

# ANTIBACTERIAL AND DIASTASE ENZYME ACTIVITIES OF HONEY *Apis mellifera* FROM INDONESIA

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## ABSTRACT

The quality of monofloral honey from *Apis mellifera* as an antibacterial can be influenced by the activity of the diastase enzyme and the secondary metabolites from the nectar source. Therefore, this study aimed to compare the activity of the diastase enzyme and the effectiveness of monofloral honey derived from *Apis mellifera* bees as a natural antibacterial agent against *Staphylococcus aureus* and *Escherichia coli*. Sampling for monofloral honey was carried out from nine different nectar sources, namely *Acacia carpa*, *Calliandra calothyrsus*, *Nephelium lappaceum*, *Melaleuca laucadendron*, *Ceiba pentandra*, *Mangifera indica*, *Coffea robusta* 1, *Coffea robusta* 2, and *Hevea brasiliensis*. Furthermore, diastase content was determined using UV-Vis spectrophotometry and the bacterial inhibition zone using the disc diffusion method. The principal component analysis (PCA) was used to analyze the clustering of diastase enzyme and antibacterial activity. The results showed that the highest diastase activity in monofloral honey was *Mangifera indica*, *Nephelium lappaceum*, and *Coffea robusta* 2 at 20.00 DN. This was followed by *Nephelium lappaceum*, *Ceiba pentandra*, and *Hevea brasiliensis* at 10.00 DN, *Acacia carpa* at 6.67 DN, *Coffea robusta* 1 at 5.00 DN, and *Calliandra calothyrsus* 4.00 DN. The clear zones for *Staphylococcus aureus* on *Coffea robusta* 2, *Acacia carpa*, *Nephelium lappaceum*, *Coffea robusta* 1, *Ceiba pentandra*, *Hevea brasiliensis*, *Nephelium lappaceum*, *Calliandra calothyrsus*, and *Mangifera indica* were 19.47, 18.53, 17.73, 17.03, 16.12, 16.10, 16.03, 15.73, and 14.73 mm, respectively. Additionally, the clear zones for *Escherichia coli* on *Ceiba pentandra*, *Coffea robusta* 2, *Acacia carpa*, *Coffea robusta* 1, *Melaleuca laucadendron*, *Mangifera indica*, *Hevea brasiliensis*, *Calliandra calothyrsus*, and *Nephelium lappaceum* were 27.93, 26.13, 24.60, 24.53; 24.53, 24.07, 21.90, 21.60, and 21.53 mm, respectively. In conclusion, clustering analysis was conducted based on nectar sources to evaluate antibacterial and diastase activity. The clusters identified are cluster 1 consisting of *Hevea brasiliensis*, cluster 2 including *Mangifera indica*, and *Nephelium lappaceum*. Others are cluster 3 consisting of *Acacia carpa* and *Calliandra calothyrsus*, and cluster group 4 including *Nephelium lappaceum*, *Ceiba pentandra*, *Coffea robusta* 1, and *Coffea robusta* 2. Therefore, it was necessary to carry out antibacterial testing of other bacteria, specifically *Salmonella typhi*, and determine the minimum inhibitory concentration (MIC) of honey with the best antibacterial activity in various concentration variations.

**Keywords:** antibacterial, clustering, diastase enzyme, monofloral honey

## INTRODUCTION

The quality of honey can be affected by several factors, such as during harvest, water content, color, taste, and aroma of honey. Harvesting honey necessitates careful timing when it is ripe and the bees are starting to close the nectar cells. The water content of honey also affects the quality by containing approximately 17-21% water. 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 produced by bees during the ripening process and is used to assess the quality of honey (Sukmawati *et al.* 2015).

Based on data from Riskesdas (2018), the prevalence of diarrhea in Indonesia is 37.88% and has increased in 2019 to 40% in toddlers (RI Ministry of Health 2020). Furthermore, the age group with the highest prevalence of diarrhea was 1 to 4 years (11.5%) and infants (9%). According to the WHO (2018), gastroenteritis in the general population has a mortality rate of 525,000 deaths per year in children under 5 years with an

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incidence rate of 1.7 million. This condition makes gastroenteritis the second leading cause of death due to infectious diseases. Acute gastroenteritis is diarrhea that lasts up to 14 days and is characterized by an increase in stool volume, frequency, and water content. The most common causes of diarrhea are infections in the form of viruses, bacteria, and parasites. It is often caused by an infectious disease from *Staphylococcus aureus* and *Escherichia coli* bacteria, which is still a major problem in public health (Muziburrahman *et al.* 2022).

*Staphylococcus aureus* is a round gram-positive bacteria arranged such as clusters of grapes and is quite active in its division, which is commonly found in burns and hospitals (Yuliati 2017). On the other hand, *escherichia coli* is a gram-negative rod-shaped bacterium and can cause various diseases, such as diarrhea, which has an incubation period of 10-24 hours before showing symptoms (Pratiwi 2018). Antibacterial compounds have been widely used to treat diseases caused by bacteria. It is imperative to adopt preventive measures to hinder the escalating resistance of bacteria to drugs, as over time, these pathogenic bacteria can develop resistance to antibacterial compounds (Panjaitan *et al.* 2018).

