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Research Article

Analysis of Biochemical Composition of Honey and itsAnti-Oxidant,Phytochemicaland Anti-Bacterial Properties

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ABSTRACT

Honey mainly consists of sugars and water. Apart from sugars, honey also contains several vitamins especially B complex and vitamin C, together with a lot of minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc are also present. Honey has been used for its healing, nutritional and therapeutic properties since ancient times. Its antibacterial potentials even against multi-drug resistant bacteria, such as *Staphylococcus aureus*, *Bacillus cereus, Bacillus subtillis, Escherichia coli, Staphylococcus albus*and *Shigellasonnei* has been proved. The nutritional composition, minerals, antibacterial, and antioxidant properties of honey will be reviewed here. There is no available information on Biochemical and antioxidant properties on Bangladeshi honey, so we investigate honey for better understanding biochemical properties of honey. Honey samples were collected from the Sundarbon, Khulna, Bangladesh. The levels of P^H, Moisture, Ash, Vitamins, minerals, and ascorbic acid, ascorbic acid equivalent antioxidant content (AEAC), sugar, protein, anti- microbial activity and antioxidants were determined in the honey samples using ferric reducing antioxidant power (FRAP), ABTS radical scavenging Activity and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assays. The total phenolic and flavonoid were determined by Folin-Ciocalteu and colorimetric assay method. 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. This review covers the composition, physico-chemical properties and the most important uses of natural honey in human diseases.

Keywords: Honey, Antioxidant, sugars, vitamin –c, minerals, Anti-bacterial properties.

INTRODUCTION

Honey is a complex mixture of 82.0% carbohydrates (sucrose, fructose, maltose), 0.3% protein, 17.0% water and 0.7% minerals, vitamins and antioxidants [1].Apart from sugars, honey also contains several vitamins, especially- B complex and vitamin C, together with a lot of minerals. Some of the vitamins found in honey include ascorbic acid, pantothenic acid, niacin and riboflavin; while minerals such as calcium, copper, iron, magnesium, manganese, phosphorus, potassium and zinc are also present [2]. Honey contains at least 181 constituents [3, 6]. The other constituents of honey are amino acids, antibiotic-rich inhibine, proteins and phenol antioxidants [7].It also contains other bioactive substances such as phenolic constituents, flavonoids, organic acids, carotenoid-derived compounds, nitric oxide (NO) metabolites, amino acids and proteins [8,9].Evidence indicates that some varieties of honey contain kynurenicacid (a tryptophan metabolite with neuroactive activity) which may contribute to its antimicrobial properties [10].The presence of enzymes such as glucose oxidase, diastase, invertase, phosphatase, catalase and peroxidase has also been documented in honey [11].High levels of ascorbic acid, catalase, peroxidase, flavonoids, phenolic acids,

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and carotenoids ensure a high level of antioxidants in honey [12]. Honey consumption by humans has been reported to increase total plasma antioxidant and reducing capacity, which can be protective to human health.In Bangladesh, honey is produced and consumed on a large scale. Sundarbons,which is the largest mangrove forest in the world, consists of 334 plant species and is ideal for giant honey bees (Apisdorsata) and honey collectors. However, there is still a lack of information on the comparative physicochemical and biochemical properties of different types of Bangladeshi honeys.The objective of the current study was to investigate the biochemical and antioxidant properties of Bangladeshi honeys of monofloral and multifloral origin.

Materials and Methods

Sample Collection and Preparation

Honey sample were collected freshly in sterile containers from the Sundarbon, Khulna, Bangladesh. All samples were collected freshly in sterile containers (level with numbers, place and date of collection) and stored at ambient temperature until analysed. Unwanted material such as wax sticks, dead bees and particles of combs were removed by straining the samples through cheesecloth before analysis.

Physical analysis

p^H

A 10% (w/v) solution of honey was prepared in milliQwater (Millipore Corporation, Massachusetts, USA) for pH measurement using a pH meter (Elico pH analyzer, Elico Pvt Ltd., Mumbai, India). The moisture content was determined using a refractometric method. In general, the refractive index increases with an increase in the solid content of a sample. The refractive indices of honey samples were measured at ambient temperature using an Atago handheld refractometer (KRUSS, HRH30, Germany), and the measurements were further corrected for a standard temperature of 20°C by adding the correction factor 0.00023/°C.

Moisture content

Proximate compositions of the honey samples were determined using the methods of AOAC (1990; 2000). For moisture content, 2.0g of each sample was dried to constant weight in hot air oven at 70°C and the moisture was calculated on dry basis [13].

