**Food Research 5 (2) : 498 - 507 (April 2021)**
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

# Antioxidant and antimicrobial properties of Indo-Malayan stingless bee (*Heterotrigona itama*) honey from different seasons and distribution of flowers

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***Article history:***
Received: 28 September 2020
Received in revised form: 12 November 2020
Accepted: 18 January 2021
Available Online: 30 April 2021

***Keywords:***
Honey,
Stingless bee,
Antioxidant,
Antimicrobial,
Seasons,
Flowers

***DOI:***
https://doi.org/10.26656/fr.2017.5(2).546

**Abstract**

The quality of honey produced by stingless bees can be affected by a variety of factors. Different seasons may have an influence on stingless bee behaviour while different flower sources may affect the properties of stingless bee honey. Thus, this study aimed to determine the antioxidant and antimicrobial properties of stingless bee honey from different multifloral sources collected during dry and rainy seasons. Honey was collected from hives placed in two different areas with multifloral (area A and B). The difference between these areas was area A only planted with two types of flowers (with stevia) and area B with more than two flowers. Pollen in the honey samples was identified to confirm the seasonal variations. The antioxidative potential of the honey samples was determined using total phenolic content (TPC), DPPH and FRAP assays and total carotenoid content (TCC). Antimicrobial properties were analysed using well diffusion method (mm) against the foodborne pathogens *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium. The results revealed that honey harvested during the dry season showed higher antioxidant properties compared to those harvested in the rainy season. The highest antioxidant activities were measured in honey samples taken from area B. Nevertheless, honey harvested in both seasons showed antimicrobial properties against all tested food pathogens, with the dry season samples showing the highest inhibition. Surprisingly, only honey from area A present showed no antimicrobial effect against *E. coli*. This study showed that the antioxidant and antimicrobial properties of multifloral stingless bee honey are strongly affected by seasonal differences and the distribution of the flowering plant.

## 1. Introduction

Throughout history, honey has always been described as a natural product with great importance for human health. It has good culinary qualities and also contains remarkable nutritional and therapeutic properties (Siddiqui *et al*., 2016; Marfo *et al.*, 2016; Biluca *et al.,* 2020; Keng *et al.,* 2017). The chemical and nutritional properties of stingless bee honey differ significantly from honey produced by *Apis mellifera* (Duarte *et al*., 2012; Menezes *et al.*, 2013). Honey produced by stingless bees has a lower viscosity, darker colour and stronger acid taste compared to honey from *Apis* spp. (Garedew *et al*., 2003). The composition and properties of honey are strongly affected by the bee species, edaphoclimatic conditions and pollen source (Gheldof and Engeseth, 2002). Ávila *et al*. (2019) has concluded stingless bee honey can be clustered according to the source of pollen. Hence, designing the source of pollen has become a new method in stingless beekeeping to produce stingless bee honey with different properties. One of the potential pollen sources may come from the stevia plant (*Stevia rebaudiana*), a non-caloric natural sweetener (Kąkol *et al*., 2012). Our observations indicated that stingless bees foraged on the pollens of

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eISSN: 2550-2166 

stevia plants. Stevia plants contain stevioside and rebaudioside A (Abou-Arab *et al.,* 2010; Halim *et al.,* 2019). The effects of pollen from stevia plants on the properties of honey are still unclear and need to be explored. It may be possible that such honey may also contain these compounds which need to be elucidated. A study by Razak *et al.* (2019) has also found the stevioside in stingless bee honey collected from plant area with stevia.

Seasonal variation has been indicated as one of the major factors that affect the properties of honey. Nascimento and Nascimento (2012) and Oyerinde *et al.* (2014) showed that the rainy season not only negatively affect honey yields but also may affect the properties of the honey itself. As the Malaysian state of Terengganu is well-known for its tropical monsoon climate, which consistently occurs from November until March (Sang *et al*., 2016), information on the effect of this climate on the quality of the harvested honey is much needed.

