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Physicochemical and antioxidant properties of Indian monofloral honeys

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Abstract

The present study deals with the investigation of erstwhile undocumented six samples of indian honey from six different floral origins - moringa (Moringa oleifera), wild Acacia (Robina pseudoacacia), mustard (Brassica spp.), litchi (Litchi chinensis), jamun (Syzgium cumini), and solai (Plectoranthus Rugosus) for its quantification and comparison of antioxidative capacity by chemometrics. The average total polyphenol and flavonoid content of honeys varied from 10.6 ± 0.68 (moringa) to 35.4 ± 1.8 mg (lychee) gallic acid equivalent and 1.53 ± 0.38 (wild acacia) to 15.67 ± 0.83 (lychee) mg of quercetin per 100 g respectively. Dark brown jamun honey with ABS450 value of 2011.6 mAU exhibited the most potent DPPH (2, 2-diphenyl-1- picrylhydrazyl) radical scavenger (IC50 = 27.48 ± 0.48 mg/mL) and a FRAP (ferric reducing ability of plasma) value of 365 ±2.1 µM Fe (II)..

Key Words: DPPH, FRAP assay, principal component analysis, total phenolic content.

Introduction

Honey is a naturally occurring substance that beekeepers collect has been gathered by bees and that bees gather, alter by mixing with certain ingredients, lay down, dry, store, and let grow in honeycombs. Honey is comprised of a diverse combination of carbs (namely sucrose, fructose, and maltose) accounting for 82.0% of its composition. Additionally, honey contains 0.7 percent of mineral, 0.3 percent of protein, 17.0 percent of water and also contains vitamins, minerals and antioxidants and In addition, there are little amounts of amino acids, vitamins, and several other components present in low concentrations. [1]. Honey that has just been extracted is typically characterized by a thick and sticky consistency with a density larger than that of water, a low surface tension, a hygroscopic nature, a relatively poor heat conductivity, and a variety of colors that are essentially shades of golden amber. Honey is a substance that occurs naturally and is created by honeybees from blossom nectar that is gathered from a range of plants. Honey is widely recognized globally for its exceptional medicinal and health benefits [2]. Depending on its botanical source, honey is widely recognized in several contexts in terms of composition, flavor, and color. Depending on a region's geography and nectar sources, honey's composition varies. The constituent elements of honey exhibit a consistent distribution across various honey samples. However, the chemical and physical characteristics of natural honey are subject to variation based on the botanical origins of the honey [3,4]

Six distinct honey samples collected from several places within the nation. The reason for conducting this research originated from the recognition that honey samples obtained from distinct floral origins and geographical regions might demonstrate differences in their physicochemical properties and antioxidant capacity, with monofloral honey being more prone to such variations. The antioxidant and physiochemical characteristic of honey samples originating from India have not been investigated or documented thus far. Various varieties of honey have been discovered to possess the ability to mitigate chronic or degenerative illnesses associated with oxidative stress, as well as exhibit antioxidant qualities that surpass the potency of vitamins A, C, and E. A variety of methodologies have been employed to assess the efficacy of antioxidants [5]. Nevertheless, several approaches yield inconsistent and unpredictable results [6]. Polyphenols and flavonoids are considered to be a noteworthy group of constituents present in honey that has antioxidant properties. In addition, different honeys have different phenolic profiles and antioxidant potentials due to variables including botanical source, region of origin, time of year, and weather. The antioxidant activity of honey is a crucial characteristic that is greatly influenced by the presence of many compounds such as flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, carotenoids, and chemicals resulting from Maillard reactions [7]. Research on the elemental composition of honey has been conducted for many centuries. However, the investigation of honey phenolics, namely honey flavonoids, which play a role in the medicinal benefits of honey, notably its antioxidant properties, is a more recent area of study. The overall antioxidant properties of honey are determined by the presence of many bioactive elements, including both enzymatic and non-enzymatic components. [8-10]. Since the inception of research on the fundamental composition of honey many centuries ago, there has been a growing fascination in honey phenolics, or more accurately, the compounds present in honey that belong to the phenolic class. In certain instances, the quantity of phenolic compounds may function as a reliable indication of

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the total antioxidant activity. The phenolic components found in honey mostly consist of flavonoid, aglycone, and phenolic acid derivatives, including benzoic and cinnamic acids and their respective esters. The phenolic content of honey varies in accordance with the flora present in the regions visited by bees. Given this information, it has been proposed that phenolic chemicals with a molecular structure of 6 carbon atoms are viable chemical markers o figure out where honey comes from and what plants it comes from. Flavonoids are the primary phenolic compounds found in floral honeys, and the composition of these compounds might vary depending on the specific flower from which the honey is derived [11]. Consequently, unifloral honeys are expected to exhibit unique profiles of flavonoids. The investigation of honey phenolics, namely the quantification of phenolic components, can provide valuable insights about its overall antioxidant activity to a certain degree [12]. Research on the elemental makeup of honey started several centuries ago. Honey is known to contain phenolic compounds. Honeys' antioxidant activity is strongly correlated with their phenolic content, specifically their total phenolic concentration [13,14] In order to enhance our comprehension of honey as a potential reservoir of nutraceuticals and to ascertain their genuineness, it is imperative to conduct a comprehensive analysis of phenolic compounds and other constituents present in honey that may exhibit antioxidant properties. A multitude of research have been undertaken to examine the physicochemical characteristics of various types of honey sourced from different regions around the globe.