Ash content

Ash content was determined by drying 5.0g of honey samples in porcelain crucibles at 105°C for 3 hrs in hot air oven. The dried samples were ignited in a furnace at 550–600°C to constant weight, cooled and weighed [14].

Biochemical Analysis

Total sugar

The sucrose content of each honey sample was determined based on the refractometricmethod (Atago handheld refractometer, ATAGO, N-1α, Japan). Briefly, the honey samples were suspended in milliQ water to make a solution of 20% (w/v) concentration. The percentage of sucrose content was measured in g/mL honey.

Determination of reducing sugars and non-reducing sugar (sucrose) contents

The estimation of reducing sugars was carried out using the Layne-Enyonmethod as described in AOAC [15]. About 2.6 g of honey was weighed and transferred to a 500 mL volumetric flask. Five milliliters (5 mL) of standardized Fehling's solutions A and B were transferred to a 250mL Erlenmeyer flask containing 7.0 mL of water and 15.0 mL of honey solution. The Erlenmeyer flask was heated and 1.0 mL of methylene blue (0.2%) was added. Titration was carried out by adding the diluted honey solution until the indicator decolorizes.

The non- reducing sugar (Sucrose) content was determined by inversion, adding 10 mL of dilute HCl, 50 mL of diluted honey solution and water in a 100 mL volumetric flask. The solution was then heated in a water bath, cooled and diluted to the mark. Finally, the Layne-Enyon method was applied and the sucrose content was obtained by difference.

Determination of glucose content: Glucose content of the honey samples was determined by enzymatic oxidation with glucose oxidase reagent (Randox Laboratories Ltd., UK). Twenty microlitres (20 µL) of the sample or standard was allowed to react with 2.0 mL of the reagent, mixed well and incubated for 10 min at 37°C. The absorbance of the sample (A_{sample}) and standard ($A_{standard}$) was read against a reagent blank within 60 min. Glucose concentration was calculated as follows:

Glucose content (mg/dL) $=(A_{sample}/A_{standard})$ x Conc. of standard $=(A_{sample}/A_{standard})$ x 100 (mg/dL)

Determination of fructose content

Fructose content was determined using the resorcinol reagent method [16]. To a solution of the honey sample, 1.0 mL resorcinol reagent was added and mixed thoroughly, and then 1.0 mL of dilute HCl was added. Standard solutions containing 0.2, 0.4, 0.6, 0.8 and 1.0 mg/ mL and made up to 2 mL with distilled water was also treated with 1.0 mL of the resorcinol reagent and 1.0 mL of diluted HCl as above. A blank solution was also prepared along with the standard and treated in the same manner. The test solution, the standard and blank were then heated in a water bath at 80°C for about 10min, the solution was then removed from the water bath, cooled by immersing in tap water for 5min and then the absorbance of both the test and standard solution were read against the blank solution at 520 nm within 30 min. The fructose contents of the honey samples were then extrapolated from a standard curve prepared using the absorbance of the standard.

Determination of fat content

Fat content was determined following extraction using rob ring tube or Majonnier fat extraction apparatus [17]. Five grams (5.0 g) of the honey sample was weighed in the extraction apparatus and mixed thoroughly with 2.0 mL of 99%ethyl alcohol. Then 10.0 mL of dilute HCl (prepared by adding 11 volumes of water to 25 volumes of concentrated HCl) was added and mixed well. The tube was then set in a water bath held at 70–80°C and shaken frequently at intervals for 30–40 minutes. The fat extraction apparatus was then filled to half its volume capacity with alcohol and cooled. Twenty five mililitres (25.0 mL) of ethyl ether was then added, shaken vigorously and allowed to stand until the upper liquid was practically clear. The ether extract was then drawn off by passing through a filter (using a plug of cotton in the stem of the funnel just enough to allow free passage of ether extract) into a pre-weighed 125 mL beaker, and was then dried on a water bath. The liquid remaining in the tube was re-extracted twice each with only1.0 mL of ether. A similar pre-weighed beaker was then used as counter poise at 100°C. The beakers were then cooled in desiccators to constant weight and the fat content calculated as follows:

% of fat content = 100× (weight of the extract / sample weight)

Determination of protein content

The total protein content of honey was determined by Lowry's method [18] of protein estimation, which is based on the formation of a copper-protein complex and the reduction of phosphomolybdate and phosphotungstate present in Folin-Ciocalteau reagent to hetero polymolybdenumblue and tungsten blue, respectively. Bovine serum albumin (BSA) (0–100 µg/ml) was used as a standard for preparing the calibration curve.

