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## THE POTENTIAL OF FOREST HONEY (*Apis* spp.) FROM TIMOR ISLAND AS ANTIBACTERIAL AGAINST PATHOGENIC BACTERIA IN FISH CULTURE

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### ABSTRACT

This study aims to assess the potential of forest honey *Apis* spp. from Timor Island as an antibacterial on pathogenic bacteria, *Aeromonas hydrophilla* and *Vibrio alginolyticus* in cultured fish. There were six types of honey tested in this study of which active compound, total glucose, pH, and water content of each honey were determined. The chemical contents of the honey were examined for alkaloid using Culvenor-Fitzgerald method, saponin with foam test, terpenoid and steroid with Lieberman-Burchard method, tannin with addition of FeCl<sub>3</sub>, and flavonoid addition of HCl and Mg powder. Total glucose was measured using spectrophotometer, water content with gravimetry, and pH with pH-meter. The antibacterial activity test of the honey was done using disc method without dilution. Results showed that all forest honey from Timor Island contained alkaloid, saponin, steroid, and terpenoid, except Kefa honey which did not have steroid and terpenoid. The water content of the honey ranged from 15.70% to 26.65%, total glucose of 71.16% to 80.58%, and pH of 3.84 to 4.06. The forest honey also had antibacterial activity against *A. hydrophilla* and *V. alginolyticus* with different inhibition zones.

**KEYWORDS:** *Aeromonas hydrophilla*; antibacterial; honey; *Vibrio alginolyticus*

### INTRODUCTION

Honey is a viscous liquid produced by honey bees from flower nectar (Yuliati, 2017). Based on the nectar source, honey can be distinguished as monoflora and polyflora. The former is produced from one major plant and the latter from the nectar of several types of plants. Forest honey is a polyflora one produced by wild bees *Apis dorsata*. These bees usually live in the forest and depend their various food sources from forest plants such as flower nectar or tree sap. Due to diverse food sources, the honey has better quality due to honey content of more complex compounds. Forest honey produced by the wild bees is also very good for health since it contains natural antibiotics.

Several studies have proved the potential of the forest honey from Indonesia as an antibacterial against pathogenic Gram-positive or Gram-negative bacteria in human. Yuliati (2017) did an *in vitro* study which revealed the antibacterial superiority of forest

honey from Sumbawa against bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosae*. Honey from Bandung and Riau Islands have also been proved to have antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Dewi *et al.*, 2017). Furthermore, bitter and sweet black honey (forest honey from Kalimantan) has antibacterial activity against *E. coli* and *S. aureus* (Fitrianiingsih *et al.*, 2014).

Honey has been studied for its applications as a pathogenic antibacterial in human. Nevertheless, reports on its utilization as antibacterial in fish culture is currently limited. Considering the antibacterial feature of the honey, Testing the antibacterial activity of forest honey against *Aeromonas hydrophilla* and *Vibrio alginolyticus* infecting cultured fish is a researchable topic. Salosso (2017) tried to study the antibacterial activity of Timorese honey from Soe against *A. hydrophilla* and *V. alginolyticus* and its active compounds. Her finding revealed that the honey from Soe contained active compounds such as flavonoid, triterpenoid, and saponin. The compounds can inhibit the growth of *A. hydrophilla* and *V. alginolyticus* in all tested concentrations. However, this study was only limited to the forest honey from Soe and not from other places.

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Timor Island is one of the large islands in East Nusa Tenggara Province, Indonesia. The forest on the island is known to produce high-quality forest honey, such as forest honey from Soe, Kefa, Ampang, South Ampang, Naikliu, and others. The forest honey from Timor Island is mostly traditionally produced by the local communities and has not been studied about their chemical contents and natural benefits. This study assessed the potential of forest honey *Apis* spp. from Timor Island as antibacterial on pathogenic bacteria, *Aeromonas hydrophila* and *Vibrio alginolyticus* in cultured fish.

## MATERIALS AND METHODS

### Honey Collection

Forest honey was purchased from honey retailers who bought the honey from local honey finders originated from three regencies in Timor Island: North Central Timor (Kefa), South Central Timor (Soe), and Kupang Regency (Ampang, South Ampoang, and Naikliu). Commercial honey, *madu rasa*, was used as a comparison honey. The paper disc containing 30 µg of nobiicin antibiotic was used as a control.

### Phytochemical Analysis

#### Alkaloid test

Honey sample of 2 mL was mixed with 3 mL of chloroform and three drops of ammonia. The chloroform fraction was separated and acidified in 10 drops of 2N H<sub>2</sub>SO<sub>4</sub>. Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) fraction was taken and added with Dragendroff reagent. The presence of alkaloid was indicated with formation of brownish (reddish) orange deposition (Hanani, 2014).

