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Honeybee honey and stingless bee honey quality characteristics and their anti-cancer potential in HeLa cells

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Abstract

Honey is one of the best natural foods produced by bees. In the present study, samples of processed–honeybee honey and processed stingless bee honey were analysed. The study aimed to compare their phytochemical, antioxidant and physicochemical properties and also to compare their anticancer potential towards HeLa cells. Honey samples were first analysed for total phenolic content, total flavonoid content, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxymethylfurfural (HMF) and diastase activity in order to determine their phytochemical, antioxidant and quality characteristics, respectively. They were then analysed to investigate anticancer properties in the sample using 3-(4, 5-dimethylthiazol-2 -yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Results revealed that honeybee honey had significantly ($p<0.0001$) higher total phenolic content as compared to stingless bee honey at 635.48(29.68) and 368.11(17.75) mg GAE/kg, respectively. The total flavonoid content of honeybee honey was 45.11(5.44) and that of stingless bee honey was 64.25 (7.54) mg CEQ/kg ($p>0.05$). The HMF of honeybee honey was 83.4 mg/kg and the diastase activity of stingless bee honey was 5.1 DN. On the other hand, the HMF of stingless bee honey and diastase activity of honeybee honey were undetectable in the current study. The 50% cell inhibition activity (IC_{50}) of honeybee honey was reported at 13.75 mg/mL, while a value for stingless bee honey could not be obtained. In conclusion, processed honeybee honey is better than processed stingless bee honey in terms of phytochemicals, antioxidant and anticancer potential. However, processed stingless bee honey shows better honey quality characteristics based on HMF and diastase activity.

1. Introduction

Honey can be defined as a very saturated aqueous solution containing numerous carbohydrates such as fructose, glucose, maltose, sucrose and other oligosaccharides and polysaccharides (Sakać *et al*., 2019). Honey is commonly used as a sweetener (Badolato *et al*., 2017) and is widely known to offer numerous benefits such as antimicrobial (Chua *et al*., 2013; Boussaid *et al*., 2018), wound healing (Rao *et al*., 2016; Boussaid *et al*., 2018) and anti-inflammatory properties (Chua *et al*., 2013; Deng *et al*., 2018; Sakač *et al*., 2019).

The physical properties of honey, such as ash content, colour, pH, enzyme activity, electrical conductivity and taste, vary depending on the honeybee species, the origin of floral, temperature and geographical origin (Deng *et al*., 2018; Khan *et al*., 2018; Sakač *et al*., 2019). Phytochemical compounds such as phenols and flavonoids have been reported to be diverse according to their geographical and honey origins (Machado De-Melo *et al*., 2017; Chew *et al*., 2018). Studies by Alvarez-Suarez *et al*. (2010) and Moniruzzaman *et al*. (2013a) have reported that dark-coloured honey tends to have higher phenolic content. Meanwhile, high phenolic content indicates strong antibacterial and antioxidant properties (Deng *et al*., 2018). Phenolic compounds in honey are a possible indicator of honey floral origin (Badolato *et al*., 2017).

Honey is a natural antioxidant source that reduces

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the risk of heart disease, cancer, immune system deficiency, cataracts and various inflammatory processes (Boussaid *et al*., 2018). Furthermore, antioxidant compounds can act as a defence against free radicals (Gül and Pehlivan, 2018). It has been reported by Aumeeruddy *et al*. (2019) that there are inconsistencies in raw (unprocessed) and processed kinds of honey' antioxidant activity. One factor that may affect this condition is the formation of melanoidin and antioxidant products formed after the final stage of the Maillard reaction.

Physicochemical can be used as a parameter to measure the quality of the honey. Physicochemical such as ash, moisture, water activity, pH, reducing sugar, free acidity, colour, hydroxymethylfurfural (HMF) and diastase activity is being called quality parameter as they can be used as a tool to measure the quality of honey. Diastase is an enzyme found in honey other than invertase. Processed honey might have lower diastase activity, as it is typically treated with heat to lower the moisture content. Pasias *et al*. (2017) reported that diastase activity can be negatively affected by heat treatment, similarly to that of HMF. Moussa *et al*. (2012) reported that diastase activity is considered a quality factor. Honey age and storage condition are reflected through their diastase level (Wanjai *et al*., 2012). During the storage, heat treatment, and feeding of honeybees, diastase activity can decrease. Inadequate processing or storage conditions might be a factor affecting the low content of diastase activity (Moussa *et al*., 2012). In addition, Wanjai *et al*. (2012) reported that diastase can be denatured when honey is heated during processing and storage.

