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Physico-chemical characteristics, antibacterial, and antioxidant activities of genuine forest honey from East Kalimantan

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Abstract

In the dense tropical forests of Kalimantan, *Apis dorsata* produces forest honey with pollen diversity that has the potential to have unique antibacterial and antioxidant properties. This study is aimed to report the physicochemical characteristics, antioxidant activity and antibacterial analysis of forest honey from East Kalimantan. A total of nine samples of forest honey were purchased from local buyers of several regions. The power of hydrogen (pH), water content, diastase enzymes, fructose levels, and glucose levels were measured. Antioxidant activity was measured with ABTS assay. Antibacterial activity was conducted against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *S. enterica* serovar Typhi. Fourier Transform Infra-Red (FTIR) identified antibacterial functional groups. Honey samples from tropical forests in East Kalimantan had pH between 2.93±0.06 and 3.83±0.06, a moisture content between 18.44±0.64 and 25.67±0.29%, fructose levels between 7.85±0.05 and 17.73±0.13%, and glucose levels between 1.24±0.16 and 17.34±0.14%. Goa Tembenus honey showed the highest IC_{50} antioxidant activity and the highest Diastase Enzyme activity. Goa Tembenus and Bongan honey showed good antibacterial activity against Gram-positive and Gram-negative. Minimum inhibitory levels of honey were found at concentrations of 25% and 30%. Honey from tropical forests in East Kalimantan has H_2O_2 as an antibacterial component.

1. Introduction

Forest honey, harvested from *Apis dorsata* bee, has the potential as a source of micro-nutrition, i.e., antioxidant (Tuksitha *et al.,* 2018). The honey-producing areas in East Kalimantan are mainly located in the Berau and Paser districts (Muslim, 2014). Forest-based honey potentials were also identified in several other regions in East Kalimantan, such as North Penajam Paser Regency, Kutai Kertanegara Regency, and West Kutai Regency. Forest bee host trees, namely Banggeris (*Kompassia excelsa*), are scattered in the forests of East Kalimantan (Muslim, 2014). However, forest honey in East Kalimantan, in general, is still not well populated and maintained, due to several factors such as the maintenance of *A. dorsata*, the timely journey to harvest honey, and the difficulty of accessing the location of honeybee hives in a deep jungle. Forest honey differs in the source of pollens obtained, final product thickness, and apparent color (Sofia *et al.,* 2017; Tanleque-Alberto *et al.,* 2019). The forests of East Kalimantan have the characteristics of humid tropical forests and have various types of tropical plants. The advantages of forest honey from East Kalimantan are in terms of taste, color, and purity (Muslim, 2014; Da Silva *et al.,* 2016; Wulandari, 2017).

However, one of the weaknesses of forest honey is standardization. Forest honey is prone to be falsified by irresponsible parties to gain excess profits (Rachmawaty, 2011; Adalina, 2017). This research seeks to support the identification and authentication of forest honey produced in East Kalimantan. The purity parameters of honey may consist of physicochemical properties, sugar contents, antioxidant and antibacterial activities, and diastase enzyme activity (Rahman *et al.,* 2013; Da Silva *et al*., 2016; Heard *et al*., 2017).

The purpose of this research was to determine the physicochemical characteristics, antioxidant activity,

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antibacterial, and functional group analysis. The observations included pH, water content, diastase enzyme activity, sugar content, antioxidant activity, antibacterial activity against four bacteria, and functional group analysis.

2. Materials and methods

2.1 Samples and materials

Nine forest honey samples were directly purchased from harvesters between 1st and 20th July 2019 in several regions in East Kalimantan, consisting of Goa Parung Honey (MP1), Goa Tembenus Honey (MT2), ICHI Conservative Honey (MI3), Muara Lawa Honey (ML4), Bongan Honey (MB5), Long Bagun Honey (MLB6), Bukit Bengkirai Honey (MBB7), Muara Bengkal Honey (MMU8), Lebak Cilong Honey (MLC9). Commercial honey (MK) was separately purchased (HNI, Indonesia). All honey samples were preserved in a dark room at ambient temperature (25±3°C) before being analyzed.

