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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF HONEY *Trigona* sp IN NORTH LOMBOK DISTRICT

Erwan[*1)], IKG. Wiryawan[1)], Syamsuhaidi[1)], DK. Purnamasari[1)], Sumiati[1)], M. Muhsinin[1)]
[1)] Faculty of Animal Husbandry, University of Mataram, Jalan Majapahit No. 62-Mataram-NTB, Indonesia

*Corresponding author: apiserwan@gmail.com

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ABSTRACT

Honey is known to contain several chemical compounds that have functions as an antibacterial, anti-inflammatory, wound healing, anticancer, and antioxidant. Honey has antibacterial effectiveness against S. aureus, S. typhi, and E. coli bacteria and has high antioxidant levels. This study focused on exploring the antibacterial and antioxidant activity of Trigona sp honey cultivated in North Lombok Regency, NTB, and Apis cerana and Apis dorsata honey as comparisons. The results showed that the three types of honey had antibacterial activity by forming various inhibition zones. These results also show that Trigona, A. cerana and A. dorsata honey are very potential to be consumed for various purposes, especially to improve health and immunity during the Covid-19 pandemic.

***Key words**: Trigona sp, apis cerana, apis dorsata, antibacterial, antioxidant*

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INTRODUCTION

Stingless bees (*Trigona* sp) are bees that do not have a sting. Morphologically the *Trigona* sp the same as other bees, namely members of the class *Insecta* and family *Apidae*. Bee *Trigona* sp live in tropical and subtropical areas of the world and are social (live in groups). The main product produced by bees *Trigona* sp are honey, pollen, propolis and wax (Kwapong et al., 2010). The advantages of the bee *Trigona* sp can be used as food crop pollinators (Giannini et al., 2015) and in Australia the bee *Trigona* sp has been used as a pollinator for macadamia nuts, mangoes and watermelons (Dollin et al., 2015). In addition, other advantages possessed by *Trigona* sp are having high adaptability to the environment, does not require a large area for cultivation and is not easy to escape from the nest. The advantages of *Trigona* sp this causes people in North Lombok Regency to cultivate *Trigona* sp intensively and proven to be able to increase people's income.

Request for *Trigona* sp honey from North Lombok district continues to increase every year. This is because this honey has a very specific taste and aroma, so it is very popular with consumers. Besides containing food substances that are very complete, making honey a source of energy that nourishes the body. The increase in demand for honey is also due to the honey produced having good quality, increasing public awareness about the efficacy of honey that can cure various diseases, as a raw material for making cosmetics and the many types of food and beverages circulating lately that are added with honey such as milk + honey, honey-flavored juice to the possibility of mineral water + honey.

Several minerals are contained in honey such as magnesium, potassium, potassium, sodium, chlorine, sulfur, iron and phosphate. Honey also contains vitamins, such as vitamin E and vitamin C as well as vitamins B1, B2 and B6 (Winarno, 2002). In addition, honey also contains antibiotic substances that are useful for fighting pathogenic bacteria that cause infectious diseases. This is because the growth of some microorganisms associated with disease or infection can be inhibited by honey (Willix et al., 1992). According to (Mundo et al., 2004), honey can inhibit the growth of pathogenic bacteria such as *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. This can be seen from the zone of inhibition produced by honey given to the media that has been planted with these bacteria. In addition, honey can also inhibit the damage of packaged turkey meat that has been done by Antony et al., (2006). By adding honey in a certain concentration, packaged turkey pieces have a longer shelf life than packaged turkey pieces without the addition of honey. From the two results of this study, it can be seen that honey can function as an antibacterial.

From some of this information, research on the antibacterial and antioxidant activity of *Trigona* sp needs to be done to prove the benefits of *Trigona* sp honey for health. The purpose of this study was to determine the antibacterial and antioxidant activity of *Trigona* sp which is cultivated in North Lombok Regency, NTB Indonesia and as a reference to support the superior product innovation cluster of North Lombok Regency.

MATERIALS AND METHODS

Sample Collection

The sample that will be used in this study is *Trigona* sp honey and as a comparison is Apis cerana dan Apis dorsata honey taken from a group of bees in North Lombok Regency.

