ANTIOXIDANT ACTIVITY OF *Homotrigona fimbriata* PROPOLIS EXTRACT

Rabiatul Demiati[1], Rendri Arista Avimaro[2], Paula Mariana Kustiawan[3*]
[1]*Undergraduate Program of Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Jl. Ir. H. Juanda No.15, Samarinda, Kalimantan Timur 75124*
[2]*Rumah Sahabat Kelulut Samarinda Group, Lempake District, Samarinda, East Kaliamantan.*
[3]*Bee Research Group, Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur Jl. Ir. H. Juanda No.15, Samarinda, East Kalimantan 75124*
**Email Corresponding: pmk195@umkt.ac.id*

Submitted: August 29, 2023 ***Revised: December 4, 2023*** ***Accepted: January 3, 2024***

ABSTRACT

Antioxidant compounds have a role in protecting the body from free radicals, Propolis from *Homotrigona fimbriata* species is widely used as an alternative to natural healing in East Kalimantan. The secondary metabolite compounds in propolis contain many flavonoids and phenolic compounds. These compounds can be utilized to fight free radicals. The purpose of this study was to determine the antioxidant content of stingless bee (*Homotrigona fimbriata*) propolis extract. This was a quantitative study of the antioxidant potential of propolis extract. The benchmark antioxidant activity was measured based on its ability to counteract free radicals using the DPPH method. The results of this study showed the presence of secondary metabolites of alkaloids, flavonoids, tannins, and saponins as well as antioxidant activity of *Homotrigona fimbriata* propolis extract with a value of IC_{50} 95,8 ppm as strong category. This can be caused by differences in geographical location, and various resins found in the trees around the beehive will affect antioxidant activity.

Keywords: *Homotrigona fimbriata*, antioxidant, stingless bee

INTRODUCTION

In daily activities, the human body can be exposed to free radicals from cigarette smoke, vehicle fumes, X-ray radiation, and gamma rays. Exposure to large quantities of free radicals can damage normal tissue, resulting in disruption of DNA production, blood vessels, lipid layers, and cell damage (Meidhiyanto, Uddin, and Sofia, 2016). An imbalance (oxidative stress) between free radicals and antioxidants interferes with the functioning of the immune system.

Free radicals are defined as unstable, highly reactive, short-lived particles with one or more unpaired electrons that remove electrons from other molecules in the body to achieve stability by compromising the integrity of lipids, proteins, and DNA against levels of oxidative stress, such as neurodegenerative diseases, diabetes, cardiovascular disease, premature aging, and even cancer (Phaniendra, Jestadi and Periyasamy, 2015). To prevent the accumulation of free radicals that can trigger the development of cancer, antioxidant compounds are needed to neutralize, reduce, and inhibit the formation of new free radicals in the body by becoming electron donors for free radicals so that free electrons in free radicals pair up and stop body damage. (Arnanda & Nuwarda, 2019).

Antioxidant compounds protect the body from free radicals as a counterweight and can balance free radical atoms by means of sufficient electrons from unpaired free radical atoms as a result of their ability to limit the chain reaction process. (Julfitriyani, Runtuwenw and Wewengkang, 2016). Antioxidants are substances that delay the oxidation process and

have a detrimental effect on the body, such as accelerating the premature aging process of the skin. Antioxidants can be found in natural ingredients, one of which is kelulut bee propolis. Bees are not only known as honey producers but are also capable of producing other bee products such as propolis, royal jelly, and bee pollen. Bees species in Indonesia include *Apis mallifera, A. andreniformis, A. dorsata, and Trigona* sp. (Mahani, Rokim, A Karim, 2011).

Propolis is a bee glue used to protect individuals from predators. Propolis is a resinous and sticky substance collected from tree sap by bee colonies and mixed with beeswax and the bee enzymes themselves. Propolis is hard and easily damaged at low temperatures. Propolis becomes sticky and rubbery above 45°C, and starts to melt at 60°C-100°C (Wagh, 2013). *Trigona* sp. known as stingless bee. This bee is capable produce more propolis compared to honey production. Potential of stingless bee as production propolis of 500 gram/colony for production time 3 months, meanwhile honey production is only 250 grams/day colony (Riendriasari and Krisnawati, 2017).

