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Original Article

PHYSICOCHEMICAL, PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF WILD HONEY COLLECTED AT MANGROVE AND MOUNTAIN AREAS IN SABAH, MALAYSIAN BORNEO

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ABSTRACT

Objective: The aim of this study was to determine the physicochemical, phytochemical content and antimicrobial properties of selected honey of Sabah, Malaysian Borneo.

Methods: A standardized protocols were used to evaluate the physicochemical properties of selected honey of Sabah while the phytochemicals content (phenolics and flavonoids) were determined using Folin-Ciocalteau and aluminium colorimetric methods. Antimicrobial properties were evaluated using disc diffusion assay.

Results: For 80% methanol extract, old Upper Mountain honey contained the highest free acidity, conductivity, total phenolic and flavonoid contents with the values 23.84 ± 0.42 ml/g, 0.61 ± 0.01 mS/cm, 9.71 ± 0.01 mg gallic acid equivalent (GAE)/g and 7.76 ± 0.02 mg rutin equivalent (RU)/g, respectively. Antimicrobial activity showed strong inhibition by old Upper Mountain honey extract (80% methanol extract) with the value of 6.00 ± 0.01 mm at concentration of 100% against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). The same trend of phytochemicals content and antimicrobial activity was also observed in absolute methanol extracts.

Conclusion: The present results suggested that wild raw honey collected at mangrove and mountain area in Sabah contained a wide range of phytochemical compounds which has the potential for human health.

Keywords: Wild honey, Physicochemical, Phytochemicals, Antimicrobials.

INTRODUCTION

Honey has been practically used as human domestic needs for food and sweet substance since many years ago [1]. It is a sweet natural product that produced by honey bee from floral nectar, transform through the hypopharyngeal gland that secretes enzyme and store in the honeycomb to mature [2]. Honey has been getting numerous attentions due to its ability to act as antibacterial agent [3].

Medicinal properties of honey have been documented as one of the oldest medical application [4]. Honey has been found to heal surgical wounds, burns, minor cuts, sore throats and laryngitis [5]. It is capable to sterilize the infected wound in human [6]. This is due to the moisture content, humidity and viscosity in honey that prevent the growth of bacteria on the infected wound [7]. Honey also known to display anti-inflammatory, antioxidant, anti-proliferative and anti-carcinogenic properties[8].

The presence of hydrogen peroxide in honey has been shown to contribute to the antimicrobial properties [9]. The acidic nature form, low level of hydrogen peroxide plus the presence of phenolic and flavonoid compounds in honey might also help in tissue growing and repairing process [10]. Previous studies suggested that enzyme glucose oxidase is responsible for the antibacterial properties of honey [11]. This enzyme might caused the nectar in the honey sac undergoes chemical changes and transformed the glucose into gluconic acid and hydrogen peroxide [9]. Non-peroxides compounds such as phenolic and flavonoid compounds have been shown to inhibit the growth of pathogenic bacteria by disturbing the function of the cell membrane [12]. This is due to the fact that phenolic and flavonoid compounds possess antioxidant activities, which exerted antibacterial effect in honey [13]. Previous studies showed that phenolic and flavonoid phytochemicals in flower nectar might affect the antibacterial properties due to possible correlation with their botanical resources and origin [13]. This was supported by the difference amount of phenolic contents present in Gelam and Coconut honey which exerted a wide range of antibacterial properties that might be caused by the variation of floral sources [14].

Honey is believed to have inhibitory effect against up to 60 species of bacteria, including aerobes and anaerobes, gram-positives and gram-negatives [9]. This was supported by increasing reports on the effectiveness of honey extracts as antibacterial agent against *Staphylococcus aureus* and *Streptococcus pyogenes* [14]. However, lack of comprehensive scientific reports on honey as antibacterial agent led to problem in current modern medicine [15]. This happens due to inconsistency of honey extracts to prevent the growth of selected microorganisms [11]. The purpose of this study is to evaluate the physicochemical and phytochemicals contents, and investigate the correlation with antimicrobial properties of selected honey of Sabah, Malaysian Borneo.