Therefore, the purpose of the present study was to investigate the physical, biochemical, and antioxidant properties of monofloral honeys in order to shed light on their therapeutic potential. With a concentration on their polyphenol content, they possess antibacterial, probiotic growth-promoting, and anti-inflammatory properties. In addition to other benefits, this would open up a world of opportunities for using Indian honey for medicinal purposes. This would open up a world of possibilities for using Indian honey for medicinal purposes along with other advantages.

Material and Methods

Chemicals

DPPH (2, 2-diphenyl-1-picrylhydrazyl) and TPTZ (2, 4, 6-tri (2-pyridyl)-s-triazine) were purchased from E. Merck India Pvt. Ltd., Kolkata, India. Folin–Ciocalteu's phenol reagent and quercetin were obtained from Sigma Aldrich Chemical Co., Milwaukee, Wis., U.S.A. All absorbance were recorded with the spectrophotometer (L6100013).All the samples used were of Analytical grade.

Honey samples

A total of 6 mono floral honey samples have been obtained from various locations in India. A few representative samples demonstrated the most common varieties available in India. Samples of honey weighing 250 g that were packaged and sealed in glass bottles and kept at 4°C were bought from a nearby market. There were six distinct categories for the honey sample, namely moringa (Moringa oleifera), wild Acacia (Robina pseudoacacia), mustard (Brassica spp.), litchi (Litchi chinensis), jamun (Syzgium cumini), and solai (Plectoranthus Rugosus) were classified as monofloral honeysAll of the samples were kept at a temperature range of 0 to 4 degrees Celsius and were analyzed within three months. The honey samples were held at ambient temperature overnight prior to being analysed

Serial no.	Sample labelling	Local name	Botanical origin	Geographical origin
1	M1	Moringa Honey	Moringa oleifera	Bihar and Jharkhand
2	A1	Wild acacia Honey	Robina pseudoacacia	Valley of Himalayas
3	L1	Lychee Honey	Nephelium litchi	Bihar
4	J1	Jamun honey	Syzgium cumini	Overall region
5	M2	Mustard Honey	Brassica spp.	Punjab
6	S1	Solai Honey	Plectoranthus Rugosus	Kashmir

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Physical analysis Colour

The study involved doing visual observations to determine the colors of samples. The color of honey is the major attribute used for its categorization. The color of honey has a natural variation including a broad spectrum of tones, spanning from pale yellow to amber, dark amber, and even black. The samples were subjected to a cleaning process and afterwards heated in a water bath at a temperature of 40°C to ensure that all samples were in a liquid state. Following this, the color of each sample was compared to a set of color charts given by Kornerup and Wancher.

pH

The pH of a 10% (w/v) solution of honey, which was made in double distilled water, was determined using a pH meter, where the pH was measured after adding 10 grams of honey to 75 milliliters of CO2 free distilled water in a 250 ml beaker [15].

Specific gravity

The specific gravity of the substance was determined by employing a specific gravity bottle with a volume of 25 ml. Subsequently, the bottle was filled with conductivity water that had undergone triple distillation using alkaline KMn04. The full bottle was then positioned within a water thermostat that had been adjusted to the desired temperature.

Once the bottle reached its equilibrium temperature, which took approximately one hour, it was removed from the thermostat. Subsequently, the exterior surface of the bottle was meticulously cleansed using a towel.

The weight of the bottle and its contents was measured using an electrical balance.

Electrical conductivity (EC)

The electrical conductivity (EC) of a honey solution (on a dry matter basis) was determined at a temperature of 20ºC. The solution was prepared by dissolving 20 grams of honey in 100 milliliters of deionized water, and the EC was measured using a conductivity meter. The measurements were conducted in triplicate and expressed in ms/cm [16].

Total sugar

The quantification of the overall sugar content in the honey samples was conducted using spectrophotometry, specifically employing the anthrone technique. A mixture was prepared by combining 1 mL of a honey solution with a concentration of 20% (w/v) and 4 mL of anthrone reagent, which consisted of 200 mg of anthrone dissolved in 100 mL of ice cold 95% H2SO4. The resulting mixture was then subjected to a boiling water bath for a duration of 8 minutes. Following a process of quick cooling, the measurement of absorbance was conducted at a wavelength of 630nm. The quantification of total sugar content was determined by utilizing the standard curve derived from D-glucose

Total protein

The protein determination in this study was conducted using the Bradford technique, as described by Bradford in 1976 [17]. A 0.1 mL solution of protein extract, specifically derived from a honey sample with a concentration of 50% w/v, was subjected to the addition of 5 mL of Coomassie Brilliant Blue. This Coomassie Brilliant Blue solution was prepared by dissolving 200 mg of Coomassie Brilliant Blue G-250 in 100 mL of 95% ethanol (C2H5OH), followed by the addition of 200 mL of 85% H3PO4.The solution that was obtained was further diluted to achieve a final volume of 2 liters. The Coomassie Brilliant Blue dye undergoes complex formation with proteins. The absorbance at 595 nm was measured after a 120 second incubation period, using a bovine serum albumin standard solution (10-100 µg/0.1 mL) in 0.15 M NaCl as the reference.

