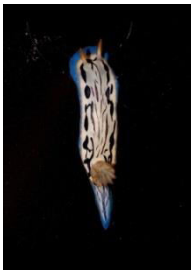

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Characterization of Honey Produced by *Apis cerana indica*, Reared in the College Campus, Coimbatore, Tamilnadu, India

Sruthy S., Abisha T.J. and Pawlin Vasanthi Joseph*

Department of Zoology, Nirmala College for Women, Coimbatore – 641018, Tamilnadu, India

**Corresponding Author*

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Abstract: The composition of honey depends mainly on climatic and environmental conditions and the diversity of the plants from which they are harvested. Due to its unique taste, nutritional value and health promoting properties, honey has a valued place in the human diet. The physicochemical, biochemical and antibacterial properties of Indian honey samples with special reference to their non-conformity was evaluated. The results revealed that no honey samples showed near to ideal characteristics of Bureau of Indian Standards (BIS). Honey samples were collected from the bee boxes of Nirmala College for Women, Coimbatore, India. The collected samples were stored at ambient temperature until analysed. A commercially available Patanjali honey was obtained from local market of Wayanad, Kerala, India for comparison. The physical and biochemical parameters of the apiary honey were much lower than the market honey. The Apiary honey showed zone of inhibitions against microbes whereas anti-microbial activity was absent in the market honey. The hydrogen peroxide scavenging assay and the 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assay was high in the apiary honey. The study concludes that the values obtained for all physical and biochemical parameters were within the range recommended by Codex Alimentarius Commission.

Keywords: Biochemical parameters, Zone of inhibitions, Hydrogen peroxide scavenging assay, ABTS radical scavenging assay, Apiary honey

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Introduction

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants, which honey bees collect, transform and combine with specific substance of their own, store and leave in the honey comb to

ripen and mature (Codex Alimentarius Commission, 2001). The bees produce honey in order to serve as their source of food in times of scarcity or during harsh weather conditions (James *et al*., 2009). The composition of honey mainly depends on climatic and environmental conditions and the diversity of the plants from which they are harvested (Cimpoiu *et al.*, 2013). Natural honey is one of the most widely sought products due to its unique nutritional and medicinal properties which are attributed to the influence of the different groups of substances it contains (Buba *et al.*, 2013).

Melissopalynology is the most frequently used method for the determination of honey botanical and geographical origin (Cotte *et al.*, 2004; Ponnuchamy *et al.*, 2014). Apitherapy or therapy with bee products is a therapeutic practice recorded in ancient times. Due to its unique taste, nutritional value and health promoting properties, honey has a valued place in the human diet. The health promoting properties mainly come from the presence of other than sugar components: enzymes, peptides, free amino acids, vitamins, organic acids, flavonoids, phenolic acids and other phytochemicals and minerals (Terrab *et al.*, 2003).

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 (Ajibola *et al.*, 2012). The various floral honey contains varying amount of minerals and trace elements. Polyphenols are an important group of compounds which is responsible for the appearance and functional properties of honey (Kenjeric *et al.*, 2007).

The physicochemical, biochemical and antibacterial properties of Indian honey samples with special reference to their non-conformity was evaluated (Kavapurayil *et al.*, 2014). The results revealed that no honey samples showed near to ideal characteristics of Bureau of Indian Standards (BIS). Some samples clearly indicated conditions of improper handling. All honey samples were found to have proper amounts of polyphenols as well as exhibited variable antimicrobial abilities.

The objective of the present work was to evaluate the physical, biochemical, anti-bacterial and antioxidant properties of honey produced by *Apis cerena indica* reared in the college campus, Coimbatore, Tamilnadu, India.

Materials and Methods

Sample Collection:

Honey samples were collected from the bee boxes of Nirmala College for Women, Coimbatore, India. The collected samples were stored at ambient temperature until analysed. A commercially available Patanjali honey was obtained from local market of Wayanad, Kerala, India for comparison.

Physical and Biochemical analysis:

pH, Moisture content (White *et al.*, 1962), and Ash content (Ranganna, 1986) was determined.

