Fisheries Journal, 14 (2), 439-448 (2024)
http://doi.org/10.29303/jp.v14i2.791

STUDY OF CHEMICAL COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF KEFA FOREST HONEY STORED AT DIFFERENT TIMES AGAINST THE BACTERIA *AEROMONAS HYDROPILLA* AND VIBRIO ALGINOLITYCUS

Kajian Senyawa Kimia Dan Aktivitas Antibakteri Madu Hutan Kefa Yang Disimpan Pada Waktu Berbeda Terhadap Bakteri Aeromonas hydropilla Dan Vibrio Alginolitycus

Yuliana Salosso[1*], Aris Senjata Ndena[1], Andi Taufika Rahma[2], Asriati Djonu[1]

[1]Aquaculture Program, Nusa Cendana University, [2]Fish Quarantine Station, Quality Control and Safety of Kupang Fishery Products

*Correspondence email : yulianasalosso@staf.undana.ac.id

(Received March 8th 2024; Accepted April 22th 2024)

ABSTRACT

Forest honey is a type of polyflora honey produced by wild bees of the *Apis dorsata* type. Kefa is the capital of North Central Timor Regency as one of the forest honey producing areas. This research aims to determine the stability of the chemical compound content and antibacterial activity of kefa forest honey stored at different times, against the bacteria *Aeromonas hydropilla* and *Vibrio alginolitycus* which are pathogenic bacteria in fish farming. Tests for the chemical compound content of honey include tests for alkaloid compounds (Culvenor-Fiztgerald method), saponins (foam test), terpenes and steroids (Lieberman-Burchard method), tannins (FeCl3 addition method) and flavonoids (HCl and Mg powder reagent addition method). The spectrophotometer method is used to measure the total sugar content, the gravimetric method to measure the water content and the pH meter to measure the pH of honey. The antibacterial activity of honey was tested using the disc method without dilution. The results showed that kefa forest honey taken at different times showed the same active compound content or did not change. The longer it is stored, the sugar, water and pH content of forest honey from Kefa decreases, but the decrease is not too big. Kefa forest honey, both new and stored for 6 months and 1 year, still has antibacterial activity against the bacteria *A. hydropilla* and *V. alginolitycus*.

Keywords: *Aeromonas hydropilla*, Antibacterial, Kefa Forest Honey, Chemical Compounds, *Vibrio alginolitycus*

ABSTRAK

Madu hutan merupakan jenis madu poliflora yang diproduksi oleh lebah liar jenis *Apis dorsata*. Kefa merupakan ibu kota Kabupaten Timor Tengah Utara sebagai salah satu daerah penghasil madu hutan. Penelitian ini bertujuan untuk mengetahui kestabilan kandungan senyawa kimia dan aktivitas antibakteri madu hutan kefa yang disimpan pada waktu yang berbeda, terhadap bakteri *Aeromonas hydropilla* dan *Vibrio alginolitycus* yang merupakan

bakteri patogen pada budidaya ikan. Uji kandungan senyawa kimia madu meliputi uji senyawa alkaloid (metode Culvenor-Fiztgerald), saponin (uji busa), terpen dan steroid (metode Lieberman-Burchard), tannin (metode penambahan $FeCl_3$) serta flavanoid (metode penambahan pereaksi HCl dan Mg serbuk). Metode spectrofotometer digunakan untuk mengukur kandungan total gula, metode gravimetri untuk mengukur kandungan air dan pH meter untuk mengukur pH madu. Pengujian aktivitas antibakteri madu dilakukan dengan metode cakram tanpa pengenceran. Hasil penelitian menunjukkan bahwa madu hutan kefa yang diambil pada waktu yang berbeda menunjukkan kandungan senyawa aktif yang sama atau tidak mengalami perubahan. Semakin lama disimpan, kandungan gula, air dan pH madu hutan asal kefa mengalami penurunan, namun penurunannya tidak terlalu besar. Madu hutan asal kefa, baik yang baru maupun yang telah disimpan selama 6 bulan dan 1 tahun tetap memiliki aktivitas antibakteri terhadap bakteri *A. hydropilla* dan *V. alginolitycus.*

Kata Kunci: *Aeromonas hydropilla*, Antibakteri, Madu Hutan Kefa, Senyawa kimia*, Vibrio alginolitycus*

INTRODUCTION

East Nusa Tenggara Province is one of the provinces where there is a lot of honey production (Salosso, 2019a), especially forest honey which has the potential to be an antibacterial in fish farming. Forest honey is a type of polyflora honey produced by wild bees of the Apis dorsata type (Muslim, 2014; Salosso, 2019b). One of the forest honey producing areas in NTT is Kefa which is the capital of North Central Timor Regency.

