

## Analysis of Antioxidant Properties and Volatile Compounds of Honeys from Different Botanical and Geographical Origins

(Analisis Antioksidan dan Sebatian Meruap Madu daripada Asal Usul Botani dan Geografi yang Berbeza)

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Received: 5 May 2021/Accepted: 20 August 2021

### ABSTRACT

Honey has been consumed since ancient time due to its nutritional and therapeutic values. Studies showed that honey possesses antioxidant properties which can inhibit oxidation and cell damage in the body. However, the chemical contents and antioxidant properties of honeys are varied, depending on botanical and geographical origins of honey. In this study, we analysed the total phenolic content (TPC), total flavonoid content (TFC), antioxidant properties (DPPH, ABTS, FRAP and TAOC) and volatile profiles of several commercial honeys originated from Malaysia, Turkey, and Yemen. The results showed that sample H4 (Pine honey) from Turkey was the highest in TPC (0.84 µg GAE/mg honey), ABTS (63.15% inhibition) and FRAP (0.45 µg FeSO<sub>4</sub> equivalent/mg honey) values, while H2 (Acacia honey) from Malaysia showed the highest values in TFC (0.11 µg quercetin equivalent/mg honey) and DPPH (45.13 mg/mL IC<sub>50</sub>). Meanwhile, H5 (Marai honey) from Yemen recorded the highest TAOC value (24.14 µg ascorbic acid equivalent/mg honey). Twenty-four volatile compounds were identified using gas chromatography-mass spectrometry (GC-MS), among others are 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl, linoleic acid ethyl ester, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, and 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one which contribute to chemical characteristics of certain honeys. In regards to the TPC, TFC, and antioxidant assays, the honey samples were ranked based on the chemical properties level as follows: H4 (Pine honey) > H2 (Acacia honey) > H7 (*Kelulut 2*) > H3 (*Kelulut 1*) > H6 (Sumar honey) > H1 (*Tualang* honey) > H5 (Marai honey). This finding increases the knowledge of the chemical compositions, volatile compounds, and antioxidant activities of several commercial honeys derived from different botanical and geographical origins.

Keywords: Antioxidant properties; flavonoids; honeybee; phenolics; stingless bee; volatile compounds

### ABSTRAK

Madu telah digunakan sejak zaman dahulu disebabkan nilai nutrisi dan terapeutiknya. Kajian menunjukkan bahawa madu mempunyai kandungan antioksidan yang boleh menghalang pengoksidaan dan kerosakan sel dalam badan. Walau bagaimanapun, kandungan kimia dan antioksidan madu adalah berbeza-beza, bergantung kepada punca botani dan geografi madu tersebut. Dalam kajian ini, kami telah menganalisis jumlah kandungan fenol (TPC), jumlah kandungan flavonoid (TFC), sifat antioksidan (DPPH, ABTS, FRAP dan TAOC) dan profil sebatian meruap daripada beberapa madu komersial yang berasal dari Malaysia, Turki dan Yaman. Keputusan kajian menunjukkan bahawa H4 (madu Pain) dari Turki adalah paling tinggi bagi TPC (0.84 µg GAE/mg madu), ABTS (63.15% penghambatan) dan FRAP (0.45 µg FeSO<sub>4</sub>/mg madu), manakala H2 (madu Akasia) dari Malaysia menunjukkan nilai tertinggi dalam TFC (0.11 µg kuersetin/mg madu) dan DPPH (45.13 mg/mL IC<sub>50</sub>). Sementara itu, H5 (madu Marai) dari Yaman mencatatkan nilai TAOC tertinggi (24.14 µg asid askorbik/mg madu). Dua puluh empat sebatian meruap telah dikenal pasti menggunakan kromatografi gas-spektrometri jisim (GC-MS), antara lain adalah 4H-piran-4-one, 2,3-dihidro-3,5-dihidroksi-6-metil, asid linolik etil

ester, 2,5-dimetil-4-hidroksi-3(2H)-furanon, dan 2,4-dihidroksi-2,5-dimetil-3(2H)-furan-3-one yang menyumbang kepada ciri-ciri kimia bagi madu-madu tertentu. Berdasarkan kepada TPC, TFC dan ujian antioksidan, sampel madu disusun mengikut tahap sifat kimia seperti berikut: H4 (madu Pain) > H2 (madu Akasia) > H7 (Kelulut 2) > H3 (Kelulut 1) > H6 (madu Sumar) > H1 (madu Tualang) > H5 (madu Marai). Hasil kajian ini dapat menambah pengetahuan tentang komposisi kimia, sebatian meruap dan aktiviti antioksidan bagi beberapa madu komersial yang berasal daripada punca botani dan geografi yang berbeza.

Kata kunci: Fenol; flavonoid; kelulut; lebah madu; sebatian meruap; sifat antioksidan

## INTRODUCTION

Honey is a natural sweet substance produced by both honeybees and stingless bees. There are 10 species of honeybees which belongs to the genus *Apis* (Gupta et al. 2014), and over 500 species of stingless bee have been identified worldwide (Michener 2013). Honey produced by stingless bees is different from that honeybee's honey in terms of its colour, taste, and viscosity. Bees collect nectar from plants, then transform and combine it with their own substances and store it in honey comb till ripen and mature (Biluca et al. 2017). Based on the floral source of nectar, honeys can be classified into monofloral or polyfloral origin. Monofloral honey is predominantly derived from one plant species but minor nectar can also be derived from other plants. Besides, polyfloral honey involves several sources of plant species and none of which is predominant (Gašić et al. 2014).