Antibacterial Activity

The antibacterial activity test of honey was carried out using the good diffusion method (Garriga et al., 1993). This antibacterial test will use four types of bacteria including *S. aureus, S. typhi* and *E. coli* bacteria. In the first step, all the tools used were sterilized by wrapping the tools to be used with paper and sterilized in an autoclave at a temperature of 121 ^{o}C with a pressure of 15 psi (per square inch) for 15

minutes. The regeneration of bacteria used was, one needle of bacterial culture from the agar isolate was inserted into 7.5 mL of TSB and incubated at 37 ^{o}C for ± one night. In addition, TSA solid media was heated until it melted, cooled to a temperature of ±40 ^{o}C. Next, put the regenerated bacteria into the TSA with a percentage of 1%, homogenized, poured into a petri dish and allowed to solidify.

After the test culture media is solid, then insert the paper disc that has been given honey infiltration which has been diluted into several concentrations with a diameter of 6 mm above the surface of the bacterial media using tweezers and slightly pressed. The infiltration process is carried out by dripping ± 20 µl of antibacterial material (Zakaria et al., 2007). The concentrations used are 100%, 50% and 25%. After that, the media was incubated for 18-24 hours at 37 ^{o}C in an incubator. Then the diameter of the clear zone formed on the media was measured using a caliper on various sides.

The concentration of the honey solution is made by using water as a solvent because water is polar and honey is polar, so honey can dissolve well in water. In addition, the use of water was chosen to avoid bacterial death due to solvent toxicity.

The presence of antibacterial activity in these honey was indicated by the formation of a clear zone around the paper disc which was given absorption of honey with various predetermined concentrations. Based on the measurement of the clear zone formed, we can determine the antibacterial power of each honey, which is classified based on the criteria of antibacterial strength by (Davis and Stout, 1971) in (Dewi, 2010), including the inhibition zone formed above 20 mm (antibacterial resistance). Categorized as very strong), 10-20 mm (categorized as strong), 5-10 mm (categorized as moderate) and below 5 mm (categorized as weak).

Antioxidant Activity

Antioxidant testing using the DPPH method is a simple method for determining the antioxidant content of a material by using 1, 1-diphenyl-2-picrylhydrazil (DPPH) as a detecting compound (Hanani et al., 2005). This method was chosen because it is a simple method, already standardized). and requires a small sample as a detecting compound Asih et al., (2012). Kedare and Singh (2011) said that DPPH is a free radical compound that is stable and can react with hydrogen atoms derived from the antioxidants of a material to form reduced DPPH. Tests were carried out at concentrations of 500 ppm, 250 ppm, 125 ppm and 62.5 in a microplate. The ratio of honey solution with DPPH solution in this test is 1:1. The total solution in the test container was 200 L consisting of 100 L of extract solution and 100 L of DPPH solution (125 M in ethanol). Giving a honey solution of 1,000 g/mL will produce a concentration of honey in the microplate of 500 g/mL. Negative control was made by mixing 100 L of ethanol with 100 L of DPPH solution. After being homogeneous, the test container containing the solution was incubated in the dark for 30 minutes and the light absorption was measured with an elisa reader at max 517 nm. Antioxidant activity was determined by calculating the percentage of DPPH free radical scavenging by the extract with the formula:

$$\% \text{ inhibition} = \{(A-B)/A\} \times 100\%$$

Information:
A= negative control absorption (without honey)
B = absorption of honey

The inhibition data obtained were then processed using the regression equation obtained from the relationship between honey concentration and the percent inhibition to determine the effective concentration at the level of 50% (EC50).

RESULTS AND DISCUSSION

Honey's Antibacterial Activity

Testing the antibacterial activity of *Trigona* honey was intended to determine how much inhibition of *Trigona* honey was

on the growth of *S. aureus, S. typhi* and *E. coli* bacteria. As a comparison, antibacterial activity was also tested on Cerana and Dorsata honey, as well as testing using a negative control (-). The results of antibacterial testing of *Trigona*, cerana and dorsata honey against S. aureus, S. typhi and E. coli bacteria are presented in Table 1.

Table 1. Antibacterial Test Results of *Trigona*, Cerana, and Dorsata Honey against S. aureus, S. typhi and E. coli bacteria.

