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Determination of Color, Phenolic Acid, Flavonoid and Antioxidant Capacity of Honey Samples from Western Ghats of Maharashtra

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KEYWORDS

Honey samples, Pearson's correlation, Folin-Ciocalteu, DPPH

ABSTRACT:

Objective: The aims of this study were to quantitatively analyze the color intensity, phenolic, flavonoid, and antioxidant properties of honey samples collected from different regions OF Western Ghats of Maharashtra. Also to describe the relationship between these parameters using Pearson's correlation and determined how these parameters can differentiate between various honey types.

Experimental: Honey samples from five distinct locations of Maharashtra's Western Ghats were examined for activity. The Pfund technique was total phenolic and flavonoid content, colour, and antioxidant used to determine colour, the aluminium chloride method for flavonoids, the Folin-Ciocalteu method for phenolic assessment, and the DPPH assay for antioxidants.

Results: The colour findings found varied from 164 to 280mm Pfund, flavonoid 0.0020-0.0215 mg/ml, and phenols 0.07-0.233 mg/ml. Antioxidant activity was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavengers, and the IC50 varied from 16 to 29 g/ml. Statistical research revealed that there were positive associations between colour intensity, flavonoid concentration, phenolic content, and antioxidant activity.

Discussion: The colour findings were 164-280mm Pfund, flavonoids 0.0020-0.0215 mg/ml, phenols 0.07-0.233 mg/ml, and antioxidant activity 16-29 g/ml. Honey's complex makeup, interactions between diverse antioxidant chemicals, and possible synergistic linkages between them can all play a role in overall antioxidant capacity.

Conclusion: This is the first study classifying color, flavonoids, phenolics and antioxidants properties of Western Maharashtra honey. These parameters varied in different types of honey, where some honey sample was the richest in color, flavonoids, phenolics and antioxidants among other samples. A strong correlation was observed between the color, flavonoids, phenolics and antioxidants of honey. We can conclude that Sumer honey was the best among the analyzed samples in terms of high color, phenolics and antioxidants.

1. Introduction

Honey is a natural food product that, in addition to being nutritious, has excellent medicinal capabilities due to the presence of bioactive compounds.The major antioxidants identified in honey, phenolic chemicals, have been extensively studied among indicators. More than 150 phenolic substances, including phenolic acids and flavonoids, have been studied in honey.

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have been extensively studied among indicators. More than 150 phenolic substances, including phenolic acids and flavonoids, have been studied in honey. [3].

Honey contains phytochemicals and other compounds such as organic acids, vitamins, and enzymes that may act as a source of dietary antioxidants. The antioxidant capacity of honey is arguably its most significant attribute, and it is influenced by the presence of flavonoids, ascorbic acid, catalase, peroxidase, phenolic acids, carotenoids, and Maillard reaction products. [4].

Honey has a high amount of antioxidants due to its high quantities of flavonoids, phenolic acids, ascorbic acid, catalase, peroxidase, and carotenoids [5]. Honey's antioxidant effects are obtained from both enzymatic (e.g., catalase, glucose oxidase, and peroxidase) and nonenzymatic (e.g., carotenoids, amino acids, ascorbic acid,-tocopherol, proteins, Maillard reaction products, flavonoids, and phenolic acids) components. The quantity and kind of antioxidants in honey are heavily influenced by the floral source or honey variety, and a link between antioxidant activity and total phenolic content has been established.

In the case of persistent wound infections. Honey is a natural food product that, in addition to its nutritional value, has excellent medicinal benefits due to the presence of bioactive compounds. In general, physiologically active chemicals in honey may be split into two groups: antibacterial and antioxidant, both of which are powerful health promoters.

[1] Even though honey is created from the same floral and geographical origin, the texture, colour, and content of the honey might vary depending on the bee type, soil, and weather circumstances.

[6] Honey's antibacterial initiators are distinctive because of its high sugar content, hydrogen peroxide, low water activity, and the presence of strong acids, flavonoids, and phenolic acids[3].

Polyphenols are a diverse group of chemical substances that include flavonoids (flavanols, flavanones, anthocyanidin, flavonols, flavones, chalcones, and isoflavones) and non-flavonoids (phenolic acids).

[3] Flavonoids are naturally occurring chemical compounds with a low molecular weight that are mostly water soluble. The flavonoids are then categorised according to the degree of oxidation of the C ring: flavonols, flavanones, isoflavones, flavanols, flavones, flavanonols, with flavones, flavanols, and flavonols being the most numerous in honey.

[3] Honey's two main antibacterial components, methylglyoxal and hydrogen peroxide, may eliminate biofilms with or without sugar.[9] Several research have demonstrated that honey has antioxidant, anti-inflammatory, antibacterial, antiviral, antiulcerative, antilipid, and anticancer activities. These effects are mostly related to the phenolic chemicals found in honey, such as flavonoids, which have antioxidant and radical-scavenging characteristics.

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2. Methods

Sample	Region
1	Mahabaleshwar
2	Gaganbavda
3	Radnagriha
4	Kohalpur
5	Amewadi

Table 1 Honey Sample collecton

2.1 Color Determination of honey samples:

The Pfund classifier was used to determine the colour intensity of the honey samples. Colour criteria defined by the United States Department of Agriculture (USDA) were used to measure each sample. Honey samples were combined and centrifuged at 3200 rpm/5 min after being diluted to 50% with distilled water. A spectrophotometer was used to measure the absorbance at 635 nm.

