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Research Paper

# Identification of Active Chemical Compounds of Honey from Some Regions in Indonesia

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**Abstract**
Bees produce honey from plant nectar, plant secretions, and excretions of plant-sucking insects. Indonesian local honey contains active compounds that have the potential effect as antioxidant and anticancer. The composition and biological effects of honey vary depending on the flower sources; seasonal and environmental factors can also influence the composition and the physical products. This research was conducted to identify the chemical compounds found in several honey samples produced by beekeepers in Indonesia with LCMS/MS method and to determine the profiles of the honey from Indonesia with the Chemspider and MassBank Database. Honey samples were collected from several regions in Indonesia. The results of the analysis showed that the honey's diastase number vary from region to region and showed that the HMF contents are relatively low. The compounds that were allegedly found through LCMS/MS analysis include and have been traced based on literature studies had bioactive activity and beneficial to health, include: millefin (potential for treating heart disease and cancer), mangiferin (anti-inflammatory, anti-diabetes, immunomodulators, anti-tumor, antioxidants), rhamnetin (anti-inflammatory), tricin (antioxidant-like), acacetin (inhibit tumor angiogenesis agents), aurantiamide acetate (antiviral or anti-inflammatory, therapeutic agent for the treatment of influenza), salvigenin (controlling inflammation, acute and chronic pain), brucine (modulates anti-inflammatory and analgesic properties), dehydrocostus lactone (anti-inflammatory), santonin (anthelmintic activity), dimethylesculetin (bilirubin clearance), imidazole 4-acetic acid (neuropharmacological properties), propafenone (antiarrhythmic), yohimbine (affected sexual performance), Velutin (anti-inflammatory), narigenin (linked to cardiovascular disease protection). Eventually, honey is is such a natural product with a number of salient therapeutic properties. However, there are still components that were found but their roles cannot be described in detail. Therefore, it is recommended that further meticulous studies should bring to light the other hidden properties of the honey compounds.

**Keywords**
Active compounds, Honey, Indonesian Fruit, Longan, Rambutans

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## 1. INTRODUCTION

Honey is a natural product that is sweet and aromatic, has high nutritional value, and affects human health. Honey contains antioxidants, antibacterial, anti-inflammatory, and antimicrobial properties, and has the effect of healing wounds (Alvarez-Suarez et al., 2013). Sumarlin et al., 2019 reported that Longan honey has decreased HEp-2 cell inhibitory activity after fractionation. Indonesian local honey, namely trigona honey, kaliandra honey, rambutan honey, and longan honey, can be used as a supplement for laryngeal cancer patients. Trigona sp, kaliandra, rambutan, and longan honeys can be used as a supplement for lung cancer patients (Sumarlin, 2019b). Most of the health promoting properties of honey are only achieved by application of rather high doses of honey such as 50 to 80 g per intake. This information shows that honey has a wide range of positive effects on nutrition and health (Gündoğdu et al., 2019).

The Result of research Boussaid et al., 2018 to Tunisian honey concluded that the six Tunisian honeys were characterized by the prevalence of total polyphenols, total flavonoids and total carotenoids, so eventually, honey is such a natural product with a number of salient therapeutic properties. Besides the geographical origin of honey has previously been studied by many researchers around Europe, especially in Slovenia, Romania, Spain, Denmark and Portugal (Bertoncelj et al., 2011; Daud et al., 2020; Stolzenbach et al., 2011; Feás et al., 2010), in Africa mainly in Morocco, Burkina Fasan and Algeria (Terrab et al., 2002; Meda et al., 2005; Ouchemoukh et al., 2007), in South America mainly in Argentina, Cuba and Brazil (Alvarez-Suarez et al., 2010; Chirife et al., 2006; Moreira et al., 2010),

and in Australia and New Zealand (Ajlouni and Sujirapinyokul, 2010; Vanhanen et al., 2011). The authorshave determined the physicochemical parameters including water content, pH, conductivity and sugar composition. Theyfound that the geographical area influences and distinguishesthe physicochemical properties of honey to a large extent (Boussaid et al., 2018).

The identifying these compounds are of extreme importance in describing beneficial health properties and each sample of honey may exhibit different variations and different phenolic compounds because the composition of these phenolics in stingless bee honey depends on the geographical location, type of plant that the bee collected the nectar, storage, climate, temperature, species of bee (de Oliveira et al., 2018). Therefore, honey produced in Indonesia also has different characteristics from honey produced in other countries.

However, comprehensive data on the composition of honey have not been maximally obtained. The chemical composition of honey can be tested using various conventional methodologies and modern instruments, including LCMS/MS (Liquid Chromatography-Mass Spectrophotometry/Mass Spectrophotometry). In this study, active compounds' identification was carried out by using LCMS/MS and the data analyzed by the Human Metabolite Database (HMDB) and MassBank Database.

