Physicochemical and Antioxidant Properties of Honeys from the Sundarbans Mangrove Forest of Bangladesh

M Rabiul Islam1, Tahmina Pervin1, Hemayet Hossain2, Badhan Saha3, and Sheikh Julfikar Hossain1

1Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna 9208, Bangladesh
2BCSIR Laboratories and 3Institute of Food Science and Technology, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka 1205, Bangladesh

ABSTRACT: This study evaluated the physicochemical, nutritional, antioxidant, and phenolic properties of ten honey samples from the Sundarbans mangrove forest, Bangladesh. The average pH, electrical conductivity, total dissolved solid, ash, moisture, hydroxymethyl furfural, titrable acidity, and absorbance were 4.3, 0.38 mS/cm, 187.5 ppm, 0.14%, 17.88%, 4.4 mg/kg, 37.7 meq/kg, and 483 mAU, respectively. In the honeys, the average contents of Ca, Cu, Fe, K, Mg, Mn, and Na were 95.5, 0.19, 6.4, 302, 39.9, 3.4, and 597 ppm, respectively, whereas Cd, Cr, Pb, and Ni were not found. The average contents of total sugar, protein, lipid, vitamin C, polyphenols, flavonoids, and anthocyanins in the honeys were 69.3%, 0.8%, 0.29%, 107.3 mg/kg, 757.2 mg gallic acid equivalent/kg, 43.1 mg catechin equivalent/kg, and 5.4 mg/kg, respectively. The honeys had strong 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity, reducing power and total antioxidant capacity. High-performance liquid chromatography analysis of the honey fractions revealed the quantification of six polyphenols namely, (+)-catechin, (−)-epicatechin, −-caumeric acid, syringic acid, p-caumeric acid, trans-cinnamic acid, and vanillic acid at 194.98, 330.34, 74.64, 218.97, 49.55, and 118.84 mg/kg, respectively. Therefore, the honeys in the Sundarbans are of excellent quality and a prospective source of polyphenols, and antioxidants.

Keywords: antioxidant, honey, nutrients, polyphenols, the Sundarbans

INTRODUCTION

Sundarbans, the world’s largest contiguous tract of mangrove forest, is located in the South-Western regions of Bangladesh. This mangrove ecosystem produces about 50% of the total production of honey in the country (1). Harvesting the honey from the Sundarbans is open to the public from April to June. Among the various plant species in the Sundarbans, the flowering periods of 12 ~ 13 plant species are synchronizing with the time of honey collection. At that time, the giant honey bee, Apis dorsata, collects nectars mainly from the flowers of Acanthus ilicifolius, Aegiceras majus, Avicennia alba, Avicennia officinalis, Brugiera gymnorrhiza, Ceriops decandra, Cynometra ramiflora, Excoecaria agallocha, Heritiera fomes, Rhizophora mucronata, Sonneratia apetala, Sonneratia caseolaris, and Xylocarpus mekingsis, and store the honey in the combs built in an open place on the branches of the trees (2). The physicochemical characteristics of these multi-floral honeys are possibly different from those of other honeys around the world due to a unique floral composition, geographical origin, and environmental conditions.

Simple sugars, such as glucose (31%) and fructose (38%), are the major components in honeys, whereas proteins, phenolic compounds, free amino acids, carotenoids, organic acids, minerals, enzymes, vitamins, and aroma compounds constitute the minor components (3-5). Reportedly, honey has more than 500 active components and is considered as part of many traditional medicines and cultures. These components contribute to anti-bacterial, anti-oxidant, anti-inflammatory, anti-browning, anti-allergic, anti-parasitory, anti-ulcer, anti-tumor, and anti-viral activities (5,6). Vitamins such as phyllochinon (K), thiamin (B12), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxin (B6), folic acid (B9), ascorbic acid (C), and α-tocopherol (E) are present in small amounts in honey, and their contribution to the recommended daily intake is marginal (4,5). The physicochemical characteristics of honeys from different regions of the world have been studied in Malaysia (7), Algeria (8), Portugal (9), and India (10). These characteristics include moisture content, electrical conductivity, reducing and non-re
ducing sugars, free acidity, and hydroxymethylfurfural (HMF) which refer to the quality criteria of honey as specified in the EC Directive 2001/110 (11). At present, the antioxidant potential of honey is also being considered as a useful quality criterion. Honey with high antioxidant potential must have high amounts of functional components. The content of polyphenols and flavonoids contribute to the antioxidant capacity of honey (6). Amino acids, ascorbic acid, carotenes, flavonols, organic acids, protein, selenium, α-tocopherol, glucose oxidase, catalase, and peroxidase are also antioxidants in honey (4-6, 12). It was reported that compared to rats fed with fructose, honey-fed rats had higher plasma α-tocopherol levels, higher α-tocopherol/triacylglycerol ratios, lower plasma nitrate levels, and lower susceptibility of the heart to lipid peroxidation (13). Selenium is an essential trace element especially for 1 to 15 years old children (5). Incorporating into selenoproteins, selenium is involved in various cellular processes such as removal of peroxides, reduction of oxidized proteins and membranes, and regulation of redox signaling (14). Antioxidant compounds inhibit the pathogenesis of various diseases including cataract, cancer, diabetes, inflammation, atherosclerosis, cardiovascular, and neurodegenerative diseases (15). Recently, studies on the physicochemical and antioxidant properties of both monofloral and multifloral honeys from different parts of Bangladesh have been conducted except for the honeys of the Sundarbans (16,17). Every year, natural honey is collected from the Sundarbans, and it is popularly consumed in South-Asian countries, especially in Bangladesh and India, whereas no reports showed detailed study of the physical, nutritional, mineral, antioxidant properties as well as polyphenolic compounds in the honeys.

MATERIALS AND METHODS

Chemicals and reagents
The 1,1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Arbutin, benzoic acid, bovine serum albumin, caffeic acid, (+)-catechin hydrate, trans-cinnamic acid, p-coumaric acid, ellagic acid, (−)-epicatechin, trans-ferulic acid, Folin-Ciocalteu’s phenol reagent, gallic acid, hydroquinone, kaempferol, myricetin, quercetin, rosmarinic acid, rutin hydrate, syringic acid, vanillic acid, and vanillin were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Acetonitrile, acetic acid, ascorbic acid, diethyl ether, dimethyl sulfoxide (DMSO), ethanol, HCl, H₂SO₄, and methanol were obtained from Merck (Darmstadt, Germany).

Honey samples
Ten composite samples of honeys namely S1, S2, S3, S4, S5, S6, S7, S8, S9, and S10 were collected from the honey collectors in the Sundarbans from March to July in 2015. Two composite samples were collected each month from different parts of the Sundarbans. The collected honey samples were taken in the laboratory and kept in a refrigerator at 4°C in air tight glass containers.