One way to prevent the emergence of bacterial resistance to synthetic antibacterials is the use of natural ingredients having antibacterial activity, including honey (Hanifa *et al.* 2020). The production of honey occurs through the transformation of nectar, a process that involves the reduction of water content within both the bee's body and the beehive. The various types of honey that exist and their potential to be used as an antibacterial agent promote this study. Some of the compounds present in honey are proteins, flavonoids, and low pH allowing for the inhibition of bacterial growth. According to Nayik and Nanda (2015), light-colored honey contains less phenol than dark-colored.

The phytochemical content of each honey varies depending on the origin of the nectar from the honey. This content is useful as an antioxidant, antibacterial, anti-inflammatory, antiviral, and antidiabetic agent, as well as affects the human body's metabolism properly, hence, they have the potential to improve health and prevent various diseases (Liu *et al.* 2013). Honey is classified as monofloral (one type of plant) or multi floral (several types of plants) depending on the origin of its nectar (Soares *et al.* 2018; Zhang *et al.* 2019). Presently, monofloral honey from *Apis mellifera* bees from Indonesia has antibacterial compounds. The effectiveness of suppressing bacterial growth varies in relation to the content of active antibacterial compounds found in honey, as well as the extractive substances derived from nectar. A total of 9 honey specimens of the monofloral species *Apis mellifera* from Indonesia were characterized in this study. Therefore, this study aimed to determine the activity of the diastase enzyme and the effectiveness of monofloral honey derived from *Apis mellifera* bees as a natural antibacterial agent against *Staphylococcus aureus* and *Escherichia coli*.

## MATERIALS AND METHODS

### Materials

The materials used in this study included 9 specimens of *Apis mellifera* honey, distilled water, starch, iodine, NaCl, acetate buffer, *Staphylococcus aureus* ATCC6538 and *Escherichia coli* ATCC8739 bacterial cultures, Nutrient Broth (NB) and Mueller Hiton Agar (MHA) media, ciprofloxacin, and aquabides.

### Sample Preparation

Samples of monofloral honey from Indonesia, as shown in Table 1, were stored in the Laboratory of the Biochemistry Master's Study Program, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University.

Table 1 Origin of monofloral honey samples used

Species	Nectar Plant	City	Province
<i>Apis mellifera</i>	<i>Acacia carpa</i>	Tanjung Jabung	Jambi
	<i>Calliandra calothyrsus</i>	Sukabumi	West Java
	<i>Nephelium lappaceum</i>	Subang	West Java
	<i>Melaleuca leucadendron</i>	Sragen	Central Java
	<i>Ceiba pentandra</i>	Pati	Central Java
	<i>Mangifera indica</i>	Pasuruan	East Java
	<i>Coffea robusta</i> 1	Malang	East Java
	<i>Coffea robusta</i> 2	Jember	East Java
	<i>Hevea brasiliensis</i>	Jember	East Java

## Water Content

The water content of monofloral honey was measured using a refractometer according to the Indonesian National Standard (2018). After dripping one drop of honey sample onto the prism, it was closed and pointed towards the light, and the result was read from the blue line on the white scale.

## Diastase Enzyme Activity

Analysis of the diastase enzyme activity of monofloral honey was performed according to the Indonesian National Standard (2018). A total of 5 g honey was added to 2.5 mL acetate buffer solution pH 5.3 and 1.5 mL of 0.5 M NaCl solution, homogenized into a 25 mL volumetric flask, and adjusted to the mark by adding distilled water. Subsequently, 10 mL of the mixture was heated in a water bath at 40°C. After 15 minutes, 5 mL of starch solution was added and homogenized. Every 15 minutes, 1 mL of the sample mixture was added to 10 mL of 0.0007 N iodine and homogenized, and then 25 mL volume of distilled water was added. The absorbance value was measured at  $\lambda$  660 nm and the initial limit of reaction time starts from the addition of the iodine indicator. Furthermore, the collection of the solution was continued until the value of  $A < 0.235$  was obtained within a certain time interval.

Diastase enzyme activity (DN) was calculated by the following formula:

$$DN = \frac{300}{t} \quad (1)$$

Where:

DN = diastase enzyme activity

t = time used to reach the value of  $A < 0.235$

## Antibacterial Activity

The antibacterial activity of monofloral honey was analyzed according to the method of Devi (2022) with modifications. Furthermore, bacterial cultures of *Staphylococcus aureus* and *Escherichia coli* rejuvenated in 50 mL nutrient broth (NB) media were used as tested strains for antibacterial testing. Each strain was incubated for 24 hours at 37°C and the culture that grow were subjected to the disk diffusion test. The Mueller Hinton Agar (MHA) media used in the test were sterilized using an autoclave at 121°C for 15 minutes. Furthermore, the MHA media was then poured into a sterile Petri dish until solidified and the bacterial culture was streaked on the MHA media with a known OD value. Sterile discs were left overnight to absorb the honey sample to be tested at a concentration of 100% and then dried in an oven at 45°C for 2 hours. The prepared discs were placed on a bacterial culture scratch on MHA and incubated at 37°C for 24 hours. Furthermore, the clear zone formed around the disc was measured with a Vernier caliper. Ciprofloxacin at a concentration of 100 ppm (0.1%) and 25  $\mu$ L of blank distilled water was used as the positive and negative control, respectively.