Determination of Carbohydrate content

Carbohydrate contents of the honey samples were determined by calculation (by difference) as follows:

%Carbohydrate=100 %–(%Moisture+%Crude Fat+%Crude Protein+%Ash)

Phytochemical activity of Honey

Total phenolic content: Phenolic compounds from honey samples were detectedby a modified spectrophotometric Folin-Ciocalteu method [19]. Briefly, 1 mL honey solution (0.2 g/ml)was mixed with 1 mL Folin and Ciocalteu's phenolreagent. After 3 min, 1 mL 10% Na2CO3 solution wasadded to the mixture and adjusted to 10 mL withdistilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm by a T 60 UV/VIS spectrophotometer (PG Instruments Ltd, UK). Ascorbic acid was used to calculate the standard curve (5, 10, 15, 20 and 25µg/mL, r2=0.9767). The estimation of the amount of phenolic compounds was carried out in triplicate. The results were reported as the mean ± standard deviations and were expressed as mg of Ascorbic acid equivalents per gmhoney.

Total Flavonoid content

Total flavonoids the total flavonoid concentration of each honey sample was determined according to the colorimetric assay developed by Zhishen [20]. 1 mL honey solution (0.5 g/ml) was mixed with 4 mL distilled water. At baseline, 0.3mL NaNO2(5%, w/v) was added. After five min, 0.3 mL AlCl3 (10% w/v) was added, followed by the addition of 2 mL NaOH (1 M) six min later. The volume was immediately increased to 10 mL by the addition of 2.4 mL distilled water. The mixture was vigorously shaken to ensure adequate mixing, and the absorbance was read at 510 nm. A calibration curve was prepared by using a standard solution of quercetine (50,100,150,200 and 250 µg/mL, r2=0.9966). The results were also

expressed as mg quercetine equivalents (CEQ) per gm honey.

Determination of Vitamins content

Determination of Vitamin C (Ascorbic Acid)

Determination of ascorbic acid content was done following the method described by Ferreira et al. [21]. Briefly,the honey sample (100 mg) was mixed with 10 ml 1% metaphosphoric acid for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml 2, 6-dichlorophenolindophenol (DCPIP) 0.005%, and the absorbance was measured within 30 min at 515 nm against a blank. The content of ascorbic acid was calculated on the basis of the calibration curve of authentic L-ascorbic acid (50, 100, 200 and 400µg/ml; $Y = 3.2453X - 0.0703$; $r^2 = 0.9440$) and the results were expressed as mg ascorbic acid/kg honey.

Determination of Vitamin A

The determination of Vitamin A is carried out Colorimetricmethod.Through this method, carotenoids and vitamin A of honeywere determined [22].Carotenoids were determined at 450 nm. After evaporating the ether phase of the honey solution, dissolved the honey sample with 1 ml of hexane. Determine the O.D. of this phase at 450 nm.The hexane phase obtained earlier is taken again and concentrated in a vacuum. Redissolve the honey in chloroform. Then, to the volume of chloroform, add four volumes of the trifluoroacetic acid reagent prepared by mixing 1 v of trifluoroacetic acid with three volumes of chloroform. Then, observe the DO at 620 nm.

Determination of Vitamin Thiamin (B1) and Riboflavin (B2)

The Thiamine and Riboflavin contents of Honey were determined using Okwu and Josiah's method [23, 24, 25]and results were expressed in milligrams per 100 grams.

Determination of minerals content

Atomic absorption spectrometry (Analyst 200, Perkin Elmer, and Waltham, MA, USA) was used to determine mineral content of Honey(K, Na, Mg, P, Ca) in central science Laboratory, Rajshahi University [26,27, 28,29]. The content of selected metals copper (Cu), nickel (Ni), iron (Fe), manganese (Mn), zinc (Zn), Lead (Pb), chromium (Cr), cobalt (Co) and cadmium (Cd) were determined at 324.8, 232.0, 248.3, 279.5, 213.9, 237.4, 357.9, 240.7 and 228.8 nm, respectively, and using air-acetylene flow where the acetylene flow was done in triplicate using flame(AAS) atomic absorption spectroscopy [30,31,32].