Bees use propolis to cover the holes and cracks in their hives, as a coating that is useful for protecting beehives from external disturbances such as insects, moths, rats, weather, and as an antibacterial (Rosana, Ahwan and Qonitah, 2021). Honey bees use propolis as an ingredient for nesting, as well as for maintaining the level of bacteria and fungi (Fatoni *et al.*, 2008; Bankova, Popova, and Trusheva, 2014). Consuming bee products can also prevent us from being attacked by viruses (Batistuta, Aulia and Kustiawan, 2021)

Since 2000, there have been approximately 300 chemical compounds in stingless bee propolis, such as flavonoids, terpenes, and phenols (Huang *et al.*, 2014). The antioxidant content of propolis is due to the presence of bioactive flavonoid and phenolic compounds (Segueni *et al.*, 2016). Flavonoid and phenolic compounds in propolis depend on the existence of a region and the species of bees (Chan, Cheung and Sze, 2013).

In research conducted by (Khairunnisa, Mardawati and Putri, 2020) it is known that the IC_{50} value of propolis with water solvent is 1149.75 ppm, with 70% ethanol solvent is 846.27 ppm and with methanol solvent is 477.01 ppm which in the blois classification has very weak, very weak and weak antioxidant intensity.

Ethanol, propylene glycol, and water are the solvents commonly used for propolis extraction. Extraction using different concentrations of ethanol produced different amounts of the active components. Ethanol is distinguished by its polarity based on the dielectric constant, which increases the polarity of the solvent (Anjum *et al.*, 2019). Extraction generally involves maceration, reflux, and percolation. Each extraction method requires different temperatures, solvents, and extraction times. The extracted components in the sample affected by extraction time (Wijaya, Novitasari and Jubaidah, 2018)

Differences in characteristics, bee species, the ability of bees to fly, various kinds of resins found in trees around the beehive, and the solvent used for extraction affect the resulting product (Aziz, Yuliawan and Kustiawan, 2021). Based on the above background, the purpose of this study was to determine the antioxidant content in the propolis extract of the kelulut bee (*Homotrigona fimbriata*) cultivated in the Lempake Village, North Samarinda District. There is limited research on this species of stingless bee propolis.

RESEARCH METHODS

Materials

The research was conducted using stir bars, water baths (faithful model DK-2000-IIIL), porcelain cups, maceration vessels, erlenmeyer, spatulas, vials, filter paper, aluminum foil, rotary evaporators (buchi R-100), measuring cups, cuvette, UV-Vis spectrophotometer (Thermo scientific genesis 10s), analytical balance (Fujitsu FS-Q 20KG × 0.1gram), micropipette (scilogex 10-1000 µl), separatory funnel, test tube, and vortex. The material used is propolis, which comes from the Lempake sub-district, North Samarinda sub-district. 96% ethanol, distilled water, ascorbic acid (Vitamin C). DPPH with methanol (p.a.)was used as reagent.

Research Procedure

1. Propolis Extraction

The 500 grams of *Homotrigona fimbriata* propolis was crushed and 96% ethanol extract was added. Maceration was then carried out with stirring and soaking for 120 hours or 5 days with daily mixing, then filtered using a Buchner funnel and filter paper. The filtrate was separated from the dregs and placed into an Erlenmeyer flask. Particle size, type of solvent, time, ratio of materials and solvents, and temperature are things that affect the maceration process (Chairunnisa, Wartini and Suhendra, 2019). The residue obtained was added with ethanol solvent again. The filtrate was evaporated into a thick extract, and the yield was calculated.

