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Effect of Maja (*Aegle marmelous*) Leaf Extract and Trigona Honey on Glucosidase Activity Inhibition

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ABSTRACT

This study aims to determine the effect of the maja leaf extract and trigona honey on the α-glucosidase enzyme inhibition, antioxidant activity, and phytochemical type of trigona honey. It is a completely randomized study with natural ingredients, which are traditionally used for diabetic treatment in South Sulawesi. These Maja leaf extracts were mixed with raw trigona honey under several proportions: F1 (only containing trigona honey), FII (trigona honey mixed with maja leaves extract in ratio of 1:1), FIII (only containing maja leaves extract), FIV (trigona honey and maja leaves extract in ratio of 2:1) and FV (trigona honey and maja leaves extract in ratio of 1:2). The five formulas were tested for the phytochemical content (flavonoid, alkaloid, tannin, triterpenoid, steroid, saponin, and quinones), antioxidant activity by DPPH method and followed by α-glucosidase enzyme inhibition test. The phytochemical test found that trigona honey only contained flavonoid and tannin compounds, whereas maja leaf extract and its mixture (with trigona honey) obtained a positive result of flavonoid, tannin, steroid, and saponin contents. Meanwhile, the antioxidant activity results are categorized as follows (IC_{50}): FI (2,524 ppm) has a very weak antioxidant activity, FII (196 ppm) has a weak antioxidant activity, FIII (201 ppm) and FIV (225 ppm) have very weak antioxidant activities, FV (147 ppm) has a moderate antioxidant activity. The results of the α-glucosidase inhibition test show that the highest value was in FV (300.74 ppm), then followed by FIII (493.54 ppm), and FIV (847.95 ppm). On the other hand, FI and FII formulas were considered unable to inhibit α-glucosidase enzymes. Therefore, adding Maja leaf extract into the trigone honey might improve its potential use for managing diabetes.

Keywords: α-glucosidase, antioxidants, extract, maja leaves, trigona honey

INTRODUCTION

The number of deaths related to diabetes reached 1.5 million globally in 2012 and keeps climbing. In Indonesia, the prevalence in 2018 has increased by 2 % from 2013 (RISKESDAS 2018). Food intake affects the amount of insulin needed to meet blood glucose target. Carbohydrate in diet affects postprandial blood glucose levels and it is a major determinant of food-related insulin requirements (Tiwari *et al.* 2013). However, once someone has diabetes, medicine is needed to control the blood glucose. The available hypoglycemic agents cause digestive discomfort and might not able to prevent diabetes complication. Thus, the use of natural or herbal ingredients which potentially act as inhibitors of the α-glukosidase enzyme might offer better blood glucose control for people with diabetes (Lisiswanti & Faris *et al.* 2017).

Trigona honey (*Biroi*) and maja leaves (*Aegle marmelos*) are two natural inggridients native from Indonesia which have the potentials for the treatment of degenerative diseases. Although they have been used as traditional medicine (diabetes), but the development using scientific approach has been scarce. Research by Sumarlin *et al.* (2015) on the bioactivity from combination of Trigona honey with the methanol extract of namnam leaves reported that the combination of these two natural ingredients has the potential as an antioxidant and antibacterial substances. Furthermore, a combination of honey and black seed (*Nigella sativa*) is able to accelerate wound healing compared to the single use of each ingredient where its effect is almost similar to the drug phenytoin (Javadi *et al.* 2018).

Trigona honey also possesses bioactive compounds that potentially act as anti-diabetic substance according to Ali *et al.* (2020). Trigona

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honey called as *Kelulut* or Tualang honey in Malay produced by *Heterotrigona itama* bee contains phenolic and flavonoids compounds that are able to inhibit the activitiy α-amylase and α-glukosidase enzymes. Flavonoids protect cell from damage and re-stimulate insulin receptor sensitivity in order to produce insulin (Susanti *et al.* 2020). The α-glukosidase enzyme, which is present in the mucosa of the small intestine, breaks down disaccharides into simple sugars, previously in the form of complex polysaccharides that get broken down by α-amylase enzyme (Febrinda 2013).