Hydromethylfurfural (HMF) is naturally produced during the heat-treatment of food containing sugar and can be used as a heat indicator in honey (Boussaid *et al*., 2018). It was reported that HMF can serve as an indicator of the freshness of honey (Al-Ghamdi *et al*., 2017). HMF can also be produced from the result of the long storage of honey (Shapla *et al*., 2018). Fructose, which is more sensitive than glucose, will form HMF as a result of the normal acidity reaction (Al-Ghamdi *et al*., 2017). Sakać *et al*. (2019) had reported that 5-sulfoxymethyfurfural, which is a cytotoxic and mutagenic compound, can be produced from the conversion of HMF. If honey with high content of HMF is consumed, this might cause the growth of cancer cells.

Many researchers have reported that honey exhibits anticancer effects, such as antitumor (Can *et al*., 2015) and antimutagenic properties (Aumeeruddy *et al*., 2019). These reports were based on the raw honey samples, but not processed honey. World Health Organization (WHO, 2018) reported that cancer is the second leading cause of death worldwide. In 2018, it caused 9.6 million deaths. Drawbacks to chemotherapeutic drugs have stimulated an alternative search for new treatments that are less harmful (Yaacob *et al*., 2013). *In vitro* was done in the current study to observe the reaction of the honey samples with cancer cells. The HeLa cancer cell is a cervical cancer cell being used in the current study to investigate the anticancer potential of processed honeybee honey and stingless bee honey.

One limited study has used processed honeybee honey and stingless bee honey as samples. Guerrini *et al*. (2009) reported that due to less knowledge being available for stingless bee honey, there is a lack of institutional quality standards which would be helpful to the honey industry. In addition, not much has been reported about the anticancer potential of processed honey. As previously reported, HMF can be converted into the cytotoxic and mutagenic compound. Honey that has been processed might have been exposed to heat treatment which might lead to the high content of HMF. This leads to question about the anticancer potential in processed honey, which supposedly has high HMF content due to it being exposed to heat during processing. The current study aimed to compare the phytochemicals (total phenolic content and total flavonoid content), antioxidant (free radical scavenging activity) and honey quality indicator (hydroxymethylfurfural and diastase activity) of processed honeybee honey and stingless bee honey, and also to document the potential for anticancer properties of processed honeybee honey and stingless bee honey.

2. Materials and methods

2.1 Samples

Raw or unprocessed honeybee honey and stingless bee honey samples were obtained from a honey farm in Marang, Terengganu, Malaysia. These samples were sent to MARDI Terengganu to be processed using the dehydration method (hydration machine). The moisture content of the samples was reduced to 20%.

2.2 Total phenolic content

The Folin-Ciocalteu method which was previously modified by Khalil *et al*. (2012) was used in the determination of phenolic content. A volume of 1 mL of Folin-Ciocalteu's phenol reagent and 1 mL of diluted honey (0.2 g/mL) were mixed. After 3 mins, 1 mL of 10% sodium carbonate solution was added and adjusted to 10 mL using distilled water. The reaction mixture was kept in dark for 90 mins before absorbance was recorded at 725 nm using a UV/Vis spectrophotometer

(Spectroquant® Pharo 300). A standard curve was plotted using gallic acid at concentrations of 20, 40, 60, 80 and 100 µg/mL. The final result was expressed in mean value±standard deviation in mg of gallic acid equivalents (GAEs) per kg of honey.

2.3 Total flavonoid content

The colourimetric assay method was adapted from Khalil *et al*. (2012). Firstly, 1 mL (0.2 g/mL) of properly diluted honey was mixed with 4 mL distilled water before adding 0.3 mL (5% w/v) sodium nitrite. This was followed by the addition of 0.3 mL of (10% w/v) aluminium chloride after 5 mins. After 6 mins, 2 mL of 1 M of sodium hydroxide solution was added and immediately marked up to 10 mL total volume with 2.4 mL distilled water. The mixture was shaken vigorously before taking the absorbance reading at a wavelength of 510 nm. The calibration curve was plotted using catechin (20, 40, 60, 80 and 100 µg/mL) and the results are expressed in mg CEQ/kg of honey.