Proline content

The determination of proline content was conducted by employing a color comparison method following the application of ninhydrin, utilizing a proline standard. The content was represented as a ratio relative to the mass of honey that was examined. The proline content was assessed utilizing the methodology established by Ough [18]. A solution of honey, with a volume of 0.5 mL and a density of 0.05 g/mL, was combined with 1 mL of formic acid (80%), 1 mL of ninhydrin solution (3% in ethylene glycol monomethyl ether), and subjected to vigorous shaking for a duration of half an hour. The combination was subjected to a boiling water bath for a duration of 10-15 minutes, after which it was then moved to a bath maintained at a temperature of 70 ºC for a period of ten minutes. A 5 mL solution containing 50 percent 2-propanol in water was subsequently introduced, and the resulting combination was allowed to cool. After 40-45 minutes after being removed from the 70 ºC

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water bath, the absorbance was measured at 510 nm using spectrometer. The amino acid proline was employed as a standard in order to generate the calibration curve.

Total lipid content

Using 100 mL of hexane, lipid was extracted from 50 g of honey. The honey's total lipid content was determined gravimetrically [19].

Total free fatty acid content

The determination of proline content in honey samples' total free fatty acid content was conducted using the cupric acetate technique with some modifications, as described by Ghosh et al. (2005) [20]. The lipid component of the honey, as outlined in the procedure, was solubilized in 5 mL of isooctane. Subsequently, a volume of 1 mL of cupric acetate-pyridine reagent, consisting of a 5% cupric acetate solution with a pH of 6 regulated using pyridine, was introduced. The two stages were forcefully combined for a duration of 90 seconds using a vortex mixer. Subsequently, the combination was let to remain undisturbed for approximately 15-20 seconds, during which time the aqueous phase distinctly separated from the isooctane. The measurement of absorbance at a wavelength of 710 nm was conducted for the isooctane phase. The calibration curve was generated using palmitic acid as the standard.

Ash content

The ash content of the honey samples was evaluated by a process including the placement of 5 g of the samples in a crucible. The crucible was then subjected to a temperature of 620 ºC for a duration of 6-8 hours within a muffle furnace. The ash measurements were conducted in triplicate, and the resulting mean value was represented as a percentage, as outlined by the AOAC (1990) [21] .The mineral content of the honey samples was assessed by the process of ashing, followed by the determination of the key mineral components using photometry and absorption spectrophotometry techniques.

Metal Analysis

A solution consisting of 5 millilitres of nitric acid (0.1 M) and 1 millilitre of hydrogen peroxide (30%, v/v) was combined with the ash obtained from a 5 gram honey sample. The resulting mixture was mixed and thereafter subjected to heating on a hotplate until it reached a state of near-complete dryness. A volume of 2 mL of hydrochloric acid (0.1 M) was introduced into the solution, which was then diluted to a final volume of 10 mL using distilled water. The concentration of metal was evaluated in triplicate using inductively coupled plasma optical emission spectrometry. The selection of reference wavelengths for each metal was made in order to prevent any potential interferences with the other elements that were analyzed. The samples used for analysis consisted only of HNO3 and H2O2, and a standard stock solution with a concentration of 50 ppm for each element was used for reference reasons. The results were presented in terms of milligrams of metal per kilogram of honey.

HMF

A honey sample weighing five grams was carefully measured and placed into a beaker with a volume of 50 milliliters. Subsequently, about 25 milliliters of water were added to the beaker and well mixed with the honey sample. The solution was transferred into a volumetric flask with a capacity of 50 ml. Subsequently, 0.5 ml of Carrez solution I was introduced, followed by the addition of 0.5 ml of Carrez solution II.The solution was diluted by adding water to get the desired concentration. The solution was passed through a filter paper, and the initial 10 ml of the resulting filtrate was discarded. A volume of 5 milliliters was extracted from the solution and transferred into separate test tubes. A volume of 5 ml of water was introduced into a test tube and well mixed, therefore creating a sample solution. A reference solution was prepared by adding 5 ml of a 0.2% sodium bisulphite solution to the second test tube and thoroughly mixing it. The absorbance of the sample solution was measured against the reference solution at wavelengths of 284 nm and 336 nm using the Hach Lange DR6000 UV-VIS Spectrophotometer. The results were reported in milligrams per kilogram (International Honey Commission, 2009).

Total free Acid

The determination of free acidity was conducted using potentiometric titration. Prior to conducting the study, the honey samples underwent homogenization in a water bath and subsequent filtration using gauze. Subsequently, a solution of phenolphthalein in alcohol was introduced subsequent to the dissolution of ten grams of honey in 75 mL of distilled water. The solution was subjected to titration using a 0.1 N sodium hydroxide (NaOH) solution. The acidity level was determined by multiplying the volume of NaOH utilized in the titration by a

factor of 10, resulting in the milliequivalent of acid per kilogram of honey. The outcome was quantified in terms of free acidity, measured in milliequivalents of acid per kilogram of honey.

Moisture content

A homogenized sample weighing 5 grams was placed in a flask and afterwards immersed in a water bath maintained at a temperature of 50 degrees Celsius in order to facilitate the dissolution of sugar crystals. The solution that had been made was allowed to cool to the ambient temperature, then it was gently mixed before being carefully positioned on the prism of the Abbe refractometer. The refractive index was measured at a temperature of 20°C for a duration of 2 minutes. Three measurements were conducted for each honey sample, and the average value was calculated.

