**JSMARTech**
Journal of Smart Bioprospecting and Technology **p-ISSN**: 2686-0805 **e-ISSN**: 2714-7894
April 2024, VOL 05 NO 01 **DOI**:https://doi.org/10.21776/ub.jsmartech.2024.005.01.20

# Diastase Enzyme Activity and Antibacterial Effectiveness to Gastroenteretis by *Apis mellifera* Honey from Indonesia

**Rara Annisaur Rosyidah[1*], Akhmad Endang Zainal Hasan[1], Dimas Andrianto[1]**

[1]Department of Biochemistry, IPB University, Bogor 18860, West Java, Indonesia

**Submission**: 19 March 2024; **Revised**: 16 April 2024; **Accepted**: 25 April 2024

***Corresponding author**: Rara Annisaur Rosyidah; **e-mail**: raraannisaurrosyidah@gmail.com

***ABSTRACT.*** *The quality of monoflora honey (Apis mellifera) as an antibacterial can be influenced by the activity of the diastase enzyme and the secondary metabolites of the nectar source. This study aims to compare the activity of the diastase enzyme and the effectiveness of monoflora honey derived from Apis mellifera bees as a natural antibacterial agent against Salmonella typhi. The sampling method for monoflora honey from nine different nectar sources (acacia, calliandra, rambutan, eucalyptus, randu, mango, robusta coffee 1, robusta coffee 2, and rubber). The results showed that the highest diastase enzyme activity in monoflora honey was mango, rambutan, and robusta coffee 2 at 20.00 DN, eucalyptus, randu, and rubber at 10.00 DN, acacia at 6.67 DN, robusta coffee 1 5.00 DN, and calliandra 4.00 DN. Zone of inhibition for S. typhi on robusta coffee 2, robusta coffee 1, randu, eucalyptus, acacia, mango, rubber, rambutan, and calliandra respectively at 18.53; 16.70; 15.27; 14.70; 13.20; 11.20; 11.17; 9.47; and 6,80. The minimum concentration inhibition was 8 µg/mL.*

***Keywords:*** *antibacterial, diastase enzyme, Apis mellifera honey.*

## INTRODUCTION

Honey harvesting must be done at the right time when the honey is ripe and the bees begin to close their hive cells by 85% so that the honey is ready for storage. Honey is rich in simple carbohydrates because the enzymes diastase and invertase are added when the nectar is sucked and regurgitated. The diastase enzyme is used to assess the quality of honey produced by bees [1].

Based on data from [2], the prevalence of diarrhea in Indonesia is 37.88% and has increased in 2019 to 40% in toddlers [3]. Globally, according to [4] in the general population gastroenteritis has a mortality rate of 525,000 deaths per year in children under 5 years with an incidence rate of 1.7 million children each year which causes the second most deaths due to infectious diseases.

*Salmonella typhi* is a Gram-negative bacterium that causes diseases such as typhoid fever, gastroenteritis, asymptomatic carriers, and bacteremia. *Salmonella* strains that invade the epithelial lining cause enteritis and diarrhea [5]. Over time, these pathogenic bacteria can become resistant, so it is necessary to take precautions to keep the bacteria from becoming increasingly resistant to drugs [6]. Honey comes from nectar which reduces the water content in the bee's body and in the beehive. The various types of honey and the potential of honey to be used as an antibacterial agent prompted this research. The compounds present in honey are carbohydrates (fructose and sucrose), flavonoids, and a low pH that inhibits bacterial growth. Light-colored honey contains less phenol [7].

Honey is classified *as* monoflora (one type of plant) or multifloral. (several plant species) depend on the origin of the honey nectar [8]. So far, the monoflora honey bioactive compound from the *Apis mellifera* bee from Indonesia has antibacterial compounds. This study characterized 9 honey specimens of the monoflora species *Apis mellifera* from Indonesia. Therefore, this study aims to determine the activity of the diastase enzyme and the effectiveness of monoflora honey derived from the *Apis mellifera* bee as a natural antibacterial agent.

## RESEARCH METHODS

### Sampling

Samples of monoflora honey from Indonesia are shown in Table 1 which is stored in the Biochemistry Masters Study Program Laboratory.

