Research Article [Kumar *et al.*, 4(12): Dec., 2013]
CODEN (USA): IJPLCP **ISSN: 0976-7126**

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Study of Physicochemical parameters and Antioxidant in Honey collected from different locations of India

H. M. Manu Kumar [1]*, A. P. Ananda[2], Deepa Vishwanathan[1] and Siddagangaiah[3]
1, Pristine Laboratories, Kodigehalli Gate, Bangalore, (Karnataka) - India
2, Ganesh Counsultancy & Analytical Services, Hebbal Industrial Area, Mysore, (Karnataka) - India
3, AGMARK Grading Laboratory, Karnataka State Agricultural Marketing, Board, Bangalore, (Karnataka) - India

Abstract

Four varieties of honey collected from the different regions of India for the investigation of physicochemical and antioxidant. The study revealed that all the samples showed pH, Moisture, EC, TDS, HMF, Colour intensity, total sugar, reducing sugar and sucrose range (3.30-4.13, 15.69-17.23%, 152.33-371.66 µS/cm, 101-241.33 mg/l, 1.75-27.87 mg/Kg, 106.60-1592 mAU, 64.88-73.08%, 62.24-70.24% and 1.76-2.58%) respectively. Among the variety polyflora forest (PFf) is the best showing pH (4.13 ± 0.02), Moisture (17.23± 0.01%), Electrical Conductivity (EC, 371.66 ± 0.68 µS/cm), Total Dissolved Solids (TDS, 241.33 ± 0.68 mg/l), Hydroxyl Methyl furfural (HMF, 1.75 ± 0.00 mg/Kg), Colour intensity (1592.00 ±2.93 mAU) and total sugar, reducing sugar and sucrose contents were 65.03 ± 0.05 %, 62.24 ± 0.29 % and 2.25 ± 0.09 % respectively. Even PFf showing minerals Ca, Mg, Na, K, Fe, Zn and polyphenol (2119.28 ± 0.34 mg/Kg), flavonoids (975.50 ± 0.24 mg/Kg), flavonols (588.30 ± 0.33 mg/Kg), flavones (387.26 ± 0.22 mg/Kg) content was found to be more than monoflora, polyflora and processed one. Compare to other varieties PFf extract was markedly a more potent DPPH free radical scavenging activity and reductive Capacity. From this it concluded that the honey PFf variety was the best than the remaining.

Key-Words: Honey, Physicochemical, Antioxidant activity, DPPH, Minerals

Introduction

The composition, flavour and colour of honey vary considerably depending on its botanical source. European legislation[1] defines various honey types and the requirements for labelling. Composition of honey varies depending upon the geographical and the nectar sources of a region. The quality of the honey depends upon its physicochemical and sensory properties. Hence knowledge about its constituents is essential in judging its quality[2]. Honey is a sweet, viscous fluid produced by honeybees (*Apis melliflera*) using the nectar of flowers. In general, the composition of honey contains approximately 70-80% sugar, mainly fructose and glucose. Water, minerals, vitamins, traces of protein and antioxidants. Ancient Egyptians, Assyrians, Chinese, Romans and Greeks have traditionally used honey as a medicinal remedy, for the management of wound healing, skin ailments and various gastrointestinal diseases[3].Characterisation of honey gained importance as it is a common food source for humans.

*** Corresponding Author**
E.mail: manu8anu@gmail.com

Recent studies revealed that phenolic compounds present in the honey can act as potent antioxidants compare to other constituents like vitamins C and E[4,5]. Many researchers have reported the antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria[6-8]. The antioxidant properties of honey are derived from both enzymatic (e.g., catalase, glucose oxidase and peroxidase) and nonenzymatic substances (e.g., ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, Maillard reaction products, flavonoids and phenolic acids[9, 7-8]. The amount and type of these antioxidants are largely dependent on the floral source and a correlation between antioxidant activity with total phenolic content has been established[9, 7-8]. Honey has been discovered for the treatments of bacterial infections by medical profession, particularly, where conventional modern therapeutic agents are failing[10]. Like other saturated sugar syrups and sugar pastes, honey has an osmolarity sufficient to inhibit microbial growth[11]. It has been reported that honey stimulates

monocytes in cell cultures to release the cytokines TNF, alpha, IL- 1 and IL - 6, the cell messengers activates the many facets of the immune response to infection[12]. In addition, to stimulate leucocytes honey provides a supply of glucose, which is essential for the respiratory burst in macrophages that produces hydrogen peroxide, the dominant component of their bacterial destroying activity[10].The acidity of honey may also assist in the bacteria destroying action of macrophages as pH inside the phagocytes value is involved in killing ingested bacteria[10].

