato M, Nagamitsu T, Momose K, Sakai S, Yumoto T, Mohamad SU, Hamid AA, dan Yamane S. 2001. Six-year population fluctuation of giant honeybee *Apis dorsata* (Hymenoptera: Apidae) in a tropical lowland dipterocarp forest in Sarawak. *Annals Entomol Soc America*. 94:545–549.

Kahono S, Nakamura K, Amir M. 1999. Seasonal migration and colony behavior of the tropical honeybee *Apis dorsata* F. (Hymenoptera: Apidae). *Treubia*. 31(3)283-297

Lo N, Gloag RS, Anderson DL, Oldroyd BP. 2010. A molecular phylogeny of the genus *Apis* suggests that the giant honey bee of the Philippines, *A. breviligula* Maa, and the plains honey bee of Southern India, *A. indica* Fabricius, are valid species. *Systematic Entomology.* 35:226-233.

Marliana S, Venty S, Suyono. 2005. Skrining fitokimia dan analisis kromatografi lapis tipis komponen kimia buah labu siam (*Sechium edule* Jacq. Swartz) dalam ekstrak etanol. *Jurnal Biofarmasi* 3(1):26-31.

Molyneux, P. 2004. *The use of the stable free radical Diphenyl picrylhydrazyl (DPPH) for estimating antioxidant activity*. [Original Article].

Mokosuli YS, Pelealu J, Tulung M, Mandey LC. Pharmacological Bioactivity Honey Bee Venom Apis nigrocincta Smith and *A. dorsata* Binghami Endemic to North Sulawesi. International Journal of Science and Engineering Investigations. 2013; 2(18):25-33

Mokosuli YS. Aktivitas antioksidasi dan antikanker ekstrak kulit batang Langsat (Lansium domesticum L.). [Tesis]. Sekolah Pascasarjana Institut Pertanian Bogor, 2008.

Mokosuli, Y. S. , Repi, R. A, Worang, R, L. Tahun 2017. Potensial antioxidant and anticancer effect of *Apis dorsata* Binghami Crude Venon from Minahasa, North Sulawesi, Journal of Entomology and Zoologi Studies 2017; 5 (2): 112-119.

Momose K, Yumoto T, Nagamitsu T, Kato M, Nagamasu H, Sakai S, Harrison RD, Itioka T, Hamid AA, Inoue T. 1998. Pollination biology in a lowland dipterocarp forest in Sarawak, Malaysia. I. characteristics of the plant-pollinator community in a lowland dipterocarp forest. *American J Bot*. 85(10):1477–1501. ISSN 1537-2197.

Prabowo , A. Y , T . Estiasi, i. Purwatiningrum. 2014. Umbi gemili (Dioscorea esculenta L.) sebagai bahan pangan mengandung senyawa bioaktif: kajian pustaka.Jurnal Pangan dan Agroindustri 2 (3):129-135.

Robinson, T. 1995. *Kandungan Senyawa Organik Tumbuhan Tinggi.* Diterjemakan oleh Prof. Dr. Kosasih Padnawinata. Bandung: ITB.

Svehla, G. 1990. *Buku Teks Analisis Anorganik Kualitatif Makro dan Semimikro.* Edisi Kelima. Penejerma Setiono, L. dan A.H. Putjaatmaka. Jakarta: PT Kalman Mmedia Pustaka.

Sangi, M.; Runtuwene, M.R.J.; Simbala. H.E.I. Dan Makang, V .M.A. 2008 Analisis Fitokimia Tumbuhan Obat Di Kabupaten Minahasa Utara. *Chemistry* Progress. Vol 1, hlm: 47-53

Salatino A, Teixeira ÉW, Negri G, Message D. 2005. Origin and chemical variation of Brazilian propolis. *eCAM* 2:33–38.

Thompson, E. B. 1985. Drug Bioscrening. America Greceway Publising Compani. Inc. Pp. 40, 118.

Tamat, S.R T. Wikanta, L.S, Maulina. 2007. Aktifitas antioksidan dan toksisitas senyawa bioaktif dari ekstrak rumput laut hijau Ulva retuculata Forsskal.Jurnal Ilmu Kefermasian Indonesia 5(1):31-36.

Ulfah, Sonia. "Uji Aktivitas Antioksidan Ekstrak Daun Rambutan (*Nephelium lappaceum* Linn) dengan

***indonesian. biodivers.j.***
**Vol. 2, No. 1: 18-29**
**ISSN : 2722-2659**

Metode DPPH (2,2-*difenil*-1-*pikrilhidrazil*)". *Skripsi*. Jakarta: Fakultas Kedokteran dan Ilmu Kesehatan UIN Syarif Hidayatullah, 2015.

Widyasari, A. R. 2008. Karakteristik dan Uji Antibakteri Senyawa Kimia Fraksi n- Heksana Dari Kulit Batang Pohon Angsret (*Spathoda campanuala eauv*). Skripsi Tidak Diterbitkan. Malang. Jurusan Kimia Universitas Briwijaya.

Yuliana, Renita, dkk. "Daya Antimikrobia Sarang Lebah Madu *Trigona* spp terhadap Mikrobia Patogen". *Bioedukasi* 8, no.1 (Februari 2015): h. 67-72.