### Diastatic Enzyme Activity

A total of 5 grams of honey was added to 2.5 mL of acetate buffer solution pH 5.3 and 1.5 mL of 0.5 M NaCl solution, then added distilled water to 25 mL. Then 10 mL of the mixture was heated in a water bath at 40°C. After 15 minutes, 5 mL of

starch solution was added. Every 15 minutes interval 1 mL of the mixture was added 10 mL of 0.0007 N iodine and added distilled water in 25 mL. Furthermore, the absorbance value was measured at λ 660 nm. The collection of the solution was continued until the value of A <0.235 was obtained within a certain time interval. Calculation of diastase enzyme activity (DN) is calculated by the following formula [9]:

$$DN = \frac{300}{t}$$

Note: DN = diastase enzyme activity
t = time used to reach the value of A<0.235

**Table 1.** Origin of the *Apis mellifera honey samples* used.

| Plants of Nectar Origin | City | Province |
|---|---|---|
| Acacia | Cape Jabung | Jambi |
| Kaliandra | Sukabumi | West Java |
| rambutans | Subang | West Java |
| Eucalyptus | Sragen | Central Java |
| Randu | Starch | Central Java |
| Mango | Pasuruan | East Java |
| Robusta coffee 1 | Poor | East Java |
| Robusta coffee 2 | Jember | East Java |
| Rubber | Jember | East Java |

## Antibacterial Activity

*Salmonella thypi* bacterial cultures were rejuvenated in Nutrient Broth (NB) media. The test was carried out in triplicate on each test bacteria and honey. Cultures that successfully grow are then tested in a disk diffusion test. Mueller Hiton Agar (MHA) media was heated at 80ºC and homogenized with a stirrer. Next, it was sterilized using an autoclave at 121ºC within 15 minutes. Wait for the MHA media until it reaches a temperature of around 35-37ºC then pour it into a sterile petri dish until it solidifies. Bacterial cultures were streaked onto MHA media using sterile cattonbud. The sterile disc was left for 24 hours to absorb the honey sample to be tested with a concentration of 100% (100,000 µg/mL), then dried in an oven at 40ºC for 2 hours. The prepared discs were placed on the bacterial culture scratches on MHA and then incubated at 37ºC for 24 hours. The clear zone formed around the disc is measured with a caliper. The positive control used the antibiotic ciprofloxacin at a concentration of 100 µg/mL (0.1%) and the negative control used a blank of 25 µL of distilled water each [10].

## Minimum Inhibitory Concentration (MIC)

Wait for the MHA media until it reaches a temperature of around 35-37˚C and then pour it into a sterile Petri dish until it solidifies. Bacterial cultures were streaked onto MHA media using a sterile cotton bud. The sterile discs were left for 24 hours to absorb samples of acacia honey, eucalyptus, randu, robusta coffee 1, and robusta coffee 2 with concentrations of 8, 40, 200, 1000, and 5000 µg/mL (0.008; 0.004; 0.2; 1; and 5%), then dried in an oven at 40ºC for 2 hours. The prepared discs were placed on the bacterial culture scratches on MHA and then incubated at 37ºC for 24 hours. The clear zone formed around the disc is measured with a caliper. The positive control used the antibiotic ciprofloxacin at a concentration of 100 µg/mL (0.1%) and the negative control used a blank of 25 µL of distilled water each [11].

## Data Analysis

The research data were analyzed using a one-way ANOVA test with a level of significance $\alpha$ = 0.05 with SPSS version 25. If the *p*-value produced in the ANOVA test is <0.05 then the data is said to have a significant difference and must be tested further. Test analysis continued with Duncan's test analysis. This analysis was conducted to determine the effect of significant differences in each monoflora honey.

# RESULTS AND DISCUSSIONS

## Diastatic Enzyme Activity

The average results of diastase enzyme activity in monoflora honey can be seen in Figure 8. The highest diastase enzyme activity in mango, rambutan, and Robusta 2 coffee was 20.00 DN, while the lowest was in calliandra honey of 4.00 DN. The diastase enzyme activity of eucalyptus, randu, and rubber honey was 10.00 DN, acacia was 6.67 DN, and robusta coffee 1 was 5.00 DN.

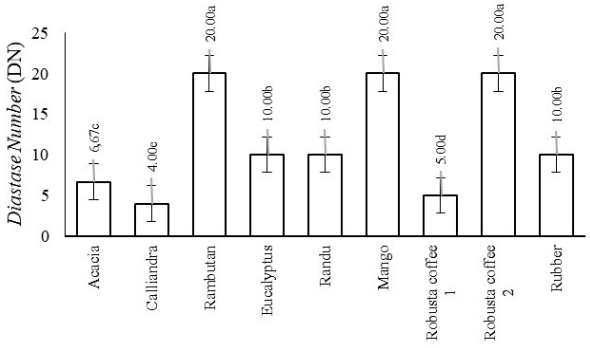


**Figure 1.** Diastase enzyme activity value of *Apis mellifera* honey. Different lowercase letters indicate statistically significant differences (α=0.05).

