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THERAPEUTIC EFFECTS OF HONEY PRODUCED BY *APIS CERANA INDICA*, REARED IN THE COLLEGE CAMPUS, COIMBATORE, TAMILNADU

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ABSTRACT

Honey is a natural supersaturated sugar solution, made by bees from the nectar of flowers. Honey consumption by humans has been reported to increase total plasma antioxidant and reducing capacity, which can be protective to human health. The aim of the present work was to characterize the honey produced by *Apis cerana indica* reared in the college campus with the commercially available honey in the market. Physical and Biochemical analysis, vitamin, Phenolic and Flavonoid content, anti microbial property and antioxidant activity of honey was determined. The anti microbial and antioxidant activity of honey produced by Apis cerana indica was higher when compared to the commercial honey. The commercial honey showed high values for the physical, biochemical analysis, vitamin content, phenolic and flavonoid content. No zone of inhibition was observed for the antimicrobial activity of commercial honey. Honey is used by human beings as food and medicine. It possesses anti-bacterial, anti-inflammatory, and anti-oxidant properties that may be beneficial for combating multidrug resistant bacteria as well as for preventing chronic inflammatory processes.

KEYWORDS: Honey, anti microbial, antioxidant, phenolic and flavonoid content, zone of inhibition, *Apis cerana indica*.

INTRODUCTION

Honey is a natural supersaturated sugar solution, made by bees from the nectar of flowers. There are basically two types of honey, apiary and forest honey. Honey produced by the honeybees *Apis cerana indica* and *Apis mellifera*, in apiaries and collected by modern extraction methods are called apiary honey. They are transparent and free from foreign materials. In contrast, those produced by rock bee, *Apis dorsata*, or by wild nest of Apis cerana indica in forests and collected by the crude method of squeezing the comb are known as forest honey.[1]

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. 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. The other constituents of honey are amino acids, antibiotic-rich inhibine, proteins and phenol antioxidants.[3] It also contains other bioactive substances such as phenolic constituents, flavonoids, organic acids, carotenoid-derived compounds, nitric oxide (NO) metabolites, amino acids and proteins.[4]

Evidence indicates that some varieties of honey contain kynurenic acid (a tryptophan metabolite with neuroactive activity) which may contribute to its antimicrobial properties.[5] The presence of enzymes such as glucose oxidase, diastase, invertase, phosphatase, catalase and peroxidase has also been documented in honey.[6] High levels of ascorbic acid, catalase, peroxidase, flavonoids, phenolic acids, and carotenoids ensure a high level of antioxidants in honey.[7] Honey consumption by humans has been reported to increase total plasma antioxidant and reducing capacity, which can be protective to human health. Although the composition of honey can be variable and is primarily dependent on its floral, geographical and entomological source, certain external factors, such as seasonal and environmental factors and processing also play an important role.[8]

The physicochemical and microbiological characteristics of honey collected during the dry and rainy seasons in the different phytogeographical areas of Benin were studied.[9] These results have shown that these honeys meet international standards and their characterization

will make it possible to obtain Beninese quality labels. Characterized the physical, biochemical and antioxidant properties of south Algeria honey samples.[10] This study showed that south Algerian honey samples have high antioxidant potentials, as indicated by their high phenolic and flavonoid content. Honey has been shown to have significant antimicrobial activity. The antimicrobial- and wound-healing efficacy of honey is very much region, and season specific. Not all honeys have the same antimicrobial potency due to the variation in the pH, sugar content, concentration of the active principles, storage conditions, and different susceptibility. The aim of the present work is to characterize the honey produced by *Apis cerana indica* reared in the college campus with the commercially available honey in the market.

MATERIALS AND METHODS

Experimental design: The apiary unit of Nirmala College was established in the year 2018. The units were placed at 3 distinct points of the college with a distance of 200 mts between the units. The apiary unit is maintained by the Department of Zoology. The bee boxes are provided with sugar syrup to compensate the lack of floral blooms at different times of the year. Honey sample was collected from the bee boxes. The collected samples were stored at ambient temperature until analysed. A commercially available honey Patanjali honey was obtained from the local market of Wayanad, Kerala.