## Data Analysis

Each experiment was carried out in triplicate, and the diastase enzyme activity and antibacterial activity resulting in each replicate were written as the mean  $\pm$  standard deviation ( $n=3$ ). The one-way ANOVA test was used to analyze data on all honey samples with a 0.05 level of significance ( $\alpha$ ) using SPSS version 25. A p-value  $< 0.05$  indicated that the data were significantly different, and should be further tested. Duncan's test was used to determine the effect of significant differences in each monofloral honey. Furthermore, the

classification of monofloral honey based on diastase enzyme and antibacterial activity was determined by the principal component analysis (PCA) chemometric method with Minitab 16.

## RESULTS AND DISCUSSION

### Water Content

Among the 9 samples analyzed, the water content of honey adhered to predetermined standards. *Coffea robusta* 2 honey had the lowest water content at 17.50%, while *Calliandra calothyrsus* had the maximum at 22.00% determined by SNI. The moisture content of *Nepheleium lappaceum*, *Melaleuca laucadendron*, *Ceiba pentandra*, *Mangifera indica*, *Coffea robusta* 1, *Acacia carpa*, and *Hevea brasiliensis* were 21.50%, 20.50%, 20.50%, 19.50%, 19.00%, 18.50%, and 18.00%, respectively. The ANOVA statistical analysis supported this result, showing a significant difference in the water content of the 9 monofloral honeys ( $p$ -value  $<0.05$ ). This showed that differences in plants as a source of nectar had an impact on the water content of honey. The results of Duncan's test ( $\alpha=0.05$ ) on

the water content of 9 monofloral honeys showed 8 groups, namely (1) *Calliandra calothyrsus*, (2) *Nepheleium lappaceum* L., (3) *Melaleuca laucadendron* L. and *Ceiba pentandra* (L.) Gaertn., (4) *Mangifera indica*, (5) *Coffea robusta* 1, (6) *Acacia carpa*, (7) *Hevea brasiliensis* M., and (8) *Coffea robusta* 2. These groups were categorized based on the proximity of their water content, as shown in Figure 1.

The water content in honey determines the stability of fermentation because it allows easy fermentation over time. Therefore, honey needs to be subjected to heat treatment to prevent fermentation by sugar-tolerant yeasts and maintain its liquid state (Singh and Singh 2018). Relative humidity (Rh) in Indonesia ranges from 60–90%, indicating that the water content in honey is approximately 18.3–33.1%. Honey is hygroscopic, and must be stored in a place that is not permeable to air. According to Pujiarti *et al.* (2021), signs of a fermentation process are the foam and gas produced by honey. Hasan *et al.* (2020) reported that the antibacterial activity of multiflora honey from Riau RHU4 has the lowest water content of 16.45% and RHI2 honey of 20.56%.

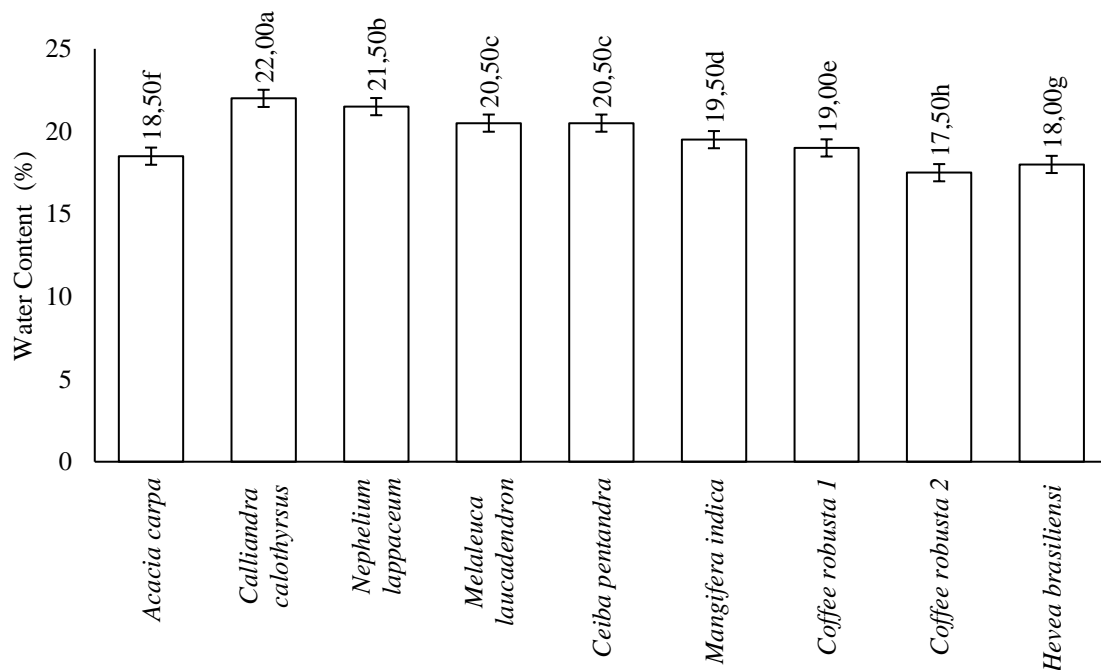


Figure 1 The water content of monofloral honey

Note: Different lowercase letters show statistically significant differences ( $\alpha=0.05$ ).

