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Evaluation of Phenolic and Antioxidant Activities in Honey Samples Collected from *Apis mellifera* Colonies

Rajpoot Gajendra Singh[1], Kumar Rajesh[2*], Kumari Rekha[3] and Hajam Younis Ahmad[4]

[1]Government Gandhi College Balaji Mihona, Bhind, Gwalior, Madhya Pradesh, India
[2]Department of Biosciences, Himachal Pradesh University, Shimla, Himachal Pradesh, India
[3]Government Degree College, Sarkaghat, Mandi, Himachal Pradesh, India
[4]Department of Life Sciences and Allied Health Sciences, University Institute of Sciences, Sant Baba Bhag Singh University, Punjab, India

**Corresponding Author*

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Abstract: Honey is a natural food product produced when the nectar and sweet deposits from plants are gathered, modified and stored in the honeycomb by honey bees. From ancient times honey has been used in therapeutic purposes especially in cough, colds, skin wounds and various gastrointestinal diseases. In this study multifloral honey samples were investigated in order to assess their total phenolic content by Folin-Ciocalteau method and their potential antioxidant activity by 2,2 diphenyl-1-picrylhydrazyl (DPPH) and ABTS free radical scavenging methods. Results of this study revealed that all honey samples possess total phenolic and antioxidant activity irrespective of climatic or floral variation.

Keywords: Honey, Phenolic content, Antioxidant activity, DPPH

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Introduction

Honey and its medicinal uses were mentioned in the old Ayurveda books of India. It is mentioned in Rig Veda (2000-3000 BC) that Vishnu (Indian God) took the form of the blue bee on a lotus flower (Kumar and Agrawal., 2016). Honey is a source of energy and may be used for the treatment of various diseases. This beneficial role is contributed to both antibacterial and anti-inflammatory properties of honey, regarding high osmolarity, acidity and content of hydrogen peroxide. The antibacterial property of honey is well known and documented as well (Molan, 1992a, b; Taomina *et al*., 2001, Weston, 2000). Antioxidant capacity of honey depends on the floral and geographical origin, climatic conditions, processing, storing and handling of honey. The greatest influence on the antioxidant activity of honey has been contributed to its botanical origin (Frankel *et al*., 1998; Gheldof *et al*., 2002; Al-Mamary *et al*., 2002; Beretta *et al*., 2005; Shivani *et*

al., 2021). Many researchers have reported that antioxidant activity is strongly correlated with phenolics content. Also, the antioxidant activity of dark honeys is higher than of the light honeys (Al-Mamary *et al*., 2002; Aljadi and Kamaruddin, 2004; Berreta *et al*., 2005). Honey serves as a source of natural antioxidants (Vit *et al*., 1997; Antony *et al*., 2000; Nagai *et al*., 2001; Gheldof *et al*., 2002; Al-Mamary *et al*., 2002; Aljadi and Kamaruddin, 2004; Beretta *et al*., 2005; Kucuk *et al.,* 2007), which play an important role in food preservation and human health by combating damage caused by oxidizing agents e.g. oxygen, namely reducing the risk of heart disease, cancer, immune-system decline, cataracts, different inflammatory processes, etc. (The National Honey Board, 2003). The antioxidants present in honey include -- (i) enzymatic: catalase (Schepartz, 1966), glucose oxidase, peroxidase and (ii) non-enzymatic substances: ascorbic acid, a-tocopherol (Crane, 1975), carotenoids, amino acids, proteins, organic acids, Maillard reaction products (Al-Mamary *et al.,* 2002; Gheldof *et al.,* 2002; Schramm *et al.,* 2003; Aljadi and Kamaruddin, 2004). Honey possesses more than 150 polyphenolic compounds, including flavonoids, flavonols, phenolic acids, catechins, and cinnamic acid derivatives. Honey contains a variety of phenolics and represents a good source of antioxidants, which makes it a good food antioxidant additive and increases its usability potential in ethnomedicine (Al-Mamary *et al.,* 2002; Aljadi and Kamaruddin, 2004; Beretta *et al*., 2005). The total phenolic content of natural samples, such as plants and honey, reflects, to some extent, the total antioxidant capacity of the sample (Beretta *et al.,* 2005). Several studies for the identification and quantification of antioxidant components of honeybee products have been reported (Ferreres *et al*., 1991; Gheldof *et al.,* 2002; Buratti *et al*., 2007). Many methods for determining the antioxidative activity in honey have been used, e.g., determination of total phenolic content (Beretta *et al.,*2005). Available literature indicates that until now there have been just few researches to determine the phenolic content and antioxidant activity of honey (Hołderna-Kędzia and Kędzia, 2006). In the present study, total phenolic and antioxidant activities of honey samples were carried out to analyse and validate quality of honey samples.