1. Phytochemical test

Used phytochemical test because it can detect unlimited bioactive components only on secondary metabolites, however to the primary metabolites that provide functional biological activity, such as proteins and peptides (Alfian and Susanti, 2012). Phytochemical test was carried out using Khairunnisa, Mardawati and Putri (2020) method with modifications.

a. Alkaloid
A propolis sample (0.5 g) was added to the Dragendroff reagent. The presence of alkaloids is indicated by the formation of a brown precipitate.

b. Flavonoid
Flavonoids was carried out by dissolving the extract in ethanol then adding 2 drops of H_2SO_4 2N solution and then homogenizing. The presence of flavonoids is indicated by a change in color to yellow, red and brown (Anastasia, 2017).

c. Saponin
The propolis sample (0.5 g) was heated in a water bath containing 20 ml of distilled water. The filtrate was homogenized and allowed to stand for 15 minutes. The presence of saponins indicates the formation of a stable foam.

d. Tannin
The 0.5 g of the propolis sample then, add the filtrate to a 1% $FeCl_3$ solution. The presence of tannins is indicated by the formation of dark blue or blackish green.

2. Antioxidant determination

Antioxidant activity was determined using the DPPH method (Damanis, Wewengkang, and Antasionasti, 2020). The maximum wavelength of DPPH was determined by taking 3 mL of DPPH solution and then adding methanol p.a to the calibration limit, homogenizing, and observing the absorption at a wavelength of 400-800 nm. The extract concentration was determined by weighing 1 mg of each formula dissolved in 10 ml of methanol p.a (1000 ppm). Pipette solution as much as 0.025 ml; 0.05 ml; 0.075 ml; 0.01 ml; In a 10 ml volumetric flask add DPPH to each pipette series 3 ml then add methanol p.a ad calibration limit. Ascobic acid was used as the positive control. Antioxidant activity tests were performed for 30 minutes. Blank solution, propolis extract solution, and Vitamin C solution were incubated at 37°C in the dark. Then, it was measured at the maximum wavelength obtained using a uv-vis spectrophotometer. The absorbance results were searched for the percent inhibition of free radical activity (Rahmawati, Muflihunna and Sarif, 2016)

Data Analysis

The data obtained were analyzed based on antioxidant activity, an inhibitory concentration value of 50, or Inhibition Concentration (IC_{50}) calculated using Excel software, and a linear regression equation was constructed.

RESULT DAN DISCUSSION

Propolis extraction

Maceration of 500 grams of propolis dissolved in 96% ethanol yielded a thick brownish yellow extract of 5.9 grams.

Phytochemical determination

The results of the phytochemical test (Table I)Table I showed that the kelulut bee propolis extract (*Homotrigona fimbriata*) had secondary metabolites, such as alkaloids, as indicated by the appearance of brown precipitates, flavonoids by the appearance of a yellow color, saponins by the presence of foam of more than 1 cm for 10 minutes, and tannins by the appearance of a green color. black or blue-black in the sample.

Table I. Phytochemical Test Results of *Homotrigona fimbriata* Propolis Extract

Compound	Reagent	Transformation
Alkaloid	Dragendroff reagent	A brown precipitate
Flavonoid	H_2SO_4	Color change to yellow
Saponin	Aquadest solvent	Formed stable foam
Tannin	Reagent $FeCl_3$	Color change to green

Antioxidant activity

The dpph method was used to determine the antioxidant activity of the propolis extract. This method was chosen because it has several advantages, including easy, simple, fast, good sensitivity for certain polarity samples, and only requires a small sample. The test method for antioxidant activity against DPPH radicals was found to be the most effective and efficient among the three test methods, while the FIC method was the least effective and efficient because of its sensitivity, which is very low and the strength is less than 20% (Maesaroh, Kurnia and Al Anshori, 2018).

The active substances contained in propolis were extracted by maceration extraction using several polar solvents. This is because of the polar nature of the flavonoids; therefore, the solvent used was also polar. The solvent used was 96% ethanol. (Hakim & Saputri, 2020). The activity of antioxidant compounds is visually indicated by a change in color from purple to yellow (Shekhar, 2014).