The fermentation process in yeast can be significantly influenced by the high water content in honey, leading to a decline in its overall quality. Moreover, the shelf life of honey is affected by its water content and a higher water content results in a relatively short shelf life. According to Hasan *et al.* (2020), honey can absorb up to 33% water by weight in moist air. Good-quality honey has a low water content, indicating that thicker honey has a lower water content (Swari *et al.* 2019). Honey harvested at an older age contains less water than at a younger age. Generally, at harvest ages <14 days, the water content of honey is relatively high. According to Nasharuddin *et al.* (2022), Indonesian honey is often fermented due to its high water content of >26%. The Indonesian National Standard (2018) reported that the quality requirement of water content in honey was a maximum of 22%. A previous study showed a correlation between the water and sugar content of honey, and the honey crystallization process was natural (Nasharuddin *et al.* 2022).

### Diastase Enzyme Activity

The average results of diastase enzyme activity in monofloral honey were shown in Figure 2. The highest diastase enzyme activity in *Mangifera indica*, *Nepheleium lappaceum*, and *Coffea robusta 2* was 20.00 DN, while the lowest was in *Calliandra calothyrsus* at 4.00 DN. Furthermore, the diastase enzyme activity of *Melaleuca laucadendron*, *Ceiba pentandra*, and *Hevea brasiliensis* was 10.00 DN, whereas *Acacia carpa* and *Coffea robusta 1* were 6.67 DN and 5.00 DN, respectively.

These results were supported by ANOVA statistical analysis which showed a significant difference between the 9 monofloral honey ( $p$ -value <0.05) based on their diastase enzyme activity. This showed that differences in plants as a source of nectar affected it. It also showed the impact of the differences in plants as a source of nectar. The results of Duncan's test ( $\alpha=0.05$ ) on the activity of the diastase enzyme from 9 monofloral honey showed 5 groups, namely (1) *Mangifera indica*, *Nepheleium lappaceum* L., and *Coffea robusta 2*, (2) *Melaleuca laucadendron* L., *Ceiba pentandra* (L.) Gaertn, and *Hevea brasiliensis* M., (3) *Acacia carpa*, (4) *Coffea robusta 1*, and (5) *Calliandra calothyrsus* M. These monofloral honey were categorized based on their proximity to the activity of the diastase enzyme they possess, as shown in Figure 2.

The diastase enzyme is one of many different types found in every variety of honey and contributes to its nutritional value. Bees introduce the diastase enzyme during the ripening of the honey. This enzyme breaks the glucosidic bonds in starch and other oligo- and polysaccharides into simple sugars. According to Singh and Singh (2018), adequate heating and storage are crucial to retaining the market value of honey since the diastase enzyme is extremely sensitive to heat.

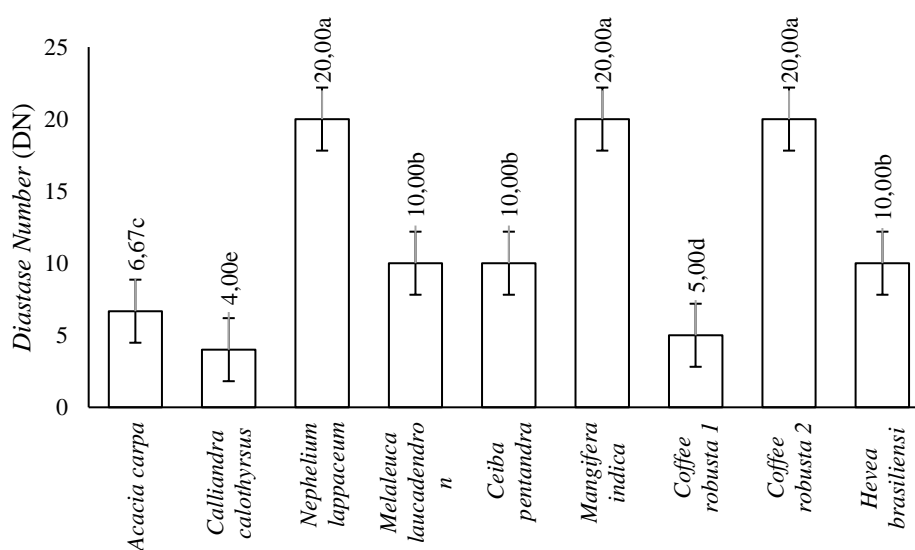


Figure 2 The diastase enzyme activity value of monofloral honey

Note: Different lowercase letters show statistically significant differences ( $\alpha=0.05$ ).