2.4 Assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay method was adapted from Khalil *et al*. (2012) and Adjimani and Asare (2015) with few modifications. The stock solution of DPPH was prepared by dissolving 2.37 mg DPPH into 80 mL of 100% methanol and marked up to 100 mL using 100% methanol. Quercetin was used as positive control and honey samples were prepared with DMSO to the concentration of 10 mg/mL. Negative control of pure DMSO was loaded into the eight rows. A volume of 200 µL of DPPH stock was loaded into each well plate and incubated for 30 mins in dark. Absorbance was taken at 517 nm using an ELISA reader. The following formula of percentage radical-scavenging activity (RSA) was used to calculate the DPPH activity in the sample:

$$\%RSA = \frac{A\ DPPH\text{-}AS}{A\ DPPH} \times 100$$

Where As = the absorbance of the solution when sample extract was added at a particular level and A_{DPPH} = the absorbance of DPPH solution

2.5 Hydroxymethylfurfural

The method for the determination of hydroxymethylfurfural (HMF) was adapted from White (1979) in the International Honey Commission (2009). Honey was diluted by dissolving 5 g honey with 25 mL distilled water. Then, 0.5 mL of Careez solution I was added and mixed well, followed by the addition of 0.5 mL of Carrez solution II. The first 10 mL of filtrate was rejected, followed by pipetting 5 mL of filtrate into two test tubes (sample solution and reference solution). For the sample solution, 5 mL of distilled water was added, while for the reference solution, 5 mL of 0.2% sodium bisulphite was added. The mixture was mixed well and the absorbance of the sample against reference was read at 284 nm and 336 nm using UV/Vis spectrophotometer (Spectroquant ® Pharo 300).

2.6 Diastase activity

The Schade *et al.* (1985) method provided by the International Honey Commission (2009) was adapted for diastase determination. A value of 10 mL of honey solution was pipetted into the flask and placed in a 40°C water bath with the second flask containing 10 mL of starch solution. After 15 mins, 5 mL of starch solution was pipetted into the honey solution. A timer was started, and for the first 5 mins, 0.5 mL of aliquots was rapidly added into 5 mL of diluted iodine solution. The amount of water (determined in starch standard solution determination) was added, mixed and immediately taken the absorbance of each separate solution at 660 nm against blank distilled water. Then, a 10 mL sample solution was added into 5 mL of water and mixed thoroughly. After that, 0.5 mL of this solution was rapidly added into a 5 mL dilute iodine solution. The absorbance reading was taken repeatedly until it reached the range of 0.456 – 0.155.

2.7 Assay of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)

In vitro cytotoxicity assay was done following standard MTT assay of ATCC which was adapted from Akbari and Javar (2013). A volume of 100 µL of previously grown cell suspensions was harvested, counted and inoculated (8×10^4 cells/well) into a 96-well plate. It was then incubated in a 5% CO_2 incubator at 37°C (24 hrs). The original culture medium was replaced with 100 µL of prepared concentration of the sample and further incubated in the 5% CO_2 incubator at 37°C for 72 hrs. After that, 20 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added into each well before further incubation for another 4 hrs. Tetrazolium salt in MTT solution was reduced into insoluble formazon crystals by viable cell mitochondria during incubation. The formazon crystals formed were equivalent to the number of existing viable cells. After 4 hrs, 120 µL medium was removed and replaced with 100 µL of DMSO, which helped in the solubility of the crystals. Samples' optical density (OD) was measured using a spectrophotometer at a wavelength of 570 nm. The following formula was used to obtain the percentage of cell inhibition:

$$\frac{OD\ control - OD\ sample}{OD\ control} \times 100$$

Where OD sample = optical density of sample and OD control = optical density of control

The cytotoxicity effect of honey samples on HeLa cell lines was determined from measurement of $IC_{50,}$ where 50% of the cell population is killed by the cytotoxic dose when compared to untreated control. It was determined from the graph plot of percentage inhibition against the concentration of honey samples.