Honey Type	Repeat	Antibacterial Activity (cm)		
		S. Aureus	*S. Typhi*	*E. Coli*
Control (-)		-	-	-
Trigona	1	-	2.9	1.9
	2	-	2.6	1.8
	3	-	2.6	1.7
Average		-	2.7	1.8
Cerana	1	-	2.4	1.7
	2	-	2.4	1.9
	3	-	2.0	1.7
Average		-	2.3	1.8
Dorsata	1	0.7	3.0	0.3
	2	0.2	3.0	0.3
	3	0.4	2.6	0.3
Average		0.4	2.9	0.3

Based on the antibacterial test results of honey at a concentration of 100%, it appears that *Trigona* and cerana honey has no antibacterial activity against gram-positive bacteria *S. aureus*, while dorsata honey has antibacterial activity with the formation of an inhibition zone ranging from 0.2-0.7 cm with an average of 0.2-0.7 cm. an average of 0.4 cm or 4.3 mm but still in the weak category, namely below 5 mm (Davis and Stout, 1971; Dewi, 2010) Against gram-negative bacteria *S. typhi*, the three types of honey have antibacterial activity by forming various inhibition zones.

The antibacterial activity of *Trigona* honey forms an inhibition zone ranging from 2.6 to 2.9 cm or an average of 2.7 cm or 27 mm, honey cerana ranges from 2.0 to 2.4 cm or an average of 2.3 cm or 23 mm and the widest zone of inhibition on the antibacterial activity of dorsata honey was 2.6-3.0 cm or an average of 2.9 cm or 29 mm. The three types of honey have an antibacterial activity which is categorized as very strong (inhibition zone > 20 mm) (Davis and Stout, 1971; Dewi, 2010). On the other hand, the antibacterial activity of dorsata honey against gram-negative bacteria E coli is classified as weak because the inhibition zone formed is below 5 mm, namely 0.3 cm or 3.0 mm (Davis and Stout, 1971; Dewi, 2010).

Trigona and cerana honey have the same antibacterial activity against E coli bacteria with an average inhibition zone of 1.8 cm or 18 mm and are classified as strong (10-20 mm) (Davis and Stout, 1971; Dewi, 2010). The antibacterial activity of *Trigona*, Apis cerana and Apis dorsata honey as well as the inhibition zone formed can be seen in Figure 1.

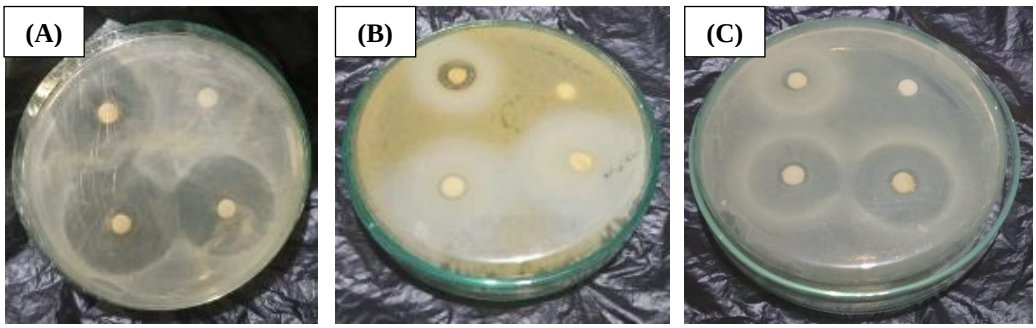

Figure 1. Antibacterial activity of *Trigona*, Apis cerana, and Apis dorsata honey against (A) S. aureus, (B) S. typhi, and (C) E coli bacteria

Honey is a sweet liquid produced by worker bees with raw materials derived from plant nectar or extrafloral nectar containing glucose, fructose, vitamins, minerals and other nutrients which are the main energy source in the colony (Kwapong et al., 2010; Roubik et al., 2013; Roubik, 1989; Sihombing, 2005). Honey is divided into several types based on the flower essences taken by the bees, including kapok flower honey, longan flower honey, rambutan flower honey, multiflora honey, forest honey, mahogany flower honey and others (Yunianto, 2010).