Fractionation of honey
Five grams of each sample was placed in a beaker to make 50 g, and the honey was mixed thoroughly. Then, 20 g of honey was placed in an airtight container. It was then extracted by adding 200 mL of 100% diethyl ether and vigorous shaking the mixture for 30 min. Hereafter, the mixture was filtered through Whatman filter paper no. 1. The filtrate was air-dried, and the extract was stored at 4°C in a refrigerator as the diethylether fraction. Similarly, ethanol, methanol, and distilled water fractions were successively prepared following the same procedure using the residues on the filter paper. The diethyl ether, ethanol, methanol, and water fractions were designated as DEH, ETH, MEH, and DWH, respectively. Finally, 10 mg of the solid was dissolved in 1 mL DMSO (10 mg/mL) to determine the total antioxidant capacity and amounts of different polyphenols.

Determination of the physicochemical properties of honeys
The pH of the honeys was determined according to the method described by the International Honey Commission (18). Electrical conductivity (EC) and total dissolved solid (TDS) were measured according to the harmonized methods of the European Honey Commission (19). The ash content of the honeys was determined as described by Piazza et al. (20). The moisture content of the honeys was determined according to the method followed by Association of Official Analytical Chemists (AOAC) (21). HMF content in the honeys was determined according to White (22). Titrable acidity (TA) of the honeys was determined according to the method of AOAC (23). The color intensity of honeys was determined using the method of Beretta et al. (24). The absorbance (ABS) was taken at 450 and 720 nm, and intensity was calculated using the formula, ABS = (ABS₄₅₀−ABS₇₂₀) × 1,000 mAU.

Determination of the nutritional properties of honeys
The total carbohydrate of the honeys was determined by the titrimetric method (25). Protein contents were calculated by the Lowry et al. (26) method. Total lipids were determined by extracting the honey with chloroform : methanol (1:2) (27). The vitamin C content was determined as described by Plummer (28) using 2,6-di-chlorophenolindophenol with minor modifications, and
was expressed as mg ascorbic acid/kg honey.

Mineral contents in the honey samples were estimated as described by Hoenig and de Kersabiec (29) with slight modifications. The concentrations of Ca, Cd, Cr, Cu, Fe, Pb, Mg, Mn, Ni, and Zn were determined by flame atomic absorption spectrophotometry. One g of honey was placed in a 50 mL flask and 15 mL of HNO₃ and HClO₄ as a ratio of 2:1 was added. The mixture was heated in a fume hood (Esco Frontier Acid Digestion, ESCO Pte. Ltd., Singapore) on a hot plate (model VWR, VELP Scientifical, Frankfurt, Germany). Generation of white fumes from the flasks indicated the completion of digestion, and the flasks were allowed to cool. These digested samples were transferred into 100 mL volumetric flasks, and the volume was adjusted to 100 mL by adding distilled water. Then, the extract was filtered with filter paper (Whatman no. 42), and the filtrate was collected in labeled plastic bottles. The solutions were analyzed for the content of elements using an atomic absorption spectrophotometer (Shimadzu AA-7000, Shimadzu Corporation, Kyoto, Japan) with suitable hollow cathode lamps. The concentrations of different elements in honeys were determined by the corresponding standard calibration curves obtained by using standard analytical reagent grade solutions of the elements, Ca, Cd, Cr, Cu, Fe, Pb, Mg, Mn, Ni, and Zn. A 0.5 M chloride solution containing 20% trichloroacetic acid and 10% lanthanum chloride (w/v) was added to the sample used for Ca measurement to prevent interference by coexisting elements. A 0.5 M chloride solution containing 10% lanthanum chloride was added to the sample used for Mg measurement. Digested honeys were used to determine the concentration of Na and K using a flame photometer.

Determination of total polyphenols (TPH), flavonoids (TF), and anthocyanins

The concentration of TPH in the honeys was determined according to the Folin-Ciocalteu method (30) with gallic acid (GA) as the standard and expressed as gallic acid equivalents (mg GAE) in the honey. The TF content in the honeys was determined by the colorimetric assay described by Zhishen et al. (31). The results were expressed as (+)-catechin equivalents (mg CE). Total anthocyanin was estimated using the method described by Fuleki and Francis (32), and the results were expressed as μg/g honey.

DPPH free radical scavenging activity

The reaction mixture (total volume, 3 mL), consisting of 0.5 mL of a 0.5 M acetic acid buffer solution at pH 5.5, 1 mL of 0.2 mM DPPH in ethanol, and 1.5 mL of a 50% (v/v) ethanol aqueous solution, was shaken vigorously with the honey according to Blois (33). After incubation at room temperature for 30 min, the amount of remaining DPPH was determined by measuring the absorbance at 517 nm. Mean values were obtained from triplicate experiments.

Reducing power capacity

The reducing power of the honeys was determined according to the method of Oyaizu (34). Briefly, different concentrations of the honeys were mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1% potassium ferricyanide solution. After incubation at 50°C for 20 min, the mixtures were mixed with 2.5 mL of 10% trichloroacetic acid followed by centrifugation at 650 g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of this solution was measured at 700 nm. Ascorbic acid served as the positive control.

Total antioxidant capacity (TAC)

The TAC assay was done according to the method described by Prieto et al. (35). The tubes containing honey or a honey fraction and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were incubated at 90°C for 90 min. After cooling at room temperature, the absorbance was measured at 695 nm against a blank. The antioxidant capacity was expressed as mg ascorbic acid equivalents (AAE/g honey or fraction) and GAE/g honey or fraction.

