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Original Research Paper

Biological Science

PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERISTICS OF APIS CERANA HONEY FROM COORG DISTRICT, KARNATAKA, INDIA

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KEYWORDS :

INTRODUCTION

Honey is one of the natural substances produced by honey bees from nectar of different flowers [1]. It is one of the most valued complex food, containing a variety of phytochemicals with health benefits. The chemical constituents of a particular honey depend on plant resources, native bees. climatic condition, soil type, geography and environmental condition of the region[2-3]. However, carbohydrate and water are the main composition of honey apart from proteins, amino acids, minerals, vitamins, enzymes, phenolic acids and organic acids. Physico- chemical analysis of honey is important to the honey industry, as these factors are intimately related to storage quality, granulation, texture, flavor, and the nutritional and medicinal quality of honey[4]. Honey possesses valuable nourishing, healing and prophylactic properties and these properties can be interpreted by its physical and chemical composition. The quality of honey is mainly analyzed by its physical, chemical and biological potentials, mainly moisture, electrical conductivity, ash, sugar, proteins, secondary metabolites, and its antioxidant, antimicrobial, anti –inflammatory, anticancer properties.

Coorg is one of the leading honey producing regions in Karnataka State, where *Apis cerana* is the main species of honey bees generally practiced for honey production commercially[5]. The different agro climatic conditions and diversified floral resources of this district is exceptional forvhoney production. Coorg is a hotspot for agro forestry with a lot of major bee flora, including spices, hence, the area is found to be potential for commercial beekeeping. Several scientists have been evaluating the physicochemical and biological characteristic of honey and its therapeutic properties from different geographical regions across the globe[6-10]. There is not much report on the biological properties of honey from Karnataka. Thus, the aim of this study is to analyze the physicochemical and biological activities of *Apis cerana* honey collected from Coorg district of Karnataka State to provide a scientific evidence of its health benefits in disease management.

MATERIALS AND METHODS

Collection of honey samples

Ten honey samples were collected during 2020 to 2021, directly from the beehive of *Apis cerana* from different places of Coorg district, Karnataka State such as Talakaveri, Baghamandala, Madkeri, Somavarpet, Kutta, Kushalnagara, Sahanivarsanthe, Ponnampet, Virajpet and Nagarahole. Honey samples were stored at room temperature to study its physical, chemical and biological properties[11].

1. Physical analysis

The total moisture was evaluated by refractive index with distilled water as reference[12]. Ash percentage was calculated by burning 5gms of each sample in a muffle furnace at 500°C for 3-4 hours, ash was cooled and weighed[12]. The pH value of honey samples was measured using a digital pH meter with a solution prepared with 10g of honey in 50ml of distilled water[13]. Free acidity was determined by titrimetric method, where ten gm of honey sample was mixed with 50ml of distilled water and filtered[13]. The solution was stirred with magnetic stirrer and titrated by adding 0.1N NaOH (pH 8.5). Acidity (milli equivalent of acid per kg of honey) was determined as 10 times the volume of NaOH used for titration. Total solid was calculated with 10 grams of honey sample dissolved in 20ml of warm distilled water, mix well and centrifuge, the sediment was dried and weighed[13].

2. Chemical analysis

Total Sugars -

Anthrone reagent was prepared in ice cold H_2SO_4 and added to honey solution. The solution was incubated in boiling water for 10mins and absorbance was measured at 630nm. A standard curve was prepared by using glucose[13].

Total proteins -

Honey sample was diluted with distilled water, to which copper reagent was added and incubated for 10mins at room temperature. Later 0.5ml of Folin's reagent was added and incubated again at room temperature for 30mins and absorbance was measured at 660nm. Bovine Serum Albumin was used to prepare the standard curve[14].

Total phenols-

The total phenol concentration in honey samples were determined by using Folin-Ciocalteu method[15]. Honey solution was prepared by dissolving 1 gm of honey with 10ml of distilled water and filtered. 1ml of 0.2N Folin- Ciocalteu reagent and 1.5ml of 0.7M sodium carbonate solution was added and adjusted volume to 15ml with distilled water. After incubation in dark at room temperature for 1 hour, the absorbency of reaction mixture was measured at 725nm. Gallic acid was used as standard to produce the calibration curve.