The use of honey by mankind can be traced back from 8,000 years ago as pictured by a cave painting in Valencia, Spain (Purbafrani et al. 2014). The ancient civilizations have been reported to utilize honey in various applications. For instance, the ancient Vedic civilization used honey for treating insomnia, cough, skin disorder such as wound and burns, eye ailment to prevent cataract, and for keeping healthy teeth and gum (Eteraf-Oskouei & Najafi 2013). In ancient Egypt, honey was used to heal burn together with Aloe vera and tannic acid as prescribed in the Smith papyrus, an Egyptian text dated back between 2600 and 2200 B.C. (Pećanac et al. 2013). Meanwhile, the ancient Greeks believed that consumption of honey could help one to live longer (Arawwawala & Hewageegana 2017). In the Islamic medicine, honey is considered as a healthy food since the holy Quran mentioned about its potential therapeutic values. The great Muslim scientist and physician, Avicenna had recommended honey as one of the remedies to treat tuberculosis (Asadi-Pooya et al. 2003).

The therapeutic values of honey have been contributed by its phenolic compounds (Cianciosi et al. 2018). These compounds are responsible for the antioxidant property of honey with their ability to scavenge

free radicals. Excessive free radical in cellular system can cause oxidative damage to the cells and break down the essential macromolecules such as nucleic and amino acids, protein, and lipid. This may lead to the development of diseases such as cancer, metabolic disorders, and cardiovascular dysfunction (Rahal et al. 2014). The composition of phenolic compounds in honey depends on the source of the floral origin. Phenolic compounds and other bioactive substances are transferred from plant's nectar to honey as a final product, making its composition are highly determined by the floral origin (Kaškonienė & Venskutonis 2010). Moreover, honey composition is influenced by seasonal and environmental factors in the geographical area, as well as the processing method of honey (Manyi-Loh et al. 2011).

The source of floral origin mainly determines the diversity of compounds found in honey. Therefore, the presence of certain volatile compounds could be used as floral markers to differentiate certain monofloral honeys. Pattamayutanon et al. (2017) have listed 2,2-dimethyl butanal, hexadecane, tetradecane, and pentadecane as floral markers for longan honey (*Apis mellifera*) from Thailand. While Seisonen et al. (2015) suggested 2H-pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-(9CI) as a compound marker for Lindin honey from Estonia. Volatile compounds could also contribute to the bioactivities of honey, especially the antioxidative effect due to their natural radical scavenging potential (Manyi-Loh et al. 2011). In the present study, the total phenolic contents (TPC), total flavonoid content (TFC), antioxidant properties (DPPH, ABTS, FRAP and TAOC) and volatiles profile of several commercial honeys derived from different botanical and geographical origins were analysed.

## MATERIALS AND METHODS

### SAMPLE COLLECTION

A total of seven honey samples were used in this study (Table 1). Samples H1-H3 were obtained from Federal

Agricultural Marketing Authority (FAMA) Kedah, Malaysia. Samples H4 were from Turkey and samples H5-H6 were from Yemen, which all samples were acquired from the market in Malaysia. Sample H7 was obtained from the Malaysian Agricultural Research and Development Institute (MARDI), Selangor, Malaysia. All

samples were obtained or purchased between September and November 2018 and were kept at 4 °C until further analysis. The information on the bee species and floral type of samples H1-H3, and H7 were provided by FAMA and MARDI, respectively. While the information for samples H4-H6 were stated on the product label.

TABLE 1. Description of honey samples analysed in this study

Samples	Name	Species	Floral type	Geographical origin	Source
H1	Tualang honey	<i>Apis dorsata</i>	Monofloral ( <i>Koompassia excels</i> )	Malaysia (Kedah)	FAMA
H2	Acacia honey	<i>Apis mellifera</i>	Monofloral ( <i>Acacia mangium</i> )	Malaysia (Kedah)	FAMA
H3	Kelulut 1	<i>Heterotrigona itama</i>	Polyfloral	Malaysia (Kedah)	FAMA
H4	Pine honey	<i>Apis mellifera</i>	Monofloral ( <i>Pinus</i> spp)	Turkey (not mentioned)	Market
H5	Marai honey	<i>Apis mellifera</i>	Polyfloral	Yemen (not mentioned)	Market
H6	Sumar honey	<i>Apis mellifera</i>	Monofloral ( <i>Vachellia tortilis</i> )	Yemen (not mentioned)	Market
H7	Kelulut 2	<i>Heterotrigona itama</i>	Polyfloral	Malaysia (Terengganu)	MARDI

FAMA: Federal Agricultural Marketing Authority, MARDI: Malaysian Agricultural Research and Development Institute

#### REAGENTS AND CHEMICALS

ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), potassium persulfate, sodium phosphate, ammonium molybdate, gallic acid, Folin-Ciocalteu reagent, sodium carbonate, quercetin hydrate, sodium acetic acid, ascorbic acid and 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma-Aldrich (Steinheim, Germany), 2,2'-diphenyl-1-picrylhydrazil (DPPH) from Merck KGaA (Darmstadt, Germany), ferric chloride from R & M Chemical (Essex, England) and dimethyl sulfoxide (DMSO) from Fisher Scientific (Loughborough, England). All reagents and chemicals were of analytical grade.