Determination of phenolic compounds in the fractions

Detection and quantification of selected phenolic compounds in the fractions were determined by high-performance liquid chromatography (HPLC)-diode-array detection (DAD) analysis as described by Jahan et al. (36) with some modifications. The analysis was carried out on a Dionex UltiMate 3000 system equipped with a quaternary rapid separation pump (LPG-3400RS, Thermo Fisher Scientific, Waltham, MA, USA) and photodiode array detector (DAD-3000RS, Thermo Fisher Scientific). Separation was performed using an Acclaim® C18 (5 μm) Dionex column (4.6×250 mm, Thermo Fisher Scientific) at 30°C with a flow rate of 1 mL/min and an injection volume of 20 μL. The mobile phase consisted of acetonitrile (solvent A), acetic acid solution pH 3.0 (solvent B), and methanol (solvent C) with the gradient elution program of 5%A/95%B (0–5 min), 10%A/90%B (6–9 min), 15%A/75%B/10%C (11–15), 20%A/65%B/15%C (16–19 min), 30%A/50%B/20%C (20–29 min), 40%A/30%B/30%C (30–35 min), and 100%A (36–40 min). The UV detector was set to 280 nm for 22 min, changed to 320 nm for 28 min, again changed to 280 nm for 35 min, and finally to 380 nm for 36 min and held for the rest of the analysis period while the diode array detector was set at an acquisition range from 200 nm to 700 nm. For the preparation of the calibration curve, a standard stock solution was prepared in methanol containing ar-
butin, (−)-epicatechin (5 μg/mL each), gallic acid, hydroquinone, vanillic acid, rosmarinic acid, myricetin (4 μg/mL each), cafffeic acid, syringic acid, vanillin, trans-ferulic acid (3 μg/mL each), p-coumaric acid, quercetin, kaempferol (2 μg/mL each), (+)-catechin hydrate, ellagic acid (10 μg/mL each), trans-cinnamic acid (1 μg/mL), rutin hydrate (6 μg/mL), and benzoic acid (8 μg/mL). A solution of the fraction was prepared at a concentration of 10 mg/mL. Prior to HPLC analysis, all the solutions (mixed standards, sample, and spiked solutions) were filtered through a 0.20 μm syringe filter (Sartorius AG, Göttingen, Germany) and then degassed in an ultrasonic bath (Hwashin, Seoul, Korea) for 15 min. Data acquisition, peak integration, and calibrations were calculated with the Dionex Chromeleon software (version 6.80 RS 10, Dionex, Sunnyvale, CA, USA).

Statistical analysis
Statistical analysis was performed using SPSS (version 16, SPSS Inc., Chicago, IL, USA). Results were expressed as mean±standard deviation (SD) for a given number of observations, n=3∼10. One way analysis of variance (ANOVA) followed by least significant difference (LSD) multiple comparison post-hoc tests were used to analyze the statistical difference. Differences with \( P \)-values <0.05 were considered statistically significant.

RESULTS AND DISCUSSION
Physicochemical properties
The physicochemical properties of honey are attributed to its characteristics, tests, quality, and functional parameters. The average pH, EC, TDS, ash, moisture, HMF, TA, and \( \text{ABS}_{450} \) of the honeys were 4.3, 0.38 mS/cm, 187.5 ppm, 0.14%, 17.88%, 4.4 mg/kg, 37.7 meq/kg, and 483 mAU, respectively (Table 1). The Codex Alimentarius (37) set up the standard quality criteria of honeys for authenticity, and that includes physical, nutritional, and chemical properties. The pH values of the analyzed honeys ranged from 3.9 to 4.4, and none of them exceeded the allowed limit of 3.2 to 5.0 set by the Codex Alimentarius (37). The Codex Alimentarius is an index of freshness of the honeys, and reflects the ability to inhibit the growth of microorganisms. These values were similar to previously reported honeys from Bangladesh, pH 3.2~4.5 (16), and also from other countries such as Brazil (38) and India (10). The honeys showed smaller EC values than the maximum limit of 0.8 mS/cm from the Codex Alimentarius (37), suggesting that they were nectar honeys. Our study also showed that honey samples with the highest EC had the highest TDS. Ranging from 0.09 to 0.18%, the ash content were lower than the allowed limit of 0.6% for floral honeys, indicating the honeys were clear and free from adulteration. The moisture content in the analyzed honeys ranged from 13.5 to 19.9%, which was less than the maximum limit of 20% (37). Moisture is one of the most important factors that determine the quality of honeys. Moisture content determines the growth of osmotolerant microorganisms in honeys. Low moisture prevents the growth of microorganisms resulting in the protection of quality and increases the shelf-life of honey, whereas high moisture shows adverse effects. Therefore, honeys of the Sundarbans exhibited low moisture content and thus were of good quality. The HMF content was determined to know the freshness and quality of the honeys. All the analyzed honeys showed HMF levels within the allowed limits of 40 mg/kg (37) that demonstrated their freshness and good quality. Reportedly, HMF is absent in fresh honey whereas various factors such as aging, processing, temperature, pH, and floral source influence its levels. Free

| Sample no. | pH   | EC (mS/cm) | TDS (ppm) | Ash (%) | Moisture (%) | HMF (mg/kg) | TA (meq/kg) | \( \text{ABS}_{450} \) (mAU) |
|------------|------|------------|-----------|---------|--------------|-------------|-------------|-----------------|
| S1         | 4.4±0.1<sup>c</sup> | 0.4±0.0<sup>b</sup> | 200±0<sup>b</sup> | 0.15±0.0<sup>b</sup> | 18.9±0.2<sup>cde</sup> | 5.4±0.1<sup>b</sup> | 31.3±0.1<sup>b</sup> | 477±1<sup>abc</sup> |
| S2         | 4.3±0.0<sup>c</sup> | 0.4±0.0<sup>b</sup> | 200±0<sup>b</sup> | 0.15±0.0<sup>b</sup> | 18.9±1.9<sup>cde</sup> | 4.9±0.1<sup>ef</sup> | 32.5±1.7<sup>ef</sup> | 431±4<sup>c</sup> |
| S3         | 4.4±0.1<sup>c</sup> | 0.5±0.1<sup>b</sup> | 225±1<sup>b</sup> | 0.18±0.1<sup>b</sup> | 17.8±3.2<sup>cde</sup> | 4.3±0.0<sup>cd</sup> | 32.5±3.5<sup>ef</sup> | 429±1<sup>abcd</sup> |
| S4         | 4.3±0.1<sup>c</sup> | 0.4±0.0<sup>b</sup> | 200±0<sup>b</sup> | 0.15±0.0<sup>b</sup> | 18.3±0.5<sup>cde</sup> | 3.7±0.0<sup>def</sup> | 34.4±0.8<sup>cd</sup> | 580±9<sup>de</sup> |
| S5         | 4.3±0.0<sup>c</sup> | 0.5±0.1<sup>b</sup> | 225±1<sup>b</sup> | 0.18±0.1<sup>b</sup> | 19.9±0.5<sup>cde</sup> | 4.6±0.1<sup>cde</sup> | 38.1±2.6<sup>ab</sup> | 449±6<sup>e</sup> |
| S6         | 4.2±0.1<sup>b</sup> | 0.4±0.0<sup>b</sup> | 200±0<sup>b</sup> | 0.15±0.0<sup>b</sup> | 17.5±1<sup>cde</sup> | 4.9±0.1<sup>cde</sup> | 38.1±0.9<sup>cde</sup> | 557±6<sup>bc</sup> |
| S7         | 4.3±0.0<sup>c</sup> | 0.4±0.1<sup>ab</sup> | 175±1<sup>ab</sup> | 0.12±0.1<sup>ab</sup> | 16.0±2<sup>ab</sup> | 4.0±0.0<sup>cd</sup> | 41.9±2.6<sup>cd</sup> | 431±5<sup>c</sup> |
| S8         | 4.3±0.0<sup>c</sup> | 0.3±0.0<sup>c</sup> | 150±0<sup>c</sup> | 0.09±0.0<sup>c</sup> | 19.5±1.10<sup>de</sup> | 3.9±0.3<sup>c</sup> | 40.6±2.6<sup>ab</sup> | 426±8<sup>e</sup> |
| S9         | 4.3±0.0<sup>c</sup> | 0.3±0.0<sup>c</sup> | 150±0<sup>c</sup> | 0.09±0.0<sup>c</sup> | 13.5±0.6<sup>cde</sup> | 4.9±0.1<sup>cde</sup> | 32.5±1.1<sup>c</sup> | 456±10<sup>c</sup> |
| S10        | 3.9±0.0<sup>c</sup> | 0.3±0.0<sup>c</sup> | 150±0<sup>c</sup> | 0.09±0.0<sup>c</sup> | 18.8±1<sup>cde</sup> | 3.6±0.2<sup>c</sup> | 41.8±0.9<sup>cde</sup> | 593±6<sup>c</sup> |
| Average    | 4.3±0.1 | 0.4±0.0 | 188±10 | 0.14±0.0 | 17.9±0.8 | 4.4±0.2 | 37.7±1.8 | 483±7 |