Modern research has shown that honey may possess anti-inflammatory activity and stimulate immune responses within a wound. The therapeutic importance of certain types of honey has been attributed to its antibacterial agents and in some countries approved for the market as a therapeutic product. Medihoney® and Active Manuka® honey can currently be purchased as wound healing medicates in Australia and New Zealand[13]. The aim of this work was to evaluate the quality of some samples of honey from the selected regions from the point of view of physicochemical properties and content of heavy metals and comparing these samples with others, analyzed honeys in literature, from countries which do not belong to locations with heavy pollution. Our work was also aimed to find some relationships among individual groups of honey and the correlation among individual constituents.

Material and Methods

Honey Samples

Four local honey samples derive directly from beekeepers in Bihar (Monoflora-MF), South Delhi (Polyflora-PF), Sirsi (Polyflora forest-PFf) and Bangalore (Processed-Pro) through Pristine laboratories, Bangalore on April 2013. All of the honey samples were stored at room temperature (22–24 °C) in airtight plastic containers until analysis.

Chemicals and Reagents

Ascorbic acid, 1,1-diphenyl-2-picrylhydrazyl, Hydroxymethylfurfural were procured from Hi-media, Mumbai**.** Folin–Ciocalteu's reagent, Gallic acid, BHT, Tannic acid and Rutin were procured from Sigma-Aldrich. Sodium carbonate (Na_2CO_3), Aluminum chloride ($AlCl_3$), Sodium nitrite ($NaNO_2$) and Sodium hydroxide (NaOH) were purchased from Merck. All chemicals used were of analytical grade.

Physical Analysis

The pH measured by pH meter 341350A-P (Extech instruments) for 10% solution, EC and TDS were measured by conductivity meter 341350A-P (Extech instruments) for 20% solution, moisture (Refractive Index), HMF (Spectrophotometer), Sugar analysis (Titration) were determined using an IS method[14]. The colour intensity and colour analysis were determined according[15].

Analysis of Antioxidant Properties

Extract preparation

Six ml each of the different varieties of honey was dissolved with 2ml of methanol made up to 60ml with water and left overnight. The mixtures were filtered using Whatman No. 1 filter paper and stored in a refrigerator for the analysis.

Determination of Total Phenolic Content

The concentration of phenolics in honey samples was estimated using a modified spectrophotometric Folin-Ciocalteu method[16]. 1 ml of honey extract was mixed with 1 ml of (1:1) FC reagent. After 3 min, 1 ml of 10% Na_2CO_3 solution was added to the mixture and made to 10 ml with distilled water. The reaction was kept in the dark for 15 min, after which the absorbance was read at 725 nm using a UV/VIS spectrophotometer (UV-1800, Shimadzu, Japan). Gallic acid was used to plot a standard concentration curve 20, 40, 60, 80 and 100 µg/mL. The concentration of phenolic compounds was measured in triplicate. The results were expressed as mg of gallic acid equivalents (GAEs) per kg honey.

Determination of Total Flavonoids Content

The total flavonoid content in each honey sample was measured using the colorimetric assay developed by Zhishen[17]. 1 ml of honey extract was mixed with 4 ml of distilled water. Then 0.3 ml of $NaNO_2$ 5% w/v was added. After five min 0.3 ml of $AlCl_3$ 10% w/v was added followed by 2 ml of NaOH (1M) leave for 6 min. The volume was made up to 10 ml with distilled water. The mixture was vigorously shaken to ensure adequate mixing and the absorbance was read at 510 nm. A calibration curve was created using a standard solution of Rutin 20, 40, 60, 80 and 100 µg/mL. The results were expressed as mg rutin equivalents per kg of honey.

Determination of Total Flavonols Content

The method of Kumaran and Karunakaran[18] was used with modifications. One ml of honey was dissolved with 1ml of ethanol, made upto 100ml with water and left overnight. It was filtered, centrifuged and the supernatant was collected. One ml of honey extract + 1ml of 2% $AlCl_3$ (in ethanol) + 1ml of sodium acetate solution (2g in 40ml of water) were thoroughly mixed together and left in a water bath at 20°C for 10 mins. The absorbance was read at 440nm against the reagent blank that contained 1ml of ethanol. The same procedure was followed for the standard rutin (1mg/ml) which was diluted to the concentrations 20, 40, 60, 80 and 100 µg/ml.