Two Gram-positive strains of *S. aureus* and *B. cereus* and two Gram-negative strains of *Escherichia coli* ATCC 35218 and *S. enterica* serovar Typhi ATCC 140281 were obtained from the Faculty of Medicine, Faculty of Pharmacy, Faculty of Agriculture, and Laboratory of Agricultural Microbiology at Mulawarman University. Chemical materials were purchased, including Mueller Hinton Agar (MHA) (Merck, USA), Nutrient agar (Merck, USA), Nutrient Broth (Merck, USA), sodium chloride (NaCl) solution (Merck, USA), sterile distilled water, Anthrone (Merck, USA), sodium oxalate (Merck, USA), calcium carbonate ($CaCO_3$) (Merck, USA), fructose (Pudak, Indonesia), glucose (Pudak, Indonesia), Pb-acetate (Pudak, Indonesia), 70% ethanol (PIM Pharmaceuticals, Indonesia), absolute ethanol (Merck), $FeCl_3$ (Merck), H_2SO_4 (Merck), HCl 2 N (Merck), $K_2S_2O_8$ (Merck, USA), ABTS (Sigma Aldrich, USA), ethanol pa (Fulltime, China), and vitamin C (Sigma Aldrich, USA).

The tools used were UV-VIS Spectrophotometry (Eppendorf, Germany), FTIR Spectrophotometer (Thermo Scientific Nicolet IS10, Madison, WI), incubators (Memmert), autoclaves (Hirayama), ovens (Binders), pH meters (Hanna), scales (Hanna), Mettler Toledo), hotplate (Thermoline), vortex (Thermoline), micropipette (Transferpette), water bath (Memmert), and glassware.

2.2 Statistical analysis

This research was conducted with the Purposive Sampling method, involving nine forest honey from various places in East Kalimantan, Indonesia. Each sample was tested in triplicate. Average measurement and standard deviation were recorded before non-factorial ANOVA was conducted. Fisher Least Significant Difference was used at alpha (α) equaled 5%.

2.3 pH and water content

Honey pH measurement was done using a Mettler Toledo pH meter. The instrument was calibrated using a standard solution of pH 4.00 and 7.00, and the water content in the sample was measured by the gravimetric method, according to Sudarmadji *et al.* (2010).

2.4 Sugar levels

Anthrone Reagent Method was used to measure sugar levels (Sartika, 2011). Honey sample carefully weighted at 14.5 g and added with 100-200 mL of water and 1 g of $CaCO_3$. The solution was boiled for 30 mins. During boiling, water was added so that the volume remains the same. After chilling at ambient temperature (25±3°C), the addition of a 3 to 5 mL Pb-acetate solution was conducted until the solution became clear. After filtration of the solution with Whatman filter paper No. 2, sodium oxalate 1 g was added. About 0.1% Anthrone reagents were prepared by dissolving 1 g Anthrone in concentrated H_2SO_4 until the volume reached 50 mL. Glucose and fructose standard solutions were made by weighing 200 mg of glucose and fructose in 100 mL of distilled water taken 10 mL diluted to 100 mL. Preparation of a standard curve was carried out employing a standard solution of glucose and fructose pipetted into a test tube 0.0 (blank), 0.2, 0.4, 0.6, 0.8, 1.0 mL. Each solution was added with 5 mL of Anthrone reagents, heated at 100°C for 12 mins, then the absorbance was read at the maximum wavelength, and interpolation was conducted.

2.5 Antioxidant activity

ABTS method was used to measure the antioxidant activity (Salampe, 2019). Samples were taken as much as 20 µL, 30 µL, 40 µL, 50 µL, and 60 µL. To each sample, 1 mL of ABTS solution (2,2-Azinobis (3- ethyl benzothiazoline)-6-sulfonic acid) was added and diluted to reach a volume of 5 mL with absolute ethanol (Fulltime, China). The final sample concentration was 100 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm. As a standard, a vitamin C stock solution was set at 200 ppm. Furthermore, from the stock solution each taken 200 µL, 250 µL, 300 µL, 350 µL, and 400 µL, added with 1 mL of ABTS solution, then diluted to a volume of 5 mL with ethanol absolute (Fulltime, China) to obtain a concentration of one ppm, 1.25 ppm, 1.5 ppm, 1.75 ppm, and two ppm. The absorbance of samples and standards was measured by UV-Vis spectrophotometry at a wavelength of 752 nm.