EC, electrical conductivity; TDS, total dissolved solid; HMF, hydroxymethylfurural; TA, titrable acidity; \( \text{ABS}_{450} \), absorbance at 450 nm. Values represent the means±SD (n=3∼10). Values with different letters (a-f) within the same column differ significantly (\( P < 0.05 \)) through one way ANOVA followed by LSD multiple comparison post-hoc test.
Ascorbic acid is one of the non-enzymatic antioxidants reported to be present in honey. It is found at concentrations ranging from 0.134 to 0.146% total fat from unifloral honey, with an average value of 0.29% (Table 2). Khalil et al. (41) noted that the content of ascorbic acid in the investigated honeys ranged from 0.7 to 0.8%, and their average value was 0.29% (Table 2).

The lipid content in the honeys is another important component, as it may contribute to the protein and pollen contents. Mangroves contain high amounts of lipids, which are free fatty acids like palmitic acid, oleic acid, and linoleic acid. The lipid content in the investigated honeys ranged from 4.2 to 6.6% of the total content (41).

Table 2. Nutrient compositions of the honeys

| Sample no. | Reducing sugar (%) | Non-reducing sugar (%) | Total sugar (%) | Total proteins (%) | Lipid (%) | Vit. C (mg/kg) | Ca (ppm) | Cu (ppm) | Fe (ppm) | K (ppm) | Mg (ppm) | Mn (ppm) | Na (ppm) | Zn (ppm) |
|------------|--------------------|------------------------|-----------------|------------------|-----------|---------------|-----------|-----------|-----------|---------|---------|---------|---------|---------|
| S1         | 62.6±0.7<sup>a,b,d</sup> | 7.6±0.1<sup>b</sup> | 70.2±0.9<sup>a,b</sup> | 0.7±0.1<sup>a</sup> | 0.26±0.1<sup>c</sup> | 89.9±7.7<sup>a</sup> | 113.3±1.9<sup>a</sup> | 0.53±0.1<sup>d</sup> | 7.6±0.4<sup>c</sup> | 230±11<sup>c</sup> | 35.4±0.8<sup>d</sup> | 5.7±0.2<sup>d</sup> | 620±51<sup>c</sup> | Bcl<sup>1</sup> |
| S2         | 59.7±0.9<sup>a</sup> | 7.7±0.2<sup>b</sup> | 67.6±0.1<sup>a</sup> | 0.7±0.1<sup>a</sup> | 0.25±0.2<sup>c</sup> | 96.8±1.1<sup>a</sup> | 103.8±1.6<sup>c</sup> | 0.02±0.1<sup>c</sup> | 4.5±0.3<sup>c</sup> | 319±16<sup>c</sup> | 29.4±0.7<sup>d</sup> | 5.5±0.2<sup>c</sup> | 585±35<sup>c</sup> | Bcl<sup>1</sup> |
| S3         | 60.4±0.7<sup>a,b</sup> | 7.1±0.3<sup>b</sup> | 67.5±0.9<sup>a</sup> | 0.7±0.6<sup>abc</sup> | 0.31±0.1<sup>c</sup> | 113.8±7.9<sup>a</sup> | 99.3±3.5<sup>ab</sup> | 4.19±0.1<sup>bc</sup> | 5.8±0.2<sup>c</sup> | 298±24<sup>c</sup> | 37.3±0.2<sup>abc</sup> | 3.5±0.2<sup>c</sup> | 598±46<sup>c</sup> | Bcl<sup>1</sup> |
| S4         | 60.2±0.9<sup>a</sup> | 6.2±0.1<sup>c</sup> | 66.2±0.3<sup>a</sup> | 0.7±0.1<sup>ab</sup> | 0.27±0.2<sup>c</sup> | 117.6±9.8<sup>a</sup> | 95.1±1.2<sup>c</sup> | 1.33±0.3<sup>cd</sup> | 2.7±0.6<sup>bc</sup> | 316±15<sup>c</sup> | 42.0±0.4<sup>abc</sup> | 2.0±0.1<sup>c</sup> | 576±53<sup>c</sup> | Bcl<sup>1</sup> |
| S5         | 64.9±0.9<sup>c</sup> | 6.5±0.2<sup>c</sup> | 71.6±1.0<sup>c</sup> | 0.9±0.2<sup>ab</sup> | 0.28±0.3<sup>c</sup> | 89.9±9.7<sup>ab</sup> | 81.0±1.6<sup>c</sup> | 0.19±0.2<sup>c</sup> | 5.4±0.3<sup>c</sup> | 328±23<sup>c</sup> | 30.4±0.3<sup>c</sup> | 7.0±0.3<sup>c</sup> | 597±29<sup>c</sup> | 0.3±0.05<sup>c</sup> |
| S6         | 66.2±0.8<sup>c</sup> | 6.3±1.0<sup>c</sup> | 72.6±0.8<sup>c</sup> | 1.1±0.3<sup>c</sup> | 0.19±0.1<sup>c</sup> | 117.6±9.8<sup>a</sup> | 95.1±1.2<sup>c</sup> | 1.33±0.3<sup>cd</sup> | 2.7±0.6<sup>bc</sup> | 316±15<sup>c</sup> | 42.0±0.4<sup>abc</sup> | 2.0±0.1<sup>c</sup> | 576±53<sup>c</sup> | Bcl<sup>1</sup> |
| S7         | 67.5±0.7<sup>c</sup> | 4.5±0.2<sup>c</sup> | 68.5±5.6<sup>c</sup> | 0.8±0.3<sup>c</sup> | 0.33±0.0<sup>c</sup> | 110.7±19.0<sup>c</sup> | 93.9±1.2<sup>c</sup> | 0.03±0.0<sup>c</sup> | 3.1±0.4<sup>c</sup> | 399±42<sup>c</sup> | 40.7±0.4<sup>cd</sup> | 2.3±0.1<sup>c</sup> | 608±42<sup>c</sup> | 0.2±0.01<sup>c</sup> |
| S8         | 65.6±0.4<sup>c</sup> | 4.9±1.0<sup>c</sup> | 70.4±0.9<sup>c</sup> | 0.9±0.4<sup>c</sup> | 0.29±0.1<sup>c</sup> | 103.6±7.9<sup>c</sup> | 88.5±1.1<sup>c</sup> | 0.03±0.0<sup>c</sup> | 6.1±0.4<sup>c</sup> | 297±17<sup>c</sup> | 52.5±0.7<sup>c</sup> | 2.1±0.1<sup>c</sup> | 664±37<sup>c</sup> | Bcl<sup>1</sup> |
| S9         | 64.3±0.6<sup>c</sup> | 4.7±0.2<sup>c</sup> | 68.7±2.4<sup>c</sup> | 0.6±0.2<sup>c</sup> | 0.40±0.1<sup>c</sup> | 103.6±7.9<sup>c</sup> | 113.4±2.0<sup>c</sup> | 0.34±0.04<sup>c</sup> | 9.7±0.6<sup>c</sup> | 273±24<sup>c</sup> | 44.8±0.3<sup>c</sup> | 1.1±0.1<sup>c</sup> | 596±43<sup>c</sup> | Bcl<sup>1</sup> |
| S10        | 66.4±0.8<sup>c</sup> | 5.2±1.1<sup>c</sup> | 70.1±0.9<sup>c</sup> | 0.7±0.1<sup>c</sup> | 0.17±0.2<sup>c</sup> | 138.4±19.5<sup>c</sup> | 66.8±0.8<sup>c</sup> | 0.02±0.0<sup>c</sup> | 8.8±0.5<sup>c</sup> | 297±16<sup>c</sup> | 25.5±0.3<sup>c</sup> | 0.9±0.1<sup>c</sup> | 513±32<sup>c</sup> | Bcl<sup>1</sup> |
| Average    | 63.3±1.5 | 6.1±0.1 | 69.3±1.6 | 0.8±0.1 | 0.29±0.1 | 107.3±10.8 | 95.5±1.4 | 0.19±0.02 | 6.4±0.4 | 302±23 | 39.9±0.6 | 3.4±0.2 | 597±41 | 0.3±0.05 |