Total flavonoids -

The total flavonoid content of honey samples was measured using aluminium chloride method[16]. One ml of honey solution was mixed with 0.5ml of 1.2% aluminium chloride and 0.5ml of 120mM potassium acetate. The volume was made to to 10ml with distilled water. The solution was incubated for 30mins at room temperature. Subsequently, the absorbance was measured at 415nm keeping quercetin as standard for calibration curve.

3. Biological analysis

Antioxidant activity by DPPH method -

The free radical scavenging activity in honey was determined by DPPH (2, 2-diphenyl -1- picrylhydrazyl) method. Honey was diluted with distilled water and different concentrations of honey solution were mixed with 2ml of 0.1mM DPPH prepared in methanol. The mixture was incubated in dark at room temperature for 30 minutes and absorbency was calculated at

517 nm using spectrophotometer[17]. The analysis was performed in triplicate and the percentage of free radical scavenging activity of each sample was calculated as: % inhibition = [Abs (blank) – Abs (sample) /Abs (blank)] x 100

Antibacterial activity by well diffusion method -

Antibacterial activity of different concentrations of honey samples was studied against four microorganisms from gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and gram-negative (*Pseudomonas aeruginosa*, and *Eschiershea coli*) bacteria. One ml of diluted bacterial culture was spread on petri plates prepared with Muller Hinton agar media. Approximate 0.6 cm diameter wells of were engraved with sterile cork borer and filled with test samples. The plates were incubated for 24 hours at 37°C, subsequently the inhibition zones were measured[18]. Tetracycline was used as positive control.

Anti-inflammatory activity by inhibition of protein denaturation assay –

Anti-inflammatory activity of honey solution was studied by using inhibition of albumin denaturation technique according to[19]. The reaction mixture consists of 1ml of honey solution and 1% bovine albumin fraction. The mixture was incubated at 37°C for 20 minutes, after cooling the samples the turbidity was measured at 660nm using UV Spectrophotometer. Aspirin was used as a positive control and percentage of protein denaturation was calculated.

RESULT AND DISCUSSION

The result of physical analysis of honey samples collected from different location of Coorg district is summarized in table 1. The moisture percentage of honey sample is a significant factor in assessment of honey quality. It depends on storage condition and the environmental factors of the region where the honey samples collected[20]. In the present study, the moisture content of the sample studied varied from 10.61% in Madkeri sample to 20.25% in Nagarahole sample, which is in the permitted limit of Codex Alimentarius. Honey with less moisture content can be stored for a long time and can prevent microbial growth. The ash was found significantly highest in honey sample collected from Talacauvery (0.80%) and least in Kushalnagara (0.32%) honey. Generally light colored honey gives low content of ash compared to dark colored honey [21].

The pH of honey samples ranged from 3.68 to 4.5 in honey from Virajpet and Talacauvery respectively. Normally, honey pH varies between 3.5 to 5.5, which depends on chemical components. The growth of bacteria can be inhibited in honey with low pH. In this study, free acidity was found to be less in ponnampet (17.10 meq.kg^{-1}) and higher in Kushalnagara (25.64 meq.kg^{-1}) honey sample which are within and the prescribed limits of 40 meq kg as proposed by Codex Alimentarius Commission [22]. The determination of free acidity depends on the organic acids present in honey samples[23]. According to Codex Alimentarius Commission[22], the maximum limit of free acidity was 50 meq.kg^{-1}. The highest total solid was recorded 89.59% in a honey sample from Madkeri.

Table 1: Physical analysis of *Apis cerna* honey of Coorg district

Sl. No.	Place of collection	Color	Moisture (%)	Ash (%)	pH	Acidity Meq.kg-1	Total Solid (%)
1	Talakaveri	Dark brown	15.78	0.80	4.5	22.5	84.20
2	Baghamandala	Dark yellow	15.47	0.68	4.30	19.30	84.50
3	Madkeri	Light amber	10.61	0.46	4.33	18.15	89.59
4	Somavarpet	Brorwn	16.66	0.70	4.26	20.26	83.35
5	Kutta	Dark amber	11.80	0.54	3.98	19.50	81.00
6	Kushalnagara	Dark brown	18.18	0.78	4.33	25.64	85.25
7	Sahanivarsanthe	Light amber	15.00	0.32	4.04	23.10	88.30
8	Ponnampet	Light yellow	20.18	0.58	4.9	17.10	87.52
9	Virajpet	Light amber	18.51	0.62	3.68	23.33	82.49
10	Nagarahole	Dark yellow	20.25	0.42	3.88	20.25	79.32