MATERIALS AND METHODS

Honey samples

Four types of selected honey of Sabah, Malaysia namely; young and old Mangrove; as well as young and old Upper Mountain were collected from the west part of Sabah, Malaysian Borneo. Manuka honey (From New Zealand) was used as positive control while Potiukan and Tropical honey (local farm honey from mix floral resources) were also used as comparison. The difference between old and young type of Mangrove and Upper Mountain honeys were due to the duration of storage, where basically old honey was harvest and kept in for maturity since 2008 while the young honey was harvested and kept in for maturity since early 2010. Mangrove and Upper Mountain honey were collected in Kota Belud, Sabah and Pitas, Sabah coastline area; respectively.

Sample extraction

Samples (7 g) were diluted with 70 ml of solvent extracts (80% methanol or absolute methanol) and vacuum-filtered through a Whatmann No.5 filter paper. The filtrate was subjected to vacuum

rotary evaporation at 40^{0}C for 1 h at room temperature to remove the solvent. The concentrated extract was put in the desiccator until the extract was free from solvent [4, 16]. The extracts were tested for their phytochemical and antimicrobial properties.

Physicochemical study

Physicochemical properties of the honey samples were observed according to the following methods. The moisture and dry matter contents were determined by using oven and weight-scale reading type as adapted from previous method [17]. pH was measured using pH meter and free acidity was determined by means of titration method [17, 18]. The electrical conductivity was measured at 27^{0}C in which the sample solution was prepared using double distilled water [18]. Colour intensity was measured at 450 nm using UV-spectrophotometer [17, 18].

Determination of total phenolic content

Total phenolic content of the honey was determined using Folin-Ciocalteau's method [14, 19, 20, 21]. 100 µl of extract was mixed with 0.75 ml of Folin-Ciocalteu's reagent (previously diluted 10-fold with distilled water); vortex for 2 min, 0.75 ml of sodium bicarbonate added to the mixture and allowed to stand for 90 min at room temperature. The mixture was then transferred to a cuvette and the absorbance was measured at 725 nm using UV-spectrophotometer. The total phenolic content of the samples were expressed as gallic acid equivalents in one gram of sample (mg of GAE/ g of honey).

Determination of total flavonoid content

Total flavonoid content of honey was determined using aluminium chloride colorimetric method [20, 22, 23, 24] with slight adjustments. 100 µl of honey extract was added to 4 ml of distilled water and 0.3 ml of 5% natrium nitrite ($NaNO_2$) was immediately added. After 5 min, 0.6 ml of 10% aluminium chloride ($AlCl_3$) was added and after 6 min, 2 ml of 1M sodium hydroxide (NaOH) and 2.1 ml of distilled water were added before thorough mixing. The mixture was transferred to a cuvette and the absorbance was measured against a blank at 510 nm using UV-spectrophotometer. Total flavonoid contents of the samples were expressed as rutin equivalents in one gram of sample (mg of RE/ g of honey).

Antimicrobial activity

Preparation of honey solutions

Honey solutions were prepared prior to the experiment by diluting the crude extracts at different concentrations (v/v) (25%, 50%, 75% and 100%; in distilled water). This was done by dissolving the respective volumes: 0.25 ml, 0.50 ml, 0.75 ml and 1.0 ml of each honey into corresponding volumes of sterile distilled water to give a 1 ml preparation. The vials were stored in a refrigerator set at 4^{0}C for future usage.

Preparations of the bacterial inoculums

The test microorganisms were obtained from the School of Science and Technology, University Malaysia Sabah. Three strains of the gram-positive bacteria were *Staphylococcus aureus* (*S.aureus*), *Bacillus cereus* (*B.cereus*) and *Bacillus subtilis* (*B.subtilis*) while two strains of the gram-negative bacteria were *Escherichia coli* (*E.coli*) and *Salmonella enteritidis* (*S.enteritidis*). One single colony of each type of the bacteria (from the nutrient agar stock culture) was inoculated and transferred into a 10 ml sterile nutrient broth. The broth cultures were incubated at 37^{0}C for 24 hours [10].

Antimicrobial assay

Antimicrobial activity was determined using disc diffusion method [10, 25, 26]. A total of 100 µl of the bacterial culture was spread on solid Mueller Hinton agar (MHA) plates. For screening, sterile six mm diameter of Whatmann no.5 filter paper was soaked with each of honey solutions at different concentration and placed into the surface of the inoculated media agar plates. The agar plates were incubated at 37^0 C for 24 hours. The diameter of the inhibition zones were measured in millimeter (mm). The positive controls (Ampicillin and Canamycin) and negative control (distilled water) were used for comparison.