Total sugars (Rao and Deshpande, 2005), Reducing and non-reducing sugars (AOAC, 1990), Glucose (Duxbury, 2006), Fructose (Saxena *et al.*, 2010), Fat Content (Saxena *et al.*, 2010), Protein (Lowry *et al.*, 1951) and Carbohydrate content were analysed. The

total phenolic content was determined by using spectrophotometric Folin-Ciocalteu method (Singleton *et al.*, 1999). Total flavonoid content was determined by colorimetric assay (Zhishen *et al.*, 1999).

Vitamin C content was determined using the method described by Ferreira *et al.* (2009). The determination of vitamin A was carried out using Colorimetric method (Sullivan and Carpenter, 1993). Thiamine and Riboflavin content of honey was determined by using Okwu and Josiah's method (Okwu and Josiah, 2006).

Antioxidant activity:

Determination of DPPH Radical Scavenging Activity:

The antioxidant activity of the honey sample was measured by using the method described by Brand-Williams *et al.* (1995) with slight modifications. 0.5 ml of 0.1 mM DPPH solution in methanol was mixed with honey sample of varying concentrations (50, 100, 150, 200 and 250 µg/ml). Corresponding blank sample was prepared and L-Ascorbic acid (25-500 µg/ml) was used as reference standard. Mixture of 0.5 ml methanol and 0.5 ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 520 nm after 30 min in dark using UV-Vis spectrophotometer. The % scavenging was calculated using the formula:

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

where Ac is the absorbance of the control and As is the absorbance of sample.

Determination of Ferrous reducing antioxidant power:

Various concentrations (50, 100, 150, 200 and 250 µg/ml) of honey sample were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$], 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 and 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 antioxidant effect of honey samples was determined using ABTS radical cation decolourization assay according to the method of Shirwaikar *et al.* (2006). The ABTS radical cation was produced by reacting ABTS solution (7 mM) with ammonium persulfate (2.45 mM) and the mixture was allowed to stand in dark at room temperature for 12-16 h before use. The honey sample solution of varying concentrations (50, 100, 150 200 and 250 µg/ml) were taken and 0.3 ml of ABTS solution was added. The absorbance (A) was read at 745 nm and the % scavenging was calculated as follow:

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

where Ac is the absorbance of control and 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 *et al.* (1989). A solution of H_2O_2 was prepared in phosphate buffer (0.1 M, pH 7.4). 2.4 ml of honey sample of varying concentrations (50, 100, 150, 200 and 250 µg/ml) was added to 0.6 ml of H_2O_2 solution (0.6 ml, 43 mM). Blank solution contains

sodium phosphate buffer without H_2O_2. The absorbance value of the reaction mixture was recorded at 230 nm and the % scavenging was calculated as follow:

% scavenging activity = Ac – As / Ac × 100

where Ac is the absorbance of the control and As is the absorbance of the sample.

Antibacterial activity of honey:

Antibacterial activity of honey was measured using agar well diffusion method described by Perez *et al.* (1990). Antibacterial activity was assessed against four bacterial strains, *Staphylococcus aureus, Escherichia coli, Bacillus* and *Shigella.* Ampicillin was taken as positive control. Zone of inhibition was measured.

Results and Discussion

pH values of honey samples were measured and obtained results confirmed that honey was acidic with pH 3.38 ± 0.02 for apiary honey and 4.43 ± 0.05 for market honey (Table 1). Studies revealed that flower honey has usually low pH values ranging from 3.3 to 4.3 (Khalil *et al.*, 2001; Bogdanov, 2003). The low pH indicates that honey is acidic. The acidity of the honey sample may be due to the presence of organic acids such as gluconic acid and also due to phosphate and chloride ions (Nanda *et al.*, 2003).

Table 1 shows moisture content for apiary and market honey as 21.37 ± 0.36 and 19.61 ± 0.11 g/100g, respectively. The moisture content of honey samples from Bangladesh ranged from 17.19 to 19.19 per cent (Islam *et al.*, 2012) which is lower than our apiary honey. According to Codex Alimentarius Commission (2001), 20.0 per cent is considered as the international standard for honey moisture content. The honey collected from our apiary fall within the range of international standard moisture content.