#### Saponin test

Honey sample of 2 mL was put into a flask, then mixed with 10 mL of distilled water, boiled for 2-3 min, and then cooled. Afterwards, the solution was shaken vigorously for 10 min. Formation of stable foam after 10 min indicated the presence of saponin (Hanani, 2014).

#### Triterpenoid and steroid test

Honey sample was dropped on the drop plate and added with Lieberman-Buchardt reagent. Presence of terpenoid indicated by the red color formation or steroid with green color (Hanani, 2014).

#### Tannin test

Honey sample of 2 mL was boiled for several mins., then added with 2-3 drops of 1% FeCl<sub>3</sub>. The presence of tannin was indicated by a dark blue or greenish-black color (Hanani, 2014).

### Flavonoid test

Honey sample of 2 mL was extracted in 5 mL of methanol. The filtrate was then added with two drops of 2 N HCl and strongly shaken, added with magnesium powder, and strongly re-shaken. Flavonoid was indicated by a high intensity of foam and the solution changes to yellow-orange or dark red (Hanani, 2014).

### Analysis of pH, Water Content, and Honey

pH was determined using a pH-meter, total glucose measurement used spectrophotometer, and water content of the honey used gravimetry.

### Antibacterial Test of the Honey

#### Solid and semi-solid TSA media preparation

Media used for the growth of bacteria *A. hydrophila* used trypticase soy agar (TSA) with addition of 0.5% NaCl and *V. alginolyticus* used TSA media with addition of 2% NaCl. These media were used for antibacterial test and made two layers, solid TSA, and semi-solid TSA. The former had been made a day before the antibacterial test was done using the TSA as labeled, while the latter was made at the time the test was done using 70% TSA as labeled.

The solid TSA was autoclaved and poured into a petri dish as much as 10 mL and left up to solid and kept in the refrigerator in an upside-down position. The semi-solid media were autoclaved and cooled down to 50°C, then added with *A. hydrophila* on 0.5% TSA media and *V. alginolyticus* on 2% TSA at a density of 10<sup>6</sup> cells/mL, and each of them was poured as much as 10 mL into the solid TSA, then left down frozen. Afterward, it was ready to do the disc test (Salosso, 2017).

#### Disc Test for Antibacterial of Honey

The sterile paper disc was dipped into the original honey (without dilution). After 30 minutes, the disc paper was attached to the TSA media inoculated with *A. hydrophila* and *V. alginolyticus*. Measurements were done after 24-hour inoculation at 37°C by observing whether clear zone occurs around the paper disc or not.

## RESULTS AND DISCUSSION

### The Active Compound of the Honey

Qualitative test of the active compounds of the honey from Timor Island is presented in Table 1. This study demonstrates that all forest honey from Timor Island contain alkaloid, saponin, and steroid and terpenoid, except Kefa honey which does not contain steroid and terpenoid.

Commercial honey contains only alkaloid. The active compound content of the forest honey from Timor Island is not quite different from that of forest honey from Aceh, Seulawah (Aceh Besar) and Trumon (South Aceh) which also contains saponin and terpenoid, but no alkaloid and steroid (Fadhmi *et al.*, 2015). However, Forest honey from Timor Island has different active compound compared to Nigerian honey which did not contain alkaloid and saponin, but contain tannin and flavanoid (Adeyemo *et al.*, 2017).

The chemical compounds of the forest honey from Soe in this study contained an alkaloid, steroid, terpenoid, and saponin. In fact, different from those found by Salosso (2017) which were only flavonoid, triterpenoid, and saponin. This evidence reflects that active compound content of the honey might be influenced by seasons as both types of forest honey from Soe were collected from the same locality but at different times. Francois *et al.* (2017) also found similar result where honey from Benin Republic holds alkaloid, tannin, and flavonoid in dry season, and alkaloid, tannin, flavonoid, and terpenoid and steroid in rainy season.

The difference in active compounds content of the honey could be the result of different nectars taken by different bees (Ma'ruf *et al.*, 2018). Moreover, Parwata *et al.* (2010) stated that honey had different composition of chemical compounds based on the food/nectar sources of the bee. This difference could result in different antibacterial activity of the honey.

**Water Content, pH, and Total Glucose**

Total sugar, water content, pH of the honey from Timor Island is provided in Table 2.

**Water Content**

The water contents in the forest honey from Timor Island were ranged from 15.70% to 26.65% (Table 2). This content was the highest compared to that of

found by Nadhila (2014) of 15%-21%. Ampoang honey had the lowest water content of 15.70%, which was lower than that of the commercial one since this honey has had water reduction process and well packed in sales. Honey from Kefa, Naikliu, South Ampoang, and Soe have not been treated for water reduction process so that it has higher water content than the commercial one.

