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Research Article

QUANTIFICATION OF SECONDARY METABOLITES IN *CERANA* HONEY AND ITS POTENTIAL ANTIOXIDANT PROPERTY

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ABSTRACT

The present study was conducted to estimate the secondary metabolites which include, total phenols, flavonoids, carotenoids and ascorbic acid contents in multi-floral *cerana* honey samples collected from three different beekeepers (2 samples from each site) in Kanakapura Taluk of Bengaluru Karnataka, India. The samples were collected between January to March 2017 and they were also subjected to free radical scavenging assay. The results revealed the presence of appreciable amounts of total phenols (118 ± 72 mg GAE/100 g), flavonoids (0.69 ± 0.36 mg QE/100g), carotenoids (0.62 ± 0.29 mg β-carotene/100 g), ascorbic acid (9.52 ± 3.91 mg/100 g) in the tested samples. The correlation between total phenols (r^2 = -0.880) and ascorbic acid (r^2 = -0.290) contents with free radical scavenging activity has clearly indicated the superior quality of honey as a natural antioxidant.

Keywords: Honey, Phenols, Ascorbic acid, Antioxidant property

INTRODUCTION

Honey is a naturally synthesized viscous food by bees extracted from floral nectar. It is considered as a healthy food with high nutritional value and medicinal properties[1-3]. Carbohydrates and water are the major primary constituents along with few minor compounds which include organic acids, minerals, proteins, enzymes, and secondary metabolites[4]. The secondary metabolites such as phenols, flavonoids, ascorbic acids, and carotenoids are reported to be responsible for the major health benefits of honey, though present in small quantity[5]. Thus, the composition of secondary metabolites is an important parameter with respect to their medicinal benefits including antioxidant property. The quality and properties of honey mainly depend on the geographical condition, climatic condition, and availability of the floral resources[6]. The comparative biochemical characterization of different kinds of honey from other regions of the world has been carried out extensively[7-8]. In India, although honey has been valued as traditional medicine, there are no ample investigations regarding the biochemical characteristics of locally available multi-floral *cerana* honey. Thus the aim of the study was to determine the composition of biologically important components of multi floral *cerana* honey samples with respect to their antioxidant properties.

MATERIALS AND METHODS

Honey Samples

Six multi floral *cerana* honey samples, two each from three beekeepers were collected from Kanakpura Taluk of Bengaluru Rural District, Karnataka, India, in between January to March 2017 and were stored in airtight bottles at room temperature till analysis.

Total Phenolic Content

The total phenolic contents were determined by following Folin-Ciocalteu assay with some modifications[9]. 0.1 mL of the diluted honey sample (100 mg/mL) was mixed with 0.5 mL of Folin-Ciocalteu phenol reagent and was incubated for five minutes. 10 ml of 7.5% Sodium carbonate solution was added to the mixture and was incubated for another 90 minutes at 25^{0}C temperature. After incubation, the absorbance of the sample solution was measured at 750 nm. Gallic acid was used as the standard to derive the calibration curve. Total phenolic content was expressed as mg Gallic acid equivalents (GAE).

Total Flavonoid Content

Total flavonoid content was measured by Aluminium chloride colorimetric assay[10]. 0.1 mL of the diluted honey sample (500 mg/mL) was mixed with 4 mL of distilled water. 5% sodium nitrite was added followed by the addition of 0.6 ml10% Aluminium chloride after five minutes. After six minutes 2 ml 1M Sodium hydroxide was added and the volume was made up to 10 ml with distilled water. The absorbance of the solution was measured at 510 nm. Quercetine was used as the standard to derive the calibration curve. The total flavonoid content was expressed as mg quercetin equivalents (QE).

Total Carotenoid Content

500 mg of the honey sample was homogenized with 10 ml of 80% acetone. The homogenized solution was centrifuged at 3000 rpm for 10 minutes. The supernatant was made up to 10 ml using 80% acetone. Absorbance was read at 450 nm[11]. β-carotene was used as the standard to derive the calibration curve. The total carotenoid content was expressed as mg of β-carotene equivalents.