## 2. EXPERIMENTAL SECTION

### 2.1 Materials

In this study, honey samples were collected from several Indonesian regions, including Lombok, Papua (Wamena), and West Java. The types of bee also vary, including trigona honey (*Tetrogonula biroi, Geniotrigona insica*), *Apis cerana, Apis dorsata*. Types of plants that are used as nectar sources include longan, and rambutan. Chemicals: metanol p.a. 99.8% (FULLTIME), n-hexane p.a. 98.5% (FULLTIME), ethyl acetate p.a. 99.5% (Smart Lab), TLC silika gel 60 $F_{254}$ (Merck), ethanol p.a. 96% (Merck, Germany), $Na_2SO_4$ anhydrous p.a (Merck), iodine p.a. (Sigma Aldrich), acetonitrile p.a. (Merck, Germany). Instruments: *Rotary evaporator (Heidolph)*, LCMS/MS (Waters, USA), UV-Vis Spectrophotometer (Thermo Scientific)

### 2.2 Methods

#### 2.2.1 Honey Extraction (Dananjaya et al., 2013)

Honey sample (100 gram) was dissolved in 300 mL methanol. The mixture was stirred using a magnetic stirrer for 30 minutes. After left to stand for 24 hours, the residue and the filtrate was separated using filter paper and then the filtrate was concentrated using a rotary evaporator at a temperature of 64°C. The resulting concentrate was called as crude extract.

#### 2.2.2 Hydroxy Methyl Furfural (HMF) Level, According to Indonesian National Standard 3545:2013

Five grams of sample was precisely weighed (up to 1 mg precision) in a small beaker glass, and then transferred into a 50 mL volumetric flask, and added with distilled water up to 25 mL volume. Carez I solution (0.50 mL) was then added to the flask, shaken until it dissolved completely, and then 0.50 mL Carez II solution was added into the flask and shaken again until it was dissolved completely. The mixture was diluted with distilled water to the ring graduation mark. To remove the foam formed on the surface, the mixture was added with a drop of alcohol. The mixture was then filtered with a filter paper, and the first 10 mL of filtrate was discarded.

The filtrate was pipetted into two test tubes (5 mL each). One of the tubes was then added with 5 mL distilled water (as sample) and the other tube was added with 5 mL of 0.20% sodium bisulfite (as reference/comparator). The tubes were shaken until completely mixed and the absorbance of the sample to the reference in 1 cm cell was determined at 284 and 336 nm. If the absorbance was higher than 0.6, in order to get an accurate result, the sample was diluted with distilled water as appropriate. As for the reference, if the absorbance was higher than 0.6, it was diluted with 0.1% $NHSO_3$ solution. The HMF level can be calculated by multiplying the absorbance value by the dilution factor.

$$\text{HMF level} = \frac{(\text{Absorbance at 284 nm - Absorbance at 336 nm}) \times 14.97 \times 5}{\text{sample (gram)}}$$

#### 2.2.3 Diastase Enzyme Activity, According to Indonesian Nati onal Standard 3545: 2013

The amount of 5 g sample was put into a 20 mL beaker, followed by 10-15 mL distilled water and 2.5 mL acetate buffer that were added in a cold state. The solution was stirred until the sample was completely dissolved. Then, the solution was transferred into a 25 mL volumetric flask containing 1.5 mL of NaCl solution and diluted to the ring graduation mark with distilled water. To determine the absorbance of the sample, 5 mL starch solution was pipetted into the sides of a test tube containing 10 mL of the diluted sample solution at the bottom (try not to mix the two solutions). The tube was then incubated in a water bath (temperature 40°C ± 0.2°C) for 15 minutes, then the content was mixed by moving the test tube back and forth in an oblique position while running a stopwatch. In exactly 5 minutes, 1 mL of the sample mixture was added quickly into 10 mL of dilute iodine in 100 mL Erlenmeyer and mixed evenly. After that, the mixture was diluted until the volume was the same as the previous one, and the absorbance value was determined with a UV-Vis spectrophotometer.

Reading was recorded at 860 nm or 600 nm in a 1 cm cell. The reaction time was measured from the mixing of the starch with the diluted honey sample to the addition of iodine. The sample mixture was continued to be taken at 5 minute or 10 minute interval until the absorbance value was <0.235. The absorbance value was plotted against the time. The time needed to reach the absorbance value (A) = 0.235 can be determined using the graph. If this value is divided by 300, it will show the diastase enzyme's activity or diastase number.

#### 2.2.4 Liquid-Liquid Partition (Dananjaya et al., 2013)

Crude honey extract was diluted with 200 mL water-methanol (3:7) and then poured into a separating funnel and followed by the addition of 100 mL n-hexane. The funnel was then shaken for 5 minutes and left to stand. There would be separation of 2 phases. The n-hexane upper fraction was separated into another container. The water-methanol lower fraction was added again with n-hexane, and the process was repeated for several times until an exact color was obtained for the upper n-hexane fraction of honey. In another separation, water-methanol dillution of crude honey was added with 100

mL of ethyl acetate and partitioned using the same above method. The separated fractions (water-methanol, n-hexane, and ethyl acetate fractions) were added with 5 grams of $Na_2SO_4$ anhydrous. After removing the $Na_2SO_4$ through filtering, the fractions were concentrated using a rotary evaporator (n-hexane fraction at 48°C, ethyl acetate fraction at 54°C, water-methanol fraction at 64°C).