These wild bees usually live in forests so their food source is a variety of plants that grow in the forest, so the quality of the honey produced is better because it comes from the nectar of various plants (Muslim, 2014). Forest honey produced by wild bees contains natural antibiotics produced by wild bees so it has the potential to be developed as an antibacterial for both humans and fish farming.

Aeromonas hydropilla is one of the bacteria that often attacks freshwater farmed fish (Triyaningsih *et al.,* 2014; Orsi *et al.,* 2017) which causes the disease Motile Aeromonas Septicemia or MAS (Susandi *et al.,* 2017; Rosidah *et al.,* 2019a), which can cause up to 100% mortality (Rosidah *et al.,* 2019b). Meanwhile, Vibrio alginolyticus is a bacterial pathogen in marine fish farming which can cause economic losses (Unisa *et al.,* 2017), which is known as the disease Vibriosis (Scarano *et al.,* 2014; Noerbaeti, 2016).

Lukistyowati & Kurniasi (2011), explained that *A. hydropilla* often causes disease outbreaks with high mortality rates reaching 80% - 100% in a short period of time, namely 1-2 weeks. The characteristics of goldfish affected by MAS disease are changes in skin color, lesions on the skin, bleeding and bruising or ulcers on the muscles (Salosso *et al.,* 2020; Susandi *et al.,* 2017; Latih & Najlah, 2013). Likewise, Noerbaeti (2016) explains that while still a major concern in the fishing industry, vibriosis is a serious and potentially fatal condition. This bacterium is opportunistic, can act as a primary or secondary pathogen which causes massive deaths at the hatchery and rearing stages of marine fish.

The antibacterial ability of forest honey has been proven by several previous researchers, both honey from Indonesia and other countries. For honey from Indonesia, Dewi *et al.* (2017) have proven the antibacterial activity of some native bee honey from Bandung and the Riau Islands against Staphylococcus aureus and Escherichia coli bacteria. Fadhmi *et al.,* (2015) have also proven the antibacterial activity of forest honey from Seulawa (West Aceh) and Trumon (South Aceh) against Staphylococcus aureus bacteria. For honey from other countries, Kalidasan *et al.,* (2017) have proven the antibacterial activity of honey from Chetheri Malai, Harur, Tamil Nadu, India against the bacteria Staphylococcus aureus, Micrococcus luteus,

Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella flexneri, Klebsiella pneumonia, proteus mirabilis, Pseudomonas fluorescens and Actobacter baumani. Furthermore, Hegazi *et al.* (2017) also proved the antibacterial activity of 10 types of honey from Saudi Arabia against the bacteria Staphylococcus aureus, Staphylococcus mutans, klebsiella pneumonia, Escherichia coli and Pseudomonas aeruginosa.

Salosso (2019b) has proven the antibacterial activity of several forest honeys from NTT against the bacteria *Aeromonas hydropilla* and Vibrio alginolitycus and one of the honeys that has the largest inhibition zone is forest honey from Kefa. However, to develop forest honey from kefa, there are still many things that need to be studied. One of them is the stability of the activity and compound content so that the shelf life of honey can be known which can be used as an antibacterial in fish farming. This research aims to determine the stability of the chemical compound content and antibacterial activity of kefa forest honey stored at different times, against the bacteria *Aeromonas hydropilla* and Vibrio alginolitycus.

METHODS

Place and time of research

Antibacterial tests were carried out in the microbiology laboratory of the Fish Quarantine Station, Quality Control and safety of Kupang Fishery Products and tests for the active compound content of honey were carried out in the Chemistry laboratory of the Faculty of FKIP, Nusa Cendana University, Kupang. Meanwhile, analysis of the pH, water and sugar content of honey was carried out at the Agricultural Technology Laboratory, Brawijaya University, Malang. This research was carried out in 2022.