These results were supported by ANOVA statistical analysis which showed that the nine monoflora honeys were significantly different (p<0.05) based on their diastase enzyme activity. This shows that differences in plants as a source of nectar affect it. The results of Duncan's test (α=0.05) on the activity of the diastase enzyme from nine monoflora honeys showed that there were 5 groups, namely (1) mango honey,

rambutan, and robusta coffee 2; (2) eucalyptus, opium, and rubber honey; (3) acacia honey; (4) Robusta coffee honey 1; and (5) calliandra honey; which are grouped based on their proximity to the activity of the diastase enzyme they possess (Fig. 1).

The diastase enzyme is added by the bees during the honey ripening process. The diastase enzyme is able to break down the glucosidic bonds in oligo and polysaccharides, namely starch into simple sugars. The diastase enzyme is very sensitive to heat so proper heating and storage are very important to maintain the market value of honey. Diastatic activity is measured as *Diastase Number* (DN). Loss of diastase activity occurs with increasing temperature. Based on the measurement results, nine samples had *a diastase number* (DN) according to the 2018 SNI standard. This is due to the ability of bees to produce saliva with higher diastase enzyme values [12].

The results of measuring the activity of the diastase enzyme in Figure 8 show that mango, rambutan, and Robusta 2 coffee honey had the highest diastase values, while calliandra honey had the lowest diastase values. Based on the measurement results, nine samples had *a diastase number* (DN) according to the 2018 SNI standard. This is due to the ability of bees to produce saliva with the higher the value of the diastase enzyme, the more bee saliva contained in honey [13].

Honey usually does not meet commercialization requirements after being overheated, reheated or/and stored for a long time because the activity diastase has fallen below the quality prerequisite limit . According to the [9], the quality requirements for the value of honey diastase enzyme activity are more than 3 DN. The activity of the diastase enzyme increased with increasing pH, but a further increase in pH resulted in a decrease diastase enzyme activity. Decreasing diastase enzyme activity at pH 4.6 changed to 5.6. Low pH with increase temperature, causing a change in the activity of the diastase enzyme is low, whereas at a higher pH it causes a decrease in the activity of the diastase enzyme with an increase in temperature [14].

**Antibacterial Activity**

According to [15] antibacterial activity is classified as weak or does not have antibacterial activity if the resulting inhibition zone is ≤12 mm, medium is 13-17 mm, and strong is ≥18 mm. The smallest inhibition zone for *S. typhi* was 6.80 mm in calliandra honey, while the smallest inhibition zone was 18.53 mm in Robusta 2 coffee honey (Figure 2). Positive control on the three bacteria showed a clear zone formed.

These results were supported by ANOVA statistical analysis which showed that the nine monoflora honeys were significantly different ($p<0.05$) based on their antibacterial activity. This shows that differences in plants as a source of nectar affect the inhibition zone. The results of Duncan's test ($\alpha=0.05$) on the inhibition zone of *S. typhi* from nine masu monoflora showed that there were 8 groups, namely (1) robusta coffee honey 2; (2) Robusta coffee honey 1; (3) opium honey; (4) eucalyptus honey; (5) acacia honey; (6) mango and rubber fight; (7) rambutan honey; and (8) calliandra honey; which are grouped based on the proximity of their inhibition zones (Fig. 2).

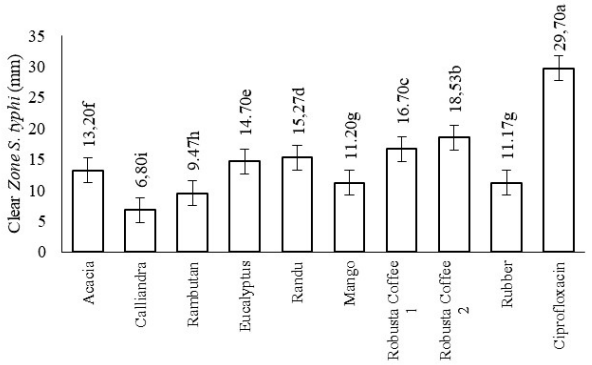


**Figure 2.** Antibacterial activity of monoflora honey against *S. typhi*. Different lowercase letters indicate statistically significant differences ($\alpha=0.05$).