Honey produced by *Trigona* sp has a sweet taste mixed with a sour taste, thus distinguishing it from honey produced by bees of the Apis genus with a sweet taste. Honey bee *Trigona* sp. wrapped in propolis stored in pots by forming lumps or piles of granules. Bee *Trigona* sp. is a group of bees that do not have a stinger 191, small body size, can produce honey even though the production is lower than the Apis genus but the production of pollen and propolis is the highest (Michener, 2007) so that this type of bee is preferred by the community for cultivation.

Products produced by bees *Trigona* sp includes honey, pollen, and propolis which enclose a mixture of pollen and nectar along with the saliva and enzymes present in the worker bee's mouth, which is beneficial to human health. Pollen is the main source of protein, vitamins, minerals, and other nutrients needed by bees (Michener, 2007; Sihombing, 2005). Pollen contains 15-30% protein, while nectar contains 50% sugar and other compounds such as lipids, amino acids, minerals, and aromatic compounds (Schoonhoven et al., 2005). Propolis is known to have antibacterial, antifungal, antiviral, and anti-biological activities such as anti-inflammatory, local anesthetic, hepatoprotective, antitumor, and immunostimulating properties (Bankova et al., 2000).

Cerana bee honey has a sweeter taste and a darker yellow color than *Trigona* honey. The smell, taste, and color of honey are influenced by the dominant source of nectar collected by bees (Pusbahnas, 2008). The honey bee *A. cerana* is categorized as a commercial local bee, small in stature, has the nature of easy to move from the nest when disturbed, is more resistant to pests and predators, can adapt to the tropics, and is more efficient in collecting nectar from thousands of scattered plants (Lewis, 2021; Saepudin et al., 2011). The cerana bee can roam in search of food less than a radius of 1 km (Hitaj et al., 2018; Saepudin et al., 2011). A. cerana is less favored by the community for breeding because it has a vicious and stinging nature.

Unlike the *Trigona* and cerana bees, the dorsata bee is a type of forest bee that is often referred to as a giant honey bee which until now scientists have not succeeded in cultivating, this is because the Asian honey bee lives in the forest and makes a nest with only one comb. hanging from tree branches and branches, open ceilings, and rocky cliffs. In addition, this forest honey bee is large, which is twice the size of the cerana bee and is wild. Different types of bees,

habitats, and food sources will affect the smell, color, taste, and efficacy of the honey produced, especially the antibacterial activity of honey.

The mechanism of antibacterial action is divided into four ways, namely (1) the mechanism of action of antibacterial through inhibition of cell wall synthesis, (2) mechanism of antibacterial action through cell membranes, (3) mechanism of action of antibacterial through protein synthesis, and (4) mechanism of action of antibacterial through inhibition of synthesis. Nucleic acids (Huda, 2013; Mietzner, 2017).

Based on the results of antibacterial testing, *Trigona* sp honey and cerana honey did not have an activity to inhibit S. aureus bacteria, while dorsata honey had inhibitory activity even though it was classified as weak. S. aureus is a Gram-positive bacterium with a spherical shape with a diameter of 0.7-1.2 m, arranged in irregular clusters like grapes, facultative anaerobes, not spore-forming, and immobile. These bacteria grow at an optimum temperature of 37 ^{o}C, but form pigments best at room temperature (20-25 ^{o}C) (Mietzner, 2017). Staphylococcus aureus is one of the largest infectious bacteria in the world. The severity of the infection also varies, ranging from minor skin infections (furunculosis and impetigo), urinary tract infections, respiratory tract infections, to the eye and Central Nervous System (CNS) infections (DeLeo et al., 2010).

According to Mietzner (2017), staphylococcus bacteria have different sensitivities to antibacterials, many influencing factors include that S aureus is one of the bacteria that can produce lactamase enzymes. This enzyme will eliminate antibacterial power, especially penicillin groups such as methicillin, oxacillin, penicillin G and ampicillin. The presence of these enzymes will damage the -lactam ring so that antibiotics become inactive, so it is suspected that this is the cause of antibacterial in *Trigona* and cerana honey not being able to inhibit the growth of S. aureus bacteria, even if it can be inhibited by antibacterial dorsata honey but its strength is weak.