Collection and Testing of Active Compounds in Forest Honey from Kefa

In this research, forest honey from Kefa was used which had been stored at different times. The first kefa honey has been stored for 2 weeks (K1), the second has been stored for 6 months (K2) and the third has been stored for 1 year (K3). Measuring the storage time of honey was carried out to determine changes in the activity and chemical compound content of honey so that a period of 2 weeks was determined which represents newly harvested honey, 6 months and 1 year. All this honey was taken from the same place but at different times and stored at room temperature. The three types of forest honey from Kefa that had been stored at different times were tested for their active compound content using the method proposed by Hanani (2014) which includes testing alkaloids, saponins, phenolics, tannins, terpenoids and steroids and flavonoids.

Analysis of pH, Water and Sugar Content of Honey

The total sugar content of the three types of kefa honey was measured using a spectrophotometer and the water content was measured using the gravimetric method while the pH value was measured using a pH meter.

Antibacterial Test of Honey using the Disc Method

Three types of honey stored for different durations were tested for antibacterial activity against the bacteria *A. hydropilla* and V. alginolitycus using the disc method. For tests on *A. hydropilla* bacteria, TSA (Tripticase Soy Agar) media was used with the addition of 0.5% NaCl, while for V. alginolitycus, TSA media was used with the addition of 2% NaCl. The TSA media used is divided into two layers, namely solid and semi-solid. The solid TSA layer was prepared one day before the antibacterial test and the semi-solid layer was prepared on the day of the test using 70% of the concentration stated on the label. The bacteria A. hydrophila and V. alginolyticus were added to each medium according to the appropriate NaCl concentration.

Then, sterile paper discs were soaked in each honey sample. After 30 minutes, the paper discs were placed on TSA media that had been inoculated with the appropriate bacteria. Measurements were carried out after incubation for 24 hours at 28°C by measuring the clear zone around the paper disc.

RESULTS

Active Compound Content of Kefa Forest Origin

The results of the qualitative test for the active compound content of forest honey from Kefa can be seen in Table 1.

Table 1. Test Results for the Active Compound Content of Forest Honey from Kefa

Num.	Treatment	Active Compound Contain					
		Flavanoid	Alkaloid	Steroid	Terpenoid	Tanin	Saponin
1	K1	-	+	-	-	-	+
2	K2	-	+	-	-	-	+
3	K3	-	+	-	-	-	+

Kefa honey stored at different times showed the same active compound content or did not change (Table 1). Starting from honey that is stored for 2 weeks, 6 months and 1 year after collection, everything contains alkaloids and saponins. This shows that even though forest honey from Kefa is stored for 1 year, the active compounds do not change. Thus, the storage time does not affect the chemical compound content of forest honey from Kefa.

Water Content, pH, and Total Honey Sugar

The test results for total sugar content, water content and pH of forest honey from Kefa can be seen in Table 2.

Table 2. Total Sugar Content, Water Content and pH of Forest Honey from Kefa

Honey types	Sugar Total (%)	Water (%)	pH
Kefa honey I	72,60	26,65	4,06
Kefa honey 2	72,45	25,11	3,93
Kefa honey 3	72,20	24,81	3,77

In Table 2, it can be seen that the longer it is stored, the sugar, water and pH content of forest honey from Kefa decreases, but the decrease is not too big. This shows that even though honey is stored for 1 year, it doesn't experience much change. As Wulandari (2017) said, low water content will protect honey from damage for a relatively long period of time.

Antibacteria activity of honey

The results of the antibacterial test of forest honey from Kefa taken at different times against the bacteria *V. alginolyticus* and *A. hyropilla* can be seen in Figure 1.