The fractions were further separated using Thin Layer Chromatography method (TLC 1) (TLC Silica gel plate 60 F254) and monitored under UV lamps at 254 nm and UV 365 nm. The separation was continued using Gravity Column Chromatography Fractionation. The fractionation results were separated again by TLC (TLC 2). The fractions with the same stain at TLC 2 were combined, and the solvent was evaporated. These were the final results of the separation process, which would next be identified using LCMS/MS Maharani et al., 2016 and the honey sample compounds were analyzed with the database on the MassBank website.

### 2.2.5 Analysis LCMS/MS (Maharani et al., 2016)

A total of 1 mg of active honey fraction was weighed and dissolved in methanol. Ten $\mu$L of the sample was taken and injected into LCMS/MS through C-18 column (2 x 150 mm) with a 0.2 mL/minute flow rate. The chromatogram and mass spectrophotometer results were then analyzed using the MassLynx software (Version 4.1). In order to identify the structure of the chemical compounds detected on the LCMS/MS, the sample base peak was compared with the database of MassBank. The column or stationary phase used in LCMS/MS was the ACQUITY UPLC®BEH C18 column. This column is a reverse-phase column because the stationary phase is non polar while the mobile phase is polar. The mobile phase used was a mixture of methanol-water and acetonitrile-water; in this case, the best separation occured when using acetonitrile-water as solvent.

## 3. RESULTS AND DISCUSSION

### 3.1 Diastase Number and HMF Testing

The determination of diastase enzyme activity is an essential parameter in determining the quality of honey purity. Some of them were even quite large because they were above the Indonesian National Standard (SNI) (Table 1). Honey that has DN can be categorized as honey that has good quality. Diastase enzyme is an enzyme released by the bees during honey's ripening process so that the honey contains diastase enzyme. The low diastase enzyme activity in honey that was not even seen may presumably due to the long storage time and cooking process at a specific temperature after harvesting the honey. It is also possible for the honey to be added with liquid invert sugar to increase the honey quantity.

**Table 1.** ADiastase Number and HMF Data of the Collected Honey Samples

| CODE | Diastase Number | HMF |
|---|---|---|
| AP.LMB | 17.95±0.05 | 4.45±0.13 |
| PP.WM | 13.94±0.06 | 3.40±0.00 |
| AP.LNG | 11.82±0.14 | 1.33±0.02 |
| AP.RBT | 25.53±0.84 | 1.78±0.08 |

Notes: AP.LMB: Apis Honey from Lombok-Indonesia, PP.WM = Multiflora Honey from Wamena Papua-Indonesia, AP.LNG = Apis Honey nectar sources longan, AP.RBT = Apis Honey nectar sources longan Rambutan.

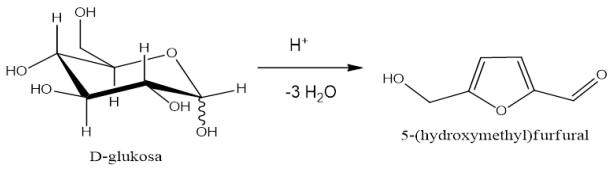


**Figure 1.** Hydroxy Methyl Furfural Formation Reaction (Kowalski et al., 2012)

According to the Honey Quality and International Regulatory Standards, diastase activity must not be less than or equal to 8, which is expressed as diastase number (DN) from the International Honey Commission. Alimentarius, 1998 has set a minimum diastase activity value of 3 for honey with naturally low enzyme content.

Diastase activity is closely related to its structure and can be modified by denaturation caused by heating. Denaturation can be considered as a discontinuous phenomenon with various intermediate states or transitions between the natural or original state and the fully denatured state (Cheftel et al., 1989).

Analysis of Hydroxy Methyl Furfural (HMF) levels of honey samples collected (Table 1) showed that all honey samples had low HMF levels of around 1.09-4.43 mg/kg and were below the Indonesian National Standard value threshold. HMF results from dehydration of carbohydrate or C6 sugar molecules in acid environment and can be accelerated by heating during processing or storage (Figure 1). HMF should not be higher than 15 mg/kg. If the DN is equal to or higher than 8, the limit for HMF is 60 mg/kg.

HMF levels can indicate honey deterioration by overheating or invert sugar (a mixture of equal parts of glucose and fructose that results from hydrolysis of sucrose) addition. Both of these treatments will increase the levels of HMF (Winarno, 1992).