The moisture content measurements were ascertained by correlating the refractive index of the honey with the respective values provided in a standardized reference table.

Determination of total polyphenol content

The Folin-Ciocalteu test was utilized by Dhar et al. (2011) to quantify the overall polyphenol content of honey samples [22]. A volume of 1 mL of an aqueous solution containing honey (10% w/v in methanol) was combined with 5 mL of Folin–Ciocalteu's phenol reagent (0.2N). After mixing the solution for 10 minutes, 4 mL of a sodium carbonate solution (75g/l) with a concentration of 2% and 60% methanol were added. Subsequently, the reaction mixture was subjected to incubation at ambient temperature for a duration of 60 minutes, followed by spectrophotometric analysis at a wavelength of 740 nm. The calibration curve was constructed by utilizing gallic acid within the range of 0-100 µg/l as the reference. The outcome of the polyphenol content was expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of honey.

Determination of flavonoid content.

The determination of the total flavonoid content in honey samples was conducted using the Dowd method. A 0.1 mg honey sample was dissolved in 5 ml of methanol, and subsequently, 5 ml of a 2% solution of aluminium trichloride (AlCl3) was added [23]. The resulting solution was thoroughly mixed and incubated for a duration of 10 minutes. The absorbance of all samples was measured at a wavelength of 415 nm using a spectrophotometer. A blank sample, consisting of a 5 ml honey solution with 5 ml methanol but without AlCl3, was used as a reference. The total flavonoid content was determined by comparing the absorbance values of the samples with a standard curve prepared using quercetin in the concentration range of 0–100 µg/mL. The results were expressed as milligrams of quercetin per 100 grams of honey (mg QE/100 g), and the average of three replications was reported.

Color intensity (ABS 450)

In order to determine the color intensity, honey samples were dissolved in warm double distilled water at a concentration of 50%. The resulting solution was then filtered through a Whatman no.1 paper. The optical density (OD) measurements of the honey solutions were recorded at wavelengths of 450 nm and 720 nm, respectively. The ABS450 values were quantified by calculating the discrepancy between the two absorbance measurements [24].

FRAP Assay

The FRAP values of honey samples were assessed using the methodology established by Benzie and Strain (1996) [25]. To make the FRAP reagent, a solution of 100 mL of 300 mM acetate buffer at a pH of 3.6 was combined with 10 mL of 40 mM/L TPTZ (2, 4, 6-tripyridyl-s-triazine) dissolved in 40 Mm/L HCl, along with 10 mL of 20 mM ferric chloride. A volume of 200 µL of an aqueous solution containing honey at a concentration of 10% was introduced into 1.5 mL of a freshly made functioning FRAP reagent. The spectrophotometric measurement of absorbance at a wavelength of 593 nm was conducted both immediately and after a 4-minute incubation period at a temperature of 37 ºC. The final absorbance was recorded as the change in absorbance. In order to construct a calibration curve, a standard solution of ferrous sulphate (FeSO4.7H2O) was employed, with concentrations ranging from 100 to 1000 µM/L. The measurement of the ferric reducing capacity of the honey sample was quantified by determining the FRAP value, which represents the concentration of FeII ions (in micromoles) in a 10% solution of honey.

DPPH radical scavenging activity

The evaluation of the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity in honey samples was conducted using the technique developed by meda et al. (2005), with some adjustments made to the procedure [26]. The first step was the preparation of methanolic solutions of honey samples at varying concentrations

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ranging from 25 to 100 mg/mL. A volume of 0.75 mL of the sample solution was introduced into 1.5 mL of DPPH solution that was dissolved in methanol at a concentration of 0.02 mg/mL. The absorbance of the reaction mixture was measured at a wavelength of 517 nm against a blank consisting of methanol after incubating for a duration of 15 minutes at room temperature. The IC50 value for each honey sample was calculated by analyzing the graph depicting the relationship between sample concentration and the percentage of DPPH radical inhibition. The percentage of inhibition DPPH radical was calculated as [(Blank absorbance-sample absorbance)/sample absorbance] x 100.

Hydroxyl radical scavenging
The hydroxyl radical scavenging activity of honey samples was assessed using the methodology outlined by Singh et al. (2002) [27]. Separate screw capped tubes were used to contain aqueous honey solutions with varying amounts of polyphenols (50, 100, and 150 µg GAE). A solution containing 1 mL of Iron EDTA (consisting of 0.1% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA (0.018%), and 1 mL of dimethyl sulphoxide (0.85% in 0.1 M phosphate buffer with pH 7.4) was prepared. The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and maintaining it in a water bath at a temperature range of 80 – 90 ºC for a duration of 15 minutes. A volume of 1 mL of Trichloroacetic acid (TCA) at a low temperature was introduced to halt the ongoing reaction. To prepare the Nash reagent, a mixture was created by combining 75 g of ammonium acetate with 3 mL of glacial acetic acid and 2 mL of acetyl acetone. The resulting solution was then diluted to a final volume of 1 L using distilled water.A volume of 3 milliliters of Nash reagent was subsequently introduced and subjected to incubation for a duration of 15 minutes at ambient temperature, resulting in the formation of a yellow hue. Subsequently, the absorbance of the reaction mixture was measured at a wavelength of 412 nm relative to the reagent blank. The calculation of hydroxyl radical scavenging activity was performed using the following: [1 - (sample absorbance \ blank absorbance)] X100.