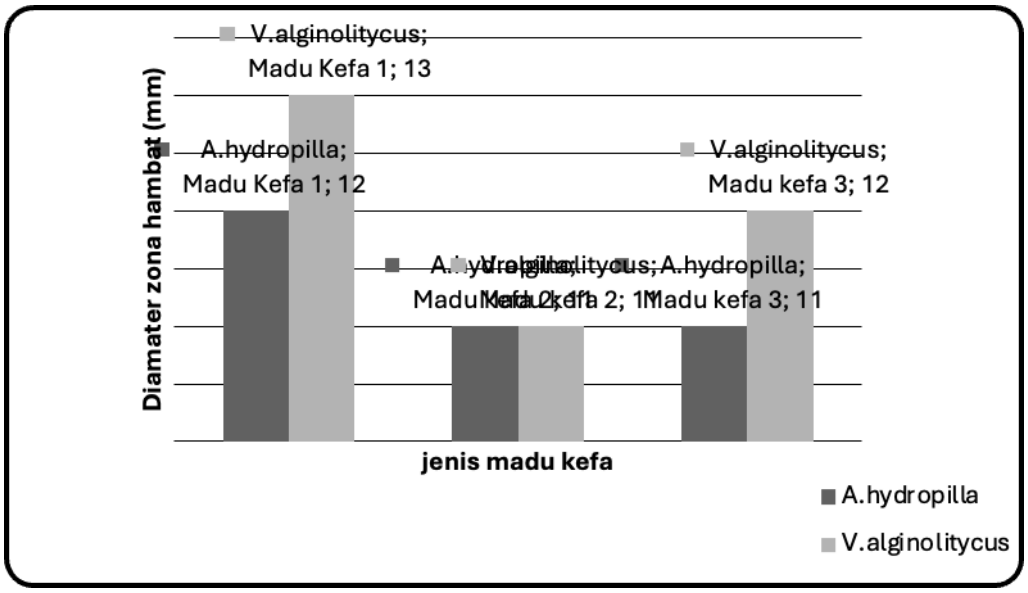

Figure 1. Antibacterial Test Results of Forest Honey from Kefa Taken at Different Times Against the Bacteria *V. Alginoliticus* and *A. hydropilla*

Kefa forest honey, both new and stored for 6 months and 1 year, still has antibacterial activity against the bacteria *A. hydropilla* and V. alginolitycus which are pathogenic bacteria in fish (Figure 1). If we compare the antibacterial activity of the three kefa honeys against the two pathogenic bacteria in fish, it can be seen that the highest activity occurs in the bacteria V. alginolitycus. This shows that the strength of honey's antibacterial activity also depends on the type of bacteria being challenged, even though both bacteria are Gram negative bacteria. As stated by Pelczar & Chan (2005), the factors that influence the antibacterial activity of antibacterial compounds are the concentration or intensity of the antibacterial substance, the number of microorganisms, temperature, microorganism species, and the presence of foreign organic materials which can reduce the effectiveness of the antibacterial compound, as well as pH.

DISCUSSION

Based on the nectar source, honey has a different composition of active compounds. These differences are thought to influence differences in honey's antibacterial activity (Parwata *et al.,* 2010). The active compound content of forest honey from Kefa (Table 1) is different from the active compound content in honey originating from Nigeria, namely that it does not contain alkaloids and saponins but contains tannins and flavonoids (Adeyemo *et al.,* 2017). Likewise, the active compound content of forest honey from Aceh is Seulawah honey (Aceh Besar) and Trumon (South Aceh), both of which contain saponins and terpenoids (Fadmi *et al.,* 2015). Differences in the active compound content of honey can be caused by differences in the nectar of each bee (Ma'ruf *et al.,* 2018).

Kefa forest honey has the potential to be antibacterial because of its alkaloid and saponin content and can be used even if it has been stored for 1 year. Alkaloids have the ability to act as antibacterials through the mechanism of destroying the peptidoglycan components of bacterial cells. With this, the cell wall layer does not form completely, causing bacterial cell death (Ajizah, 2004). Likewise, saponins work as antibacterials by disrupting the stability of bacterial cell membranes, causing damage to the cell membrane which ultimately results in bacterial cells experiencing lysis (Kurniawan & Aryana, 2015).

Forest honey from Kefa, both freshly harvested and stored for 6 months and 1 year, has a high total sugar content, namely around 72.20% - 72.60%. The total sugar content of forest honey from Kefa is not much different from the total sugar content of forest honey from Soe, namely 74.37% (Salosso, 2019a) and rock honey from Timor, namely 74.22% (Salosso, 2019c). However, it is higher than natural stone honey, namely only 66.2% (Salosso, 2019c). Even though all the islands are part of NTT, such as Soe and Kefa, they have slightly different honey sugar content, this can be caused by the differences in the climate of Timor Island and Semau Island which affects the types of plants that grow around the forests where the honey nests. Likewise, when compared with the total sugar content of honey from other provinces, rubber tree honey from Central Bangka district contains 74.77% total sugar (Evahelda *et al.,* 2017). This difference can be caused by differences in honey collection locations, where each location has plants or forests which are different sources of nectar. As stated by Parwata *et al.* (2010) that honey has a different composition of chemical compounds including total sugar based on the nectar feed source.