Chemical analysis

The composition of honey is complex as it contains about 80-85% of sugar, 10-15% protein and many other constituents in small amount[24]. Sugar depends on the sugar concentration of nectar in flowers. In the present study, sugar content was found highest in Nagarahole (91.7%) sample followed by Kutta (91.2%) and Bagamandala (90.4%). Moderate sugar concentration was recorded in the honey sample of Somavarpet (88.1) and Ponnampet (82.0). Comparatively low content of sugar was revealed from Madkeri (79.2%), Virajpet (78.5%), Kushalnagara (78.2%), Shanivarsanthe (75.2 %) and Talacauvery (73.8 %). Likewise, protein content of honey samples studied ranged between 0.93μg/ml to 0.19μg/ml, from honey samples of Talacauvery and Kushalnagara respectively, which mainly depends on the pollen present in honey samples. The phenol content was found to be highest in dark colored honey 0.88 μg/ml from Nagarahole, 0.84 μg/ml from Talacauvery and 0.79 μg/ml from Kushalnagara honey samples compare to 0.25 μg/ml and 0.18 μg/ml reported from Ponnampet and Madkeri honey sample which was found to be light brown in color. Similarly, flavonoids concentration fromTalacauvery and Nagarahole samples were reported to be highest 0.29 μg/ml and 0.24 μg/ml, respectively, compared to light colored honey samples from Ponnnampet (0.16 μg/ml) and Madkeri (0.15 μg/ml). Phenol and flavonoids are an important compound in honey, concern to its color and biological properties[25]. The result obtained from chemical analysis of honey samples collected from Coorg is detailed in table 2.

Table 2: Chemical analysis of *Apis cerna* honey of Coorg district

Sl. No.	Place of collection	Total Sugar(%)	Protein (μg/ml)	Phenols (μg/ml)	Flavonoids (μg/ml)
1	Talakaveri	73.8	0.93	0.84	0.29
2	Baghamandala	90.4	0.45	0.51	0.19
3	Madkeri	79.2	0.60	0.18	0.15
4	Somavarpet	88.1	0.48	0.40	0.20
5	Kutta	91.2	0.21	0.45	0.17
6	Kushalnagara	78.2	0.19	0.79	0.19
7	Sahanivarsanthe	75.2	0.40	0.51	0.18
8	Ponnampet	82.0	0.33	0.25	0.16
9	Virajpet	78.5	0.58	0.55	0.18
10	Nagarahole	91.7	0.53	0.88	0.24

Biological analysis

Free radicals released during metabolism, react with cell components and damage the cell, which leads to various diseases. Antioxidants obtained from the foods can interrupt the damage caused by these radicals. The antioxidant property depends on the color of honey where the darker honey has significant antioxidant potential [26-27]. According to the present research, DPPH react with antioxidant agent present in honey samples, measured in terms of decrease in its absorbance at 517nm. The result signifies that the honey samples collected from Talakaveri, Nagarahole, Kushalnagara and Virajpet with IC_{50} values of 23.58 μg/ml,

32.90 µg/ml, 40.32 µg/ml and 43.42 µg/ml respectively has the highest ability of free radical scavenging potential. Whereas, the samples from Somavarpet, Shanivarasanthe, Kutta, Bagamandala, Madkeri and Ponnampet showed a moderate activity when compared to standard Gallic acid (Figure 1).