The water content of honey could be different with different localities. Honey water content of rubber tree nectar from Central Bangka Regency was 24.25% (Evahelda *et al.*, 2017), while the water contents of Trumon and Seulawah honey from Aceh were 22.05% and 19.81% (Fadhmi *et al.*, 2015), respectively. The water contents of bitter and sweet black honey from Central were 16.19% and 15.40% (Fitrianiingsih *et al.*, 2014), respectively. In general, the water content of some types of honey from Indonesia ranged from 17.8% to 21.0% (Dewi *et al.*, 2017). The difference in the water content of the honey is affected by several factors, such as climate, post-harvest handling, nectar type collected, honey maturity, production techniques, and storage (Baroni *et al.*, 2009).

**Total Glucose**

Honey holds high total sugar, and dominantly consists of glucose and fructose (Nayik & Nanda, 2015). Forest honey from Timor Island has high total sugar, 71.16%-80.58%. Total sugar content of Timorese honey was not quite different from the total sugar content of honey from rubber tree nectar of Central Bangka regency, 74.77% (Evahelda *et al.*, 2017). Honey has also different chemical compounds, including total sugar based upon food source of the nectar (Parwata *et al.*, 2010).

According to Evahelda *et al.* (2017), the high sugar content will make the honey to be more viscous or concentrated so that it has hygroscopic feature. This honey characteristic is determined by fructose since fructose is more soluble than glucose (Buba *et al.*,

Table 1. Active compounds in forest honey from Timor Island

Honey types	Active compound tests					
	Flavonoid	Alkaloid	Steroid	Terpenoid	Tanin	Saponin
Kefa honey	-	+	-	-	-	+
Ampoang honey	-	+	+	+	-	+
Naikliu honey	-	+	+	+	-	+
South Ampoang honey	-	+	+	+	-	+
Soe honey	-	+	+	+	-	+
Commercial honey	-	+	-	-	-	-

Table 2. Percentages of water content and total glucose and pH level of forest honey from Timor Island

Honey types	Water (%)	Total glucose (%)	pH
Honey from Kefa	26.65	72.60	4.06
Honey from Ampoang	15.70	80.58	3.95
Honey from Naikliu	26.65	71.16	4.00
Honey from South Ampoang	23.02	75.2	3.85
Honey from Soe	23.52	74.37	3.84
Commercial honey	21.18	78.53	3.87

2013). Moreover, according to Erguder *et al.* (2008), sugar content of honey consisting of mixture of glucose and fructose causes the honey to osmotic feature and can inhibit bacterial growth.

### Honey pH

The pH of forest honey from Timor Island ranged from 3.84 to 4.06. This range tends to be the same as that of from other places in Indonesia, such as randu honey with pH of 3.8, rambutan honey with pH of 4.21, longan honey with pH of 4.48, kaliandara honey with pH of 4.37 (Chayati, 2008), and rubber tree honey from Bangka, 3.92 (Evahelda *et al.*, 2017). Similarly, honey from India has pH of 4.1 (Veeraputhiran *et al.*, 2013).

Despite having a similar pH range, there is a difference in honey pH among honey from Timor Island. This difference could result from differences in mineral and acid content of the honey (Gulfranz *et al.*, 2010). According to Buba *et al.* (2013), the mineral content of the honey is influenced by soil condition, geographic position, climatic condition where the nectar source plants grow.

Honey of low pH can inhibit the growth of various bacteria since bacteria can grow in neutral or alkaline pH conditions. Acidity gives high impact on the growth and the survival of bacterial cells. Each species has an optimum range of acidity for growth. When pH declines to the lowest limit for bacterial growth, not only bacterial cells stop growing, but the bacteria will lose their ability to survive as well.

### Antibacterial Activity

Antibacterial test of the forest honey from Timor Island against *V. alginoliticus* and *A. hyropilla* can be seen in Figure 1. It is apparent that all types of forest honey from Timor Island possess antibacterial activity against *A. hydropilla* and *V. alginoliticus*, but the diameters of inhibition zone are different.

Against *V. alginoliticus*, the inhibition zones of all honey types from Timor Island were all higher than that of commercial honey, madu rasa (11 mm), except that south Ampoang honey has lower inhibition zone (9 mm). All these inhibition zones are still lower than that of antibiotics (22.5 mm). Similarly, against *A. hydropilla*, the inhibition zones produced by all forest honey from Timor Island were higher than that of the commercial honey (8 mm), but lower than that of antibiotics (16.5 mm). Thus, all types of forest honey from Timor island are potential to be considered as antibacterial against *A. hydropilla* and *V. alginoliticus*. The bigger the inhibition zone produced by the honey is, the higher the antibacterial activity.