Fruit of maja plant can be eaten directly, while the leaves and shoots are consumed as vegetables or salads in various countries in Asia. In Indonesia, they are consumed as cooking spices and used to reduce appetite (Nigam & Nambiar 2015). Maja plants contain many phytochemicals such as carotenoids, phenolics, alkaloids, pectin, tannins, coumarins, flavonoids, and terpenoids (Manandhar *et al.* 2018). Research by Mudi *et al.* (2017) showed that the glucose-lowering effect of the Maja leaf and fruit extracts were related to the effect of increased insulin sensitivity by lowering insulin resistance in type 2 diabetic rats.

Research on the mixing of trigona honey and maja leaf exract aims to determine the effect of Maja (*Aegle marmelous*) leaf extract and proportion of trigona honey on inhibition of glucosidase activity as antidiabetes. It is expected to produce a positive synergistic effect on the content of phytochemical compounds, antioxidant activity, and α-glucosidase enzyme inhibitory activity. Therefore, this study is expected to provide some information related to the antioxidant and antidiabetic activity of mixed in trigona honey and maja leaf extract.

METHODS

Design, location, and time

This study used a completely randomized design. The study involved several stages and test. The research samples, maja leaves and stingless bee honey (raw), were studied at two different locations. The extraction and antioxidant activity test were conducted in the biopharmaceutical laboratory at Hasanudin University. Then, the phytochemical and α-glucosidase enzyme inhibitory analysis of the sample was analyzed in biopharmaceutical laboratory of IPB University. The samples were sent in amber screw bottle to prevent antioxidant damage with normal temperature from Hasandin University to IPB University. The study was conducted from September to November 2021.

Material and tools

The main materials for this study were maja leaves (*Aegle marmelous*) from Makassar and stingless bee honey (*Trigona biroi* sp) from Bone-Bone village, North Luwu of South Sulawesi. These materials were processed with 96% ethanol solvent, filter paper, hot water, 0.05 Mg powder, concentrated HCl 1 ml, amyl alcohol, glacial CH_3COOH, concentrated H_2SO_4, 10 ml of chloroform, ammonia, H_2SO_4 2M; Dragendorf, Meyer, and Wagner reagents; distilled water, HCl 1N, $FeCl_3$ 1%, 0.002% DPPH 6 ml, 0.004% DPPH 12 ml, methanol, ascorbic acid, p-nitrophenyl-D-glucopyranoside, 0.1M phosphate buffer pH 7.0, α-glucosidase, dimethyl sufoxide (DMSO), 1% acarbose solution, and 2 NHcl.

The instruments or tools used in this study included a rotary evaporator (Buchi R220, Germany), blenders (Signora, Indonesia), hot plates (Thermo SP 131320-33q, USA), dropper pipettes, digital scales (Denver instrument SI-234, USA), spectrophotometer (Shimadzu 1800, Japan), microplates, and microplate readers (biotek ELx808, USA).

Procedures

Extraction of maja leaves and phytochemical testing. Seven hundred grams of fresh maja leaves was put in an oven and dried at temperature of 50°C for 8 h to obtain 100 g of dried maja leaves. These were observed every 2 h. Next, these dried leaves were grounded into fine powder (simplisia) using a simplisia grinding machine (memmert, USA) with water content <10% (Sumarlin *et al.* 2015). These simplisia powder was then soaked with 96% ethanol and put into maceration process for 24 h. After the process finished, the sample was filtered to obtain the first filtrate result (Edison *et al.* 2020). Then, the maja leaves residue was macerated again with ethanol solvent for 9 h, in order to obtain the second filtrate result. As the last step, the filtare was evaporated by using a rotary evaporator at 49°C with pressure 50 bar for 5 h to obtain a thick extract as much as 31 g which was used in the experiment with ethanol content of the extract not exceeding 1% v/v (20°C) (Sumarlin *et al.* 2015).