Statistical analysis
The experiments were conducted in triplicate, and the outcomes were reported as the mean values together with the standard deviation (SD). The statistical distinctions indicated by letters were derived from a one-way analysis of variance (ANOVA) followed by duncan's test ($p < 0.05$). Pearson's correlation coefficient (r) was used to establish correlations in bivariate linear analyses, with a significance level set at $p < 0.01$. The data analysis was performed using Microsoft Office Excel 2007 and the SPSS version 16.0 software.

Results and discussion
This chapter presents the findings of examinations into the different physical and chemical properties of honey samples gathered from diverse locations in India and its neighboring states. The majority of the different honey sample collections were done throughout the two main honey flow seasons, namely summer and fall. An effort has been made to research how various physico-chemical properties of honeys are impacted by seasonality, temperature, and geographical variation. Additionally, correlations between various traits as well as their connections to various altitudes were looked into. It has also been investigated how temperature and time of day affect honey preservation. These attributes have all been used to analyze the nutritional value of honey. Results associated with the physico-chemical characteristics of the honey samples under investigation are presented in a concise manner through the utilization of tables and graphs.

Physical analysis Colour
The honey samples used in these studies ranged in colour from light white to dark amber, with many shades in between (Table 2). Samples taken in the summer, from May to June, were bright yellow. The samples taken in the fall (September–October) were mostly darker, mostly in amber shades (Table 2). Samples with higher moisture content were lighter while those with lower moisture content were darker in colour.

pH values
Analysed honey samples were discovered to have an acidic nature. According to Table 5, their mean pH values ranged from 3.2 ± 0.3 (mustard) to 4.52 ± 0.2 (jamun). In general, it was discovered that honey has an acidic character, regardless of the diverse botanical and geographic origins of the substance.

Specific gravity
Honey density, measured by specific gravity, differed from water density by roughly 50% and was also influenced by the honey's water content. In comparison to B. Juncea honey (1.35), honeys from monofloral had mean specific gravities ranging from 1.27 ± 0.002 (mustard) to 1.43± 0.001 (wild acacia) (Table 2) (Singh & Bath 1997) [28].

Electrical conductivity

The current investigation likewise showed a linear relationship between ash concentration and electrical conductivity. According to Table 2, the EC values of the honey samples that were analyzed ranged from 0.21 ±0.02 ms/cm (acacia) to 0.95 ± 0.05 ms/cm (jamun).

Total Sugar

The anthrone technique was used to assess the total sugar content of monofloral honey, which ranges from 57.5±0.5 (mustard) to 68.4± 0.9% (acacia).The result were shown to be similar with previous Indian honey reports that varied from 43.3 to 66.7% . The conversion of sugar into inorganic acid may have contributed to the low total sugar level [29].

Total protein

The average protein content of the honey samples that were examined exhibited a range of 870 ± 7 (in mustard) to 1758 ± 6 µg/g (in wild acacia) as determined by the Bradford technique (Table 2). The protein content of honey exhibited a progressive decline in the following sequence: wild acacia > solai > litchi > jamun > moringa > mustard.

Proline content

The presence of proline in honey serves as an indicator of its quality, as well as a potential marker for sugar adulteration. Specifically, if the proline level in honey goes below a threshold value of 183 mg/kg, it suggests the presence of sugar adulteration.. All of the honey samples that were examined showed good proline levels (Table 2), which ranged from 210 mg/kg (in jamun) to 503 mg/kg (in acacia), showing that there had been no adulteration

TABLE 2: Physical parameter of honey sample

Floral origin	**pH**	**Specific gravity**	**Electrical conductivity (ms/cm)**	**Colour**	Total sugar (%)	Total protein (in ug/g)	Proline content (mg/kg)	Total lipid (MG/100g)	Total free fatty Acid (mg/100g)	Ash content (%)	**Moisture (%)**	**Free Acdity(meq/kg)**	**HMF(mg/kg)**
Moringa Honey	**4.25 ± 0.15 e**	**1.41 ± 0.005 d**	**0.65 ± 0.004c**	**Amber**	59.7 ± 0.4c	983 ± 9b	346.8 ± 8d	18.6 ± 0.3d	9.1 ± 0.0b	0.16 ± 0.003b	17.75±0.15b	24.68±0.11c	5.95 ±0.10d
Wild acacia Honey	**3.69 ± 0.05 c**	**1.43 ± 0.001 d**	**0.21 ± 0.05a**	**Light yellow**	68.4 ± 0.9e	1758 ± 6e	503 ± 8f	15.2 ± 0.1b	9.3 ± 0.01 c	0.09 ± 0.001a	**16.59.± 0.3a**	20.82±0.09a	**2.49 ±0.07b**
Lychee Honey	**3.95 ± 0.1d**	**1.30 ± 0.002 b**	**0.81 ± 0.02d**	**Light amber**	57.0 ± 0.6a	1212 ± 9c	309.2 ± 10c	20.5 ± 0.2e	12.5 ±0.03d	0.31 ± 0.002d	**18.30±0.21c**	25.09±0.07d	**4.97 ±0.13c**
Jamun honey	**4.52 ± 0.2f**	**1.35 ± 0.004 c**	**0.95 ± 0.05f**	**Dark brown**	61.5 ± 0.9d	986 ± 7b	210 ± 5a	14.8 ± 0.2a	8.2 ± 0.01 a	0.71 ± 0.002e	19.58±0.12e	**25.23±0.22e**	**6.93 ±0.09e**
Mustard Honey	**3.20 ± 0.3a**	**1.27 ± 0.002 a**	**0.87 ± 0.01e**	**Amber brown**	57.5 ± 0.5a	870 ± 7a	229.5 ± 6b	17.5 ± 0.1c	9.4 ± 0.03 c	0.27 ± 0.001c	18.53±0.23c	24.58±1.95c	**1.23 ±0.17a**
Solai Honey	**3.42 ± 0.21 b**	**1.41 ± 0.000 e**	**0.33 ± 0.02b**	**White to pale**	58.3 ± 0.8b	1680 ± 5d	413 ± 9e	15.6 ± 0.1b	9.5 ± 0.01 c	0.17 ± 0.001b	19.08±0.28d	23.67±2.03b	6.87 ±0.06e