2.8 Statistical analysis

Data are presented as mean (SD) with triplicate analysis, followed by either independent t-test or One-way ANOVA using SPSS software. In all analyses, p, 0.05 was taken to indicate statistical significance.

3. Results and discussion

3.1 Total phenolic content

It has been reported that total phenolic content is associated with the antioxidant activity of honey. Previously Chua *et al*. (2013) reported that the phenolic acid content in honey can affect the flavour and colour of honey. Total phenolic compounds are strongly dependent on the plant species where the nectar was collected (Giordano *et al*., 2018).

The results obtained in the present study are shown in Table 1. The phenolic content for stingless bee honey was in agreement with the results reported by Abu Bakar *et al*. (2017) for Malaysian stingless bee honey, which ranged from 357.14 – 520.83 mg GAE/kg. In Serbian *Apis mellifera* honey (Gašić *et al.*, 2014) and Manuka honey (Bundit *et al*., 2016), the total phenolic contents were 30 – 1390 mg GAE/kg and 657.91±83.16 mg GAE/ kg, which is in agreement with the total phenolic content of honeybee honey obtained in the current study.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of honeybee honey and stingless bee honey

	Samples		
	Honeybee honey	Stingless bee honey	p-value
TPC (mg GAE/kg)	635.48±29.68	368.11±17.75	0.000
TFC (mg CEQ/kg)	45.11±5.44	64.25±7.54	0.056

A previous study reported Iranian *Apis mellifera* honey recorded 60.1 – 744.9 mg GAE/kg (Mahmoodi-Khaledi *et al*., 2016) total phenolic content, which is also in agreement with the total phenolic content of honeybee honey reported in the current study. However, Can *et al*. (2015) reported the level of phenolic content for Turkish Acacia honey at 160.2±27.0 mg GAE/kg, which is considered lower than the results obtained in the current study. Malaysian Acacia honey had a total phenolic content of 1965.00±63.23 mg GAE/kg, as reported by Chua *et al*. (2013), which is markedly higher compared to that of the current study. The high value of phenolic content reported by Chua *et al*. (2013) might be due to the presence of organic acids which affect the antioxidant capacity of the honey.

The total phenolic content of stingless bee honey of the current study was higher compared to a previous study by Renneh *et al*. (2018) and Tufail Ahmad *et al.* (2019), which reported that the total phenolic content for stingless bee honey was in the range of 58.6 to 235.28±0.6 mg GAE/kg. In contrast, the total phenolic content for Malaysian stingless bee honey reported by Kek *et al*. (2014) was higher than the current study at 791.5 mg GAE/kg. However, Malaysian stingless bee honey in the current study is in agreement with studies by Biluca *et al*. (2016) and Jimenez *et al*. (2016), which reported that the total phenolic content of Brazil and Mexico stingless bee honey ranged from 103 – 980 mg GAE/kg and 258.5 – 401 mg GAE/kg, respectively. A high level of phenolic content in both kinds of honey in the current study indicates their potential as antioxidants. Different levels of phenolic content in different types of honey are due to botanical differences (Moniruzzaman *et al*., 2013a; Mahmood-Khaledi *et al*., 2016; Giordano *et al*., 2018).

The higher total phenolic content of honeybee honey as compared to stingless bee honey in the present study might be due to more dark-coloured which tend to exhibit higher phenolic content (Alvarez-Suarez *et al*., 2010; Moniruzzaman *et al*., 2013a) and that honeybee honey has higher potential as an antioxidant (Deng *et al.*, 2018). Machado De-Melo *et al.* (2017) and Chew *et al.* (2018) have clearly suggested that the variations in phenolic compounds in different honey were contributed by geographical and honey origin. In addition, the source of flower and climate change are the key contributors to the physicochemical properties of honey (Puškadija *et al.* 2007; Ulusoy *et al.* 2010; Mahmood 2018). In relation to previous studies, phenolic content can contribute to health benefits such as anticarcinogenic, anti-inflammatory, antiatherogenic, antithrombotic, immune-modulating and analgesic activities (Eteraf-Oskouei and Najafi, 2013). It can be concluded that honeybee honey in the current study has a higher potential for health benefits than stingless bee honey.