#### ANALYSIS OF TOTAL PHENOLIC CONTENT (TPC)

Total phenolic content (TPC) was measured according to Biluca et al. (2016) with slight modification. Briefly, 20  $\mu$ L (10 mg/mL) of the samples was inserted into a 96-well plate and added with 50  $\mu$ L Folin-Ciocalteu (F-C) reagent. The F-C reagent was diluted with distilled water with a ratio of 1:10. The mixture was left for 3 min before 100  $\mu$ L

(60 g/L) of  $\text{Na}_2\text{CO}_3$  was added. Finally, the plate was left in the dark for 60 min before the absorbance was read at 750 nm using VersaMax™ microplate reader. Gallic acid was used to calculate standard curve (3.90, 7.81, 15.62, 31.25, 62.5, and 250  $\mu$ g/mL;  $r^2 = 0.9827$ ), and the TPC values were expressed as  $\mu$ g of gallic acid equivalents (GAEs) per mg of honey.

#### ANALYSIS OF TOTAL FLAVONOID CONTENT (TFC)

The total flavonoid content (TFC) was determined according to the method by Ranneh et al. (2018) with slight modification. Briefly, 2% of  $\text{AlCl}_3$  solution in methanol was prepared in the beaker before transferred to the 50 mL centrifuge tube. Then, 100  $\mu$ L (10 mg/mL) of samples were inserted into 96-well microplates and added with 100  $\mu$ L 2% v/v  $\text{AlCl}_3$  solution. The plate was incubated in the dark for 1 hour and the absorbance was measured at 430 nm using VersaMax™ microplate reader. Quercetin was used to calculate standard curve (3.90, 7.81, 15.62, 31.25, 62.5, and 250  $\mu$ g/mL;  $r^2 = 0.9998$ ). The TFC values were expressed as  $\mu$ g of quercetin equivalent per mg of honey.

## ANALYSIS OF 2,2'-DIPHENYL-1- PICRYLHYDRAZIL (DPPH)

The DPPH assay was carried out according to Salonen et al. (2017). One gram of honey was dissolved in 1 mL methanol. This stock solution was then further diluted to eight different concentrations (1.95, 3.90, 7.81, 15.62, 31.25, 62.5, 125, and 250 mg/mL) with methanol. The samples were then centrifuged at 10000 rpm for three min at 4 °C (Eppendorf 5415R centrifuge, Hamburg, Germany). Then, 60 µL of each concentration of samples was mixed with 60 µL of DPPH (0.1 mg/mL methanol) in the 96-well plates and incubated at room temperature for 30 min. The absorbance was measured at 517 nm using VersaMax™ microplate reader. Methanol was used as a blank and the percentage of the radical-scavenging assay (RSA) was calculated as:

$$\%RSA = [\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}] / [\text{Absorbance}_{\text{blank}}] \times 100$$

The graph of these percentages against various concentrations (7.81, 15.62, 31.25, 62.5, 125, and 250 mg/mL) was then plotted and the results are expressed as IC<sub>50</sub> mg/mL (required concentration of honey sample for 50% inhibition of free radicals). Butylated hydroxytoluene (BHT) was used as a reference.

## ANALYSIS OF FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

Ferric reducing antioxidant power (FRAP) was measured according to Habib et al. (2014). FRAP reagent was prepared with the ratio of 10:1:1 of solution 1 (300 mM acetate buffer), solution 2 (10 mM TPTZ) and solution 3 (20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O), respectively. Then, 10 µL of the sample (10 mg/mL) were loaded to each well of the 96-well plate and added with 300 µL of FRAP reagent. The plate was incubated in the dark at 35 °C and the absorbance was read at wavelength 470 nm using VersaMax™ microplate reader after 4 min. Iron sulfate (FeSO<sub>4</sub>) was used to calculate standard curve (25, 50, 100, 200, 600 and 800 µg/mL; r<sup>2</sup> = 0.9950). FRAP values were expressed as µg of FeSO<sub>4</sub> equivalent per mg of honeys.

## ANALYSIS OF TOTAL ANTIOXIDANT CAPACITY (TAOC)

Phosphomolybdate assay procedure as described by Jan et al. (2013). The reagent solution was prepared by mixing 28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulphuric acid. One millilitre of the reagent and 0.1 mL of the sample (10 mg/mL) were loaded into a 1.5 mL microcentrifuge tube and were incubated at 95 °C for 90 min. After the tube was cooled to room temperature, 300 µL of each mixture was transferred to

the 96-well microplate and the absorbance was analysed at 695 nm using VersaMax™ microplate reader. Ascorbic acid was used to calculate standard curve (3.90, 7.81, 15.62, 31.25, 62.5 and 250 µg/mL; r<sup>2</sup> = 0.9971), and the values were expressed as µg of ascorbic acid equivalent per mg of honey.