Materials and Methods

Fresh honey samples were obtained directly from beekeepers from different locations in Gwalior Chambal region of Madhya Pradesh, India. Samples were filtered, properly labelled and stored in air tight containers at room temperature.

All the chemicals and reagents used in honey analysis were of analytical grade. DPPH (1,1 diphenyl-2-picrylhdrazyl), ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) diammo-nium salt, Folin-Ciocalteau reagent, gallic acid, phosphate-buffered saline and methanol were obtained from Fluka, Germany. Potassium persulfate and sodium carbonate were purchased from POCH, Poland. For absorbance measurements an UV‑VIS spectrophotometer Unicam UV2-100 was used.

Total phenolic content (TPC) of honeys was analysed by using Meda *et al*. (2005) method. Honey solution with a concentration of 1 g/10 ml of distilled water was centrifuged (3000×g) and filtered. After that, 0.5 ml of the resultant solution were mixed with 2.5 ml of 0.2N solution of Folin-Ciocalteau reagent and 2 ml of sodium carbonate solution (75 g/l). After incubation in dark at room temperature for 2 h, the absorbance of the reaction mixture was measured at λ=760 nm using a UV-VIS spectrophotometer. The standard curve was produced for gallic acid within the concentration range from 20 to 200 mg/l (R2=0.9998, y=0.1254x-0.1424) The total phenolic content was expressed as mg gallic acid equivalents per100 g of honey sample (mgGAE/100 g). The scavenging activity against 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical of honey was estimated according to the procedure described by Turkmen *et al*. (2005) with some modifications. 2 g honey sample was dissolved in 10 ml of distilled water, centrifuged (3000×g) and

filtered. Then, 0.75 ml of the solution were mixed with 2.25 ml of 0.1 mmol/l methanol solution of DPPH 1,1–diphenyl-2-picrylhydrazyl. The control test was made with distilled water in place of honey solution. The reaction mixtures were vortex-mixed well and incubated for 60 min at room temperature in the dark. Absorbance was measured at λ=517 nm against methanol, using a UV-VIS spectrophotometer. Antioxidant activity was expressed as a per cent inhibition of DPPH radical and calculated as follow:

Antioxidant Activity [%] = (Abs control-Abs sample)/Abs control×100%

The antioxidant activity of honey samples in the reaction with stable ABTS radical cation was determined according to Baltrusaityte *et al*. (2007). ABTS was obtained in the reaction of a 2 mmol/l stock solution of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt in phosphate-buffered saline with potassium persulfate. The mixture was left to stand for 24 h. Prior to analysis the ABTS+ solution was diluted with phosphate buffer saline to produce a solution with absorbance of 0.80±0.03 at λ=734 nm. 2 g honey sample was dissolved in 10 ml of distilled water, centrifuged (3000×g) and filtered. Then, 0.1 ml of the solution was mixed with 6 ml of ABTS solution, vortex-mixed well and after 15 min absorbance was measured at a wavelength of 734 nm. The control test was made with distilled water in place of honey solutions. Antioxidant activity was expressed as per cent inhibition of ABTS, calculated from the same equation as for DPPH.

Statistical Analysis:

The data obtained were subjected to statistical analysis using Sigma plot Software, after taking necessary transformations, wherever applicable.

Results and Discussion

Forty eight honey samples from four different districts were analysed in order to assess their phenolic content and antioxidant activity. The result showed that all the samples tested possess phenolic content, free radical scavenging activity and antioxidant activity with slight variation.

Phenolic content:

The average phenolic content of honey samples obtained from Gwalior, Guna, Morena and Shivpuri was found to be 65.0±0.38, 63.8±0.49, 54.01±0.45 and 60.0±0.47 mg, respectively (Table 1). No statistical difference was found in the phenolic content of Gwalior and Guna honey samples. Maximum phenolic content was obtained in Gwalior Market samples. However, phenolic content of samples obtained from Morena was significantly lower than all other locations ($P<0.05$). Total phenolic contents of the honey samples used in this study are similar to those reported by Vit *et al*. (2009) for Venezuelan *Apis mellifera* honeys (38.15 to 182.10 mgGAE/100 g, with an average of 93.50 ± 51.62 mgGAE/100 g). The values of phenolic contents in this study are, however, higher ($P<0.05$) than those reported by Adetuyi *et al*. (2009) for *Apis mellifera* honey samples in Owo community, Ondo State in southwest Nigeria (0.75 to 2.85 mgGAE/100 g). The phenolic contents obtained in this study are also higher ($P<0.05$) than those observed in selected Czech honey; 3.92 ± 0.13 to 16.71 mgGAE/100 g and in some honeys from Poland, 7.17 ± 0.13 to 20.16 ± 1.68 mgGAE/100 g (Kaskoneine *et al*., 2009). It was earlier reported that, in honey samples from Burkina Faso, total phenolic contents varied from 32.59 to 114.75 mgGAE/100 g with a mean of 74.38 ± 20.54 mgGAE/100 g. Phenols are reported to have antioxidant capacities that are much stronger than those of vitamins C and E (Oboh *et al*., 2009). According to Blasa *et al*. (2006), raw honey contains copious amounts of compounds such as flavonoids and other polyphenols which may function as antioxidants. Honey polyphenols are said to originate from nectar, pollen or propolis (Makawi *et al*., 2009).

Antioxidant Activity:

(a) *Radical Scavenging Activity (DPPH)*:

Table 1: Total Phenolic Content (mg GAE/100g) of honey samples collected from different locations in Madhya Pradesh

S. No.	Gwalior Market	Guna	Morena	Shivpuri
1.	63.5±0.44	63.4±0.75	53.4±0.75	59.3±0.55
2.	64.3±0.40	63.5±0.40	53.5±0.40	59.7±0.42
3.	65.2±0.61	63.4±0.23	54.7±0.23	60.5±0.31
4.	64.4±0.38	64.2±0.23	54.2±0.32	60.5±0.15
5.	64.6±0.20	63.7±0.61	54.3±0.41	60.4±0.51
6.	64.3±0.61	63.2±0.27	53.7±0.34	59.9±0.27
7.	66.1±0.17	63.4±0.64	53.4±0.48	60.1±0.80
8.	65.9±0.70	64.3±0.52	54.3±0.42	60.2±0.61
9.	64.2±0.55	64.5±0.57	54.5±0.38	59.9±0.38
10.	64.9±0.17	64.3±0.62	54.3±0.53	60.3±0.62
11.	65.0±0.21	63.3±0.53	53.3±0.47	59.4±0.52
12.	64.9±0.17	64.4±0.61	54.4±0.61	59.8±0.51
Mean	65.0±0.38	63.8±0.49	54.0±0.45	60.0±0.47

Values expressed as Mean ± S.E.

The radical scavenging activity in 48 honey samples varied from 46% to 70% in the DPPH reaction system. The results showed that samples from Gwalior market have maximum average free radical scavenging activity i.e. 70.00±0.52 followed by 52.00±0.64 in Guna samples, 49.00±0.66 in Morena samples and 46.00±0.65 per cent in Shivpuri samples (Table 2). However, it was observed that except for Gwalior region samples, per cent radical scavenging activity was statistically similar among the samples for all the three locations.

The difference in radical scavenging activity of Gwalior samples can be due to dark color (long preserved samples) as it has already been reported that dark colored honey tended to be highly active in the reaction with DPPH (Meda *et al.,* 2005; Blaska *et al.,* 2006; Bertoncelj *et al.,* 2007). Antioxidant capacity was reported higher for darker honey samples (Frankel *et al.,* 1998; Chen *et al.,* 2000; Nagai *et al.,* 2001; Gheldof and Engeseth, 2002) as well as in honey with higher content of water (Frankel *et al.,* 1998; Aljadi and Kamaruddin, 2004). It has also been observed that the composition and antioxidant capacity of honey depend on the floral source used to collect nectar; seasonal and environmental factors, as well as processing may also have an effect on honey composition and antioxidant activity (Frankel *et al.,* 1998; Chen *et al.,* 2000; Al-Mamary *et al.,* 2002; Gheldof and Engeseth, 2002; Gheldof *et al.,* 2002).

