Research Article

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Physical, Chemical and Biological Characteristics of Sri Lankan Bee Honey Varieties

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ABSTRACT

Objectives: Honey has abundant medicinal properties. It is formed by floral nectar, swaps of plants, and the secretions of bees. The current study determined the physical, chemical, and biological characteristics of Sri Lankan raw bee honey varieties.

Methods: A honey stock/library was created using local honey produced by *Apis cerana*, and *Apis dorsata*. Colour, conductivity, cell constant and pH were determined under physical tests while free acidity, water content and hydroxymethylfurfural content were determined under chemical tests. Evaluation of biological activities includes checking for antimicrobial activity, antioxidant activity and enzymatic activity.

Results: The pH values recorded for *A. dorsata* and *A. cerana* honey ranged from 4.14–5.55 and 4.54–6.33, respectively. The electrical conductivity of all the samples ranged from 0.39-2.59 mS cm^{-1}. The free acidity of honey samples of *A. dorsata* was between 25-81.7 meq/kg and that for *A. cerana* was between 40- 82.7 meq/kg. The hydroxymethylfurfural content of honey samples of *A. dorsata* and *A. cerana* were 9.38-49.50 mg/kg and 7.49-53.94 mg/kg, respectively. The two honey varieties were similar in chemical and physical characteristics tested except for water content where the water content of honey from *A. dorsata* was significantly higher than that of *A. cerana*. All honey samples investigated had antibacterial activity against gram-negative bacteria *E. coli* and gram-positive bacteria *S. aureus.* A. cerana honey had higher antioxidant activity than *A. dorsata* honey while *A. dorsata* honey had higher enzymatic activity than *A. cerana* honey.

Conclusions: The present study reveals that the physiochemical properties of two honey varieties were similar except for water content while biological properties were significantly different. This study indicates that honey from both *A. dorsata* and *A. cerana* of Sri Lanka has potential therapeutic activities that can be used to develop natural drug formulations.

Keywords: Honey, Sri Lanka, *Apis cerana, Apis dorsata*, physicochemical characteristics, biological characteristics

INTRODUCTION

Honey is a natural sweet substance possessing unique nutritional and medicinal properties [1]. It is produced mainly from the nectar of blossoms by the honeybees. The nectar is collected, modified by combining it with a specific substance and stored inside the beehive to ripen and mature [2]. The flavor, colour, aroma, texture and chemical composition of honey varies greatly based on its floral source, and seasonal and environmental factors [3].

Honey comprises a mixture of carbohydrates, proteins, amino acids, vitamins, antioxidants, minerals and other compounds [4,5]. Honey is well-known for its health benefits. It has been used for the treatment of insomnia [6], used in the wound healing and healing of burns, ulcers, infected wounds and open wounds [1,7]. Certain types of honey can help prevent bacterial infections. Honey can be used as first-line treatment for infections with bacteria that are resistant to many currently available antibiotics and may even help reverse bacterial resistance to antibiotics. Honey can be mixed with other remedies and consumed or rubbed onto the skin [1]. The antibacterial activity of honey results from its high acidity or low pH, osmolarity, the content of hydrogen peroxide (H_2O_2) and non-peroxide components [2,8].

Phenol compounds, flavonoids, vitamin C, carotenoids and certain enzymes (glucose oxidase, catalase) present in honey contribute to its antioxidant properties [9]. The antioxidant potency of honey is strongly correlated not only with the concentration of total phenolic present but also with its colour, with dark-coloured honey having higher total phenolic contents and consequently, higher antioxidant capacities [5].

Usually, honey can be used as a single compound or as a combination with other compounds for the treatment of several diseases. Though it has been incorporated into traditional medicine, many countries have rediscovered the therapeutic activity of honey, extending its applications to modern medicine [1]. Consequently, during the last decades, much research has been carried out focused on Manuka honey [10] which is not found in Sri Lanka. Sri Lanka is a tropical country rich in biodiversity. Two bee species namely, *Apis cerana* and *Apis dorsta* are the major producers of raw bee honey in Sri Lanka. There is a significant research gap in investigations into raw honey produced by these Sri Lankan bees. The current study is a preliminary investigation aimed at the initial comparative physicochemical and biological characterization of raw honey produced by honeybee species present in Sri Lanka. The present findings demonstrated that the two honey varieties are similar in chemical and physical characteristics tested while the biological characteristics tested were significantly different in the two honey varieties. This study indicates that honey from both *A. dorsata* and A. *cerana* has the future potential to be developed as new drug formulations.