Formula determination. The formula for this study was created from maja leaf extract and trigona honey. The ratio used was based on a comparison of the content of active compounds contained in trigona honey and maja leaf extract, which has an antidiabetic potential. Research by Ali *et al.* (2020) stated that from honey concentration of 80 g/ml and 100 g/ml, there was an inhibition of α- glukosidase enzyme of more than 50% in Trigona Itama honey. The most common feed for these bees are mangrove, multi-fruits and *tualang*. In line with this research, (Phuwapraisirisan *et al.* 2008) succeded in isolating new active compounds called anhydroaegelin, aegelinoside A and aegelinoside B, where the most potential compound for inhibiting the α-glukosidase enzyme was anhydroaegelin, under a concentration of 10 g/ml with an IC_{50} inhibitory value of 35.8 M by using an 0.5 mg/ml extract. The LD50 for maja leaf extract was 10 g/kg (Phuwapraisirisan *et al.* 2008). The researchers concluded that the required materials for testing the inhibition of α-glukosidase enzyme were 100 g/ml or equivalent to 0.1 mg/ml of trigona honey and 500 g/ml or equivalent to 0.5 mg/ml of maja leaves extract as seen in Table 1.

Test of flavonoid content. A total of 50 mg sample was weighed then added with 100 ml of hot water and boiled for about 5 min, and then filtered to obtain 5 ml filtrate. The powder 0.05 mg of Mg, 1 ml of concentrated HCl, and amyl alcohol was added to the produced filtrate. The sample created different colours such as red, yellow, or orange on the amyl alcohol layer if the test had a positive result (Edison *et al.* 2020).

Table 1. Proportions of mixed trigona honey and maja leaves formula used in this study

Formula	Trigona honey (raw) (mg/ml)	Maja leaves extract (mg/ml)
I (1:0)	0.1	-
II (1:1)	0.1	0.5
III (0:1)	-	0.5
IV (2:1)	0.2	0.5
V (1:2)	0.1	1.0

The proportion of comparisons is based on studies that have succeeded in obtaining a positive effect on the alpha-glucosidase enzyme test namely

Ali *et al.* (2020) using 0.1 mg/ml trigona honey and Phuwapraisirisan *et al.* (2008) using Maja leaves extract 0.5 mg/ml

Test of triterpenoid/steroid content. A total of 50 mg sample was weighed then added with 10 drops of glacial CH_3COOH and 2 drops of concentrated H_2SO_4. This solution was then shaken slowly and let for several minutes. The sample presented particular colors with red or purple colors indicated triterpenoids content while blue or green colors indicated steroid content (Edison *et al.* 2020).

Test of saponin content. A total sample of 50 mg was weighed and added with 10 ml water and shaken for 1 min then added with 2 drops of 1 N HCL. The saponin content was indicated by the foam formation that remained stable for 7 min (Edison *et al.* 2020).

Test of tannin content. A total of 50 mg sample was weighed and added with 10 drops of 1 % $FeCl_3$. Then, an observation was done where the sample produced green, red, purple, blue or solid black colours when the sample had positive result (having tannin content) (Edison *et al.* 2020).

Test of quinon content. A total of 50 mg sample was weighed and added with 2 drops of 1 M NaOH. Then, the sample produced red colour which indicated that there was a positive result (having quinon content) (Edison *et al.* 2020).

Antioxidant determination by DPPH methods (Sumarlin et al. 2015). A total of 2 ml sample was put into a test tube then added with 2 ml of 0.002 % DPPH. Next, the samples were homogenized and incubated for 30 min in a dark room and absorption was read at 517 nm with a UV-VIS spectrophotometer. The measurement was repeated three times. For standard curve ascorbic acid standard was prepared at a concentration of 0.5, 1, 2, 4, and 8 ppm with the same treatment as the test sample. The absorbance value obtained was then used to determine the percentage of inhibition value. Then, the result from calculations was entered into a regression calculation where IC_{50} value (Inhibition Concentration) was obtained when the percent inhibition value was 50% (Sumarlin *et al.* 2015).