According to Ichsan *et al.* (2022), in the chemical test with the diastase enzyme, 17 out of 22 samples were found to have good quality because they met the SNI requirement. The Diastase Number (DN) value ranged from 0.64 to 23.11, while for fake honey, the value of DN was 0, indicating that it was undetectable. The absence of the diastase enzyme in a honey sample can serve as an indicator of potential adulteration, suggesting that the sample may be classified as fake honey. Such samples solely consist of sugars without the presence of the diastase enzyme. Furthermore, Tulandi (2019) showed that storage temperature greatly affected the quality of honey. There was a significant difference in diastase enzyme activity in each treatment at storage temperature. The best storage temperature for honey was 26°C (room temperature) compared to hot (50°C) and cold ( $\pm 5^\circ\text{C}$ ) temperatures.

The activity of this enzyme decreased with storage and heating time, as indicated by its measurement through DN. It is observed that diastase activity decreases as the temperature rises, and the decline becomes more significant with higher temperature increments. The diastase activity decreases quickly during isothermal heating at a constant temperature but increases once again as the number of heating cycles increases (Singh and Singh 2018). According to Harjo *et al.* (2015), diastase activity in rubber and rambutan honey was 11.89 DN and 11.58 DN, respectively. The presence of the diastase enzyme in honey can be attributed to the bee's ability to produce saliva. Consequently, a higher value of the diastase enzyme indicates a greater concentration of bee saliva contained in honey.

A decrease in the value of the diastase enzyme activity can occur in the storage process before testing. Therefore, it is advisable to conduct diastase enzyme testing shortly after the honey is harvested to ensure accurate assessment. The activity of the diastase enzyme is an indicator of the quality of honey because it can indicate its processing and storage time. According to Pujiarti *et al.* (2021), storage can significantly affect the inactivation of enzymes. Honey products usually do not meet requirements for commercialization after they have been heated, reheated, or stored for a long time, because the diastase activity is below the quality requirements. According to the Indonesian National Standard

(2018), the quality requirements for the value of honey diastase activity are more than 3 DN.

### Antibacterial Activity

The smallest clear zone for *Staphylococcus aureus* was 14.73 mm in *Mangifera indica*, while the largest was 19.47 mm in *Coffea robusta 2*. The clear zones for *Acacia carpa*, *Melaleuca laucadendron*, *Coffea robusta 1*, *Ceiba pentandra*, *Hevea brasiliensisensi*, *Nephelium lappaceum*, and *Calliandra calothyrsus* were 18.53 mm, 17.73 mm, 17.03 mm, 16.12 mm, 16.10 mm, 16.03 mm, and 15.73 mm, respectively, as shown in Figure 3. The smallest clear zone for *Escherichia coli* was 21.53 mm in *Nephelium lappaceum*, while the largest was 27.93 mm in *Ceiba pentandra*. The values for *Coffea robusta 2*, *Acacia carpa*, *Coffea robusta 1*, *Melaleuca laucadendron*, *Mangifera indica*, *Hevea brasiliensisensi*, and *Calliandra calothyrsus* were 26.13 mm, 24.60 mm, 24.53 mm, 22.53 mm, 22.07 mm, 21.90 mm, and 21.60 mm, respectively, as shown in Figure 4.

The positive control on both bacteria showed that a clear zone was formed. This is consistent with the ANOVA statistical analysis, showing that the 9 monofloral honey were significantly different ( $p$ -value  $< 0.05$ ) based on their antibacterial activity. The results of Duncan's test ( $\alpha = 0.05$ ) on the inhibition zone of *Staphylococcus aureus* bacteria from 9 monofloral honey showed 6 groups, namely (1) *Coffea robusta 2* and *Acacia carpa*, (2) *Acacia carpa* and *Melaleuca laucadendron* L., (3) *Melaleuca laucadendron* L. and *Coffea robusta 1*, (4) *Coffea robusta 1*, *Ceiba pentandra* (L.) Gaertn, and *Nephelium lappaceum* L., (5) *Ceiba pentandra* (L.) Gaertn, *Hevea brasiliensisensi* M., *Nephelium lappaceum* L., and *Calliandra calothyrsus* M., and (6) *Mangifera indica*. These monofloral honey were grouped based on the proximity of their inhibition zones, as shown in Figure 3. Furthermore, Duncan's test at  $\alpha = 0.05$  on the inhibition zone of *Escherichia coli* bacteria showed that 4 groups, namely (1) *Ceiba pentandra* (L.) Gaertn, (2) *Coffea robusta 1*, (3) *Acacia carpa* and *Coffea robusta 2*, and (4) *Melaleuca laucadendron* L., *Mangifera indica*, *Hevea brasiliensisensi* M., *Calliandra calothyrsus* M., and *Nephelium lappaceum* L. They were groups based on the proximity of their inhibition zones, as shown in Figure 4.