2.6 Diastase enzyme activity

The method used refers to a standardized method (SNI 3545: 2013). Honey was taken 10 mL mixed with 5 mL of starch solution in a test tube heated with a water bath at 40±2°C for 15 mins. At each 5 minute intervals, 1 mL of the mixture was taken, and 10 mL of iodine solution was added. The remaining mixture was then added to water until it reaches the initial volume. Absorbance was read at a wavelength of 660 nm. Repetition of the treatment was carried out until it reaches to an A value <0.235. The results obtained were plotted to obtain the absorbance value curve with time (mins). The simple formula for measuring DN was DN = 300/t, where DN was the value of the Diastase Enzyme activity, and 't' was the time required until the Absorbance value was reached <0.235 (A).

2.7 Antibacterial activity

This method refers to previous research (Astrini, 2014). Each strain inoculum was resuspended at 35°C in sloped agar. One loop of the bacteria was put into the Nutrient Broth in a test tube, then was incubated for 24 hrs at 37°C. Bacterial concentrations were adjusted to 0.5 10^{-7} CFU/mL with MacFarland Standard Solution. Media Mueller Hilton agar (MHA) was prepared on a petri dish, and then wells were made with a diameter of 6 mm. Each well was dripped with honey as much as 50 µL. The negative control used was 0.9% NaCl, while chloramphenicol 30 µg/mL was used as a positive control. The Petri dishes were incubated for 24 hrs at a temperature of 37°C.

2.7.1 Minimum inhibitory concentration (MIC)

Forest Honey Minimum Inhibitory Level showed the lowest concentration of honey that inhibited Gram-positive and negative bacteria at a concentration of 1 x 10^7 CFU/mL. The concentration of honey used was 25%, 30%, 35%, and 40%.

2.8 Functional group analysis with FTIR

Irnawati (2020) method was used to determine the FTIR spectrum of the samples. The FTIR (Thermo Scientific Nicolet IS10, Madison, WI) instrument was controlled with OMNIC (Thermo Scientific) software. Honey samples were dropped on the FTIR sample site and then measured. The sampling technique used was horizontal attenuated total reflectance (HATR) using ZnSe crystal. Each sample was scanned three times at a resolution of 8 cm^{-1} at wavenumbers between 4000 and 650 cm^{-1}. Then, the spectrum obtained was recorded as absorbance values at each data point.

3. Results and discussion

Forest honey was obtained from traditional harvesters originating from nine areas in three districts in East Kalimantan. The acquisition price for each honey was varied, ranging between 100 and 200 thousand rupiahs per liter. All honey samples were obtained directly from the honey harvesters. It is a common belief in the community that the honey purchased from the local seller was genuine. The price of forest honey was relatively higher than commercial honey on the market because of more properties and unique harvesting. Other factors that make the price of forest honey expensive were that the manual harvesting process and the long journey in a deep jungle to harvest the honey (Sofia *et al.,* 2017).

3.1 pH value

The pH value ranged between 2.93±0.06 and 3.83±0.06 (table 1). The highest value was obtained from MK honey (3.83±0.06), and the lowest value was from MMU8 honey (2.93±0.06). Each type of honey had a different pH value. This was caused by environmental factors and various types of food and nest conditions. (Yesserie, 2015) Manuka and Tenerife honey have the same pH as in this study. Both honey were more acidic than other types of honey, as reported earlier (Bentabol Manzanares *et al*., 2014; Deng *et al.,* 2018). The acidic nature of honey could affect antimicrobial activity and might have an essential role in the shelf life of honey (Mandal *et al.,* 2010).

3.2 Water content

Honey content ranged from 18.44% to 25.67% (Table 1), while the honey SNI requirement is at a maximum of 22% (SNI 3545: 2013). The water contents of Honey MT2, MI3, ML4, MB5, MLB6, MBB7, MMU8, and MLC9 were higher than SNI requirements. Forest honey had a reasonably high water content due to the nature of a humid, tropical forest area with high rainfall. This statement was in line with Bogdanov (2011), stating that honey has hygroscopic properties. So, when harvesting forest honey in the morning, there will be indirect absorption of water vapor from the surrounding environment. The absorption of water vapor by honey occurs continuously until the packaging stage. The water content value of Kalimantan forest honey reported in this study was almost the same as other forest honey, 19.2% (Alzahrani *et al.,* 2012). Other studies indicated lower honey water levels, 11.59±0.12% (Moniruzzaman *et al.,* 2013).