Determination of flavones

The total amounts of flavones in all the varieties of honey investigated was determined by the difference between the total flavonoids contents and the total flavonol contents.

Reducing power assay

The method of Pulido *et al*[19] was used with modifications. 2.5ml of extract was mixed with 2.5ml of sodium phosphate buffer (0.2M pH 6.6) and 2.5ml of potassium ferricyanide (1% in water) in a test tube and reacted for 20 min at 50ºC.The mixture was cooled using crushed ice and 0.5ml of trichloroacetic acid (10% in water) was added and the set up was centrifuged for 10 min. One ml of the supernatant was collected and an equal volume of water was added 0.2ml of 0.1% ferric chloride. The absorbance was read at 700nm against the reagent blank. Rutin was used as the standard. Increased absorbance reading indicates increased reducing power.

DPPH Free radical scavenging activity

The scavenging activity of DPPH free radicals developed according to the method reported by Gyamfi et al[20]. Fifty microliters of the honey extract in methanol, yielding 100 µg/ml in each reaction, was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 µl) only was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, read the absorbance at 517 nm BHT was used as standard. The percent inhibition was calculated from the following equation:

% Inhibition = [Absorbance of control - Absorbance of test sample]/ [Absorbance of control] X 100

Minerals and Metal analysis

Inductively coupled plasma–optical emission spectrometer (ICP-OES Perkin Elmer. USA) was used in the analysis of minerals and metals. Sample was made to ash and dissolves in 10% nitric acid, filtered and made up to 100 ml and fed to ICP-OES. Instrument is calibrated using multi standard elements (Perkin Elmer Life & Analytical Sciences US) with 10 % nitric acid as sample blank.

Statistical Analyses

Assays were performed in triplicate, and the results were expressed as mean values with standard deviations (SD). The significant differences represented by letters were obtained by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) *post hoc* test ($p < 0.05$).

Results and Discussion

Honey is naturally acidic irrespective of its geographical origin, which may be due to the presence of organic acids that contribute to its flavor and its stability against microbial spoilage. The pH of honey samples is important during the extraction process because it affects the texture of honey as well as its stability and shelf life[21]. All of the tested honey samples were acidic in nature, with pH values that varied between 3.30 to 4.13 (Table 1). These values were similar to those previously reported for other honey samples from India, Brazil, Spain and Turkey, which were reported to have pH between 3.49 and 4.70 [22, 23]. A highly acidic honey sample indicates the possible fermentation of sugars into organic acids. None of the investigated samples exceeded the allowed limit (3.7-4.5), which may be considered as an index of freshness of all honey samples.The moisture content in the investigated honey samples was between 14.56% to 17.23%. Which are within the limit (≤20%) recommended by the international quality regulations [1, 24] (Table 1). Water content is very important for the shelf life of honey during storage [25] and can lead to undesirable honey fermentation due to osmotolerant yeasts, which form ethyl alcohol and carbon dioxide [26]. EC is one of the most important factors for determining the physical characteristics of honey [27]. It is also an important physicochemical measurement for the authentication of unifloral honeys [28]. The EC values of samples were within the allowed parameters (lower than 800 µS/cm) (Table 1). The values of EC change when the amount of plant pollen decreases. According to [29], the nectars from some plants are "stronger" (gives more mineral and energy) than others, and even low contamination of honey with "stronger" nectar can modify its sensory and physicochemical properties. TDS is a measure of the combined content of all inorganic and organic substances in honey, results demonstrate that there is a good correlation between EC and TDS.Hydroxymethylfurfural (HMF) is an essential parameter used to indicate honey quality. HMF formation results from the acid-catalyzed dehydration of hexose sugars. In fresh honey it is present in trace amounts and its concentration increase with storage and the prolonged heating of the honey. With the exception of a sample (PFf) that contained 1.75 mg/kg of HMF, the HMF concentrations of the remaining honey samples MF, PF and Pro were similar, ranging from 19.96 to 27.87 mg/kg respectively (Table 1). Notably, all HMF concentrations were within the recommended range set by the Codex Alimentarius [30] at 80 mg/kg.