Inhibitor α-glukosidase test (Widowati et al. 2015). The first solution to be reacted was Control Blank Solution (B0), Blank Solution (B1), Control Sample Solution (S0), and Sample Solution (S1). The substrate preparation was made by dissolving p-nitrophenyl α-D-glucopyranoside in 0.1 M phosphate buffer (pH 7.0). Meanwhile, the α-glucosidase enzyme solution was made by dissolving 1 mg of α-glucoside in 100 ml of phosphate buffer (pH 7.0). The blank solution consisted of 10 µl of Dimethyl sulfoxide (DMSO) solution, 50 µl of 0.1 M phosphate buffer (pH 7.0), 25 µl of p-nitrophenyl α-D-glucopyranose acted as substrate, and 25 µl of -glucosidase enzyme solution. The difference between the blank and the control blank was that the blank control did not use the α-glucosidase enzyme.

Preparation of Sample Control Solution (S0) and Sample Solution (S1) was carried out by dissolving maja leaf extract and trigona honey in buffer. The sample reaction mixture consisted of 10 µl extract, 50 µl 0.1 M phosphate buffer (pH 7.0), 25 µl p-nitrophenyl -αD-glucopyranoside 0.5 mM as a substrate, and 25 µl of α-glucosidase enzyme solution. The difference between the sample and the control sample is that the control sample did not use the α-glucosidase enzyme.

The positive control applied in this test was acarbose with concentration of 1%. A sample of comparison was made from Glucobay tablet dissolved in distilled water and 2 NHcl (ratio of 1:1) under concentration of 1% (w/v) and centrifuged. A total of added 10 µl of supernatant was taken and added into the samples. Each reaction sample was mixed into different microplates and the solution on the microplate was incubated at 37°C for 30 min.

As the next phase, the solution was added with Na_2CO_3 to stop the reaction and the inhibitory activity was read with a microplate reader at 410 nm. The inhibitory power of the extract was calculated as percentage (%) of inhibition as follows:

$$Inhibition(\%) = [(K - (S1 - S0)/K)]\ X\ 100\%$$

Where:
K: Absorbance of Blank (B1) - Absorbance of Blank (B0) = Absorbance of Control Sample (S0)
S1: Absorbance of Sample (Sumarlin *et al.* 2015).

Data analysis

The data collected were compiled with Microsoft Excel 2010 and qualitative phytochemical test results are presented in descriptive analysis. The antioxidant activity and α-glucosidase enzyme inhibition tests were analyzed using regression formula in Microsoft Excel 2010 for the IC_{50} value.

RESULTS AND DISCUSSION

Phytochemical test

There are phytochemical compounds found in plants that are able to counteract free radicals, such as phenolic, flavonoids, coumarin derivatives and other compounds (Febrinda *et al.* 2013).

As shown in Table 2, flavonoid was identified in trigona honey, maja leafs extract and mixture of both materials. Most green plants have a high flavonoid content compared to non green plants (Widotiasari 2016). In addition, Ali *et al.* (2020) reported that trigona honey has flavonoid compounds including trigona honey from bees of *Itama* spp. Flavonoids act as antioxidants, inhibitors of α-glucosidase enzymes, and antibacterials substance. A similar study was proposed by Ningrum *et al.* (2019) who also obtained a positive test result for flavonoid content in maja leaves. Flavonoids have been shown to be a powerful agent to reduce the pathogenesis of diabetes and its complications. The antidiabetic mechanism of flavonoids was to reduce apoptosis and insulin resistence and increase insulin secretion and GLUT 4 translocation. Regular consumption of cyanidin inhibits glucosidase and amylase, reducing the absorption of carbohydrates in the intestine (Al-Ishaq *et al.* 2019). Alkaloid compounds can also act as an antioxidant (Sulasiyah 2018). However, there was no identified alkaloid compound content in all formulas.

Meanwhile, for tannin compound, all formulas produced a positive reaction. Tannins are among phenolic compounds that give bitter and astringent/chelate taste and they are able to aggromerate protein, amino acid and alkaloids (Julianto 2019). Tannins from plants are well known antioxidants. Tannins enhance glucose uptake and inhibit adipogenesis, thus having potentials for treatment of diabetes type 2 (Kumari & Jain 2012).