3.3 Diastase enzyme activity

The minimum activity of the enzyme diastase in

Table. 1 Physicochemical property of Forest Honey in East Kalimantan

Honey	pH	Moisture (%)	Fructose (%)	Glucose (%)	IC_{50} (ppm)	Diastase Number (DN)
MP1	3.63±0.06^{b}	20.39±0.20^{c}	7.85 ±0.05^{i}	3.73± 0.22^{f}	177.25±0.88	17.65
MT2	3.33±0.06^{cd}	22.42±0.35^{b}	14.61±0.08^{c}	15.43±0.08^{c}	117.46±0.40	21.43
MI3	3.20±0.10^{e}	22.13±0.88^{b}	17.73±0.13^{b}	17.34±0.14^{b}	395.69±2.20	14.29
ML4	3.37±0.06^{c}	23.00±0.63^{b}	13.46 ±0.08^{d}	12.97±0.11^{d}	404.75±2.26	13.64
MB5	3.03±0.06^{f}	22.59±1.40^{b}	11.18 ±0.14^{f}	7.52±0.24^{e}	371.35±2.46	10.71
MLB6	3.03±0.06^{f}	23.16±0.30^{b}	8.77 ±0.22^{g}	2.80±0.11^{g}	395.72±1.60	13.04
MBB7	3.23±0.06^{de}	25.67±0.29^{a}	8.30 ±0.18^{h}	1.45±0.05^{h}	538.77±0.94	17.65
MMU8	2.93±0.06^{f}	25.63±0.65^{a}	8.36 ±0.10^{h}	1.24±0.16^{i}	214.01±0.24	15.79
MLC9	3.30±0.10^{cde}	25.44±1.13^{a}	12.37±0.12^{e}	7.39±0.05^{e}	250.52±0.83	12.5
MK	3.83±0.06^{a}	18.44±0.64^{d}	20.63±0.20^{a}	18.67±0.21^{a}	584.13±3.20	13.04

Values expressed as mean±SD (n = 3). Numbers on the same row followed by the same letter indicate no significant difference in the LSD test at the α level of 5%.

honey is three and is expressed by the diastase number (DN) (SNI 3545: 2013). DN on the Schade scale means total grams of starch hydrolyzed in 1 hr at 40°C per 100 g honey (Codex Alimentarius Commission, 2001) Levels of diastase enzymes from Forests in East Kalimantan ranged between 10.71 and 21.43 DN. This value is above the minimum amount that has been set (SNI 3545: 2013). The lowest diastase enzyme activity was in MB5 forest honey, while the highest DN was in MT2 honey (Table 1) Diastase enzymes are formed during the process of maturation of honey. This enzyme is only found in pure honey or fresh honey. In other studies, the DN values of multiflora honey samples from Santa Fe province of Argentina ranged from 11.2±0.6 to 25.8±0.9 (Tosi *et al*., 2008). Diastase enzyme testing can also be used as an indicator of honey heating treatment (Purwanti, 2015). Diastase enzyme activity decreases when honey is heated at temperatures over 40°C (Purnamasari, 2015).

3.4 Sugar level

The main components of honey are water and sugar. The types of sugar found in honey are glucose and fructose, ranging from 70 to 80%, while water is from 10 to 20%—other minor components identified as organic acid minerals, vitamins, enzymes, proteins, and volatile components. The reducing sugar content, which is considered glucose, has a minimum requirement of 65% (SNI 3545: 2013). The average glucose content in all forest honey samples from East Kalimantan is between 7.8% and 20.6% for fructose and between 1.2% and 18.7% (Table 1). In comparison, buckwheat honey from the USA had glucose content of 27-35% and fructose of 31-39% (Pasini *et al.,* 2013). The sugar content of manuka honey was 60.7 g/100 g (Deng *et al.,* 2018). Malaysian honey sugar content was 71.2 g/100 g (Moniruzzaman *et al.,* 2013). The amount of fructose and glucose in both manuka and Malaysian honey were higher than 60 g/100 g.

All honey samples, according to SNI standards (SNI 3545: 2013) fallen below the minimum level of reducing sugar of 65%. Many factors cause sugar content in honey to decrease. Some of the factors that can affect the reducing sugar content of honey are moisture content, and when harvesting (Sudjatmiko, 2011). Unprocessed forest honey generally does not meet the SNI requirements of the water content of not more than 25% (Sartika, 2014).