MATERIALS AND METHODS

Honey sample collection and stock

Raw honey samples were collected in sterile glass jars from the two honey-producing bee species of Sri Lanka, namely *Apis cerana* (eastern honeybee/meemassa/commercial honeybee) and *Apis dorsata* (Bambara/rock bee) from all around Sri Lanka (including wet, dry and intermediate climatic zones). Altogether fourteen honey samples were collected of which seven (C, D, G, H, I, K, L) were from *A. cerana* and seven (A, B, E, F, J, M, N) from *A. dorsata*. Except for sample A, the other samples of *A. dorsata* were collected from the wet zone.

Honey-producing species, geographical location (GPS data) of the area in which the sample was collected, common flower type/s available in the vicinity, and the date and weather information were collected for each sample. Collected raw honey samples were stored in airtight sterile containers at room temperature for short-term use.

Physical tests

pH: The pH of each sample was measured using a pH meter in triplicates.

Colour: The absorbance of raw honey samples was measured at 560 nm using a UV spectrophotometer and the average of three replicates was used to assign the colour according to the standard table [11].

Conductivity: The electrical conductivity (EC) of each honey sample was measured following the harmonized methods of the international honey commission (IHC) and values were expressed in mS/cmh [11].

Chemical Tests: Free acidity, water content and hydroxymethylfurfural content were determined according to harmonized methods of the IHC.

Free acidity: For the free acidity testing, 10 g of honey sample was dissolved in 75 mL of carbon dioxide-free water in a 250 mL beaker while stirring with a magnetic stirrer. The calibrated pH electrode was immersed in the diluted honey solution and the pH value was recorded. The solution was titrated with a standardized NaOH (0.1 M) up to pH 8.30 and completed within 2 minutes. Each measurement was recorded in triplicates. Free acidity was expressed as milliequivalents (millimoles acid/kg honey) which is equal to the average volume of 0.1 M NaOH in milliliters multiplied by 10 [11].

Water content: Water content was determined using the refractive index method. Homogenized honey samples were closed tightly and placed in a 50°C water bath until the sugar crystals dissolved. It was cooled to room temperature with stirring. The surface of the prism was covered with the sample evenly and the refractive index was -measured after two minutes. Results were reported as an average of two measurements. The corresponding moisture content was read from the table according to Stefan et al. [11].

Hydroxymethylfurfural (HMF) content: HMF content was determined using the following method in IHC. A sample of 5.0 g of honey was dissolved in 25 mL of water and that solution was transferred into a 50 mL volumetric flask. Then 0.5 mL of Carrez solution I (15 g of potassium ferrocyanide ($K_4Fe(CN)_6 \cdot 3H_2O$) in 100 mL of distilled water) was added and mixed well. After that, 0.5 mL of Carrez solution II (30 g of Zn $(CH_3CO_2)_2 \cdot 2H_2O$) in 100 m of water) was added and again mixed well. The final volume was made up to 50 mL with water. Foam formation was suppressed by adding a drop of ethanol. The solution was filtered using Whatman papers. While rejecting the first 10 mL, the filtrate was pipetted out to each of the two test tubes (18 x 150 mm). The sample solution was prepared using 5.0 mL of water which was added to one of the test tubes and was mixed well. The reference solution was prepared using 5.0 mL of 0.2% sodium bisulfite solution added to the second test tube and mixed well. The absorbance of each sample solution was determined against the reference solution in 10 mm quartz cells at 284 and 336 nm within one hour.

HMF in mg/kg = (A_{284} - A_{336}) x 149.7 x 5 x D/W

Where: A_{284} is the absorbance at 284 nm and A_{336} is the absorbance at 336 nm. D is the dilution factor and W is the weight of the sample (12)·(13).

Biological Tests

Antibacterial activity: The antibacterial activity of honey was determined using the spread plate method. McFarland Standards (0.5) were used to standardize the approximate number of bacteria that were inoculated per plate. Five concentrations of honey samples including 0.2 g/mL, 0.4 g/mL, 0.6 g/mL, 0.8 g/mL and 1 g/mL were used for the investigation of antibacterial activity while 0.1 mg/mL gentamycin was used as the positive control.