Comparing among honey types from Timor Island, the highest antibacterial activity against *V. alginoliticus* was found in the forest honey from Naikliu (13 mm), while the highest antibacterial activity against *A. hydropilla* was found in the forest honey from Kefa (12 mm). It reveals that the antibacterial activity of each honey is different depending upon the type of honey, geographic position, and flower where the end product comes (Rio *et al.*, 2012).

The potential of antibacterial in the honey could result from several mechanisms, i.e. presence of radical hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) compound that can kill the pathogenic microorganisms (Johnston *et al.*, 2018; Nadhilla, 2014; Carina *et al.*, 2014), high acidity of the honey to reduce growth and survival of the bacteria (Johnston *et al.*, 2018; Carina *et al.*, 2014; Nadhilla, 2014; Erguder *et al.*, 2008), high sugar content inhibiting the bacteria to grow and develop (Carina *et al.*, 2014; Erguder *et al.*, 2008), and presence of antibacterial organic compounds (Carina *et al.*, 2014).

Antibacterial activity of forest honey has been proved by previous researchers. For pathogenic bacteria in human, forest honey can inhibit the growth of *Staphylococcus aureus* (Ma'ruf *et al.*, 2018; Adeyemo *et al.*, 2017; Nadhilla, 2014; Rio *et al.*, 2012; Hegazi *et al.*, 2017), *Escherichia coli* (Adeyemo *et al.*, 2017;

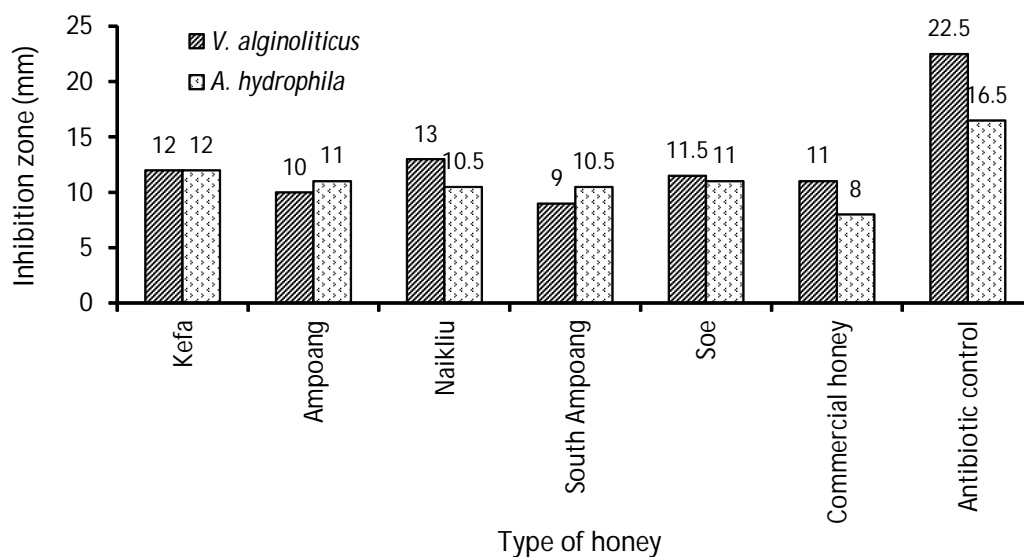


Figure 1. Antibacterial activity of forest honey from Timor Island against *V. alginoliticus* and *A. hydrophila*.

Aurongze & Azim, 2015; Rio *et al.*, 2012; Hegazi *et al.*, 2017), *Salmonella typhi*, *Shigella Sonneie*, *Vibrio furnissii*, *Yersinia pestis*, *Campylobacter jejuni* (Aurongze & Azim, 2015).

Antibacterial activity of forest honey against pathogens in fish is also shown by previous researchers. Stratev *et al.* (2015) has proved that rapa honey and royal jelly from Bulgaria are potential as an antibacterial against *A. hydrophila*. Ramalivhana *et al.* (2014) also found that honey from South Africa has antibacterial activity against *A. hydrophila*. Salosso (2017) reveals the antibacterial activity of Timor honey from Soe against *A. hydrophila* and *V. alginolyticus*.

## CONCLUSION

Different forest honey from five areas in Timor Island contained alkaloid, saponin, steroid, and terpenoid, except that Kefa honey which did not hold steroid and terpenoid. The water content of all honey ranged from 15.70% to 26.65%, total glucose 71.16% to 80.58%, and pH 3.84 to 4.06. It also had antibacterial activity against *A. hydrophila* and *V. alginolyticus* with different inhibition zones.

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