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Phenolic Profiles, Antioxidant, Antibacterial Activities and Nutritional Value of Vietnamese Honey from Different Botanical and Geographical Sources

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Abstract: Honey is a natural product made by honeybees, its composition depends on factors such as climate, soil and plant source. In this study, the nutritional parameters, phenolic composition, antioxidant activity and antibacterial ability of 30 different types of honey of different botanical and geographical origins in Vietnam were investigated. The study focused on the characterization and evaluation of the influence of plant origin and geographical location on physical–chemical properties and biological activities (antioxidant and antibacterial). The obtained results show that all honey samples meet quality standards according to international standards and Vietnamese standards, except for some exceptions recorded in moisture, 5-hydroxymethylfurfural (HMF) value and ash. These samples were explored for the detection of 13 polyphenols by using high-performance liquid chromatography (HPLC). The classification of honey samples collected from different regions and botanical sources was performed by principal component analysis (PCA), and it was observed that certain phenolic compounds contributed to the identification of honey samples. In addition, the correlation between physicochemical properties, chemical composition and biological activity of most honeys was also first clarified in this study. Overall, our data provide an overview data set and essential results in creating a database on the world honey trait map.

Keywords: physicochemical properties; chemical composition; antioxidant activity; biological properties; principal component analysis

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1. Introduction

Honey is a natural food with a long history of use as a natural sweetener or functional food in many cultures worldwide to support health [1]. Honey is proven to be a good product capable of promoting many beneficially biological actions for human consumption, such as antibacterial, anti-inflammatory, antioxidant activities and wound healing [2–4]. The biological effects of honey are mainly due to the variety of its chemical, physical, and biological components. Accordingly, honey is a product produced by honeybees, and its main component is a complex of carbohydrate chains (accounting for 95% of the dry weight of honey) [5]. Alternatively, it can be considered a natural supersaturated sugar solution with primarily glucose and fructose (1.2:1 ratio) and 1% of sucrose in total. However, the fructose: glucose ratio is mainly dependent on the source of nectar. Therefore, the composition and properties of each honey show significant differences according to the

origin of the plant species where the bees get the nectar. In addition, honey also contains many auxiliary components such as proteins, enzymes (invertase, glucose oxidase, catalase, phosphatases), organic acids (gluconic acid, acetic acid, etc) and amino acids, vitamins (ascorbic acid, niacin, pyridoxine, etc), lipids, phenolic acids, flavonoids and minerals [6–8].

Currently, the quality of honey is usually based on its physicochemical, sensory and microbiological properties. The type of nectar (plant origin), geographical origin (climatic conditions), and processing method are the factors affecting the physicochemical properties of honey. In addition, there have been many reports showing the high efficiency of using physicochemical parameters such as moisture, sugar composition, pH, acidity, ash, HMF, and colour to distinguish different types of honey [9–12]. Many research groups have recognised the diversity of chemical composition, biological activity and antibacterial ability of honey. Ginnie Ornella Lai Moon Dor and colleagues (2014) performed an evaluation on honey in Mauritius and found the best 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability in eucalyptus honey (The half-maximal inhibitory concentration—IC_{50} = 31.24 ± 0.75 mg/mL) and honey Longan flower bee (IC_{50} = 31.30 ± 0.85 mg/mL). In particular, the study showed a positive correlation between the total polyphenol content (TPC = 74 mgGAE/100 g) and total flavonoid content (TFC = 11.5 QEmg/100 g) in the antioxidant capacity of this honey [13]. Another study on honey in Thailand reported that coffee flower honey had phytochemicals (TPC = 734.76 mgGAE/kg and TFC = 178.31 mgQE/kg) and the highest DPPH free radical inhibitory activity compared to other honey, such as longan flower honey, lychee flower honey, etc. This study demonstrated the potential of honey for use as an effective antibacterial, antioxidant, and anti-tyrosinase therapy [14]. The report, after evaluation of honey collected in Japan, noted that DPPH free radical inhibition of eucalyptus honey and chamomile honey was the most effective (IC_{50} 65.09 mg/mL and 62.2 mg/mL) and the highest TPC value (66.45 mgGAE/100 g) was observed in eucalyptus honey [7]. For studies on the correlation between physicochemical components and biological activities of honey, many studies have recorded a very close correlation between TPC composition and TFC's ability to inhibit DPPH free radicals [15]. The reviews that suggest the correlation obtained between antioxidant capacity and TPC indicated that phenolic compounds are mainly responsible for the antioxidant effects of honey and the antioxidant activity of phenols mainly arises from their redox properties. Phenolic composition and quantities of phenolic compounds among honeys based on different floral sources, geographical factors, environmental factors, seasonal factors and nectar collection methods. The main influencing factor is flower origin, while processing, handling and storage have little impact on honey's structure and phenolic composition. In addition, phenolic acids and flavonoids are also used as biological markers to identify the botanical origin of honey [16].

With natural climatic conditions supporting fruit trees' growth, honey in Vietnam has become more diverse, prosperous, and abundant than in some other countries. From the northern region to the southern region, depending on the local flora characteristics, some great honeys well known throughout Vietnam are recorded, such as longan flower honey, coffee flower honey, lychee flower honey, mint flower honey, coconut flower honey, rubber honey, Melaleuca flower honey, rambutan flower honey, mangrove flower honey. However, despite the diversity in honey types, the beekeeping industry is in its infancy because it has been only focused on production efficiency instead of fully exploiting honey's characteristics in each locality. In addition, to our best knowledge, there has not been a general study or in-depth analysis of physicochemical properties, chemical composition, biological activity and antibacterial ability of domestic honey in Vietnam. Therefore, this study is considered the first to evaluate and provide detailed data sets on essential parameters of different types of honey in Vietnam. The data were collected, analysed by qualitative and quantitative methods (Ultraviolet-visible absorption spectroscopy (UV-Vis) and High-performance liquid chromatography (HPLC)) and then statistically processed according to the Pearson correlation evaluation model and principal component analysis (PCA) to pro-

vide reliable conclusions about the correlation between quality criteria, biological activity, chemical composition for plant origin and geographical location of honey.

2. Materials and Methods

2.1. Honey Samples

Thirty (commercial) honey samples were purchased from various suppliers and conducted in February 2022 at the Institute of Applied Technology and Sustainable Development, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam. Information on the botanical and geographical origin of the honey samples is listed in Table 1 and Figure 1. Honey samples were analysed within two months after being collected at the research unit. Manuka honey was used as the standard for comparison. After collection, all honey samples were stored in glass bottles and refrigerated at 4–5 °C until analysis.

Table 1. Classification of honey types and regional sources.

Honey Code	Sample	Scientific Name	Geographic Regions
HN1 (n = 3)	Longan flower honey	*Dimocarpus longan*	Ben Tre
CF2 (n =3)	Coffee flower honey	*Coffea arabica*	DakLak
HV3 (n = 3)	Lychee flower honey	*Litchi chinensis*	Bac Giang
BH4 (n = 3)	Mint flower honey	*Mentha arvensis*	Ha Giang
HD5 (n = 3)	Coconut flower honey	*Cocos nucifera*	Ben Tre
CS6 (n = 3)	Rubber leaf honey	*Hevea brasiliensis*	Gia Lai
HT7 (n = 3)	Melaleuca flower honey	*Melaleuca alternifolia*	Ca Mau
CC8 (n =3)	Rambutan flower honey	*Nephelium lappaceum*	Dong Nai
SV9 (n =3)	Mangrove flower honey	*Rhizophora mangle*	Nam Dinh
HK10 (n =3)	Acacia flower honey	*Acacia (Robinia pseudoacacia)*	Thai Nguyen
Manuka (Control)	Manuka Honey	*Leptospermum scoparium*	New Zealand

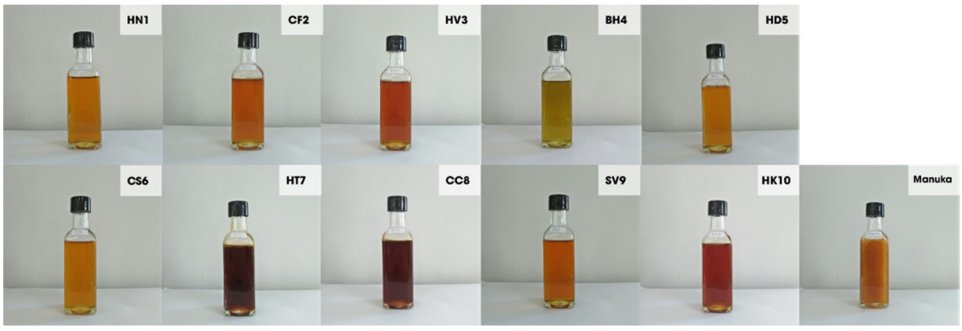


Figure 1. Colours of honey samples.

2.2. Physical-Chemical Analysis

Moisture content was determined by drying 5 g of each honey sample at 105 °C until a constant mass was recorded. The total soluble solids content was measured with a refractometer (ATAGO PR-101Alpha). A few drops of honey were applied to the refractive prism of the instrument, and the value displayed on the screen was then recorded (AOAC, 2000). Free acidity was determined by titration method (AOAC, 2000) using a potentiometric Mehtrom Eco Titrator (Switzerland). The water activity (a_w) was determined using a Novasina AG CH-8853 Lachen instrument (Switzerland). The ash content was determined according to the methods of AOAC (2000). Honey samples were ashed at 600 °C ± 25 °C for 3 h using a calcination device SGIFITECH-FTMF-701 (SCI Finetech—Korea).

2.3. Hydroxymethylfurfural (HMF)

The HMF content was determined using the standard AOAC procedure (AOAC, 2000). Five grams of honey dissolved in 25 mL of distilled water was measured for its absorbance at wavelengths 284 and 336 (using Aligent Cary 60 UV instrument, visible spectrometer, Santa Clara, CA, USA) with 0.2 $NaHSO_3$ as the reference solution. The absorbance should be less than 0.6, and it must be diluted with 0.2% $NaHSO_3$ solution if it is greater than 0.6. HMF is calculated using the formula:

$$HMF\left(\frac{mg}{kg}\right)\frac{(OD_{284}-OD_{336})\times 149.7\times 5\times \mathrm{D}}{W} \quad (1)$$

where D is the dilution factor, and W is the sample mass.