## ANALYSIS OF 2,2'-AZINO-BIS

## (3-ETHYLBENZOTHIAZOLINE-6-SULPHURIC ACID) (ABTS)

Free radical scavenging activity of the honey sample was determined using ABTS assay according to Silva et al. (2013) with slight modification. ABTS+● solution (7mM) was first prepared by dissolving 38 mg of 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphuric acid) diammonium salt (ABTS) in 10 mL distilled water and added by 10 mL of 20 mM potassium persulfate. The solution was incubated in the dark for 12 h prior to use at room temperature and underwent dilution (by adding distilled water) to reach absorbance of 0.68 (± 0.008485) at 743 nm. Then, 10 µL (10 mg/mL) of samples were transferred into a 96-well plate and added by 300 µL of ABTS+● solution. The plate was incubated for 10 min at room temperature and the absorbance was read at 743 nm wavelength using VersaMax™ microplate reader. DMSO was used as a blank. The values were calculated as:

$$\text{Percentage (\%)} \text{ of inhibition} = [(\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{blank}}] \times 100$$

## GC-MS ANALYSIS

GC-MS analysis was performed according to Mohamad Shah et al. (2013) with minor modification. Initially, honey was weighed (0.5 g) into a microcentrifuge tube and mixed with 2.5 mL methanol. The mixture was vortexed for 3 min before centrifuged at 2500 rpm for 5 min. Finally, the mixture was filtered using a syringe filter (0.22 µm) into a GC vial for analysis.

GC-MS analysis was performed using a Shimadzu QP2010 ULTRA coupled with mass spectrometer. The column was an Rtx-5MS fused-silica capillary column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) with helium as a mobile phase at a flow rate of 50 mL/min. The injection volume was 5 µL volume using a split mode at an injector temperature of 300 °C. The oven temperature was held (5 min) at 40 °C and ramped to 160 and 280 °C (15 min hold) with rate 4 and 5°C/min, respectively. The total run time for each sample was 74 min. All peaks were identified based on mass matching (≥ 90%) from the NIST libraries. Only compounds with 90% or greater spectral matching accuracy were reported.

## STATISTICAL ANALYSIS

Assays were performed in triplicate ( $n = 3$ ) and the results were expressed as the mean values with standard deviation (SD). The significant difference ( $p < 0.05$ ) represented by different letters were obtained by one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT). Pearson product-moment coefficient test was used to determine the relationship of variables (TPC, TFC, DPPH, ABTS, FRAP and TAOC). All statistical analysis was conducted using SPSS software version 15.

## RESULTS AND DISCUSSION

In this study, seven samples of honey from different types and sources were analysed for their total phenolic content (TPC) and total flavonoid content (TFC), antioxidant properties and volatile compounds. Two honey samples i.e. H3 and H7 (*Kelulut*) were produced by *Heterotrigona itama*, the highly propagated stingless bee (*Meliponini*) species in Malaysia. The other honey samples were produced by the honeybee (*Apini*) subfamily derived from Malaysia, Turkey and Yemen (Table 1). The TPC and TFC values are presented in Figure 1.