The solidified labeled nutrient agar (NA) plates were divided into four parts as H_1, H_2, G and D where H_1 and H_2 for honey samples, G for 0.1 mg/mL gentamycin solution and D for negative control (sterile deionized distilled water). The spread plates were made by evenly spreading 0.1 mL standardized bacterial suspension under aseptic conditions. Wells were cut into the agar using a sterile cork borer with an 8 mm diameter and sealed with 0.1 mL agar. After the plates were solidified, honey samples were transferred into both wells that were labeled H_1 and H_2. 0.1 mL sterile deionized distilled water was used as a blank for every plate and 0.1 mL of 0.1 mg/mL gentamycin solution was used as the positive control. All plates were sealed with parafilm and incubated at 37°C for 24 hours. The diameters of zones of inhibition of the wells were obtained by measuring them in at least two directions perpendicular to each other. The mean of diameters of the inhibition zone for each well and honey sample was calculated [14,15].

The Chi-square test was used to analyze the significant differences among the inhibitory zones for bacteria between *A. dorsata* and *A. cerana* honey samples at different concentrations. A probability of less than 0.05 was considered significant.

DPPH Assay: Ethanol extract of honey was prepared by adding 1000 µL ethanol to 1.00 g of each honey sample, mixing by vortexing and sonicating for 10 minutes at room temperature. All samples were centrifuged at 6000 rpm for 15 minutes and the supernatant was collected. The extracted honey samples were used to determine the antioxidant activity of honey using DPPH assay [16,17].

The ethanol extract of honey samples was dissolved in methanol and a 10000-ppm stock solution was prepared. That 10000-ppm stock solution was used to prepare 5000 ppm, 1250 ppm, 1000 ppm, 500 ppm and 250 ppm concentrations of honey solutions. 2 mL of each solution was mixed with 2 mL of DPPH (0.1 mM). After the mixtures were shaken and left for 30 minutes at room temperature in the dark, the absorbance was measured at 517 nm and ascorbic acid was used as the positive control.

The percentage of the radical scavenging activity (RSA) was calculated based on the following equation [11,16].

DPPH scavenged (%) =100- (A_{sample} –$As._{blank}$) / (A_{cont} –$Ac._{blank}$) ×100

A_{cont}, A_{sample} $Ac._{blank}$ and $As._{blank}$ are the absorbance values (at 517 nm) respectively for the negative control, sample, negative control blank and sample blank.

Total phenolic content: Total phenolic content was determined using Folin Ciocalteau Phenolic Content Quantification Assay Kit 400. 150 µL of 0.1 g/mL honey solution was mixed with 500 µL Folin-Ciocalteu reagent (10%) for 5 minutes and then 1500 µL of a Na_2CO_3 (7.5%) solution was added. All samples were incubated at room temperature in darkness for 30 minutes, and their absorbance was read at 765 nm using a spectrophotometer. Total phenolic content is expressed as mg GAE/kg of honey from a calibration curve. Gallic acid was used to calculate a standard curve (20, 40, 60, 80 and 100 µg/mL). All samples were analyzed in triplicate. The concentration of total phenolic compounds in honey samples was determined as milligrams of gallic acid equivalent by using the following equation,

A= (c×v)/m

Where, A is total phenol content (mg/g GAE), c is the concentration of gallic acid in mg/mL, v is the volume of extract (honey sample) and m is the mass of the honey sample (g) [16-19].

Enzymatic activity: Alpha-amylase activity (diastase) activity was determined by methods developed by Schade, J. E et. Al. Preparation of honey sample as follows. 10.0 g of honey was dissolved in a small amount of water and 5 mL of acetate buffer. This was transferred to a 50 mL volumetric flask containing 3 mL of 0.5 M NaCl solution. The NaCl must not be added before the honey is dissolved in the buffer, as diastase activity is rapidly lost at pH values below 3.9 or 4.0 in the presence of NaCl [20].

For the determination of the activity, 10 mL of the 20% honey solution and 5 mL of the starch solution were used. The reaction solutions were placed into the 40^0C water bath for 15 minutes. The digestion was initiated by mixing the honey and starch solutions. Periodically, 0.5 mL of the digestion mixture was removed, and 5 mL of the iodine solution was added

quickly. Each time of sampling was noted. Distilled water was used instead of the starch solution and the same procedure was followed to prepare the blank solution. Absorbance was measured with reference to the blank solution at 660 nm using a spectrophotometer. The absorbance value was taken from the same time intervals up to the absorbance value reaching 0.235. The unit of diastase activity established for the method is the amount of enzyme which will convert 0.01 g of starch to the prescribed endpoint in 1 hour under the test conditions. Results are expressed in units per gram of honey [20].