(b) *Antioxidant Activity (ABTS)*:

The antioxidant activity in ABTS reaction system was analyzed for 48 honey samples and reported to be much lower than DPPH reaction system. The per cent antioxidant activity varied between 40 to 54%. The data showed that Gwalior market samples have maximum average antioxidant activity in ABTS reaction system the value being 53.14±0.44 followed by 46.17±0.52 in Shivpuri samples, 45.75±0.40 in Guna samples and minimum antioxidant activity was observed in Morena samples i.e. 40.78±0.40 per cent (Table 3). However, no statistical difference was observed in the value of antioxidant activity among the

Table 2: Radical scavenging activity DPPH (%) of honey samples collected from different locations in Madhya Pradesh

S. No.	Gwalior Market	Guna	Morena	Shivpuri
1.	70.02±0.66	51.58±0.68	49.18±0.75	45.51±0.92
2.	70.17±0.60	52.34±0.65	48.87±0.84	46.87±0.89
3.	69.47±0.38	52.18±0.54	50.68±0.73	46.68±0.73
4.	69.81±0.67	52.31±0.63	48.21±0.77	44.43±0.76
5.	69.93±0.65	51.44±0.46	49.18±0.61	46.47±0.58
6.	69.71±0.45	51.44±0.75	48.88±0.73	45.91±0.78
7.	70.59±0.42	53.39±0.52	49.51±0.81	44.43±0.38
8.	68.76±0.26	51.35±0.66	49.29±0.51	45.59±0.55
9.	70.82±0.67	52.21±0.63	49.54±0.54	46.59±0.59
10.	69.61±0.25	52.29±0.68	47.68±0.42	46.47±0.58
11.	70.71±0.59	51.54±0.62	48.39±0.64	45.65±0.61
12.	70.06±0.69	51.93±0.86	48.59±0.74	47.40±0.38
Mean	70.00±0.52	52.00±0.64	49.00±0.66	46.00±0.65

Values expressed as Mean ± S.E.

Table 3: Antioxidant activity ABTS (%) of honey samples collected from different locations in Madhya Pradesh

S. No.	Gwalior Market	Guna	Morena	Shivpuri
1.	54.19±0.53	45.58±0.37	40.61±0.46	46.48±0.45
2.	52.60±0.38	46.41±0.39	41.61±0.59	45.44±0.38
3.	54.39±0.39	45.59±0.38	39.62±0.59	44.82±0.56
4.	52.67±0.48	46.14±0.59	41.14±0.56	45.44±0.39
5.	53.45±0.38	45.28±0.33	38.41±0.35	45.98±0.72
6.	52.38±0.42	46.41±0.39	41.38±0.39	46.74±0.43
7.	53.26±0.47	45.60±0.35	40.36±0.41	46.46±0.43
8.	52.57±0.39	45.47±0.36	41.31±0.48	47.47±0.69
9.	52.55±0.50	45.60±0.35	40.63±0.46	46.43±0.51
10.	54.23±0.49	44.82±0.57	42.82±0.54	44.82±0.57
11.	52.64±0.38	46.43±0.36	40.66±0.43	47.36±0.45
12.	52.69±0.47	45.65±0.39	40.81±0.59	46.65±0.62
Mean	53.14±0.44	45.75±0.40	40.78±0.49	46.17±0.52

Values expressed as Mean ± S.E.

samples of various locations. It was observed that freshly collected honey samples have lower antioxidant activity as compared to those preserved for longer period (Market samples)

The difference in antioxidant activity can be due to the dark color of samples. Beretta *et al.* (2005)and Bertoncelj *et al.* (2007) suggested that dark colored honey obtained from forest, honeydew and buckwheat had highest antioxidant activity and pale or light color honey of Acacia had low antioxidant activity. In the literature, several studies for the identification and quantification of antioxidant components of honeybee products have been reported (Ferreres *et al.,* 1994; Gheldof *et al.,* 2002; Buratti *et al.,* 2007).

Conclusion

The antioxidant and phenolic activities of honey are the important parameters for the validation of honey quality. In the current study, all honey samples were found to have both these activities hence are suitable from consumption and health point of view.

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References

Adetuyi F.O, Ibrahim TA, Ojei J and Ogundahunsi GA. (2009) Total phenol, tocopherol and antibacterial quality of honey *Apis mellifera* sold in Owo community, Ondo State, Nigeria. Afr J Biotech. 8: 1305-1309.

Aljadi AM and Kamaruddin MY. (2004) Evaluation of the phenolic contents and antioxidants capacities of two Malaysian floral honeys. Food Chem. 85: 513-518.

Al-Mamary M, Al-Meeri A and Al-Habori M. (2002) Antioxidant activities and total phenolics of different types of honey. Nutr Res. 22: 1041-1047.

Antony SM, Han IY, Rieck JR and Dawson PL. (2000) Antioxidative effect of Maillard reaction products formed from honey at different reaction times. J Agric Food Chem. 48: 3985-3989.