Evahelda et al., 2015 stated that the increase in HMF levels in honey indicates a decrease in reducing sugar levels in honey due to the dehydration process. HMF test parameters become an indicator of honey's freshness, heating process, and honey storage time. Honey that is still fresh or newly harvested has very little or even has no HMF (Bogdanov et al., 2004). Honey with HMF below 10 mg/kg indicates that honey is still fresh, and honey with HMF of 30-100 mg/kg indicates that honey has been stored for a long time (Gabor and Goian, 2006). Based on the above statement, the honey sample in this study was classified as fresh honey (Table 1). The presence of HMF in sugar-based products is influenced by the type of honey, pH value, acid content, moisture and exposure to light (Kesić et al., 2017).

Honey is considered both nutritional and medicinal, although the presence of certain constituents, for example, heavy metals (even in trace amounts), some alkaloids, and HMF and its derivatives may contribute to honey's toxicity (Islam et al., 2013; Sanna et al., 2000). HMF is a cyclic aldehyde produced by sugar degradation through the Maillard reaction (a non-enzymatic browning reaction) during food processing or long storage of honey (Bastos et al., 2012).

In addition to exerting detrimental effects (mutagenic, genotoxic, organotoxic and enzyme inhibitory), HMF, which is converted to a non-excretable, genotoxic compound called 5- sulfoxymethylfurfural, is beneficial to human health by providing antioxidative, anti-allergic, anti-inflammatory, anti-hypoxic, anti-sickling, and anti-hyperuricemic

effects. Therefore, HMF is a neo-forming contaminant that draws great attention from scientists (Shapla et al., 2018).

Previous studies have reported that honey stored at low temperatures and/or under fresh conditions has low or minimal HMF concentrations, while aged and/or honey stored at comparatively higher or medium temperature has high HMF concentrations. In addition to storage conditions, the use of metallic containers and honey floral sources are critical factors affecting HMF levels (Shapla et al., 2018). Hence, higher HMF concentration is indicative of poor storage conditions and/or excess heating of honey (Fallico et al., 2004; Khalil et al., 2010).

### 3.2 Extraction and Liquid-Liquid Partitioning in Honey

The extraction method aims to dissolve the compounds in the sample which have the same polarity using an organic solvent. Methanol is a universal solvent because in addition to being able to extract polar components, it can also extract non polar components such as wax and fat (Houghton, 1998). In addition, if you use water as a honey solvent and then add organic solvents or direct addition of non polar and semi polar solvents to the honey, the honey will coagulate and it is will form a gel that difficult to separate. This is because the addition of organic solvents causes the protein in honey to denaturation, the natural structure of the protein will be damaged and then form a gel matrix by balancing the interactions between proteins and protein-solvents in honey.

The methanol extract of the sample honey was then separated by the liquid-liquid partition method. The principle of this method is the separation of compounds that have differ in solubility between two immiscible solvents and it is separate compounds based on their polarity. The liquid-liquid partitioning process is carried out in stages starting with the addition of n-hexane (non polar) and water which then forms two phases, the upper phase or the n-hexane phase separated. The second stage, the lower phase is followed by the addition of ethyl acetate (semi polar) and forms two phases again, namely the upper phase in the form of ethyl acetate and the lower phase in the form of a water phase or a polar phase. The next step, the ethyl acetate phase and water phase of honey were separated respectively so that the honey sample would be obtained respectively non polar extract, semipolar extract, and polar honey extract. The samples were continued for the LCMS/MS test.

### 3.3 LCMS/MS Analysis of APLMB Honey

The ethyl acetate fraction of APLMB sample also showed seven peaks (Figure 2), which indicates the presence of certain compounds in the fraction. However, search analysis for the alleged compound through online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found four suspected compounds (Table 2).

From the results of analysis, there were some potential compounds, and Tebufenozida was among them. Tebufenozide was in the group of potential compounds from the ethyl acetate extract of APLMB honey. Tebufenozide is a new caterpillar control agent that poses minimal hazards, chemically and mechanically, to non target organisms and the environment. Millefin was also found in the list of the potential compounds that belongs to the sesquiterpenoid group. It was reported that sesquiterpenoids can play a significant role in human health, both as part of a balanced diet and as a pharmaceutical agent, because of their potential for treating heart disease and cancer (Chadwick et al., 2013).