Statistical analysis

All experiments were carried out in three replicates in three independent experiments. Correlations among data obtained were analysed using Pearson's coefficient. The results were presented as mean ± standard deviation (SD). The level of statistical significance was set at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Physicochemical properties

Physicochemical properties of selected honey of Sabah were analysed and the results were shown in **Table 1**. Colour intensity of the honey was mainly related to the presence of phenolic and flavonoid compounds [27]. It was observed that the colour of honey ranged from light amber to dark amber. The colour intensity of the samples were highest in Manuka honey followed by old Upper Mountain honey > old Mangrove honey > young Upper Mountain honey and Upper Mountain honey > Potiukan honey > Tropical honey. The result of this study showed that dark amber colour honey displayed higher intensity as compared to light colour except for Potiukan. This was in agreement with Jasna *et al.* [2] who reported that light colour Slovenian honey showed low intensity. Meanwhile, Terrab *et al.,* [28] reported that dark colour of honey contained higher phenolic content as compared to light colour honey.

All samples of selected honey from Sabah were found to be in acidic form. The pH ranged from 4.02 – 4.26 with Tropical honey recorded the highest pH with the value of 4.26 ± 0.08, while Manuka honey recorded the lowest pH with the value of 4.02 ± 0.24. This was similar with earlier literature which reported that the pH of Malaysian honey were in the ranged between 3.55 to 4.91 [29]. Low pH of honey allowed it to act as potential antibacterial agent [21]. This was supported by the fact that optimum pH growth for bacteria ranged from 7.00 to 7.50 [30].

Acidity of honey is due to the presence of organic acids such as gluconic acid and some inorganic ions such as phosphate and sulphate [31]. Free acidity of selected honey of Sabah, Malaysia were in the ranged between 22.00-24.84 ml/g with young Upper Mountain recorded the highest at 24.84 ± 0.44 ml/g, followed by Manuka, old Upper Mountain, old Mangrove, young Mangrove, Tropical and Potiukan. These values were very well within the allowed limits (50 meq/kg) [29]. The variation in acidity among the samples might due to the difference in terms of composition of the compounds present and harvesting season [29].

Electrical conductivity of selected honey was highest in Manuka honey with the value of 0.64 ± 0.04 mS/cm, followed by old Upper Mountain > young Upper Mountain > old Mangrove > young Mangrove > Potiukan > Tropical. Old Upper Mountain honey showed the highest value which might be due to the presence of sodium chloride from the substrate resources [32]. This was in disagreement with Alvarez-Suarez et al., [4] who reported that the Black Mangrove honey showed the highest electrical conductivity due to the coastal climate of which the honey was produced. Previous study also showed that sodium chloride is not affected by the substrate conditions and could well penetrate into the leaves through special glands in mangrove plants [4].

Moisture contents of honey samples ranged from 20.40% to 23.50%, with Tropical honey recorded the highest moisture content with the value of 23.50 ± 1.05% while young Mangrove honey recorded the lowest moisture content with the value of 20.40 ± 0.68%. The results for moisture content in this study were within the values found in Malaysian honey (between 16% and 23%) [29] and higher than those in European region [18], which confirms that moisture content might be affected by tropical climatic conditions [30, 31, 32].

The reason of having high moisture content in honey sample was due to accumulation of moisture content from the actual plant and surrounding weather [33]. As for the matter of dry content, the results showed that young Mangrove honey recorded the highest dry matter content with the value of 79.60 ± 0.19%, followed by old mangrove honey > young Upper Mountain honey > Manuka honey > old Upper Mountain honey > Potiukan honey > Tropical honey.