The antibacterial activity of monoflora honey against *S. typhi* was classified as strong to resistant or had no effect because the diameter of the inhibition zone produced was in the range of 6.80 mm to 18.53 mm.

The high sugar concentration in honey (75% monosaccharides and 10-15% disaccharides) can induce osmosis in bacteria. As a result, cells shrink due to dehydration and are unable to survive in a hypertonic sugar solution [16]. Optimal growth of most microorganisms occurs at a neutral pH, ranging from 6.5 to 7.5, while honey's pH is 3.2 to 4.5 caused by gluconic acid [17]. Biological compounds are transmitted from the nectar to the honey which has the health-promoting properties of honey. Phenolic compounds are found at high levels in honey and may contribute to its antibacterial activity [18].

Based on studies of the mechanism of antibacterial action of the phenolic acids found in honey, among others, caffeic acid causes oxidative stress, chlorogenic acid causes cytoplasmic and nucleotide leakage as a consequence of higher membrane permeability [19], ferulic acid causes damage cell membranes are associated with morphological variations, gallic acid causes intracellular leakage as a result of disruption of cell membranes and increased

pore formation [20], p-kumaric acid causes disruption of cell membranes and bacterial DNA binding, and syngasic acid causes membrane dysfunction cells [21].

According to [22], types of polyphenols such as apigenin have beta-lactamase inhibitory activity. Some bacteria, such as *S. aureus* and *E. coli,* have beta - lactamase enzymes that can break down the beta-lactam ring of antibiotics , making them inactive. This process begins with the breaking of the CN bond in the beta-lactam ring which prevents the antibiotic from binding to the transpeptidase protein. As a result, the antibiotic loses its ability to inhibit the formation of the bacterial cell wall. Large molecular phenolic compounds are able to inactivate essential enzymes in bacterial cells even at very low concentrations.

**Value of Minimum Inhibitory Concentration (MIC)**

The Minimum Inhibitory Concentration (MIC) carried out in this study used the diffusion method on *Mueller Hiton Agar* (MHA) media. The minimum inhibitory concentration test was carried out on 5 honeys that had the top 5 antibacterial activities out of the 9 tested honeys. The concentrations used were 8, 40, 200, 1000 and 5000 µg/mL. The MIC value for each type of honey will vary depending on the active content in a sample dissolved in water.

The smallest inhibition zone for *S. aureus* was 0.73 mm in acacia honey with a concentration of 8 µg/mL while the largest inhibition zone was 12.50 mm in Robusta coffee honey 1 with a concentration of 5000 µg/mL. The smallest inhibition zone for *E. coli* was 0.40 mm in Robusta coffee honey 2 with a concentration of 8 µg/mL while the largest inhibition zone was 18.57 in Robusta coffee honey 2 with a concentration of 5000 µg/mL. The smallest inhibition zone *for S. typhi* was 0.20 mm for 8 µg/mL honey, while the largest was 5.33 mm for Robusta coffee 2 honey with a concentration of 5000 µg/mL (Table 2).

This study used the diffusion method to test the minimum inhibitory concentration (MIC) on acacia honey, eucalyptus, randu, robusta coffee 1 and robusta coffee 2. The diffusion method has the ability to measure the sensitivity of a sample and estimate the minimum inhibitory concentration that can visually inhibit bacteria, has high flexibility, and is easy to apply [11]. In this study, the minimum inhibitory concentration (MIC) was tested at concentrations of 8, 40, 200, 1000 and 5000 µg/mL. The presence of a clear zone formed indicates the inhibition of the sample against bacteria based on the minimum concentration. Table 2 shows that the minimum inhibition zone diameters of acacia honey, eucalyptus, randu, robusta coffee 1 and robusta coffee 2 have a diameter of less than 18.57 mm.