Values represent the mean±SD (n=3∼10). Values with different letters (a-i) within the same column differ significantly (P<0.05) through one way ANOVA followed by LSD multiple comparison post-hoc test.

1Below detection level.
2Not significant.
content of the honeys in the Sundarbans ranged from 89.9 to 138.4 mg/kg with an average value of 107.3 mg/kg (Table 2). A high content of ascorbic acid indicates a high antioxidant capacity of honey (43).

Reportedly, mineral content is an important index of possible environmental pollution and a potential indicator of the geographical origin of honey. Mineral contents in the honey of the Sundarbans are shown in Table 2. In this study, a total of twelve elements were quantified, and they were: Ca, Cd, Cr, Cu, Fe, Pb, K, Mg, Mn, Na, Ni, and Zn. Among the minerals, Na was the highest with a mean value of 597 ppm followed by K (302 ppm), Ca (95.5 ppm), Mg (39.9 ppm), Fe (6.4 ppm), Mn (3.4 ppm), Cu (0.19 ppm), and Zn. The content of Na was the highest probably because the honeys were produced in a coastal saline environment. Toxic elements (Cd, Cr, Pb, and Ni) were not detected in these honeys probably because the food web of the honeybees was not contaminated with the elements. The honeys contained higher amounts of Ca, Fe, Mg, and Na than those of the honeys of Portugal (9), Mexico (44), etc. It is well known that mineral elements are involved in various physiological and metabolic processes, especially in bone formation, blood clotting, muscles contraction, and enzymes activity. Therefore, honey is popularly used as a good source of nutritional supplements.

Total polyphenols, flavonoids, and anthocyanins contents

Reportedly, polyphenols, flavonoids, and anthocyanins are the major bioactive compounds in foods and beverages that contribute significantly to the taste, texture, color, and functional properties. Cimpoiu et al. (45) reported that the appearance and functional properties of honey depend on the content of total polyphenols. Alvarez-Suarez et al. (40) measured the content of total polyphenols to determine the floral origin of honeys. The average polyphenols, flavonoids, and anthocyanins contents of the honeys in the Sundarbans were 757.2 mg GAE/kg, 43.1 mg CE/kg, and 5.4 mg/kg, respectively (Table 3). The content of phenolics in these honeys was similar to that of the strawberry tree honey (789.7 mg GAE/kg) (24), but it was higher than that of the honeys from Cuba (40), Burkina Fasa (43), Algeria (46), and Malaysia (47). Islam et al. (16) reported that the polyphenols content ranged from 152.4 to 688.5 mg GAE/kg as detected in the honeys from different parts of Bangladesh. Flavonoids, and anthocyanins are low molecular weight phenolic compounds. The total flavonoids content of the honeys ranged from 25.1 to 63.9 mg CE/kg with the mean value of 43.1 mg CE/kg (Table 3). The honeys collected in July showed the highest contents of polyphenols, flavonoids, and anthocyanins (Table 3). The flavonoids content in the honeys from the Sundarbans was higher than that of Cuban (40), Burkina Fasa (43), Turkish (48), and Malaysian (49) honeys. However, the variation in the content of polyphenols, flavonoids, and anthocyanins may be due to floral types, climatic conditions, types of bee species, and harvesting period.