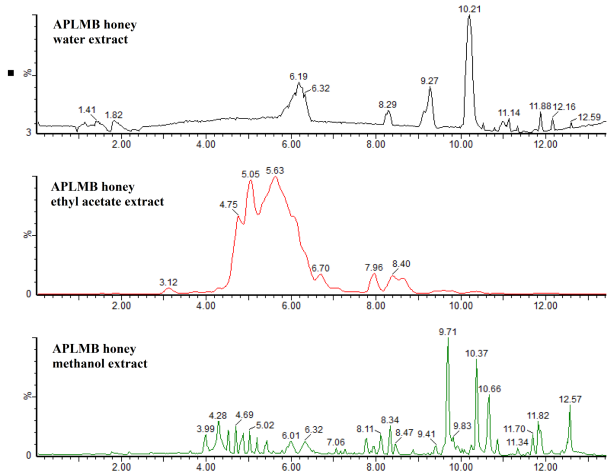


**Figure 2.** LCMS/MS Chromatogram of APLMB Honey Extracts

The methanol extracts of the APLMB sample showed more peaks, i.e. 24 peaks. Mangiferin was one in the list, where mangiferin-treated cells resulted in a significant increase in cell survival under $H_2O_2$ stress that gives mangiferin a useful perspective in preventing oxidative stress-related diseases (Lou et al., 2012). Mangiferin is also found to have various bioactivities, such as anti-inflammatory (Carvalho et al., 2009), anti-diabetes (Muruganandan et al., 2005), immunomodulators (Guha et al., 1996), anti-tumor (Noratto et al., 2010; Rajendran et al., 2008), and antioxidants (Dar et al., 2005; Barreto et al., 2008). It can promote endothelial cell migration during angiogenesis and may have promising preventive and therapeutic potential in vascular disease (Daud et al., 2020).

Rhamnetin was also in the list of the potential compounds from the methanol extract, which can be used as a candidate for natural compound in developing new anti-inflammatory drugs (Belchor et al., 2017). The study results by Zhang et al., 2015 suggested that rhamnetin can improve the recovery of cognitive deficits caused by TBI (Traumatic Brain Injury). The mechanism may be related to the inhibition of inflammation oxidative stress in the hippocampus.

Tricin is another compound from the methanol extract that exerts anti-inflammatory effect through a mechanism that involves the TLR4/NF-$\kappa$B/STAT signaling cascade (Shalini et al., 2015). Tricin has long been recognized to have antioxidant-like health benefits (e.g., Watanabe, 1999; Kwon et al., 2002; Kwon and Kim, 2003; Lu et al., 2006; Duarte-Almeida et al., 2006; Hasegawa et al., 2008; Mu et al., 2008) because of its potent inhibition of lipoperoxidation and its sparing effect on vitamin E in erythrocyte membranes (Rice-Evans et al., 1997; Pietta, 2000); as an antiviral (Li et al., 2005; Sakai et al., 2008); antihistamines Kuwabara et al., 2003; as well as in immunomodulatory activities (Liang et al., 1997; Wang et al., 2004) and antitubercular (Gu et al., 2004).

Acacetin, one of the potential compounds from the methanol extract, inhibits tyrosine phosphorylation of Stat-1 and Stat-3, and VEGF expression in cancer cells. Overall, acacetin inhibits stat signaling and suppresses angiogenesis in vitro, ex vivo, and in vivo, and therefore, acacetin can inhibit tumor angiogenesis agents and their growth (Bhat et al., 2013). In other studies, acacetin was reported to

**Table 2.** Potential Compounds from LCMS/MS Analysis of APLMB Honey Extract

| APLMB Honey Extract | Potential Compounds |
|---|---|
| Methanol | Mangiferin [4.69] |
| | 2'-Deoxyinosine-5'-monophosphate [5.43] |
| | Brucine [6.32] |
| | Rhamnetin [7.06] |
| | Tricin [8.47] |
| | Tebufenozide [9.41] |
| | Acacetin [10.37] |
| | 3,7-Dihydroxy-3',4'-dimethoxyflavone [10.66] |
| | Aurantiamide acetate [11.34] |
| | Salvigenin [11.82] |
| | 3',7-Dimethoxy-3-hydroxyflavone [12.57] |
| Ethyl Acetate | 2,4-dimethylphenylformamid [3.12] |
| | Tebufenozide [4.75] |
| | Brucine [5.63] |
| | Millefin [7.95] |
| Water | Brucine [6.19] |

provide a synergistic effect when administered together with oxacillin or ampicillin, while the antibacterial activity and resistance regulation of acacetin against clinical isolates of MRSA (Methicillin-resistant *Staphylococcus aureus*) are thought to be able to control MRSA infections (Cha et al., 2014).

Aurantiamide acetate (compound E17), that was reported by Zhou et al., 2017 as an active compound found in the root extract of B. cusia, was also estimated to contained in the methanol extract of APLMB honey. Although research showed that aurantiamide acetate has antioxidant and anti-inflammatory properties, its effects and mechanisms function as antiviral or anti-inflammatory. However, Zhou et al., 2017. also showed that aurantiamide acetate isolated from B. cusia root has potent antiviral and anti-inflammatory effects on IAV-infected cells through inhibition of the NF-$\kappa$B pathway. Therefore, aurantiamide acetate could be a potential therapeutic agent for the treatment of influenza.

Salvigenin is one of the active flavonoids in plants. Salvigenin could reduce inflammation. In a test, in the group receiving Salvigenin at 100 mg/kg, the inflammation was significantly lower than in the control group ($P < 0.05$). Thus, Salvigenin has a dose-dependent analgesic effect that is useful in controlling inflammation, acute and chronic pain (Mansourabadi et al., 2016).