Physical analysis

pH (pH meter), Moisture content[11] and Ash content[12] was determined.

Biochemical analysis

Total sugar: The total sugar in the honey samples were estimated using anthrone method.[13] Sugar in the presence of sulphuric acid gets dehydrated to furfural or hydroxymethylfurfural, which later react with anthrone (dihydro oxanthrancene) to yield a bluish-green colour complex that has an absorption maximum at 620nm. Approximately 2g of honey sample was taken individually and homogenized with known volume of 5% TCA. The homogenate was then centrifuged at 2500 rpm in a centrifuge for 10 min and supernatant was filtered. To the filtrate, athrone reagent was added, placed in a serological water-bath for 10-15 min for color development. The distilled water served as the control. The colored product was measured for their optical density at 620nm in spectrophotometer.

$$\text{Total carbohydrate} = \frac{\text{OD of the sample} \times \text{standard value}}{\text{weight of the sample taken} \times 100}$$

Reducing sugars and Non reducing sugars: The estimation of reducing sugar was carried out using the Layne -Eynon method.[14] About 2.6g of honey was weighed and transferred to a 500 ml volumetric flask. Five millilitres (5ml) of standardized Fehlings solutions A and B were transferred to a 250ml Erlenmeyer flask containing 7ml of water and 15ml of honey solution. The Erlenmeyer flask was heated and 1ml 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 content was calculated as = Amount of total sugars - amount of reducing sugars.

Determination of glucose content: Glucose content of the honey samples was determined by enzymatic oxidation with glucose oxidase reagent. 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) × 100 (mg/dL)

Determination of fructose content: Fructose content was determined using the resorcinol reagent method as described as AOAC (1990; 2000). 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 content 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.[15] 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 millimetres (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 125ml

beaker, and was then dried on a water bath. The liquid remaining in the tube was re-extracted twice each with only 1.0ml 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:

$$\% \text{ of fat content} = \frac{100 \times \text{weight of the extract}}{\text{sample weight}}$$

Determination of protein content: The total protein content of honey was determined by Lowry's method.[16] A known amount of sample was weighed and homogenized with 10% trichloroacetic acid, to precipitate the protein. It was centrifuged at 4000 rpm for 5minutes to get a clear supernatant. The precipitate was dissolved in 1N NaOH made up to 10ml with the same. 1ml of this was taken and treated with 5ml alkaline $CuSo_4$ for 5 mins. Then, 0.5ml of folinciocaltaeu reagent was added. A blank was prepared and used. The O.D was taken after 20 min at 650nm. A standard was prepared by dissolving 1ml of bovine serum albumin (BSA) in 10ml of 1N NaOH and made up to 100ml in a standard flask. Standard graph was prepared using bovine serum albumin as standard.

$$\text{Total protein in honey} = \frac{\text{OD of sample} \times \text{standard value}}{\text{Weight of the sample} \times \text{volume of extract}}$$

Determination of carbohydrate content: Total carbohydrate was estimated by the anthrone method. Approximately 2g of honey sample was taken individually and homogenized with known volume of 5% TCA. The homogenate was then centrifuged at 2500 rpm in a centrifuge for 10 min and supernatant was filtered. To the filtrate, athrone reagent was added, placed in a serological water-bath for 10-15 min for color development. The distilled water served as the control. The colored product was measured for their optical density at 620nm in a UV- VIS spectrophotometer. D-glucose was used as the standard for consumption of the results.

$$\text{Total carbohydrate} = \frac{\text{OD of the sample} \times \text{standard value}}{\text{weight of the sample taken} \times 100}$$

Phyto-chemical constituent of honey

Total phenolic content: Phenolic compounds from honey samples were detected by a modified spectrophotometric Folin-Ciocalteu method.[17] Briefly, 1 mL honey solution (0.2 g/ml) was mixed with 1 mL Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL 10% Na_2Co_3 solution was added to the mixture and adjusted to 10 mL with distilled 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. 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 deviation and were expressed as mg of Ascorbic acid equivalents per gm honey.