Honey from a stingless bee was reported to have antimicrobial and antioxidant properties (Ahmad *et al.,* 2019; Biluca *et al.* 2020) and is suggested as an alternative medicine to help in wound healing (Al-Mamary *et al*., 2002; Mulu *et al*., 2004). Previous studies have shown that the antibacterial and antifungal properties of stingless bee honey are contributed by the low pH, osmotic pressure (Mandal and Mandal, 2011; Zainol *et al*., 2013; Garedew *et al*. 2003; Hasali *et al.,* 2015; Ahmad *et al.* 2019), total phenolic and flavonoid content present in the honey (Khalil *et al.,* 2011; Iurlina *et al.,* 2019). Moreover, Hasali *et al*. (2015) stated that the antibacterial and antifungal properties of stingless bee honey have originated from the presence of lactic acid bacteria (LAB) strains.

Furthermore, the pollen source has also been indicated to influence the antioxidant properties of honey (Moniruzzaman *et al*., 2013). Thus, apart from studying the antioxidant properties of stingless bee honey, the present research is also intended to study the antimicrobial properties of multifloral honey produced by stingless bees that gathered pollen in areas with and without stevia plants.

## 2. Materials and methods

### *2.1 Stingless bee honey samples*

Fresh honey from stingless bee (*Heterotrigona itama*) with different pollen sources was collected from two different sites (with and without stevia) at Pusat Tunas Stevia (PTS) plant nursery, Besut, Terengganu during the dry (April to July 2017) and rainy (September to November 2017) seasons. Sterile pipettes and glass bottles were used for the honey sampling process. Extracted honey samples were placed in sterile, enclosed sterilized opaque glass containers wrapped with aluminium. Samples were then stored at 4$^{o}$C until further analysis.

### *2.2 Dry and rainy season identification*

Sample collection of stingless bee honey involved two phases, which were the dry and rainy seasons. Hygrometers were placed at both sampling sites few days before sample collection according to the method of de Figueiredo-Mecca *et al*. (2013), Nascimento *et al*. (2012) and Puškadija *et al*. (2007). Relative humidity and temperature were recorded at 1-hour interval for 3 hrs. The data collected was compared with data from the Terengganu Meteorological Department, Malaysia to determine the season.

### *2.3 Pollen identification*

The main objective of pollen identification is to determine the pollen distribution of stingless bee (*Heterotrigona itama*) honey samples. The multifloral source of stingless bee honey was analysed using the pollen identification method by Marcos *et al.* (2015) with some modifications. In this method, five stingless bees with pollen on their hind tibia were captured per area for 6 days and then kept in separate glass jars. Pollens were collected from the stingless bees' hind tibia using a needle and then suspended in 1 mL of distilled water. One drop of suspended pollen was been placed onto a glass slide and covered with a coverslip. The pollens were observed under 100× magnification using a light microscope. Photos of the pollens were taken and used for visual identification.

### *2.4 Total phenolic content (TPC)*

The total phenol (TPC) content test was determined using a modified version of Folin-Ciocalteu's phenol reagent (Singleton *et al*., 1999). Samples of 1 gram of stingless bee honey were weighed in test tubes wrapped with aluminium foil and were diluted with 10 mL of distilled water. One millilitre of diluted honey samples was transferred using a micropipette and mixed with 5 mL of 10% (v/v) Folin-Ciocalteu's reagent and incubated for 5 mins at room temperature (25±2°C). After 5 mins, 4 mL of 75% w/v aqueous sodium carbonate solution was added and the mixture was further incubated for 2 hrs. The absorbances of the samples were then measured at 765 nm using a UV-Vis (double beam) spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan). The TPC of each sample was reported as the mean value of triplicate assays and expressed in mg gallic acid equivalents (GAE)/kg of

honey sample.

*2.5 Total carotenoid content*

The total carotenoid content of the honey samples was determined as previously reported by Ferreira *et al*. (2009) using ß-carotene as the standard (Boussaid *et al*., 2014). The samples were mixed with an acetone-hexane mixture (6:4) and shaken vigorously at room temperature. The mixture was measured at 450 nm using a UV-Vis (single beam) spectrophotometer (Spectroquant Pharo 300, USA).

*2.6 Free radical scavenging ability*

The free radical scavenging activity of the honey samples was determined using the 1,1- diphenyl-2-picrylhydrazyl (DPPH) assay (Boussaid *et al*. 2014). Samples were added with the DPPH and then incubated for 60 min at room temp (25±2°C). The absorbance of the samples and control were measured at 517 nm with a UV-Vis spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan). The radical inhibition measurements were expressed as a percentage (%) of DPPH inhibition (Boussaid *et al*., 2014).

