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PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND PALYNOLOGICAL ANALYSIS OF SOME MULTIFLORAL HONEYS FROM GARHWAL HIMALAYA

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ABSTRACT

In this study multifloral honey samples collected from different localities of Garhwal Himalaya were analyzed for their botanical origin, phenolic content and antioxidant activity by using standard methods. The botanical origin revealed fifty-seven pollen types in the samples. The total phenolic content expressed as gallic acid equivalent ranged from 0.125 to 4.18 mg/100gm. High phenolic content were found in multifloral honeys with pollen types such as Prunus persica, Pyrus pashia, Citrus sinensis, Aesculus indica, Rhododendron arboreum and Brassica campestris. The antioxidant activity ranged from 1.68 to 8.70 mg/ml and strongly correlated with their phenolic content. The Garhwal Himalayan honeys can be used as a source of potential antioxidants to food materials to give them additional function or might be helpful in preventing oxidative stress.

KEY WORDS

Antioxidant activity, Apis cerana-indica, Garhwal Himalaya, multifloral honey, phenolic content, pollen types

INTRODUCTION

Cells produce free radicals during metabolism which in turn being unstable may damage the cells. Free radicals have a harmful effect by certain synthetic compounds having many side effects. This makes scientists to keep exploring natural sources of antioxidants with multifunctional potential as alternatives for toxic synthetic antioxidants, to avoid the metabolic pathways of any oxidation [1]. To prevent the oxidative stress caused by these free radicals produced during metabolism, sufficient number of antioxidants required to be consumed.

Honey serves as a source of natural antioxidants, which are effective in reducing the risk of heart disease, cancer, immune-system decline, cataract, different inflammatory processes, etc. [2]. It is rich in antioxidants such as vitamin C, phenolic acids, flavonoids and is a valuable natural product being used since the earliest times in history [3, 4]. Honeybees visit flowers of different crops, vegetables, medicinal and fruit plants to collect pollen and nectar. These plants are known as bee plants or bee flora. Pollen as food is used by honeybees and nectar as raw material to make honey. Traditionally honey has been used by the people of Garhwal Himalaya as food and as medicine in the treatment of cough. Garhwal Himalaya is highly rich in plant wealth due to its varied eco-climatic conditions. Many of these plants possess antioxidant compounds in varying quantities. Phenolic acids and flavonoids are the main antioxidants in apiary products [5, 6]. The antioxidant capacity of honeys depends on the plant source used by honeybees to collect nectar and pollen. The phenolic compounds present in honey are directly related to botanical sources, such as pollens, nectars, resins and oils that are Collected by the bees, and consequently, honeys from different floral origins possess distinct bioactive properties [7] while Other substances present in honey such as organic acids, amino acids, proteins, enzymes, lipids, flavonoids and vitamins are also responsible for its biological

properties (including antioxidant and antibiotic activities) [8, 9, 10, 11, 12].

The major purpose of this work was to evaluate the antioxidant activity of multifloral Garhwal Himalayan honeys produced by *Apis cerana-indica.*

MATERIALS AND METHODS

The study was conducted in Garhwal Himalaya during the year 2014-2015. Garhwal Himalaya situated in the central part of the Western Himalaya lies between the latitudes 29° 31'9" N and 31° 26'5" N and longitudes 77° 33'5" E and 80° 6'0" E with a total area of 29,089 km^2. Twenty-one honey samples were collected directly from inhabitants of rural areas of Garhwal Himalaya. The samples were stored at room temperature in dark before analysis. To confirm their botanical origin, all of the samples were subjected to melissopalynological analysis [13]. The total phenolic content was determined by standard methods [14].

The antioxidant activity of honey samples was analyzed by using the 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH). For stock solution, 0.05 gm honey was dissolved in 50 ml methanol. 0.00395 gm of DPPH dissolved in 100 ml of methanol was used as control. The honey samples were dissolved in methanol at concentrations ranging from 0.1 to 0.9 mg/ ml., 3 ml of DPPH solution was mixed with each concentration of honey solution. After the mixtures were left for 30 min at room temperature in the dark, the absorbance of the remaining DPPH was measured in spectrophotometer at 515 nm [15] and converted into the percentage of antioxidant activity (AA) using the formula:

$AA\% = 100 - \{[(Abs_{sample} - Abs_{blank}) \times 100]/Abscontrol\}$

The blank consisted of 1ml methanol with 3 ml of the DPPH solution. The radical scavenging activity was expressed as IC50 (the concentration of the honey sample mg/ml, required to scavenge 50% of DPPH) presented in Table 1. T test was used to determine the statistical significant difference between phenolic content and IC_{50} value.