Colour intensity is related to the presence of pigments, such as carotenoids and flavonoids, which are known to have antioxidant properties [31].The colour intensity of increase with storage period. PF, PFf and Processed samples showed higher values of optical density at 450 nm. Sample MF had the lowest value of 106.6mAU which was in the range of fresh honey. Colour intensity values of the investigated samples ranged from 106.60-1592.00 mAU (Table 1). The Reducing Sugars content of the honeys analyzed ranged from 62.2 -70.24% and Sucrose is 1.76 -2.58% (Table 2). None of the samples exceeded the highest limit set for total sugar content by the European community directive [1].Minerals as inorganic elements function as co-factors in enzyme catalyzed reactions, regulation of acid-base balance, nerve conduction, muscle irritability and structural elements of the body. Minerals are typically resistant to heat, and therefore, processing does not cause a negative impact on the mineral content of honey. Although these quantities are typically quite low, these elements are still important for human function and are not found in most other sweeteners (Table 3).Metals are notable for their tendency to accumulate in select tissues of the human body and their overall potential to be toxic at high levels of exposure. This exposure can occur through a variety of routes and one of them is ingestion involuntarily through the food. In this purpose MF, PF, PFf and Pro honey samples were investigated (Table 3). Phenolic acids and polyphenols are plant derived secondary metabolites. Dark coloured honeys are reported to contain more phenolic acid derivatives but less flavonoids than light coloured ones [32]. The antioxidant activity of natural honeys depends largely on their chemical composition, such as phenolics, flavonoids, enzymes, organic acids, amino acids, Maillard reaction products, ascorbic acid, carotenoids, as well as their origins [33, 34]. Thus, phenolics or polyphenols are one of the most important classes of compounds found in honey. The total concentration of phenols in honey is highly dependent on its plant source. The concentration of polyphenols determined was 890.5-2119.28 mg/kg. While PFf sample shows high polyphenol content (Table 4).

Flavonoids are the largest group of polyphenolic compounds found in higher plants and synthesized from the shikimic acid and malonic acid pathways [35]. Flavonoids possess free radical scavenging activities which prevent oxidative cell damage, have anti-inflammatory, anticancer activities as well as protection against the different levels of carcinogenesis. The PFf sample contained the highest amount (975.50 mg/kg) of flavonoids and total flavonoids range obtained was 111.83-975.5 mg/kg (Table 4). Flavonols are phytochemical compounds found in high concentrations in a variety of plant-based foods and beverages. Based on their structure (3-hydroxyflavone backbone), flavonols are classified as flavonoids that include the following compounds: quercetin, kaempferol, and myricetin. The results of the flavonols contents were 61.3-588.3 mg/kg, of which Processed contains lowest value of 61.3 and PFf contains the highest of 588.3 mg/kg (Table 4). The free radical scavenging activities of honey samples were measured using the DPPH assay. The reduction of DPPH radicals can be observed by the decrease in absorbance at 517 nm. Different honey samples reduced DPPH radicals significantly. The values of percent decolorization of DPPH radicals are shown in the Fig 1. Among the four honey samples PFf show higher inhibition while MF shows lower. Reducing power assay is a novel method that is used in the assay of the antioxidant activities of various medicinal plants and it employs the reduction of $Fe3^+$ to $Fe2^+$. This is because antioxidants are strong reducing agents. The reducing power of all the samples as shown in Figure 2. The reducing power of all the PFf shows highest activity compared to other samples.

Conclusion

In conclusion, the result of this study indicated that honey samples derived form four different region of India, were mostly at good quality. This is the first study to attempted to investigate the physicochemical and antioxidant properties of different regions of Indian honeys more elaborately. All the samples analyzed, contained significant quantities of minerals, indicating their nutritive potentials. This study showed that Indian honey samples have high antioxidant potential, as indicated by their high phenolic, flavonoid and flavonols contents. The samples had strong reducing power and inhibitory actions on DPPH radical, indicating their antioxidant properties. Finally, the study highlights the effect of regional flora on the composition and antioxidant potentials of honey.

Acknowledgement

I am grateful to Deepa Vishwanathan, Proprietor of The Pristine Laboratories, Bangalore Certified AGMARK laboratory and Approved by Government of India for providing the opportunity for carryout this research project.