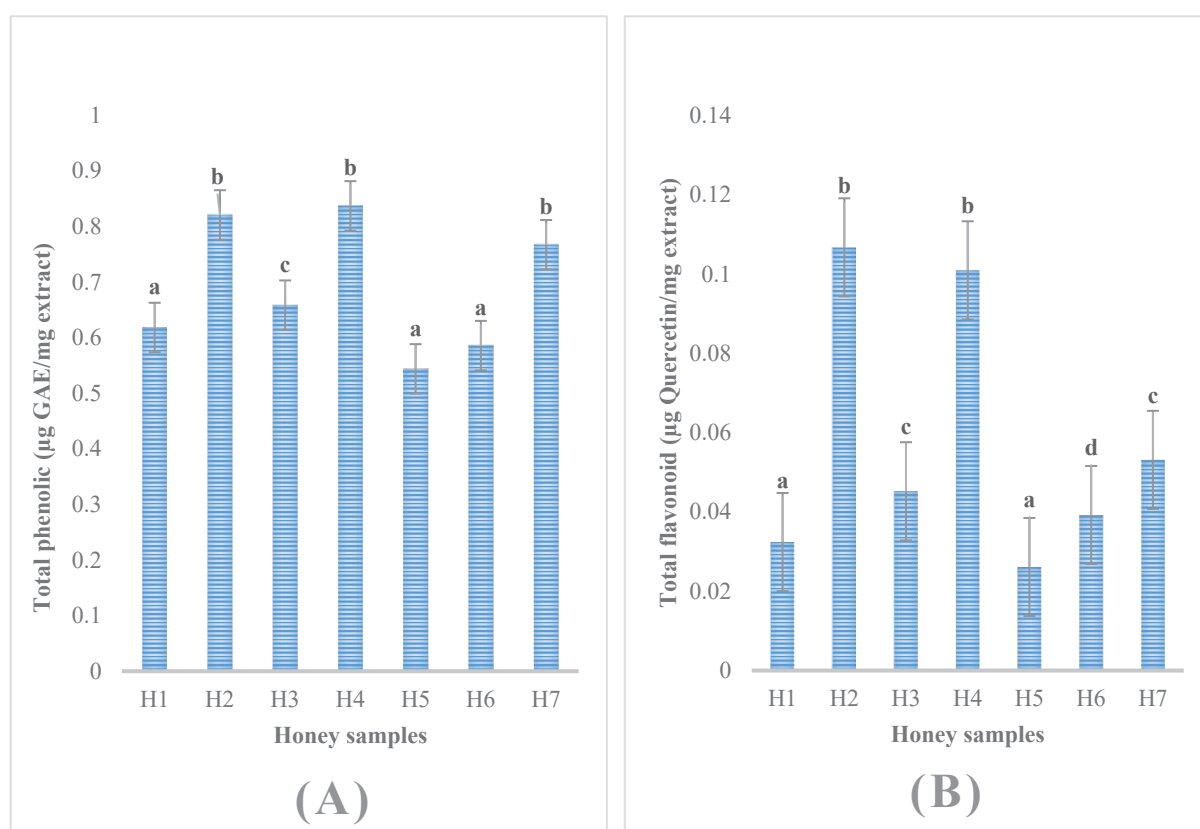


FIGURE 1. (A) total phenolic content (TPC); (B) total flavonoid content (TFC) of several honey samples (H1: *Tualang*; H2: *Acacia*; H3: *Kelulut* 1; H4: *Pine*; H5: *Marai*; H6: *Sumar*; H7: *Kelulut* 2). Values presented are mean  $\pm$  SD ( $n = 3$ ). Bars having different letters are significantly different ( $p < 0.05$ )

Generally, the phenolic compounds are classified into two main families: phenolic acids with their related derivatives; and flavonoids. Flavonoids itself can be further classified in a number of subfamilies such as flavones, flavonols, isoflavones, flavanones, and flavanonols (Ciulu et al. 2016). The TPC values found in

this study range between  $0.54 \pm 0.16$  and  $0.84 \pm 0.07$   $\mu\text{g}$  GAE/mg honey (gallic acid equivalent per milligram of honey) with the highest value shown by H4 (*Pine* honey) followed by H2 (*Acacia* honey) and H7 (*Kelulut* 2) which were significantly different ( $p < 0.05$ ) from other samples. However, a lower range was found for TFC value (0.03

$\pm 0.01$  to  $0.11 \pm 0.24$   $\mu\text{g}$  quercetin equivalent/mg honey) with the significant highest value ( $p < 0.05$ ) was recorded in H2 (Acacia honey) followed by H4 (Pine honey).

Moniruzzaman et al. (2013) reported that the TPC values of their *Tualang* and Acacia honey samples collected from the tropical rain forests of Kedah and Johor (Malaysia) are  $0.35 \pm 0.81$  and  $0.19 \pm 0.84$   $\mu\text{g}$  gallic acid equivalent/mg honey, respectively. While the TFC values are  $0.07 \pm 0.74$  and  $0.02 \pm 1.73$   $\mu\text{g}$  catechin equivalent/mg honey for *Tualang* and Acacia honey, respectively. Both TPC and TFC values reported by Moniruzzaman et al. (2013) are lower compared to our present study, except for the TFC value of *Tualang* honey. In another study of samples collected from the forests of Kedah and Johor (Malaysia), the *Kelulut* honey showed a significantly stronger antioxidant capacity compared to *Tualang* honey, which the result was correlated to the

higher TFC and TPC level in *Kelulut* honey (Ranneh et al. 2018). The variations of chemical composition and level inferred that floral origins (Cianciosi et al. 2018) and environmental/seasonal factors (Cheung et al. 2019) contribute to the phenolic and flavonoid content in honey.

Antioxidant properties of honey samples were investigated by DPPH, ABTS, FRAP, and TAOC assays. DPPH and ABTS assays are based on the free radical scavenging properties of honey and the decolorization of DPPH and ABTS radicals occurs during the presence of antioxidant compounds. Meanwhile, FRAP and TAOC assays measure the potential of honey as a reducing agent. In the presence of a reducing agent (antioxidant),  $\text{Fe}^{3+}$ TPTZ complex and Molybdenum VI are reduced to  $\text{Fe}^{2+}$ TPTZ complex and Molybdenum V. The results of the antioxidant properties of honeys are presented in Figure 2.