Further, salvigenin was reported to cause significant reduction in fasting serum glucose, triglycerides, total cholesterol, HbA1c, and increased plasma insulin and HDL levels in diabetic rats. Increasing the insulin secretion could be the mechanism for the antidiabetic effect of salvigenin. The antidiabetic and cardioprotective effects show that salvigenin, as a flavonoid compound, can be used to reduce diabetes and its cardiovascular complications (Sadeghi et al., 2016).

The results showed that brucine has the function as a receptor antagonist. Also, brucine induces a rapid and sustained increase in intracellular [$Ca^{2+}$], impairs the mitochondrial membrane's potential, and triggers the apoptotic process of HepG2 cells, modulates anti-inflammatory and analgesic properties (Yin et al., 2003).

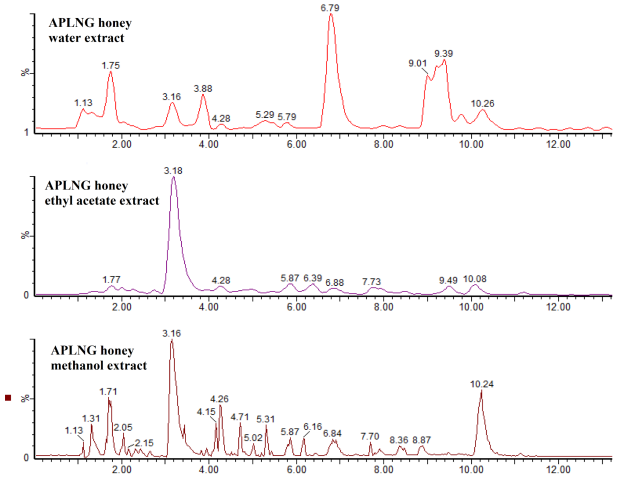


**Figure 3.** LCMS/MS Chromatogram of APLNG Honey Extracts

### 3.4 LCMS/MS Analysis of APLNG honey

In the APLNG sample, the water fraction showed 11 peaks (Figure 3), which indicates the presence of certain compounds in the fraction. However, searches on the online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found five suspected compounds (Table 3).

In the APLNG sample, the ethyl acetate fraction showed nine peaks, indicating certain compounds in the fraction. However, searches on the online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found five suspected compounds (Table 3).

Dehydrocostus lactone (DHE), a natural sesquiterpene lactone, has been used to treat various diseases with its anti-inflam matory activity. It has recently caused widespread interest in researchers because it has anticancer properties in several types of carcinoma. These findings provide pharmacological evidence for the development of DHE as a

**Table 3.** Potential Compounds from LCMS/MS Analysis of APLNG Honey Extract

| APLNG Honey Extract | Potential Compounds |
|---|---|
| Methanol | 2-amino-3-(4-hydroxy-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan -2-yl]oxyphenyl)propanoic acid [1.13]<br>N-Fructosyl phenylalanine [1.31]<br>dehydrocostus lactone [2.05]<br>4-hydroxyquinoline [3.16]<br>2-Hydroxyphenylacetic acid [4.15]<br>m-Anisic-acid [4.26]<br>Imidazole-4-acetate [4.71]<br>Dimethylesculetin [5.02]<br>Salsolinol [5.31]<br>Indole-3-carboxyaldehyde [5.87] |
| Ethyl Acetate | 4-hydroxyquinoline [3.18]<br>Dehydrocostus lactone [4.28]<br>Indole-3-carboxyaldehyde [5.87]<br>Santonin [6.39]<br>Dimethylesculetin [7.73] |
| Water | 2-amino-3-(4-hydroxy-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan -2-yl]oxyphenyl)propanoic acid [1.15]<br>Indole-3-carboxyaldehyde [3.16] |

potential agent against glioma (Wang et al., 2017).

Santonin is a compound responsible for plants' anthelmintic activity and has been used for many years as a drug to treat parasitological diseases (Sakipova et al., 2017). Dimethylesculetin is a compound that has a role in the Yin Chin (*Artemisia capillaris*, a traditional herbal medicine) activities in accelerating in vivo bilirubin clearance (Huang et al., 2004). Masuyama et al., 2016) state that dimthylesculetin treatment during pregnancy could prevent maternal hypertension, glucose intolerance and hyperlipidemia, and fetal overgrowth in high-fat diet (HFD)-induced obese pregnant mice. Dimethylesculetin suppressed the mRNA expression of gluconeogenic genes, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, and lipogenic genes, sterol regulatory element-binding protein 1 and stearoyl-CoA desaturase 1, and enhanced CAR-mediated transcription.

In the APLNG sample, the methanol fraction showed 18 peaks, indicating certain compounds in the fraction. However, searches on the online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found 10 suspected compounds (Table 3).

