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In vitro anti-diabetic activity of stingless bee honey from different botanical origins

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Abstract

The number of patients with diabetes mellitus is increasing at an alarming rate and this situation has triggered the interest of searching natural product as an alternative treatment. Stingless bee honey containing a diverse array of bioactive compounds is commonly utilized as a functional food and also found to possess various therapeutic effects including anti-diabetic through α-amylase and α-glucosidase inhibition. However, the composition level of bioactive compounds varying of geographical origins and botanical sources of honey leads to different enzyme inhibition abilities. Therefore, this study explored the total phenolic, total flavonoid, α-amylase and α-glucosidase inhibition activities of stingless bee honey from various botanical origins. In this study, stingless bee honey was collected from 6 different botanical origins namely, acacia, coconut, mangrove, starfruit, multifruit and multiflower plant. Honey from tualang tree was used as a positive control. Phenolic and flavonoid contents as well as α-amylase and α-glucosidase inhibition activities of honey were studied spectrophotometrically. Stingless bee honey from mangrove was found to have the highest phenolic content (141.74±0.03 mg GAE/100 g). The honey collected from coconut origin showed the highest flavonoid content with the value of 51.33±0.02 mg RE/100 g and also achieved the highest percentage inhibition against α-glucosidase (68.33% at 100 µg/mL). Furthermore, tualang honey and honey samples from mangrove, coconut and Acacia tree were found to have strong α-amylase inhibition abilities as their inhibition percentages were more than 70.00% at 100 µg/mL. This study showed that the presence of flavonoid and phenolic compounds in honey from different botanical origins yielded different degree of α-amylase and α-glucosidase inhibition and also recommended the uses of stingless bee honey in diabetes treatment.

1. Introduction

Diabetes mellitus is one of metabolic diseases characterized by chronic hyperglycemia that triggers the carbohydrate, fat and protein metabolism disorders in the human body, leading to long-term damage and dysfunction of various organs (Alberti and Zimmet, 1998; Rahmawati *et al*., 2019). In 2017, approximately 451 million of the population were affected by this disease and it had brought up to 5 million deaths in the worldwide population in the same year (Rutebemberwa *et al*., 2013). In addition, the number of patients is predicted to increase by years up to 693 million in 2045 (Cho *et al*., 2018). Failure in recognizing diabetes treatment as priority became the major factor that leads to morbidity and even increases the rate of mortality globally (Shaw *et al*., 2010). However, the high cost of disease treatment has led to a financial burden to patients with diabetes, especially those in developing countries. Therefore, there is an urge to encourage people to search for an alternative way such as lifestyle modification including a change of dietary intake to enhance the treatment of patients suffering from diabetes.

Honey, a sugary secretion extracted from honeycombs comprises more than 200 constituents including the main substances such as fructose and glucose (Eteraf-Oskouei and Najafi, 2013). It is known as one of the natural products that have potential health benefits to human and among a variety of honey, stingless bee honey is considered as one of the food substances that used for natural remedies. Basically, it is produced by the stingless bees from the family of *Meliponini*, which made up of various types of genera such as *Melipona*, *Trigona*, and *Heterotrigona* whereas among the stingless bee species, *Geniotrigona thoracica*,

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Heterotrigona itama, *Lepidotrigona terminata* and also *Tetragonula laeviceps* are reared commercially in Malaysia (Mohd Rafie *et al*., 2018). Stingless bee honey has been reported to contain rich phytochemical contents and possess anti-inflammatory, antimicrobial, antioxidant, anticancer and antiseptic properties as well as to boost the immune system (Eteraf-Oskouei and Najafi, 2013; Abd Jalil *et al*., 2017; Akhir *et al*., 2017). Furthermore, it displays specific anti-diabetic role in the inhibition of α-amylase and α-glycosidase enzyme activity by controlling the glucose content in the human body (Campone *et al*., 2014).

However, the nutritional content, physiochemical properties and variety of bioactive compounds of honey might be affected by several factors such as geographical origins, their botanical sources and environmental factor (Majid *et al*., 2019; Lim *et al*., 2019). Thus, this study was aimed to determine phenolic and flavonoid contents as well as to evaluate the α-amylase and α-glucosidase inhibition activities of honey collected from the stingless bee, *Heterotrigona itama* in different botanical origins.