Total flavonoid content: Total flavonoid content was determined by colorimetric assay.[18] 1ml honey solution (0.5g/ml) was mixed with 4ml distilled water. To it 0.3ml of 5 % $NaNo_2$ was added and the volume was made up to 10ml with distilled water. The solution was mixed and absorbance was measured against the blank at 510nm. The results were expressed as mg quercetin equivalents (QE).

Determination of vitamin content

Determination of vitamin C (Ascorbic acid): Determination of ascorbic acid content was done following the method described by[19]. 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 dichlorophenol indophenol (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 L-ascorbic acid and the results were expressed as mg ascorbic acid/kg honey.

Determination of vitamin A: The determination of Vitamin A is carried out by Colorimetric method. Through this method, carotenoids and vitamin A of honey were determined.[20] Carotenoids were determined at 450 nm. After evaporating the ether phase of the honey solution, dissolve 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. Re-dissolve the honey in chloroform. Then, to the volume of chloroform, add four volumes of the tri fluoro acetic acid reagent prepared by mixing 1 v of trifluoro acetic acid with three volumes of chloroform. Then, observe the OD at 620 nm.

Determination of Vitamin Thiamin (B1) and Riboflavin (B2): The Thiamine and Riboflavin contents of Honey were determined using[21] method and results were expressed in milligrams per 100 gram.

Antibacterial activity of honey

Antibacterial activity of honey was measured using agar well diffusion method. Antibacterial activity was assessed using bacterial strains such as *Staphylococcus aureus, Escherichia coli, Bacillus and Shigella*. Nutrient agar medium was prepared on the petri plates and was left on sterile surface until the agar has solidified. The plates were swabbed with sterile cotton. Wells (10mm diameter and 2cm apart) were made in each of the plates using sterile cork borer. Then the wells were filled with 5mg/ml, 10mg/ml and 15 mg/ml of honey respectively. Ampicillin was taken as positive control. Petri plates were then incubated for 18-24 hrs at 37˚C in an

incubator. At the end of incubation period, the zone of inhibition was measured.

Analysis of antioxidant activity of honey

Determination of DPPH Radical Scavenging Activity: 0.5 ml of 0.1mM DPPH solution in methanol was mixed with plant extract solution of varying concentrations (50, 100, 150, 200, 250 µg/ml). Corresponding blank sample was prepared and L-Ascorbic acid (25-500µg/ml) was used as reference standard. Mixture of 0.5ml methanol and 0.5ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 520nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\text{Inhibition \%} = \text{Ac-As/Ac} \times 100$$

Where Ac is the absorbance of the control As is the absorbance of the sample.

Determination of reducing power as Reducing Power assay: Various concentrations (50, 100,150, 200, and 250 µg/ml) of extracts were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(Cn)_6$] (1%), and then the mixture was incubated at 50°C for 30 min. Afterward, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increased reducing power.

Determination of ABTS radical scavenging Activity: The ABTS radical cation (ABTS+) was produced by reacting ABTS solution (7mM) with 2.45mM of ammonium persulfate and the mixture was allowed to stand in dark at room temperature for 12 – 16 hours before use. Honey extract solution of varying concentrations was tested (50, 100,150, 200 and 250 µg/ml), with 0.3mL of ABTS solution. The absorbance (A) was read at 745nm and the percentage inhibition was calculated as follows,