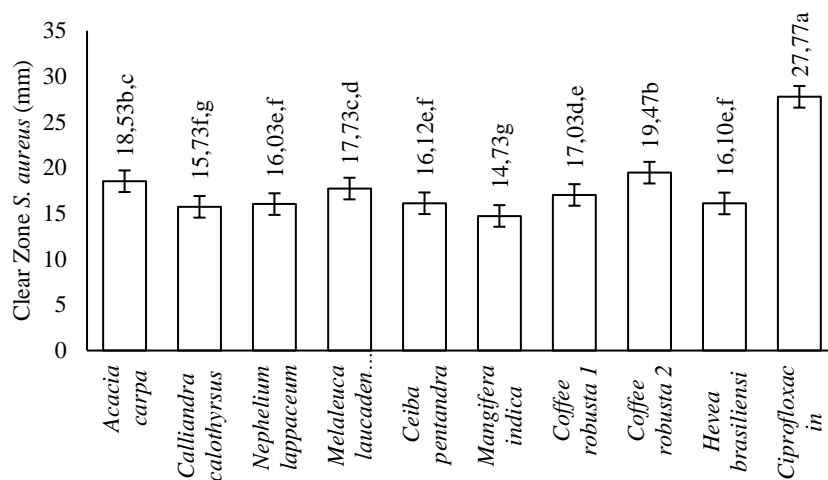


Figure 3 The antibacterial activity of monofloral honey against *Staphylococcus aureus*  
 Note: Different lowercase letters show statistically significant differences ( $\alpha=0.05$ ).

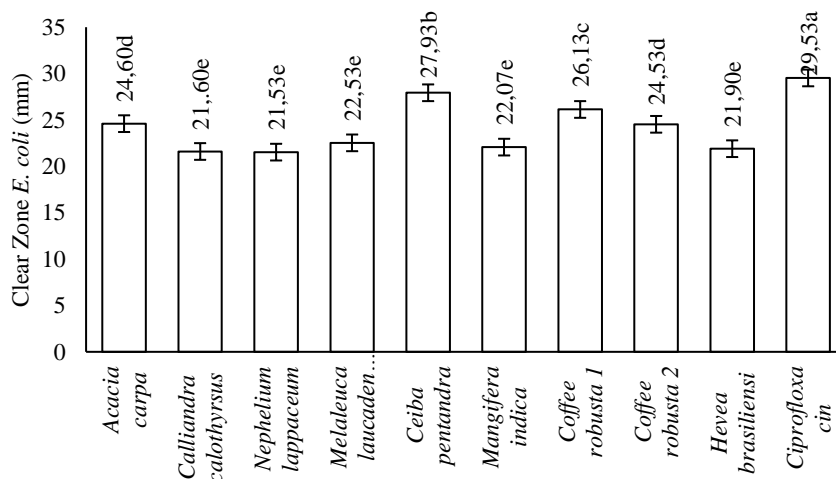


Figure 4 The antibacterial activity of monofloral honey against *Escherichia coli*  
 Note: Different lowercase letters show statistically significant differences ( $\alpha=0.05$ ).

The clear zone value is determined by subtracting the diameter of the disc used from the measured clear zone diameter. The effectiveness of bacterial inhibition by honey is influenced not only by physicochemical characteristics but also by its bioactive compounds. Furthermore, the content of bioactive compounds is obtained from nectar sources that are taken and processed by honey bees (Hasan *et al.* 2020).

The monofloral honey antibacterial test was carried out on two test bacteria, namely *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative). Furthermore, antibacterial activity was determined based on the diameter of the clear zone formed after incubation. This test used the antibiotic ciprofloxacin (100 ppm) as the positive control. According to the Clinical Laboratory Standard

Institute (CLSI) (2013), antibacterial activity is classified as weak or lacking when the resulting inhibition zone is  $\leq 12$  mm. Similarly, it is classified as medium and strong when the inhibition zone is 13-17 mm and  $\geq 18$  mm, respectively. Based on this classification, the test results showed that monofloral honey had moderate to strong antibacterial activity against *Staphylococcus aureus*. This was because the diameter of the resulting inhibition zone was in the range of 14.73 mm and 19.47 mm. The antibacterial activity of monofloral honey against *Escherichia coli* was classified as strong because the diameter of the resulting inhibition zone was greater than 18 mm, ranging from 21.53 mm to 27.93 mm.

According to Hasan *et al.* (2020), the smallest inhibition zone of *Staphylococcus aureus* for multi-

floral honey from Riau was 7.84 mm for 20% RHI1 honey, while the largest for RHI2 80% honey was 19.25 mm. The smallest inhibition zone of *Escherichia coli* in RHI1 honey with a concentration of 40% was 1.22 mm, while the largest inhibition zone in PLW1 60% honey was 4.58 mm. According to Kaligis *et al.* (2020), the minimum inhibitory concentration (MIC) of forest honey toward *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* is 12.5%, 12.5%, and 25%, respectively. In this study, there was no minimum bactericidal concentration (MBC) in either forest or black honey. Furthermore, the antibacterial effect of forest honey is superior to that of black honey by a small margin.