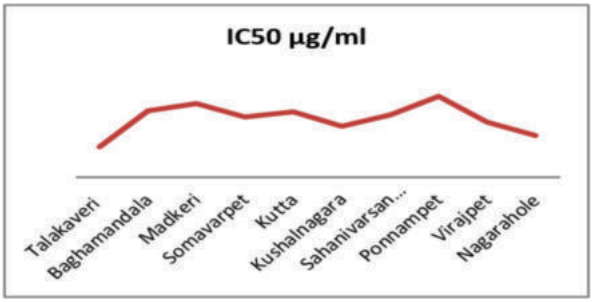

Figure 1: IC50 values of DPPH radical scavenging activity

According to the result obtained from the antibacterial activity of honey samples collected from different places of Coorg district, the highest activity was recorded in gram-negative bacteria such as *Pseudomonas aeruginosa* and *Eschiershea Cali*. The maximum zone of inhibition was observed in samples collected from Kutta, Kushalnagara, Virajpet and Nagarahole, whereas, the honey samples from Talakaveri, Baghamandala and Sahanivarsanthe revealed the moderate zone of inhibition. Ponnampet, Madkeri and Somavarpet honey samples were reported less activity when compared to tetracycline, a positive control. Furthermore, less zone of inhibition was observed in all the honey samples against *Staphylococcus aureus* and *Enterococcus faecalis*, the gram-positive bacteria (Table 3).

Table 3: Zone of inhibition at the concentration of 100µg/ml of honey samples

Sl. No.	Place of collection	Staphylococcus aureus	Enterococcus faecalis	Pseudomonas aeruginosa,	Eschiershea coli	Standard Tetracycline
1	Talakaveri	--	10.0	17.0	18.0	20.0
2	Baghamandala	--	10.5	19.5	18.0	24.5
3	Madkeri	--	--	---	17.5	22.0
4	Somavarpet	--	---	---	17.5	25.0
5	Kutta	8.0	11.5	20.5	19.5	22.5
6	Kushalnagara	--	12.0	21.5	19.0	25.0
7	Sahanivarsanthe	8.5	---	19.0	15.0	23.0
8	Ponnampet	--	—	----	14.5	24.0
9	Virajpet	9.0	11.5	21.0	20.0	20.5
10	Nagarahole	9.0	12.0	20.0	18.0	23.0

Anti-inflammatory activity

Anti- inflammatory activity of honey samples collected from different locations of the Coorg district by inhibition of protein denaturation method reported the significant activity in all the honey samples. Aspirin was used as standard anti-inflammatory compound shows 54.93% of inhibition in protein denaturation at the concentration of 500µg/ml, which was dose dependent inhibition of albumin denaturation [28].

According to the present investigation, it is proved that honey sample collected from Talakaveri, Bagamandala, Nagarahole and Kutta has potentially inhibited the denaturation of albumin with 42.45%, 41.98%, 41.22% and 40.48% respectively. The samples from Virajpet, (38.94%), Somavarpet (36.80), Madkeri (33.08%) and Ponnampet (32.47%) reported the moderate activity, followed by the samples from Shanivarsanthe and Kushalnagara (Figure 2). The variation in physical and chemical properties of honey depending on floral source.

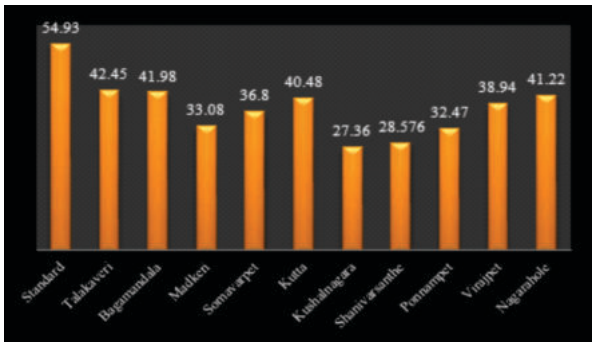

Figure 2: Percentage of protein denaturation inhibition

The present result is in correlation with phenol and flavonoids content, where phenolics play a key role in therapeutic properties of natural products[29]. However, honey is rich in carbohydrates with low pH and high acidity, which decreases the bacterial growth[30]. Honey has been used in many pharmaceuticals as a wound healer, in cough syrup and to cure number of oxidative, inflammatory and infectious diseases. From this study it is evident that honey is a good source of antioxidant, antibacterial and anti-inflammatory compounds. Furthermore, the separation of active compounds responsible for biologically beneficial properties may help may help as remedial to several diseases and safeguard the human health.

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