The content of triterpenoid was not found in this study because the samples did not produce blue colour, instead it produced green colour as an indication of a positive result of steroid content. Research by Sumarlin *et al.* (2018) that tested triterpenoid content in honey and its combination with namnam leaves also did not produce a positive result of triterpenoid only in honey, but it had positive result in mixture of namnam leaves and honey. The green colour was formed when the sample was treated with glacial CH_3COOH and concentrated H_2S0_4 in formulas of FII, FIII, FIV and FV, which indicated a positive result for steroid content. As for the F1 formula that contained only trigona honey, it did not indicate any steroid content. Thus, the addition of maja leafs extract provided a strong steroid content in the formulas. This result aligned with the research of Sumarlin *et al.* (2018) where the steroid content in rambutan honey was not found in the honey sample only but it was apparent in namnam leaf extract and in the mixture of namnam leaf extract with rambutan honey. Steroids are part of saponin family namely sapogenins, which is an antioxidant known to have an effect on the treatment of diabetes (Astuti *et al.* 2012).

There was no saponin content in the F1 with only trigona honey. Saponin was only found in maja leaf extract. Hence, the formulas with maja leaf extract (FIII, FIV, and FV) were able to produce a positive test of saponin. Saponin, as a glucosidase enzyme inhibitor, inhibits the breakdown of carbohydrates into glucose. Saponins works through regeneration of pancreas, which causes an increased number of pancreatic β-cells and the Langerhans Island, so that insulin secretion can help reduce blood glucose levels. The regeneration of pancreatic β-cells occurs due to the presence of quiescent cells in the pancreas, which have ability to regenerate (Susanti *et al.* 2020). Saponin, which gives an effect in the bubble formation, can cause hemolysis in red blood cells. Another example of saponin glycosides is liquorice, with its expectorant and anti-inflammatory activities, or diosgin compound, which is important for formation of glucocorticoid and for steroid hormones as progesterone (Julianto 2019). Due to the content of saponin in maja leaf extract, the leaves are known and used as a traditional anti-fertility agent (Vinita Bisht 2017).

Quinone content was identified by red colour formation in sample treated with 1 M NaOH. None of the formulas formed a red colour, whether in trigona honey sample, maja leaf extract, or in the mixture of both. Research of Sumarlin *et al.* (2018) also obtained the same results where there was an absence of quinone

Table 2. Result of phytochemical test on mixtures of maja leaf ethanol extract with trigona honey

Test result	Formula				
	FI	FII	FIII	FIV	FV
Flavonoid	+	+	+	+	+
Alkaloid	-	-	-	-	-
Tanin	+	+	+	+	+
Triterpenoid	-	-	-	-	-
Steroid	-	+	+	+	+
Saponin	-	+	+	+	+
Quinon	-	-	-	-	-

FI: Trigona honey (raw) (1:0)
FII: Trigona honey (raw): Maja leaves extract (1:1)
FIII: Maja leaves extract (0:1)
FIV: Trigona honey (raw): Maja leaves extract (2:1)
FV: Trigona honey (raw): Maja leaves extract (1:2)

content in rambutan honey mixed with namnam leaves extract. Phytochemical compound in honey is influenced by the dominant type of plants consumed by the bees.

Antioxidant activity test

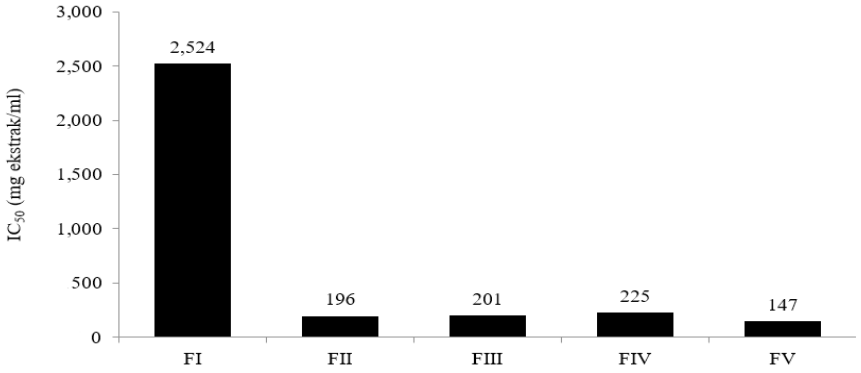
The antioxidant activity was determined by IC_{50} (inhibition concentration) value as it indicates the ability of the extract to inhibit free radicals' activity (2,2-diphenyl-1-picryhydrazyl or DPPH) that is marked by colour change in the solution from light purple to yellow to mark the oxidation of free radicals. The color change was then measured quantitatively to obtain absorption used to find the IC_{50} value. The IC_{50} value was obtained by employing a linear regression formula, which stated the relationship between antioxidant fraction concentration (x) and percent of inhibition (y) (Purwanto *et al.* 2017).