References

1. Council Directive of the European Union. Council directive 2001/110/EC of 20 December 2001 relating to honey. *Off. J. Eur. Communities*, 47–52, (2002).
2. Anupama, D; Bhat, K K; Sapna, V. K. Sensory and physicochemical properties of commercial

samples of honey, Food Research International, Vol. 36 (2): 183–191, (2003).

3. Abell D.C., Friebe H., Schweger C., Kwok A.S.K., Sporns P. Comparison of processed unifloral clover and canola honey. *Apidologie*, *27*, 451-480, (1996).
4. Vinson, J A; Dabbagh, Y A; Serry, M M; Jang, J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. Journal of Agricultural and Food Chemistry 43 (11): 2800–2802, (1995).
5. cao, G; Sofic, E; Prior, R L. Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. Free Radical Biol. Med 22 (5): 749–760, (1997).
6. Lusby PE, Coombes AL.Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res 36: 464-467, (2005).
7. Gheldof, N.; Engeseth, N.J. Antioxidant capacity of honeys from various floral sourcesbased 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, (2002).
8. Mundo MA, Padilla-Zakour OI, Worobo RW. Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. Int J Food Microbiol; 97: 1-8, (2004).
9. Al, M.L.; Daniel, D.; Moise, A.; Bobis, O.; Laslo, L.; Bogdanov, S. Physico-chemical and bioactive properties of different floral origin honeys from Romania. *Food Chem, 112*, 863–867, (2009).
10. Molan, P.C., Betts, J. Using honey dressings: The Practical Considerations. *Nurs Time*., 96(49): 36-37, (2000).
11. Chirife, J., Herszage, L., Joseph, A., Okoh, E.S. In Vitro Study of Bacterial Growth Inhibition in Concentrated Sugar Solutions: Microbiological Basis for the use of Sugar in Treating Infected Wounds. *Antimicrob Agents Chemother*., 23: 766 -773, (1983).
12. Tonks, A., Cooper, R.A., Price, A.J., Molan, P.C., Jones, K.P. Stimulation of tnfalpha Release in Monocytes by Honey. *Cytokines*., 14: 240-242, (2001).
13. Adeleye I.A., Opiah L. Antimicrobial activity of extracts of local cough mixtures on upper respiratory tract bacterial pathogens. *West Indian Medical Journal*, *52* (3), 188-190, (2003).
14. Indian Standard. Extracted honey – specification (second revision). IS 4941:1994.
15. White, J.W. Instrumental color classification of honey: Collaborative study. *J. AOAC, 67*, 1129-1131, (1984).
16. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-ciocalteu reagent. *Methods Enzymol.*, *299*, 152–178, (1999).
17. Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem. 64*, 555–559, (1999).
18. Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of *Phyllanthus species* from India . *Lebens-Wiss Technol*. 40: 344-352, (2007).
19. Pulido R, Bravo L, Saura CF. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem*., (48): 396-3402, (2000).
20. Gyamfi MA, Yonamine M, Aniya Y. Free radical scavenging action of medicinal herbs from Ghana Thonningia sanguinea on experimentally induced liver injuries. *Gen Pharmacol* 32: 661- 667, (1999).
21. Terrab, A.; Díez, M.J.; Heredia, F.J. Characterisation of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chem. 79*, 373–379, (2002).
22. Azeredo, L.C.; Azeredo, M.A.A.; Souza, S.R.; Dutra, V.M.L. Protein contents and physicochemical properties in honey samples of *Apis mellifera* of different floral origins. *Food Chem. 80*, 249–254, (2003).
23. Saxena, S.; Gautam, S.; Sharma, A. Physical, biochemical and antioxidant properties of some Indian honeys. *Food Chem.*, *118*, 391–397, (2010).
24. Codex Alimentarius Commission. Revise codex standard for honey. *Codex Standards* 12–1982, Rev.1 (1987), Rev.2 (**2001**)1, COMESA/FDHS 002:2004.
25. Terrab, A.; Gonzále, M.; González, A. Characterisation of Moroccan unifloral honeys using multivariate analysis. *Eur. Food Res. Technol. 218*, 88–95, (2003).