Gram-negative bacteria tend to be more sensitive to the antibacterial substances contained in honey than Gram-positive. They also have hydrophilic groups that easily bind to polar compounds, making the antibacterial activity of honey more effective against Gram-negative bacteria than Gram-positive (Wadi 2022). Furthermore, the study conducted with monofloral honey from Indonesia showed that *Escherichia coli* was the most influential bacteria. This can be attributed to osmosis, facilitated by the high sugar content of honey, causing water to flow out of the cells, such that the resulting pure and undiluted honey prevents the growth of germs. According to Almasaudi (2021), Dehydration also causes cells to shrink, which hinders their ability to survive in a hypertonic sugar solution, thereby reducing the antibacterial potential.

The vast majority of microbes normally grow in neutral environments with pH values between 6.5 and 7.5. One of the most distinguishing characteristics of the antibacterial qualities of honey is its acidity, which ranges between pH 3.2 and 4.5. Its acidity is caused by the presence of a significant organic acid (gluconic) that is present in the sample at a concentration of 0.5% (w/v). The natural glucose oxidase enzyme converts glucose into glycogen acid, which is a particularly effective antibacterial agent. Furthermore, low pH in pure and undisturbed honey can add to its antibacterial capabilities, when diluted in food or other body fluids (Almasaudi 2021).

Cianciosi *et al.* (2018) reported that flavonoids and phenolic acids were among the broad group

of compounds known as polyphenols and distinguished by their phenolic structures. These biological components are converted from nectar to honey and play a significant role in the health benefits of honey. The presence of numerous phenolic chemicals in honey provides a plausible explanation for its antibacterial properties (Gunes *et al.* 2017).

Several studies explored the antibacterial activity of phenolic acids in honey. Among these acids, caffeic was found to induce oxidative stress and chlorogenic acid increases membrane permeability, resulting in cytoplasmic and nucleotide leakage (Gorniak *et al.* 2019). Furthermore, gallic acid induces intracellular leakage as a result of the breakdown of cell membranes and increased hole formation (Shi *et al.* 2016). P-coumaric acid disrupts cell membranes and bacterial DNA brushing, and syringic acid causes cell membrane dysfunction (Borges *et al.* 2013). The antibacterial mechanisms of flavonoids found in honey include apigenin and kaemferol, which can inhibit DNA gyrase. Galanin inhibits peptidoglycan synthesis from ribosomes and luteolin inhibits FAS-I in mycobacteria and inhibits DNA helicase DnaB and RecBCD. On the other hand, myricetin inhibits DNA B helicase, pinocembrin induces cell lysis (Collins *et al.* 2019), and catechins contribute to the generation of H<sub>2</sub>O<sub>2</sub> (Das *et al.* 2015).

### Honey Clusterization

The results of the score plot on the antibacterial activity of *Staphylococcus aureus* and *Escherichia coli* and diastase activity show 4 main clusters of monofloral honey from Indonesia with different nectar sources (Figure 5). The first cluster (Figure 5a) consists of *Hevea brasiliensis* from Jember. The second cluster (Figure 5b) consists of *Mangifera indica* from Pasuruan and *Nephelium lappaceum* from Subang. The third cluster (Figure 5c) consists of *Acacia carpa* from Tanjung Jabung and *Calliandra calothyrsus* from Sukabumi. Finally, the fourth cluster (Figure 5d) consists of *Melaleuca laucadendron* honey from Sragen, *Ceiba pentandra* from Pati, *Coffea robusta* 1 from Malang, and *Coffea robusta* 2 from Jember.