**Table 2.** Value of minimum inhibitory concentrations (MIC).

| Sample | Conc. (µg/mL) | *S. aureus* (mm) | *E. coli* (mm) | *S. typhi* (mm) |
|---|---|---|---|---|
| **Acacia** | 5000 | 13,70a | 16,67a | 3,57a |
| | 1000 | 8.50b | 14,33b | 2,57b |
| | 200 | 4,43c | 4,63c | 2.43c |
| | 40 | 1.60d | 2,73d | 1,27d |
| | 8 | 0.73e | 1.07e | 0.37e |
| **Eucalyptus** | 5000 | 6,63a | 17,43a | 4.03a |
| | 1000 | 4,30b | 15,10b | 3,43b |
| | 200 | 3.53c | 3,10c | 2.57c |
| | 40 | 2,30d | 1.37d | 1.57d |
| | 8 | 1.53e | 1.40e | 1.53e |
| **Randu** | 5000 | 11,23a | 13,20a | 5,23a |
| | 1000 | 4,13b | 10,83b | 4,27b |
| | 200 | 3,37c | 2.40c | 3,37c |
| | 40 | 2,60d | 1,33d | 1,23d |
| | 8 | 1.70e | 0.63e | 0.20e |
| **Robusta coffee 1** | 5000 | 12.50a | 18,33a | 4,40a |
| | 1000 | 4,73b | 15,23b | 2,73b |
| | 200 | 3,10c | 5,53c | 1.37c |
| | 40 | 2,20d | 3,43d | 0.37d |
| | 8 | 1.57e | 1,13e | 0.30e |
| **Robusta coffee 2** | 5000 | 9,70a | 18,57a | 5,33a |
| | 1000 | 5,40b | 15,90b | 4,23b |
| | 200 | 3,47c | 4,20c | 3,23c |
| | 40 | 1.73d | 1.50d | 2,20d |
| | 8 | 0.97e | 0.40e | 1,13e |

The results of the inhibition zone measurements in Table 2 show that the concentration dilution in the honey treatment did not have a major effect on the results of the inhibition zones formed. This is because the inhibition zone that is still formed at a concentration of 8 µg/mL indicates an indication of very active bioactive compounds in honey, so the concentration of honey needs to be reduced to determine the effectiveness of the inhibition on the three tested bacteria. In addition, the relationship between high diastase enzyme activity and low water content indicates greater inhibition of bacterial growth [23]. Antibacterial selectivity and inhibitory effect on the tested bacteria were divided into narrow-spectrum antibacterials and broad-spectrum antibacterials. Narrow spectrum antibacterials have the ability to inhibit the growth of certain gram bacteria, while broad spectrum ones can inhibit the growth of gram positive and Gram-negative bacteria [11].

The difference in the results of the inhibition zones could be due to differences in the content of bioactive compounds in each honey. The physicochemical characteristics of honey are not the only factors that influence the ability of bacterial inhibition, but the bioactive compounds in honey have a greater influence. The content of

bioactive compounds comes from nectar collected and processed by honey bees [23].

Based on how it works, antibacterial properties are divided into bactericidal and bacteriostatic. Bactericidal antibacterials are substances that work to kill bacteria, while bacteriostatic work to inhibit bacterial growth. Some antibacterial substances are bacteriostatic at low concentrations, but bactericidal at high concentrations [11].

## CONCLUSION

The highest diastase enzyme activity was mango honey, rambutan and Robusta coffee 2 of 20.00 DN. The greatest antibacterial activity against *S. typhi* namely Robusta 2 coffee with 18.53 mm. The minimum concentration to inhibit *S. aureus, E. coli,* and *S. typhi bacteria* was at a concentration of 8 µg/mL. This research needs to be tested further regarding the analysis of the specific antibacterial content of 9 monoflora honeys using *High-Performance Liquid Chromatography* or *Liquid Chromatography-Mass Spectrometry* to confirm the compound acting as an antibacterial.

## ACKNOWLEDGMENT

The authors are grateful to the Department of Biochemistry, IPB University, for the support provided during this study

## CONFLICT OF INTEREST

We declare that there is no conflict of interest.

## REFERENCES

[1] Sukmawati, Noor A, and Firdaus. 2015. Analysis of the quality of Mallawa honey based on physical-chemical parameters. *Ind. J. Chem. Res.* **2015.** 2015(3): 259-262.

[2] Basic Health Research Results. **2018.** Jakarta (ID). Indonesian Ministry of Health Research and Development Agency.

[3] Ministry of Health of the Republic of Indonesia. *Indonesia Health Profile*. **2020.** Jakarta (ID): Directorate General of Disease Control and Environmental Health.

[4] World Health Organization (WHO). 2018. Diarrhoeal Disease. (accessed 2 Sept 2021). Downloaded from URL: https://www.who.int/en/news-room/fact-sheets/detail/diarrhoeal-disease.