Differences in honey water content are influenced by several factors such as climate factors, post-harvest handling, type of nectar collected, honey maturity level, production and storage processes (Baroni *et al.,* 2009). This has been proven by the difference in water content of forest honey from Kefa which ranges from 24.81-26.65% to other places. The water content of honey from kefa is greater than the water content found by Nadhila (2014), which is around 15-21% and Stratev *et al.* (2015) namely 16.8%. Likewise, the water content of bitter black honey and sweet black honey from Central Kalimantan is 16.19% and 15.40% respectively (Fitrianingsih *et al.,* 2014), as well as some honey from Indonesia ranging from 17.8 – 21.0%. (Dewi *et al.,* 2017). However, it is not much different from the water content of honey from rubber tree nectar from Central Bangka district, which is 24.25% (Evahelda *et al.,* 2017).

The pH value of forest honey from Kefa, both new and stored, is around 3.77 - 4.06. The pH range value of forest honey from Kefa tends to be the same as the pH value of honey originating from other places in Indonesia, such as rubber honey from Bangka, which is 3.92 (Evahelda *et al.,* 2017), kapok honey has a pH value of 3.8, longan 4. .48, rambutan 4.21 and Kaliandara honey 4.37 (Chayati, 2008). Likewise, in other countries, honey from India has a pH value of 4.1 (Veeraputhiran *et al.,* 2013) and Rafe honey from Bulgaria has a pH value of 3.232 (Stratev *et al.,* 2015). According to Gulfraz *et al.* (2010), differences in the pH value of honey can be caused by differences in the acid and mineral content of honey. Furthermore, according to Buba *et al.* (2013), the mineral content of honey is influenced by geographical location, climatic conditions and the soil where the plants that are the source of nectar grow.

Based on storage time, honey that has just been harvested or has not been stored for a long time has higher antibacterial activity, namely 13 mm for V. alginolitycus bacteria and 12 cm for *A. hydropilla* bacteria. Although the active compound content, total sugar, water and pH value of the three types of Kefa honey tested did not change much during storage, the antibacterial activity was better in honey that had just been harvested or had not been stored for too long.

The size of the inhibition zone produced shows the antibacterial power of the honey. When compared with the inhibition zone produced by musi rawas forest honey against E. coli bacteria at the same concentration, namely 100% (pure honey) which reaches 31 mm (Huda, 2013), then the antibacterial power of kefa forest honey against V. alginolitycus bacteria and *A. hydropilla* is still much lower at only 13 mm and 12 mm. Likewise, when compared with the inhibition zone produced by honey from Bandung against S. aureus bacteria ranging from 21.33 mm - 13.76 mm and E. coli ranging from 14.93 mm - 19.67 mm (Dewi *et al.,* 2017). Likewise, honey from Tamil Nadu, India, produced against 10 types of gram-negative and

gram-positive bacteria, namely Konbu honey ranging from 22-34, Malan honey ranging from 17-26, commercial honey 17-22 (Kalidasan *et al.,* 2017).

The antibacterial activity of honey, whether freshly harvested or stored, can be triggered by several factors such as high sugar content, high acidity levels (Johnstond *et al.,* 2018; Carina *et al.,* 2014; Nadhilla, 2014), and the presence of organic compounds. which has antibacterial properties (Carina *et al.,* 2014). Apart from that, this activity can also be caused by the presence of hydrogen peroxide radical compounds (H2O2) which can eliminate pathogenic microorganisms (Johnstond *et al.,* 2018; Nadhilla, 2014; Carina *et al.,* 2014).

The growth of various types of bacteria can be prevented by honey because it has a low pH value. Where bacteria can grow at neutral or non-acidic pH (Garedew *et al.,* 2003). The acidity level greatly influences the growth and survival of bacterial cells. When growing, each bacteria has an optimum acidity level. A decrease in pH to the lowest limit can cause the growth of bacterial cells to stop and they lose their ability to live.

