



Studies on floral sources, phenolic, flavonoid, proline contents and antioxidant capacity of honey from the Khirsoo area of Pauri, Uttarakhand, India

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ABSTRACT

Present study was carried out to determine the floral sources, phenolic, flavonoid, proline contents and antioxidant capacity of 24 honey samples collected from the Khirsoo area of the Pauri district, Uttarakhand (India). The phenolic, flavonoid, proline contents, and antioxidant capacity of the honey samples ranged from 36.83 to 106.66 mg GAE/100g, 3.99 to 20.56 mg QE/100g, 27.3 to 621.22 mg/kg and 16.24 to 25.96 mg AAE/100g, respectively. Maximum quantity of phenolic, flavonoid contents and antioxidant capacity was found in Thapla honey representing the dominant floral sources *Aesculus indica*, *Rhododendron arboreum*, *Myrica esculentum*, *Berberis aristata*, *Coriandrum sativa*, Brassicaceous and Rosaceous members. Highest proline content was found in Gwaad honey, extracted in the month of November with dominant bee forage species *Prunus cerasoides*, *Sesamum indicum*, *Impatiens balsaminea*, *Zea mays* and *Luffa* spp. Study has shown that there is a positive correlation between the antioxidant capacity and phenolic contents ($R=0.844$) as well as in between antioxidants and the flavonoid contents ($R=0.694$). The observation revealed that the region has a good potential of bee forage species and quality of honey is good for dietary and medicinal purpose.

Key words: Antioxidant, Flavonoid, Phenolic, Pollen spectra, Proline

Bees and plants are co-evolved during middle cretaceous period (Michener 1974), they share a special relationship with each other. The hymenopterans is a group that generally feeds on nectar and pollen for energy and protein sources respectively to fulfill their food requirement, while the bees in course of getting their food by wandering from flower to flower act as the primary pollinators for about three-quarters of crops those requiring animal pollination (Free 1993, Krell 1996, Klatt *et al.* 2014, Pashte and Kulkarni 2015). Rearing of honey bees for production of honey is one of the widespread agricultural activities done in India (Nayik and Nanda 2015).

Honey is a matrix of vegetal origin processed by diverse types of bees of Apidae, Hymenoptera, by feeding on nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects (Codex Alimentarius Commission 2001). Melissopalynological studies are important tools in determining the origin of honey and floral sources upon which bees' foraged (Ohe *et al.* 2004) as the floral component gives a direct impact on

the sugar and secondary metabolites of honey. Pollen grains have several valuable phytochemicals such as carotenoids, steroids, terpenoids and flavonoids (Kao *et al.* 2011). Floral origin also has a direct impact on phenolic and flavonoid contents of honey (Martos *et al.* 2000).

Honey composition and physico-chemical properties are wholly dependent on the processing, storage, floral sources and climatic conditions of region (Saxena *et al.* 2010). Chemically, honey is mostly dominated by carbohydrates in the form of fructose and glucose and other minor constituents of honey such as organic acids, mineral salts, vitamins, proteins, enzymes, volatile chemicals, phenolic acids and flavonoids (Gheldof *et al.* 2002, Nayik and Nanda 2015). Most of the phenolic compounds found in honey are in the form of flavonoids (Viuda-Martos *et al.* 2008). The phenolic compounds present in honey act as natural antioxidants, due to which honey is used as a food preservative (Codex Alimentarius 2001, Gheldof *et al.* 2002). Antioxidant capacity of honey is mainly due to the phenolic compounds, free amino acids (proline), and there is a high correlation between phenolic contents and antioxidant capacity of honey (Alzahrani *et al.* 2012, Meda *et al.* 2005).

Standard honey contains 11 to 21 free amino acids with proline as a major component (White and Doner 1980) which also participate in antioxidant capacity of honey. Proline is known to be an energy source for protein synthesis in

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invertebrates (Osanai and Yonezawa 1986) and appears to have a major role in insect flying metabolism (Bursell *et al.* 1974). Present study was aimed to analyze the floral sources, phenolic, flavonoid, proline contents and antioxidant capacity of 24 honey samples collected from Khirsoo area of Pauri district in Uttarakhand Himalaya, India.