26. Bogdanov S., Martin P., Lüllmann C. Harmonisedmethods of the European Honey Commission, Apidologie, extra issue, 1–59, (1997).
27. Serrano, S.; Villarejo, M.; Espejo, R.; Jodral, M. Chemical and physical parameters of Andalusian honey: Classification of citrus and eucalyptus honeys by discriminant analysis. *Food Chem.*, *87*, 619–625, (2004).
28. Mateo, R.; Bosch-Reig, F. Classification of spanish unifloral honeys by discriminantanalysis of electrical conductivity, color, water content, sugars, and pH. *J. Agric. Food Chem.*, *46*, 393–400, (1998).
29. Persano, O.L.; Piazza, M.G.; Sabatini, A.G.; Accorti, M. Characterization of unifloral honeys. *Apidologie*, *26*, 453–465, (1995).
30. Codex Alimentarius, Alinorm 01/25. Draft Revised Standard for Honey at Step 8 of the Codex Procedure; EU Directive /1/110/2001 of 02/12/2001 (L 10/47), (2000).
31. Frankel, S.; Robinson, G.E.; Berenbaum, M.R. Antioxidant capacity and correlation characteristics of 14 unifloral honeys. *J. Apicult. Res.*, *37*, 27–31, (1998).
32. Amiot M.J., Aubert S., Gonnet M., Tacchini M. Phenolic composition of honeys: preliminary study on identification and group quantification, Apidologie 20, 115–125, (1989).
33. Meda, A.; Lamien, C.E.; Romito, M.; Millogo, J.; Nacoulma, O.G. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activiity. *Food Chem. 91*, 571–577, (2005).
34. Socha, R.; Juszczak, L.; Pietrzyk, S.; Fortuna, T. Antioxidant activity and phenolic composition of herb honeys. *Food Chem.*, *113*, 568–574, (2009).
35. Rupasinghe, H.P.V. The role of polyphenols in quality, post harvest handling and processing of fruits. Ed: Paliyath, G, Lurrie, S, Murr, D. Handa A. Post Harvest Biology and Technology of Fruits, Vegetables and Flowers. Wiley-Blackwell Publishers, 260-281, (2008).

Table 1: Physical parameters (pH, moisture content, EC, TDS, color characteristics and HMF contents) of Indian honey

Sample	pH	Moisture content (%)	Electrical Conductivity (EC) µS/cm	Total dissolved solids (TDS) mg/l	HMF Content (mg/kg)	ABS450 (mAU; 50 w/v)
MF	3.81 ±0.00 a	16.47 ±0.00 a	152.33 ± 0.93 a	101.00 ±0.44 a	19.96 ±0.00 a	106.60 ±0.20 a
PF	3.80 ± 0.00 b	15.69 ± 0.00 b	210.66 ± 0.51 b	141.00 ±0.44 b	25.64 ± 0.01 b	504.10 ± 0.01 b
PFf	4.13 ± 0.02 a	17.23 ± 0.01 a	371.66 ± 0.68 a	241.33 ±0.68 a	1.75 ± 0.00 a	1592.00 ± 2.93 a
Pro	3.30 ± 0.04 a	14.56 ± 0.00 a	220.66 ± 0.51 a	151.33 ±0.68 a	27.87 ± 0.01 a	714.06 ± 0.01 a
Mean	3.75 ± 0.01	15.97 ± 0.01	238.38 ± 0.65	158.66 ± 0.56	18.73 ± 0.01	729.19 ± 0.78

Means are compared by using One way ANOVA-*Post Hoc* Multiple Comparisons. In each column, values with different letters (superscripts) indicate significant differences ($p < 0.05$).

Table 2: Reducing and non-reducing sugar content of Indian honey

Sample	Total Reducing sugar content mean ± SD%	Reducing sugar mean ± SD%	Sucrose mean ± SD%
MF	73.08 ±0.07 a	70.24 ± 0.15 a	2.58 ± 0.03 a
PF	64.88 ± 0.14 b	63.05 ± 0.06 b	1.76 ± 0.01 a
PFf	65.03 ± 0.05 a	62.24 ± 0.29 a	2.25 ± 0.09 a
Pro	72.02 ± 0.03 a	68.98 ± 0.24 a	2.50 ± 0.06 a
Mean	68.75 ± 0.07	66.12 ± 0.18	2.27 ± 0.04

Means are compared by using One way ANOVA-*Post Hoc* Multiple Comparisons. In each column, values with different letters (superscripts) indicate significant differences ($p < 0.05$).