According to Evahelda *et al.* (2017) the hygroscopic properties of honey indicate that the honey is thick or thick due to the high sugar content. Fructose plays a role in determining the hygroscopic properties of honey. This is caused by the nature of fructose which is more soluble compared to glucose (Buba *et al.,* 2013). Furthermore, according to Erguder (2008), the sugar content in honey which consists of a mixture of glucose and fructose causes honey to have osmotic properties and can inhibit the growth of bacteria.

Hydrogen peroxide in honey is produced enzymatically and has antibacterial properties. The presence of the radical compound hydrogen peroxide (H2O2) which kills pathogenic microorganisms (Fadhami, 2014). Furthermore, Yuliati (2017), explained that the antimicrobial process of hydrogen peroxide has the ability to oxidize and form hydroxyl free radicals which are more toxic than peroxide so that damage to bacterial cells occurs more easily (Yuliati, 2017).

CONCLUSSION

The conclusions of the research are explained as follows

1. Kefa honey taken at different times shows the same active compound content or has not changed.
2. The longer it is stored, the sugar, water and pH content of forest honey from Kefa decreases, but the decrease is not too big
3. Forest honey from Kefa, both new and stored for 6 months and 1 year, still has antibacterial activity against the bacteria *A. hydropilla* and V. alginolitycus.

ACKNOWLEDGEMENT

Penulis mengucapan terima kasih kepada Kepala Stasiun Karantina Ikan, Pengendalian Mutu dan keamanan Hasil Perikanan Kupang yang telah memberi kesempatan untuk menggunakan Fasilitas Laboratorium mikrobiologi untuk pelaksanaan kegiatan penelitian ini.

REFERENCES

Adeyemo, R. O., Torimiro, N., Akinola, S. A., Lawal, S. K., Abolarinwa, T. O., & Adewoye, W. O. (2017). Study on antibacterial efficacy of Different Honey Types in South Western Nigeria Against Wound Associated Bacteria. *Journal of Aphyterapy*, *2*(1), 15- 19.

Ajizah, A. (2004). Sensitivitas *Salmonella typhimurium* Terhadap Ekstrak Daun *Psidium guajava* L. *Bioscientie*, *1*(1), 31-8.

Baroni, M. V., Arrua, C., Nores, M. L., Fayé, P., Díaz, M. D. P., Chiabrando, G. A., & Wunderlin, D. A. (2009). Composition of honey from Córdoba (Argentina): Assessment of North/South Provenance by chemometrics. *Food Chemistry, 114*(1), 727–733.

Buba., Fatimah., Gidado, A., & Shugaba, A. (2013). Analysis of biochemical composition of honey sampel from Nort-East Nigeria. *Journal of Biochemistry and Analytical Biochemistry, 2*(3), 1–7.

Carina, L., Soledad, V., & Marina, B. (2014). Antibacterial activity of honey: A review of honey around the world. *Journal of Microbiology and Antimicrobials, 6*(3), 51-56.

Chayati, I. (2008). Sifat Fisikokimia Madu Monoflora dari daerah Istimewa Yogyakarta dan Jawa tengah. *Agritech, 28*(1), 1-7.

Dewi, M. A., Kartasasmita, R. E., & Wibowo, M.S. (2017). Uji Aktivitas antibakteri beberapa madu asli lebah asal Indonesia Terhadap *Staphylococcus aureus* dan *Escherichia coli. Kartika Jurnal Ilmu Farmasi, 5*(1).

Erguder, B. I., Kilicoglu, S. S., Namuslu, M., Kilicoglu, B., Devrim, E., Kismet, K., & Durak, I. (2008). Honey Prevent Hepatic Damage Induced by Obstruction of the Common Bile Duct. *World J Gastroenterol, 12*(23), 3729-3732.

Evahelda, E., Pratama, F., Malahayati, N., & Santoso, B. (2017). Sifat Fisik dan Kimia Madu dari Nektar Pohon Karet di Kabupaten Bangka Tengah, Indonesia. *AGRITECH, 37*(4), 363-368.