The IC_{50} value in antioxidant activity is divided into five categories; the IC_{50} value of less than 0.05 mg/ml (<50 ppm) as a very strong antioxidant group, the IC_{50} value in the range of 0.05–0.1 mg/ml (50–100 ppm) as a strong antioxidant group, the IC_{50} value in the range of 0.1–0.15 mg/ml (100–150 ppm) as a moderate antioxidant group, the IC_{50} value in the range of 0.15–0.2 mg/ml (150–200 ppm) as a weak antioxidant group,and the IC_{50} value in the range of more than 0.2 mg/ml (>200 ppm) as a very weak antioxidant group (Susana *et al.* 2018).

Figure 1 shows that F1 obtained IC_{50} value of 2,524 ppm, which means that the antioxidant activity of sample trigona honey was very weak; meanwhile, only FIV (a mixture with extra/more trigona honey than the maja leaf extract) had IC_{50} value of 225 ppm and FIII (maja leaves extract without a mixture) had IC_{50} value of 201 ppm, which means that the antioxidant activity found in the two formulas were in very weak. Stronger antioxidant activity was found in FII (a mixture of trigona honey and maja leaf extract in equal ratio) with IC_{50} value of 196 ppm. Meanwhile, the FV (a mixture of trigona honey and maja leaf extract with a higher ratio of maja leaf extract) had IC_{50} value of 147 ppm and it was the moderate category.

Muruke's reseach (2014) found that antioxidant activity of trigona honey was 4.19 mg/ml or 4.190 ppm. Meanwhile, the higher antioxidant activity from maja leaf extract was in accordance with Wilujeng *et al.*'s (2020) research that found a strong antioxidant activity in maja fruit extract with IC_{50} value of 0.107µg/ml.

The formulas with potential antioxidant (<200 ppm) in the study were found in FII and FV that showed addition of maja leaf extract to trigona honey gave a positive effect to the antioxidant activity. This result also aligned with the previous phytochemical test result in all formulas added with maja leaf extract that were found to have various phytochemical compounds. Antioxidants can stop the free radical auto oxidation in lipid oxidation (Susana *et al.* 2018). Increased free radicals activity is associated with various diseases, such as diabetes, cancer, cardiovascular diseases or other degenerative diseases. Degenerative disease such as diabetes



FI: Trigona honey (raw) (1:0)
FII: Trigona honey (raw): Maja leaves extract (1:1)
FIII: Maja leaves extract (0:1)
FIV: Trigona honey (raw): Maja leaves extract (2:1)
FV: Trigona honey (raw): Maja leaves extract (1:2)

Figure 1. The result of IC_{50} test of antioxidant activity from maja leaves ethanol extract and trigona honey

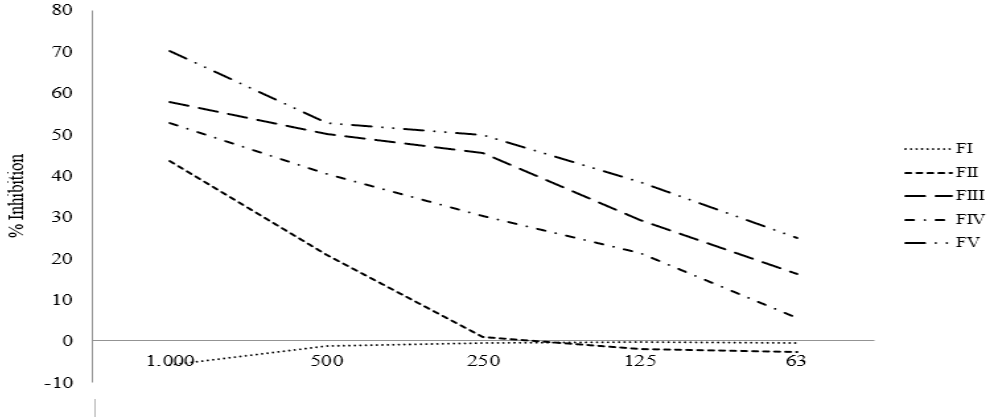
can be caused by the presence of reactive and unstable free radicals that attack macromolecules causing oxidative stress in mitochondria which will stimulate glucose intolerance. Meanwhile, the state of glucose intolerance in diabetics can result in production of free radicals, characterized by inflammatory oxidation and increased activity of (NAD(P)H oxidase that induces the formation of reactive free radicals. Free radicals increase the expression of TNF- (Tumor Necrosis Factor) and exacerbate stress. Increased oxidative stress, TNF- and cytokines, such as interleukins, can cause insulin resistance by decreasing insulin receptor autophosphorylation (Husain & Kumar 2012).