2.4. Colour

The colour of honey samples was measured based on reflectometry using a Chroma CR-400 m (Konica Minolta Sensing, Inc., Osaka, Japan). The reflectance of the entire visible spectrum (360–780 nm) was recorded at the wavelength range of 10 nm. The CIE colour parameters used were L* (Brightness: L* = 100 for white and 0 for black), a* (red/green axis: positive a* is red and negative a* is green), b* (yellowness/blueness axis: positive b* is yellow and negative b* is blue). The device will display these parameters on the screen after the sample measurement is finished.

2.5. Total Sugar

A total of 2.5 g of honey was diluted with 125 mL of distilled water and then heated at 80 °C for 15 min, then allowed to cool at room temperature. A total of 10 mL of 10% $Pb(CH_3COO)_2$ was added to the mixture, and the mixture was evenly shaken and then allowed to settle. A total of 5 mL Na_2HPO_4 of the mixture was allowed to settle, then filtrated with filter paper. The filtrate was collected and made up to 500 mL with distilled water. A total of 100 mL of filtrate solution was added to a 250 mL conical flask with the addition of 15 mL of 33% HCl. The mixture was then heated in a water bath at 100 °C for 15 min and then allowed to cool at room temperature. The mixture was neutralized by 30% NaOH and then made up to 250 mL. A volume (10 mL) of the mixture solution was mixed with 25 mL Fehling A and 25 mL Fehling B, then boiled for 30 min. After cooling, copper oxide precipitate (red colour) was obtained, filtered, and washed with distilled water. The precipitate was dissolved in 10 mL of 5% $Fe_2(SO_4)_3$. The mixture was then titrated with 0.1 N $KMnO_4$ solution until the appearance of persistent dark pink colour for 1 min. The volume of $KMnO_4$ solution was recorded and calculated for the total sugar content.

2.6. Phytochemical Screening

Apply qualitative analytical methods to detect secondary metabolites and phytochemical compounds present in honey. Honey samples were qualitatively tested based on selected from the methods of T.N. Pham et al. (2020) and G. Edo (2022) [17,18] (Table 2).

Table 2. Methods for qualitative phytochemical compounds.

Compounds	Implementation Methods	Observation
Alkaloid	Sample + HCl + Dragendorff	Yellow precipitation
Flavonoid	Sample + NaOH 1% -> A A + HCl 1% -> B	A: Dark yellow B: Colour loss
Saponin	Sample + 1 mL distilled water and a few drops of olive oil -> boiled 90 °C	The solution turns into a milky emulsion
Tannin	Sample + $Pb(C_2H_3O_2)_2$	Yellow precipitation
Steroids	Sample + 1 mL $CHCl_3$ + 1 mL CH_3COOH	Red–brown
Phenolic	Sample + $FeCl_3$ 1%	Green or blue–green

Based on the degree of reaction and the colour expression of the solution after the reaction: (-): There is no such compound in the sample. (+): A group of compounds that exist in the sample.

2.7. Total Polyphenol Content

Total polyphenol content was determined using the Folin–Ciocalteu method based on the previous report of Tri Nhut Pham et al. (2020) with correction [19]. First, the honey was diluted to the right concentration. Next, 0.3 mL of diluted honey was put into a test tube, and 1.5 mL of Folin—Ciocalteu solution (10%) was added and homogenized using a vortex device. After allowing the solution to stabilize for 5 min, 1.2 mL of Na_2CO_3 (7.5%) was added and mixed well. Then, the mixture was incubated at room temperature in the dark for 30 min and then the absorbance was recorded at 765 nm using a UV-Vis spectrometer (Evolution 60S—THERMO—USA). The polyphenol content (mg GAE/mL sample solution) was calculated using the equation y = ax + b of the gallic acid standard curve.

2.8. Total Flavonoid Content

The total flavonoid content was determined using the aluminium chloride colorimetric method reported by Tri Nhut Pham [19]. First, the honey was diluted to the right concentration. A total of 2 mL of diluted honey was placed in a test tube and 0.1 mL of 10% aluminium chloride ($AlCl_3$) solution and 0.1 mL of 0.1 mM potassium acetate solution was added. The mixture was placed in the dark at room temperature. Then, the mixture was determined optically at 415 nm using a UV-Vis spectrophotometer (Evolution 60S—THERMO—USA). The total flavonoid content of the sample is presented as mgQE/100 g sample.

2.9. Phenolic Compound Analysis by U-HPLC

The conjugated phenolic acids were extracted from honey according to the study of Quang Vinh Nguyen [20]. The honey was centrifuged to remove the residue, then weighed and dissolved honey with an aqueous solution of pH = 2 in a ratio of 1:5 (honey: water). Samples were extracted with SPE solid phase, eluted with MeOH, then filtered through a 0.45 µm filter, and then injected into the UHPLC—Thermo-Ultimate 3000 UPLC system (Thermo Scientific-USA) with a volume of 2 µL. The analysis was performed on a PFP-C18 column maintained at 30 °C (150 × 4.6 × 3 mm), a particle size of 5 µm and connected to a tracking UV detector at 265 nm. The elution was performed with a solvent system consisting of (A) methanol and (B) water acidified with 0.1% H_3PO_4. Separations were performed using a segmented linear gradient as follows: From 0 to 0.5 min 95% B, from 0.5 to 8.0 min 95–83%B, from 8.0 to 10.0 min 83–70%B, from 10 to 15 min 70–55%B, 15–20 min 55–5%B, 20 to 22 min 5–95%B, 22 to 26 min 95%B with flow rate 0.2 mL/min. The procedure time was maintained for 26 min. Standard substances used included: Gallic acid, catechin, Chlorogenic acid, Caffein, Epicatechin gallate, Vitexin, Salicylic acid, Rutin, Apigetrin, Quercitrin, Quercetin, Kaempferol and Apigenin. Phenolic compounds were determined by comparing the retention times, peak areas and UV-Vis spectra of the respective standards.

2.10. DPPH Free Radical Scavenging Ability

The DPPH free radical scavenging capacity was referenced as reported by Tri Nhut Pham [21]. Dilute the honey to the appropriate concentration and then add 0.5 mL to the test tube. Next, add 1.5 mL of DPPH (OD_{517} nm = 1.1 ± 0.02) to the test tube, mix well and incubate in the dark for 30 min. Then, the sample was analysed for optical density at 517 nm using a UV-VIS spectrophotometer (Evolution 60S—THEMO—USA). The results are recorded based on the IC_{50} value, which is the concentration at which the sample can reduce 50% of DPPH free radicals.

$$\text{DPPH}\ (\%) = \frac{\text{Abs}_C - \text{Abs}_T}{\text{Abs}_C} \times 100 \quad (2)$$

In there:
Abs_C: Optical absorbance of the control sample
Abs_T: Optical absorbance of the specimen

2.11. ABTS Free Radical Scavenging Ability

ABTS free radical scavenging capacity was referenced as reported by Tri Nhut Pham [21]. First, ABTS free radical solution was prepared by adding 10 mL of 7.4 mM ABTS solution to 10 mL of 2.6 mM $K_2S_2O_8$ solution and incubating in the dark for 24 h. Then, it was diluted with ethanol and the absorbance of the solution at 734 nm to 1.1 ± 0.02 was adjusted. The honey was diluted to the appropriate concentration, and then 0.5 mL was added to the test tube. Next, 5 mL of ABTS solution (OD_{517nm} = 1.1 ± 0.02) was added into the test tube, shake well and incubate in the dark for 30 min. Measure the optical absorbance at 734 nm using a UV-VIS spectrophotometer (Evolution 60S—THERMO—USA). The results are recorded based on the IC_{50} value, which is the concentration at which the sample can reduce 50% of ABTS free radicals.

$$\text{ABTS } (\%) = \frac{\text{Abs}_C - \text{Abs}_T}{\text{Abs}_C} \times 100 \quad (3)$$

In there:
Abs_C: Optical absorbance of the control sample
Abs_T: Optical absorbance of the specimen

2.12. Antibacterial Activity

Testing the antibacterial activity of honey by agar plate diffusion method was carried out as described by M. Balouiri et al. (2016) [22]. The antibacterial activity of honey was carried out based on the agar plate diffusion method in bacterial strains, including *Escherichia coli* NRRL B-409 and *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Honey samples were prepared at a concentration of 90% (*w*/*v*) in MH medium and filtered through a 0.22 µm filter. The bacterial broth was spread evenly on a Petri dish containing the MHA medium. Holes were drilled in the agar plate (6 mm in diameter), then 90 µL of honey solution was put into the agar wells on the Petri dish, incubated at 4 °C for 8 h so that the antibacterial active substance from the agar well diffuses into the environment then the Petri dish was incubated at 37 °C for 24 h. The control used was the antibiotic ampicillin. The antibacterial activity of honey was determined by measuring the antibacterial ring diameter (mm).

2.13. Statistical Analysis

To verify the differences or similarities between plants and regions, statistical analyses are used based on the results of the physicochemical analysis. Minitab software version 20.3 (Sydney, Australia) and Origin Pro version 2016 (OriginLab Corp., Northampton, MA, USA) were used to perform the ANOVA statistical analysis. The data represent the mean value of 3 replicates ± standard deviation with a significance level of $p \leq 5\%$. All data were normalized, and similarity between samples was calculated using Pearson correlation. Principal components analysis (PCA) is used to reduce (or eliminate) data duplication and produce the most representative variables by combining the original variables in a single data set of variables with the same dimension.

3. Results and Discussion

3.1. Phytochemical Screening

Thirty honey samples and manuka honey (control) were screened for the presence of secondary metabolites, including alkaloids, flavonoids, saponins, sulphates, tannins, steroids and phenolic compounds. The screening results presented in Table 3 showed no presence of tannins and steroids in all honey samples. The foaming test showed that 6 out of 11 types of honey (coffee flower honey, lychee flower honey, melaleuca flower honey, rambutan flower honey, mangrove flower honey and manuka honey) had a positive reaction with saponins. This result is similar to the previous report when recording the presence of saponins in longan flower honey, lychee honey and rubber honey in Mauritius and Indonesia [13,23]. In addition, the reaction showing the presence of phenolics,

and flavonoids was found to be positive in all tested honey samples. Nwankwo et al. (2014) also recorded similar results about the presence of phenolic and flavonoid when evaluating honey in Nigeria [24]. This indicated that all Vietnamese honey contained valuable phytochemical compounds.