Fadhmi., Mudatsir., & Syaukani, E. (2015). Perbandingan daya Hambat Madu Seulawah dengan Madu Trumon terhadap Bakteri *Staphylococcus aureus* Secara In Vitro. *Jurnal Biotik, 3*(1), 9-14.

Fitrianingsih, S. P., Khairat, A., & Choestrina, R. (2014). Aktivitas antibakteri madu Pahit dan Madu Hitam manis terhadap *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal Farmasi Galenika, 1*(2), 32-37.

Garedew, A., Schmolz, E., & Lamprecht, I. (2003). The Antimicrobial Activity of Honey of the Stingless Bee *Trigono* spp. *Journal of Apicultural Science, 47*(1), 37-48.

Gulfraz, M., Ifftikhar, F., Asif, S., Raja, G. K., Asad, M. J., Abbasi, K. & Zeenat, A. (2010). Quality assement and antimicrobial activity of various honey types of Pakistan. *African Journal of Biotecnology, 9*(41), 6902-6906.

Hanani, E. (2014). Analisis Fitokimia. Penerbit buku Kedokteran EGC, Jakarta. 262 hal.

Hegazi, G. A., Al Guthami, F. M., Al Gethami, A. F. M., Abd Allah, F. M., Saleh, A. A., & Fouad, E. A. (2017). Potential antibacterial activity of some Saudi Arabia honey. *Veterinary World, 10*, 233-237.

Huda, M. (2013). Pengaruh madu terhadap Pertumbuhan bakteri Gram Positif (*Staphylococcus aureus*) dan bakteri Gram Negatif (*Escherchia coli*). *Jurnal Analis Kesehatan, 2*(2), 250-259.

Johnston, M., McBride, M., Dahiya, D., Owusu-Apenten, R., & Nigam, P. S. (2018). Antibacterial activity of Manuka honey and its components: An overview. *AIMS Microbiology, 4*(4), 655–664.

Kalidasan, G., Saranraj, P., Ragul, V., & Sivasakhti, S. (2017). Antibacterial activity of natural and commercial Honey-A Comparative study. *Advances in Biological research, 11*(6), 365-372. https://doi.org/10.5829/idosi.abr.2017.365.372

Kurniawan & Aryana. (2015). Binahong (*Cassia alata*) as Inhibition of *Escherrichia coli* Growth. *J. majority, 4*(4), 100-104.

Lukistyowati, I., & Kurniasih. (2011). Kelangsungan hidup ikan mas *Cyprinus carpio* yang diberi pakan ekstrak bawang putih *Allium sativum* dan diinfeksi *Aeromonas hydrophilla*. *Jurnal Perikanan dan Kelautan, 16*, 144–160.

Ma'ruf, M., Mawaddah, G. A., Eriana, N. N. A., Swari, F. I., Aslamiah, S., & Lutpiatina, L. (2018). Madu Lebah Kelulut *(Trigona spp.)* Dalam Aktifitas Terhadap Bakteri *Staphylococcus aureus* Resisten. *Jurnal Skala Kesehatan Politeknik Kesehatan Banjarmasin, 9*(1).

Muslim, T. (2014). Potensi madu Hutan sebagai Obat dan Pengelolaannya di Indonesia. *Prosiding Seminar Balitek KSDA*. 3 Desember 2014, hlm. 67-82.

Nadhilla N. F. (2014). The Activity of antibacterial agent of Honey against *Staphylococcus aureus*. *Jurnal Majority, 3*(7), 94-101.

Noerbaeti, E., Pattah, H., & Nuraini, W. (2016). Potensi Ekstrak Daun Gamal *Gliricidia sepium* Sebagai Antibakteri *Vibrio* sp. dan *Flexibacter maritimum. Jurnal Teknologi Budidaya Laut, 6,* 43-49.

Orsi, R. O., Santos, V. G. D., Pezzato, L. E., De carvalho, P. L. P. F., Teixeira, C. P., Freitas, J. M. A., Padovani, C. R., Sartori, M. M. P., & Barros, M. M. (2017). Activity of Brazilian Propolis against *Aeromonas hydropilla* and its effect on Nila tilapia Growth, hematological and non-specific immune response under bacterial infection. *Anais da Academimia Brasileira de Cencias, 89*(3), 1785-1799.