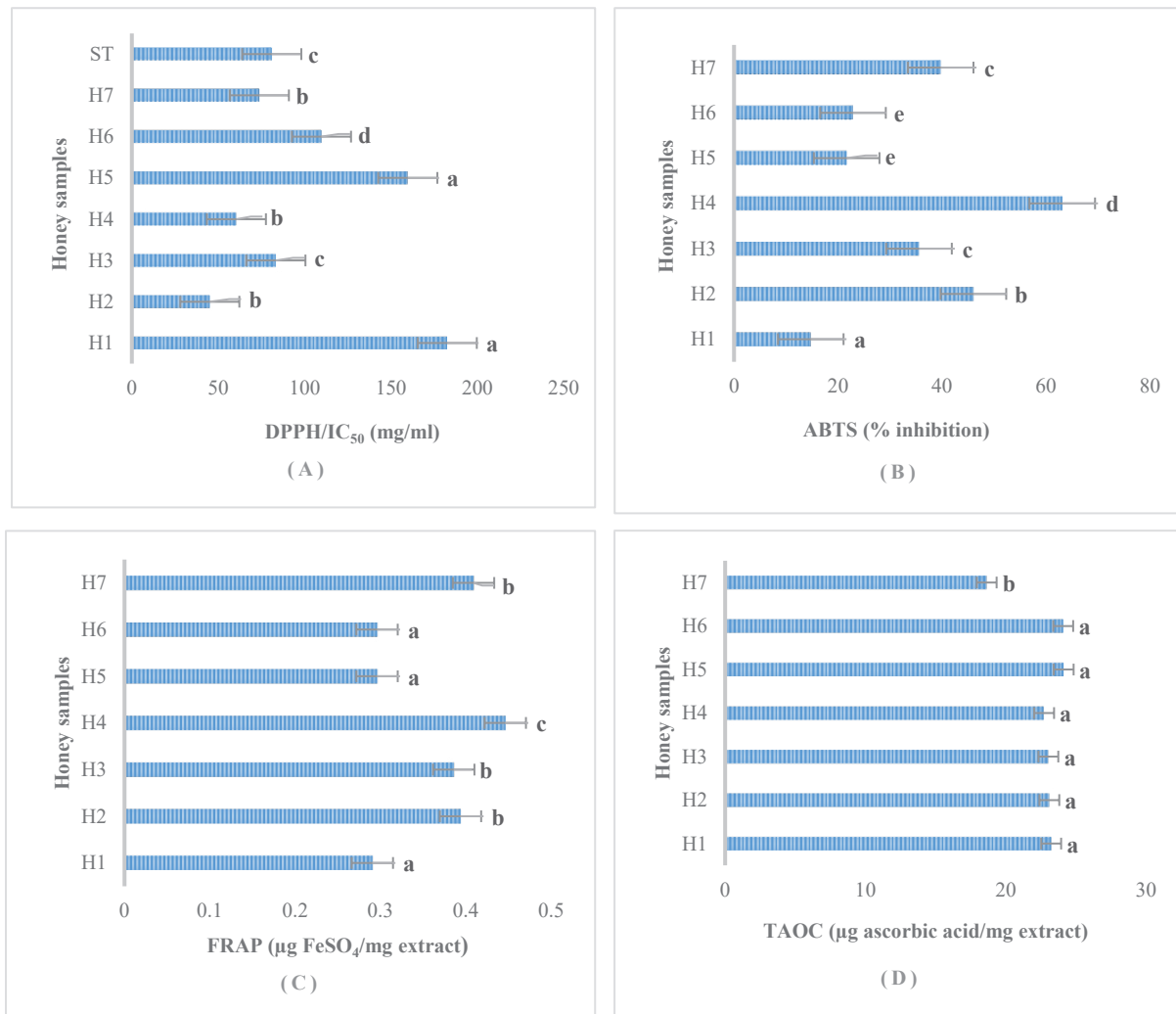


FIGURE 2. Antioxidant properties of (A) DPPH; (B) ABTS; (C) FRAP; and (D) TAOC of several honey samples (H1: *Tualang*; H2: Acacia; H3: *Kelulut* 1; H4: Pine; H5: Marai; H6: Sumar; H7: *Kelulut* 2). Values presented are mean  $\pm$  SD ( $n = 3$ ). Bars having different letters are significantly different ( $p < 0.05$ )

The DPPH IC<sub>50</sub> values (Figure 2(A)) indicate the concentration of honey that has caused 50% scavenging of free radical, which means the lower the IC<sub>50</sub> value, the higher the antioxidant properties of the honey. Our result shows that the DPPH IC<sub>50</sub> values range from 45.12 (H2) to 182.56 mg/mL (H1). Sample H3 (*Kelulut* 1) showed an antioxidant activity (IC<sub>50</sub> = 83.38 mg/mL) comparable to the reference butylated hydroxytoluence (BHT) (IC<sub>50</sub> = 80.98 mg/mL). H2 (Acacia honey) showed the highest activity (IC<sub>50</sub> = 45.12 mg/mL), followed by H4 (Pine honey) (IC<sub>50</sub> = 60.50 mg/mL), H7 (*Kelulut* 2) (IC<sub>50</sub> = 73.80 mg/mL), H6 (Sumar honey) (IC<sub>50</sub> = 109.76 mg/mL), H5 (Marai honey) (IC<sub>50</sub> = 159.74 mg/mL) and H1 (*Tualang* honey) (IC<sub>50</sub> = 182.56 mg/mL). Samples that possess IC<sub>50</sub> values ranging from 10 to 50 mg/mL is considered to have strong antioxidant activity, 50 to 100 mg/mL is intermediate, and > 100 mg/mL is considered as weak (Phongpaichit et al. 2007). Therefore, in this study, H2 (Acacia honey) possesses a strong antioxidant activity. While H4 (Pine honey), H7 (*Kelulut* 2), and H3 (*Kelulut* 1) shows intermediate antioxidant activity. The lowest antioxidant activity corresponds to H1 (*Tualang* honey) and H5 (Marai honey). The free radical scavenging activity of the honey samples was also evaluated through ABTS assay and reported as % inhibition. In accordance with DPPH results, H2 and H4 exhibited the best ABTS inhibition activity (46.06 and 63.15%) among the samples ( $p < 0.05$ ). The findings from both assays are also consistent with the TPC and TFC results, indicating that the samples with higher TPC and TFC values would also have higher antioxidant free radical scavenging properties (Gašić et al. 2014; Ranneh et al. 2018).