Table 1: Antioxidant activity and phenolic content of different multifloral honey samples

[1]IC: Inhibitory Concentration, [2]GAE: Gallic acid equivalent

Sample	Name of the Place/ District	Altitude	IC 50 Value	Phenols (mg GAE/100 gm)[2]
H1	Badeth village (Rudraprayag)	1326m	5.42	0.125
H2	Ushara village (Rudraprayag)	2286m	5.08	0.237
H3	Chilond village (Rudraprayag)	1980m	3.06	3
H4	Kurchoi village (Chamoli)	1995m	8.70	2.21
H5	Gadagu village (Rudraprayag)	1708m	2.44	0.538
H6	Chaumasi village (Rudraprayag)	1897m	3.36	0.72
H7	Balawala (Dehradun)	612m	2.80	1.67
H8	Joshimath (Chamoli)	2035m	5.63	1.61
H9	Bhunal village (Rudraprayag)	1811m	6.92	4.18
H10	Raigdi village (Chamoli)	2200m	3.25	2.18
H11	Karchi village (Chamoli)	2206m	4.73	0.853
H12	Brambari village (Rudraprayag)	1276m	4.11	3.53
H13	Jaltalla village (Rudraprayag)	1286m	2.26	2.32
H14	Guptkashi (Chamoli)	1474m	2.53	0.524
H15	Akhori village (Tehri)	2006m	2.48	1.55
H16	Khunnu village (Rudraprayag)	1582m	1.68	2.22
H17	Tyuri village (Rudraprayag)	1712m	3.27	1.92
H18	Kirora Malla village (Rudraprayag)	2057m	2.50	2.82
H19	Bhainsari (Rudraprayag)	920m	2.57	0.853
H20	Ambari village Vikasnagar (Dehradun)	471m	2.96	0.755
H21	Tugasi village (Chamoli)	2722m	5.70	2.43

RESULTS AND DISCUSSION

Melissopalynological analysis

A total of 57 pollen types, belonging to 28 botanical families from 21 honey samples were identified and presented in Table 2 with their frequency classes and presence in the samples. Some of the bee forage plants of the area are mentioned in plate 1. All the samples were multifloral in their origin. Multifloral honey is prepared from the nectar of many flowers. High pollen diversity found in honeys represents the good mix of seasonal nectar and pollen plants. The frequency classes [13] of pollen types are described as follows:

1. **Secondary pollen type-** Pollen of eleven plants emerged as secondary source in studied samples. Some of them are *Brassica campestris*, *Aesculus indica*, *Eucalyptus* spp., *Pogostemon benghalense*, *Prunus persica*, *Pyrus pashia* and *Zea mays* (Table 2).
2. **Important minor pollen-** Pollen of forty three plants emerged as important minor source consisting mainly of *Abelmoschus esculentus*, *Ageratum conyzoides*, *Berberis asiatica*, *Citrus aurantifolia, Citrus sinensis*, *Colebrookia oppositifolia*, *Coriandrum sativum Cucurbita maxima*, *Juglans regia, Myrica esculenta Pyracantha crenulata*, *Rhododendron arboreum, and Viburnum grandiflorum*.
3. **Minor pollen-** Pollen of seventeen plants emerged as minor source. Important of are *Amaranthus* spp., *Commelina benghalensis, Grewia optiva*, *Indigofera heterantha*, *Lyonia ovalifolia*, *Murraya koenigii*, *Rubus ellipticus*, *Rubus foliosus*, *Rumex hastatus* and *Woodfordia fructicosa* (Table 2).

Total phenol content and antioxidant activity

The samples were analyzed to assess their antioxidant activity and total phenolic content. Polyphenols are secondary metabolites present in foods from plant origin. There are three main types of polyphenols, the flavonoids, phenolic acids and tannins that are potent antioxidants. These compounds are considered the main substances promoting health benefits [16, 17]. The phenolic content in the honeys ranged from 0.125 to 4.18 mg GAE/100gm. The lowest value was determined in Sample H1 collected from Badeth village with an average of 0.125 mg GAE/100gm honey and highest phenolic content was found in Sample H9 collected from Bhunal village with an average value of 4.18 mg GAE/100gm honey. In the present study, the phenolic content of honey samples made by *Apis cerana-indica* were higher than that of previous reported values on *Apis mellifera* honey from Nigeria (0.75 to 2.85 mg/100g GAE) [18] and (2.0-39.0 mgGAE/1000gm) on Romanian *Acacia* honey [19].