3.5 ABTS antioxidant levels

Honey is flower pollen collected by bees. Therefore, honey's bioactive component depends on the type and quality of flowers that can be accessed by bees (Al-Brahim and Mohammed, 2020). Naturally, flower extracts have many active compounds as a form of protection against natural conditions, parasites, and bacteria. Some active compounds in honey are then characterized as antioxidants. Components responsible for the presence of antioxidant effects on honey are flavonoids, catalases, phenolic acids, peroxidases, carotenoids, and non-peroxide compounds (Kamilatussaniah and Yuniastuti. 2016). For example, cardamom flowers contain hydroxycinnamic, aromatic antioxidants from the phenylpropanoid group (Pramitha *et al.,* 2016). The antioxidant power found in flower extracts collected by bees depends on the antioxidant group and stereoisomer of the chemical compounds (Tanleque-Alberto *et al.,* 2019). Some factors influence this antioxidant activity, including the source of interest used to produce nectar and environmental factors, including weather, climate, and processing (Tuksitha *et al*., 2018; Al-Brahim and Mohammed, 2020)

One of the honey that has the most potent antioxidant that had been studied was manuka honey from New Zealand (Deng *et al.,* 2018). Forest honey from East Kalimantan has IC_{50} antioxidant levels ranging between 117.46±0.40 and 584.13±3.20 ppm (Table 1).

The most potent antioxidant, or the lowest IC_{50} value, was found in MT2 forest honey, and the highest was in MK honey. Manuka honey had an EC_{50} antioxidant content of 82.4 mg/mL (Deng *et al.,* 2018).

The antioxidant content of honey depends on nectar and phenolic compounds (Bertoncelj *et al.,* 2007; Khalil *et al.,* 2012). Antioxidant activity correlates with the color of honey. Darker honey colors tend to have higher phenolic and antioxidant compounds. (Tuksitha *et al.,* 2018; Dżugan *et al.,* 2020). MT2 forest honey has a darker color compared to other forest honey. Polyphenol compounds, flavonoids play an active role in the capture of free radicals and, in the body functions as an antioxidant, anti-inflammatory, inhibits the growth of microbes (Adawiah *et al.,* 2015). Forest honey from East Kalimantan is expected to help maintain the immune system from the free radical attack.

3.6 Antibacterial activity

An antibacterial activity can be seen from the welling zone in the agar medium that has been dropped with honey. The clear area shows inhibitory activity against gram-positive and gram-negative bacteria. The effectiveness of inhibition on each test bacteria differs from one another depending on the type of honey and type of bacteria (Dżugan *et al*., 2020). Inhibitory zone diameters of all types of honey against *S. aureus, B. cereus, E. coli*, and *S. enterica* serovar Typhi bacteria can be seen in Table 2. Some other factors that influence differences in antibacterial activity are the age of honey and the suspension state of the test bacteria. Some honey showed vigorous antibacterial activity on Gram-positive bacteria. Some others had antibacterial activity on Gram-negative bacteria.

Forest honey from East Kalimantan has a resistance between 0.3 and 2.6 mm against *B. cereus* bacteria. The inhibition zone formed against the *S. aureus* bacteria is between 0.3 and 9.2 mm. The inhibitory power for *E. coli* is between 0.1 and 8 mm. for *S. enterica* serovar Typhi bacteria, the tasteless zone formed is between 0 and 2.6 mm. The best inhibition is obtained from MT2 honey, which has inhibition of around 9.2 mm for *S. aureus* and 8 mm for *E. coli*. For the record, there is a sustainability problem of MT2 honey. This honey is obtained from forests in the Penajam Paser Utara area, in which the location is situated in the new Indonesian capital region. Other honey, which has good inhibition against bacteria, is MB5, which has inhibition of 7.1 mm against *S. aureus* and 7.4 mm against *E. coli*. This honey comes from Bongan, West Kutai Regency, East Kalimantan. Honey MT2 and MB5 had the highest activity among the honey samples studied. Some honey from Arabic had inhibitory zone activity against *E. coli* ranging between 5±0.6 and 14.00±1.0 mm and against *S. aureus* ranging between 9.67±1.0 and 20.33±2.1 mm (Ghramh *et al.,* 2019)

The bacterial cell wall structure is the cause of differences in antibacterial responses (Anyanwu, 2011). Osmotic pressure, acidity, hydrogen peroxide, and phytochemical contents influence the antibacterial activity of honey. The minor antibacterial components included 3,4.5-trimethoxybenzoic acid, syringic acid, methyl syringic, terpenes, pinocembrin, and benzyl alcohol (Rahman *et al.,* 2013). Honey from tropical forests of East Kalimantan has antibacterial activity caused by low pH and hydrogen peroxide.