Antioxidant activity

The free radical scavenging activities of the honeys were measured using the DPPH free radical assay (Table 3). All the honeys dose-dependently increased the DPPH free radical scavenging activity and from the dose-dependent curves the concentrations of honeys, which scavenged 50% of DPPH free radical called inhibition concentrations 50 (IC50) were calculated (Fig. 1A). All the honeys from the Sundarbans had smaller IC50 values, which meant stronger DPPH free radical scavenging activities than the Indian (10), Algerian (46), and Malaysian (47) honeys. Therefore, the honeys produced in the Sundarbans have strong antioxidant activity because of their

| Sample No. | Polyphenols (mg GAE/kg) | Flavonoids (mg CE/kg) | Anthocyanins (µg/g) | % DPPH scavenging at 40 mg/mL | Reducing power at 6 mg/mL |
|------------|------------------------|----------------------|---------------------|-----------------------------|-------------------------|
| S1         | 715.9±24.56            | 33.1±0.8             | 5.1±0.2             | 62.7±1.3                    | 0.62±0.02               |
| S2         | 665.2±5.6              | 29.2±1.2             | 4.1±0.3             | 61.1±0.6                    | 0.63±0.06               |
| S3         | 604.4±12.2             | 25.1±2.5             | 3.6±1.4             | 70.1±1.0                    | 0.61±0.02               |
| S4         | 859.5±36.8             | 61.2±6.0             | 5.8±0.2             | 72.2±0.7                    | 0.58±0.03               |
| S5         | 732.6±78.4             | 33.3±1.2             | 4.1±1.1             | 75.7±1.3                    | 0.62±0.06               |
| S6         | 833.5±69.3             | 60.2±3.8             | 5.9±0.1             | 73.5±0.8                    | 0.78±0.01               |
| S7         | 768.1±26.4             | 40.9±1.7             | 6.1±0.1             | 74.6±0.9                    | 0.77±0.03               |
| S8         | 733.9±0.9              | 40.9±0.8             | 5.1±0.1             | 76.2±1.8                    | 0.67±0.04               |
| S9         | 772.2±13.9             | 42.5±2.1             | 6.3±0.3             | 72.8±0.6                    | 0.63±0.03               |
| S10        | 886.2±40.5             | 63.9±0.4             | 7.4±0.2             | 61.8±0.7                    | 0.75±0.03               |
| Average    | 757.2±30.8             | 43.1±2.1             | 5.4±0.2             | 70.1±0.9                    | 0.67±0.03               |

GAE, gallic acid equivalent; CE, (+)-catechin equivalent; DPPH, 1,1-diphenyl-2-picrylhydrazyl. Values represent the mean±SD (n=3−10). Values with different letters (a-h) within the same column differ significantly (P<0.05) through one way ANOVA followed by LSD multiple comparison post-hoc test.
Physicochemical Properties of Mangrove Honeys

high potential in scavenging free radicals. This may be
due to the presence of high amounts of polyphenols as
well as other functional components in these honeys.

Reducing power is one of the important measurements
of antioxidant activity. The more antioxidant compounds
reduce the more oxidized form of ferric iron (Fe\(^{3+}\)) to fer-
rous iron (Fe\(^{2+}\)). In the present study, the reducing pow-
ers of the honeys were determined using the potassium
ferricyanide reduction method as shown in Table 3. All
the honeys showed nearly similar reducing power, and
they had dose-dependent effects (data not shown). This
may be due to the presence of nearly similar amounts of
polyphenols since there is a strong positive correlation
between the content of polyphenols and reducing power
(50). The total antioxidant capacity was expressed as the
mg AAE/g honey, and as the mg GAE/g honey as shown
in Fig. 1B. The mean total antioxidant capacity of the
honeys was 107.26±8.87 mg AAE/g and 175±18.63 mg
GAE/g honey. The antioxidant capacity of fresh honeys
in the Sundarbans is comparable to the antioxidant ca-
pacity of mangrove apple, S. apetala (51). Hence, it is im-
portant to determine the antioxidant power of honey as
an eligible parameter for quality. Polyphenols, flavonoids,
anthocyanins, and vitamins along with various compo-
nents in honey synergistically contribute to the intrinsic
antioxidant capacity.

Though honey collectors start to collect the honeys
from the Sundarbans from April to July of each year, they
usually do not store it monthly. Collected honeys are
usually piled up in large vessels, stored, and then sold
with the name of Sundarbans’ honey. Twenty grams of
composite honey was successively fractionated into di-
eethyl ether, ethanol, methanol, and distilled water, and
the yields were 0.07±0.01, 29.53±0.43, 55.25±1.42, and
5.95±0.91%, respectively. Most of the components in
the honeys from the Sundarbans are hydrophilic where-
as nearly 1% is composed of lipophilic components. Fig.
2A shows the total antioxidant capacity of one gram of

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**Fig. 1.** Antioxidant activity of the honeys. (A) Inhibitory concentration 50 (IC\(_{50}\)) for scavenging DPPH free radicals by the honeys
(AAE: ascorbic acid, positive control); (B) comparison of total antioxidant capacity of the honeys (AAE: ascorbic acid equivalent,
GAE: gallic acid equivalent). Data were presented as mean±SD (n=3–9). Different letters (a–e) indicate significant differences when
compared with each other of the same type (IC\(_{50}\), AAE or GAE) at \(P<0.05\) using one way analysis of variance (ANOVA) followed
by LSD multiple comparison post-hoc test.

**Fig. 2.** (A) Antioxidant capacity of different fractions of the honeys; (B) distribution of antioxidant capacity of one gram fresh honey
in different fractions. AAE, ascorbic acid equivalent; GAE, gallic acid equivalent. Data were presented as mean±SD (n=3–5). Different
letters (a–d) indicate significant differences at \(P<0.05\) when compared with each other of the same type (AAE or GAE) according
to one way analysis of variance (ANOVA) followed by LSD multiple comparison post-hoc test. DEH, diethyl ether; ETH, ethanol;
MEH, methanol; DWH, distilled water fractions of the honeys.
the different fractions. Among the fractions, DEH showed the highest total antioxidant capacity followed by DWH, ETH, and MEH. Fig. 2B shows the distribution of total antioxidant capacity of one gram raw honey in different solvent fractions. It showed the highest antioxidant capacity included in MEH (174.2 mg GAE/g honey or 81.9 mg AAE/g honey) followed by ETH (100.8 mg GAE/g honey or 47.4 mg AAE/g honey) and DWH (21.2 mg GAE/g honey or 10 mg AAE/g honey).