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Values are expressed as mean ± SD.
Values that are accompanied by distinct superscript letters inside each column are significantly different at $p < 0.05$ by Duncan's test
Total lipid content and total free fatty acid content

The mean total lipid content of honey samples was measured gravimetrically and found to be in the range of 20.5 ± 0.2 (litchi) to 14.8 ± 0.2 mg/100g (jamun). The free fatty acid content was highest in litchi (12.5 ± 0.03) and lowest in moringa (9.1 ± 0.00 mg/100g of honey) as well.
Ash content

The ash content % serves as an indicator of the mineral composition and is regarded as a quality criterion that can provide insights into the potential botanical source of honey. The study observed that the average ash level of honey samples exhibited a range of 0.14 ± 0.003 % (in moringa) to 0.72 ± 0.002 % (in jamun) (Table 2). The value under consideration is within a similar range when compared to the reported values from Turkey (0.25%), Spain (0.22%) (Perez et al., 2007), and Romania (0.03-0.40) (Al et al., 2009).

Metal analysis
Honey exhibits a very modest mineral composition, generally ranging from 0.1% to 0.2% in flower honey and 1% or more in honeydew honey. The primary aim of this investigation was to determine the potential influence of plant ecology on the trace metal composition of honeys. This information may then be utilized as bio-markers to authenticate the geographical origin of the honey.

HMF
The assessment of honey freshness and/or overheating commonly relies on the evaluation of two established markers, namely the Hydroxymethylfurfural (HMF) content and diastase activity. In accordance with international regulations, it is mandated that a minimum diastase activity of 8 on the Gothe's scale and a maximum amount of 40 mg/kg of HMF be adhered to. The quantity of hydroxymethylfurfural ranged from 4.97 to 6.93 mg/kg in the honey samples analyzed, as indicated in Table 8. The content of HMF serves as an indicator of the level of freshness in honey. The levels of HMF ranged from 4.97 to 6.93 mg/kg, which is significantly lower than the maximum limit of 40 mg/kg (80 mg/kg for honeys produced in tropical climates) mandated by international honey regulations. The measurement of HMF is a crucial indicator of honey quality, resulting from the dehydration of hexoses, with a special emphasis on fructose, under the influence of acid catalysts.

Free acidity:
The free acidity of honey might potentially be attributed to the equilibrium between organic acids, such as gluconic, pyruvic, malic, and citric acids, and their respective lactones or internal esters, as well as some inorganic ions like phosphate and chloride. The total acidity value with a range spanning from 20 to 25 meq/kg, as presented in Table 2. According to a resolution made by the European Union Council, the upper threshold for free acidity is set at 50.00 milliequivalents per kilogram. Variations in geographical contexts, harvesting procedures, and storage conditions may account for discrepancies between the results of various studies and our own results.

Moisture content
The moisture level of the honey samples ranged from 17.08% to 19.58% (w/w), which is well below the maximum limit of 20% (w/w) set by European legislation and International standards for ensuring honey quality. The moisture level of honey is a crucial factor in assessing its quality, since it directly influences the stability of honey and its capacity to resist spoiling caused by yeast fermentation during storage

Total polyphenol content
The quantification of the overall polyphenol concentration in honey was conducted utilizing gallic acid as the reference standard. The amount of GAE (gallic acid equivalent) per 100 g in moringa honey ranged from 10.6 ± 0.68 mg to 44.9 ± 2.1 mg in lychee honey, as shown in Table 9 and Figure 6. The observed ranking of total phenolic content in the analyzed honeys was as follows: jamun > lychee > solai > acacia > mustard > moringa. In the current investigation, it was determined that the overall polyphenol content was notably greater compared to the levels reported in previous studies conducted by Berreta et al. in 2005 [24] and Bertoncelj et al. in 2007 [30], specifically in acacia and clover honey samples. The authors of the later study revealed similar levels of total phenolic content in lime, chestnut, fir, spruce, buckwheat, honeydew, dandelion, chicory, and sulla honey. However, much higher levels were identified in strawberry and several African honeys.