2. Materials and methods

2.1 Sample preparation

The sample chosen in this study was stingless bee honey from *H. itama* sp. The stingless bee honey samples were collected from six botanical origins including Acacia tree, mangrove tree, coconut tree, multiflower plant, multifruit plant and starfruit tree while honey from tualang tree was used as a positive control. Then, the honey samples were stored at 4ºC for further analysis.

2.2 Determination of total phenolic content

Folin-Ciocalteu method was conducted to determine the total phenolic content of honey samples (Yap *et al*., 2015; Istasse *et al*., 2016). The absorbance was measured spectrophotometrically at 725 nm. A calibration curve ($y = 0.009x - 0.0217$; $R^2 = 0.9914$) was used to calculate the total phenolic content. The experiment was carried out in triplicate and the results were expressed as mg of Gallic acid equivalent (GAE) per 100 g of honey.

2.3 Determination of total flavonoid content

Total flavonoid content was determined based on the method as stated in the study of Islam *et al*. (2017) with some modification. The absorbance was measured spectrophotometrically at 510 nm. A calibration curve ($y = 0.0021x + 0.004$; $R^2 = 0.9869$) was used to calculate the total flavonoid content and the result was expressed as mg of Rutin equivalents (RE) per 100 g of honey.

2.4 Determination of α-amylase inhibition assay

α-amylase inhibition activity was carried out based on the method of Inoue *et al.* (2007) with some modification. The reaction mixture containing 500 µL of appropriate dilutions (20-100 µg/mL) of the honey sample, 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) and porcine pancreatic α-amylase (0.5 mg/mL) was incubated at 25ºC for 10 mins. Then, 500 µL of 1% starch solution in 0.02 M sodium phosphate buffer was added to the mixture and the mixture was incubated at 25ºC for another 10 mins. After incubation, dinitro salicylic acid (DNSA) solution was added to the mixture. The reaction was stopped by incubating the mixture in boiling water bath for 5 mins and then left to cool at room temperature. A total of 10 mL of distilled water was added to dilute the reaction mixture. The absorbance was measured spectrophotometrically at 540 nm. The reference sample was tested in the same way with the exception of the test sample and the result was expressed as percentage inhibition.

2.5 Determination of α-glycosidase inhibition assay

α-glucosidase inhibitory activity was conducted according to the study of Apostolidis *et al.* (2007) with some modification. 100 µL of α-glucosidase (0.5 mg/mL) in 0.1 M phosphate buffer (pH 6.0) solution was added to 150 µL of appropriate dilutions (20-100 µg/mL) of extracts and incubated at 25ºC for 10 mins. Then, the mixture was incubated at 25ºC for another 5 mins after the addition of 100 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside in 0.1 M phosphate buffer (pH 6.9) solution. After incubation, the absorbance was measured spectrophotometrically at 405 nm. The reference sample was tested in the same way with the exception of the test sample and the result was expressed as percentage inhibition.

2.6 Statistical analysis of the data

50% inhibition (IC_{50}) values of α-amylase and α-glucosidase activities were determined from the regression analysis. Correlation analysis was conducted to determine the relationship between the flavonoids, phenolic compounds and inhibition activities of α-amylase and α-glucosidase.

3. Results and discussion

3.1 Total phenolic content

Total phenolic content in this study was ranged from 77.52±0.02 to 141.74±0.03 (mg GAE/100 g) at the concentration of 100 µg/mL (Table 1). Stingless bee honey from mangrove origin showed the highest phenolic content with the value of 141.74±0.03 (mg

GAE/100 g); followed by the honey from coconut tree with the second highest phenolic content of 101.41±0.06 (mg GAE/100 g). The descending trend of total phenolic content in honey samples from other botanical origins were shown as follow: Acacia (99.74±0.08 mg GAE/100 g) > tualang (89.19±0.01 mg GAE/100 g) > starfruit (85.74±0.02 mg GAE/100 g) > multiflower (83.08±0.04 mg GAE/100 g) > multifruit (77.52±0.02 mg GAE/100 g). The composition of honey varies depending on the botanical origins and the result of this study was in agreement with the study of Shamsudin *et al*. (2019). In general, polyphenols in honey play an important role to maximize the efficiency of some biological activities that take place in the human body (Hoffman and Gerber, 2015). Therefore in this study, the presence of polyphenol compounds in honey might responsible for the α-amylase and α-glycosidase inhibition activity.