MATERIALS AND METHODS

The study was carried out in the Khirsoo area (29°45' to 30°15' north latitudes and 78°29' to 79°25' east longitudes) of Pauri Garhwal, Uttarakhand (India) during the years 2015 and 2016. The study area falls at an elevation from 600–1700 m and represents a submontane and montane type of vegetation. The honeybee (*Apis cerana-indica*) also known as Indian honey bee is most commonly found in the area. A total twenty four honey samples were collected directly from the inhabitants at the time of extraction from the domesticated hives of *Apis cerana-indica*. During field survey floral samples for reference slides around the apiaries were collected and name of collection sites, season of honey extraction recorded (Table 1).

Pollen analysis of honey samples was carried out by

studying the reference pollen slides microscopically by following Erdtman (1960) and categorized into different frequency classes such as predominant pollen (>45% of pollen grains of one type counted), those having several types such as secondary pollen (16-44%), important minor pollen (3-15%) and minor pollen (<3%) by following Louveaux (1970). Almost all pollen grains were identified by these methods, a few species which could not be identified manually, have been taken help of pertinent local flora and published data (Wodehouse 1935, Erdtman 1952, Maheshwari 1986). Absolute pollen count of each honey sample was carried out by using haemocytometer and categorized under different groups (Table 1.)

Phenolic contents of the honey samples were determined following Silici *et al.* (2010). Results were calculated using a standard curve of gallic acid ($R^2=0.995$) and expressed in mg gallic acid equivalent (GAE)/100g honey. Flavonoid contents of the honey samples were determined following Zhishen *et al.* (1999), and results were calculated using the standard curve of quercetin ($R^2=0.973$) expressed in mg QE/100g. Antioxidant capacity of samples was determined (Silici *et al.* 2010), and results were expressed in mg AAE/100g

Table 1 Details of the collection site, extraction period and APC of honey samples

Honey sample	Elevation range	Collection site (Villages name)	Extraction month	Absolute pollen count/10gm honey	Different groups	Floral origin
HS-Rai-01	600-1000	Raibaadi	July	9502	II	Multifloral
HS-Ait-02	-do-	Aithana	August	41875	III	Multifloral
HS-Mar-03	-do-	Maroda	May	95937	IV	Multifloral
HS-Gaj-04	-do-	Gajeli	August	201875	V	Multifloral
HS-Kaf-05	-do-	Kafooli	June	41875	III	Multifloral
HS-Mus-06	1200-1400	Musooli	May	43312	III	Multifloral
HS-Mus-07	-do-	Musooli	October	85027	IV	Multifloral
HS-Kho-08	-do-	Khola	July	11562	III	Multifloral
HS-Kho-09	-do-	Khola	March	55937	IV	Multifloral
HS-Bal-10	-do-	Balodi	November	11562	III	Multifloral
HS-Bal-11	-do-	Balodi	May	17505	III	Multifloral
HS-Bha-12	-do-	Bhainshkott	August	15035	III	Multifloral
HS-Mar-13	-do-	Markhoda	July	25825	III	Multifloral
HS-Mar-14	-do-	Markhoda	October	61687	IV	Multifloral
HS-Cha-15	-do-	Chakoli	November	98965	IV	Multifloral
HS-Kot-16	Above-1400	Kothagii	May	26875	III	Multifloral
HS-Kot-17	-do-	Kothagii	July	95935	IV	Multifloral
HS-Gwa-18	-do-	Gwaad	August	13125	III	Multifloral
HS-Gwa-19	-do-	Gwaad	November	13437	III	Multifloral
HS-Gwa-20	-do-	Gwaad	March	21656	III	Multifloral
HS-Khi-21	-do-	Khirsoo	May	83331	IV	Multifloral
HS-Tha-22	-do-	Thapla	May	84965	IV	Multifloral
HS-Min-23	-do-	Mindaan	July	25368	III	Multifloral
HS-Min-24	-do-	Mindaan	November	85937	IV	Multifloral

*Group I <2000; Group II 2000 - 10 000; Group III 10 000-50 000; Group IV 50 000- 100 000 and Group V > 100 000, which indicates extremely poor, poor, rich, very rich and extremely rich amount of pollen count in honey respectively (Jose *et al.* 1989).