FRAP and TAOC assays measure the antioxidant properties based on the reducing power of the samples. In the present study, the FRAP value is between  $0.29 \pm 0.06$  (H1) and  $0.45 \pm 0.02$  (H4) FeSO<sub>4</sub> equivalence in milligram of honey. In contrast to DPPH and ABTS results, the second-highest value was H7 (*Kelulut* 2) and not H2 (Acacia honey). The TAOC which was determined using phosphomolybdate assay, however, shows different results from the other antioxidant assays. The imported honey from Yemen which are Marai (H5) and Sumar (H6) recorded the highest TAOC values with  $24.14 \pm 0.43$  and  $24.11 \pm 0.04$  ascorbic acid equivalence in milligram of honey, respectively. However, this result was not statistically significant with other samples except for H7 (*Kelulut* 2) which showed significantly lower value ( $p < 0.05$ ).

Pearson product-moment coefficient test was conducted to identify the correlation between variables of phenolic compounds and antioxidant activities. As shown in Table 2, TPC and TFC had a strong positive correlation with ABTS and FRAP and a strong negative correlation with DPPH. A similar correlation was also found by Khalil et al. (2012) and Moniruzzaman et al. (2014) suggesting that phenolic compounds are important antioxidant factors. However, this does not imply that other compounds are not involved in the prevention of oxidation processes. As evidenced by a weak and very weak negative correlation between TAOC, TPC, and TFC, a non-phenolic antioxidant may also responsible for the antioxidant activity of honey (Bertoncelj et al. 2007; Bogdanov 2012).

TABLE 2. Correlation coefficient results with Pearson product-moment coefficient test

	TPC	TFC	FRAP	ABTS	TAOC	DPPH
TPC	1	.916**	.907**	.899**	-.498	-.833*
TFC	.916**	1	.778*	.881**	-.132	-.812*
FRAP	.907**	.778*	1	.947**	-.544	-.873*
ABTS	.899**	.881**	.947**	1	-.323	-.865*
TAOC	-.498	-.132	-.544	-.323	1	.388
DPPH	-.833*	-.812*	-.873*	-.865*	.388	1

\* Correlation is significant at  $p < 0.05$

\*\* Correlation is significant at  $p < 0.01$

Profiling and identification of the volatile compounds was conducted using a gas chromatography-mass spectrometry (GC-MS). Based on a comparison of the mass spectra with the NIST library, 24 peaks of the volatile

compounds with 90% or greater spectral matching accuracy have been identified from the honey samples (Table 3). These volatiles belong to different chemical classes such as oxygenated compound (aldehyde, ketones,

TABLE 3. Major volatile compound in honey samples

Peaks	RT (min)	Molecular formula	[M-W](Frag. MS <i>m/z</i> )	Compounds	Samples
1	3.152 – 3.291	CH <sub>6</sub> N <sub>2</sub>	46 (14,15,22,28,31,42,46)	Hydrazine, methyl	H3,H5
2	3.363 – 3.434	CH <sub>2</sub> O <sub>2</sub>	46 (12,19,29,31,46)	Formic acid	H1,H2
3	3.366	C <sub>2</sub> H <sub>6</sub> N <sub>2</sub> O <sub>2</sub>	90 (30,33,44,45,49,60,61)	Methanamine, N-methoxy-N-nitroso	H3
4	3.514 – 3.674	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	60 (2,15,18,26,43,55,60)	Acetic acid	H1,H3
5	4.381 – 4.412	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	86 (15,27,29,39,50,55,57,71,85)	Butane, 1,2:3,4-diepoxy-, (-/+)	H3,H4
6	4.514	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	86 (14,26,29,31,38,50,55,59,68,85)	2,2'-Bioxirane	H3
7	5.093 – 5.413	C <sub>4</sub> H <sub>10</sub> O <sub>3</sub>	106 (15,26,31,33,41,45,59,75,77,105)	Glycolaldehyde dimethyl acetal	All except H4
8	5.686 – 6.260	C <sub>5</sub> H <sub>4</sub> O <sub>2</sub>	96 (14,24,29,34,39,40,49,67,96)	Furfural	All
9	7.203 – 7.394	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98 (14,24,27,39,41,48,53,55,61,69,79,81,85,98)	2-Furanmethanol	All
10	8.586	C <sub>6</sub> H <sub>12</sub> O <sub>3</sub>	132 (29,31,41,45,53,59,69,72,85,101,102,131)	Furan, tetrahydro-2,5-dimethoxy	H4
11	9.248 – 9.300	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	110 (27,38,39,44,51,63,77,93,95,110)	Ethanone, 1-(2-furanyl)	H3,H4,H6
12	9.527	C <sub>4</sub> H <sub>4</sub> O <sub>2</sub>	84 (27,29,33,39,41,49,55,56,67,83,84)	2(5H)-Furanone	H5
13	10.069 – 10.195	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>	98 (26,35,41,51,55,57,66,69,72,79,96,98)	1,2-Cyclopentanedione	H1,H2,H4,H7
14	10.832 – 11.997	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144 (43,45,55,59,73,84,97,101,144)	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	H3,H7
15	11.398 – 11.472	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	110 (15,27,29,39,51,53,61,69,81,95,110)	2-Furancarboxaldehyde, 5-methyl	All
16	12.596	C <sub>8</sub> H <sub>8</sub> O	120 (29,39,51,65,66,77,89,91,118,120)	Benzene acetaldehyde	H7
17	17.014	C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>	128 (15,29,31,43,45,57,60,72,85,87,100,128)	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	H1
18	20.403	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144 (39,43,46,55,58,69,73,87,97,101,115,126,144)	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	H2
19	21.769	C <sub>5</sub> H <sub>12</sub> O <sub>3</sub>	120 (15,29,41,47,48,59,60,75,89,90,119)	Ethane, 1,1,2-trimethoxy	H5
20	23.993 – 25.389	C <sub>6</sub> H <sub>6</sub> O <sub>3</sub>	126 (29,38,41,50,68,69,80,97,109,126)	5-Hydroxymethylfurfural	All
21	46.973	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282 (43,55,69,83,97,111,123,137,165,179,222,264)	9-Octadecenoic acid, (E)	H4
22	47.335	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308 (41,55,67,81,95,109,123,136,150,220,263,308)	Linoleic acid ethyl ester	H1
23	56.230	C <sub>44</sub> H <sub>90</sub>	618 (29,57,71,113,211,267,351,407,491,533,618)	Tetratetracontane	H7
24	60.546	C <sub>20</sub> H <sub>38</sub> O <sub>2</sub>	310 (43,55,69,82,96,109,137,166,194,235,267,310)	Z-14-Octadecen-1-ol acetate	H7