50% transmittance value = 60/t ×0.10/0.001×1.0/2.0 = 300/t

Invertase activity was determined using the Siegenthaler method [21,22]. The honey samples were prepared by dissolving 5.0 g of honey in a buffer solution in a 25 mL flask. It was kept in the refrigerator for 1 day. The volume of 5.0 mL of substrate solution was placed in a test tube at 40^0C. At starting time, 0.50 mL of honey solution was added. The samples were then mixed and incubated at 40^0C. After exactly 20 minutes 0.50 mL of stop solution (363.42g of tris-hydroxymethyl aminomethane dissolved in water and diluted to 1L. The pH adjusted to 9.5 with 3M hydrochloric acid) was added and mixed. For the blank, 5.0 mL of substrate solution was incubated at 40^0C at the same time. The volume of 0.50 mL of stop solution was added and mixed and 0.50 mL of the honey solution was added. The solutions were cooled to room temperature and within one hour the absorbance was measured for the samples and the blank solution in 1cm cells at 400 nm. The amount of p-nitrophenol (μM) produced corresponds to the amount of substrate (μM) utilized. Invertase (1U/Kg) = 1 μmoL PNPG / minutes × kg honey).

Invertase in U/kg = $6\times0.05\times0.05298\times104\times A_{400} = 158.94\times A_{400}$

Where U is one international unit with a defined utilization of 1μM per minute.

Invertase Number (IN) = $21.64 \times A_{400}$ where 21.64= slope of linear regression of IN (y-axis) on A_{400} (x-axis) [21].

Results

Physical Tests

The average absorbance values of honey samples at 560 nm, relevant colour, pH and conductivity are expressed in Table 1. The average absorbance range lies between 0.342 - 2.386. According to Stefan et al. three colours can be seen between the above absorbance range, namely Extra Light Amber (ELA), Extra white (EW) and Light Amber (LA). The color of extra light amber (ELA) was shown by 64% (n=9) of the samples used for this experiment.

The pH values lie between 4.14 – 5.55. According to the methods of IHC, this method is valid for the determination of the EC of honey samples within the range of 0.1 – 3 $mS.cm^{-1}$ [15]. All the EC values obtained for the samples used in this experiment were within that range (0.39- 2.59 $mS.cm^{-1}$).

Chemical tests

The calculated free acidity value, water content and HMF of each honey sample of *A. dorsata* and *A. cerana* are presented in Table 2.

The free acidity of honey samples of *A. dorsata* varied between 25- 81.7 meq/kg. The free acidity of honey samples of *A. cerana* was between 40 – 82.7 meq/kg, except in the sample K (119 meq /kg). Comparatively, honey samples of *A. dorsta* had more water content compared to honey samples of *A. cerana.*

Table 1: The results of physical tests of honey samples of *Apis dorsata* and *Apis cerana.*

Species	Sample	Average absorbance	Colour names	pH	Conductivity (mS/cm)
Apis dorsata	A	0.762	ELA	5.49	1.98
	B	0.326	EW	4.52	0.39
	E	1.706	LA	5.55	0.41
	F	1.356	ELA	4.69	0.53
	J	0.322	ELA	4.25	0.41
	M	0.978	ELA	4.89	2.59
	N	1.747	LA	4.70	0.69
Apis cerana	C	1.364	ELA	5.06	0.89
	D	1.146	ELA	5.42	1.32
	G	2.386	LA	5.48	0.60
	H	0.342	EW	5.24	0.82
	I	0.823	ELA	6.33	0.17
	K	1.354	ELA	5.94	2.21
	L	1.369	ELA	4.54	0.86

Table 2: The results of chemical tests of honey samples of *Apis dorsata* and *Apis cerana.*

Species	Sample	Free acidity meq/kg	RI	Water(g/100g)	HMF content(mg/kg)
Apis dorsata	A	42	1.4859	20.2	15.02
	B	25	1.4742	25	32.58
	E	73	1.4765	24	9.38
	F	80.7	1.4550	>25(#)	16.07
	J	81.7	1.4693	>25(#)	49.50
	M	47.3	1.4691	>25(#)	34.93
	N	47.7	1.4779	23.6	26.00
Apis cerana	C	43	1.4867	20	19.96
	D	48	1.4871	19.8	15.32
	G	41.3	1.4811	22.2	16.77
	H	61.67	1.4792	23	55.59
	I	40	1.4927	17.6	7.49
	K	119	1.4838	21	15.42
	L	82.7	1.4848	20.6	31.99