Table 3. Phytochemical identification results of different types of honey.

Sample	Qualitative Indicators					
	Alkaloids	**Flavonoids**	**Saponin**	**Tannins**	**Steroid**	**Phenolics**
HN1	+	+	+	-	-	+
CF2	+	+	+	-	-	+
HV3	+	+	+	-	-	+
BH4	+	+	-	-	-	+
HD5	-	+	-	-	-	+
CS6	+	+	+		-	+
HT7	-	+	+	-	-	+
CC8	-	+	+	-	-	+
SV9	-	+	+	-	-	+
HK10	-	+	-	-	-	+
Manuka	+	+	+	-	-	+

HN1: Longan flower honey; CF2: Coffee flower honey; HV3: Lychee flower honey; BH4: Mint flower honey; HD5: Coconut flower honey; CS6: Rubber leaf honey; HT7: Melaleuca flower honey; CC8: Rambutan flower honey; SV9: Mangrove flower honey; HK10: Acacia flower honey; Manuka: Manuka Honey (Control).

3.2. Physicochemical Properties

Moisture is one of the important criteria for evaluating the quality of honey. Too much water in honey causes the formation of yeasts (resulting in the formation of ethyl alcohol and carbon dioxide). The product is fermented under the influence of natural oxidation to form acetic acid and water, creating a sour taste [25]. In addition, the moisture value is also shown to have a close correlation with the dry matter content (a measure to determine the total content of inorganic and organic substances, including molecular, ionized, microparticles, or suspension) in honey.

The results of moisture analysis in honey samples are presented in Table 4. In general, the moisture content of honey ranged from 10.78–23.96%. Melaleuca honey had the highest moisture content of 23.71%, and longan flower honey showed the lowest moisture content of 10.78%. Except for samples of longan flower honey, melaleuca flower honey and rambutan flower honey, the others were evaluated with moisture content below 20%, which is the maximum acceptable limit according to the Codex standard for honey [26]. The moisture content of longan flower honey and lychee flower honey in Vietnam had values of 10.78 $\pm$ 0.35% and 15.45 $\pm$ 0.33%, respectively, which were lower than those collected in Thailand (20.11. $\pm$ 1.16% and 19.97 $\pm$ 0.85%) [10]. While Indonesian rubber honey had a relatively high moisture content (22.5–30%), the moisture content of rubber honey in Vietnam experienced a lower value of 16.86 $\pm$ 0.21% [23]. When compared with the control sample, which is manuka honey, it was found that Vietnamese honey samples had almost higher moisture content (except for longan honey samples). However, in general, the obtained analytical results showed similarities with previous studies on honey from countries with similar climate and soil conditions to Vietnam. The moisture content of honey was reported to range from 17.86 to 19.06% in Malaysia, 17.8 to 21.7% in Thailand, and from 18 to 26% in Indonesia [27–29]. The difference in moisture content of honey was influenced by various factors such as the time of extraction, the maturity of the hive, and the climatic conditions [21]. At the same time, the moisture value showed a significant influence on the dry matter content (°Brix), which was a tool to determine the presence of organic and inorganic matter in honey products. The TSS value of Vietnamese honey showed the lowest value, with 70.99% for rambutan flower honey and 84.04% for mint flower honey. This result was consistent with the previous record of Mexican honey (65.42–85.42) and Brazilian honey (79.4–83.4) [3,12]. Many previous studies indicated that the low moisture

levels of honey were more resistant to the fermentation process, thereby the shelf life of the honey product was enhanced [30,31]. The results of the moisture value could be a useful tool for beekeepers and honey producers to have appropriate methods to optimize the preservation and storage of products.

Table 4. Physical-chemical parameters of the honey samples.

Sample	Physical-Chemical Parameter						
	Moisture (%)	Brix	HMF (mg/kg)	Total Sugar (%)	Acidity (meq/kg)	Ash (%)	a_w
HN1	10.78 ± 0.35^{g}	79.22 ± 0.94^{cd}	28.38 ± 0.82^{de}	88.60 ± 1.12^{a}	12.79 ± 0.51^{h}	0.52 ± 0.02^{d}	0.700 ± 0.002^{a}
CF2	22.38 ± 0.27^{b}	74.60 ± 0.22^{f}	48.15 ± 0.81^{b}	60.60 ± 1.52^{gh}	35.85 ± 0.43^{d}	0.18 ± 0.02^{l}	0.618 ± 0.002^{e}
HV3	15.45 ± 0.33^{d}	81.03 ± 0.41^{b}	58.58 ± 0.71^{a}	71.68 ± 1.31^{cd}	20.29 ± 1.01^{e}	0.60 ± 0.01^{b}	0.601 ± 0.001^{f}
BH4	14.15 ± 0.26^{e}	84.04 ± 0.36^{a}	30.46 ± 2.00^{d}	84.50 ± 0.72^{b}	14.94 ± 0.49^{g}	0.38 ± 0.02^{g}	0.599 ± 0.002^{fg}
HD5	13.94 ± 0.36^{e}	73.88 ± 0.27^{fg}	27.97 ± 1.47^{de}	68.03 ± 1.05^{e}	17.36 ± 0.56^{f}	0.30 ± 0.02^{i}	0.683 ± 0.001^{b}
CS6	16.86 ± 0.21^{c}	76.82 ± 0.34^{e}	12.37 ± 1.35^{h}	62.50 ± 1.39^{g}	15.32 ± 0.34^{g}	0.46 ± 0.01^{e}	0.632 ± 0.002^{cd}
HT7	23.96 ± 0.38^{a}	73.15 ± 0.65^{h}	34.90 ± 1.83^{c}	65.47 ± 1.23^{f}	48.21 ± 0.53^{a}	0.41 ± 0.02^{f}	0.640 ± 0.002^{c}
CC8	23.71 ± 0.29^{a}	70.99 ± 0.42^{i}	49.49 ± 0.84^{b}	70.87 ± 0.90^{d}	42.25 ± 0.24^{b}	0.56 ± 0.01^{c}	0.622 ± 0.002^{e}
SV9	15.90 ± 0.35^{d}	78.58 ± 0.24^{d}	28.03 ± 0.84^{e}	59.68 ± 1.03^{h}	13.37 ± 0.16^{h}	0.64 ± 0.02^{a}	0.600 ± 0.001^{fg}
HK10	16.63 ± 0.28^{c}	79.22 ± 0.63^{cd}	25.85 ± 1.23^{f}	58.90 ± 1.57^{h}	41.17 ± 0.42^{c}	0.35 ± 0.02^{h}	0.624 ± 0.002^{de}
Manuka	12.79 ± 0.36^{f}	79.84 ± 0.39^{c}	22.43 ± 0.98^{g}	73.38 ± 1.31^{bc}	20.18 ± 0.43^{e}	0.22 ± 0.01^{k}	0.592 ± 0.001^{fg}

Different letters after values in the same column represent statistically significant differences between samples ($p < 0.05$). HN1: Longan flower honey; CF2: Coffee flower honey; HV3: Lychee flower honey; BH4: Mint flower honey; HD5: Coconut flower honey; CS6: Rubber leaf honey; HT7: Melaleuca flower honey; CC8: Rambutan flower honey; SV9: Mangrove flower honey; HK10: Acacia flower honey; Manuka: Manuka Honey (Control).

Honey is primarily acidic, largely due to its gluconic acid content, which is formed by the action of glucose oxidase (GOx) during the separation and conversion to glucose [32]. In particular, free acidity is an indicator of interest when evaluating the quality of any honey. Free acidity represents the presence of organic acids and some other compounds in honey (lactones, esters and inorganic ions) [33]. This index also indicates the presence of chemical components that can donate large amounts of H^+ atoms, such as proteins, phenolic acids and vitamin C [34]. In addition, it is also a factor to help evaluate the quality and freshness of honey as the increase of this index is proportional to the time when the fermentation process of sugars into organic acids occurs. The results of the assessment of free acid content in honey in Vietnam are presented in Table 3. The free acidity value of Vietnamese honey samples ranged from 12.79 to 48.21 meq/kg. Longan flower honey had the lowest acidity value of 12.79 meq/kg, while melaleuca honey showed the highest value of 48.21 meq/kg. Compared to manuka honey, most Vietnamese honey had the same or lower acidity.

Meanwhile, the free acidity values of coffee flower honey (35.850 meq/kg), melaleuca flower honey (48.21 meq/kg), rambutan honey (42.25 meq/kg), and honey acacia bee (41.17 meq/kg) were significantly higher than the control sample. In general, the free acidity of each analyzed sample was not greater than 50 meq/kg. This result was within the allowable limit (<50 meq/kg) according to the international honey standard (Codex Alimentarius) and the law of the European Union (<40 meq/kg). Previously, Paulic et al. (2020) also noted that the free acid content in honey samples collected in Romanian ranged from 16–31.6 meq/kg [34]. Ömer Erturk et al. (2018) reported the free acidity value of honey samples collected in Turkey from 8.8–12.3 meq/kg [11]. For example, when compared with longan flower honey and longan flower honey of Thailand (17.60 meq/kg and 27.84 meq/kg), the acidity value of Vietnamese honey showed lower values (12.79 meq/kg and 20.29 meq/kg) [10]. The differences in free acidity values between different types of honey as well as between different countries could be greatly influenced by flower origin or by differences in honey harvesting time [35]. Similar to the moisture value, the data on the free acid level was also an effective indicator for the producer to be more proactive in controlling the quality of honey products.