3.6.1 Minimum inhibitory concentration (MIC)

Further testing of the antibacterial activity of honey MT2 and MB5 was carried out with the Minimum Inhibitory Concentration (MIC) technique for *S. aureus* and *E. coli bacteria*. The method of testing MIC is the same as testing antibacterial activity, but this test is conducted by setting some concentrations of honey to get

Table 2. Bacterial inhibition zone of East Kalimantan forest honey

Honey	Inhibition zone (mm)			
	Gram-positive (+)		Gram-negative (-)	
	B. cereus	*S. aureus*	*E. coli*	*S. enterica* serovar Typhi
MP1	2.6±0.22	3.6±0.09	7.9±0.13	2.6±0.21
MT2	1.2±0.12	9.2±0.15	8.0±0.35	1.1±0.13
MI3	2.0±0.06	1.4±0.24	5.6±0.28	0.0±0.00
ML4	1.0±0.12	2.5±0.29	7.2±0.28	0.0±0.00
MB5	0.3±0.14	7.1±0.05	7.4±0.38	0.0±0.00
MLB6	0.0±0.00	2.3±0.24	5.3±0.25	0.0±0.00
MBB7	0.3±0.14	0.3±0.18	2.8±0.20	0.0±0.00
MMU8	0.0±0.00	1.1±0.10	0.1±0.13	0.6±0.13
MLC9	0.5±0.17	1.4±0.24	5.3±0.24	0.0±0.00
MK	0.9±0.13	2.0±0.08	3.1±0.41	0.0±0.00

Values expressed as mean±SD (n = 3).

the lowest concentration that still shows inhibition of bacterial growth (Mandal *et al.,* 2010)

The concentrations used in the MIC test in this study were 20%, 25%, 30%, and 40%. The concentration was obtained after several experiments (Table 3). It was reported that MT2 honey obtained a minimum inhibitory concentration of 2.7 mm at a concentration of 25%, against *S. aureus* bacteria and 0.8 mm at a concentration of 25% against *E. coli* bacteria. In MB5 honey, MIC for *S. aureus* and *E. coli* bacteria was established at 30% honey concentration.

Table 3. MIC of forest honey against Gram-positive and Gram-negative bacteria

Honey	Concentration	Inhibition zone (mm)	
		S. aureus	*E.coli*
MT2	MT2 40%	5.2±0.25	3.5±0.61
	MT2 30%	3.7±0.26	1.4±0.12
	MT2 25%	2.7±0.25	0.8±0.29
	MT2 20%	0.0±0.00	0.0±0.00
MB5	MB5 40%	3.1±0.17	1.2±0.29
	MB5 30%	2.0±0.06	0.5±0.06
	MB5 25%	0.0±0.00	0.0±0.00
	MB5 20%	0.0±0.00	0.0±0.00

Values are expressed as mean±SD (n = 3)

Similar research has also been carried out by (Voidarou *et al*., 2011; Packer *et al*., 2012). The minimum concentration against *P. aeruginosa* bacteria of Heather honey, Manuka honey, and Khadikraft honey was 10%. Local honey has inhibition of 11% to the respective bacteria. Buckwheat honey from the USA was effective against Gram-positive bacteria. From the research results obtained, MIC values of 15 and 20% respectively for *S. aureus*. In Ethiopian wheat honey, MIC was higher observed for *K. pneumoniae* than *S. aureus* and *E. coli* (Hammond *et al*., 2016). Research (Dżugan *et al.,* 2020) Buckwheat honey has a minimum inhibitory level in *S. aureus* and *E. coli* bacteria by 25%. Based on the above comparison, Kalimantan forest honey has the same minimum inhibition potential as Polish buckwheat honey.

3.6.2 Honey antibacterial potential compared with chloramphenicol antibiotics

The results of testing the antibacterial activity were compared with those of the 30 µg/mL chloramphenicol antibiotic (Table 4). Furthermore, these results were calculated by comparing the percent inhibition of honey against antibiotics. The percent inhibition of honey varies between 1% and 46% in all types of bacteria and honey types. MT2 honey has the highest inhibition of 46% equivalent to 13.86 µg chloramphenicol against *S. aureus* bacteria and 39% against *E. coli* bacteria. MB5 honey has 36% inhibition, equivalent to 11.76 µg chloramphenicol against *S. aureus* and *E. coli*. Other researchers report that 1 mL of bitter honey C and D were equal to 21.87 and 18.38 µg chloramphenicol (Astrini, 2014). It was concluded that East Kalimantan tropical forest honey had lower inhibition compared to other commercial bitter honey. The more moderate antibacterial content is due to the high moisture content of East Kalimantan tropical forest honey.