3.2 Total flavonoid content

The flavonoid contents in honey are partly obtained from the flavonoid in pollens, which comes from the hydrolysis of certain substances in the bee saliva (Oliveira *et al*., 2017). Moreover, high flavonoids compounds indicate a high level of antioxidants in honey (Khalil *et al*., 2010). The total flavonoid content of honeybee honey and stingless bee honey are shown in Table 1. In comparison with Malaysian honeybee honey, as reported in previous studies by Moniruzzaman *et al*.

(2013b), the total flavonoid content reported in the current study is a bit higher than that of the previous study with a value ranging from 21.95 – 37.39 mg CE/ kg. In addition, Ranneh *et al*. (2018) and Selvaraju *et al*. (2019) reported the total flavonoid contents of Malaysian stingless bee honey as ranging from 97.88 – 101.5 mg CE/kg and 10.18 – 12.68 mg CE/kg, respectively. In comparison with previous studies, the flavonoid contents of stingless bee honey in the current study are lower than the values reported by Ranneh *et al*. (2018) but higher than the values reported by Selvaraju *et al*. (2019). Oliveira *et al*. (2017) reported the total flavonoid content of Brazilian stingless bee honey ranged from 302.4 – 2797.3 mg QE/kg, which was higher compared to Malaysian stingless bee honey in the current study.

However, in comparison with the previous study for honeybee honey in Brazil, the flavonoid content in the current study is higher than the result reported. Kadri *et al*. (2017) previously reported that the flavonoid contents of *Apis mellifera* honey in Brazil ranged from 2.82 – 3.22 mg QE/kg (centrifuged) and 7.07 – 8.65 mg QE/kg (pressed). Their study showed that the extraction process can affect the phenolic content in honey, which might be due to more pollen present in pressed honey. In the present study, raw honeybee honey and stingless bee honey were processed using the dehydration method. Apart from processing methods, the flavonoid content in honey varies according to bee species and floral origin (Machado De-Melo *et al*., 2017; Oliveira *et al*., 2017; Chew *et al*., 2018). Commercial Mauritian honey studied by Aumeeruddy *et al*. (2019) reported total flavonoid content at 80.94±1.49 mg RE/g, notably higher than the total flavonoid content reported in the current study.

The presence of flavonoid content in both honey showed the potential antioxidant characteristics of the samples. Statistically, the values obtained are not significantly different, indicating that the total flavonoid contents of both kinds of honey are considered similar.

3.3 Free radical scavenging activity (DPPH)

The DPPH scavenging activity of honeybee honey and stingless bee honey are expressed in percentages, as shown in Table 2. The results indicated that DPPH % of honeybee honey and stingless bee honey reached 34.19% and 19.80%, respectively. Malaysian Acacia honey (honeybee honey) and Malaysian stingless bee honey from a previous study by Chua *et al*. (2013) and Selvaraju *et al*. (2019) reported a DPPH value of 29.98% and a range of 27.12 – 67.66%, respectively. Both studies agreed with the results obtained in the current study.

When compared to Malaysia Tualang honey from a previous study by Khalil *et al*. (2012), DPPH inhibition percentage activity was recorded at 34.93±2.44%, which is in agreement with the results obtained in the current study (34.19%). Can *et al*. (2015) reported values of 152.40±62.00% for Turkish Acacia honey, which are higher than those of the current study. Moreover, the study by Jimenez *et al*. (2016) reported DPPH scavenging activity of Mexican stingless bee honey ranged from 15.65 – 19.04%, which is in agreement with the result obtained for stingless bee honey in the current study (19.80%).