Table 1: Physical analysis of apiary honey and market honey

SL. No	Parameters	Apiary honey	Market honey
1	pH	3.38 ± 0.02	4.43 ± 0.05
2	Moisture (g/100g)	19.61 ± 0.11	21.37 ± 0.36
3	Ash (g/100g)	0.22 ± 0.05	0.39 ± 0.09

Values are Mean ± SD

In the present study, ash content of apiary honey is 0.22 ± 0.05 and market honey is 0.39 ± 0.09 g/100g, respectively (Table 2). Ash content also determines the floral origin of honey (Bogdanov, 2003). The ash content of honey obtained in this study were within the limits of <0.6 g/100g specified by international norms (Codex Alimentarius Commission, 2001), Furthermore, the study conducted by Sahinler *et al.* (2004) and (Buba *et al.*, 2013) revealed that the honey produced from colonies fed with sugar syrup showed low ash content.

The values of total sugars ranged from 49.58 ± 0.55 g/100g in apiary honey and 79.07 ± 0.91 g/100g in market honey (Table 2). The reducing and non-reducing sugar contents of apiary honey are 61.99 ± 2.50 and 3.84 ± 0.92 g/100g and market honey are 82.23 ± 3.01 and 4.02 ± 1.13 g/100g, respectively (Table 2). The values obtained for total sugars in the present study were lesser than the values reported by Shobham *et al.* (2017). These values are comparable to those of Portuguese honey which range within 64.5–80.0% (Feas *et al.*, 2010). The values of

Table 2: Biochemical analysis of apiary honey and market honey

S. No.	Parameters	Apiary Honey	Market Honey
1	Total sugar (g/100g)	49.58 ± 0.55	79.07 ± 0.91
2	Reducing sugar (g/100g)	61.99 ± 2.50	82.23 ± 3.01
3	Non reducing sugar (g/100g)	3.84 ± 0.92	4.02 ± 1.13
4	Glucose (g/100g)	27.5 ± 1.17	37.3 ± 1.64
5	Fructose (g/100g)	30.3 ± 0.33	38.41 ± 0.79
6	Fat (g/100g)	0.06 ± 0.04	0.23 ± 0.10
7	Protein (g/100g)	2.30 ± 1.36	3.98 ± 1.93
8	Carbohydrate (g/100g)	77.81 ± 1.73	92.31 ± 2.36
9	Total phenol (mg/100g)	14.47 ± 0.97	19.67 ± 1.28
10	Total flavonoid (mg/100g)	55.23 ± 2.96	63.60 ± 3.31

Values are Mean± SD

non- reducing sugars for the honey samples in the current study were within the range of ≤ 5 g/100g as suggested by Council Directive of the European Union (CDEU, 2002).

The results showed that there were no significant differences between glucose and fructose content of examined honey samples. The glucose and fructose content for market honey was 37.3 ± 1.64 and 38.41 ± 0.79 g/100g and for honey from apiary unit was 27.5 ± 1.17 and 30.3 ± 0.33 g/100g, respectively (Table 2). It indicates that fructose is higher than that of glucose. This observation shows that fructose is the major sugar in the examined samples and, it is in agreement with the earlier observations (White and Doner, 1980). Fructose and glucose constitute the primary sugars in all honey samples, and in honey of good quality the fructose content should exceed that of glucose (Zafar *et al.*, 2008).

The fat content of honey samples investigated in this study is 0.06 ± 0.04 g/100g for honey from apiary unit and 0.23 ± 0.10 g/100g for market honey (Table 2). Reports indicating that honey contains little or no fat are available in the literature (Singh and Bath, 1997). The fat content of the honey samples falls within the range of 0.1 to 0.5 g/100 g (Buba *et al.*, 2013). The results indicate that honey contains less amount of fat and therefore not considered as a good source of lipid (Singh and Bath, 1997).