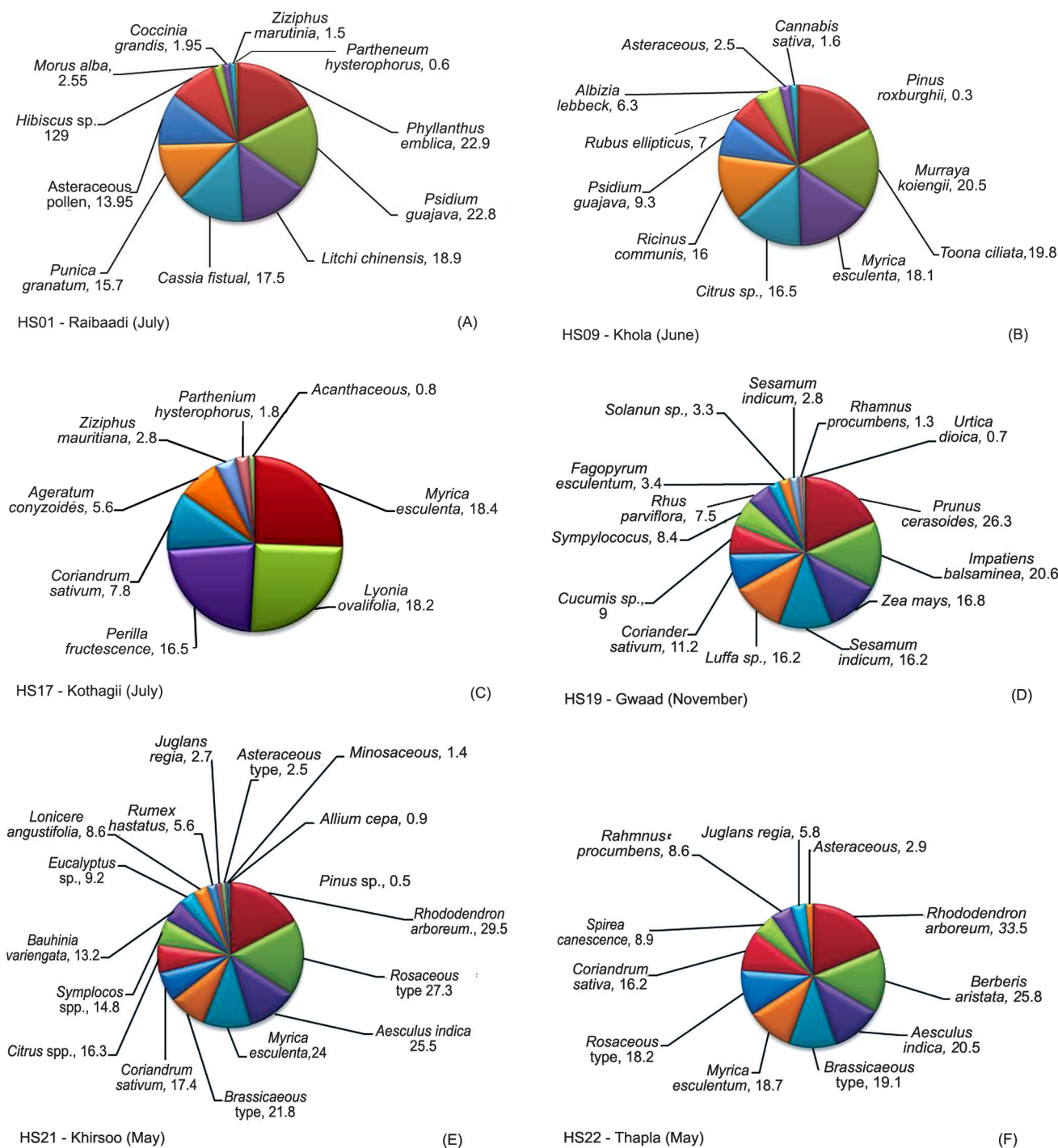


Fig 1 A-F Details of few pollen spectra of collection sites.

honey. We followed Ough (1969) for determination of proline content using the following formula:

$$\text{Proline} \left(\frac{\text{mg}}{\text{Kg}} \right) = \frac{ES}{EA} \times \frac{E1}{E2} \times 80$$

where, ES = Absorbance of the sample solution, EA = Absorbance of the proline standard solution (average of two readings), E1 = mg proline taken for the standard solution, E2 = Weight of honey in g, 80 = Dilution factor.

Statistical analysis was carried out in triplicate, and the results were expressed as mean and standard deviation (SD).

Correlations were established using Pearson's correlation coefficient (R) in bivariate linear correlations (P < 0.01) to determine the relationship between the two variables was calculated using SPSS software.