[5] Radji M. *Textbook of Microbiology: A Pharmacy and Medical Student Guide*. **2016.** Jakarta (ID): EGC.

[6] Panjaitan R, Darmawati S, and Prastiyanto M. Antibacterial activity of honey against multi-drug resistant *Salmonella typhi* and methicillinresistant *Staphylococcus aureus* bacteria. **2018** Unimus FMIPA Education National Seminar. Semarang, Indonesia: Semarang: Muhammadiyah University of Semarang. pp. 71-77.

[7] Nayik GA, and Nanda V. Physico-chemical, enzymatic, mineral and color characterization of three different varieties of honey from Khasmir valley of India with a multivariate approach. *J Food Nutr Sci*. **2015.** 65(2): 101–108.

[8] Zhang YZ, Chen YF, Wu YQ, Si JJ, Zhang CP, Zheng HQ, and Hu FL. Discrimination of the entomological origin of honey according to the secretions of the bee (*Apis cerana* or *Apis mellifera*). *FoodRes Int.* **2019.** 116:362–369.

[9] Indonesian National Standard 8664:2018. *Standardization of Honey Quality*. **2018.** Jakarta (ID): National Standardization Body.

[10] Devi AR. Test the effectiveness of honey from four species of bees as an antibacterial agent [thesis]. **2022.** Bogor (ID): Bogor Agricultural Institute.

[11] Chairunisa F. Antibacterial activity of the extract and 70% ethanol fraction of red betel leaves (*Piper crocatumi*) against *Escherichia coli bacteria* pBR322 [thesis]. **2019.** Bogor (ID): Bogor Agricultural Institute.

[12] Singh I, and Singh S. Honey moisture reduction and quality. *J. Food. sci. Technol.* **2018.** 55(10): 3861-3871.

[13] Harjo SST, Radiati LE, and Rosyidi D. Comparison of rubber honey and rambutan honey based on water content, diastase enzyme activity, and hydroxymethylfurfural (HMF). *J. Anim. Prod. Sci. Techol*. **2015.** 10(1): 18-21.

[14] Kamboj R, Sandhu RS, Kaler RSS, Bera MB, and Nanda V. Optimization of process parameters on hydroxymethylfurfural content, diastase and invertase activity of coriander honey. *J. Food Sci Technol*. **2019.** 56(7): 3205-3214.

[15] Clinical Laboratory Standard Institute. *Performance Standard for Antimicrobial Susceptibility Testing*. **2013.** Twentieth Information Supplement. USA.

[16] Hasan AEZ, Andrianto D, Rahmania A, Ahmada IGC, and Abdillah RM. Effect of heating on flavonoid concentration in honey harvested from across the Indonesian archipelago. *Online Pharmacology*. **2021.** 2021(3): 615-623.

[17] Almasaudi S. The antibacterial activity of honey. *Saudi J Biol Sci*. **2021.** 28(2021): 2188-2196.

[18] Gunes N, Aydin L, Beleni D, Hranitz JM, Mengilig S, and Selova S. Stress responses of honey bees to organic acid and essential oil treatment against varroa mites. *J. Apicultur. Research*. **2017.** 56(2): 175-181.

[19] Gorniak I, Bartoszewski R, and Kroliczewski J. Comprehensive review of antimicrobial activities of plant flavonoids. *Phytocem Rev.* **2019.** 18(1): 241-272.

[20] Shi C, Zhang X, Sun Y, Wang M, Song Y, Zheng Z, Chen Y, Liu X, Jia Z, Dong R, Cui LU, and Xia X. Antimicrobial activity of ferulic acid against *Cronobakter sakazakii* and possible

mechanism of action. *Foodborne Pathog Dis.* **2016.** 13(4): 196-204.
[21] Borgen A, Ferreira C, Saavedra MJ, and Simoes M. Antibacterial activity and mode of action of ferulic and gallic acid against pathogenic bacteria. *Microbial Drug Resistance*. **2013.** 19(4): 256-265.
[22] Simanjuntak AR. Virtual screening of natural polyphenolic compounds as beta-lactamase inhibitors for treating beta-lactam antibiotic resistance [thesis]. **2021.** Bogor (ID): Bogor Agricultural Institute.
[23] Hasan AEZ, Herawati H, Purnomo, and Amalia L. Physicochemistry of multiflora honey from Riau and its potential as an antibacterial for *Escherichia* coli and *Staphylococcus aureus*. *Chem. Prog*. **2020.** 13(2): 81-90.