The antibacterial power of honey has different activities against various microorganisms, the activity against infected wounds will depend on the osmolarity and acidity of honey (Nuriza et al., 2005; Rajeswari et al., 2010). This is related to the content of hydrogen peroxide and phenolic compounds sourced from flower nectar. The ability to fly and the flexibility to choose feed and absorb nutrients that are wider and varied in the forest causes the honey produced to have a higher quality than the honey produced by cultivated bees such as *Trigona* and cerana bees. Based on the results of research Huda (2013) showed that there was the antibacterial activity of Rawas Forest honey against *S. aureus* bacteria at a concentration of 10% to 100% with an inhibition zone of 10.60 mm to 31.60 mm. It was further stated that the high sugar content of honey was able to inhibit the growth and development of bacteria, due to osmotic pressure. The strong interaction between water and sugar molecules leaves little water molecules available for bacteria, if the water decreases drastically the bacteria will lose their ability to live (Huda, 2013; Zulhawa et al., 2014).

The three types of honey have antibacterial activity against *S. typhi* bacteria with the formation of an inhibition zone. The widest zone of inhibition resulted from the antibacterial activity of Dorsata honey. S. typhi is a gram-negative rod-shaped bacterium, has an outer membrane component composed of lipopolysaccharide, and can function as endotoxin, and is a bacterium that causes typhoid fever (Hudri, 2014; Sri, 2009) which is a systemic infectious disease with long-lasting fever and accompanied by inflammation that can damage the intestines and liver. As reported by (Hanani et al., 2005) in recent years, antibiotic resistance has emerged in several bacterial strains that threaten public health. S. typhi bacterial strains include strains that are resistant to many types of antibiotic drugs (Hanani et

al., 2005) including ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracyclines. The results of the study stated that honey with concentrations of 90 and 100% inhibited the growth of MDR S. typhi bacteria by forming inhibition zones of 11.4 mm and 13.4 mm, respectively.

The antibacterial activity of *Trigona* and cerana honey against E coli bacteria are included in the strong category (10-20 mm) (Davis and Stout, 1971 in Dewi, 2010). However, the antibacterial activity of Dorsata honey against E coli bacteria is in the weak category (< 5 mm) in the strong category (10-20 mm) (Davis & Stout, 1971 in Dewi, 2010). Escherichia coli is a gram-negative enteric bacterium that is large, rod-shaped, non-sporing, and lives in the digestive tract of humans and animals. Usually *E coli* attacks the organs of the digestive tract which causes acute diarrhea in children under 2 years old and causes infections in the urinary tract and causes meningitis in premature and neonatal infants (Samapta and Sagiran, 2003).

According to (Huda, 2013; Willix et al., 1992), the ability of honey to inhibit the growth of E. coli bacteria is due to the presence of the enzyme glucose oxidase which can increase its antibacterial ability by converting glucose in honey into glyconic acid and hydrogen peroxide. Hydrogen peroxide can denature proteins and inhibit the synthesis or function of nucleic acids present in E. coli bacteria. In addition, it can not be separated from the content of active compounds contained in it. According to Mietzner (2017), the mechanism of the active compounds in honey resembles several ways of working antibacterial in general which are divided into four ways. Several suitable means such as inhibition of protein and nucleic acid synthesis. In addition, it is also influenced by several factors, namely osmolarity, pH, the activity of peroxide and non-peroxide compounds, flavonoid compounds, essential oils, and various other organic compounds, as well as antibacterial properties as well. Influenced by the effect of high osmolarity, low water activity, low pH so that the acidity of honey becomes high (Huda, 2013). Mietzner (2017) added that the antibacterial properties of honey are influenced by the presence of a lysozyme-like compound that has an antibacterial activity which is now known as inhibin, in which various microbes are very sensitive to inhibin. The level of lysozyme in honey is very dependent on the type, age, and condition of the honey.

Antioxidant Level

Increased production of free radicals that are formed due to factors such as air pollution, environment, stress, and UV radiation results in an inadequate body defense system, so a supply of antioxidants from outside the body is needed. Honey is an alternative source of antioxidants that are obtained in a natural and safe form which is needed to balance the production of antioxidants in the body. The test results of antioxidant levels from *Trigona*, cerana, and dorsata honey are presented in Table 2.