A mixture of propolis solution and DPPH solution at a concentration of 25 ppm formed a faded purple color, then at concentrations of 50 and 75 the color changed from purple to faded yellow, while at a concentration of 100 ppm a thick yellow color was seen. The mixture of vitamin C solution and DPPH solution also changed color from deep purple to dark yellow, which was directly proportional to the high concentration of propolis extract. This change was caused by the reduction in absorbance of the DPPH molecule. The color changes according to the number of captured electrons. Free radicals that do not have an electron pair are purple. The color change is due to the bond between the DPPH electrons and hydrogen atoms, which indicates the ability of antioxidants to capture free radicals, which is directly proportional to the increase in the concentration of propolis extract. (Karim, Jura and Sabang, 2015) In measure the absorbance value of all solutions, starting from the blank solution, propolis extract, and Vitamin C, the absorbance value was measured using UV-Vis spectrophotometry. After setting the UV-Vis spectrophotometer at a wavelength of 517 nm (maximum length of DPPH), the absorbance of the blank solution (DPPH) was measured, propolis extract solution at concentrations of 25, 50, 75,100 ppm, and Vitamin C solution at concentrations of 1, 2, 4, 6, and 8 ppm. The following is a linear regression equation for the antioxidant activity of a solution of propolis extract and Vitamin C.

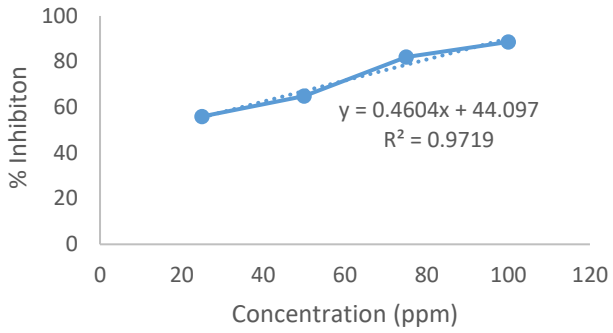

Figure 1. Antioxidant activity of *H. fimbriata* propolis extract

After obtaining the linear regression equation, the IC_{50} value of propolis **Figure 1** and vitamin C **Figure 2** is 95.884 ppm and 9.296 ppm vitamin C has a very strong antioxidant value because vitamin C is a natural antioxidant compound that is often used as a comparator compound in testing antioxidant activity.

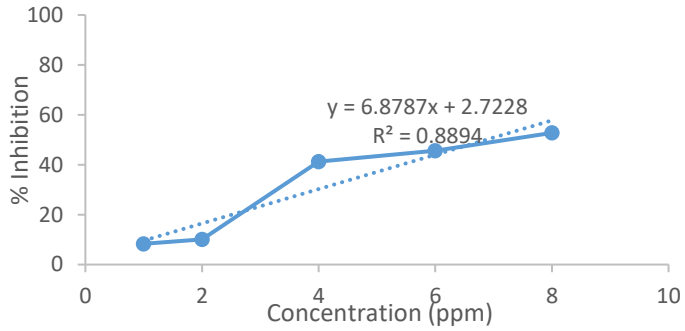

Figure 2. Antioxidant Activity of Vitamin C

Natural antioxidant compounds from stingless bee products are relatively safe and do not cause toxicity (Kustiawan *et al.* 2021). Different species exhibit different antioxidant activities (Kegode, Ndungu and Kiatoko, 2023). This is influenced by the preference of bees to collect certain types of plant resin.

CONCLUSION

The propolis extract of the kelulut bee (*Homotrigona fimbriata*) is suspected to contain positive alkaloids, flavonoids, saponins and tannins after a phytochemical test using the test tube method. IC_{50} propolis extract of 95.884 µg/ml based on the blois classification shows that propolis extract has strong antioxidants

ACKNOWLEDMENT

We thank the Institute for Research and Community Service, Universitas Muhammdiyah Kalimantan Timur, who funded this research through a Lecturer and Student Collaborative Research Grant.

DAFTAR PUSTAKA

Alfian, R. and Susanti, H. (2012) 'Penetapan Kadar Fenolik Total Ekstrak Metanol Kelopak Bunga Rosella Merah (Hibiscus sabdariffa Linn) Dengan Variasi Tempat Tumbuh Secara Spektrofotometri', *Pharmaciana*, 2(1). Available at:

https://doi.org/10.12928/pharmaciana.v2i1.655.