RT: Retention time; [M-W]: Molecular weight; H1: *Tualang*; H2: *Acacia* honey; H3: *Kelulut 1*; H4: *Pine* honey; H5: *Marai* honey; H6: *Sumar* honey; H7: *Kelulut 2*



esters, alcohol, and carboxylic), hydrocarbon (aliphatic, aromatic, and cyclic) and heterocyclic compounds (pyran and furan derivatives). Furan derivatives which are produced as a result of sugar degradation (Aljohar et al. 2018) are found as the most encountered compound with furfural, 2-furanmethanol, 2-furancarboxaldehyde, 5-methyl and 5-hydroxymethylfurfural (HMF) detected in all samples. HMF can be found in sugar-containing food and is associated with sugar degradation due to heat or long storage. This compound is commonly low and minimal concentration in honey (<40 or <80 mg/kg for honeys from tropical region) but when HMF is detected at higher level than the concentration limit, this indicates a poor storage condition, or exposure to high temperature and excessive heat treatment (Shapla et al. 2018). However, in this study, quantitative study was not conducted to compare HMF level among the samples.

Of the 24 volatile compounds that have been identified, only 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (DDMP) and 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one have been reported to have antioxidant activities (Kanzler et al. 2016; Wong & Kern 2011). In the present study, DDMP was only detected in sample H2 (Acacia honey). This compound has been reported in several foodstuffs including prunes (Čechovská et al. 2011), heated pear (Hwang et al. 2013), whole wheat bread (Bin & Peterson 2016), and honey (Awasum et al. 2015). Besides antioxidant activity, DDMP has also been associated with autonomic nerve activity of rats (Beppu et al. 2012).

The antioxidant compound 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one was only detected in the sample H3 (*Kelulut* 1) and H7 (*Kelulut* 2). Chukwu et al. (2007) reported that 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one was found in the *Sysepalum dulcificum* fruit extract. Therefore, this compound could be derived from a certain plant species which its nectar consumed by the stingless bees. Nevertheless, though both honey samples were produced by the same species of stingless bee *Heterotrigona itama*, their volatiles profile are different. This implies that the foraging activities of the same bee species is different and depends on the geographical and botanical origins.

#### CONCLUSION

In this study, Pine honey from Turkey (H4) showed the highest phenolic contents and antioxidant properties followed by Acacia honey (H2), *Kelulut* 2 (H7), *Kelulut* 1 (H3), Sumar honey (H6), *Tualang* honey (H1) and Marai honey (H5). A strong correlation was found between

the phenolic content and their antioxidant properties: DPPH, ABTS and FRAP except for TAOC (a negative weak correlation). In terms of volatile compounds, only DDMP and 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one have been reported possess antioxidant activities. DDMP was only identified in Acacia honey (H2), while 2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one was detected only in *Kelulut* samples. Moreover, linoleic acid and furaneol which were identified in *Tualang* honey (H1) could be considered as possible floral markers. However, further studies are required particularly related with their physicochemical properties and bioactive components for a better understanding. Overall, the present study provides valuable information on the analysis and profiling of honeys from different botanical and geographical origins.

#### ACKNOWLEDGEMENTS

The authors would like to thank the Ministry of Higher Education, Malaysia for the financial support under Fundamental Research Grant Scheme (Project No. FP035-2021 [FRGS/1/2021/STG01/UM/02/9]).

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