*2.7 Ferric reducing antioxidant power (FRAP)*

The reducing power of the honey samples were determined based on the method described by Benzie and Strain (1996). The FRAP reagent was prepared by mixing 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution with 300 mM acetate buffer (pH 3.6) and 20 mM iron (III) chloride hexahydrate. An aliquot of 200 µL of honey solution was mixed with 1.5 mL of FRAP reagent, and incubated at 37°C in a water bath for 4 mins. The absorbance of the mixture was then measured at 593 nm (distilled water as blank) using a UV- Vis spectrophotometer and compared with ferrous sulfate ($FeSO_4$) as the standard.

*2.8 Antimicrobial activity using well-diffusion method*

The effects of different pollen sources and seasons towards the antimicrobial activity of stingless bee honey (*Heterotrigona itama*) were determined using the well-diffusion method on Mueller Hilton agar against *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 13932)., *Salmonella enterica* serovar Typhimurium (ATCC 13311) and *Staphylococcus aureus* (ATCC 6538). Suspensions of 24 hrs cultures of food-borne pathogenic bacteria were prepared using saline water and the turbidity was ensured to be equivalent with 0.5 McFarland. Suspensions (100 µL) made were spread onto Mueller Hilton agar plates and then, four wells were made on the agar plates using the back of sterile tips (Ewnetu *et al*., 2013). Then, 100 µL of each sample was added to the wells. All of the plates were then incubated at 37°C for 24 hrs in an incubator and the diameter of bacterial growth was inhibition was measured.

*2.9 Statistical Analysis*

All analyses were carried out in triplicate for each sample and the experimental results were expressed in mean value ± standard deviation. Significant analysis of variance ($p < 0.05$) was performed using the Minitab statistical software (version 14).

## 3. Results and discussion

*3.1 Pollen identification from hind tibia*

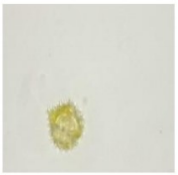
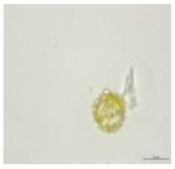
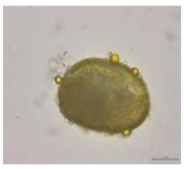
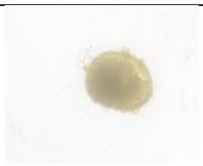
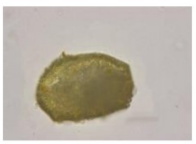
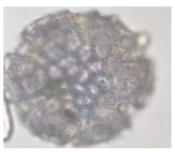
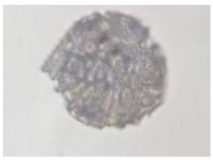
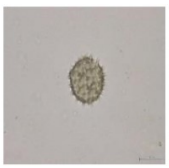
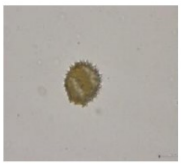
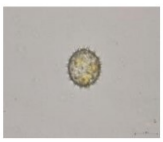
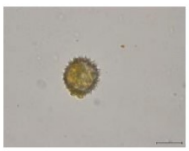
Pollens collected from stingless bee-hind tibia were compared with the reference flowers under 100X magnification using a light microscope (Table 1) for identification. The data obtained from pollen identification is important to determine the differences between the multifloral honey samples. The data obtained clearly showed that the type of pollen collected depends on the species of flowering plants surrounding the nest.