HMF is an important indicator used to evaluate the purity and freshness of honey. HMF, which is usually present in trace form in fresh honey, is an active ingredient produced from fructose (the sugar with the highest percentage in honey). This compound is gradually

formed during storage, and its formation is faster due to heating or ageing. The HMF value is also influenced by a number of other factors such as pH, temperature, time of heat exposure of honey, storage conditions and flower source. Thus, the HMF value is an indicator to identify the freshness of honey and the quality of storage conditions [12]. The results of HMF content, shown in Table 3, showed that the HMF value of Vietnamese honey had a minimum level of 12.37 mg/kg (rubber leaf honey) and a maximum of 58.58 mg/kg for lychee honey. In particular, honey samples, including longan flower honey, mint flower honey, coconut flower honey, rubber leaf honey, Melaleuca flower honey, mangrove flower honey and acacia flower honey, were considerably valuable with HMF < 40 mg/kg. In contrast, the others exhibited very high HMF values (<80 mg/kg). It can be seen that all Vietnamese honeys showed the HMF levels within the allowable limits of the Codex Alimentarius Committee—the world food standard (under WHO) (<80 mg/kg—for countries in the tropical regions). HMF values in the range of 4 to 58 mg/kg and 10 to 30.8 mg/kg were reported in the evaluation of Brazilian honey and Romanian honey, respectively [12,34]. The HMF values of Vietnamese honey showed a statistically significant difference ($p < 0.05$) among the analysed groups. This may be due to differences in flower source, harvest time and storage method of each production unit.

Monosaccharides (simple sugars—reducing sugars) make up the majority of the honey composition. Specifically, glucose and fructose account for more than 65% of total soluble solids, while the others are oligosaccharides (disaccharides, trisaccharides, tetrasaccharides—poly sugars). The results, shown in Table 3, showed that acacia flower honey had the lowest reducing sugar content at 58.90%, and longan flower honey had the highest value at 88.60%. The results of total sugar content in longan flower honey in this study were lower than those in a previous study (87.35%) [22]. The total sugar content was comparable to some Guinean honey (59–71%), Brazilian honey (67.6–72.4%) and Pakistani honey (61.7–72.4%) [36–38].

Ash content is a quality criterion that can indicate the mineral content of honey and its botanical and geographical origin. The percentage of ash content of all honey samples was distributed from 0.18% to 0.64%, with a statistically significant difference ($p < 0.05$). All honey in this study showed a value of <0.6%, complying with the Codex Alimentarius Commission—the World Food Standard (WHO). Overall, this result was consistent with previous reports that ash content could be used as a marker to distinguish honeys from different floral sources [5]. In addition, it also aids in the detection of abnormalities and defects in honey processing.

3.3. Colour

Colour is a sensory property that determines the consumer's choice of a honey product as they are immediately observable. This is also a parameter to evaluate the applicability of honey in the industry. Because lighter-coloured honeys are often used for direct consumption, darker-coloured honeys are usually used in industry due to their high nutritional value. Usually, light-coloured honeys are considerably more expensive than dark-coloured honeys [38]. However, it has been noted that honey with a dark colour and strong flavour is preferred by people in European countries. Meanwhile, consumers in North American countries prefer honey that is lighter in colour and has a white tone because of its lighter, less intense flavour [39]. The colour of 11 honey types evaluated by using the CIE Lab colour system method is shown in the data in Table 5 and Figure 1.

Table 5. Colour parameters of different types of honey.

Sample	L*	a*	b*
HN1	47.07	6.56	37.84
CF2	41.5	11.64	38.45
HV3	35.3	16.88	30.31
BH4	49.96	3.66	35.05
HD5	45.55	5.83	36.04
CS6	45.27	6.12	37.61
HT7	20.87	13.33	7.41
CC8	22.75	15.59	10.1
SV9	43.89	11.5	40.47
HK10	29.03	21.17	21.25
Manuka	25.83	3.97	10.4

HN1: Longan flower honey; CF2: Coffee flower honey; HV3: Lychee flower honey; BH4: Mint flower honey; HD5: Coconut flower honey; CS6: Rubber leaf honey; HT7: Melaleuca flower honey; CC8: Rambutan flower honey; SV9: Mangrove flower honey; HK10: Acacia flower honey; Manuka: Manuka Honey (Control).

Apparently, the colour of honey varied from pale yellow to dark amber or black. It has been suggested that honey samples with L* > 50 will be considered light honey and those with L* $\leq$ 50 will be dark [26]. The L* value represents the brightness of the sample with the value range from 0 (black) to 100 (complete whiteness). A positive b* value indicates the yellowness of the product, and a positive a* value indicates the redness. According to these colour indicators, it can be seen that the honey in this study is classified as dark honey because the L* index was from 20.87–49.96. The range of a* index was from 3.66–16.88 and the 7.41–40.47 range was assigned to b* index. In general, 7 out of 11 honey types from Vietnam exhibited yellow colour as the b* value was greater than 30. Meanwhile, melaleuca flower honey and rambutan flower honey showed L* values of 20.87 and 22.75, respectively, indicating darker colour than the others. It can be seen that Vietnamese honey showed significant variation in colour compared to previous studies on Iranian honey (L*: 18.95–21.42; a*: −0.003–0.4 and b*: 0.447–1.62), Indian honey (L* = 26.3–36.8; a* = 0.1–4.9 and b* = 3.07–14.7) and Turkish honey (L* = 27.011, a* = 2.647 and b* = 0.436) [26,38,40]. Coffee flower honey (L* = 41.5; a* = 11.64 and b* = 38.45), lychee honey (L * = 35.3; a* = 16.88 and b* = 30.31) and acacia flower honey (L* = 29.03; a* = 21.17 and b* = 21.25) of Vietnam were more bright and dark yellow compared to honey collected in Guatemala with values of L* = 3.32, a* = 1.55 and b* = 1.77 for coffee flower honey, L* = 4.20; a* = 2.85 and b* = 3.08 for lychee honey, and L* = 11.97; a* = 5.19 and b* = 24.18 for acacia honey [4]. The colour of Vietnamese acacia honey had a relatively similar colour index compared to Serbian and Romanian acacia honey [5,34].

Many studies have shown that honey with a darker colour has a better antioxidant effect than those with a lighter colour [41]. Although the commercial honey samples in this study were recognized from a single flower source, more than 50% of honey from a specific flower was technically adequate to give the recognition of that honey type. The rest of other honey compositions from different origins (<50%) can be acceptable, leading to the difference in visual colour, and phytochemical constituents in commercial honey. The honey colour is highly influenced by the origin of the flower and its composition (organic and inorganic). The content of HMF, phenolic, pollen and minerals are significant factors that possibly determine the visual colour of honey [42,43]. Harvesting method, storage time, storage temperature, and climatic conditions also contributed to a wide variety of honey colour.

3.4. Total Phenolic and Flavonoid Content

The two most common compounds are phenolic acids (non-flavonoids) and flavonoids. Based on the carbon chain classification, 16 types of phenolic compounds have been shown to be present in honey (Gallic acid, vanillic acid, syringic acid, quercetin, caffeic acid, syringic acid, epicatechin, *p*-coumaric acid, ferulic acid, rutin) [15]. Phenolic compounds are

among the most important compounds that affect not only the organoleptic characteristics but also the functional properties of honey.

The results of the analysis of the total phenolic content of commercial honey in Vietnam are presented in Table 6. The TPC of Vietnamese honey varies from 11.61 mgGAE/100 g (HD5) to 67.14 mgGAE/100 g (HK10). Statistically significant differences were observed in the majority of different honey samples. Acacia flower honey has the largest TPC (67.14 mgGAE/100 g), followed by rambutan flower honey (65.98 mgGAE/100 g), melaleuca flower honey (63.32 mgGAE/100 g) and coffee flower honey (57.81 mgGAE/100 g). These honey samples all had higher TPC content than Manuka honey (45.05 mgGAE/100 g), indicating that they have good antioxidant potential. Daniela Pauliuc et al. (2020) reported in their study that the TPC of honey in Romania ranges from 18.9 mgGAE/100 g to 23.7 mgGAE/100 g (mint honey) [34]. In a study of four native honeys in Malaysia, Moniruzzaman (2013) reported that sourwood honey had the highest TPC (58.03 mgGAE/100 g), followed by longan honey (56.35 mgGAE/100 g) and the lowest, especially rubber honey (14.45 mgGAE/100 g) [29]. The total phenolic content of lychee honey (China), coffee flower honey (Guatemala) and acacia flower honey (Hungary) was recorded by Nagai's research group (2017) as 85.2, 54.6 and 24.3 mgGAE/100 g, respectively [4].

Table 6. Total phenol and flavonoid contents of different types of honey.

Sample	TPC (mgGAE/100 g)	TFC (mgQE/100 g)
HN1	13.81 ± 0.77^{h}	12.22 ± 1.02^{h}
CF2	57.81 ± 3.04^{c}	30.16 ± 1.22^{e}
HV3	35.38 ± 1.17^{e}	31.85 ± 1.03^{e}
BH4	19.46 ± 2.08^{g}	22.56 ± 1.09^{f}
HD5	11.61 ± 0.57^{h}	9.79 ± 1.07^{i}
CS6	27.58 ± 1.26^{f}	17.53 ± 1.59^{g}
HT7	63.32 ± 1.53^{b}	65.12 ± 3.21^{b}
CC8	65.98 ± 2.32^{ab}	75.54 ± 3.95^{a}
SV9	24.23 ± 1.13^{f}	23.73 ± 0.23^{f}
HK10	67.14 ± 1.96^{a}	57.80 ± 0.58^{c}
Manuka	45.05 ± 1.25^{d}	44.09 ± 3.09^{d}

Different letters after values in the same column represent statistically significant differences between samples ($p < 0.05$). HN1: Longan flower honey; CF2: Coffee flower honey; HV3: Lychee flower honey; BH4: Mint flower honey; HD5: Coconut flower honey; CS6: Rubber leaf honey; HT7: Melaleuca flower honey; CC8: Rambutan flower honey; SV9: Mangrove flower honey; HK10: Acacia flower honey; Manuka: Manuka Honey (Control).

Similar to phenolic compounds, the flavonoids of honey are gradually becoming a criterion to help evaluate quality, distinguish plant origin, detect adulteration and evaluate their biological effects [39]. The main flavonoids found in honey are pinocembrin, apigenin, campferol, quercetin, pinobanksin, luteolin, galangin, hesperetin and isorhamnetin [34]. Flavonoids are best known for their mechanism of scavenging free radicals by neutralizing the radical reactive element. Therefore, honey with high TFC will show it has potential in antioxidant capacity and has a very effective use value.