The antioxidant activity of the honey samples was examined using the DPPH scavenging assay. DPPH radical scavenging activity was observed with all tested samples, these tested samples showed higher antioxidant activity compared to gallic acid, quercetin and ascorbic acid. The scavenging activity of all honey samples expressed as IC_{50} with respect to DPPH radical, ranged from 1.68 to 8.70 mg/ml [Table 1, Figure 1]. The results showed that the DPPH radical scavenging activity of honey samples increased gradually as the concentration increased, with the observation that complete inhibition was never reached. Decrease in absorbance of DPPH solution (that is, from purple to yellow) depends on intrinsic antioxidant activity of antioxidant as well as on speed of reaction between DPPH and antioxidant [20].

Among all samples, Sample H4 collected from Kurchoi village showed the lowest antioxidant activity (8.70 mg/ml) and Sample H16 collected in Khunnu village showed the highest antioxidant activity (1.68 mg/ml). A lower IC_{50} value in honey indicates a greater ability to neutralize free radicals [15]. In the present study, the antioxidant capacities of honey samples were within the range of previous reported values 3.17 to 8.79 mg/ml [15] and higher than 7.2 to 53.8 mg/ml [5], 10.6-12.9 mg/ml[14], 106.67 to 168.94 mg/ml[21], 4.2 to 106.72 mg/ml[22] and 12.20 mg/ml in multifloral honey [23]. These findings show that the honey samples collected in the present study have greater antioxidant potential compared to the results reported in the literature. In the present study, phenolic content for multifloral samples were lower than that of previous values 17-66 mg GAE/g (14), 250-509 mg GAE/1000gm [15], 226.16 -727.77 mg GAE/1000gm [21], 52.2- 789.6 mgGAE/1000gm [24] and 21.4 to 34.8 mg GAE/100g [25].

Table 2. Pollen spectra of multifloral honey samples showing presence and frequency class[1] of each pollen type.

Pollen type	Family	Honey samples																				
		H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21
Abelmoschus esculentus	Malvaceae	-	-	5(IP)	-	-	5(IP)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aesculus indica	Hippocastanaceae	-	15(IP)	-	13(IP)	20(SP)	-	-	-	14(IP)	16(SP)	-	-	-	-	20(SP)	-	16(SP)	20(SP)	-	-	-
Ageratum conyzoides	Asteraceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17(IP)	-
Amaranthus spp.,	Amaranthaceae	-	-	-	-	-	-	-	-	-	-	2(MP)	-	-	-	-	-	-	-	3(IP)	--	2(MP)
Berberis aristata	Berberidaceae	-	2(MP)	-	-	-	-	-	-	-	-	-	-	-	-	-	.5(MP)	2.5(MP)	-	-	-	-
Berberis asiatica	Berberidaceae	2(MP)	4(IP)	-	-	-	-	-	-	-	-	-	1(MP)	-	-	-	-	2.5(MP)	-	-	-	-
Brassica campestris	Brassicaceae	8(IP)	13(IP)	-	12(IP)	9(IP)	-	41(SP)	14(IP)	9(IP)	13(IP)	-	14(IP)	9(IP)	12(IP)	13(IP)	-	10(IP)	12(IP)	13(IP)	-	11(IP)
Caesalpinia decapetala	Caesalpiniaceae	6(IP)	-	-	-	-	-	-	-	-	-	-	6(IP)	-	-	-	-	-	-	-	--	-
Callistemon citrinus	Myrtaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39(SP)	-
Citrus aurantifolia	Rutaceae	-	-	-	-	-	-	-	11(IP)	-	-	-	-	-	-	8(IP)	-	-	-	-	-	-
Citrus aurantium	Rutaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	7(IP)	8(IP)	3(IP)	-				
Citrus medica	Rutaceae	-	-	-	-	-	-	-	-	-	-	-	8(IP)	-	-	-	-	-	-	-	-	-
Citrus pseudolimon	Rutaceae	15(IP)	-	-	-	10(IP)	-	-	-	10(IP)	-	-	8(IP)	6(IP)	8(IP)	-	3(IP)	6(IP)	8(IP)	-	-	-
Citrus reticulata	Rutaceae	-	8(IP)	-	-	-	-	-	12(IP)	-	-	-		6(IP)	-	-	3(IP)	-	-	-	-	-
Citrus sinensis	Rutaceae	13(IP)	12(IP)	-	-	8(IP)	-	-	-	12(IP)	-	-	-	6(IP)	-	-	4(IP)	6(IP)	6(IP)	-	-	-
Colebrookia oppositifolia	Lamiaceae	-	-	-	-	-	-	-	-	-	-	-	-	6(IP)	-	-	4(IP)	-	-	-	-	-
Commelina benghalensis	Commelinaceae	-	-	-	-	-	-	-	-	-	-	.5(MP)	-	-	-	-	-	-	-	-	-	.5(MP)
Coriandrum sativum	Apiaceae	5(IP)	5(IP)	-	-	5(IP)	-	-	5(IP)	3(IP)	-	-	5(IP)	-	5(IP)	-	5(IP)	4(IP)	5(IP)	-	-	-