$$\text{\% scavenging activity} = \text{Ac-As/Ac} \times 100$$

Where Ac is the absorbance of the control As is the absorbance of the sample

Determination of hydrogen peroxide scavenging activity: The hydrogen peroxide scavenging assay was carried out following the procedure of Ruch.[22] For this a solution of H_2O_2 (43 mM) was prepared in phosphate buffer (0.1 M, pH 7.4). 2.4ml of honey solution of varying concentrations (50, 100,150, 200, and 250 µg/ml) was added to 0.6 mL of H_2O_2 solution. Blank solution contains the sodium phosphate buffer without H_2O_2. The absorbance value of the reaction mixture was recorded at 230 nm and the percentage inhibition was calculated as follows,

$$\text{\% scavenging activity} = \text{Ac-As/Ac} \times 100$$

Where Ac is the absorbance of the control As is the absorbance of the sample

RESULTS AND DISCUSSION

In the present study, average pH of honey sample procured from the apiary unit was 3.91. The low pH inhibits the presence and growth of microorganisms, which may have the potential to be used as a good antibacterial agent.[23] Studies revealed that flower honey has usually low pH values ranging from 3.3 to 4.3.[24] Honey pH can provide a good indication of its origin.

The moisture content is 19.93g/100g in apiary unit and the market honey is 21.37g/100g. The low moisture content is indicative of the time of extraction of honey with respect to the maturity/ripening process of the honey, climatic factors, and storage conditions. According to codex Alimentarius commission and EU commission, 20.0g/100g is considered as the international standard for honey moisture content.

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 content. Ash content of honey in apiary unit is 0.31 g/100g and maximum in standard 0.39 g/100g. The floral origin has been reported to be responsible for the difference in ash content[25] and maximum limit of ash content is 0.6%.[26]

The total sugar content of apiary unit is 55.28 g/100g. It is less when compared to standard honey 79.07 g/100g. These values are comparable to those of honey collected in western India (42.8-60.6%).[27]

Non reducing sugar and reducing sugar content of honey contains sucrose, maltose, turanose, etc and insoluble substances like dextrin, colloids, etc. which can influence the crystallization process of the glucose/water (G/W) ratio which is considered more appropriate than the fructose/glucose (F/G) ratio for the prediction of honey crystallization. The apiary unit honey has lower value for non reducing sugar 3.89 g/100g and reducing sugar 63.89 g/100g. Our results are comparable to those obtained with India (62.2-70.2%) and Pakistan honey (57.7-70.5%)[28,29] but higher than those of Algeria (34.5-50.3%).[30] The values of Non- reducing sugars for the honey samples in the current study were within the range of ≤5g/100g provided by Council Directive of the European Union (2001).

The total carbohydrate content of market honey is higher 92.31 g/100g than the apiary unit 89.65 g/100g. Analysis of carbohydrate in honey is a quality criterion which is influenced by honey storage and heating and thus is an indicator of honey freshness. The monosaccharides,

fructose and glucose, are the main sugars found in honey; these hexoses are products of the hydrolysis of sucrose.[31]

Glucose and fructose content of apiary unit varies between 29.14 g/100g and 33.13 g/100g. Generally, the sugar spectrum of honey depends upon the sugars present in the nectar and the enzymes present in the bee and nectar.

The fat content of the honey samples investigated in this study indicates that honey contains very little amount of lipid and therefore not considered a good source of lipid.[32] The apiary unit honey has a lower value of 0.09 g/100g.The honey proteins are mainly in the form of enzymes. The apiary unit value is 2.88 g/100g and market honey is 3.98 g/100g.