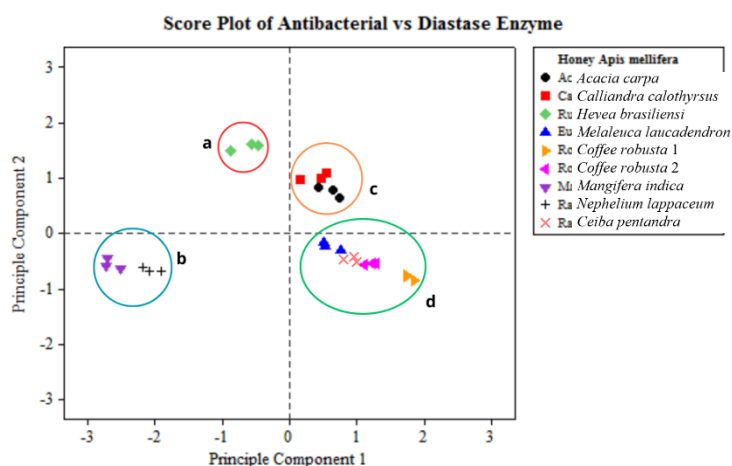


Figure 5 Score plot results on antibacterial and diastase enzyme activity

Table 2 Taxonomy of the nectar source plants used

Cluster	Species	Genus	Family	Ordo	Class	Phylum	Kingdom
1-1	<i>Hevea brasiliensis</i> M.	Hevea	Euphorbiaceae	Euphorbiales	Dicotyledoneae	Spermatophyta	Plantae
2-1	<i>Mangifera indica</i>	Mangifera	Anacardiaceae	Sapindales	Dicotyledoneae	Spermatophyta	Plantae
2-2	<i>Nephelium lappaceum</i> L.	Nephelium	Sapindaceae	Sapindales	Magnoliopsida	Spermatophyta	Plantae
3-1	<i>Calliandra calothyrsus</i> M.	Calliandra	Fabaceae	Fabales	Magnoliopsida	Magnoliophyta	Plantae
3-2	<i>Acacia crassicaarpa</i>	Acacia	Fabaceae	Fabales	Magnoliopsida	Magnoliophyta	Plantae
4-1	<i>Melaleuca laucadendron</i> L.	Melaleuca	Myrtaceae	Myrtales	Magnoliopsida	Magnoliophyta	Plantae
4-2	<i>Ceiba pentandra</i> (L.) Gaertn	Ceiba	Malvaceae	Malvales	Magnoliopsida	Magnoliophyta	Plantae
4-3	<i>Coffea robusta</i>	Coffea	Rubiaceae	Rubiales	Magnoliopsida	Magnoliophyta	Plantae
4-4	<i>Coffea robusta</i>	Coffea	Rubiaceae	Rubiales	Magnoliopsida	Magnoliophyta	Plantae

The clustering of monofloral honey derived from 9 nectar-sourced plants in Indonesia was determined using the multivariate PCA method. This analysis considered both the antibacterial activity and diastase enzymes. The results showed that there are groups with similarities and some exhibit dissimilarities. The PCA method is used to simplify data and enhance readability by transforming the data linearly to form a new coordinate with maximum variance (Ghani *et al.* 2016).

The arrangement of taxonomy is based on phylogenetic and chemotaxonomy. In this context, plants that share a close kinship tend to possess similar types and homologies of secondary metabolite content. The identified chemical compounds have antibacterial activity. For example, cluster 1 is the genus *Hevea*, which contains flavonoids (Evahelda *et al.* 2017). Cluster 2 is of the Sapindales order, and it contains acetophenone, butanoic acid, and benzyl alcohol (Rohman 2017). Cluster 3 belongs to the Fabaceae family, containing protocatechuic, gallic, caffeic, and ascorbic acid (Obisioiu *et al.* 2021). Cluster 4 belongs to the Magnoliopsida class, which is characterized by aromatic

compounds, such as sesquiterpenes, isoflavonoids, and benzylisoquinone alkaloids (Chassagne *et al.* 2021).

The differences in honey characteristics, such as taste, aroma, color, and composition, can be attributed to the diverse range of plants whose nectar is collected by bees for honey production. These variations subsequently influence the antibacterial activity of honey. Furthermore, several factors contribute to the antibacterial activity of honey, namely the types of flowers that take their nectar from different areas and their high sugar content (Arumsari *et al.* 2019).

## CONCLUSION

In conclusion, the resulting diastase activity in monofloral honey includes *Mangifera indica*, *Nephelium lappaceum*, *Coffea robusta* 2 at 20.00 DN, *Nephelium lappaceum*, *Ceiba pentandra*, and *Hevea brasiliensis* at 10.00 DN, *Acacia carpa* at 6.67 DN, *Coffea robusta* 1 at 5.00 DN, and *Calliandra calothyrsus* 4.00 DN. The inhibition zones for *Staphylococcus aureus* on *Coffea robusta* 2, *Acacia carpa*, *Nephelium lappaceum*, *Coffea robusta* 1, *Ceiba pentandra*, *Hevea brasiliensis*, *Nephelium lappaceum*,

*Calliandra calothyrsus*, and *Mangifera indica* were 19.47, 18.53, 17.73; 17.03, 16.12, 16.10, 16.03, 15.73, and 14.73 mm, respectively. Furthermore, the inhibition zones for *Escherichia coli* on *Ceiba pentandra*, *Coffea robusta* 2, *Acacia carpa*, *Coffea robusta* 1, *Melaleuca laucadendron*, *Mangifera indica*, *Hevea brasiliensis*, *Calliandra calothyrsus*, and *Nephelium lappaceum* were 27.93, 26.13, 24.60, 24.53, 24.53, 24.07, 21.90, 21.60, and 21.53 mm, respectively. In this study, clusterization analysis was based on the antibacterial and diastase activity, namely cluster 1 consisting of *Hevea brasiliensis*, cluster 2 comprising *Mangifera indica* and *Nephelium lappaceum*, cluster 3 containing *Acacia carpa* and *Calliandra calothyrsus*, as well as cluster 4 involving *Melaleuca laucadendron*, *Ceiba pentandra*, *Coffea robusta* 1, and *Coffea robusta* 2. Therefore, it is necessary to carry out antibacterial testing of other bacteria, specifically *Salmonella typhi*, and determine MIC of honey with the best antibacterial activity in various concentration variations.

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#### AUTHORS' CONTRIBUTION

Rara Annisaur Rosyidah designed and conducted all the experiments, analyzed the data, performed the data visualization, and wrote the manuscript. Akhmad Endang Zainal Hasan and Dimas Andrianto assisted in conducting the experiments, performed the data visualization, and wrote the manuscript. All authors have read and approved the final manuscript.

#### CONFLICT OF INTEREST

The authors declare that there are no competing interests. This current study does not involve experiments on animals or human subjects and the data are available from the authors.

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