Corylus jacquemontii	Corylaceae	-	-	-	1(MP)	-	-	-	-	-	1(MP)	-	-	-	-	-	-	-	-	-	-
Cucurbita maxima	Cucurbitaceae	-	-	15(IP)	-	-	9(IP)	-	-	-	-	7(IP)	-	-	-	-	-	-	-	7(IP)	-	6(IP)
Dalbergia sissoo	Fabaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	13(IP)	-
Eucalyptus spp.,	Myrtaceae	-	-	-	-	-	-	42(SP)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fagopyrum dibotrys	Polygonaceae	-	-	-	-	-	-	-	-	-	-	5(IP)	-	-	-	-	-	-	-	-	-	5(IP)
Grewia optiva	Tiliaceae	-	-	-	-	-	-	-	2(MP)	-	-	-	-	2(MP)	2(MP)	1(MP)	1(MP)	-	-	-	-	-
Impatiens scabrida	Balsaminaceae	-	-	-	-	-	-	-	-	-	-	3(IP)	-	-	-	-	-	-	-	-	-	3(IP)
Impatiens bicornata	Balsaminaceae	-	-	-	-	-	-	-	-	-	-	3(IP)	-	-	-	-	-	-	-	-	-	3(IP)
Indigofera heterantha	Fabaceae	-	-	-	-	-	-	-	-	-	-	.5(MP)	-	-	3(IP)	-	-	-	-	-	-	.5(MP)
Juglans regia	Juglandaceae	-	-	-	3(IP)	1(MP)	-	-	3(IP)	2(MP)	3(IP)	-	3(IP)	3(IP)	-	4(IP)	-	3(IP)	2(MP)	-	-	-
Lagenaria siceraria	Cucurbitaceae	-	-	6(IP)	-	-	9(IP)	-	-	-	-	7(IP)	-	-	-	-	-	-	-	7(IP)	-	6(IP)
Litchi chinensis	Sapindaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25(SP)	-
Luffa acutangula	Cucurbitaceae	-	-	6(IP)	-	-	9(IP)	-	-	-	-	7(IP)	-	-	-	-	-	-	-	7(IP)	-	6(IP)
Luffa cylindrica	Cucurbitaceae	-	-	6(IP)	-	-	7(IP)	-	-	-	-	7(IP)	-	-	-	-	-	-	-	7(IP)	-	6(IP)
Lyonia ovalifolia	Ericaeae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(MP)	-	-	-	-
Medicago sativa	Fabaceae	1(MP)	-	2(MP)		-	2(MP)	-	-	-	-	-	-	-	-	-	-	-	-	1(MP)	-	-
Melia azedarach	Meliaceae	-	-	-	-	-	-	-	-	-	-	-	-	4(IP)	11(IP)	-	3(IP)	-	-	-	-	-
Murraya koenigii	Rutaceae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1(MP)	-