Pfund = -38.70 + 371.39 –Abs[6]

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2.2 Determination of phenolic components:

1ml of honey was combined with 1ml of folin ciocalteu reagent. After 3 minutes, add 1ml (10% sodium carbonate solution) and adjust to 10 ml with purified water. The response was held in the dark for 90 minutes. The absorbance was measured at 725 nm.[7]

2.3 Extraction of phenols:

0.4g honey samples + 10ml solvent (10mM EDTA, 1% L-ascorbic acid, 2M NaOH). By adding 2.8 ml of 7.2 M HCL and vortexed for 5-10 seconds before being extracted twice with 10ml ethyl acetate the reaction was acidified.

The organic layer was mixed and dried until the solvent was completely evaporated. 1.5 ml of 25% methanol was used to dissolve the residue. HPLC was used to filter and analyse the sample.[8]

2.4 Determination of flavonoids content:

Honey solutions with a concentration of 0.2g/ml were produced. A 5% aluminium chloride solution was combined with 2ml of the stock solution. Incubate for 30 minutes. At 437nm, absorbance was measured. [7]

2.5 Extraction of flavonoids-

In 50ml of water, dissolve 10g of honey. Add 0.2M HCL and a 30% NaOH solution. Using ethyl acetate, extract three times.

The organic layer was dried, and the residue was redissolved in 5 mL of methanol. HPLC was used to filter and analyse the sample. [8]

2.6 Radical scavenging activity [DPPH assay] :

In the investigation, the scavenging activity against 1,1-diphenyl-2picrylhydrazil(DPPH) radical was utilised. 0.75 mL of 0.1g/mL honey solution in warm water was combined with 1.5mL of 0.09mg/mL DPPH in methanol.

After 5 minutes incubation at 25 °C in a water bath, absorbance at 517nm was measured against a distilled water with blank sample of honey solution. In addition, the absorbance of a radical blank was measured using 0.75 cc of pure water.

Honey's radical scavenging activity was reported as a percentage inhibition of the DPPH radical and was determined as follows:

RSA(DPPH inhibition, %)=[(AB-AT)/AB] x100

AB= absorbance of radical blank (without honey)

AT= absorbance of test sample (with honey).[10]

3. Results

Sample	Color	Color (mmPfund)	Total Phenolic Content	Total Flavonoid Content	Radical -Scavenging activity (IC_{50})
1	Dark amber	217.18	0.22 ±0.01	0.0158 ±0.007506	21
2	Dark amber	254	**0.1950** ±0.00577	0.0020 ±0.000577	18.5
3	Dark amber	280	**0.233** ±0.032146	0.0215 ±0.001528	29
4	Amber	112	**0.07** ±0.023094	0.012 ±0.005774	16
5	Dark amber	230	**0.18** ±0.055076	0.0075 ±0.003606	16

Table 2 Color intensity, phenolic content (mg/kg), flavonoid content (mg/kg), and IC_{50} values (mg/mL) obtained from the antioxidant activity of honey samples (mean ± SD; n = 3)

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	Colour	Flavonoid	Phenolics	Antioxidant
Colour	1			
Flavonoid	0.42331282	1		
Phenolics	-0.948329348	-0.499586099	1	
Antioxidant	0.317187205	0.901872497	-0.322411321	1

Table 3 Correlation between antioxidant parameters (Pearson correlation coefficients)

The table below shows the proven correlation between colour, flavonoids, phenolics, and antioxidants. Color:flavonoids (0.42331282) and color:antioxidants (0.317187205) had a substantial positive connection, as did flavonoids:antioxidants (0.901872497).Color:phenolics(-0.948329348), flavonoids:phenolics (-0.499586099), and phenolics antioxidants (-0.322411321) had a negative relationship. This inverse relationship between antioxidants and other factors is due to the IC50 calculation, where a lower number suggests more antioxidants. The greatest relationship appears between colour and both flavonoid and antioxidant levels, showing that darker honey contains more flavonoids and phenolics, which boost antioxidant levels. According to the link between flavonoids and antioxidants, flavonoids are the most important contributors to radical scavenging activity in honey samples. A similar degree of correlations has demonstrated by Mohamed Al-Farsi et al. [7].

4. Discussion

Several published research have revealed relationships between honey properties such as colour, antioxidant activity, phenol and flavonoid concentration, and (Mohamed Al-Farsi et al) discovered the same. According to our findings, black honey samples are richer in phenols and flavonoids and have stronger antioxidant activity than honey sample 3[7]. A link was found between flavonoid content, phenolic concentration, and antioxidant activity. The Pfund technique was used to determine colour, the aluminium chloride method for flavonoids, the Folin-ciocalteu method for phenolic assessment, and the DPPH assay for antioxidants. The colour findings were 164-280mm Pfund, flavonoids 0.0020-0.0215 mg/ml, phenols 0.07-0.233 mg/ml, and antioxidant activity 16-29 g/ml. Honey's complex makeup, interactions between diverse antioxidant chemicals, and possible synergistic linkages between them can all play a role in overall antioxidant capacity.

5. Conclusion

The study discovered a link between the colour, flavonoids, phenol concentration, and antioxidant capacity of the honey samples tested. Darker honey samples included more phenolic components, whereas flavonoids boosted antioxidant activity. Based on the HPLC results, phenolic substances and flavonoids were also identified. Honey was classified based on the quantity of antioxidant activity and the colour of honey samples.

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