Total Ascorbic Acid

Total ascorbic acid content was measured by the 2, 4-dinitrophenylhydrazine (DNPH) colorimetric assay. 0.1 mL of the diluted honey sample (500 mg/mL) was taken and the volume was made to 2 mL with 4% trichloroacetic acid followed by 2% DNPH and 2 drops of 10% thiourea. After incubating for 3 hours at 37°C, the crystals formed were mixed with 2.5 mL of chilled 85% sulphuric acid. The absorbance was taken at 521 nm. The total ascorbic acid content was expressed as mg/ g of sample.

DPPH Radical Scavenging Activity

Free radical scavenging capacity of honey samples was estimated using the stable DPPH radical. 0.5 mL (10 mg/mL) of the diluted honey sample in different concentrations (100, 200, 300, 400, 500 µg) were taken in the test tubes and the volume in each test tube was made up to 1 mL with methanol. To all the tubes, 3 mL of DPPH solution was added and incubated in dark condition for 15 minutes. After incubation, the absorbance was read at 517 nm[12]. The percentage of radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

(Where, A_o = Absorbance of control, A_1= Absorbance of tested samples)

The concentration of honey required to scavenge 50% of DPPH inhibition (IC_{50} mg/mL) was calculated using the regression equation, comparing inhibitory honey concentration to that of inhibition percentage.

Statistical Analysis

Results are expressed as mean ± standard error and correlation coefficient was calculated using SPSS software.

RESULTS

Total Phenolic Content

Our results indicated that total phenolic content in the honey samples ranged from 53.5 ± 0.03 to 197 ± 2.90 with an average of 118 ± 72 mg GAE/100 g of honey (Table 1).

Total Flavonoid Content

The total flavonoid content of the honey samples ranged from 0.40 ± 0.06 to 0.97 ± 0.23 mg QE/100 g. The average value was 0.69 ± 0.36 mg QE/ 100 g of honey (Table 1).

Total Carotenoid Content

The total carotenoid content of the samples ranged from 0.40 ± 0.14 to 0.79 ± 0.09 mg β-carotene/100 g of honey. The mean carotenoid content in samples was found to be 0.62 ± 0.29 mg β-carotene/100 g (Table 1).

Ascorbic Acid Content

The ascorbic acid content in the samples ranged from 6.02 ± 0.09 to 10.25 ± 0.08 mg/100 g of honey (Figure 1). The mean value of ascorbic acid content was 9.52 ± 3.91 mg /100 mg of honey).

DPPH Radical Scavenging Activity

The lower value of IC_{50} indicates a higher antioxidant property of the sample. The IC_{50} value of samples ranged from 5.05 ± 0.06 to 31.17 ± 0.39 mg/mL of the sample. Mean value of samples was 16.62 ± 9.08 mg/mL of honey.

The samples showed a significant correlation between phenol (r^2 = -0.880), ascorbic acid (r^2 = -0.290), total DPPH scavenging activity (Table 2).

Table 1: **Total phenolic, carotenoid, flavonoid, ascorbic acid contents and IC_{50} values of honey**

Parameters	Mean ± SD	Max	Min
Total phenolic content (mg GAE/100 g)	118.48 ± 72.49	197.90	53.50
Total flavonoid contents (mg QE/100 g)	0.69 ± 0.36	0.97	0.40
Total carotenoids (mg β-carotene/100 g)	0.62 ± 0.29	0.79	0.40
Ascorbic acid (mg/ 100 g)	9.52 ± 3.91	10.25	9.02
$IC_{50}^{\#}$ (mg/mL)	17.31 ± 11.38	31.17	5.05

$^{\#}IC_{50}$ refers to Inhibitory concentration at 50%

Table 2: Correlation coefficient between total phenolic, total flavonoid, total carotenoid, ascorbic acid contents and IC_{50}value