Myrica esculenta	Myricaeae	-	-	4(IP)	-	-	4(IP)	-	-	-	-	-	-	-	-	-	-	-	-	2(MP)	-	-
Pogostemon benghalense	Lamiaceae	-	-	-	-	-	-	17(SP)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Prinsepia utilis	Rosaceae	-	-	-	8(IP)		-	-	-	-	8(IP)	-	-	-	-	-	-	-	-	-	-	-
Prunus armeniaca	Rosaceae	-	-	-	7(IP)	-	-	-	10(IP)	-	4(IP)	-	-	-	-	-	-	-	-	-		-
Prunus persica	Rosaceae	17(SP)	17(SP)	-	7(IP)	10(IP)	-	-	16(SP)	17(SP)	16(SP)	-	18(SP)	8(IP)	18(SP)	17(SP)	16(SP)	17(SP)	16(SP)	-	-	-
Pyracantha crenulata	Rosaceae	-	-	-	6(IP)	-	-	-	-	-	5(IP)	-	-	-	-	-	-	-	-	-	-	-
Pyrus communis	Rosaceae	-	-	-	6(IP)	9(IP)	-	-	16(SP)	-	4(IP)	-	-	8(IP0	-	-	-	-	-	-	-	-
Pyrus pashia	Rosaceae	14(IP)	6(IP)	-	18(SP)	9(IP	-	-	-	16(SP)	17(SP)	-	18(SP)	9(IP)	20(SP)	16(SP)	16(SP)	17(SP)	16(SP)	-	-	-
Rhododendron arboreum	Ericaceae	12(IP)	11(IP)	-	-	12(IP)	-	-	11(IP)	12(IP)	-	-	13(IP)	12(IP)	13(IP)	11(IP)	11(IP)	9(IP)	11(IP)	-	-	-
Ricinus communis	Euphorbiaceae	3(IP)	-	-	-	-	-	-	-	-	-	-	4(IP)	-	-	-	-	-	-	-	-	-
Rosa macrophylla	Rosaceae	-	-	22(SP)	-	-	16(SP	-	-	-	-	20(SP)	-	-	-	-	-	-	-	-	-	19(SP)
Rubus ellipticus	Rosaceae	-	7(IP)	-	8(IP)	-	-	-	-	2.5(MP)	5(IP)	-	-	-	-	-	6(IP)	3(IP)	-	-	-	-
Rubus foliosus	Rosaceae	-	-	-	-	-	-	-	-	2.5(MP)	-	-	-	-	-	-	-	3(IP)	-	-	-	-
Rubus niveus	Rosaceae	-	-	-	7(IP)		-	-	-	-	4(IP)	-	-	-	-	-	-	-	-	-	-	-
Rumex hastatus	Polygonaceae	-	-	-	-	-	-	-	-	-	-	-	-	1(MP)	-	-	.5(MP)	-	-	-	-	-
Sapindus mukorossi	Sapindaceae	-	-	-	-	-	-	-	-	-	-	-	-	5(IP)	-	-	9(IP)	-	-	-	-	-
Toona hexandra	Meliaceae	-	-	-	-	-	-	-	-	-	-	-	-	7(IP)	-	-	7(IP)	-	-	-	-	-
Viburnum grandiflorum	Caprifoliaceae	-	-	-	4(IP)	-	-	-	-	-	1(MP)	-	-	-	-	-	-	-	-	-	-	-
Woodfordia fructicosa	Lythraceae	4(IP)	-	-	-	7(IP)	-	-	-	-	-	-	2(MP)	-	-	-	-	-	2(MP_)	-	-	-

Zanthoxylum armatum	Rutaceae	-	-	-	-	-	-	-	-	-	-	-	-	8(IP)	-	-	8(IP)	-	-	-	-	-
Zea mays	Poaceae	-	-	34(SP)	-	-	39(SP)	-	-	-	-	38(SP)	-	-	-	-	-	-	-	38(SP)	-	32(SP)

[1]Frequency classes - Predominant pollen (PP, >45%), secondary pollen (SP, 16-45%), important minor pollen (IP, 3-15%), minor pollen (MP, <3%)

The p values given by significance are less than the level of significance which is 0.05 (Table 3a and 3b). T test between the parameters analyzed were found to be statistically significant ($p<0.05$). The phenolic profile of honeys and consequently their antioxidant capacity depend on the floral sources used to collect honey [14]. There was strong and positive relationship between the phenolic content and antioxidant activity of honey. The study showed that the samples collected from Garhwal Himalaya although with low content of phenols, besides this, the samples have a wide range of antioxidant activity.

CONCLUSION

Multifloral honey is prepared from the nectar of diverse range of plants. The melissopalynological analysis of honey samples from Garhwal Himalaya indicated that *Aesculus indica*, *Brassica campestris*, *Callistemon citrinus*, *Citrus* spp., *Eucalyptus* spp., *Juglans regia*, *Prunus persica*, *Pyrus communis*, *Pyrus pashia*, *Rhododendron arboreum* and *Zea mays* were found as important pollen producing plants. All of the samples exhibited good antioxidant activity and average phenolic content. The study observed that phenolic contents of the Garhwal Himalayan honey samples are strongly responsible for their antioxidant activity, emphasizing the need of such honeys as important nutritional source of antioxidant compounds. Thus, it can be concluded that samples produced in different parts of Garhwal Himalaya can be used as source of natural antioxidants ensuing health benefits.

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