3.7 Analysis of honey antibacterial functional group

The mechanism of honey as an antibacterial can be classified directly in the presence of hydrogen peroxide (H_2O_2) (Adawiah *et al.,* 2015). Hydrogen peroxide is a substance sensitive to heat and light, and beekeeping methods and storage conditions influence the content of H_2O_2 (Dżugan *et al*., 2020). One quick way to detect H_2O_2 is to use the FTIR instrument and compare the reading results against the H_2O_2 standard (Table 5).

Before discussing the confirmation of the presence of H_2O_2, all honey samples have similar functional group peaks (Figure 1). Absorption widens at the wavenumber between 3251.62 and 3269.84 cm^{-1}, where O-H

Table 4. Comparison of percentage inhibition to chloramphenicol antibiotics

Honey	percentage inhibition compared to 30 µg/mL chloramphenicol			
	Gram-positive (+)		Gram-negative (-)	
	S. aureus	*B. cereus*	*E. coli*	*S. enterica* serovar Typhi
MP1	18.18±0.92	12.26±2.13	38.60±1.23	15.70±2.56
MT2	46.21±1.52	5.65±1.14	39.22±3.47	6.86±1.52
MI3	6.94±2.42	9.74±0.60	27.57±2.78	0.00±0.00
ML4	12.50±2.96	4.81±1.18	35.17±2.78	0.00±0.00
MB5	35.61±0.51	1.20±1.39	36.15±3.68	0.00±0.00
MLB6	11.62±2.40	0.00±0.00	25.74±2.45	0.00±0.00
MBB7	1.52±1.80	1.20±1.39	13.73±1.96	0.00±0.00
MMU8	5.56±1.01	0.00±0.00	0.61±1.23	3.81±1.52
MLC9	7.07±2.47	2.28±1.64	26.10±2.31	0.00±0.00
MP1	10.10±0.82	4.21±1.20	14.95±4.03	0.00±0.00

Values expressed as mean±SD (n = 3).

Table 5. Comparison of FTIR spectra of East Kalimantan forest honey and hydrogen peroxide

Wavenumber (cm^{-1})											Functional group
MP1	MT2	MI3	ML4	MB5	MLB6	MBB7	MMU8	MLC9	MK	H_2O_2	
3269.84	3269.44	3269.15	3260.33	3266.89	3267.85	3256.4	3251.62	3268.63	3261.78	3124	O-H stretching (class carboxylic acid)
2931.32	2930.85	2931.62	2931.83	2932.03	2932.13	2931.98	2931.18	2931.95	2931.7	2768.74	O-H stretching (class alcohol)
1640.71	1640.13	1640.59	1640.58	1640.07	1640.34	1640.79	1640.58	1640.04	1641.21	1609.1	C=C
1414.3	1414.38	1414.45	1414.31	1414.59	1414.32	1415.68	1414.98	1414.05	1414.03		CH_3 Bending
1345.71	1345.75	1345.81	1346.56	1345.47	1345.61	1346.96	1346.66	1345.54	1345.28	1310.64	O-H bending (class phenol)
125343	1253.59	1253.63	1253.61	1254.09	1253.62	1255.57	1256.17	1253.68	1253.27		C-O-C
1026.75	1027.21	1027.07	1027.29	1027.45	1026.75	1029.31	1029.65	1027.83	1025.73		C-O
915.1	915.84	-	-	917.08	-	920.08	920.65	-	915.68	-	Substituted benzene
863.3	863.03	863.27	862.87	862.6	862.83		-	862.8	863.53	-	Substituted benzene
815.47	815.37	815.4	815.14	815.03	815.07	814.69	815.23	815.01	815.57	-	Substituted benzene
772.11	771.85	771.98	771.45	771.11	771.53		771.33	771.42	772.86	-	Substituted benzene