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

Pelczar, M. J., & Chan, E. C. S. (2005). Dasar-Dasar Mikrobiologi. Penerbit Universitas Indonesia. 997 hal.

Rosidah., Buwono, I. D., Lili, W., Suryadi, I. B., & Triandika, A. R. (2019a). Ketahanan ikan lele sangkuriang, *Clarias gariepinus* Burchell 1822 terhadap *Aeromonas hydrophila* pasca pemberian ekstrak daun kelor (*Moringa oleifera* L.) melalui pakan. *Jurnal Iktiologi Indonesia, 19*(1), 97-113. https://doi.org/10.32491/jii.v19i1.435

Rosidah., Sriati., Subhan, U., Mulyani, Y., & Dermawan, R. (2019b). The effectivess of Honey supplementation in feed for improving goldfish fingerling *Carassius auratus* Immune system against *Aeromonas hydropilla* bacteria attack. *Jurnal Akuakultur Indonesia, 18*(1), 89-100.

Salosso, Y. (2019a). Uji Antibakteri Madu Semut Asal Pulau Semau Terhadap Bakteri *Aeromonas hydrophila* dan *Vibrio alginolitycus. Jurnal Sains Dan Inovasi Perikanan*, *3*(2), 68-72.

Salosso, Y. (2019b). The Potential Of Forest Honey (*Apis* spp.) From Timor Island As Antibacterial Against Pathogenic Bacteria In Fish Culture. *Indonesian Aquaculture Journal, 14*(2), 1-8.

Salosso, Y. (2019c). Chemical composition and antibacterial activity of honey collected from East Nusa Tenggara, Indonesia on pathogenic bacteria in aquaculture. *IOP Conf. Ser.: Earth Environ. Sci.* **370** 012030. 1-7.

Salosso, Y., Rebhung, F., Sunadji., & Anggrainy, K. (2020). Application of Kefa Forest Honey as antibacterial in the treatment of common carp (*Cyprinus carpio*) infected with bacteria *Aeromonas hydropilla*. *AACL Bioflux, 13*(2), 984-992.

Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S., & De Santis, E. P. L. (2014). Antibiotic resistance of Vibrio species isolated from *Sparus aurata* reared in Italian mariculture. *New Microbiologica, 37*, 329-337.

Stratev, D., Vashin, I., Balkanska, R., & Dinkov, D. (2015). Antibacterial Activity Of Royal Jelly And Rape Honey Against *Aeromonas hydrophila* (Atcc 7965). *Journal Of Food And Health Science, 1*(2), 67-74.

Susandi F., Mulyana., & Rosmawati. (2017). Peningkatan Imunitas Benih Ikan Gurame (*Osphronemus gouramy* Lac.) Terhadap Bakteri *Aeromonas hydrophila* Menggunakan Rosella (*Hibiscus sabdariffa* L.). *Jurnal Mina Sains, 3*(2), 1-12.

Triyaningsih., Sarjito., & Prayinto, S. B. (2014). Patogenisitas *Aeromonas hydrophila* yang diisolasi dari lele dumbo *Clarias gariepinus* yang berasal dari Boyolali. *Journal of Aquaculture Management and Technology, 3,* 11−17.

Unissa, R., Neeeraj, P. S., Ayyub, Md I., & Omsa, N. (2017). Evaluation Of Antibacterial Activity Of *Achyranthes aspera* Extract Against *Vibrio alginolyticus*: An In Vitro Study. *Saudi Journal Of Pathology And Microbiology, 2*(8), 241-246.

Veeraputhiran, V., Maribah, T. P. E., & Alfred, A. 2013). Physicochemical comparison and preservative of flora and non flora Honey. *Agriculture and food Science, 3*(3), 128-130.

Wulandari, D. D. (2017). Kualitas madu (Keasaman, kadara air, dan kadar gula pereduksi) berdasarkan perbedaan suhu penyimpanan. *Jurnal Kimia Riset, 2*(1), 16-22.

Yuliati. (2017). Uji Efektivitas Larutan Madu Sebagai Antibakteri Terhadap Pertumbuhan *Staphylococcus aureus* Dan *Pseudomonas aeruginosae* Dengan Metode Disk Diffusion. *Jurnal Profesi Medika, 11*(1).