Table 1: Physicochemical properties of selected honey of Sabah, Malaysia

Honey sample	pH	Free acidity (ml/g)	Conductivity (mS/cm)	Moisture content (%)	Dry matter content (%)	Intensity (I)	Colour
Old Upper Mountain	4.05 ± 0.04	23.84 ± 0.42	0.61 ± 0.01	22.50 ± 1.51	77.50 ± 0.21	0.31 ± 0.01	Dark amber
Old Mangrove	4.08 ± 0.01	22.61 ± 1.22	0.42 ± 0.03	20.70 ± 0.04	79.30 ± 0.28	0.26 ± 0.05	Dark amber
Young Mangrove	4.06 ± 0.04	22.11 ± 1.03	0.40 ± 0.01	20.40 ± 0.68	79.60 ± 0.19	0.24 ± 0.04	Amber
Young Upper Mountain	4.05 ± 0.02	24.84 ± 0.44	0.47 ± 0.02	21.50 ± 1.32	78.50 ± 1.11	0.22 ± 0.01	Light amber
Tropical	4.26 ± 0.08	22.06 ± 0.62	0.20 ± 0.08	23.50 ± 1.02	76.50 ± 1.36	0.17 ± 0.01	Amber
Potiukan	4.22 ± 0.14	22.00 ± 0.81	0.22 ± 0.01	23.00 ± 1.04	77.00 ± 0.98	0.19 ± 0.02	Dark amber
Manuka	4.02 ± 0.24	23.05 ± 0.16	0.64 ± 0.04	21.80 ± 0.00	78.20 ± 1.02	0.44 ± 0.02	Dark amber

Values are expressed as mean ± standard deviation (SD)

Total phenolic content determination

The results of this study showed that the total phenolic content was highest in old Upper Mountain honey, followed by old Mangrove honey, young Mangrove honey, young Upper Mountain honey, Potiukan and Tropical honey for both 80% and absolute methanol extracts, respectively (**Table 2**). Variations in total phenolic contents might be due to the variation of floral sources and geographical location [34]. This was supported by the accessibility and availability of floral sources by the honey bee [4]. Upper Mountain honey was collected from mixed dipterocarp forest, Mangrove honey was collected from mangrove forest (Mangrove tree) while Potiukan and Tropical honey was collected from local farm (Menggaris tree). In the other hand, Manuka honey was collected from Manuka or tea tree (*Leptospermum scoparium* and *Leptospermum polygalifolium*). Therefore, the nectar collected might have different phenolic content due to varieties of the floral resources and also locations of the apiaries. Furthermore, highland areas are always surrounded by forest, which serves as a great botanical resource as compared to lowland areas. The present study showed that among the two extracts, the 80% methanol extract showed higher phenolic content as compared to absolute methanol extract. Addition of small amount of organic solvents to an aqueous medium creates a more polar medium which facilitates extraction of phenolic compounds as compared to mono-component solvent [34]. However, the results for total phenolic content of all the samples were lower than Gelam honey (21.4 ± 1.29 mg/ml) and Coconut honey (15.6 ± 1.05 mg/ml) [14].

Total flavonoid content determination

The results of this study showed that total flavonoid content was highest (among honey of Sabah) in old Upper Mountain honey, followed by old Mangrove honey > young Mangrove honey > young Upper Mountain honey > Potiukan > Tropical honey for 80% and absolute methanol extracts, respectively (**Table 2**). The highest total flavonoid content was shown in 80% methanolic extract of old Upper Mountain honey with the value of 7.76 ± 0.04 mg RE/g. The results obtained for the total flavonoids content in this study showed acceptable level of flavonoids content as compared with other types of honey as previously reported [34]. Variations in total flavonoid contents might be due to botanical origin and climatic conditions [23]. This was supported by the influenced of the nectar compositions collected from the flower [20]. The high humidity affected the growth and maturity of the trees which may be associated with the representations of these compounds in the honey.

Table 2: Phytochemicals contents of selected honey of Sabah in 80% and absolute methanol extractions

Honey samples	Total Phenolic Content	Total Flavonoid Content
80% methanol extracts		
Old Upper Mountain	9.71 ± 0.01	7.76 ± 0.04
Old Mangrove	7.61 ± 0.03	5.05 ± 0.00
Young Mangrove	5.03 ± 0.02	4.00 ± 0.01
Young Upper Mountain	4.86 ± 0.02	3.23 ± 0.01
Tropical	3.43 ± 0.02	2.45 ± 0.03
Potiukan	4.08 ± 0.02	2.78 ± 0.00
Manuka	10.61 ± 0.00	9.84 ± 0.00
Absolute methanol extracts		
Old Upper Mountain	9.12 ± 0.04	5.71 ± 0.01
Old Mangrove	6.00 ± 0.01	3.01 ± 0.01
Young Mangrove	4.57 ± 0.02	2.74 ± 0.00
Young Upper Mountain	4.30 ± 0.01	2.64 ± 0.02
Tropical	2.46 ± 0.02	2.27 ± 0.01
Potiukan	3.58 ± 0.01	2.37 ± 0.00
Manuka	8.81 ± 0.00	8.07 ± 0.00

Values are expressed as mean ± standard deviation (SD), [1]Total phenolic content values are expressed as mg gallic acid equivalents in 1 g of honey (mg GAE/ 1 g honey).,[2]Total flavonoid content values are expressed as mg rutin equivalents in 1 g of honey (mg RE/ 1 g honey).