Analysis of antioxidant activities

Determination of Total antioxidant activity

The total antioxidant activity of honey was eluted by using the method described by Prieto et al (1999). Honey was dissolved in methanol to obtain a concentration of 500 µg/ml. 3 ml of honeyt was placed in a test tube, 0.3 ml of reagent solution (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate, 4 mM Ammonium molybdate) was then added and the resulting mixture was incubated at 950 C for 90 minutes. After the mixture was cooled to room temperature, the absorbance of the each solution was measured by using UV-Visible spectrophotometer at 695 nm against blank. The experiment was performed in triplicate [33]. A calibration curve was constructed, using ascorbic acid (100-500 µg/ml) as standard and total antioxidant activity of honey (µg/ml) expressed as ascorbic acid equivalents.

Determination of DPPH Radical Scavenging Activity

The antioxidant properties of each honey sample were also studied by evaluating the free radical-scavenging activity of the DPPH radical. The determination was based on the method proposed by Ferreira et al. briefly; 0.8 mL honey solution (0.2 g/ml) was mixed with 2.7 mL methanolic solution containing DPPH radicals (0.024 mg/mL). The mixture was vigorously shaken and left to stand for 15 min in the dark (until their absorbances remained unchanged). The reduction of theDPPH radical was determined by measurement of the absorbance at 517 nm [33]. Butylatedhydroxytoluene(BHT) was used as reference material. The radicalscavenging activity (RSA) was calculated as the percentage of DPPH discoloration using the equation % RSA = $([A_{DPPH} - A_S]/A_{DPPH}) \times 100$, where $A_S$is the absorbance of the solution when the sample solution has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

Determination of ferrous reducing antioxidant power

The Ferrous reducing antioxidant power (FRAP) of Honey was performed according to a modified method described by Benzie& Strain [34, 35]. Briefly,

200µL properly diluted honey (0.1 g/mL) was mixed with1.5 mL FRAP reagent. The reaction mixture was incubated at 37°C for 4 min, and then the absorbance was read at 593 nm against a blank that was prepared using distilled water. FRAP reagent was pre-warmed to 37°C and was freshly prepared by mixing 10 volumes of 300mM/L acetate buffer (pH 3.6) with 1 volume of 10 mmolTPTZ solution in 40 mM/L HCl with 1 volume of 20 mM ferric chloride (FeCl3.6H2O). A calibration curve was prepared using an aqueous solution of ferrous sulphate (FeSO4.7H2O) at 100, 200, 400, 600 and 1000µmol/L. FRAP values were expressed as micromoles of ferrous equivalent (µmol Fe [II]) per kg honey.

Determination of ABTS radical scavenging Activity

The ABTS radical cation preparation: ABTS 2 mM (0.0548 gm in 50 ml) was prepared in distilled water; Potassium per sulphate 70 mM (0.0189g in 1ml) was prepared in distilledwater. 200 µl of potassium persulphate and 50 ml of ABTS were mixed and used after 2 hrs. This solution was used for the assay. To the 0.5 ml of various concentrations of methanolic honey/standard, 0.3 ml of ABTS radical cation and 1.7 ml of phosphate buffer pH 7.4 was added and the absorbance was measured at 734 nm. Vitamin C was used as positive control [36, 37]. The experiment was performed in triplicate. The inhibition was calculated in following way: I (%) = 100 x $(A_0-A_1)/A_0$ Where A_0 is the absorbance of the control, A_1 is the absorbance of the honey/standard. A percent inhibition versus concentration curve was plotted and the concentration of sample required for % 50 inhibition was determined and expressed as IC_{50} value. The lower the IC_{50} value indicates high antioxidant capacity.

Anti-Bacterial activity of honey

The bactericidal action of pure honey on many pathogenic organisms including enteropathogens such as Salmonella species, Shigella species, Escherichia coliand other gram negative organisms has also been reported [38]. Furthermore, honey has been employed to shorten the duration of diarrhoea in patients with bactericidal gastro-enteritis due to bacterial infection as well as applied to heal wounds like the conventional antibiotics and antiseptics [39, 40].Antibacterial activity of honey was measured using disc diffusion method as described by [41] with some modification. Antibacterial activity was assessed against threebacterial strainsStaphylococcus aureus(S. aureus), Escherichia coli (E. coli), and Bacillus subtilis (B. subtilis).Twenty milliliter media containing bacterial strain was poured into nutrient agar petri plats and allowed to set. After that, sterile filter paper discs (10 mm) placed onsurface of the medium followed by loading 100ll sample (10 mg/ml) dissolved in DMSO onto filter discs. The same concentration of ampicillin was also loaded as positive control. Petri plates were then incubated for 18–24 h at 37^0C in an incubator. At the end of incubation, periodZone of inhibitions was measured by zone reader.