Baltrusaityte V, Venskutonis PR and Ceksteryt V. (2007) Radical scavenging activity of different floral origin honey and beebread phenolic extracts. Food Chem. 101: 502-514.

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

Bertoncej J, Dobersek U, Jamnik M and Golob T. (2007) Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. Food Chem. 105: 822-828.

Bertoncelj J, Polak T, Kropf U, Korosec M and Golob T. (2011) LC-DAD-ESI/MS analysis of fl avonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. Food Chem. 127(1): 296-302.

Blasa M, Candiracci M, Accorosi A, Piacentini MP, Albertini MC and Piatti E. (2006) Raw Millefiori honey is packed full of antioxidants. Food Chem. 97: 217-222.

Chen L, Mehta A, Berenbaum M, Zangerl AR and Engeseth NJ. (2000) Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J Agric Food Chem. 48: 4997-5000.

Crane E. (1975) Honey: A comprehensive survey. New York, USA: Crane Russak and Company.

Ferreres F, Tomas-Barberan FA, Gil MI and Tomas-Lorente F. (1991) An HPLC technique for flavonoid analysis in honey. J Sci Food Agric. 56: 49-56.

Frankel S, Robinson GE and Berenbaum MR. (1998) Antioxidant capacity and correlated characteristic of 14 unifloral honeys. J Apicult Res. 37: 27-31.

Gheldof N and Engeseth NJ. (2002) Antioxidant capacity of honeys from various floral sources based on the determination of oxygen radical absorbance capacity and inhibition of in vitro lipoprotein oxidation in human serum samples. J Agric Food Chem. 50: 3050-3055.

Hołderna-Kędzia E and Kedzia B. (2006) Research on an antioxidant capacity of honeys. Acta Agrobotanica 59: 265-269.

Kaskoniene V, Maruska A, Kornysova O, Charezun N and Ligor M. (2009) Quantitative and qualitative determination of phenolic compounds in honey. Chemine Technologija 3: 74-80.

Kucuk M, Kolayli S, Karaoglu S, Ulusoy E, Baltac C and Candan F. (2007) Biological activities and chemical composition of three honeys of different types from Anatolia. Food Chem. 100: 526-534.

Kumar R and Agrawal OP. (2016) Honeybee Nutrition. Lambert Academic Publishing, Germany.

Makawi SZA, Gadkariem EA and Ayoub SMH. (2009) Determination of antioxidant flavonoids in Sudanese honey samples by Solid Phase Extraction and High Performance Liquid Chromatography. Elect J Chem. 6: 429-437.

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

Molan PC. (1992a) The antibacterial activity of honey: 1. The nature of the antibacterial activity. Bee World 73(1): 5-28.

Molan PC. (1992b) The antibacterial activity of honey: 2. Variation in the potency of the antibacterial activity. Bee World 73(2): 59-76.

Nagai T, Sakai M, Inoue R, Inoue H and Suzuki N. (2001) Antioxidative activities of some commercially honeys, royal jelly, and propolis. J Food Chem. 75: 237-240.

Oboh G. (2005) Effect of blanching on the antioxidant property of some tropical green leafy vegetables. Technology 38: 513-517.

Schepartz AI. (1966) Honey catalase: Occurrence and some kinetic properties. J Apic Res. 5: 167-176.

Schramm DD, Karim M, Schrader HR, Holt RR, Cardeti Buratti S, Benedetti S and Cosio MS. (2007) Evaluation of the antioxidant power of honey, propolis and royal jelly by amperometric flow injection analysis. Talanta 71: 1387-1392.

Shivani, Hajam YA, Kumar R. (2021) Physico-chemical analysis of honey samples collected from *Apis cerana* in Western Himalayan region. J Entomol Res. 45(2): 246-253.

Taomina PJ, Niemira BA and Beuchat LR. (2001) Inhibitory activity of honey against foodborne pathogens as influenced by the presence of hydrogen peroxide and level of antioxidant power. Int J Food Microbiol. 69: 217-225.

Vit P, Soler C and Tomas-Barberan FA. (1997) Profiles of phenolic compounds of *Apis mellifera* and *Melipona* spp. Honeys from Venezuela. Zeitschrift fur Lebensmittel-Untersuchung und-Forschung A 204: 43-47.

Weston RJ. (2000) The contribution of catalase and other natural products to the antibacterial activity of honey: a review. Food Chem. 71: 235-239.