As can be seen, the TFC of Vietnamese honey presented in Table 6 has a spectrum of values ranging from 9.79 to 75.54 mgQE/100 g. Rambutan flower honey showed the highest value of 75.54 mgQE/100 g, followed by melaleuca flower honey (65.12 mgQE/100 g), acacia flower honey (57.80 mgQE/100 g), lychee flower (31.85 mgQE/100 g). The TFC value of manuka honey (44.09 mgQE/100 g) is 1.7 times lower than that of rambutan flower honey (with the highest TFC value) and 4.8 times higher than that of lychee honey (with the highest TFC value). There was a similarity in the TFC results of Vietnam mint honey (22.56 mgQE/100 g) compared with the previous report of Pauliuc (2020) when evaluating the same honey produced in Romania (25.73 mgQE/100 g) [34]. The TFC result of acacia honey in this study (57.80 mgQE/100 g) was higher than that of honey in Malaysia (2.96–5.81 mg QE/100 g), Iran (2.71 mg QE/100 g) and China (9.14–12.17 mg QE/100 g) [39,44,45]. Besides, rubber leaf honey also has a higher TFC than the study in Malaysia (1.17 mgQE/100 g) [46]. In addition, the TFC of Vietnamese

honey recorded a higher value than that of Turkey (TFC from 1.3–2.8 mgQE/100 g), Romania (0.91–15.33 mgQE/100 g) and Tunisia (9.58–22.45 mgQE/100 g) [47–49].

The presence and variability of TPC and TFC in honeys are influenced by the plant origin, geographical factors and climatic conditions of the region. The report of the research team in China has shown a significant difference in TPC values between honeys of the same plant origin but in different geographical locations [45]. Research by Neupane et al. on honey in Nepal has shown that honey harvested at low altitudes (800–1500 m) has lower phenol content than honey harvested at heights of 1500 m and above [50]. An assessment of the effects of climate on honey in Kenya found a significant difference between honey produced in hot climates (35.47 mgQE/100 g) and climates with high moisture content (29.19 mg QE/100 g). Differences were also observed in TPC of honey produced in high rainfall regions (141.71 mg GAE/100 g) with honey collected in low rainfall, hot and humid climates (116.17 mg GAE/100 g) and in semiarid areas (98.37 mg GAE/100 g) [51]. The differences between the phytochemical values reported in the reports suggest that the phenolic composition of honey acts as an effective and potential factor in the desire to compare, differentiate and trace the origin of honey.

3.5. Polyphenol Compositions

With a presence above 0.8%, polyphenol components are thought to play a role in the antioxidant capacity of honey. A close correlation between the free radical scavenging activity and the content of phenolic components was also reported. In addition, many studies have recognised phenolic acids for their ability to use these components to evaluate the quality and classify honey according to its botanical origin. The characteristics and quantity of polyphenols vary according to harvest season, climatic conditions and influencing factors during processing.

In the studied honey samples, the 13 analysed polyphenol components were mostly found in all honey samples at different concentrations (Table 7 and Figure 2). Gallic acid was found to have the highest value (12.50 mg/100 g) in longan flower honey, while the lowest value was found in coconut flower honey (1.03 mg/100 g). Furthermore, it was found that the control honey (manuka) had a much lower gallic acid content (0.28 mg/100 g) than the other honey samples. Compared with other types of honey, acacia honey has a high content of catechin (104.4 mg/100 g), chlorogenic acid (140.70 mg/100 g), epicatechin gallate (18.9 mg/100 g) and salicylic acid (129.25 mg/100 g). In large quantities, salicylic acid was also observed in coffee flower honey (228.13 mg/100 g) and rambutan flower honey (131.02 mg/100 g). Caffeic acid and kaempferol dominated the polyphenol composition of coffee flower honey with the highest values of 281.44 and 11.36 mg/100 g, respectively. This result shows that the caffeic acid composition is suitable for use as a distinguishing marker for coffee flower honey. This result shows the relevance and prominence of coffee flower honey when compared with the report of the Combarros-Fuertes research group (2019) which only recorded low content of caffeic acid and kaempferol on other honeys in Spain [6]. Melaleuca honey and rambutan flower honey have the highest rutin, apigenin and quercetin content, while apigetrin predominates in lychee honey although it is found in all types of honey. Previous studies have mentioned that gallic acid is a component responsible for the antioxidant activity of honey. However, the geographical origin of honey has a great influence on the gallic acid content [17]. Chronogenic and caffeic acid have been reported to be phenolic acids found abundantly in honey and have the potential for antibacterial function [52].

Table 7. Phenolic content of honey samples.

Phenolic Content (mg/100 g)	HN1	CF2	HV3	BH4	HD5	CS6	HT7	CC8	SV9	HK10	Manuka
Gallic acid	12.50	1.15	2.99	2.92	1.03	1.79	8.40	2.88	5.70	1.53	0.28
Catechin	12.70	47.87	36.80	12.46	9.51	69.29	37.63	34.38	13.74	104.40	100.25
Chlonogenic acid	10.13	27.46	14.62	9.88	6.50	43.28	28.43	34.12	21.95	140.70	33.68
Caffein	6.94	281.44	11.78	4.21	6.66	26.06	19.91	36.31	1.85	24.03	7.89
Epicatechin gallate	1.94	11.79	12.57	2.36	2.27	8.43	9.30	9.46	4.29	18.90	64.28
Vitexin	0.00	3.46	5.65	1.34	1.27	3.23	12.90	8.89	4.03	11.97	17.73
Salicylic acid	29.49	228.13	47.68	38.43	22.39	106.31	88.81	131.02	33.23	129.25	54.76
Rutin	1.60	4.79	0.00	1.25	1.25	3.19	8.40	8.38	1.75	0.00	2.77
Apigetrin	3.72	63.54	232.34	2.32	5.02	38.28	14.18	14.07	3.96	32.43	2.51
Quercitrin	0.80	3.66	4.94	0.76	0.72	1.33	6.67	4.23	0.94	4.78	5.22
Quercetin	0.06	2.08	1.28	0.44	0.14	0.58	4.67	5.70	0.46	1.26	1.40
Kaempferol	2.68	11.36	2.16	2.16	1.79	4.89	3.17	4.13	1.61	4.56	2.63
Apigenin	0.05	0.33	0.18	0.05	0.01	0.18	0.55	0.65	0.09	0.13	0.50

HN1: Longan flower honey; CF2: Coffee flower honey; HV3: Lychee flower honey; BH4: Mint flower honey; HD5: Coconut flower honey; CS6: Rubber leaf honey; HT7: Melaleuca flower honey; CC8: Rambutan flower honey; SV9: Mangrove flower honey; HK10: Acacia flower honey; Manuka: Manuka Honey (Control).

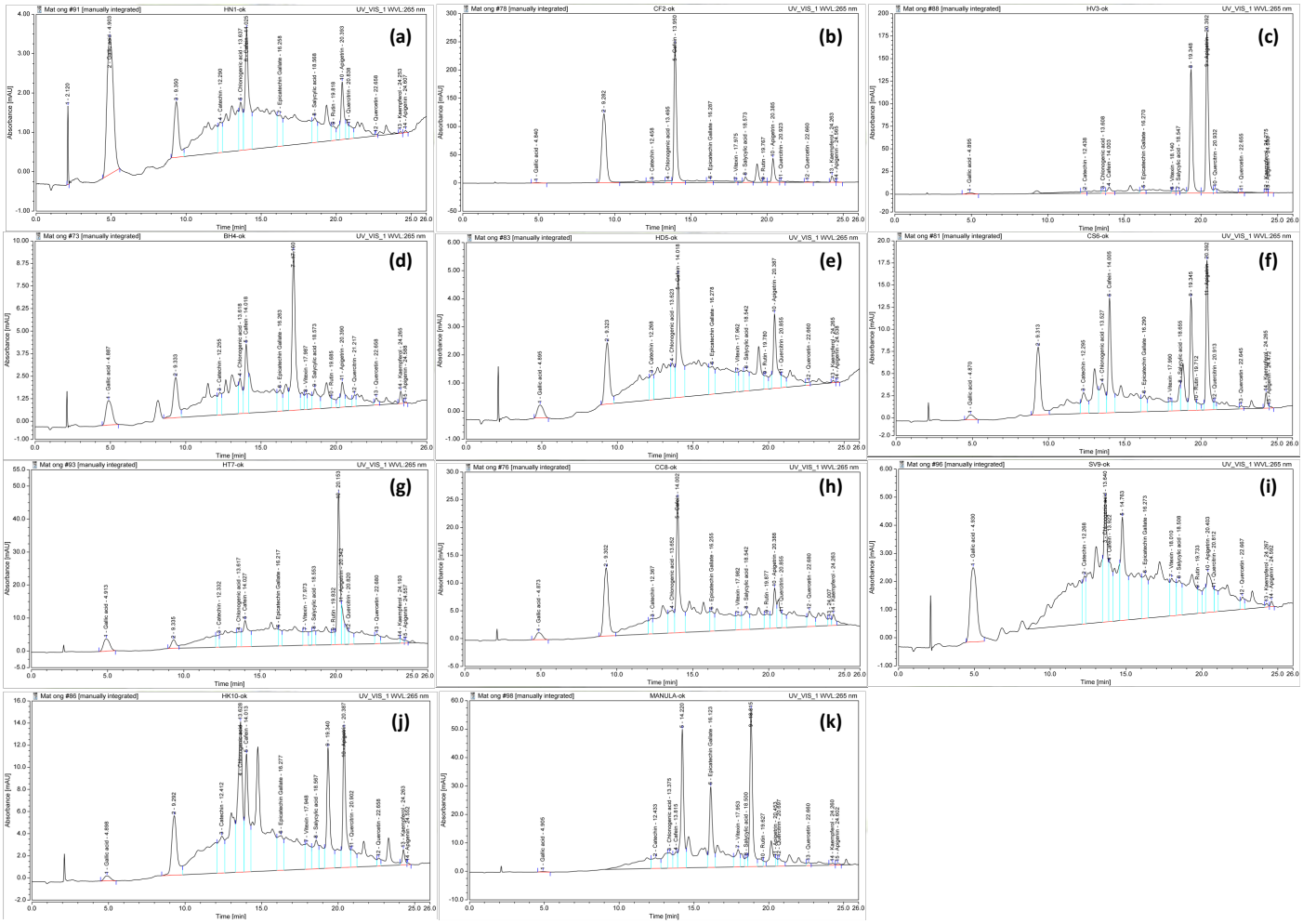


Figure 2. High-performance liquid chromatography chromatogram of (**a**) HN1; (**b**) CF2; (**c**) HV3; (**d**) BH4; (**e**) I HD5; (**f**) CS6; (**g**) HT7; (**h**) CC8; (**i**) SV9; (**j**) HK10 and (**k**) Manuka.