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Flavonoid content

The mean total flavonoid concentration of monofloral honeys exhibited a range of 1.53 ± 0.38 to 19.3 ± 1.28 mg of quercetin per 100 g honey. The greatest and lowest values were recorded in acacia and jamun honeys, respectively (refer to Table 9 and Figure 7). The order of flavonoid concentration in the investigated honeys, as determined by the $AlCl_3$ technique, was found to be as follows: jamun > lychee > mustard > moringa > solai > acacia. The flavonoid concentration detected in this study exhibited a significant increase compared to multifloral honeys sourced from Burkina Faso (Meda et al., 2005) [23] and Romanian acacia honeys (Al et al., 2009) [31]. Nevertheless, comparable values were noted in the case of sunflower and lime honeys from Romania. A strong positive connection (r = 0.816) was identified between the levels of polyphenols and flavonoids, as seen in Table 3. This finding aligns with the results reported in a prior study conducted by Al et al. in 2009.

Color intensity (ABS450)

The spectrophotometric method was used to determine the color intensity of honey solutions with a concentration of 50%. The net absorbance values ranged from 870.67 ± 132..9 mAU for pale yellow acacia honey to2011.5± 15.7 mAU for dark brown jamun honey. These results are shown in Table 10 and illustrated in Figure 9. The observed trend in the net absorbance values of the analyzed honeys indicates a reduction in the following sequence: jamun > litchi > moringa > mustard > solai > acacia. The study conducted by Berreta et al. (2005) and Bertoncelj et al. (2007) revealed that the ABS450 values of Slovenian and Italian honeys exhibited a range of 70 to 495 mAU and 25 to 3413 mAU, respectively [30]. The potential correlation between color intensity (ABS450) and the presence of pigments such as carotenoids, flavonoids, and Maillard reaction products, which are recognized for their antioxidant properties, might be explored. The results of this study indicate a strong positive correlation between color intensity and the levels of polyphenols (r = 0.971), flavonoids (r = 0.787), and the inverse of the IC50 value of DPPH (r = 0.732). This suggests that darker honeys possess effective radical scavenging properties, which can be attributed to the presence of polyphenols and flavonoids, as shown in Table 3.

FRAP assay

The FRAP assay is a commonly employed and dependable method for quantifying the overall antioxidant capacity of a substance. It operates on the principle that the sample's ability to decrease Fe^{3+} to Fe^{2+} in the presence of TPTZ results in the formation of a blue Ferrous-TPTZ complex, which exhibits maximum absorption at 593 nm. The study determined the mean FRAP values, measured in terms of Fe (II) (µM) in a 10% honey solution. Among the honey samples from india, litchi honey exhibited the greatest mean FRAP value (623.2 ± 3.5). This was followed by solai, jamun, mustard, acacia, moringa honey samples, as shown in Table 3.

DPPH assay

DPPH is a stable nitrogen-centered free radical that is often used to determine antioxidant activity. The DPPH test analyses the materials' capacity to donate hydrogen (or electrons), decolorizing the DPPH radical from purple to yellow and reducing it to its reduced state. The scavenging capacity of the honey samples under examination was shown in Table 3 as an IC50 value, which is the amount of antioxidant required to reduce the starting concentration of DPPH by 50%. This indicates that the lower the sample's IC50 value, the greater its antiradical efficacy. The research found that solai honey was the least active, with an IC50 value of 82.2 1.03 mg/mL, whereas jamun honey was the most effective DPPH scavenger, with an IC50 value of 27.48 0.48 mg/mL. The capacity of DPPH radical scavenging decreased in the following order: moringa > litchi > mustard > acacia > solai > jamun. The high positive connection between 1/IC50 and polyphenol concentration (r = 0.803) seen in Table 4 leads to the conclusion that phenolic chemicals in honey may be responsible for its antiradical efficacy

Table 3: Total polyphenol and flavonoid content of different floral origin of Honey

Floral origin	Total polyphenol content (mg of gallic acid equivalent/100g of honey)	Flavonoid content (mg of quercetin equivalent/100g of honey)	Color intensity	FRAP Values (µM Fe (II) equivalence)	DPPH radical scavenging activity (IC50 in mg/ml)	Percentage of Hydrogen scavenging Activity (150 µg)
Moringa Honey	**10.6 ± 0.68a**	**5.13± 0.23b**	**980±24.4b,c**	**152.8±2.4a,b**	**82.2 ±1.03f**	**56.5±1c**
Wild acacia Honey	**23.01 ± 2.9c**	**1.53± 0.38a**	**870.67± 132.9b**	**341 ± 1.9b,c**	**45.93± 0.75c**	**46.2±0.9a**
Lychee Honey	**35.4 ± 1.8e**	**15.67± 0.83d**	**1792.5± 96.5d**	**623.2 ± 3.5f**	**74.14± 1.28e**	**57 ± 0.8c**
Jamun honey	**44.9 ± 2.1f**	**19.3± 1.28e**	**2011.6± 15.7e**	**365 ±2.1c,d**	**21.78 ±0.48a**	**81.89±1e**
Mustard Honey	**20.2 ± 0.79b**	**5.9± 0.83c**	**1045.2± 24.2c**	**400.1 ± 1.9d**	**60.37± 1.57d**	**50.94± 1.3b**
Solai Honey	**32.45 ± 3.0d**	**1.69± 0.25a**	**449± 44.3a**	**498 ±7.1e**	**32.52± 0.56b**	**70.2±1.2d**

The data are expressed as mean standard deviation. Different letters in each column denote statistically significant differences (p 0.05).