Results

Honey is characteristically acidic, with a pH ranging between 4.2 and 4.79 (Table-1). Honey inhibits the presence and growth of microorganisms, Bangladeshi honey may have the potential to be used as good antibacterial agents. (Table-1) Showed moisture and Ash contents of honey were 15.42±0.37 (g/100g) and 0.36±0.08 (g/100g).

(Table-2) Showed the total sugar content of the honey samples was 60.32 ±0.9 (g/100g). The reducing sugar and non-reducing sugar content of honey were 72.30 ± 5.64 (g/100g) and 1.84 ± 0.79 (g/100g). The Glucose and Fructose contents of Honey were 29.56 ± 2.24 (g/100g) and 38.94 ± 0.89 (g/100g). The fat, protein and Carbohydrate Content of Honey were 0.20 ± 0.10 (g/100g), 0.68 ± 0.29 (g/100g), 84.30 ± 2.03 (g/100g). The Total phenolic and Flavonoid content of Honey were 19.47±1.04 mg/100g and 63.23±3.26 mg/100g(Table-2).

(Table-3) Showed the Vitamins content of Honey. The amount of ascorbic acid (Vit-C) and Vit-A were 21.68 ± 2.83(mg/100g) and 0.56±0.04 (mg/100g). The Thiamin (B1) and Riboflavin (B2) contents of Honey were 0.32±0.03 (mg/100g) and 0.9±0.28 (mg/100g).

The minerals content of Honey showed in (Table-4). The amount of minerals content(K, Na, Mg, P, Ca) were (0.0569, 1.6020, 0.7069, 2.0300, 3.0260) ppm/100g. The amount of metalliccontent of minerals(Cu, Ni, Fe, Mn, Zn, Pb, Cr, Co and Cd) were (0.0427, 0.0042, 0.2050, 0.0059, 0.0208, 0.0560, 0.0100, 0.0185, 0.0107) ppm/100g.

(Table-5) showed total antioxidant activity of standard ascorbic acid.Honey showed highest total antioxidant capacity and it was 1.753 (µg/mL)

calculated as Ascorbic acid equivalents was detected.

(Table-6) showed Antibacterial activity of Honey. The antibacterial activity of the diluted honey (100 %) was tested against four gram-positive (*S.aureus*,*B. cereus*,*B. subtilis* and *S.albus*) and three gram-negative (*E. coli*, *S. dysenteriae* and *S. sonnei*) bacterial strains. The methanolic mixture of honey was found with best antimicrobial effect against *S. aureus* (9.3±0.04mm zone of inhibition),*B. cereus* (7.0±0.05 mm zone of inhibition),*S. albus* (11.3±0.03 mm zone of inhibition) and *B. subtilis* (11.8±0.19mm zone of inhibition). Another antimicrobial effect against*E.coli* (9.9±0.95mm zone of inhibition), *S. dysenteriae* (6.3±0.15mm zone of inhibition), *S. sonnei* (8.6±0.07mm zone of inhibition).The reference antibiotic, Azithromycin, was taken as control which showed maximum inhibition of *B. subtilis*(30±1.33mmzone of inhibition). The highest antibacterialactivity of methanolic mixture of honey against *B. subtilis* attributed to its good therapeutic value against infection diseases (Table-6).

Total phenolic content was determinates as a standard curve used Ascorbic acid (Figure-1) and the Flavonoid was determinates as a standard curve used quercetine (Figure-2). The Flavonoid content is higher than the total Phenolic content. (Figure-3) showed the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of Honey. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 1,1-diphenyl-2-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple colour. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The IC_{50} value of the extract was 1200 µg/ml, as opposed to that of ascorbic acid (IC_{50} 250 µg/ml), which is a well-known antioxidant.

(Figure-4) showed the reductive capabilities of Honey compared to ascorbic acid. The Ferrous reducing power of Honeywas found remarkable and the reducing power of the honey was observed to rise as the concentration of Honey gradually increased.

(Figure-5) showed the ABTS radical scavenging Activity of Honey. ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacities of foods. In this assay, ABTS is converted to its radical cation by addition of potassium persulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. ABTS radical scavenging activity used standard as ascorbic acid. Honey showed highest ABTS scavenging activity and compared with ascorbic acid as standard IC_{50} value is 150 µg/ml and result IC_{50} value is 500µg/ml.

Table-1: P^H, moisture and Ash content of honey

Parameter	Amount
P^H	4.58±0.01
Moisture (g/100g)	15.42±0.37
Ash (g/100g)	0.36±0.08

Values are mean ± S.D. of triplicate analyses.