**Identification and quantification of phenolic compounds**

The ethanol, methanol, and distilled water fractions of the honeys were composed of more than 99% of the amount fractionated. Therefore, identification and quantification of individual phenolic compounds in them were analyzed by HPLC. The chromatographic separations of polyphenols in the standard, ETH, MEH, and DWH are shown in Fig. 3. The content of each phenolic compound was calculated from the corresponding calibration curve as the mean of five determinations. The amount of individual phenolic compound in fresh honey was determined using the mean value. All together, six polyphenols were identified and quantified in the honey and the order being (−)-epicatechin> syringic acid> (+)-catechin> vanillic acid> p-coumaric acid> trans-cinnamic acid. The concentration of (−)-epicatechin, syringic acid, (+)-catechin, vanillic acid, p-coumaric acid, and trans-cinnamic acid were 330.34, 218.97, 194.98, 118.84, 74.64, and 49.55 mg/kg, respectively. Thus each kilogram of honey from the Sundarbans consists of 987.3±0.3 mg of the polyphenols, nearly 1 mg polyphenols per gram of the honey. However, until now, no reports described the composition of phenolic compounds in mangrove honey. The high content of polyphenols in this mangrove honey may be due to the origin of the nectar from unique multifloral vegetations grown in the Sundarbans. Notably, the nectar of *Sonneratia apetala* contribute significantly to honey production in the Sundarbans, and the fruits of the plants have a high content of polyphenols and flavonoids (51,52). However, Moniruzzaman et al. (17) reported

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**Fig. 3.** (A) High-performance liquid chromatography (HPLC) chromatogram of a standard mixture of polyphenolic compounds. Peaks: 1, arbutin; 2, gallic acid; 3, hydroquinone; 4, (+)-catechin; 5, vanillic acid; 6, caffeic acid; 7, syringic acid; 8, (−)-epicatechin; 9, vanillin; 10, p-coumaric acid; 11, trans-ferulic acid; 12, rutin hydrate; 13, ellagic acid; 14, benzoic acid; 15, rosmarinic acid; 16, myricetin; 17, quercetin; 18, trans-cinnamic acid; 19, kaempferol. (B) HPLC chromatogram of ethanol. Peaks: 1, vanillic acid; 2, syringic acid; 3, (−)-epicatechin; 4, p-coumaric acid; 5, trans-cinnamic acid. (C) HPLC chromatogram of methanol. Peak: 1, (+)-catechin; 2, vanillic acid; 3, syringic acid; 4, (−)-epicatechin; 5, p-coumaric acid; 6, trans-cinnamic acid. (D) HPLC chromatogram of water. Peaks: 1, syringic acid; 2, (−)-epicatechin; 3, p-coumaric acid.
nine phenolic compounds namely gallic acid, chlorogenic acid, caffeic acid, benzoic acid, trans-cinnamic acid, catechin, myricetin, naringenin, and kaempferol in some monofloral honey from Bangladesh. Khalil et al. (49) reported catechin, gallic acid, caffeic acid, syringic acid, benzoic acid, naringenin, trans-cinnamic acid, and kaempferol apigenin from Malaysian honey samples. Gallic acid, caffeic acid, chlorogenic acid, myricetin, kaempferol, coumaric acid, ferulic acid, and quercetin were detected in Australian honey samples (53). The observed variations in the content of polyphenols were possibly because of the different floral sources of honeys as well as influences of climatic and edaphic characteristics.

The results revealed that the physicochemical characteristics of the honeys in the Sundarbans were excellent with achieving the standard set up for honeys according to EC Directive 2001/110 (11). The levels of the physicochemical properties of the analyzed honeys were nearly similar with those found in honeys from Cuba (40), Malaysia (47), and Mexico (54). The polyphenols content (757 mg GAE/kg) and antioxidant activity of the honeys were higher than those found in honeys from India (10), other parts of Bangladesh (17), Cuba (40), Burkina Fasa (43), Algeria (46), Malaysia (47), and Spain (55), whereas lower than strawberry tree honey (24), some Mexican multifloral and monofloral (orange blossom, bell flower, eucalyptus flower) honeys (54). However, the analyzed honeys were free from toxic elements (Cd, Cr, Pb, and Ni). Six polyphenols namely (−)-epicatechin, syringic acid, (+)-catechin, vanillic acid, p-coumaric acid, and trans-cinnamic acid were detected and quantified at 330.34, 218.97, 194.98, 118.84, 74.64, and 49.55 mg/kg honey, respectively. Each gram of honey from the Sundarbans consists of nearly 1 mg of these polyphenols with total antioxidant capacity of 296 mg GAE or 139 mg AAE. Thus the honeys produced in the Sundarbans mangrove forest could be of great use in dietary supplements as well as neutraceuticals.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Gani MO. 2001. The giant honey bee (Apis dorsata) and honey hunting in Sundarbans reserved forests of Bangladesh. Proceedings of the 37th International Apicultural Congress, Durban, South Africa. p 335.
2. Food and Agriculture Organization of the United Nations. 1994. Integrated resource development of the Sundarbans reserved forest, Bangladesh. Draft Final report on the Development of Apiculture, FAO, Rome. Report No: FAO-FO-DP/BGD/84/056.
3. Alvarez-Suarez JM, Tulipani S, Romandini S, Bertoli E, Battino M. 2010. Contribution of honey in nutrition and human health: a review. Mediterr J Nutr Metab 3: 15-23.
4. The National Honey Board. 2003. Honey-health and therapeutic qualities. https://www.biologiq.nl/UserFiles/Compendium%20Honey%202002.pdf (accessed Mar 2017).
5. Bogdanov S, Jurendic T, Sieber R, Gallmann P. 2008. Honey for nutrition and health: a review. J Am Coll Nutr 27: 677-689.
6. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA. 2008. Functional properties of honey, propolis, and royal jelly. J Food Sci 73: R117-R124.
7. Aljadi AM, Kamaruddin MY. 2004. Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. Food Chem 85: 513-518.
8. Ouchemoukh S, Louailleche H, Schweitzer P. 2007. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Control 18: 52-58.
9. Silva LR, Videira R, Monteiro AP, Valentão P, Andrade PB. 2009. Honey from Luso region (Portugal): physicochemical characteristics and mineral contents. Microchem J 93: 73-77.
10. Saxena S, Gautam S, Sharma A. 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem 118: 391-397.
11. The European Union. 2001. Council Directive 2001/110 relating to honey. Off J Eur Communities 10: 47-52.
12. Ferrereres F, Garcia-Viguera C, Tomás-Lorente F, Tomás-Barberán FA. 1993. Hesperetin: a marker of the floral origin of citrus honey. J Sci Food Agric 61: 121-123.
13. Busselborsts J, Gueux E, Rock E, Mazur A, Rayssiguiyer Y. 2002. Substituting honey for refined carbohydrates protects rats from hypertriglyceridemic and prooxidative effects of fructose. J Nutr 132: 3379-3382.
14. Papp LV, Lu J, Holmgren A, Khanna JK. 2007. From selenium to selenoproteins: synthesis, identity, and their role in human health. Antioxid Redox Signal 9: 775-806.
15. Pandey KB, Rizvi SI. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev 2: 270-278.
16. Islam A, Khalil I, Islam N, Moniruzzaman M, Mottalib A, Sulaiman SA, Gan SH. 2012. Physicochemical and antioxidant properties of Bangladeshi honeys stored for more than one year. BMC Complementary Altern Med 12: 177.
17. Moniruzzaman M, An CY, Rao PV, Hawlader MNI, Azlan SABM, Sulaiman SA, Gan SH. 2014. Identification of phenolic acids and flavonoids in monofloral honey from Bangladesh by high performance liquid chromatography: determination of antioxidant capacity. Bio Med Res Int 2014: 737490.
18. International Honey Commission. 2002. Harmonised methods of the International Honey Commission. http://www. ihc-platform.net/ihcmethods2009.pdf (accessed Mar 2017).
19. Bogdanov S, Martin P, Luellmann C. 1997. Harmonised methods of the European Honey Commission. Apidologie 28: 1-59.
20. Piazza M, Accorti M, Persano Oddo L. 1991. Electrical conductivity, ash, colour and specific rotatory power in Italian unifloral honeys. Apicatura 7: 51-63.
AOAC. 1990. *Official Methods of Analysis of AOAC International*. Association of Official Analytical Chemists, Arlington, VA, USA. p 1026-1033.