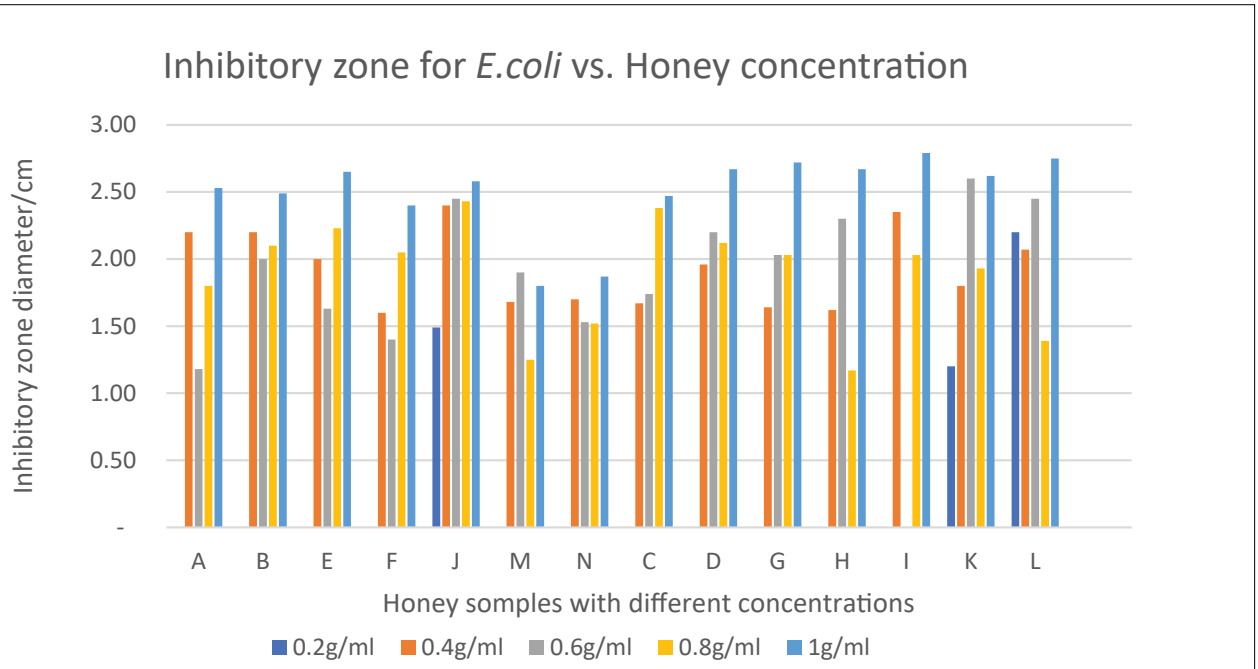

Figure 1: Inhibitory zones for *E. coli* using different honey concentrations. X axis: Honey samples with different concentrations (*A. Dorsata* honey: A, B, E, F, J, M, N; *A. cerana* honey: C, D, G, H, I, K, L), Y axis: Inhibitory zone diameter/cm.

The HMF content of honey samples of *A. dorsata* that were used in this experiment varied between 9.38-49.50 while the HMF content of honey samples of *A. cerana* lies between 7.49 -53.94. The highest HMF content was observed in *A. cerana* honey sample H (55.59 mg/kg) while the lowest was observed in *A. cerana* honey sample I (7.49 mg/kg).

Biological tests

All fourteen honey samples were evaluated for their antibacterial activity against the Gram-positive species *Staphylococcus aureus* and the Gram-negative species *Escherichia coli*. The mean inhibitory diameter after 24 hours of incubation is shown in Figure 1 and Figure 2 for each honey sample at different concentrations.

Most of the honey samples gave inhibitory zones at different concentrations (0.2 g/mL 0.4 g/mL, 0.6 g/mL, 0.8 g/mL and 1g/mL) against Gram-negative bacteria *E. coli* as well as gram-positive *S. aureus*. However, more inhibitory zones were observed against gram-negative bacteria *E. coli* compared to *S.*