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Table-2: Total sugar, reducing sugar, non reducing sugar, Glucose, Fructose, Fat, Protein, Carbohydrate, Total Phenolic content and Flavonoid content of honey

parameter	Amount
Total sugar (g/100g)	60.32±0.9
Reducing sugar (g/100g)	72.30 ± 5.64
Non reducing sugar (sucrose)(g/100g)	1.84 ± 0.79
Glucose(g/100g)	29.56 ± 2.24
Fructose(g/100g)	38.94 ± 0.89
Fat (g/100g)	0.20 ± 0.10
Protein (g/100g)	0.68 ± 0.29
Carbohydrate(g/100g)	84.30 ± 2.03
Total phenol (mg/100 gm)	19.47±1.04
Total flavonoid (mg/100 gm)	63.23±3.26

Values are mean ± S.D. of triplicate analyses.

Table-3: Vitamins content of Honey

Vitamins	Amount
Vit-C (mg/100g)	21.68 ± 2.83
Vit-A (mg/100g)	0.56±0.04
Thiamin (B1) (mg/100g)	0.32±0.03
Riboflavin (B2) (mg/100g)	0.9±0.28

Values are mean ± S.D. of triplicate analyses.

 CODEN (USA): JBPRAU

Table-4: Minerals content of Honey

Minerals of Honey	Amount in (ppm)
K	0.0569
Na	1.6020
Cu	0.0427
Fe	0.2050
Zn	0.0208
Ni	0.0042
Cd	0.0107
Pb	0.0560
Co	0.0185
Mn	0.0059
Ca	3.0260
Cr	0.0100
P	2.0300
Mg	0.7069

Table-5: Total Antioxidant activity of honey

Concentration of sample/standard(µg/mL)	Absorbance of Honey (µg/mL)	Absorbance of vitamin C (µg/mL) (Standard)
0.00	0.00	0.00
50	0.302	1.26
100	0.865	2.347
150	1.042	3.435
200	1.136	3.917
250	1.328	3.935
300	1.753	3.961

Table-6: Antibacterial activity of Honey

Bacterial Strain	Zone of Inhibition (mm)	
	Methanol (Honey) (400 µg/ml)	Azithromycin(15 µg/ml)
Bacillus cereus	7.0±0.05	28±0.78
Bacillus subtillis	11.8±0.19	30±1.33
Staphylococcus aureus	9.3±0.04	22±0.95
Escherichia coli	9.9±0.95	23±0.72
Shigelladysenteriae	6.3±0.15	26±0.53
Shigellasonnei	8.6±0.07	27±0.37
Staphylococcus albus	11.3±0.03	29±0.57

Each value is the mean±SD of triplicate measurements

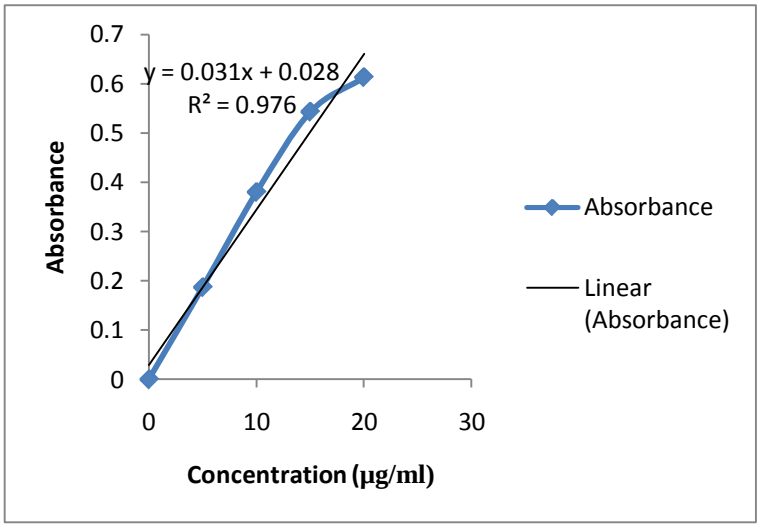

Figure-1: Standard curve of Ascorbic Acid for the determination of total phenolic compounds