3.6. Antioxidant Activity

Antioxidants are compounds that protect cells from damage caused by free radicals. These substances capture, stabilize, or neutralize reactive oxygen species (ROS) before they reach cells. In plant cells, chloroplasts are the main source of ROS production, whereas, in animals, mitochondria are the starting point of ROS. Here, single oxygens ($^1O_2$), superoxide

(O_2), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO) and hypochlorous acid (HOCl) were generated [53]. Normally, these free radicals carry out the phosphorylation and signalling processes to the cell. However, increasing the chain reaction damages biomolecules and throws off the balance between prooxidant and antioxidant substances, causing a phenomenon known as oxidative stress [54]. This phenomenon causes damage to cells, leading to the formation of a number of cardiovascular diseases, cancer and ageing. One of the solutions proposed to limit this process is the addition of exogenous antioxidant compounds from fruits, vegetables and other sources from the plant or animal kingdom. Accordingly, phenolic compounds are believed to be able to effectively scavenge free radicals by donating hydrogen atoms, electrons or metal cations as well as based on their association with acids and organic sugars [55]. In honey, phenolic compounds play an important role in their antioxidant activity. Predicting the antioxidant activity of honey is not very effective using a single test method because the oxidation reactions of honey are very complex. Therefore, it is necessary to use at least two or more methods to increase the reliability of the test as recommended by Pentós [56]. In this study, DPPH and ABTS free radical scavenging methods were used to evaluate the free radical scavenging ability of commercial honey in Vietnam.

The results presentId in Table"8 sh'w that the DPPH free radical scavenging activity presented according to IC_{50} values ranges from 89.10 to 1285.24 mg/mL. Theoretically, a lower IC_{50} value represents a better free radical scavenger hunt. In the group that may have good DPPH free radical scavenging ability, melaleuca honey has the lowest IC_{50} value (89.10 mg/mL) among the remaining domestic honey samples. Next is acacia flower honey (102.95 mg/mL), rambutan flower honey (207.48 mg/mL) and coffee flower honey (300.96 mg/mL). Manuka honey has a higher IC_{50} value (97.59 mg/mL) than Melaleuca honey (89.10 mg/mL), which indicates that the antioxidant effect of melaleuca honey is better than that of manuka honey. A statistically significant difference ($p < 0.05$) also shows the diversity in DPPH free radical scavenging capacity as well as predicts the difference between phenolics composition in honey of different origins. A study in Turkey showed that the DPPH free radical scavenging ability of honey in this country has an IC_{50} value of 20.05–152.40 mg/mL, in which chestnut honey has the best free radical scavenging capacity [57]. In the report of Nascimento (2017), honey in Brazil has a DPPH reduction efficiency ranging from 25.45 to 294.26 mg/mL [7]. In another record, the antioxidant activity of dark and light honey in south eastern Portugal was recorded with an average value of 68.17 and 27.24 mg/mL [52]. In general, most honey in Vietnam has a higher IC_{50} value than those found in other plant species.

For the free radical scavenging effect of honey based on the ABTS test, the antioxidant capacity was recorded by formulating a calibration curve with vitamin C. The results shown in Table 8 show that the lowest IC_{50} value obtained is 11.05 mg/mL (melaleuca honey), and the highest value is 149.49 mg/mL (longan flower honey). The obtained results show a fluctuation of IC_{50} value between different types of honey. In particular, it was found that melaleuca flower honey (11.05 mg/mL) and acacia honey (11.98 mg/mL) have the ability to eliminate 50% of free radicals, almost equivalent to manuka honey. This shows that the antioxidant effect of this honey is extremely potent. In addition, the results also show that these two types of honey have more effective free radical scavenging capacity when compared with previous reports of acacia honey (44.37 mg/mL) and wild carrot honey (202.26 mg/mL) originated from Germany [58]. The mean IC_{50} determined in the ABTS test was observed to be significantly lower than the mean IC_{50} determined in the DPPH test. This can be explained because the DPPH radical is only capable of reacting with lipophilic antioxidants, while the $ABTS^+$ radical is able to react with both hydrophilic and lipophilic states of the antioxidant [59]. In general, it is not correct to directly compare the free radical scavenging capacity of one honey with previous data referring to another, because the reaction conditions, experimental set-up, and sample properties of each study are different. However, based on ongoing and ongoing evaluations, it can be confirmed that phenolic compounds play an important role in the antioxidant activity of honey. Although not a

major source of antioxidants in the human diet, honey shows very well its potential as a supplement in the search for a variety of nutrients source of antioxidants. Above all, the pleasantly sweet taste of honey makes it easier for users to accept consumption in cases where they cannot receive antioxidants from plants.

Table 8. The antioxidant capacity of different types of honey.

Sample	IC_{50} DPPH (mg/mL)	IC_{50} ABTS (mg/mL)
HN1	1285.24 ± 8.33^{a}	147.49 ± 2.12^{a}
CF2	300.96 ± 4.32^{g}	41.20 ± 1.03^{f}
HV3	981.52 ± 9.12^{d}	57.23 ± 1.23^{d}
BH4	1026.74 ± 12.33^{c}	72.66 ± 1.75^{c}
HD5	1088.80 ± 15.55^{b}	121.62 ± 2.23^{b}
CS6	356.41 ± 9.44^{f}	36.49 ± 1.18^{g}
HT7	89.10 ± 2.03^{l}	11.05 ± 0.22^{l}
CC8	207.48 ± 3.34^{h}	20.64 ± 1.34^{h}
SV9	869.21 ± 4.22^{e}	48.79 ± 1.44^{e}
HK10	102.95 ± 2.39^{i}	11.98 ± 1.01^{i}
Manuka	97.59 ± 1.33^{k}	11.83 ± 0.33^{ik}

Different letters after values in the same column represent statistically significant differences between samples ($p < 0.05$). HN1: Longan flower honey; CF2: Coffee flower honey; HV3: Lychee flower honey; BH4: Mint flower honey; HD5: Coconut flower honey; CS6: Rubber leaf honey; HT7: Melaleuca flower honey; CC8: Rambutan flower honey; SV9: Mangrove flower honey; HK10: Acacia flower honey; Manuka: Manuka Honey (Control).

3.7. Antibacterial

Honey is a natural product containing large amounts of phytochemical compounds such as phenolics and flavonoids with many beneficial biological health effects, including its antibacterial activity. To evaluate the antibacterial power of honey, a good diffusion test was performed to measure the zones of inhibition produced using different concentrations of honey samples against different pathogenic microorganisms. The results of the antibacterial ability assessment of honey in Vietnam are presented in Table 9 and Figure 3.

Table 9. Average diameter (mm) of bacterial inhibition zone of different types of honey.

Sample	Zone of Inhibition in Diameter (mm)		
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
HN1	14.00 ± 1.00^{b}	11.33 ± 2.88^{bc}	10.66 ± 1.24^{bcd}
CF2	12.33 ± 0.57^{cd}	9.33 ± 0.57^{bc}	9.66 ± 0.47^{cd}
HV3	13.00 ± 1.00^{bcd}	10.33 ± 1.52^{bc}	11.33 ± 0.47^{bc}
BH4	13.33 ± 0.57^{bcd}	11.66 ± 1.52^{b}	11.66 ± 1.24^{b}
HD5	13.66 ± 1.52^{bc}	10.66 ± 2.30^{bc}	11.33 ± 0.47^{bc}
CS6	10.66 ± 0.57^{e}	9.00 ± 0.00^{c}	9.00 ± 0.81^{d}
HT7	12.00 ± 1.00^{de}	9.33 ± 0.57^{bc}	9.33 ± 0.47^{d}
CC8	12.33 ± 1.52^{cd}	11.66 ± 0.57^{bc}	11.33 ± 0.47^{bc}
SV9	12.00 ± 1.00^{de}	11.33 ± 0.57^{bc}	10.33 ± 1.24^{bcd}
HK10	10.66 ± 0.57^{e}	10.66 ± 1.15^{bc}	9.33 ± 0.47^{d}
Manuka	12.66 ± 0.57^{bcd}	9.66 ± 1.15^{bc}	9.66 ± 0.47^{cd}
Ampicillin (+)	19.66 ± 0.57^{a}	15.00 ± 1.00^{a}	16.00 ± 1.41^{a}

Different letters after values in the same column represent statistically significant differences between samples ($p < 0.05$). HN1: Longan flower honey; CF2: Coffee flower honey; HV3: Lychee flower honey; BH4: Mint flower honey; HD5: Coconut flower honey; CS6: Rubber leaf honey; HT7: Melaleuca flower honey; CC8: Rambutan flower honey; SV9: Mangrove flower honey; HK10: Acacia flower honey; Manuka: Manuka Honey (Control).