Table 3: Minerals and metal content of Indian honey

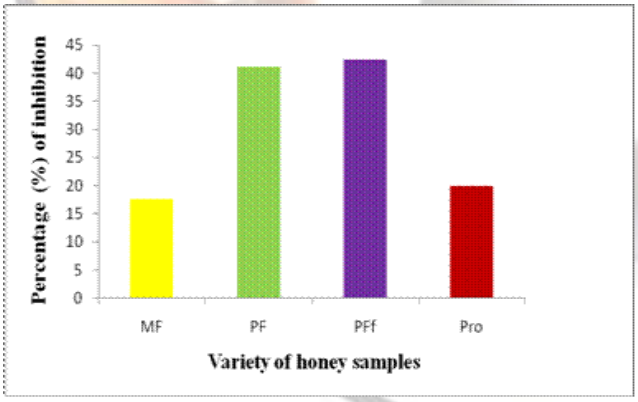
Analytes	Monoflora mg/l	Polyflora mg/l	Polyflora in Forest mg/l	Processed (MF+PF) mg/l
Ca	82.74±0.57	76.42±0.38	300.40±2.92	114.50±0.38
Mg	21.37±0.034	20.22±0.179	92.54±0.83	20.610±0.34
Na	23.42±0.036	73.33±0.34	293.36±2.04	83.33±0.64
K	116.66±0.25	176.66±0.46	1266.66±9.64	83.33±0.64
Fe	4.45±0..001	12.99±0.032	9.9±0.14	13.390±0.049
Zn	2.06±0.008	6.60±0.030	8.10±0.019	2.55±0. 007
Se	< 0.01	< 0.01	< 0.01	< 0.01
Cu	0.34±0.006	0.66±0.003	1.60±0.034	2.20±0.006
Ni	< 0.01	< 0.01	< 0.01	< 0.01
Cr	0.06±0.001	0.09±0.004	0.02±0.001	0.040±0.001
Cd	< 0.003	< 0.003	< 0.003	< 0.003
As	< 0.01	< 0.01	< 0.01	< 0.01
Hg	< 0.001	< 0.001	< 0.001	< 0.001
Pb	0.08±0.004	0.11±0.006	0.1±0.003	0.07±0.004

Means are compared by using automated instrument software Perkin Elmer Vinlab 32ICP US.

Table 4: Polyphenolic composition of 4 honey samples from different locations of India

Sample	Total polyphenols (mg $_{gallicacid}$/kg)	Flavonoids (mg $_{rutin}$/kg)	Flavonols (mg $_{rutin}$/kg)	Flavones (mg $_{rutin}$/kg)
MF	890.50 ± 0.42 d	190.38 ± 0.24 d	111.51 ± 0.25 d	78.40 ± 0.15 d
PF	995.30 ± 0.27 b	224.31 ± 0.30 c	65.50 ± 0.23 c	158.70 ± 0.18 c
PFf	2119.28 ± 0.34 c	975.50 ± 0.24 b	588.30 ± 0.33 b	387.26 ± 0.22 b
Pro	976.76 ± 0.26 a	111.83 ± 0.24 a	61.30 ± 0.32 a	50.70 ± 0.09 a
Mean	1245.46 ± 0.32	375.50 ± 0.25	206.20 ± 0.28	168.76 ± 0.16

Means are compared by using One way ANOVA-*Post Hoc* Multiple Comparisons. In each column, values with different letters (superscripts) indicate significant differences ($p < 0.05$).



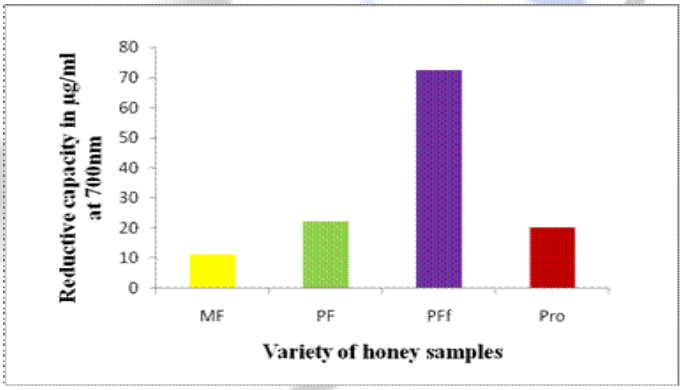


Fig. 1: Percentage of inhibition of DPPH radical scavenging activity of Indian honeys

Fig. 2: Reductive Capacity in µg at 250mg/ml concentrations of Indian honeys