Table 2. Percentage of antioxidant levels of *Trigona*, cerana, and dosata honey

Sample	Repeat	Antioxidant Level (%)	Average (%)
Trigona Honey	1	69.93	70.02
	2	70.11	
Cerana Honey	1	74.02	74.91
	2	75.80	
Dorsata Honey	1	80.60	81.23
	2	81.85	

Based on the antioxidant level test results, on average, *Trigona* honey has the lowest antioxidant content (70.02%) compared to Cerana honey (74.91%) and the highest is Dorsata honey (81.23%). The higher antioxidant content of Dorsata forest honey than cultivated honey is because the Dorsata bee is a forest bee with varied feed sources and the ability to fly Dorsata honey which is farther (> 1 km) than cultivated honey (< 1 km) (Hitaj et al., 2018; Saepudin et al., 2011) so that Dorsata honey consumes feed and absorbs more varied nutrients than *Trigona* and Cerana honey. Parwata et al., 2010) also confirmed that honey has different chemical compositions based on its nectar feed source. These differences are thought to affect differences in the antioxidant levels of honey. The antioxidant activity of the honey isolate was expressed in terms of the percentage of its inhibition against DPPH radicals. This percentage of inhibition was obtained from the difference in absorption between the absorbance of DPPH and the absorbance of the sample as measured by a UV-Vis spectrophotometer. The magnitude of the antioxidant activity is indicated by the IC_{50} value, which is the concentration of the sample solution to inhibit 50% of DPPH free radicals.

Table 3. Results of measurement of absorbance, percentage of inhibition, and IC_{50} values of honey and quercetin as blanks

No	Sample	Sample Concentration (µg/ml)	Absorbance	% inhibition	Equality Y=bx+a	IC_{50} (µg/ml)
1	Control	2.0	0.504	10.32		
2	Quercetin	4.0	0.405	27.94		
3		6.0	0.301	46.44		8.18
4		8.0	0.185	67.08		
5		10.0	0.093	83.45	Y = 7.54x+11.62	
6	Honey	2.0	0.552	1.78	R^2 = 0.999	
7		4.0	0.475	15.48		
8		6.0	0.355	36.83		6.76
9		8.0	0.198	64.77	Y = 9.902x+16.994	
10		10.0	0.109	80.6	R^2 = 0.987	

Based on Table 3, the IC_{50} value of honey isolate (6.76 g/ml) was lower than that of quercetin (8.18 g/ml), which means that the smaller the IC_{50} value, the smaller the concentration of the solution used to inhibit 50% of DPPH free radicals. This shows that honey has a stronger ability to reduce DPPH free radicals than quercetin. However, honey and quercetin have strong activity because they have an IC_{50} value of less than 200 g/ml (Wahdaningsih and Setyowati, 2011).

Honey has antioxidant compounds because honey contains vitamin C, organic acids, enzymes, phenolic acids, flavonoids, and beta carotene which are useful as high antioxidants and have antioxidant activity (Gheldof et al., 2002; Giorgi et al., 2011; Parwata et al., 2010). Phenolic compounds, flavonoids, and vitamin C can donate hydrogen atoms to DPPH free radicals to form stable reduced DPPH compounds (DPPH-H). The higher the phenolic, flavonoid, and vitamin C content, the more DPPH free radicals react so that the concentration decreases, which means the higher the antioxidant activity (Adawiah et al., 2016).

CONCLUSION

From the results of the research on the antibacterial and antioxidant activity of honey, it can be concluded that *Trigona*, cerana, and dorsata honey have an antibacterial activity which is classified as very strong against S. aureus, S. typhi, and E. coli bacteria. *Trigona* honey antioxidant levels are lower than cerana honey and dorsata honey.

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REFERENCES

Adawiah A, D. Sukandar, and A. Muawanah. 2016. Aktivitas Antioksidan dan Kandungan Komponen Bioaktif Sari Buah Namnam. *Jurnal Kimia VALENSI*. 1(2): 130-136. https://doi.org/10.15408/jkv.v0i0.3155

Samapta and Sagiran. 2003. Efek Antibakterisid Madu terhadap Escherichia coli. *Mutiara Medika: Jurnal Kedokteran Dan Kesehatan*. 3(1): 28–33. DOI: https://doi.org/10.18196/mmjkk.v3i1.1544

Antony S, J. R. Rieck, J. C. Acton, I. Y. Han, E. L. Halpin and P. L. Dawson. 2006. Effect of Dry Honey on the Shelf Life of Packaged Turkey Slices1. *Poultry Science*. 85(10): 1811–1820. https://doi.org/https://doi.org/10.1093/ps/85.10.1811

Asih I. A. R., K. Ratnayani, and I. B. Swardana. 2012. Isolasi Dan Identifikasi Senyawa Golongan Flavonoid Dari Madu K Elengkeng (Nephelium longata L.). *Jurnal Kimia (Journal of Chemistry)*. 6 (1):72-78.