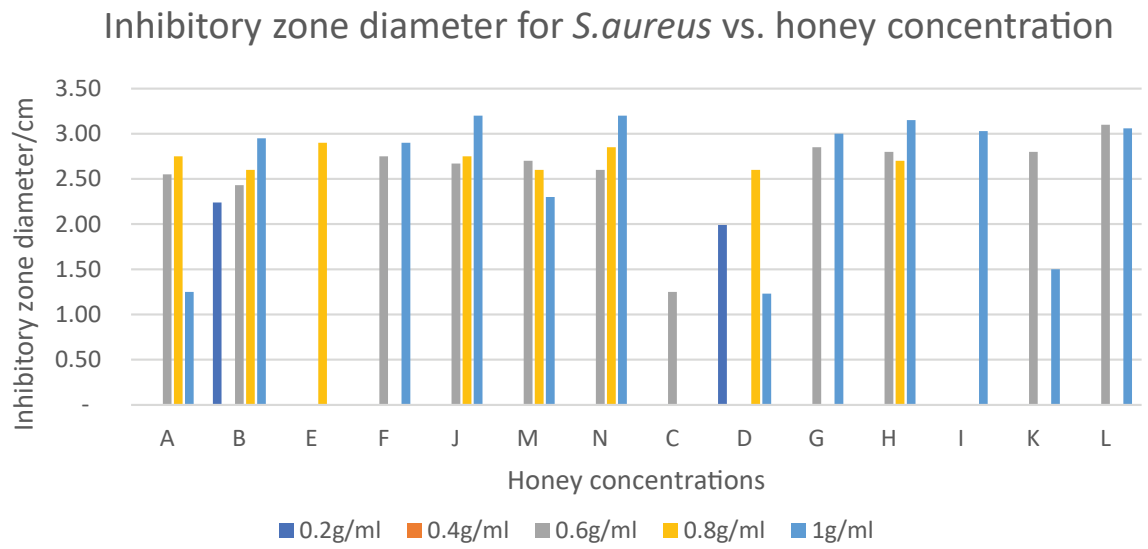

Figure 2: Inhibitory zones for *S. aureus* using different honey concentrations. X axis: Honey samples with different concentrations (*A. Dorsata* honey: A, B, E, F, J, M, N; *A. cerana* honey: C, D, G, H, I, K, L), Y axis: Inhibitory zone diameter/cm.

Table 3: Results of total phenolic content assay, alpha amylase assay and invertase assay.

Sample type	Sample	Total phenolic content GAE/kg	DN value	IN value
***A. cerana* honey**	C	355.32 ± 4.46	13.07 ± 0.18	10.67 ± 0.37
	D	1,243.06 ± 210.07	1.61 ± 0.03	1.08 ± 0.13
	G	631.72 ± 27.16	2.35 ± 0.11	2.60 ± 0.20
	H	276.16 ± 25.85	1.21 ± 0.04	3.84 ± 0.03
	I	406.25 ± 12.73	Negligible	0.33 ± 0.24
	K	1,952.34 ± 34.73	1.94 ± 0.04	5.58 ± 0.10
	L	759.49 ± 22.58	2.54 ± 0.03	3.83 ± 0.04
***A. dorsata* honey**	A	454.86 ± 26.39	2.54 ± 0.05	0.82 ± 0.04
	B	316.90 ± 27.26	2.61 ± 0.11	1.07 ± 0.36
	E	345.73 ± 12.08	1.35 ± 0.04	0.45 ± 0.21
	F	552.55 ± 27.58	Negligible	0.12 ± 0.02
	J	375.23 ± 2.12	Negligible	0.66 ± 0.69
	M	730.79 ± 37.28	3.42 ± 0.16	1.15 ± 0.39
	N	493.29 ± 24.86	4.49 ± 0.09	4.60 ± 0.16

aureus. The antimicrobial activity was increasing with honey concentration in some honey samples. The inhibitory zones for *E. coli* between *A. dorsata* and *A. cerana* honey samples at 1 g/mL concentration were significantly different (p= 0.0299, p<0.05), where honey from *A. cerana* produced the largest inhibition zone compared to honey from *A. dorsata.*

For the DPPH assay, the dry zone *A. cerana* honey sample K had the highest scavenging activity (IC_{50} =2.42 ± 0.30 mg/mL) and highest total phenolic content (1952.34 ± 34.73 GAE/Kg). A. *dorsata* honey sample F, M and *A. cerana* honey sample D had IC_{50} values of 4.95 ± 0.53 mg/mL, 4.11 ± 0.14 mg/mL, 4.53 ± 0.49 mg/mL respectively.