Tunnicliff, 1998 research suggests that imidazole 4-acetic acid (IMA) is a metabolite naturally occurring in the brain. However, it is not clear what biochemical pathway involved in its biosynthesis and disruption. However, some evidence suggests that IMA is a product from the oxidation of histamine. This compound has neuropharmacological properties, many of which are consistent with GABA A receptor activation. Indeed, IMA can potentially replace [3H] GABA from the GABA A site. IMA exhibits specific partial agonist characteristic as an adjunct to benzodiazepine binding with the GABAA receptor complex in a membrane preparation. Besides, it has an affinity for the GABAC receptor, where it appears to act as an antagonist, and possibly as a weak partial agonist. The third recognition site for IMA in the brain is the I1-imidazoline receptor.

### 3.5 LCMS/MS Analysis of APRBT honey

In the APRBT sample, the water fraction showed ten peaks (Figure 4), which indicates the presence of certain compounds in the fraction. However, searches on the online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found four suspected compounds (Table 4).

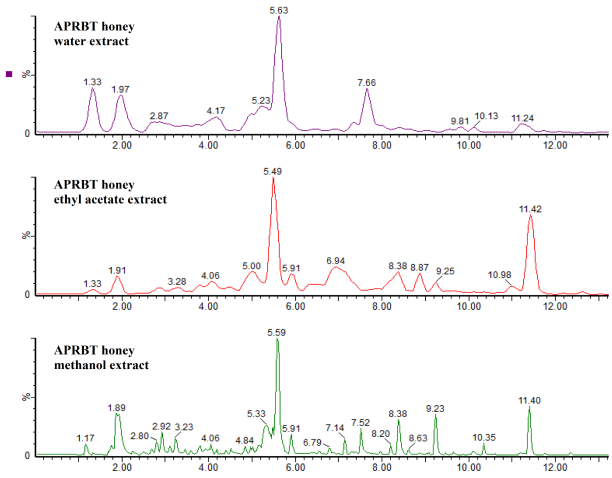


**Figure 4.** LCMS/MS Chromatogram of APRBT Honey Extracts

Propafenone is a class Ic antiarrhythmic drug (Schuff-Werner et al., 1981). It is a beta-adrenergic blocker that causes bradycardia and bronchospasm (Siddoway et al., 1984). The major metabolic process for propafenone occurs in the liver (Konz et al., 2008; Schlepper, 1987). The bioavailability and plasma concentration for propafenone

**Table 4.** Potential Compounds from the LCMS/MS Analysis of APRBT Honey Extract

| APRBT Honey Extract | Potential Compounds |
|---|---|
| Methanol | 2-amino-3-(4-hydroxy-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan -2-yl]oxyphenyl)propanoic acid [1.17] |
| | 2-Amino-N-(2,2,4,4-tetramethyl-3-thietanyl)propanamide [3.82] |
| | Cytidine [4.06] |
| | D-sphingosine [4.84] |
| | D-beta-Homophenylalanine [5.33] |
| | Propafenone [5.59] |
| | Rauwolscine [5.91] |
| | Diethanolamine [6.79] |
| | Santonin [7.14] |
| | Dihydrochalcone [8.2] |
| | Feruloylputrescine [8.38] |
| Ethyl Acetate | N-(1-Deoxy-1-fructosyl)phenylalanine [1.33] |
| | L-threonine [1.91] |
| | N-Acetylphenylalanine [3.28] |
| | 1,2,3,4-Tetrahydro-1-methyl-beta-carboline-3-carboxylic acid [4.06] |
| | Yohimbine [5.91] |
| | Velutin [6.94] |
| | 13a-Hydroxylupanin [8,38] |
| Water | N-(1-Deoxy-1-fructosyl)phenylalanine [1.33] |
| | 1,2,3,4-Tetrahydro-1-methyl-beta-carboline-3-carboxylic acid [4.17] |
| | Propafenone [5.63] |
| | 2,3-Dihydroflavone [9.81] |

**Table 5.** Potential Compounds from the LCMS/MS Analysis of PPWM Honey Extract

| PPWM Honey Extract | Potential Compounds |
|---|---|
| Methanol | 2-amino-3-(4-hydroxy-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan -2-yl]oxyphenyl)propanoic acid [1.17] |
| | 2-Amino-N-(2,2,4,4-tetramethyl-3-thietanyl)propanamide [1.71] |
| | Imidazole-4-acetate [2.92] |
| | Naringenin [4.97] |
| | Brucine [6.32] |
| | Santonin [7.32] |
| | Feruloylputrescine [8.69] |
| Ethyl Acetate | 2-amino-3-(4-hydroxy-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan -2-yl]oxyphenyl)propanoic acid [1.25] |
| | Kyunurenic acid [1.79] |
| | Imidazole-4-acetate [2.85] |
| | Indole-3-aldehyde [4.06] |
| | Tyramine [4.69] |
| | Naringenin [5.02] |
| | Propafenone [5.91] |
| | Santonin [7.29] |
| Water | 2-amino-3-(4-hydroxy-3-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan -2-yl]oxyphenyl)propanoic acid [1.17] |
| | N-Fructosyl phenylalanine [2.27] |
| | Brucine [6.34] |
| | Rauwolscine [9.79] |