Antimicrobial activity

Honey contained numerous phytochemicals such as phenolic and flavonoid compounds that possess health potential and effective as anti-bacterial agent [35].

Results for the antimicrobial study of the honey of Sabah, Malaysian Borneo showed that gram-positive bacteria (*S.aureus*, *B.subtilis* and *B.cereus*) were the most sensitive bacteria as compared to the gram-

negative bacteria. In the preliminary screening result, the results showed that old Upper Mountain honey showed promising antimicrobial effect against *S.aureus*, *B.cereus* and *B.subtilis*, with the inhibition were observed against *S.aureus* with the value of 6.00 ± 0.01 mm (at 100% concentration) in 80% methanol extract and 4.00 ± 0.04 mm (at 100% concentration) in absolute methanol extracts; respectively (Table 3-Table 5). No inhibition was observed by negative control (distilled water).

Mohapatra *et al.,* [3] revealed that the differences in the inhibitory zones were due to osmotic effect, pH, and the presence of hydrogen peroxide and phytochemicals [3]. This was supported by Agbaje *et al.,* [36] who reported that non-peroxide chemicals such as phenolic, flavonoid and methylglyoxal compounds might contributes to the antibacterial properties in honey. The antimicrobial results also showed that the honey extracts were more sensitive towards the gram-positive bacteria than gram-negative bacteria. This was in agreement with Cooper *et al.,* [37] and Mundo *et al.,* [38] who reported that raw honey displayed sensitive anti-microbial properties towards *S.aureus* as compared to *E.coli* in *in vitro* experiment. Basualdo *et al.,* [39] reported that there is a variation in the antibacterial activity of honey against different types of microorganisms. Differences of floral sources and geographical factors such as temperature, humidity and the presence of putative antibacterial agents could be one of the possibilities [40,41]. As reported by Yap *et al.,* [42] upper mountain and mangrove areas contain diverse floral resources that can be utilize by wild honey bee.

According to Melissa *et al.,* [43] the presence of unstable putative and thermobile antibacterial agents could as well become barrier and affected the sensitivity of honey extracts towards the microorganisms. Potiukan and Tropical honey only showed antimicrobial effect against *B.cereus* (**Table 4**) and no antimicrobial effects against gram-negative bacteria (*E.coli* and *S.enteritidis*) (data not shown) for 80% and absolute methanol extracts.

Antimicrobial activity of antibiotics and positive control showed stronger inhibition activity in 80% methanol extract as compared to absolute methanol extract (Table 6-Table 7).

Table 3: Antimicrobial activity of selected honey of Sabah against *S.aureus* in 80% and absolute methanol extractions

Concentration of 80% methanol extract (v/v)	Inhibition zone (mm) old Upper Mountain	Inhibition zone (mm) old Mangrove	Inhibition zone (mm) young Mangrove	Inhibition zone (mm) young Upper Mountain
25%	2.00 ± 0.03	1.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.01
50%	3.00 ± 0.00	2.00 ± 0.11	2.00 ± 0.01	2.00 ± 0.00
75%	5.00 ± 0.00	3.00 ± 0.00	3.00 ± 0.02	3.00 ± 0.00
100%	6.00 ± 0.01	4.00 ± 0.01	4.00 ± 0.01	4.00 ± 0.09
Concentration of absolute methanol extract (v/v)	**Inhibition zone (mm) old Upper Mountain**	**Inhibition zone (mm) old Mangrove**	**Inhibition zone (mm) young Mangrove**	**Inhibition zone (mm) young Upper Mountain**
25%	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.01
50%	2.00 ± 0.01	1.00 ± 0.01	2.00 ± 0.02	1.00 ± 0.02
75%	3.00 ± 0.00	2.00 ± 0.01	2.00 ± 0.07	2.00 ± 0.01
100%	4.00 ± 0.04	3.00 ± 0.01	3.00 ± 0.13	3.00 ± 0.03

Values are expressed as mean ± standard deviation (SD)

Table 4: Antimicrobial activity of selected honey of Sabah against *B.cereus* in 80% and absolute methanol extractions