Anastasia, D.S.W. et al. (2017) 'Uji Fitokimia dan Aktivitas Antioksidan dari Saurauia Bracteosa Ekstrak Saurauia Bracteosa (Saurauia Bracteosa Dc.)', *Pharmacon*, 6(1), pp. 53–61.

Anjum, S.I. *et al.* (2019) 'Composition and functional properties of propolis (bee glue): A review', *Saudi Journal of Biological Sciences*, 26(7), pp. 1695–1703. Available at: https://doi.org/10.1016/j.sjbs.2018.08.013.

Arnanda, Q.P. and Nuwarda, R.F. (2019) 'Penggunaan Radiofarmaka Teknisium-99M Dari Senyawa Glutation dan Senyawa Flavonoid Sebagai Deteksi Dini Radikal Bebas Pemicu Kanker', *Farmaka Suplemen*, 14(1), pp. 1–15.

Aziz, A., Yuliawan, V. N., and Kustiawan, P. M. (2021). Identification of secondary metabolites and antibacterial activity of non-polar fraction from Heterotrigona itama propolis. *Journal of Fundamental and Applied Pharmaceutical Science*, *2*(1), 23-33. Available at: https://doi.org/10.18196/jfaps.v2i1.12406

Bankova, V., Popova, M. and Trusheva, B. (2014) 'Propolis volatile compounds: Chemical diversity and biological activity: A review', *Chemistry Central Journal*, 8(1), pp. 1–8. Available at: https://doi.org/10.1186/1752-153X-8-28.

Batistuta, M.A., Aulia, A. and Kustiawan, P.M. (2021) 'Potensi Aktivitas Anti Virus dari Produk Alami Lebah Kelulut', *Jurnal Farmasi Udayana*, 10(2), pp. 144–148. Available at: https://doi.org/10.24843/JFU.2021.v10.i02.p06

Chairunnisa, S., Wartini, N.M. and Suhendra, L. (2019) 'Pengaruh Suhu dan Waktu Maserasi terhadap Karakteristik Ekstrak Daun Bidara (Ziziphus mauritiana L.) sebagai Sumber Saponin', *Jurnal Rekayasa Dan Manajemen Agroindustri*, 7(4), p. 551. Available at: https://doi.org/10.24843/jrma.2019.v07.i04.p07.

Chan, G.C.F., Cheung, K.W. and Sze, D.M.Y. (2013) 'The immunomodulatory and anticancer properties of propolis', *Clinical Reviews in Allergy and Immunology* [Preprint]. Available at: https://doi.org/10.1007/s12016-012-8322-2.

Damanis, F.V.M., Wewengkang, D.S. and Antasionasti, I. (2020) 'PHARMACON – Program Studi Farmasi, Fmipa, Universitas Sam Ratulangi, Volume 9 Nomor 3 Agustus 2020', 9, pp. 464–469.

Fatoni, A.M.I.N. *et al.* (2008) 'Antibacterial Activity of Propolis Produced by Trigona spp. Against Campylobacter spp.', *HAYATI Journal of Biosciences*, 15(4), pp. 161–164. Available at: https://doi.org/10.4308/hjb.15.4.161.

Hakim, A.R. and Saputri, R. (2020) 'Narrative Review: Optimasi Etanol sebagai Pelarut Senyawa Flavonoid dan Fenolik', *Jurnal Surya Medika*, 6(1), pp. 177–180. Available at: https://doi.org/10.33084/jsm.v6i1.1641.

Huang, S. *et al.* (2014) 'Recent advances in the chemical composition of propolis', *Molecules*, 19(12), pp. 19610–19632. Available at: https://doi.org/10.3390/molecules191219610.

Julfitriyani, Runtuwenw, M.R. and Wewengkang, D. (2016) 'Uji Aktivitas Antioksidan Dan Toksisitas Ekstrak Etanol Daun Foki Sabarati (Solanum Torvum)', *PHARMACON Jurnal Ilmiah Farmasi-UNSRAT*, 5(3), pp. 94–101.

Karim, K., Jura, M.R. and Sabang, S.M. (2015) 'Antioxidant Activity Test of Patikan Kebo (Euphorbia hirta L.)', *Jurnal Akademik Kimia*, 4(2), pp. 56–63.