The average invertase activity, DN value and IN of the honey samples of *A. dorsata* and *A. cerana* are expressed in Table 3. Dry zone *A. dorsata* honey sample A had the highest DN value (13.07 ± 0.18). Only sample A had higher invertase activity (78.41 ± 3.27 IU/kg) than the acceptable limit of EU standard (≥50 IU/kg). Additionally, *A. dorsata* honey showed higher enzymatic activity compared to *A. cerana* honey.

DISCUSSION

Fourteen honey samples from two bee honey varieties (*Apis dorsata* and *Apis cerana*) were used for this investigation. Since the colonies of *Apis dorsata* migrate seasonally, most

of the samples of *Apis dorsata* collected were from the wet zone except sample A which was collected from the dry zone. Honey samples of *Apis cerana* were from all three climatic zones (dry, wet and intermediate) of Sri Lanka.

There is no significant difference between the colours of the samples of *Apis dorsata* and *Apis cerana* used in this experiment. Most of the honey samples of both species were of extra light amber colour (ELA). In commercial honey production, the colour might be changed according to beekeeper's interventions, distinct ways of handling the combs and contamination by heavy metals [23].

The pH values of all the honey samples were within the acidic range of 4.14 - 6.33. The average pH of the tested honey samples of *A. dorsata* (4.87) is lower than the average pH of the tested honey samples of *A. cerana* (5.43). The pH values of honey samples were close to those previously reported in Indian, Algerian, Brazilian, Spanish and Turkish honey (between pH 3.49 and 4.70) [23-27] . Therefore, the pH variation may be due to the species of bee or the botanical origin. The fermentation process of sugars into organic acids such as acetic, gluconic, oxalic, formic, malic, succinic, pyruvic, tartaric, citric and amino acids are also responsible for the acidity of honey (28)·(29). Acidity is a factor that is related to the secretions of bees or the nectar of flowers. Acidity in the above studies was found to be 17, 28.67, 29 and 25 miliequi/kg [28]. The free acidity of our honey samples varied between 25-82.7 meq/kg. The stability and the flavor of honey depend on the higher acidity and serve as an indication of higher content of minerals [23,28].

Conductivity is a parameter that relates to the acids and the ash content of the honey [23]. The conductivity of darker honey is slightly greater than lighter honey, which indicated that darker honey has more mineral content [28]. Thus, the electrical conductivity may exhibit a noticeable natural variation and for the honey origin, ash content deeds as a quality criterion [11]. If the ash and acid contents are higher, the conductivity of honey samples is increased. The electrical conductivity (EC) of a previous study ranged from 0.53 ± 0.03 to 4.18 ± 0.05 ms/cm [25]. Except for two samples (A-1.98, M-2.59), the conductivities of other honey samples of *A. dorsata* were within the range of 0.37-0.64 ms/cm that follows the standard limit (not more than 0.8 mS/cm) [23-29].

The moisture content is one of the quality criteria of honey that determines the stability against microorganisms and the resistance to the spoilage that occurred through the fermentation process of yeast [11]. The higher the moisture content the higher probability of honey fermentation during storage. Lower moisture limits (<20%), extend the shelf life of honey [23]. Honey samples used in the Sohaimy et al study, the moisture contents of the examined honey samples (14.73 ± 0.36% to 18.32 ± 0.67%) were relatively low compared to our samples. Moreover, in a Nigerian study, the moisture content of the samples had average values of 16.00 ± 2.19 g/100 g [2]. According to our study water content of *A. dorsata* honey is significantly higher than that of *A. cerana* honey. The main reason for this might be the geographical variation.

HMF generation is a natural process that occurs with heat. At higher temperatures, this happens rapidly. Consequently, HMF content is used as an indicator for the heating and time of the storage [11]. A low amount of HMF is an indication of honey of good quality. There was no significant difference between the HMF content of *A.cerana* honey vs *A. dorsata* honey.

As a whole, most of the honey samples gave inhibitory zones at different concentrations against Gram-negative bacteria *E. coli* than Gram-positive bacteria *S. aureus*. Moreover, the antimicrobial activity was increasing with honey concentration in the majority of the honey samples tested. Our findings are in accordance with Abd-El Aal et al., where honey has shown a greater inhibitory effect on isolated gram-negative bacteria [30]. Gram-positive bacteria proved to be more susceptible to Malaysian honey compared to Gram-negative bacteria species which is in contrast to our findings [7].