Concentration of 80% methanol extract (v/v)	Inhibition zone (mm) old Upper Mountain	Inhibition zone (mm) old Mangrove	Inhibition zone (mm) young Mangrove	Inhibition zone (mm) young Upper Mountain
25%	2.00 ± 0.01	2.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01
50%	3.00 ± 0.04	3.00 ± 0.01	2.00 ± 0.03	2.00 ± 0.02
75%	4.00 ± 0.00	4.00 ± 0.06	3.00 ± 0.01	3.00 ± 0.03
100%	5.00 ± 0.03	5.00 ± 0.05	4.00 ± 0.02	5.00 ± 0.02
Concentration of absolute methanol extract (v/v)	**Inhibition zone (mm) old Upper Mountain**	**Inhibition zone (mm) old Mangrove**	**Inhibition zone (mm) young Mangrove**	**Inhibition zone (mm) young Upper Mountain**
25%	1.00 ± 0.01	1.00 ± 0.02	1.00 ± 0.00	1.00 ± 0.15
50%	2.00 ± 0.02	2.00 ± 0.01	2.00 ± 0.02	2.00 ± 0.22
75%	3.00 ± 0.03	3.00 ± 0.01	3.00 ± 0.03	3.00 ± 0.01
100%	4.00 ± 0.01	4.00 ± 0.00	4.00 ± 0.01	3.00 ± 0.21

Values are expressed as mean ± standard deviation (SD)

Concentration of 80% methanol extract (v/v)	Inhibition zone (mm) Potiukan	Inhibition zone (mm) Tropical	Concentration of absolute methanol extract (v/v)	Inhibition zone (mm) Potiukan	Inhibition zone (mm) Tropical
25%	1.00 ± 0.01	1.00 ± 0.01	25%	1.00 ± 0.01	1.00 ± 0.01
50%	2.00 ± 0.02	2.00 ± 0.02	50%	2.00 ± 0.02	2.00 ± 0.02
75%	3.00 ± 0.02	3.00 ± 0.03	75%	2.00 ± 0.01	2.00 ± 0.03
100%	4.00 ± 0.01	4.00 ± 0.04	100%	2.00 ± 0.01	3.00 ± 0.03

Values are expressed as mean ± standard deviation (SD)

Relation between physicochemical, phytochemicals and antimicrobial activity

Previous studies showed the correlation between physicochemical and phytochemicals [2] as well as phytochemicals and antimicrobial activity in honey [31, 42]. Accordingly, correlation analysis was performed and showed that there were a strong positive correlation between the colour intensity with the total phenolics and flavonoids contents with the values of ($r = 0.921$, $p<0.01$), ($r = 0.884$, $p<0.01$); respectively. Meanwhile, the antimicrobial activity and the total phenolic and flavonoid contents showed moderate positive correlation with the values of ($r = 0.711$, $p<0.01$) and ($r = 0.746$, $p<0.01$); respectively. Total phenolic content also showed strong positive correlation with total flavonoid content ($r= 0.924$, $p<0.01$) and in agreement with previous literature [44,45]. Meanwhile, moderate positive correlation was observed between total phenolic content with electrical conductivity with the value of ($r= 0.591$, $p<0.01$). The results of this study were in agreement with earlier

literature by Jasna *et al.,* [2] which showed strong correlation between phenolic and flavonoid contents in all honey samples. Total phenolic contents in Coconut and Gelam honey were contributed mainly by the flavonoid contents. Study by Nuriza *et al.,* [29], reported that the antimicrobial activity of Malaysian honey contributed mainly by polyphenol phytochemicals.

Table 5: Antimicrobial activity of selected honey of Sabah against *B.subtilis* in 80% and absolute methanol extractions