RESULTS AND DISCUSSION

Pollen analysis of honey samples revealed that all the samples are multifloral in nature (Table 1, Fig 1 A-F). We identified seventy eight pollen types represents 93 pollen taxa belonging to forty seven families. Asteraceae, Rosaceae, Myrtaceae, Mimosaceae were found dominant families.

Absolute pollen count of all samples showed rich pollen amount ranged between 9502 to 201875 pollen grains/10g of honey (Table 1). Multifloral nature of all the samples was due to presence of heterogenous vegetation in the feeding range of honeybee hives. Present findings are in line with Sahney *et al.* (2016) who reported 37 pollen types in 6 honey samples belonging to 23 families from India.

Phenolic contents (mg of GAE/100g of honey) ranged from 36.83 to 106.66 with a mean of 55.7604±1.295504 and flavonoid contents (mg QE/100g) varied from 3.99 to 21.3 with a mean of 9.854583±0.178417 (Table 2). The highest phenolic and flavonoid contents were found in Thapla honey (Sample 22) and a positive correlation (R=0.837) existed between them which is significant at the 0.01 level. This honey showed the predominance of pollen-nectar of *Aesculus indica*, *Myrica esculenta*, *Rhododendron arboreum*, *Berberis aristata*, *Coriandrum sativum*, Brassicaceous and Rosaceous members. The mean values for phenolic and flavonoid contents found in the samples were in the line with the average values reported earlier by Meda *et al.* (2005) (74.38±20.54 mg GAE/ 100g); (2.57±2.09 mg QE/100g), Sant'Ana *et al.* (2012), (61.11 - 175.39 mg GAE/ 100g); (2.94 - 10.911 mg QE/100g), Moniruzzaman *et al.* (2013), (129.16 to 341.67 mg GAE/ 100 kg); (28.83-113.06 mg QE/100 kg) and Boulanouar *et al.* 2017 (86±0.006 mg GAE/ 100g); (8±0.002 mg RE/100g) from Burkina Fasan, Brazil, Malaysia and Algeria respectively. Natural honey has been reported to contain flavonoids of approximately 2 mg/100g (Ferrerres *et al.* 1994), and in our study all samples showed the flavonoid contents higher than the 2 mg/100g.

Antioxidant capacity (mg AAE/100g) of honey samples varied from 16.23 to 25.96 with a mean of 20.64917±0.261871 (Table 2) using a standard curve of ascorbic acid. A positive correlation was found between phenolic contents and antioxidant capacity (R=0.844) and similar relation was also found between flavonoid and antioxidants (R=0.694). Thapla honey (Sample 22) showed a high positive relationship (value) between these parameters (phenolic, flavonoid contents and antioxidant capacity). Other samples such as Khirsoo honey (Sample 21) showed second highest phenolic (99.23±1.56), flavonoid contents (21.3±0.519), antioxidant capacity (25.83±0.763) and a significant correlation was found between these. Honey characteristically shows a high antioxidant property due to high phenolic and flavonoid contents and some time it may be due to the predominant amino acids. Meda *et al.* (2005) reported a positive correlation between phenolic and antioxidants AEAC (R=0.5), and found negative correlation in between flavonoid and antioxidants. In our study a positive correlation was observed between antioxidants and flavonoid (R=0.694), although correlation between the phenolics and antioxidants AAE (R= 0.844) was in line with Meda *et al.* (2005). Result of present study reveal that the high antioxidant capacity in *Apis cerana-indica* honey samples is mainly due to phenolic and flavonoid contents. The data obtained from the analysis of multifloral honeys for phenolic, flavonoid, antioxidant capacity was interpreted with the

Table 2 Details of the phenolic, flavonoid, proline contents and antioxidant capacity of honey samples