The protein analysis of honey samples showed that apiary honey contains 2.30 ± 1.36

Table 3: Vitamin content of apiary honey and market honey

S. No.	Vitamins	Apiary Honey	Market Honey
1	Vitamin C (mg/100g)	16.66 ± 2.07	21.5 ± 2.62
2	Vitamin A (mg/100g)	0.45 ± 0.03	0.89 ± 0.08
3	Thiamine(B1) (mg/100g)	0.30 ± 0.02	0.53 ± 0.06
4	Riboflavin(B2) (mg/100g)	0.86 ± 0.24	0.98 ± 0.38

Values are Mean± SD

g/100g and market honey has 3.98 ± 1.93 g/100g (Table 2). This observation is comparable to the honey analysis reported by El Sohaimy *et al.* (2015). It is well known that honey contains trace amount of proteins usually originated from pollen which is a natural and protein-rich food source (Schafer *et al.*, 2006) and some enzymes such as glucose oxidase invertase and diastase (Subrahmanyam, 2007). The protein content for five different brands of unifloral honey from the northern region of Bangladesh ranged between 0.655 and 0.744 g/100g (Buba *et al*., 2013). The significant differences observed may be ascribed to differences in the botanical origin of honey since it was reported that the diastase and the invertase enzymes varied in wide limits depending on the botanical origin of honey (Oddo *et al.*, 1999).

The total carbohydrate content of current study is 92.31± 2.36 g/100g for market honey and 77.81 ± 1.73 g/100g for honey from apiary unit (Table 2). Carbohydrates are the main constituents of honey comprising about 95% of honey dry weight. The monosaccharides, fructose and glucose, are the main sugars found in honey; these hexoses are products of the hydrolysis of sucrose.

In the present study, total phenolic and flavonoid contents in apiary honey were 14.47 ± 0.97 and 55.23 ± 2.96 mg/100g, respectively (Table 2). The phenolic and flavonoid content were 19.67 ± 1.28 and 63.60 ± 3.31 mg/100g in market honey (Table 2). Polyphenols in honey are mainly flavonoids, phenolic acid and phenolic acid derivatives. The phenolic and flavonoid content showed that the blending of different variety of nectars from different flowers leads to a superior antioxidant property in multifloral honey samples (Alvarez-Suarez *et al.*, 2010). The flavonoid content can vary between 60 and 460 µg/100g of honey and was higher in samples produced during a dry season with high temperatures (Kavapurayil *et al.*, 2014).

The honey collected from apiary unit has vitamin C 16.66±2.07 mg/100g, vitamin A 0.45±0.03 mg/100g, Thiamin 0.30±0.02 mg/100g and Riboflavin 0.86±0.24 mg/100g (Table 3). However, the values obtained for vitamin content of honey from apiary unit is lower than the market honey. The amount of vitamins in honey is generally small. The vitamin C content of honey samples available in Bangladesh is found to be lower than our honey sample (Khalil *et al.*, 2001). It was reported that the honey of *Apis mellifera* has a

Table 4: Antibacterial activity of apiary honey

Bacterial Strain	Honey Dilution (mg/ml)	Zone of Inhibition (mm)
Escherichia coli	5	8 ± 0.91
	10	11 ± 0.95
	15	13 ± 0.98
	Control (Ampicillin)	46 ± 1.42
Staphylococcus aureus	5	6 ± 0.01
	10	8 ± 0.03
	15	9 ± 0.04
	Control	31 ± 0.61
Bacillus	5	5 ± 0.03
	10	8 ± 0.06
	15	11 ± 0.15
	Control	37 ± 1.41
Shigella	5	5 ± 0.13
	10	6 ± 0.16
	15	8 ± 0.23
	Control	42 ± 0.88

Values are Mean± SD

low concentration of vitamin C, less than 5mg/100g (White, 1975) and concentration of 2.5 mg vitamin C per 100 g honey has been reported by Bogdanov *et al.* (2008).