*3.2 Pollen distribution analysis*

The distribution of pollens foraged by stingless bees varies according to the available flower source and preference by the stingless bee itself. This preferential behaviour is normally done by the scouts of stingless bees which will identify high-quality pollen before signalling to the foraging stingless bees to forage for the pollen (Real, 2012). Stingless bees identify the types of flower based on the colour of pollen and also scent (Harder *et al.,* 2004). Previous studies (Steven *et al.,* 2003; Harder *et al.,* 2004) discovered that flower characteristics such as the colour of petals colour, the colour of pollen and the size of petals influence the foraging behaviour of stingless bees. The pollen distribution analysis in the present study showed different types of pollens foraged by stingless bees at nests in both areas (A and B; Figure 1). Only two plant species were identified from the pollen collected from stingless bees in area A. The majority of the pollen was from the flowers of *Antigonon leptopus* (53%) while the remainder was from *S. rebaudiana* (47%). *A leptopus* is a flowering plant that grows with a vine structure. Stingless bees are attracted to *A. leptopus* which have smaller and more attractive pollen and petal colour compared to the white flowers produced by *S. rebaudiana*. Meanwhile, there were more (four) plant species identified from the pollen on stingless bees in the area B, which were *A. leptopus*, *Biden pilosa*, *Caudatus sulphureus* and *Orthosiphon stamineus*. *A. leptopus*

Table 1. Pollen identification from stingless bee (*H. itama*) hind tibia collected from area A and B

| Area | Reference flower | | Pollen from reference flower under 100× magnification | Pollen from stingless bee-hind tibia under 100× magnification |
|---|---|---|---|---|
| A | *Stevia rebaudiana* |  |  |  |
| | *Antigonon leptopus* |  |  |  |
| B | *Antigonon leptopus* |  |  |  |
| | *Orthosiphon stamineus* |  |  |  |
| | *Biden pilosa* |  |  |  |
| | *Caudatus sulphureus* |  |  |  |

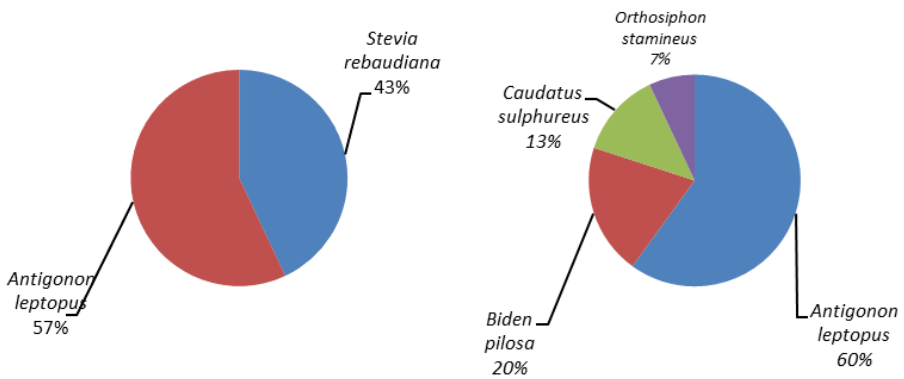



Figure 1. Pollen distribution of stingless bee nest surrounded with multifloral in area A and B for 6 days

dominated the total pollen distribution (60%) compared to other plant species.

### *3.3 Effect of environmental conditions and pollen distribution on antioxidant and antimicrobial properties of stingless bee honey*

These analyses were done to study the relationship between environmental conditions and pollen distribution on the antioxidant and antimicrobial properties of stingless bee honey. The environmental conditions of the selected area were determined based on the temperature and relative humidity. The highest recorded relative humidity (%) was at 84.30% during the rainy season while the lowest was at 51.67 % during the dry season (Figure 2) and the temperature was in the range of 28 - 31°C and 21 - 22°C for dry and rainy seasons, respectively (Figure 3). The increase in the percentage of relative humidity was because of the rainfall. The relative humidity during the rainy season was within the range as reported by the Malaysian Meteorological Department which ranged from 72% to 90% (Malaysian Meteorological Department, 2009).

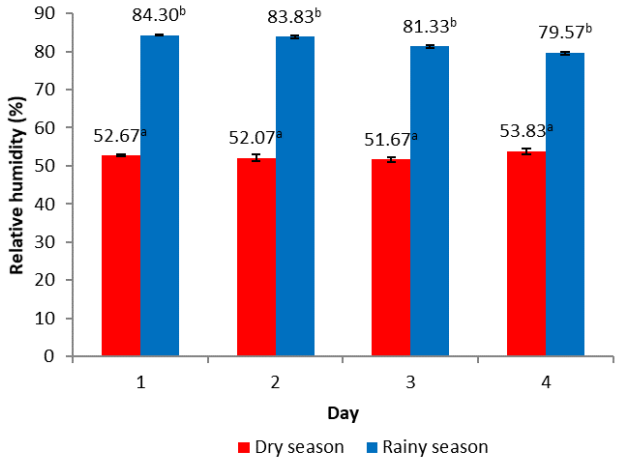



Figure 2. Relative humidity (%) for dry and rainy season at Kampung Tempinis, Jertih.
*Data are mean of triplicates (n = 3) with error bar indicating standard deviation. Mean values with different letters indicate significant difference ($p < 0.05$) between seasons.