Bankova V. S., S. L. de Castro, and M. C. Marcucci. 2000. Propolis: recent advances in chemistry and plant origin. *Apidologie*. 31(1): 3-15. https://doi.org/10.1051/apido:2000102

Davis W. W., and T. R. Stout. 1971. Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. *Applied Microbiology*. 22(4), 659–665. https://pubmed.ncbi.nlm.nih.gov/5002143

DeLeo F. R., M. Otto, B. N. Kreiswirth, and H. F. Chambers. 2010. Community-associated meticillin-resistant Staphylococcus aureus. *Lancet (London, England)*. 375(9725): 1557–1568. https://doi.org/10.1016/S0140-6736(09)61999-1

Dewi F.K. 2010. *Aktivitas antibakteri ekstrak etanol buah mengkudu (morinda citrifolia, linnaeus) terhadap bakteri pembusuk daging segar*. Skripsi. Universitas Sebelas Maret.

Dollin A. E, L. J. Dollin, and C. Rasmussen. 2015. Australian and New Guinean Stingless Bees of the Genus Austroplebeia Moure (Hymenoptera: Apidae)-a revision. *Zootaxa*. 4047: 1-73. https://doi.org/10.11646/zootaxa.4047.1.1

Garriga M., M. Hugas, T. Aymerich, and J. M. Monfort. 1993. Bacteriocinogenic activity of lactobacilli from fermented sausages. *The Journal of Applied Bacteriology*. 75(2): 142–148. https://doi.org/10.1111/j.1365-2672.1993.tb02759.x

Gheldof N, X.H. Wang, and N. J. Engeseth. 2002. Identification and quantification of antioxidant components of honeys from various floral sources. *Journal of Agricultural and Food Chemistry*. 50(21): 5870–5877. https://doi.org/10.1021/jf0256135

Giannini T. C., S. Boff, G. D. Cordeiro, E. A. Cartolano, A. K.Veiga, V. L. Imperatriz-Fonseca, and A. M. Saraiva. 2015. Crop pollinators in Brazil: a review of reported interactions. *Apidologie*. 46(2): 209–223. https://doi.org/10.1007/s13592-014-0316-z

Giorgi A, M. Madeo, J. Baumgartner, and G. C. Lozzia. 2011. The relationships between phenolic content, pollen diversity, physicochemical information and radical scavenging activity in honey. *Molecules (Basel, Switzerland)*. *16*(1): 336–347.

https://doi.org/10.3390/molecules16010336

Hanani E, A. im, R. Sekarini, and M. Kefarmasian. 2005. *Identifikasi Senyawa Antioksidan dalam spons Callyspongia sp dari Kepulauan Seribu*. Pharmaceutical Sciences and Research. 2(3):127–133. doi: 10.7454/psr.v2i3.3389

Hitaj C, D. Smith, and K. Hunt. 2018. *Honeybees on the move: Pollination services and honey production*. Agricultural & Applied Economics Association Annual Meeting At: Washington, D.C.

Huda M. 2013. Pengaruh Madu Terhadap Pertumbuhan Bakteri Gram Positif (S. aureus) dan Bakteri Gram Negatif (E. coli). *Jurnal Analis Kesehatan*, 2(2): 250-259. DOI: http://dx.doi.org/10.26630/jak.v2i2.437

Hudri F. A. 2014. *Uji efektifitas ekstrak madu multiflora dalam menghambat pertumbuhan bakteri Salmonella typhi*. Skirpsi. Universitas Negeri Islam Syarif Hidayatullah.

Kedare S. B and R. P. Singh. 2011. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4): 412–422. https://doi.org/10.1007/s13197-011-0251-1

Kwapong P, K. Aidoo, R. Combey, and A. Karikari. 2010. *Stingless bees "a training manual for stingless beeskeeping."* Unimax Macmillan LTD.