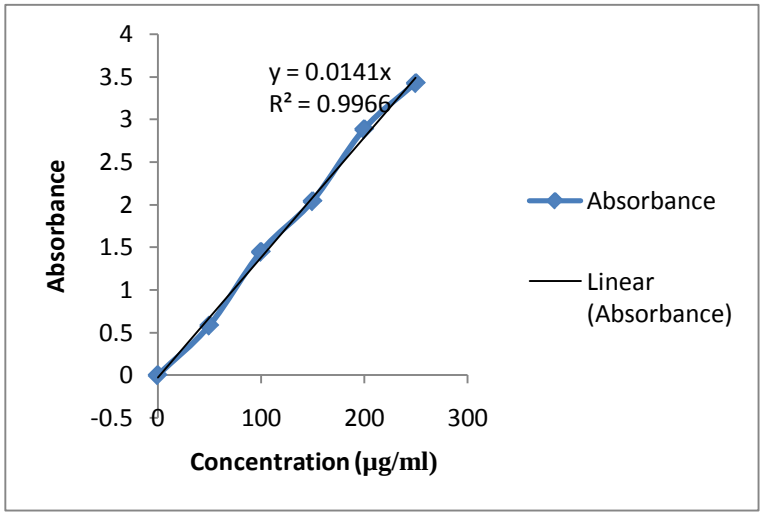

Figure-2: Standard curve of quercetine for the determination of total flavonoids compounds