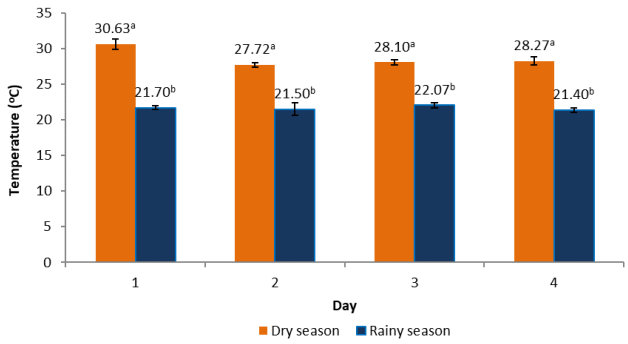



Figure 3. Temperature for dry and rainy season at Kampung Tempinis, Jertih
* Data are mean of triplicates (n = 3) with error bar indicating standard deviation. Mean values with different letters indicate significant difference ($p < 0.05$) between seasons.

From the results obtained, there were significant differences ($p < 0.05$) in the antioxidant of stingless bee honey obtained during the dry and rainy season from different flower sources (Table 2). The total phenolic content (TPC), DPPH and FRAP scavenging activity (%) in multifloral stingless bee honey from area B were higher compared to the honey collected in area A in both seasons ($p < 0.05$), where the highest was during the dry season. Compounds that may have contributed towards the antioxidant activity were phenols, catalase, glucose oxidase, phenolic acids, ascorbic acids, flavonoids, carotenoids derivatives, organic acids, Maillard reaction products, and amino acids (Alzahrani *et al*., 2012). The lower antioxidant activity in honey samples during the rainy season may be due to the negative effect of the season on foraging activity and pollen viability (Puškadija *et al*., 2007). In rainy seasons, strong winds reaching 10 to 30 knots (Abdullahi *et al.*, 2014) can cause pollens to be blown away while also disturbing the foraging activity of stingless bees (Puškadija *et al*., 2007). During the rainy seasons, when the food source is insufficient, stingless bees will forage pollen in the range of only 20 meters around the nest (Samsudin, 2016) and some of the foragers will just remain in the hive until the rains stop (Jaapar *et al.* 2018). During the dry season, a large forager bee can forage up to 2100 meters (Kuhn-Neto *et al.,* 2009). Besides, the presence of water vapour during the rainy season also gives a negative effect on the pollen and the quality of the stingless bee honey. The effects of relative humidity and temperature on the distribution of pollen was clearly explained by Aronne (1999) and subsequently affected the bee foraging activity. Puškadija *et al*. (2007) observed the most intensive bee activity occurred at a humidity of 40 to 50% while a higher relative humidity (>70%) reduced the bee scavenging activity down to 0%. These suggest that the rainy season reduce the antioxidant properties and the yield of the honey. Observation of stingless bee honey from both seasons also showed differences in the volume of yield and intensity of colour. Honey collected during the dry season was darker and of higher yield compared to those from the rainy season.

Other than the season, the high antioxidant properties of multifloral stingless bee honey samples from area B were clearly due to the higher variety, quantity and quality of flowers surrounding the nest. The plant species identified from the pollen on stingless bees in the area without stevia plants (Figure 1, Area B) may have contributed to the high antioxidant activity in the honey. Previous studies reported that alkaloids, flavonoids, tannins, terpenoids, saponins and phlobatanin in *A. leptopus* (Govindappa, 2015; Pradhan and Bhatnagar, 2016) are well-known to have potential medicinal and therapeutic properties (Kennedy and