Table 1. Total phenolic content for honey samples

Samples	Phenolic content (mg GAE/100 g)
Acacia (K1)	99.74±0.08
Coconut (K2)	101.41±0.06
Mangrove (K3)	141.74±0.03
Starfruit (K4)	85.74±0.02
Multifruit (K5)	77.52±0.02
Multiflower (K6)	83.08±0.04
Tualang (K7)	89.19±0.01

Honey samples were labelled as: K1 represents acacia tree; K2 represents coconut tree; K3 represents mangrove tree; K4 represents starfruit tree; K5 represents multifruit plant; K6 represents multiflower plant; K7 represents tualang tree. Each value was shown in mean±standard deviation (n=3).

3.2 Total flavonoid content

Total flavonoid content values were ranged from 13.71±0.02 to 51.33±0.02 (mg RE/100 g) (Table 2). Stingless bee honey from coconut origin displayed the highest flavonoid content with the value of 51.33±0.02 (mg RE/100 g) and the second highest flavonoid content value of 44.66±0.02 (mg RE/100 g) was observed in the honey sample from the mangrove tree. Total flavonoid content of honey samples from other origins was arranged in the descending order as shown follow: multifruit (41.33±0.01 mg RE/100 g) > starfruit (32.28±0.04 mg RE/100 g) > Acacia (29.42±0.01 mg RE/100 g) > multiflower (19.42±0.05 mg RE/100 g) > tualang (13.71±0.02 mg RE/100 g). The overall result showed that the stingless bee honey samples were found to have more total flavonoid content than the tualang honey and this was analogue to the study of Abu Bakar *et al*. (2017) as the total flavonoid content of *H. itama* honey collected from different locations was much higher than tualang honey. The variation on the composition of flavonoid compounds in honey that observed in this study was likely due to the floral sources but geographical locations, seasonal and environmental factors could also be one of the reasons (Kaskoniene and Venskutonis, 2010). According to Erejuwa *et al*. (2011), elimination of excess free radicals from body might be one of the preventions among diabetic patients. Hence, the result in this study was in compliance with the statement as the presence of flavonoid in all the honey samples could act as a free radical scavenger.

Table 2. Total flavonoid content for honey samples

Samples	Flavonoid content (mg RE/100 g)
Acacia (K1)	29.42±0.01
Coconut (K2)	51.33±0.02
Mangrove (K3)	44.66±0.02
Starfruit (K4)	32.28±0.04
Multifruit (K5)	41.33±0.01
Multiflower (K6)	19.42±0.05
Tualang (K7)	13.71±0.02

Honey samples were labelled as: K1 represents acacia tree; K2 represents coconut tree; K3 represents mangrove tree; K4 represents starfruit tree; K5 represents multifruit plant; K6 represents multiflower plant; K7 represents tualang tree. Each value was shown in mean±standard deviation (n=3).

3.3 α -amylase inhibition assay

All honey samples exhibited α-amylase inhibition ability at the concentrations ranged from 20 to 100 µg/mL. Based on the result obtained in Table 3, the percentage of α-amylase inhibition increased as the concentrations of honey samples increased. At a concentration of 100 µg/mL, the highest activity was observed in the tualang honey (74.83%), followed by the mangrove (72.15%), coconut (71.65%), Acacia (70.30%), multifruit (55.76%), multiflower (46.76%) and starfruit (36.78%). The percentages inhibition of α-amylase at 80 µg/mL was recorded with the values from 27.03 to 59.57% while the percentage inhibition beyond 60 µg/mL was less than 50%. Starfruit honey showed the least α-amylase percentage inhibition (0.17-36.77%) along the concentrations. IC_{50} values of the honey samples were ranged from 66.86 to 128.75 µg/mL. Tualang honey achieved the highest inhibition activity with its low IC_{50} value and this could be related to the presence of more phenolic acid in the honey. Phenolic compounds that present in tualang honey such as kaempferol, caffeic acid and p-coumaric acid were found to have anti-diabetic properties and they might contribute to the α-amylase inhibition activity (Ahmed and Othman, 2013; Bharti *et al*., 2017). However, stingless bee honey samples from mangrove, coconut and Acacia trees could also be a good α-amylase inhibitor to reduce postprandial blood glucose levels in the body because their inhibition abilities were more than 70% at 100 µg/mL.