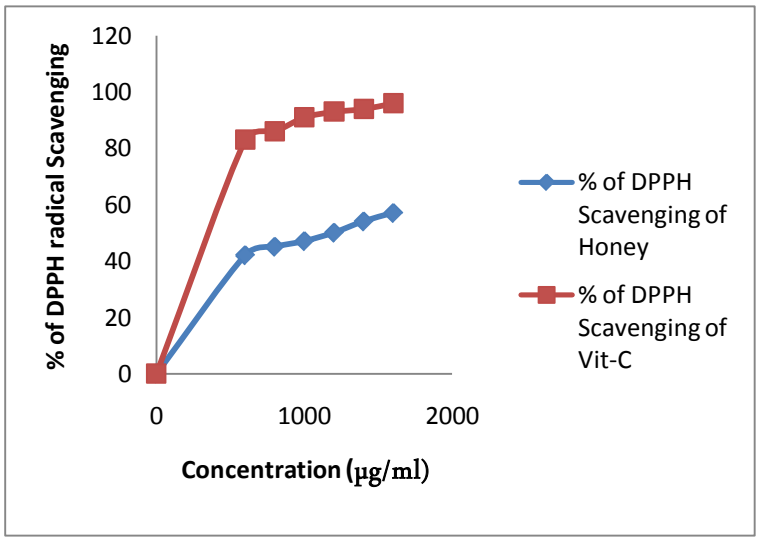

Figure-3: Determination of DPPH free radical scavenging activity of Honey

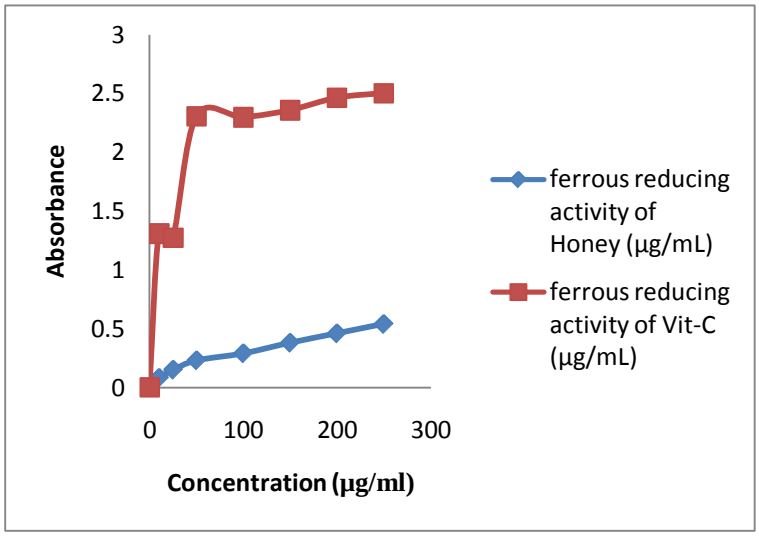

Figure-4: Determination of ferrous reducing antioxidant capacity

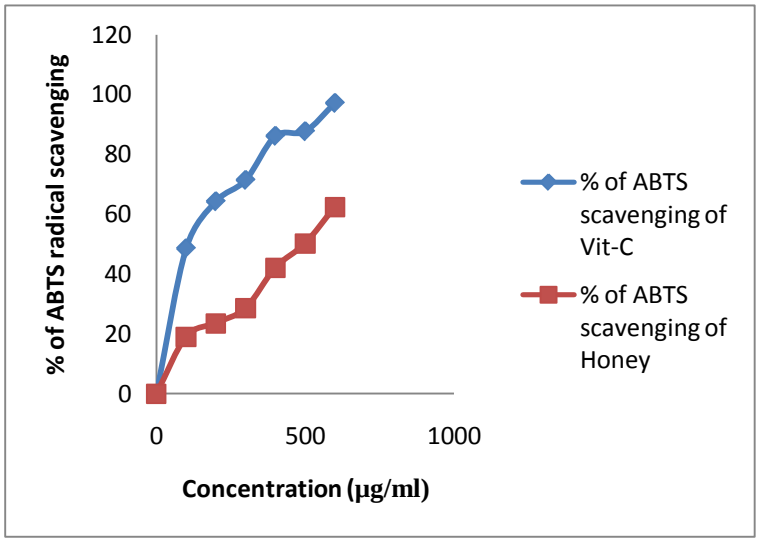

Figure-5: Determination of ABTS free radical scavenging activity of Honey

Discussion

Honey is characteristically acidic, It has low pH inhibits the presence and growth of microorganisms, Bangladeshi honey may have the potential to be used as good antibacterial agents.Moisture content is an important quality parameter, importantabove all for honey shelf-life [42, 43]. The Ash content of honey is also a parameter that is used in determining the floral origin of honey represent their mineral and trace element contents.The total sugar content of Honey especially glucose and fructose, were not converted to HMF evenafter storage at room temperature for 1.5 years; therefore, the sugar content remains high. The reducing sugar contents for the samples from the different States within the sub-region were not significantly different from each other and they all fall within the range of values reported by other scientists [44]. Honey contains non reducing sugars (sucrose, maltose, turanose, etc) and insoluble substances (like dextrin, colloids, etc.) which can influence the crystallization process, the glucose/water (G/W) ratio is considered more appropriate than the fructose/glucose (F/G) ratio for the prediction of honey crystallization [45].Generally, the sugar spectrum of honey depends upon the sugars present in the nectar and the enzymes present in the bee and nectarFructose and glucose constitute the primary sugars in all honey samples, and in honey of good quality the fructose content should exceed that of glucose [46]. The fat contents of the honey samples investigated in this studyindicating that honey contains very little amount of lipid and therefore not considered a good source of lipid [47].The honey proteins are mainly in the form of enzymes. The enzymes added include diastase (amylase), which digest starch to maltose and is relatively stable to heat and storage, and invertase (saccharase or α-glucosidase), which catalyses the conversion of sucrose to glucose and fructose.Glucose oxidase and catalase are two other enzymes added by the honey bee, which regulate the production of hydrogen peroxide H_2O_2; the H_2O_2 serve as one of the anti-bacterial factor in honey [48].The total carbohydrate contents of the honey samples from all the States were not significantly different from each other. The monosaccharaides, fructose and glucose, are the main sugars found in honey; these hexoses are products of the hydrolysis of sucrose. In addition to these sugars, 25 others have been detected in honey samples [49].The phenolic and Flavonoid content showed that the blending of different varietyof nectars from different flowers leads to a superior antioxidant property in multifloral honey samples [50].Honey contains flavonoid which is associated with a reduced risk of cardiovascular diseases (CVD).Honey contains vitamins content which may serve as sources of polyphenol and dietary antioxidant [51]. Honey contains elements such as zinc, selenium, copper, calcium, potassium, chromium, manganese, etc.Some of these minerals are reported to play vital roles in the maintenance of normal glucose tolerance and insulin secretion from the pancreatic β-cells [52].The Total antioxidant, DPPH, FRAP, and ABTS activity of Honey protect us from various disease such as hepatic and renal damage, which could be attributed to the honey's antioxidant propertiesof scavenging the reactive oxygen species or protecting the antioxidant defence mechanism [53]. Honey against both Gram positive and Gram negative bacteria is an indication of broad spectrum of activity and thus can be used to source antibiotic substances for drug development that can be used in the control of these bacterial infections [54,55]. It has both a direct and an indirect action.

Conclusion

Honey mainly consistsof sugars and water, but also contains several vitamins, especially B complex and vitamin C, together with a lot of minerals. It possesses anti-bacterial, anti-inflammatory and anti-oxidant properties that may be beneficial for combating multi-drug resistant bacteria as well as for preventing chronic inflammatory processes, such as atherosclerosis and diabetes mellitus.From the above discussion, we can conclude that the wasted honey might be used to extract bioactive compounds in reasonable yield to develop preventive medicines and floral-based medicines.

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