The total phenol content of honey in apiary unit is 16.87mg/100gm which is low when compared to market honey 19.67 mg/100gm. The total flavonoid present in apiary unit is comparatively higher than phenol. It ranges from 57.18 mg/100gm in apiary unit and standard content is 63.60 mg/100gm. Honey contains flavonoid which is associated with a reduced risk of cardiovascular diseases (CVD). The phenolic and Flavonoid content showed that the blending of different variety of nectars from different flowers leads to a superior antioxidant property in multi floral honey samples.[33]

Vitamin content: Honey contains vitamins which may serve as a source of polyphenol and dietary antioxidant.[34] 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.[35] The vitamin content of apiary unit is comparatively lower than market honey. Vitamin A and vitamin C is significantly higher than Thiamin and Riboflavin. It was reported that the honey of Apis mellifera has a low concentration of vitamin C, less than 5mg/100g[36] and concentration of 2.5 mg vitamin C per 100g honey is found in the literature.[23]

ANTI-BACTERIAL ACTIVITY OF HONEY

The Apiary honey exhibited varying levels of antibacterial activity against all the selected strains as indicated by the zone of inhibition. In the apiary unit honey showed maximum zone of inhibition for *E.coli* and *Bacillus*, which is higher when compared to *shigella* and *staphylococcus*. The medicinal importance of honey has been known since ancient times and its antimicrobial property as well as wound-healing activities is well-known long ago. The concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater is its usefulness as an antibacterial agent.[37] Zone of inhibition is totally absent in market honey.

ANTI-OXIDANT PROPERTY OF HONEY

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical. The apiary unit honey shows maximum scavenging on different concentrations of samples. DPPH is a stable free radical with deep violet colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The degree of discolouration indicates the scavenging potential of the antioxidant compound.

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential. The reduction ability ("Fe^{3+} to Fe^{2+} transformation" in terms of increasing absorbance) was found to increase with rising concentration in all the samples.[38] In this study market honey showed maximum absorbance.

The antioxidant effect of extracts was determined using ABTS (2,2–azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay. ABTS is a chromogen which changes into reduce (ABTS+) into its colourless form and the extent of decolourisation is proportional to the percent reduction of (ABTS+). ABTS is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacity of food.[39] The analysis indicates that apiary unit honey shows maximum scavenging percentage.

The hydrogen peroxide scavenging assay showed water-soluble phenolic acids as antioxidants, scavengers of hydrogen peroxide (H_2O_2). The strongest antioxidant, scavenging of H_2O_2 was exhibited by 3,4,5-trihydroxybenzoic (gallic) acid and 1,2,3-trihydroxybenzene (pyrogallol) with three hydroxyl groups bonded to the aromatic ring in an ortho position in relation to each other.[40] The analysis indicates that maximum percentage of scavenging is observed in apiary honey.

Table 1: Physico-chemical parameters of apiary honey and market honey.

Parameter	Sample	Market honey
pH	3.91±0.02	4.43±0.05
Moisture (g/100g)	19.93±0.29	21.37±0.36
Ash (g/100g)	0.31±0.07	0.39±0.09s

Values are mean ± standard deviation of the sample

Table 2: Biochemical analysis of apiary honey and market honey.

Parameter	Sample	Market Honey
Total sugar (g/100g)	55.28±0.59	79.07±0.91
Reducing sugar (g/100g)	63.89±2.87	82.23±3.01
Non- reducing sugar (g/100g)	3.89±0.97	4.02±1.13
Glucose (g/100g)	29.14±1.32	37.3±1.64
Fructose (g/100g)	33.13±0.54	38.41±0.79
Fat (g/100g)	0.09±0.08	0.23±0.10
Protein (g/100g)	2.88±1.73	3.98±1.93
Carbohydrate (g/100g)	89.65±2.13	92.31±2.36
Total phenol (mg/100gm)	16.87±1.15	19.67±1.28
Total flavonoid (mg/100gm)	57.18±3.12	63.60±3.31

Values are mean ± standard deviation of the samples

Table 3: Vitamin content of apiary honey and Market Honey.

Parameter	Sample	Market honey
Vitamin C (mg/100g)	18.5±2	21.5±2.62
Vitamin A (mg/100g)	0.53±0.05	0.89±0.08
Thiamin (mg/100g)	0.35±0.03	0.53±0.06
Riboflavin (mg/100g)	0.91±0.31	0.98±0.38

Values are mean ± standard deviation of the samples

Table 4: Anti-bacterial activity of honey samples.