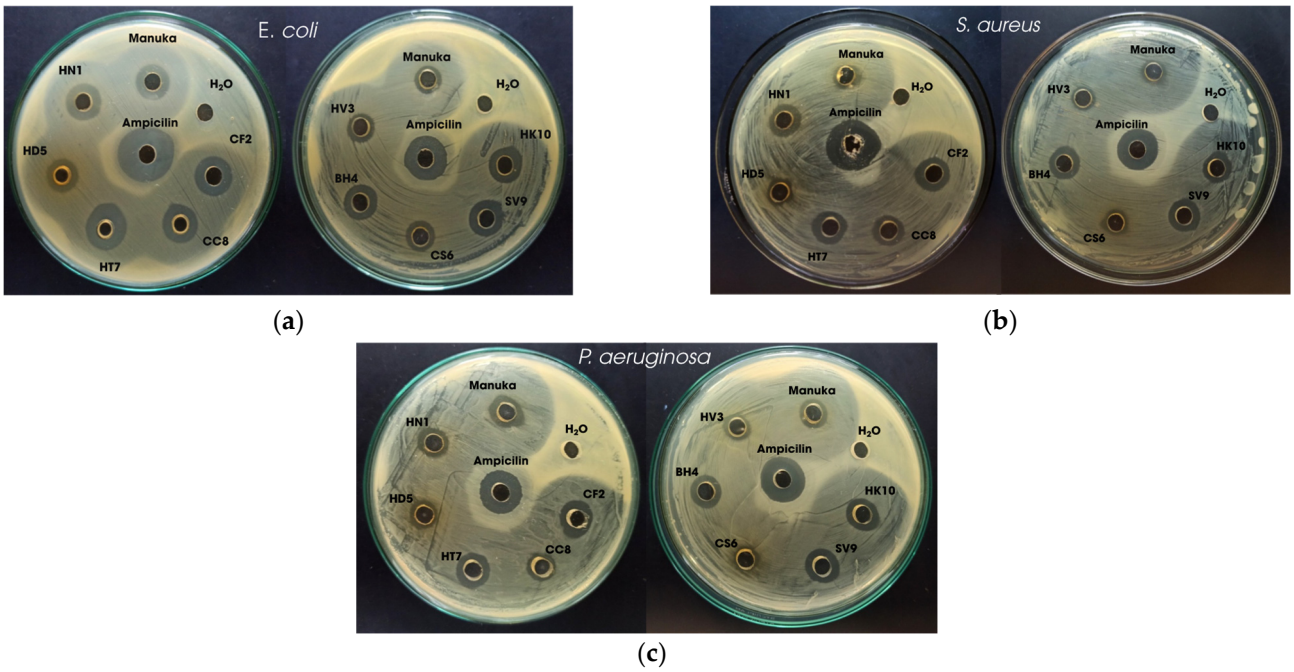


Figure 3. Antibacterial ring diameters of honeys on strains of (**a**) *E. coli*; (**b**) *S. aureus* and (**c**) *P. aeruginosa*.

Observing the results, it can be seen that most of the honey samples showed moderate resistance to *E. coli*, *P. aeruginosa* and *S. aureus* bacteria (<15 mm). For *E. coli* and *P. aeruginosa*, longan honey showed the best inhibition of bacteria (14 mm and 11.33 mm), while rubber honey recorded resistance to the activity of both these bacteria is weakest (10.66 mm and 9.00 mm). At the same time, the results of recording the activity of honey on *S. aureus* showed that mint honey showed the most effective activity against this bacterium (11.66 mm). In contrast, rubber leaf honey has the weakest antibacterial ability of all the tested samples (9.00 mm). What is interesting to be pointed out in this study is that the majority of honey samples in Vietnam have the ability to inhibit bacteria almost similar to manuka honey. The difference in bacterial inhibition zone values is explained by the influence of osmotic effects, pH, the presence of hydrogen peroxide components and phytochemicals. In addition, it has been suggested that phenolics, flavonoids and methylglyoxal compounds are the main contributors to the antibacterial properties of honey [60]. At the same time, there are also reports that the difference in plant origin and geographical factors such as humidity and temperature is one of the reasons for the difference in resistance to bacteria between different types of honey [61]. The results are similar to the study reported by Kalidasan (2017) that recorded the antibacterial ring diameters of commercial honey samples on *E. coli* and *S. aureus* strains with values of 17 and 22 (mm), respectively [62]. Yap (2014) conducted an evaluation of honey in Malaysia and recorded the optimal antibacterial ring diameter on strains of *E. coli* and *S. aureus* with values from 4.00 to 6.00 (mm) [61]. In a study on honey in India, a perfect inhibitory effect on all three strains of *E. coli*, *P. aeruginosa* and *S. aureus* was reported at 31–45 (mm) [63]. Overall, the results show that Vietnamese honey is a good potential therapeutic resource for health problems caused by common bacterial strains.

3.8. Correlation

The relationship between the physicochemical composition of honeys in Vietnam is shown in Figure 4. The correlation matrix image presents the relationship from less intimate to more intimate by the colour range from blue (−1) to red (+1). In which the positive or negative correlation between the components is expressed through the Pearson correlation coefficient (R). The results show that moisture has a very high positive correlation with free acid content (R = 0.834, red), TPC (0.764, orange), TFC (R = 0.689, orange) and the correlation

inverse to Brix (R = −0.719, blue). The results are similar to the report of Harisun (2019) which noted an inverse correlation between humidity and Brix (R = −0.719); however, did not find any relationship between moisture and TPC and TFC on honey in Johor [64]. Pentós et al. reported a positive correlation between water content and TPC (R = 0.877) when evaluated on Polish honey [56]. The negative correlation between moisture and Brix in this study is consistent with previous reports explaining that low dissolved solids content is a consequence of high moisture levels in honey [64]. Considering that, there is a very strong positive correlation between TPC and TFC (R = 0.901, red). This result is consistent with previous records of honey in Australia (R = 0.866), Tunisia (R = 0.915) and Malaysia (R = 0.958) [29,41,48].

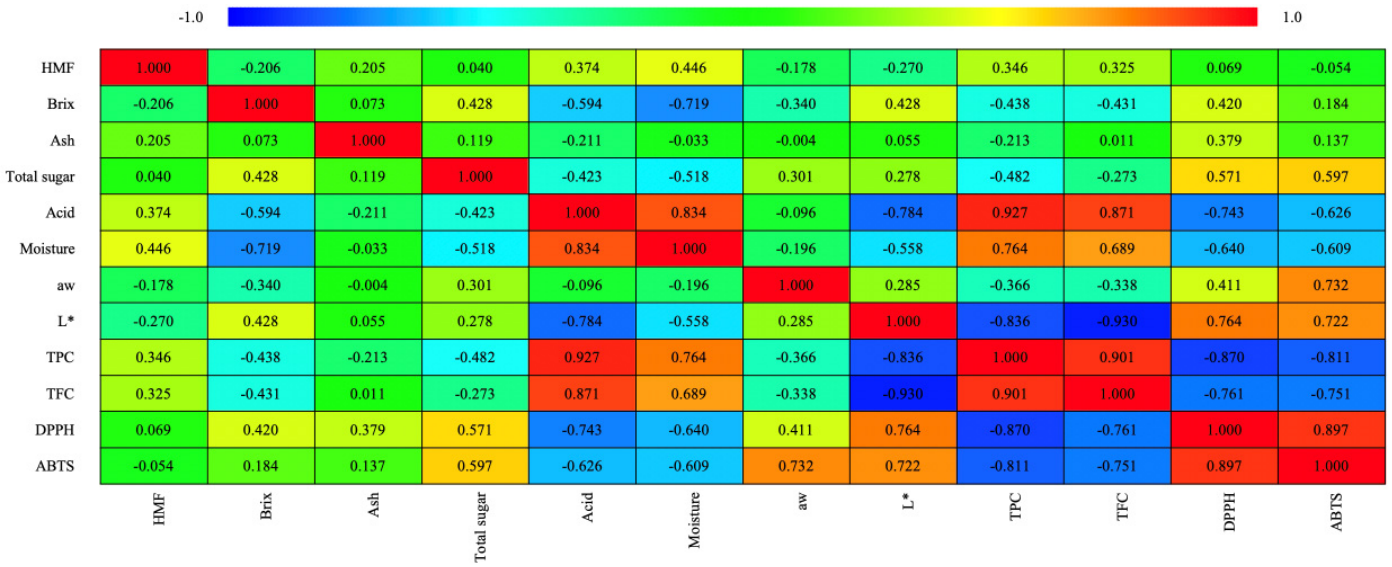


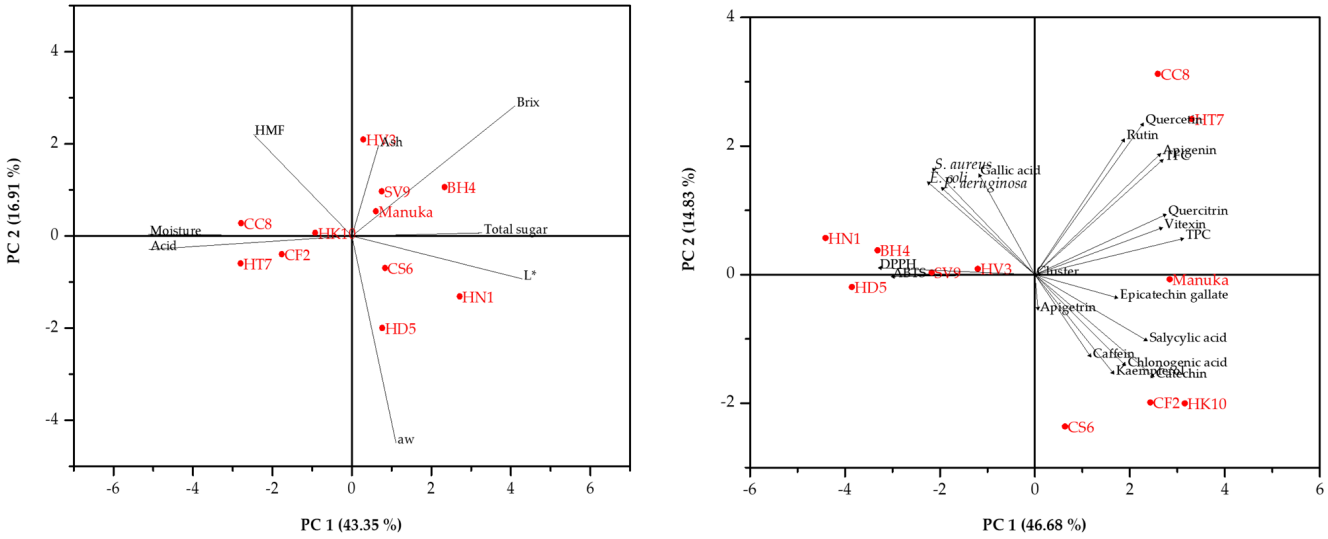
Figure 4. Correlation coefficients for antioxidant capacity versus TPC, TFC and physicochemical properties of honey.