Table 2. Free radical scavenging activity in honeybee honey and stingless bee honey

Concentration (mg/mL)	% DPPH		
	Honeybee honey	Stingless bee honey	Quercetin
100.00	21.60±27.20[a]	10.46±6.09[a]	0.07±0.00[a]
50.00	13.50±5.42[a]	23.32±1.95[a]	0.06±0.00[b]
25.00	49.64±0.99[a]	51.74±0.47[a]	0.07±0.00[b]
12.50	65.20±0.13[a]	39.75±0.11[b]	0.06±0.01[c]
6.25	56.64±1.05[a]	22.25±0.73[b]	0.07±0.01[c]
3.12	33.00±6.97[a]	11.56±1.80[b]	0.06±0.00[b]
1.56	9.50±10.43[a]	7.30±1.90[a]	0.07±0.01[a]
0.78	10.55±5.48[a]	3.41±2.05[a]	0.07±0.00[a]
0.39	5.78±0.35[a]	2.55±0.24[b]	0.06±0.01[c]

Values are expressed as mean±SD of triplicate analysis. Values with different superscripts within the same row are significantly different by One-way ANOVA with post-hoc Tukey's test.

Based on previous studies, it is apparent that the DPPH inhibition varies not solely depending on the honey type. In some study, the DPPH inhibition value between honeybee honey and stingless honey is within close range. In contrast, this may vary greatly in another study. Chua *et al*. (2013) reported that relative to TPC, TFC of honey samples was strongly correlated with the anti-oxidative activity. However, as anti-oxidative activity depends on both TPC (Deng *et al*., 2018) and TFC (Khalil *et al*., 2010), it can be concluded that again geographical origin and harvesting season are important in determining honey characteristics. The higher inhibition of DPPH in stingless bee's honey may be associated with fermentation in the hive. This was explained by Menezes *et al.* (2013) who demonstrated the association of the lactic acid bacteria in the hive and antioxidant activity in the honey.

3.4 Hydroxymethylfurfural (HMF)

HMF content in honey can indicate the degree of degradation caused by strong and/or prolonged heat treatment and inadequate or prolonged storage conditions (Sakač *et al*., 2019). Kadri *et al*. (2017) also recorded

negative reading of HMF for the honey sample that had undergone the centrifugation process. Moreover, Biluca *et al*. (2014) reported stingless bee producing rare honey due to its exotic flavour and certain characteristics which when being introduced to elevated temperature it shows resistance to the formation of HMF. This may explain why the HMF level of stingless bee honey in the current study was undetectable. The HMF level for honeybee honey obtained in the current study was 83.4 mg/kg. According to Al-Ghamdi *et al*. (2017), the international level of HMF limit has been set at 40 mg/kg; however, for honey originating from tropical countries and which has undergone processing, this was set at a limit of 80 mg/kg. This indicates that the results obtained in the current study were a bit over the standard limit reported.

Honeybee honey in the current study has higher HMF levels in comparison with Acacia (honeybee) honey from Turkey (Can *et al*., 2015), Iran (Mahmood-Khaledi *et al*., 2016) and Serbia (Sakač *et al*., 2019) with HMF levels of 12.56±5.21 mg/kg, 5.68 – 42.16 mg/kg, and 0.44 to 8.2 mg/kg, respectively. This might be due to the honey used in the previous studies being raw or unprocessed. Differences in treatments can be considered as a factor contributing to the higher HMF level reported in the current study as compared to the previous studies. The high level of HMF reported in the current study was due to the treatment done on the sample prior to analysis. Shapla *et al*. (2018) reported that the external characteristics which can affect the composition of honey include the processing involved, storage time and conditions, and seasonal and environmental factors.

3.5 Diastase activity

Diastase is a starch-digesting enzyme which includes α- and β-amylase (Sak-Bosnar and Sakač, 2012) that can be found in honey. When heated or stored, the enzyme can be denatured (Wanjai *et al*., 2012). Schade's method is based on the diastatic degradation of blue coloured starch-triiodide complex, in which the colour decreases and is measured using a spectrophotometer (Sak-Bosnar and Sakač, 2012). Diastase activity is shown in Table 3; however, it was undetectable in the honeybee honey samples. A previous study by Suntiparapop *et al*. (2012) also reported undetectable diastase activity in Thailand stingless bee honey.