It was observed that the IC_{50} of ascorbic acid was significantly lower than all honey samples. The 50% scavenging activity of ascorbic acid was 4.63 ± 0.15 µg/mL. Only four samples gave IC_{50} value within the used concentration series (0.25–5.0 mg/mL). Honey sample K (IC_{50} =2.42 ± 0.30 mg/mL) had the highest scavenging activity compared to other honey samples. The scavenging activity was increased in the order of sample K> M> D> F. All these four samples were extra light amber colour confirming that naturally darker honey has greater antioxidant properties [5].

The total phenolic content of the honey samples ranged from 1,952.34-276.16 and reveals that the phenolic content varies according to the floral source. *A. cerana* honey sample K had the highest total phenolic content (1,952.34 ± 34.73 GAE/kg). For Thai and Manuka honey, phenolic content was in the range of 210-1,519, 563-785 mg GAE/kg respectively [31]. According to Alzahrani et al. New Zealand Manuka honey had the highest phenolic content with 899.09 ± 11.75 mg GAE/kg. The antioxidant activity is increased regularly with the increasing amount of polyphenols [10]. The results of this study indicate that the samples compare favorably with samples in many parts of the world and also fall within the

limits of international standards. In the alpha-amylase assay, sample A which was collected from the dry zone showed the highest DN of 13.07 ± 0.18). The DN values of dry zone samples used in this investigation varied between (13.07-1.35). The DN values of intermediate zone samples used in this investigation varied between (4.49- 2.54). The DN values of wet zone samples used in this investigation varied between (2.54- 1.21). Mean DN value of 24.3 ± 9.3 was observed which is relatively high compared to our findings [32].

Invertase activity was highest in (78.41 ± 3.27 IU/kg) in *A. Dorsata* honey sample A (dry zone) and lowest (0.90 ± 0.18 IU/kg) in *A. cerana* honey sample H (wet zone). Only sample A had higher invertase activity than the acceptable limit of the EU standard (> 50 IU/kg). The *A. dorsata* honey samples showed higher invertase activity than *A. cerana* honey. According to a study carried out in the Czech on fresh honey, invertase activity varies at individual honey types and ranges from 0.8 to 25.9 IN. The mean value is 15.8 ± 6.3. Invertase activity is a much more sensitive indicator of heating, storage conditions and storing time of honey than is diastase or HMF [32].

Since commercial honey production is not very popular in Sri Lanka, classification of honey according to the blossom origin (monofloral, multi-floral with a predominance of some plants) or recognition of the botanical origin is difficult [33]. It would be a limitation of this study. That can be avoided through the careful inspection of the habitat of the specific group of bees unless it is impracticable.

Even though the water content is higher in the samples of *A. dorsata*, the results obtained for the other parameters determined in this investigation were interesting.

The future of this research should be concentrated on the identification of the physical, chemical, biological and medicinal properties of these Sri Lankan honey varieties according to their botanical and geographical origin while directing toward the most economical and effective outcomes. The recognized medicinal properties can be further developed into in vitro and in vivo investigations combined with animal studies that will lead to new formulations containing honey with therapeutic activity.

CONCLUSIONS

The results obtained in this investigation revealed that most of the honey samples of *A. dorsata* and *A. cerana* found in Sri Lanka belong to ELA colour. Honey samples of *A. dorsata* contained more water than the honey samples of *A. cerana*. The only observed significant physiochemical variation between the honey samples of *A. dorsata* and *A. cerana* was the water content. The present findings demonstrated the two honey varieties are similar in chemical and physical characteristics tested. Most of the honey samples gave inhibitory zones at different concentrations against Gram-negative bacteria *E. coli* than Gram-positive bacteria *S. aureus.* A. *cerena* honey samples collected from the dry zone had higher radical scavenging activity and total phenolic content than other samples. *A. dorsata* honey showed higher enzymatic activity compared to *A. cerana* honey. The current findings revealed that *A. cerana* honey had higher antioxidant activity than *A. dorsata* honey while *A. dorsata* honey had higher enzymatic activity than *A. cerana* honey. In conclusion, the biological characteristics tested were significantly different in the two honey varieties. This study indicates that honey from both *A. dorsata* and A. *cerana* has the future potential to be developed as new drug formulations.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ETHICAL APPROVAL

The research does not involve any testing with animal or humans.

AUTHOR CONTRIBUTION

TT conceived and designed the analysis, carried out the experiments, curated the data, did the formal analysis, supervised, wrote the original draft, and did the editing. WW and LV carried out the experiments, contributed data and performed the analysis. TT, NA and LV wrote and reviewed the paper.

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