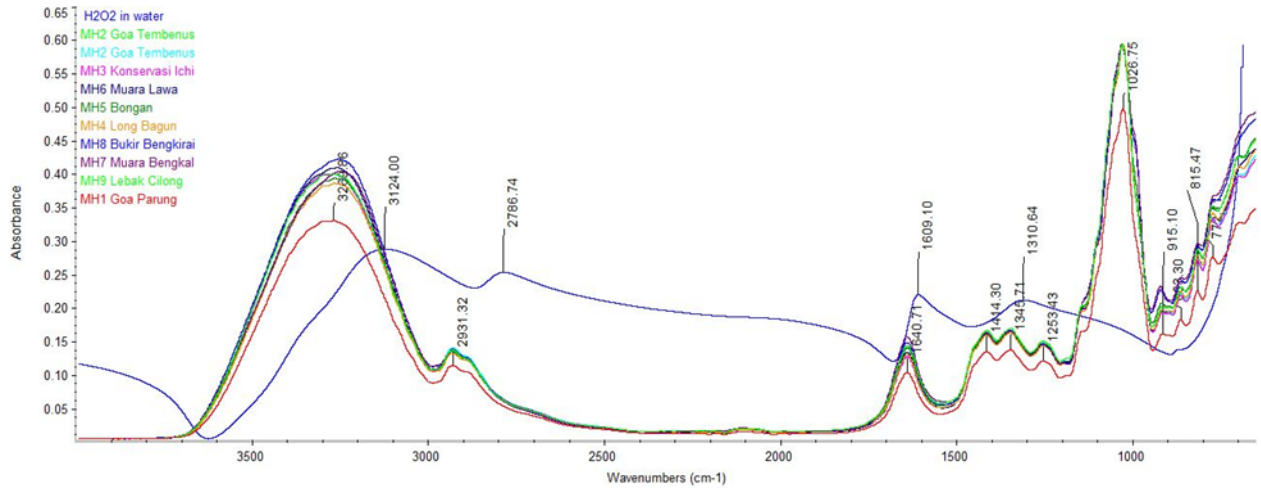

Figure 1. All spectrum of Honey compared with H_2O_2 standard

vibrations occur (Table 5). Aliphatic C-H vibrations in honey were detected at wavenumbers between 2930.85 and 2932.13 cm^{-1}. The C=C honey function group exposed from the wavenumber between 1640.13 and 1641.21 cm^{-1}. The peak wavenumber between 1414.3 and 1414.98 cm^{-1} was identified as CH_3. Vibrations of C-O compounds identified as peaks at wavenumbers between 1026.75 and 1029.65 cm^{-1}. The next peak was the aromatic ring substitution detected in the wavenumber between 920.08 and 771. 33 cm^{-1} (Table 5). From the results of FTIR spectrum readings, functional groups OH, CH aliphatic, C=C, CH_3, CO, C-O-C, and several aromatic rings identified in the honey from tropical forests of East Kalimantan.

Concerning H_2O_2 standard, all honey from tropical forests in East Kalimantan shows the presence of functional groups O-H stretching (class carboxylic acid), O-H stretching (class alcohol), and O-H bending (class phenol). Hydrogen peroxide and some non-peroxide components were the antibacterial properties of Polish and Canadian buckwheat honey (Brudzynski *et al.,* 2012; Sowa *et al.,* 2017).

4. Conclusion

Forest honey originating from several areas of East Kalimantan forest has different diastase enzyme levels, water levels, pH levels, sugar, antibacterial, and antioxidant content. However, the profile of FTIR and forest honey tends to be the same. MT2 honey has high antioxidant activity with IC_{50} values of 117.46±0.40 ppm. MT2 and MB5 honey had antibacterial activity against *S. aureus* and *E. coli* with clear zone diameters of 9.2±0.15 and 8.0±0.35 mm for MT2 honey and 7.1±0.05 and 7.4±0.38 mm for MB5 honey. Minimum inhibitory levels for Gram-negative and positive test bacteria were in the range of 25% honey concentration for MT2 honey and 30% for MB5 honey. The antimicrobial activity of 1 mL MT2 honey was equivalent to 13.86 µg chloramphenicol for the *S. aureus* test bacteria. The activity of 1 mL MT2 honey was equivalent to 11.76 µg chloramphenicol for *E. coli* test bacteria. The FTIR pattern showed that all honey samples have the same leading functional group as the H_2O_2 standard.

Conflict of interest

We declare that we do not have a conflict of interest.

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