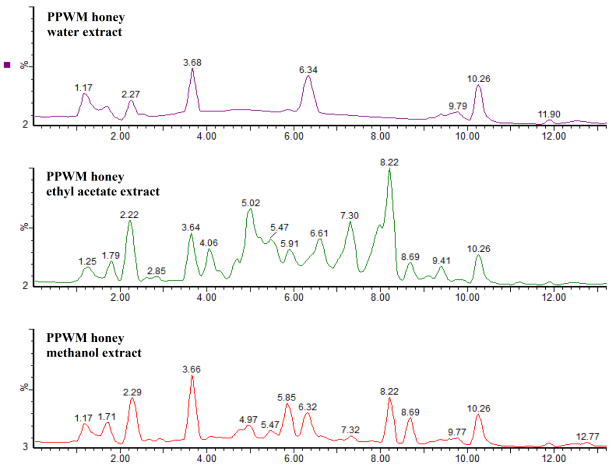


**Figure 5.** LCMS/MS Chromatogram of PPWM Honey Extracts

differ among patients undergoing long-term therapy.

In the APRBT sample, the ethyl acetate fraction showed 12 peaks, indicating certain compounds in the fraction. However, searches on the online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found seven suspected compounds (Table 4).

Yohimbine, an alpha-2-adrenoceptor antagonist characterized pharmacologically by activity in the central and peripheral nervous systems, has been used for more than a century to treat erectile dysfunction. In-depth and systematic animal studies have shown that yohimbine profoundly affected sexual performance (Morales, 2000).

Velutin, a potent anti-inflammatory flavone, was also a compound from the ethyl acetate extract of APBRT honey. Velutine effectively inhibited the expression of proinflammatory cytokines TNF-$\alpha$ and IL-6 in low micromol levels by inhibiting NF-$\kappa$B activation and phosphorylation of p38 and JNK (Xie et al., 2012).

### 3.6 LCMS/MS Analysis of PPWM honey

In the PPWM sample, the water fraction showed six peaks (Figure 5), which indicates the presence of certain compounds in the fraction. However, searches on the online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found four suspected compounds (Table 5).

In the PPWM sample, the ethyl acetate fraction showed 15 peaks, indicating certain compounds in the fraction. However, searches on the online databases of MassBank, HMDB, and MassBank of North America (MoNA) only found eight suspected compounds (Table 5).

Naringenin is one of the essential natural flavonoids, primarily found in some edible fruits, such as Citrus and tomato species (Mbaveng et al., 2014; Jadeja and Devkar, 2014; Zobeiri et al., 2018), and fibers included in the Smyrna-type Ficus carica (Soltana et al., 2018).

Despite a large amount of data regarding naringenin in vitro biological effects, only a few studies available for its use as a therapeutic molecule. However, some specific products were formulated under the pure compound supplementation and some studies used naringenin-containing complex polyphenol blends. The most promising activities appear to be linked to cardiovascular disease protection, especially in compromised patients. However, some of these data should be expanded to understand better the mechanism of naringenin in pathological or physiological conditions. Several clinical studies have been conducted so far. Further clinical studies are needed to address better the safety, efficacy, delivery, and bioavailability of naringenin in human (Salehi et al., 2019).

## 4. CONCLUSIONS

In the present study, identification of several samples of honey in Indonesia from various regions are the honey samples have different Diastase Number and the HMF contents are relatively low. There is diversity in the composition of the honey, which may be due to the various regions of origin of the honey. The compounds that were allegedly found through LCMS/MS analysis include and have been traced based on literature studies had bioactive activity and beneficial to health, include: millefin (potential for treating heart disease and cancer), mangiferin (anti-inflammatory, anti-diabetes, immunomodulators, anti-tumor, antioxidants), rhamnetin (anti-inflammatory), tricin (antioxidant-like), acacetin (inhibit tumor angiogenesis agents), aurantiamide acetate (antiviral or anti-inflammatory, therapeutic agent for the treatment of influenza), salvigenin (controlling inflammation, acute and chronic pain), brucine (modulates anti-inflammatory and analgesic properties), dehydrocostus lactone (anti- inflammatory), santonin (anthelmintic activity), dimethylesculetin (bilirubin clearance), imidazole 4-acetic acid (neuropharmacological properties), propafenone (antiarrhythmic), yohimbine (affected sexual performance), Velutin (anti-inflammatory), narigenin (link ed to cardiovascular disease protection). Eventually, honey is is such a natural product with a number of salient therapeutic properties. However, there are still components that were found but their roles cannot be described in detail. Therefore, it is recommended that further meticulous studies should bring to light the other hidden properties of the honey compounds.

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