White JW Jr. 1979. Spectrophotometric method for hydroxy-methylfurfural in honey. *J Assoc Off Anal Chem* 62: 509-514.

AOAC. 2000. *Official Methods of Analysis of AOAC International*. Association of Official Analytical Chemists, Arlington, VA, USA. p 1033.

Beretta G, Granata P, Ferrero M, Orioli M, Facino RM. 2005. Standardization of antioxidant properties of honey by a combination of spectrophotometric/fluorimetric assays and chemometrics. *Anal Chim Acta* 533: 185-191.

Lane JH, Eynon L. 1923. Determination of reducing sugars by means of Fehling’s solution with methylene blue as internal indicator. *J Soc Chem Ind* 42: 32T-36T.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265-275.

Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37: 911-917.

Plummer TD. 1987. An introduction to practical biochemistry. McGraw-Hill Book Company (U.K.) Ltd., London, UK. p 223-230.

Hoenig M, de Kersabiec AM. 1996. Sample preparation steps for analysis by atomic spectroscopy methods: present status. *Spectrochim Acta Part B* 51: 1297-1307.

Ough CS, Amerine MA. 1988. Methods for analysis of musts and wines. John Wiley & Sons, New York, NY, USA. p 196-221.

Zhishen J, Mengcheng T, Jianming W. 1999. The determination of vitamin E. *Nature* 401: 695-696.

Azeredo LC, Azeredo MAA, de Souza SR, Dutra VML. 2003. Total flavonoid content of Turkish pine honeydew honey. *J Food Chem* 88: 651-658.

Moniruzzaman M, Rahman MM, Hossain H. 2016. Nutrient and phenolic compounds in honey of different floral origins. *Food Chem* 80: 249-254.

Terrab A, Recamales AF, Hernandez D, Heredia FJ. 2004. Characterization of Spanish thyme honeys by their physicochemical characteristics and mineral contents. *Food Chem* 88: 537-542.

Alvarez-Suarez JM, Tulipani S, Diaz D, Estevez Y, Romandini S, Giampieri F, Damiani E, Astolfi P, Bompadre S, Battino M. 2010. Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. *Food Chem Toxicol* 48: 2490-2499.

Ibrahim Khalil M, Motallah MA, Anisuzzaman ASM, Sathe ZS, Hye MA, Shahjahan M. 2001. Biochemical analysis of different brands of unifloral honey available at the northern region of Bangladesh. *J Med Sci* 1: 385-388.

Buba F, Gidado A, Shugaba A. 2013. Analysis of biochemical composition of honey samples from North-East Nigeria. *Biochim Anal Biochem* 2: 139.

Meda A, Lamien CE, Romito M, Millogo J, Nacoumla OG. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chem* 91: 571-577.

Mondragón-Cortez P, Ulloa JA, Rosas-Ulloa P, Rodríguez-Rodríguez R, Resendiz Vázquez JA. 2013. Physicochemical characterization of honey from the West region of México. *CyTA J Food* 11: 7-13.

Cimpoiu C, Hosu A, Miclaus V, Puscas A. 2013. Determination of the floral origin of some Romanian honeys on the basis of physical and biochemical properties. *Spectrochim Acta A Mol Biomol Spectros* 100: 149-154.

Khalil I, Moniruzzaman M, Bokurá L, Benhanifiia M, Islam A, Islam N, Sulaiman SA, Gan SH. 2012. Physicochemical and antioxidant properties of Algerian honey. *Molecules* 17: 11199-11215.

Moniruzzaman M, Sulaiman SA, Khalil MI, Gan SH. 2013. Evaluation of physicochemical and antioxidant properties of sourwood and other Malaysian honeys: a comparison with manuka honey. *Chem Cent J* 7: 138.

Özkök A, D'arcy B, Sorkun K. 2010. Total phenolic content and total flavonoid content of Turkish pine honeydew honey. *J ApiProd ApiMed Sci* 2: 6-71.

Khalil MI, Alam N, Moniruzzaman M, Sulaiman SA, Gan SH. 2011. Phenolic acid composition and antioxidant properties of Malaysian honeys. *J Food Sci* 76: C921-C928.

Hossain SJ, Tsujiyama I, Takasugi M, Islam MA, Biswas RS, Aoshima H. 2008. Total phenolic content, antioxidative, anti-amylose, anti-glucoisidase, and antihistamine release activities of Bangladeshi fruits. *Food Sci Technol Res* 14: 261-268.

Hossain SJ, Iltekaruzzaman M, Haque MA, Saha B, Moniruzzaman M, Rahman MM, Hossain H. 2016. Nutrient compositions, antioxidant activity, and common phenolics of *Sonneratia apetala* (Buch.-Ham.) fruit. *Int J Food Prop* 19: 1080-1092.

Hossain SJ, Basar MH, Rokeya B, Arif KMT, Sultana MS, Rahman MH. 2013. Evaluation of antioxidant, anti diabetic and antibacterial activities of the fruit of *Sonneratia apetala* (Buch.-Ham.). *Orient Pharm Exp Med* 13: 95-102.

Yaoa L, Jiang Y, Singanusong R, Datta N, Raymont K. 2005. Phenolic acids in Australian *Melaleuca, Guioa, Lophostemon, Banksia* and *Helianthus* honeys and their potential for floral authentication. *Food Res Int* 38: 651-658.

Rodríguez B, Mendoza S, Itturriaga MH, Castaño-Tostado E. 2012. Quality parameters and antioxidant and antibacterial properties of some Mexican honeys. *J Food Sci* 71: C121-C127.

Vela L, de Lorenzo C, Pérez RA. 2007. Antioxidant capacity of Spanish honeys and its correlation with polyphenol content and other physicochemical properties. *J Sci Food Agric* 87: 1069-1075.