Concentration of 80% methanol extract (v/v)	**Inhibition zone (mm) old Upper Mountain**	**Inhibition zone (mm) old Mangrove**	**Inhibition zone (mm) young Mangrove**	**Inhibition zone (mm) young Upper Mountain**
25%	2.00 ± 0.01	2.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.02
50%	3.00 ± 0.01	3.00 ± 0.02	2.00 ± 0.02	2.00 ± 0.01
75%	4.00 ± 0.02	4.00 ± 0.01	3.00 ± 0.01	3.00 ± 0.02
100%	6.00 ± 0.01	5.00 ± 0.01	4.00 ± 0.02	4.00 ± 0.01
Concentration of absolute methanol extract (v/v)	**Inhibition zone (mm) old Upper Mountain**	**Inhibition zone (mm) old Mangrove**	**Inhibition zone (mm) young Mangrove**	**Inhibition zone (mm) young Upper Mountain**
25%	2.00 ± 0.00	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.03
50%	3.00 ± 0.02	2.00 ± 0.02	1.00 ± 0.01	1.00 ± 0.01
75%	3.00 ± 0.01	3.00 ± 0.02	2.00 ± 0.01	2.00 ± 0.02
100%	4.00 ± 0.00	4.00 ± 0.01	3.00 ± 0.02	3.00 ± 0.00

Values are expressed as mean ± standard deviation (SD)

Table 6: Antimicrobial activity of selected antibiotics against tested microorganisms

Inhibition zone (mm) Canamycin (µg/ml)	***S.aureus***	***B.cereus***	***B.subtilis***	***E.coli***	***S.enetritidis***
25%	12.00 ± 0.01	12.00 ± 0.01	10.00 ± 0.03	10.00 ± 0.02	12.00 ± 0.01
50%	14.00 ± 0.00	14.00 ± 0.01	12.00 ± 0.01	12.00 ± 0.01	14.00 ± 0.02
75%	16.00 ± 0.01	16.00 ± 0.01	14.00 ± 0.04	13.00 ± 0.01	15.00 ± 0.02
100%	20.00 ± 0.01	18.00 ± 0.01	16.00 ± 0.01	14.00 ± 0.01	18.00 ± 0.01
Inhibition zone (mm) Ampicillin (µg/ml)	***S.aureus***	***B.cereus***	***B.subtilis***	***E.coli***	***S.enetritidis***
25%	8.00 ± 0.01	10.00 ± 0.04	8.00 ± 0.05	6.00 ± 0.01	10.00 ± 0.01
50%	9.00 ± 0.03	12.00 ± 0.02	10.00 ± 0.03	8.00 ± 0.00	12.00 ± 0.03
75%	10.00 ± 0.01	13.00 ± 0.01	11.00 ± 0.02	10.00 ± 0.02	14.00 ± 0.01
100%	12.00 ± 0.01	14.00 ± 0.01	14.00 ± 0.02	12.00 ± 0.01	16.00 ± 0.02

Values are expressed as mean ± standard deviation (SD)

Table 7: Antibacterial activity of positive control against tested microorganisms in 80% and absolute methanol extractions

Inhibition zone (mm) Manuka (80% methanol extract)	***S.aureus***	***B.cereus***	***B.subtilis***	***E.coli***	***S.enetritidis***
25%	4.00 ± 0.02	4.00 ± 0.07	2.00 ± 0.00	2.00 ± 0.02	2.00 ± 0.01
50%	5.00 ± 0.01	6.00 ± 0.01	4.00 ± 0.01	3.00 ± 0.01	3.00 ± 0.02
75%	6.00 ± 0.09	8.00 ± 0.02	6.00 ± 0.04	4.00 ± 0.04	4.00 ± 0.03
100%	8.00 ± 0.01	10.00 ± 0.03	8.00 ± 0.06	6.00 ± 0.06	5.00 ± 0.03
Inhibition zone (mm) Manuka (absolute methanol extract)	***S.aureus***	***B.cereus***	***B.subtilis***	***E.coli***	***S.enetritidis***
25%	2.00 ± 0.11	3.00 ± 0.09	2.00 ± 0.01	1.00 ± 0.00	1.00 ± 0.01
50%	4.00 ± 0.01	5.00 ± 0.11	3.00 ± 0.00	2.00 ± 0.02	2.00 ± 0.02
75%	5.00 ± 0.00	6.00 ± 0.02	4.00 ± 0.04	4.00 ± 0.06	3.00 ± 0.02
100%	6.00 ± 0.03	7.00 ± 0.06	5.00 ± 0.01	5.00 ± 0.01	4.00 ± 0.04

Values are expressed as mean ± standard deviation (SD)

CONCLUSION

In conclusion, the present study showed that selected honey of Sabah contained a wide range of phytochemicals including phenolic and flavonoid compounds that might contributed to the antimicrobial properties. Further studies on the isolation and identification of bioactive compounds and possible mechanism of action should be done continuously as it might provide new information on the efficacy of honey as antibacterial agent.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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