Table 2. Antioxidant properties of stingless bees from different flower sources and seasons

| | Dry Season | | Rainy Season | |
|---|---|---|---|---|
| | A | B | A | B |
| TPC (mg GAE/ g) | $13.31\pm0.52^{aA}$ | $23.79\pm0.16^{aB}$ | $6.16\pm0.31^{bA}$ | $9.38\pm0.53^{bB}$ |
| DPPH (%) | $72.12\pm0.68^{aA}$ | $86.03\pm0.91^{aB}$ | $32.77\pm0.80^{bA}$ | $50.99\pm0.50^{bB}$ |
| FRAP (μg Fe(II)/ Kg) | $112.57\pm0.49^{aA}$ | $153.26\pm0.93^{aB}$ | $42.31\pm0.35^{bA}$ | $90.64\pm0.75^{bB}$ |
| Carotenoid (μg of β- carotene/mL) | $0.13\pm0.01^{aA}$ | $0.25\pm0.1^{aB}$ | $0.05\pm0.006^{bA}$ | $0.12\pm0.01^{bB}$ |

A – Multifloral stingless bee honey from area A, B – Multifloral stingless bee honey from area B. Values are expressed as mean±SD from three independent replicates of each sample. Values with a different lowercase superscript are significantly different ($p < 0.05$) between seasons while values with a different uppercase superscript are significantly different ($p < 0.05$) between flower source.

Wightman, 2011). In another study by Bartolome *et al*. (2013), the specific phytochemicals and their classes (in parentheses) that were able to be identified with antioxidant properties in *B. pilosa* were 3,5-Di-O-caffeoylquinic acid (phenylpropanoid:), 3-O-Rabinobioside (saccharide), quercetin 3-O-rutinoside and jacein (flavonoids); and chlorogenic acid (phenolic compound). *C. sulphureus* also contains phytochemical substances that contributed to the antioxidant properties of stingless bee honey. Similarly, Ameer *et al.* (2012) also identified classes of flavonoids, polyphenols, diterpenes and terpenoids present in *O. tamineus*.

Table 2 also shows a similar trend where multifloral stingless bee honey samples from area B that were collected during the dry season showed the highest total carotenoid content (0.25 μg/mL) compared to other samples. The difference in total carotenoid content in honey is strongly influenced by the flower sources. This can be seen in a study by Boussaid *et al*. (2014) who showed that honey from citrus flowers having had the highest total carotenoid content (4.72 mg/kg) while the lowest content (1.16 mg/kg) was found in honey from rosemary flower. Meanwhile, a previous study by Jimenez *et al*. (2016) on *Scaptotrigona mexicana* honey showed a very low total carotenoid content of 0.56 mg/ kg.

The antimicrobial activities of multifloral stingless bee honey from both areas against four pathogenic bacteria are shown in Table 3 for both seasons. As can be seen, there were significant differences ($p < 0.05$) in the antimicrobial activities of the honey samples from the dry and rainy seasons. Multifloral stingless bee honey samples from both areas during the dry season showed higher inhibition compared to the rainy season. Positive inhibition results against *L. monocytogenes* can be seen in the result and again, the larger inhibition was shown by honey from the dry season. Amongst the four pathogenic bacteria, the largest inhibition zone was shown against *S. enterica* ser. Typhimurium while the lowest was *E. coli*. The relatively small inhibition zone showed that *E. coli* was able to withstand the low pH and other non-peroxide antimicrobial properties of the multifloral stingless bee honey. A previous study by Ulusoy *et al*. (2010) showed a similar inhibitory trend where honey samples bees honey from different flower sources have smaller inhibition zones against *E. coli* compared to other pathogenic bacteria. However, the present study found that the multifloral stingless bee honey from area B had a larger inhibition zone against *E. coli* at 11.33 mm compared to honey samples from a previous study by Ulusoy *et al.* (2010) where the inhibition was only at 6 mm and 8 mm for honey from monofloral (lime flower) and multifloral (rhododendron, chestnut and lime), respectively.

Multifloral stingless bee honey samples from both areas during the dry and rainy seasons were able to inhibit *S. aureus* higher than those collected during the rainy season. A study by Ulusoy *et al.* (2010) showed no inhibition of honey from the lime flower, rhododendron and chestnut against *S. aureus*. This shows that stingless bee honey has a stronger antimicrobial activity compared to honey produced by *Apis* spp. In another study by Zainol *et al.* (2013), the inhibition of Gelam and Tualang honey against the *S. aureus* were 15.52 and 16.34 mm respectively, which were smaller in comparison to the result in the present study (19.33 mm).