Table 3. Characteristics of processed honeybee honey and processed stingless bee honey

Characteristics	Processed HBH	Processed SBH
HMF, (mg/kg)	83.4	Undetectable
Diastase, DN	Undetectable	5.1
IC_{50} (mg/mL)	13.75	Undetectable

Moussa *et al*. (2012) reported that factors that can decrease the diastase activity of honey are storage, the feeding of honeybees during honey flow, and heat treatment. In addition, Wanjai *et al*. (2012) also reported that diastase can be denatured after storage and heat treatment. Moreover, Wetwitayaklung *et al*. (2018) reported that a low level of diastase suggested that the sample might be inappropriately stored or processed during harvesting and also it might be due to the high ambient temperature as high temperature can increase HMF levels and decrease diastase activity. In the present study, samples were stored in a 4°C refrigerator until analysis.

Meanwhile, for stingless bee honey, the diastase number obtained was 5.1, as shown in Table 3. The results obtained are in agreement with a previous study by Nordin *et al*. (2018) who reviewed the physicochemical of stingless bee honey species which include the diastase activity with the range of 1.3 to 15.63. This showed that the diastase number of stingless bee honey in the current study is within the range reported by Nordin *et al*. (2018). Codex Alimentarius (2001) reported that diastase activity of honey that was determined after processing and/or blending is not less than 8 Schade units, while honey with low natural enzyme is not less than 3 Schade units.

In comparison to other types of honey, the diastase activity of stingless bee honey in the current study is in contrast with the diastase activity of honey reported by Morales *et al*. (2012) which ranged from 7.3 to 22.1 Schade units. Moreover, the study by Can *et al*. (2015) reported 12.60±1.07 for diastase of Acacia (honeybee) Turkish honey. The diastase activity of stingless bee honey in the current study is considered acceptable under the limit set by Codex Alimentarius (2001) of not less than 3 DN as this honey is considered a low natural enzyme. Meanwhile, the diastase activity of honeybee honey was undetectable.

3.6 MTT

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is a colourimetric cytotoxicity assay which indirectly measures the cell present through cellular metabolism (Abel and Baird, 2018). The viability was measured by the amount of blue formazan formed after the addition of MTT onto each cell, indicating the number of viable cells in the test samples (Nordin *et al*., 2018).

The present study found that the concentration of honeybee honey required for 50% cell inhibition was at 13.75 mg/mL. However, the concentrations of stingless bee honey used were unable to inhibit the proliferation of HeLa cell lines. This showed that a lower concentration

of honeybee honey was needed for 50% of HeLa cell inhibition. A previous study by Mohd Salleh *et al*. (2017) recorded IC_{50} at 13.09±2.43 mg/mL for inhibition of Malaysian Acacia honey with MCF-7 cell line, which is in agreement with the results obtained in the current study (13.75 mg/mL).

A study by Zarrinnahad *et al*. (2018) demonstrated the melittin inhibition on HeLa cell proliferation at IC_{50} of 2, 1.7 and 1.8 µg/mL at 6, 12 and 24 hrs of incubation, respectively. Abel and Baird (2018) demonstrated the cytotoxicity of thyme honey with prostate cancer cell with a value of 39.9±10.2% for the PC3 cell line and 64.9±5.8% for the DU145 cell line. The cytotoxicity of honey against cell cancer has been reported by previous studies, but no specificity of honey against cancer cell can be concluded (Abel and Baird, 2018).

Aumeeruddy *et al*. (2019) demonstrated non-correlation between total phenolic content, antioxidant activity and anticancer activity. Raw Mauritian Eucalyptus honey and commercial honey were used in their study. Lower phenolic content has been reported in Mauritian Eucalyptus honey as compared to the commercial honey, but only Mauritian Eucalyptus honey showed anticancer activity against MCF7 cell lines (Aumeeruddy *et al*. 2019). However, Portokalakis *et al*. (2016) demonstrated a high correlation of cytotoxicity of Manuka honey with its total phenolic content ($R^2 = 0.99$) and antioxidant power ($R^2 = 0.95$).

4. Conclusion

The total phenolic content of honeybee honey was significantly higher than stingless bee honey; however, there were no significant differences in total flavonoid content. Different concentrations of honey exhibit different percentages of DPPH activity. In general, the present study showed that processed honeybee honey demonstrated better antioxidant activity based on TPC, TFC and DPPH analyses. These results correspond to its anticancer potential towards HeLa cells. Interestingly, processed stingless bee honey showed better honey quality according to HMF and diastase activity.

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