Honey sample	Phenolic (mg GAE/100g)	Flavonoid (mg QE/100g)	Proline (mg/kg)	Antioxidant capacity (mg AAE/100g)
HS-Rai-01	36.83 ± 1.25	4.53 ± 0.425	68.26 ± 11.82	16.2 ± 0.05
HS-Ait-02	42.16 ± 2.02	9.09 ± 0.1	129.7 ± 23.64	17.16 ± 0.152
HS-Mar-03	38.63 ± 1.51	7.88 ± 0.08	409.6 ± 35.47	16.23 ± 0.251
HS-Gaj-04	46.96 ± 0.95	5.58 ± 0.015	157.01 ± 11.82	18.65 ± 0.132
HS-Kaf-05	72.5 ± 2.78	14.6 ± 0.298	348.16 ± 70.94	23.42 ± 0.2
HS-Mus-06	43.5 ± 0.5	8.74 ± 0.015	300.37 ± 23.64	18.22 ± 0.049
HS-Mus-07	47.96 ± 0.95	9.03 ± 0.041	368.64 ± 20.48	17.48 ± 0.0028
HS-Kho-08	44.83 ± 2.02	7.06 ± 0.208	54.61 ± 15.64	20.53 ± 0.502
HS-Kho-09	44.96 ± 0.95	3.99 ± 0.095	375.46 ± 31.28	18.06 ± 0.057
HS-Bal-10	41.16 ± 2.56	7.88 ± 0.005	320.85 ± 11.82	18.53 ± 0.602
HS-Bal-11	37.56 ± 0.51	7.49 ± 0.065	211.62 ± 11.82	18.09 ± 0.095
HS-Bha-12	49.23 ± 1.07	9.04 ± 0.041	150.18 ± 11.82	19.06 ± 0.055
HS-Mar-13	85.1 ± 1.95	10.28 ± 0.257	136.53 ± 23.64	21.98 ± 0.975
HS-Mar-14	89.83 ± 0.763	16.55 ± 0.484	191.14 ± 42.63	25.87 ± 0.037
HS-Cha-15	51.09 ± 0.95	14.4 ± 0.148	259.41 ± 96.78	20.75 ± 0.005
HS-Kot-16	44.26 ± 1.0101	4.04 ± 0.05	361.81 ± 11.82	20.3 ± 0.519
HS-Kot-17	48.3 ± 1.12	7.16 ± 0.198	27.3 ± 11.82	22.09 ± 0.3
HS-Gwa-18	45.16 ± 1.04	4.08 ± 0.005	146.37 ± 11.82	22.13 ± 0.321
HS-Gwa-19	70.96 ± 1.004	14.39 ± 0.005	621.22 ± 47.29	25.63 ± 0.378
HS-Gwa-20	50.09 ± 1.02	14.36 ± 0.29	116.05 ± 11.82	19.75 ± 0.2
HS-Khi-21	99.23 ± 1.569	21.3 ± 0.519	484.69 ± 11.82	25.83 ± 0.763
HS-Tha-22	106.66 ± 1.52	21.56 ± 0.513	320.85 ± 42.63	25.96 ± 0.0571
HS-Min-23	53.23 ± 1.07	7.23 ± 0.208	102.4 ± 20.48	21.93 ± 0.057
HS-Min-24	48.06 ± 1.006	7.25 ± 0.217	354.98 ± 11.82	21.69 ± 0.525
mean ± SD	55.76 ± 1.295	9.85 ± 0.17	250.7 ± 26.02	20.64 ± 0.26

pollen frequency classes of honey samples and proline with the harvesting period of honey. The main honey flow seasons in the state are 'Chait' (April), 'Baisakh' (May) and 'Kartik' (October) (Tiwari *et al.* 2013).

The maximum proline content 621.22 mg/kg was found in the honey collected in month of November (Sample 19) and the minimum 27.13 mg/kg in July (Sample No. 17) honey, with a mean of 250.7171±26.02333 (Table 2). Low proline in Gwaad honey (Sample no. 17) as compared to Kothagii honey (Sample no. 19), inspite of both the sites having similar elevation and vegetation, can be attributed to extraction of honey in minor honey flow season, which the inhabitants also practice besides the main honey flow season extraction and a short ripening period.

Proline content serves as a determinant of quality and ripeness of honey as well as an indicator for detecting sugar adulteration when it falls below 183 mg/kg in the honey (Bogdanov 1999). A few of our samples showed the proline content below 183 mg/kg. It may be concluded that older (mature) the honey, higher is the proline content as reported by Gerónimo and Fritz (2001). Long ripening period (ca 4-6 month) is one of the determining factors for good amount of proline content in the honey.

We conclude that the study area harbours high diversity of plants which favours the visit of many pollinators especially honeybees, thus enhancing the quality of apicultural products. The study revealed that not only phenolic but also flavonoid contents of honey are positively correlated with the antioxidant capacity. Pearson bivariate analysis proved to be effective tool in establishing the relation between phenolic, flavonoid contents and antioxidant capacity of honey.

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