Table 3 also shows positive inhibition result against *L. monocytogenes* and again, the larger inhibition was shown by honey from the dry season. Amongst the four pathogenic bacteria, the largest inhibition zone was shown against *S. enterica* ser. Typhimurium. The large inhibition zone of stingless bee honey against *S. enterica* ser. Typhimurium may be due to the optimum pH growth of *S. enterica* ser. Typhimurium being pH 7-7.5 (Podolak *et al*., 2010) which was not compatible with the much lower pH of the stingless bee honey.

Overall, this study showed a trend where the higher antioxidant values in honey exhibited the larger antimicrobial inhibition zones, and this observation is in agreement with a previous study by Ulusoy *et al*. (2010). Multifloral stingless bee honey samples from both areas during the rainy season showed a lower inhibition zone

Table 3. Diameter of inhibition zone in (mm) of stingless bee A and B from dry and rainy seasons against four pathogenic bacteria

| Pathogenic bacteria | Diameter of inhibition zone (mm) | | | |
|---|---|---|---|---|
| | Dry Season | | Rainy season | |
| | A | B | A | B |
| *Escherichia coli* | 8.67±0.58[a] | 11.33±0.58[a] | 0.00[b] | 7.33±0.58[b] |
| *Staphylococcus aureus* | 16.33±1.15[a] | 19.33±1.15[a] | 7.67±0.58[b] | 11.33±0.58[b] |
| *Listeria monocytogenes* | 11.33±0.58[a] | 14.67±0.58[a] | 5.67±0.58[b] | 8.33±0.58[b] |
| *Salmonella enterica ser.* Typhimurium | 28.00±1.00[a] | 32.33±0.58[a] | 13.67±1.15[b] | 17.33±0.58[b] |

A – Multifloral stingless bee honey from area A, B – Multifloral stingless bee honey from area B. Values are expressed as mean±SD from three independent replicates of each sample. Values with a different lowercase superscript are significantly different ($p < 0.05$) between seasons while values with a different uppercase superscript are significantly different ($p < 0.05$) between flower source.

against all of the tested bacteria compared to honey samples collected during the dry season. As previously discussed, the rainy season reduced the foraging activity of stingless bees and increased environmental humidity which negatively impacted the antimicrobial activity of stingless bee honey. The increase in humidity will also influence the pH of stingless bee honey produced in the nest. Bacteria are sensitive to the hydrogen ion concentration in their environment thus, pH plays an important role in the inhibition of bacteria. Other compounds that contribute towards the antimicrobial activity of stingless bee honey are phytochemical factors such as phenolic compounds, flavonoids, antibacterial peptides, methylglyoxal, methyl syringate, antibiotic-like derivatives and other components present in trace amounts which are classified as non-peroxide antibacterial factors (Mandal and Mandal, 2011). Plants produce a great number of secondary metabolites that have an antimicrobial activity which also contributes to the antimicrobial properties of honey (Cos *et al*., 2006) and as discussed, all of the identified plants in the study have this property. Although the multifloral honey sample from area A which planted with stevia plants showed lower antioxidant and antimicrobial activities, data from our preliminary study tested on all of the samples (unpublished data) indicated the presence of beneficial sweeteners from stevia plants (stevioside and rebaudioside A) in the honey which could potentially give additional value to the honey. A similar result was also shown by a study on stingless bees honey as reported by Razak *et al.* (2019).

## 4. Conclusion

In conclusion, different flower sources and seasons influenced the antioxidant and antimicrobial properties of multifloral stingless bee honey. The rainy season was proven to reduce antioxidant and antimicrobial properties. Meanwhile, higher flower variety and quantity increased antioxidant and antimicrobial properties. Pollination with the presence of stevia plants may increase other beneficial properties derived from the plant. The differences in antioxidant and antimicrobial activity between the two honey samples strongly showed the potential relationship between the availability of flower sources and its' effect on the antioxidant and antimicrobial activity of the stingless bee honey itself.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Acknowledgements

The authors would like to thank Universiti Malaysia Terengganu for all of the funds and support for the research and Pusat Tunas Stevia (PTS) for the contribution of samples.

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