Table 3. α -amylase inhibition of honey samples

Samples	Inhibition activity (%)				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
Acacia (K1)	1.23	9.29	39.48	46.38	70.29
Coconut (K2)	0.41	13.6	28.39	45.64	71.65
Mangrove (K3)	21.24	33.2	45.15	56.74	72.14
Starfruit (K4)	0.17	7.44	19.02	27.03	36.77
Multifruit (K5)	6.82	20.13	26.54	50.45	55.75
Multiflower (K6)	3.74	14.71	22.23	28.27	46.75
Tualang (K7)	2.39	22.64	43.02	59.57	74.82

Honey samples were labelled as: K1 represents acacia tree; K2 represents coconut tree; K3 represents mangrove tree; K4 represents starfruit tree; K5 represents multifruit plant; K6 represents multiflower plant; K7 represents tualang tree. Each value was shown in mean±standard deviation (n=3).

3.4 α -glucosidase inhibition assay

α -glucosidase inhibition ability of all honey samples was studied at the concentrations ranged from 20 to 100 µg/mL (Table 4). In this assay, stingless bee honey sourced from coconut displayed the highest α-glucosidase inhibition with 68.32% at a concentration of 100 µg/mL. This probably related to its high phenolic and flavonoid contents as recorded in this study. 13.82 to 60.60% of α-glucosidase inhibition was recorded among the honey samples at the concentration of 80 µg/mL while at the concentrations from 20 to 60 µg/mL, all honey samples achieved less than 50% of inhibition. The least percentage inhibition for α-glucosidase inhibition activity was observed in starfruit honey with the values from 1.08% to 20.52% along with the concentrations from 20 to 100 µg/mL. The lowest IC_{50} (77.69 µg/mL) was found in the honey from coconut origin whereas the honey from starfruit had the IC_{50} value greater than 100 µg/mL. Krishnasree and Mary Ukkuru (2017) showed that the stingless bee honey has a significant effect in α-amylase and α-glucosidase inhibition activities, however different types of bioactive compounds present in the honey from different botanical origins might influence the inhibition ability of honey (Shamsudin *et al*., 2019).

3.5 Correlation between bioactive compounds, α-amylase inhibition and α-glucosidase inhibition

Total phenolic content was moderately correlated with α-amylase inhibition activity and α-glucosidase inhibition activity with the r values of 0.5376 and 0.5173, respectively. Meanwhile, low positive correlations were observed between total flavonoid content, α-amylase and α-glucosidase inhibition activities with the r values of 0.1580 and 0.2327, respectively. Both flavonoid and phenolic compounds possess a positive effect on enzyme inhibition activities, but the presence of phenolic compounds contributed more on the inhibition ability of honey. The study of Devarajan and Venugopal (2012) also stated that major phenolic compounds and flavonoid in the honey extract may increase its potential anti-diabetic effects such as in α-amylase and α-glucosidase inhibition activities.

4. Conclusion

In conclusion, phenolic and flavonoid contents of stingless bee honey samples were varied in their botanical sources and the composition of honey resulted in different α-amylase and α-glucosidase inhibitory abilities as there was a positive correlation between enzyme inhibition activities (i.e. α-amylase and α-glucosidase) and bioactive compounds. Tualang honey displayed the highest α-amylase inhibition activity (74.83% at 100 µg/mL) but stingless bee honey samples from mangrove, coconut and acacia tree could potentially reduce blood glucose levels in the body, while honey from coconut origin exhibited the highest α-

Table 4. α –glucosidase inhibition of honey samples

Samples	Inhibition activity (%)				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
Acacia (K1)	1.89	4.87	12.46	34.46	38.75
Coconut (K2)	3.25	24.93	26.02	56.10	68.32
Mangrove (K3)	8.13	20.33	33.33	45.96	67.51
Starfruit (K4)	1.08	2.16	5.96	13.82	20.52
Multifruit (K5)	5.69	15.71	28.72	35.77	45.52
Multiflower (K6)	4.60	10.29	20.59	42.00	49.32
Tualang (K7)	8.13	33.06	38.75	60.60	62.60

Honey samples were labelled as: K1 represents acacia tree; K2 represents coconut tree; K3 represents mangrove tree; K4 represents starfruit tree; K5 represents multifruit plant; K6 represents multiflower plant; K7 represents tualang tree. Each value was shown in mean±standard deviation (n=3).

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glucosidase inhibition activity due to its high content of phenolic and flavonoids. Therefore, stingless bee honey is suggested as a good source containing rich bioactive constituents and highly recommended to be utilized for nutraceutical and pharmaceutical applications. Further studies concerning the identification of bioactive compounds of the stingless bee honey and their verification of enzyme inhibition activity through clinical research are needed for effective management of diabetes.

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