Hydroxyl radical scavenging activity

The hydroxyl radical is classified as an exceptionally reactive oxygen species, and it is recognized for its ability to induce lipid peroxidation and inflict substantial cellular harm by the extraction of hydrogen atoms from unsaturated fatty acids. The hydroxyl radicals produced by the combination of ascorbic acid, iron, and EDTA within an in vitro system exhibited a notable decrease in their levels as a result of the hydroxyl radical scavenging properties exhibited by the honey samples. The formation of hydroxyl radicals as a result of oxidation led to the reaction with DMSO, resulting in the production of formaldehyde. The presence of formaldehyde was confirmed by treatment with Nash reagent. An observed correlation was noted between the rise in polyphenol content and the increase in hydroxyl radical scavenging activity. The hydroxyl radical scavenging activity of jamun and solai honey was observed to be much greater, with values of 81.89 ± 1% and 70.2 ± 1.2% respectively, when tested at a concentration of 150µg honey polyphenol. This information can be seen in Table 12 and Figure 12. This observation aligns with the conclusions drawn by several researchers that have documented a correlation between dosage and efficacy in different food sources, such as sesame coat and pomegranate peel (Chang et al., 2002; Singh et al., 2002) [32]. The hydroxyl radical scavenging activity of honeys from india, when evaluated at a concentration of 150µg honey polyphenol, exhibited the following order: jamun > solai > litchi > mustard > acacia> moringa. Honey possess significant hydroxyl radical scavenging capacity, despite their relatively low levels of polyphenols and flavonoids. This observation holds true even when comparing their scavenging performance at equivalent polyphenol concentrations. This suggests that the honey has the capability to effectively reduce the rate of chain reaction caused by reactive oxygen species during lipid peroxidation.

Correlation between antioxidant properties and biochemical parameters

Table 4 displays noteworthy associations between the biochemical and antioxidant markers. As previously demonstrated (Smith, 2019), a robust and statistically significant positive correlation was seen between the levels of total phenolics and total flavonoids. The correlation coefficient (r = 0.9590) is shown in Table 4. There is a significant positive correlation (r = 0.8250) between phenolics and FRAP, suggesting that phenolics have a role in the antioxidant activity of honey. The observed association, which was shown to be statistically significant, aligns with the earlier research conducted by Saxena et al. [33], Kishore et al. [34], and Khalil et al. [35]. A substantial positive correlation (r = 0.8200) was observed between phenolics and color, as previously reported [30,36]. This finding suggests that color pigments in honey may serve as a reliable indication of its antioxidant effects. The strong positive correlation (r = 0.869) observed between phenols and DPPH activity indicates that phenolic compounds play a significant role in determining the antiradical effectiveness. This finding is consistent with the findings of Beretta et al. [24]. A significant association was identified between flavonoids and FRAP (r = 0.691), which has been previously shown by Alvarez-Suarez [37] and Khalil [35].

There exists a significant connection between color and DPPH (r = 0.9480) as well as color and FRAP (r = 0.9140), suggesting a potential substantial association between color and the antioxidant capacity of honey. This relationship has previously been seen and documented by Saxena et al. [33] and Kishore et al. [34]. The findings of this research reveal that proline had a moderate connection with phenolics (r = 0.5690), flavonoids (r = 0.5060), DPPH (r = 0.6430), and FRAP (r = 0.4730). These results suggest that proline may have a role in the antioxidant capabilities of honey.

Table 4 : Correlation matrix (pearson's correalation coefficient) between various antioxidative parameter of honey sample

	Polyphenol	flavonoid	ABS450	DPPH	FRAP
Flavonoid	**0.810**				
ABS450	**0.971**	**0.787**			
DPPH	**0.803**	**0.671**	**0.732**		
FRAP	**0.462**	**0.428**	**0.558**	**0.027**	
Hydroxyl	**-0.619**	**-0.213**	**-0.551**	**-0.591**	**0.085**

Conclusion

In this study, we examined several physicochemical and bioactive characteristics of monofloral honey samples collected from diverse regions throughout India. The samples exhibited preserved antioxidant capabilities, as shown by their elevated phenolic and flavonoid levels. The HMF concentrations in Indian honey samples were seen to be preserved, perhaps attributed to their acidic properties and low moisture content, which serve as inhibitory factors against HMF synthesis. The color of honey and its proline concentration have been identified as strong indications of its antioxidant activity. Generally, it can be seen that multifloral honeys have greater antioxidant characteristics due to their elevated concentrations of phenolics, flavonoids, AEAC, DPPH, and FRAP values in comparison to monofloral honeys. Additionally, it was shown that the honey samples derived from syzgium cumini and litchi chinesis, which are monofloral sources, had significant levels of antioxidant activity. Additional research is necessary to investigate the antioxidant constituents found in Indian honeys, with particular emphasis on the characterization and quantification of specific flavonoids and phenolic acids.

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