Inhibition α-glucosidase test

The α-glucosidase inhibition test was employed to determine the anti-diabetic effect from trigona honey, maja leaves extract, and their mixture in several different proportions. In vitro analysis of α-glucosidase enzymes tests the sample's inhibition reaction against p-nitrophenyl α-D-glucopyranosia substrate. If the tested sample has the ability to inhibit the α-glucosidase enzyme, then the resulting p-nitrophenyl will be reduced (Pratama *et al.* 2015).

In the p-nitrophenyl test, the formula turns into yellow colour when the α-glucoside enzyme hydrolyzes the substrate. The absorption of the resulted color is measured by a spectrophotometer and used to calculate the inhibition percentage (%) and then applied for IC_{50} value determination.

The results showed that the inhibition percentages were between -5.85 until 43.64% inhibition in all samples. It indicated that FI and FII formulas did not have potentials as to inhibit α-glucosidase enzymes. Meanwhile, FIII, FIV and FV were proven to have inhibition percentages above 0%. So, these results could be used to calculate IC_{50} which indicated their potential ability as an inhibitor of α-glucosidase enzyme. The research from Gurudeeban *et al.* (2012) suggested a similar result where the average inhibition percentage of maja leaves and fruit extract ranged from 72.23±0.30. In this assay, FV (a mixture of trigona honey and maja leaf extract with a higher percentage of maja leaf extract) displayed the highest α-glucosidase inhibition. This probably related to the phytochemical contents of maja leaf as recorded in this study. Similar inhibition activity of α-glucosidase enzyme in trigona honey was also shown by Rahmawati *et al.* (2019) and Ali *et al.* (2020) with the value of IC_{50} 1.917 ppm with 74.82±2.39 % inhibition. This result showed that an increase in maja leaf extract proportion added positive effect to the phytochemical content, antioxidant activity and α-glucosidase enzyme inhibitory activity in the formulas (Figure 2).



Values are presented in average value (n=3)
FI: Trigona honey (raw) (1:0)
FII: Trigona honey (raw): Maja leaves extract (1:1)
FIII: Maja leaves extract (0:1)
FIV: Trigona honey (raw): Maja leaves extract (2:1)
FV: Trigona honey (raw): Maja leaves extract (1:2).

Figure 2. The result of inhibitory α-glucosidase test

CONCLUSION

Phytochemical, antioxidant activity and α-glucosidase enzyme inhibition tests showed that the addition of maja leaf extract to trigona honey in increasing proportion were beneficial to the antioxidant activity and α-glucosidase enzyme inhibition. The addition of maja leaf extract was able to produce positive effect to the phytochemical content (flavonoids, tannins, steroids, and saponins compounds). Meanwhile, trigona honey was found to only contain alkaloids and tannins. Moreover, the antioxidant activity and α-glucosidase enzyme inhibitory activity of the formula also increased along with the addition of maja leaf extract. Further research to test more variations of proportions for obtaining a more precise dose so that it can be used for preventing degenerative diseases, such as diabetes. Therefore, based on this in-vitro study, we recommend that mixing of trigona honey and maja leaf extract may offer beneficial effects for managing diabetes.

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DECLARATION OF INTERESTS

The authors declare no conflict of interest with any party, other person or institution for this research.

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