The antibacterial activity of different concentrations (5 mg/ml, 10 mg/ml and 15 mg/ml) of apiary honey and market honey was tested against four bacterial strains (*Escherichia coli, Staphylococcus aureus, Shigella* and *Bacillus*). In apiary honey, maximum zone of inhibition is found against *E. coli* (8±0.98, 11±0.95 and 13±0.98) followed by *Bacillus* (5±0.03, 8±0.06 and 11±0.15) (Table 4). The zone of inhibition is absent in market honey which does not possess any antibacterial activity. Several authors reported that different honeys vary substantially in the potency of their antibacterial activity, which varies with the plant source (Lusby *et al*., 2005). The concentration of honey has an impact on antibacterial activity; the higher the concentration of honey the greater its usefulness as an antibacterial agent (Badawy *et al.*, 2004).

DPPH Radical Scavenging Activity is found to be increasing with concentration in apiary and market honey (Table 5). The DPPH from

Table 5: Antioxidant activity-DPPH Radical Scavenging Assay

% OF SCAVENGING			
Volume of sample and standard (µl)	Quercetin standard (µl)	Apiary honey (µl)	Market honey (µl)
50	70.38	14.56	18.47
100	68.44	23.18	26.53
150	62.62	27.39	28.45
200	58.73	35.24	39.47
250	51.45	49.57	45.32

Table 6: Ferrous Reducing Antioxidant Power

ABSORBANCE AT 700 nm			
Volume of sample and standard (µl)	Quercetin standard (µl)	Apiary honey (µl)	Market honey (µl)
50	0.321	1.163	1.352
100	0.53	0.962	1.209
150	0.786	0.824	1.173
200	0.889	0.621	1.111
250	1.021	0.293	0.764

scavenging activity of Bangladeshi honey ranged from 33.6% to 97.5% (Islam *et al.*, 2012). The honey samples from India ranged 44% to 71%. The higher the DPPH scavenging activity, the higher is the antioxidant activity of the sample (Saxena *et al.*, 2010).

Ferrous Reducing Power is found to be decreasing with concentration in both honey samples (Table 6). The ferrous reducing antioxidant capacity of honey samples is found to be high (Asaduzzaman *et al.*, 2015).

ABTS (2, 2-azino-bis-3-ethylbenzothia zoline-6-sulphonic acid) is also frequently used by the food industry and agricultural researchers to measure the antioxidant capacity of food (Asaduzzaman *et al.*, 2015). In the present study, there is increase in scavenging activity with increase in

Table 7: ABTS Radical Scavenging Assay

% OF SCAVENGING			
Volume of sample and standard (µl)	Quercetin standard (µl)	Apiary honey (µl)	Market honey (µl)
50	36.82	29.45	21.47
100	42.60	35.37	35.35
150	54.87	48.19	46.15
200	61.21	54.51	57.42
250	79.26	62.30	68.05

Table 8: Hydrogen Peroxide Scavenging Activity

% OF SCAVENGING			
Volume of sample and standard (µl)	Quercetin standard (µl)	Apiary honey (µl)	Market honey (µl)
50	39.62	23.41	19.02
100	45.47	27.07	20.51
150	56.60	31.95	25.46
200	63.57	45.12	39.02
250	75.42	59.75	44.02

concentration in both apiary and market honey (Table 7).

Some water-soluble phenolic acids were identified as antioxidants, scavengers of hydrogen peroxide. Hydrogen Peroxide Scavenging Activity is also found to increase with concentration of honey samples and apiary honey shows higher scavenging activity (Table 8). 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 (Sroka and Jerkovic, 2014).

Conclusion

Depending upon the plant origin, honey varies in their appearance and composition. The study concludes that the values obtained for all physical and biochemical parameters were within the range recommended by Codex Alimentarius Commission. Honey mainly consists of carbohydrates, but also contains vitamins; especially vitamin C. Carbohydrate is an important and relevant nutritive component, which makes it an

excellent source of energy for all age groups. The bee unit also favours pollination of some flowering plants and thereby the growth of flowering plants.

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