Kegode, T.M., Ndungu, N. and Kiatoko, N. (2023) 'Determination of total flavonoids, phenolics, and antioxidant activity of propolis from six stingless bee species in Kenya', *JSFA Reports*, 3(9), pp. 441–446. Available at: https://doi.org/https://doi.org/10.1002/jsf2.147.

Khairunnisa, K., Mardawati, E. and Putri, S.H. (2020) 'Karakteristik Fitokimia dan Aktivitas Antioksidan Ekstrak Propolis Lebah Trigona Sp', *Jurnal Industri Pertanian*, 2(1), pp. 124–129.

Kustiawan, P.M. *et al.* (2021) 'A review of botanical characteristics, chemical composition, pharmacological activity and use of scorodocarpus borneensis', *Biointerface Res. Appl. Chem*, 12(6), pp. 8324–8334. Available at:

https://doi.org/10.33263/BRIAC126.83248334

Maesaroh, K., Kurnia, D. and Al Anshori, J. (2018) 'Perbandingan Metode Uji Aktivitas Antioksidan DPPH, FRAP dan FIC Terhadap Asam Askorbat, Asam Galat dan Kuersetin', *Chimica et Natura Acta*, 6(2), p. 93. Available at: https://doi.org/10.24198/cna.v6.n2.19049.

Mahani, Rokim, A Karim, N.N. (2011) 'Keajaiban Propolis Trigona', in *1*. 1st edn. Jakarta: Pustaka Bunda, p. 90.

Meidhiyanto, R., Uddin, I. and Sofia, S.N. (2016) 'Hubungan Jumlah Leukosit Terhadap Kadar Troponin I Pada Pasien Infark Miokard', *Jurnal Kedokteran Diponegoro*, 5(4), pp. 1545–1551.

Phaniendra, A., Jestadi, D.B. and Periyasamy, L. (2015) 'Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases', *Indian Journal of Clinical Biochemistry*, 30(1), pp. 11–26. Available at: https://doi.org/10.1007/s12291-014-0446-0.

Rahmawati, R., Muflihunna, A. and Sarif, L.M. (2016) 'Analisis Aktivitas Antioksidan Produk Sirup Buah Mengkudu (Morinda citrifolia L.) Dengan Metode Dpph', *Jurnal Fitofarmaka Indonesia*, 2(2), pp. 97–101. Available at: https://doi.org/10.33096/jffi.v2i2.177.

Riendriasari, S.D. and Krisnawati, K. (2017) 'Produksi Propolis Mentah (Raw Propolis) Lebah Madu Trigona Spp Di Pulau Lombok', *ULIN: Jurnal Hutan Tropis*, 1(1), pp. 71–75. Available at: https://doi.org/10.32522/ujht.v1i1.797.

Rosana, M., Ahwan, A. and Qonitah, F. (2021) 'Uji Kualitatif Aktivitas Antioksidan Ekstrak Etanol Propolis', *Lumbung Farmasi: Jurnal Ilmu Kefarmasian*, 2(2), p. 154. Available at: https://doi.org/10.31764/lf.v2i2.5499.

Segueni, N. *et al.* (2016) 'Flavonoids from Algerian propolis', *Arabian Journal of Chemistry*, 9, pp. S425–S428. Available at: https://doi.org/10.1016/j.arabjc.2011.05.013.

Shekhar, T.. (2014) 'Antioxidant Activity by DPPH Radical Scavenging Method of Ageratum conyzoides Linn. Leaves', *Orient* [Preprint].

Wagh, V.D. (2013) 'Propolis: A wonder bees product and its pharmacological potentials', *Advances in Pharmacological Sciences*, 2013. Available at: https://doi.org/10.1155/2013/308249.

Wijaya, H., Novitasari and Jubaidah, S. (2018) 'Perbandingan Metode Ekstraksi Terhadap Rendemen Ekstrak Daun Rambui Laut (Sonneratia caseolaris L. Engl)', *Jurnal Ilmiah Manuntung*, 4(1), pp. 79–83.