The negative correlation between TPC and L* (R = −0.836, blue) in this study is similar to that reported in Polish honey (R= −0.941); however, it is opposite to that reported previously on the positive relationship when evaluated on honey in Tunisia (R = 0.964) [48]. This diversity difference shows that the amount and composition of polyphenols in honey varies and differs depending on the plant origin of the honey. The results show that, L* value is positively correlated and TPC and TFC values are negatively correlated with antioxidant capacity for both DPPH and ABTS free radical scavenging methods in this study. In particular, the results obtained in two tests, DPPH and ABTS, are presented in terms of the ability to capture 50% of free radicals, that is, the smaller the IC_{50} value, the higher the antioxidant capacity. Based on that, it can be seen that the correlation value between TPC-DPPH (R = −0.870) and TPC-ABTS (R = −0.811) shows that TPC has a high correlation with DPPH free radical scavenging activity and ABTS. Similar results were also observed in honey from Tunisia (R = −0.945), Brazilian honey (R = −0.8918) [48,65]. However, not all studies show a strong positive correlation. For example, Raneh's report noted that no correlation was found between TPC and DPPH and ABTS when evaluating Tualang and Kelulut honey in Malaysia [66]. Overall, most of the results suggest that the antioxidant capacity of honey is largely influenced by the phenolic compounds it contains.

3.9. Principal Component Analysis (PCA)

Principal component analysis (PCA) assessed the differences between honey types. This multivariate analysis helps to find critical data structures as well as observed trends and magnitudes between honey types based on physicochemical properties, phenolic compounds, antioxidant activity and antibacterial ability to serve as a basis to distinguish

honey from different geographical locations (Figure 5a,b). Lines are drawn from the centre point to the respective partitions of each principal component. Accordingly, the closer the distance between the lines and the sample points, the closer the relationship between them.



(**a**) (**b**)

Figure 5. Principal component analysis (PCA) biplot for (**a**) Physical-Chemical parameter and (**b**) Total phenolic and flavonoid contents (TPC and TFC), antioxidant activity, antimicrobial activity and phenolic compounds of different honeys.

Figure 5a depicts 61.51% of the variation in the data set of quality indicators, in which PC1 is the most important component when it explains 46.68% of the variation and PC2 accounts for 14.83%. The dispersion of ingredients showed variables closely related to PC1 including °Brix, total sugar, L*, total acid and moisture. The variables that dominate PC2 are HMF, °Brix, ash and water activity. The results showed that honeys including HN1, HV3, BH4, HD5, CS6, SV9 and manuka were characterized by positive values of PC1 and PC2. Specifically, the variables °Brix, total sugar, ash L* and water activity are the factors that make these honeys different. This result is in contrast to the previous report of Marijana B. Sakač (2019) that the variable L* can be used to distinguish acacia honey [5]. This result shows that the colour difference of honey is also influenced by the growing conditions and geographical location of the plants. It is observed that lychee honey (HV3) exhibits its strong characterization with ash value. For the remaining honeys, HK10 and CC8 are characterized by positive values of PC1 and PC2. In contrast, with acid and moisture values, CF2 and HT7 were affected by negative values of PC1 and PC2, respectively.

The influence of the parameters including total phenolic and flavonoid content (TPC and TFC), antioxidant activity, antibacterial activity and phenolic components on the specificity of honeys in this study is observed in Figure 4b. Accordingly, the PCA-biplot of the IC_{50} value recorded DPPH and ABTS free radical scavenging activity towards the negative region of both PC1 and PC2. There, the presence of honeys such as HN1, HV3, BH4, HD5 and SV9 showed that they were not characterized by their antioxidant capacity. Gallic acid is a phenol shown to have effective biological activities such as antioxidant, anticancer (against prostate and cervical cancer cells) and antibacterial [16]. This study also showed a close relationship between gallic acid and the ability to inhibit bacteria such as *S. aureus*, *E. coli* and *P. aeruginosa* expressed through the interaction site located in the positive region of PC2 and the negative region of PC1. In this region, samples HN1, HV3 and BH4 showed a relatively close correlation with gallic acid content and antibacterial ability. Considering that coffee flower honey (CF2) and acacia flower honey (HK10) have a very close correlation with components including caffeine, kaempferol,

catechin, chlorogenic acid and salicylic acid. Similarly, small gaps of variables including TFC, apigenin, quercetin and rutin can be observed for melaleuca honey (HT7). This opens up a new approach that can give coffee flower honey, acacia honey and melaleuca honey an accurate identification mark to make product traceability more convenient.

3.10. Clusters Analysis

Figure 6 shows that the similarity between honey types and geographical regions is more significant than 66.55%. Consider that groups with a similarity level of 88.85% or more include purple, green and blue groups. Longan flower honey, coconut flower honey in the South, mint flower honey, and mangrove flower honey in the North of Vietnam were classified into two groups with a similarity >99%. In this cluster, rubber leaf honey also showed high similarity to the above honey groups (>95%). In addition, for the group including melaleuca flower honey and rambutan flower honey, it was found that there was about 95% similarity in these two types of honey originating in the South of Vietnam.

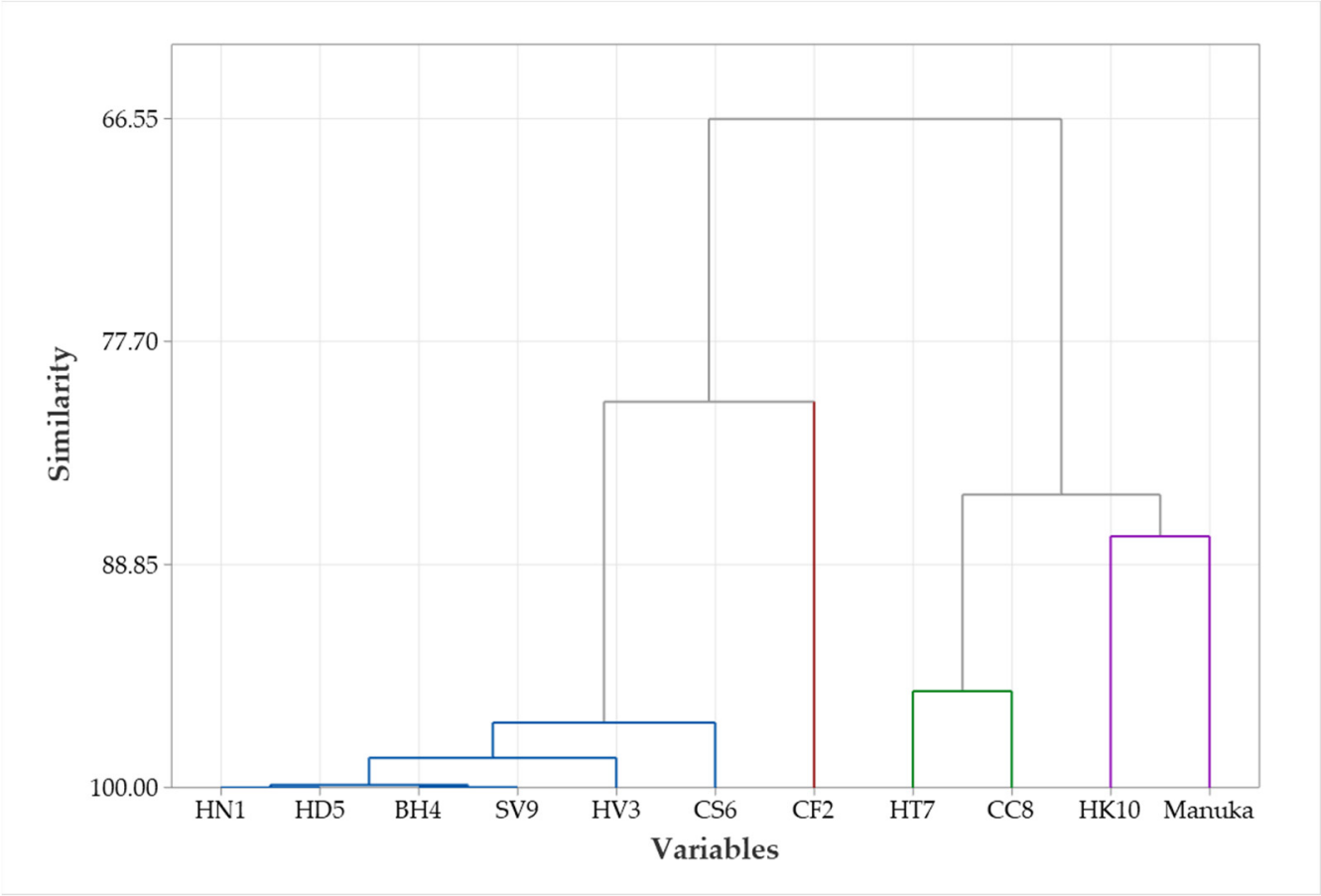


Figure 6. Dendrogram of the cluster analyses for the 30 honey samples.

The results show that some types of honey have similar identification marks and may be due to the dishonesty of the honey beekeeper or the packer. Currently, because of high operating costs, it is common for manufacturers to have little or no reliable analytical methods (such as melissopalynology, HPLC, GC-MS, etc.) to determine honey origin. Research on commercial honey in Brazil also found that the labelling of haplotype honey on the products of some producers is often carried out to add value to their products [12]. The obtained results also show that coffee flower honey and acacia flower honey exhibit specificity consistent with previous analyses that have noted the presence of unique identifiers recorded on only two these honeys.

4. Conclusions

The analytical results presented here form the first database on quality and safety parameters, phytochemical composition, antioxidant capacity and antibacterial activity of some honeys in Vietnam. Most of the analysed honeys have quality traits in accordance with international quality standards (Codex Alimentarius) and Vietnamese standards. The colour diversity is recorded and shows that the difference in colour shades is influenced by exogenous as well as endogenous factors of honey. Biologically active compounds such as polyphenols and flavonoids show the relationship between these components with antioxidant (DPPH and ABTS test) and antibacterial (*E. coli*, *S. aureus* and *P. aeruginosa*) properties of honey. In addition, the results of principle composition analysis show that polyphenol components can be used as markers of origin for some types of honey. This study provides a new perspective on applying honey and its products in several medical treatments (prevention of infection, wound healing, increased immunity, etc.). In addition, this study will provide data on the chemical properties of honey from different plant sources in the world in general and in Vietnam in particular.

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