	Phenols	Flavonoids	Carotenoids	Ascorbic acid	IC_{50}
Phenols	1.000	-0.381	-0.333	0.313	-0.880*
Flavonoids		1.000	0.195	0.126	0.577
Carotenoids			1.000	0.291	0.475
Ascorbic acid				1.000	-0.290*
IC_{50}					1.000

*significant

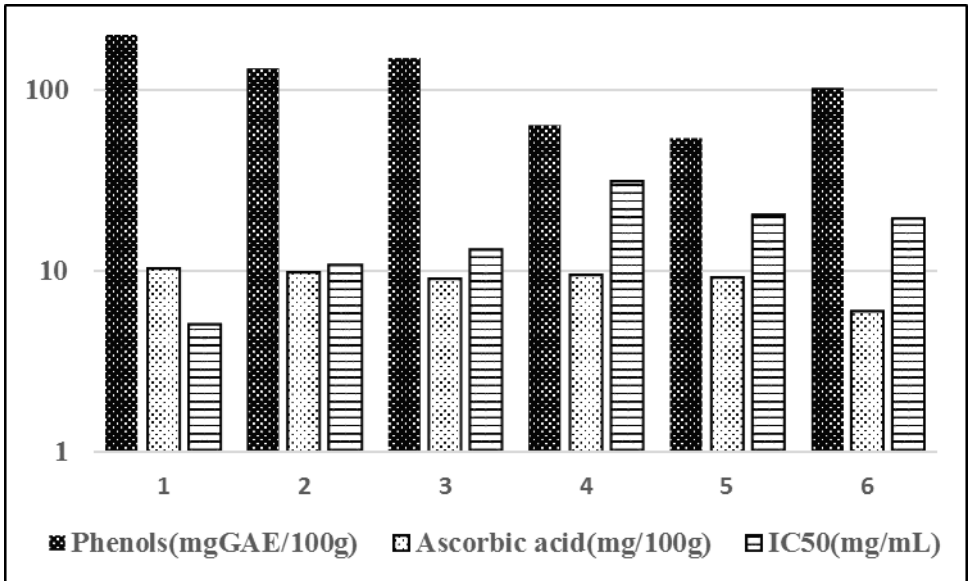

Figure 1: Total phenolic, ascorbic acid contents and IC_{50} value

DISCUSSION

Phenolic, flavonoid, carotenoid contents together with ascorbic acid and enzymes are the most important constituents of honey that are responsible for its antioxidant activity[13-14]. The present study has reported the presence of all the components in the appreciable amounts. The total phenolic content in honey is an important aromatic secondary metabolite derived from plants and contributes to the antioxidant property of honey. Variation in the phenolic, flavonoid and carotenoid contents in the samples can be attributed to the botanical origin of pollen as well as nectar in the honey[15]. Similar results in the total phenolic content ranging from 89.05 to 124.54 mg GAE/100 g, similarly carotenoid content ranging from 0.908 to 6.51 mgQE/100 g has been reported in *cerana* honey samples collected from different locations across India[16-17]. Ascorbic acid is the most important vitamin in honey with strong reducing action mainly derived from the pollen. Similar results ranging from 13.16 to1.63 mg/100 mg was reported from earlier studies[18].

Lower IC_{50} value indicates a higher antioxidant property of the sample. The activity of the free radical (DPPH) varied significantly from 31.17 ± 0.39 to 5.05 ± 0.06 mg/mL of sample (Figure 1). Many studies have indicated a wide variation in the antioxidant activity attributing to floral availability[19].

A high correlation between total phenolic content, ascorbic acid content (Table 2) with IC_{50} value indicates the effective antioxidant ability of honey.

CONCLUSION

The present study has established the presence of biologically important essential phytochemicals in the locally available multi floral *cerana* honey samples from Karnataka. The study unequivocally confirmed the antioxidant property of these honey samples as indicated by the presence of phenolic, flavonoid, carotenoid and ascorbic acid contents. Samples contained high concentrations of phenolic and ascorbic acid contents. The positive correlation between phenolic and ascorbic acid contents with free radical scavenging activity has clearly indicated the superior quality of honey as a natural antioxidant.

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