Lewis S.W. 2021. Eva Crane: Honey, 'A Comprehensive Survey.' *Bee World*. 98(3):1-1. https://doi.org/10.1080/0005772X.2020.1865626

Michener C. D. 2007. *The bees of the world* (2th ed.). The Johns Hopkins University Press.

Mietzner G. F. B. 2017. *Mikrobiologi Kedokteran Jawetz, Melnick & Adelberg = Jawetz, Melnick & Adelberg's Medical Microbiology* (25th ed.). Jakarta : EGC.

Mundo M. A, O. I. Padilla-Zakour, and R. W. Worobo. 2004. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. *International Journal of Food Microbiology*, 97(1): 1–8. https://doi.org/10.1016/j.ijfoodmicro.2004.03.025

Nuriza T., N. A. B. Halim, M. Zubaid, A. Khan, and S. Mohsin. 2005. Antibacterial activity of local Malaysian honey. *Malaysian Journal of Pharmaceutical Sciences*. 3(2): 1-10.

Parwata I. M. O. A, K. Ratnayani, and A. Listya. 2010. Aktivitas Antiradikal Bebas Serta Kadar Beta Karoten Pada Madu Randu (Ceiba pentandra) Dan Madu Kelengkeng (Nephelium longata L.). *Jurnal Kimia (Journal Of Chemistry)*. 4(1): 54-62.

Pusbahnas. (2008). *Lebah Madu Cara Beternak dan Pemanfaatannya*. Penebar Swadaya.

Rajeswari S., V. Thiruvengadam, and N. M. Ramaswamy. 2010. Production of interspecific hybrids between Sesamum alatum Thonn and Sesamum indicum L. through ovule culture and screening for phyllody disease resistance. *South African Journal of Botany*. 76: 252–258. https://doi.org/10.1016/j.sajb.2009.11.003

Roubik D, P. Vit, and S. Pedro. 2013. *Pot-Honey: A Legacy of Stingless Bees.* https://doi.org/10.1007/978-1-4614-4960-7

Roubik D. W. 1989. Ecology and Natural History of Tropical Bees. In *Cambridge Tropical Biology Series*. Cambridge University Press. https://doi.org/DOI: 10.1017/CBO9780511574641

Saepudin R, A. Fuah, and A. Luki. 2011. Peningkatan Produktivitas Lebah Madu Melalui Penerapan Sistem Integrasi dengan Kebun Kopi. *Jurnal*

Sain Peternakan Indonesia. 6 (2): 115–124. https://doi.org/10.31186/jspi.id.6.2.115-124

Schoonhoven L., J. van Loon, and M. Dicke. 2005. *Insect-Plant Biology*. Oxford University Press

Sihombing D. T. H. 2005. *Ilmu Ternak Lebah Madu*. UGM Press.

Darmawati S. 2009. Keanekaragaman Genetik Salmonella typhi. *Jurnal Kesehatan*, *2*(1): 27-33.

Wahdaningsih S and S. W. Setyowati. 2011. Aktifitas penangkap radikal bebas dari batang pakis (Alsophila glauca J.Sm). *Majalah Obat Tradisional*, *16*(3): 156–160.

Willix D. J, P. C. Molan and C. G. Harfoot. 1992. A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey. *The Journal of Applied Bacteriology*, *73*(5): 388–394. https://doi.org/10.1111/j.1365-2672.1992.tb04993.x

Winarno F. G. 2002. *Kimia Pangan dan Gizi*. Gramedia.

Yunianto M. 2010. *Meracik Sendiri Ramuan Herbal Nabi*. Pustaka Arafah.

Zakaria Z. A, H.Zaiton, E. F. P. Henie, A. M. Jais and E. N. H Zainuddin. 2007. In vitro antibacterial activity of Averrhoa bilimbi L. leaves and fruits extracts. *Int J Trop Med*. 2 (3)*:* 96–100. doi=ijtmed.2007.96.100

Zulhawa D. J, Maryani and N. H. Dewi. 2014. Daya Hambat Madu Sumbawa terhadap Pertumbuhan Staphylococcus aureus Isolat Infeksi Luka Operasi. *Biofarmasi*. 12(1): 40–44.