	Sample	Sample	Sample	Ampicillin
	5mg/ml	10mg/ml	15mg/ml	10mg/ml
E.coli	7.8±0.6	8.7±0.05	10.2±0.09	12.8±0.2
Bacillus	3.55±0.20	5.61±0.15	10.89±0.45	13.1±0.3
Shigella	1.30±0.26	3.81±0.32	6.24±0.33	18.3±0.4
S. Aureus	2.28±0.30	5.77±0.14	7.46±0.56	9.5±0.1

Values are mean ± standard deviation of the samples

Table 5: Anti-oxidant Property of honey by DPPH Radical Scavenging Assay.

DPPH Radical Scavenging Assay		
	Volume of standard& sample (µl)	**% Scavenging**
S1	50µl	70.38
S2	100µl	68.44
S3	150µl	62.62
S4	200µl	58.73
S5	250µl	51.45
SampleT1	50µl	23.57
T2	100µl	39.32
T3	150µl	45.47
T4	200µl	58.45
T5	250µl	63.54
Standard T1	50µl	18.47
T2	100µl	26.53
T3	150µl	28.45
T4	200µl	39.47
T5	250µl	45.32

S: Quercetin standard (1mg/ml)

Table 6: Anti-oxidant Property of honey by Reducing Power Assay.

Reducing power assay		
	Volume of standard & sample (µl)	**Absorbance at 700nm**
S1	50µl	0.321
S2	100µl	0.53
S3	150µl	0.786
S4	200µl	0.889

S5	250µl	1.021
Sample T1	50µl	1.233
T2	100µl	1.199
T3	150µl	1.141
T4	200µl	1.058
T5	250µl	0.858
Standard T1	50µl	1.352
T2	100µl	1.209
T3	150µl	1.173
T4	200µl	1.111
T5	250µl	0.764

S: Quercetin standard (1mg/ml)

Table 7: Anti-oxidant Property of honey by ABTS Radical Scavenging Assay.

ABTS Radical Scavenging Assay		
	Volume of standard& sample (µl)	**% Scavenging**
S1	50µl	36.82
S2	100µl	42.60
S3	150µl	54.87
S4	200µl	61.21
S5	250µl	79.26
Sample T1	50µl	26.15
T2	100µl	35.37
T3	150µl	45.12
T4	200µl	51.10
T5	250µl	62.45
Standard T1	50µl	21.47
T2	100µl	35.35
T3	150µl	46.15
T4	200µl	57.42
T5	250µl	68.05

S: Quercetin standard (1mg/ml)

Table 8: Anti-oxidant Property of honey by Hydrogen Peroxide Radical Scavenging Activity.

Hydrogen Peroxide Radical Scavenging Activity		
	Volume of standard& sample (µl)	**% Scavenging**
S1	50µl	39.62
S2	100µl	45.47
S3	150µl	56.60
S4	200µl	63.57
S5	250µl	75.42
Sample T1	50µl	33.65
T2	100µl	46.34
T3	150µl	53.17
T4	200µl	58.78
T5	250µl	63.90
Standard T1	50µl	19.02
T2	100µl	20.51
T3	150µl	25.46
T4	200µl	39.02
T5	250µl	44.02

S: Quercetin standard (1mg/ml)

CONCLUSION

The colour, aroma and consistency of honey all depend upon which flowers the bees have been foraging. The place where they live has no effect upon the quality of honey that bees make. It is only subsequent handling by humans that leads to reduction in quality; if the honey is harvested when the water content is still too high, if it is contaminated, over-heated, over-filtered or spoiled in

any other way. Honey is used by human beings as food and medicine. It possesses anti-bacterial, anti-inflammatory, and anti-oxidant properties that may be beneficial for combating multidrug resistant bacteria as well as for preventing chronic inflammatory processes, such as atherosclerosis and Diabetes mellitus.

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