



ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY OF DIFFERENT HONEY SAMPLES: A COMPARATIVE STUDY

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ABSTRACT

Objectives: The objectives of the present investigation are to evaluate potency of different honey samples for its anti-inflammatory and antioxidant activity. **Methods:** Different types of honey samples namely kashmir honey, morena honey, melghat honey and Vindhyaachal honey were evaluated for variations in activities and quality. Antioxidant activity, ash content, P^H, Moisture content and Anti-inflammatory activity were determined and compared. Anti-inflammatory activity was determined using HRBC membrane stabilization and protein denaturation methods while total Phenolic content and total flavonoid content were used to determine antioxidant potential. **Results and Discussion:** Among the different honey samples evaluated for various activities Kashmir honey showed better results of all evaluations including its anti-inflammatory and antioxidant activity. Kashmir honey showed total phenolic and flavonoid content of 307 mg GAE/100gm and 31 mg CEQ/100gm, respectively indicating its superior antioxidant potential. Ash content, pH and moisture content of Kashmir honey was 0.28 gm/100gm, 4.8 and 13.8 gm/100 gm, respectively. Honey samples of different concentrations (30, 40, 50, 60 mg/ml) were compared for its anti-inflammatory potential. Results reveal that as the concentration of honey was increased there is significant increase in anti-inflammatory activity. **Conclusion:** Due to presence of different phytochemical compounds honey exhibits multiple biological activities. From this study, it can be concluded that antioxidant and anti-inflammatory properties of honey is related to presence of phenolic acids and flavonoids.

KEYWORDS: Honey, anti-inflammatory activity, antioxidant activity.

INTRODUCTION

Since long ago, human beings have been consuming honey and now it is being used in various food products and beverages as an additive. Honey samples have variety of flavors, based on the source of the nectar collected, and accordingly, various types and grades of honey are available. Along with the usage in foods and beverages, honey is also being used to treat various ailments and is being considered as a natural cure for a wide variety of diseases. It successfully heals the wounds.^[1] burns and periodontitis.^[2] Honey possesses variety of pharmacological properties, especially antioxidant, antimicrobial anti-inflammatory activity properties.^[3] Since long back, honey is applied topically to treat infection and for wound healing.^[4,5] Honey contains at least 200 components mostly, carbohydrates and water. It also contains minerals, proteins, free enzymes, free amino acids, vitamins, organic acids, flavonoids, phenolic acids, and other phytochemicals.^[6] Inflammation not only makes the wound uncomfortable and difficult to manage, but also prevents the tissue from repairing the wound through the healing processes.

Histologically, honey reduced number of inflammatory cells present in burned tissue^[4] and in full thickness wounds.^[7] These results indicate that non-sugar components of honey are involved in an anti-inflammatory effect.^[4] The exact mechanism of anti-inflammatory action of honey has remained unknown, but it has been suggested that honey inhibits prostaglandin synthesis, which is often responsible for the heat, itchiness, swelling and pain, commonly observed characteristic associated with inflammation.^[8]

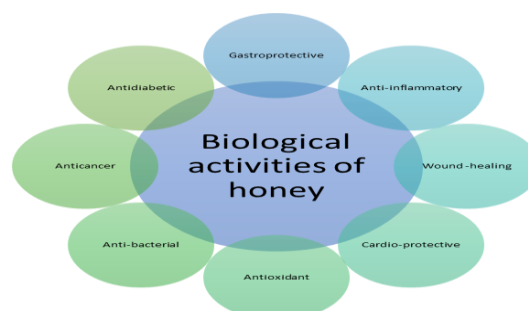


Fig. 1: Use of honey in formulation development.

MATERIALS AND METHODS

Materials: Different Honey samples namely kashmir honey, morena honey, melghat honey and Vindhyachal honey were procured from Central bee research institute, Pune. All other chemicals used were of analytical grade and purchased from oswal scintifics, pune.

Methods: Honey samples were evaluated for variations in activities and quality. Total Phenolic content, total flavonoid content, ash content, P^H , Moisture content and Anti-inflammatory activity were determined.

1. Total phenolic contents

The concentration of phenolic compounds in honey samples was determination using a modified spectrophotometric Folin-Ciocalteu method.^[9] Briefly, 1 mL of honey extract was mixed with 1 mL of Folin Ciocalteu's phenol reagent. After 3 min, 1 mL of 10% Na_2CO_3 solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm using a T-60 UV/VIS spectrophotometer (PG Instruments Ltd, UK). Gallic acid was used to calculate a standard curve (20, 40, 60, 80 and 100 μ g/mL), ($r = 0.9970$). The concentration of phenolic compounds was measured in triplicate. The results were reported as the mean $\pm$ standard deviation and expressed as μ g of gallic acid equivalents (GAE) per g of honey.

2. Total Flavonoids Content

The total flavonoid Content for each honey sample was determined using the colorimetric assay developed by Zhishen JT et al (1999).^[10] Honey extract (1 mL) was mixed with 4 mL of distilled water. At the baseline, 0.3 mL of $NaNO_2$ (5%, w/v) was added. After five min, 0.3 mL of $AlCl_3$ (10% 3 w/v) was added, followed by the addition of 2 mL of NaOH (1 M) 6 min later. The volume was then increased to 10 mL by the addition of 2.4 mL 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 catechin (20, 40, 60, 80 and 100 μ g/mL; $r = 0.9880$).

3. Ash content: Ash content was determined according to the methods of Association of Analytical Communities (AOAC), 1999; 5 g of honey was placed in combustion pots, which required preheating to darkness with a gas flame to prevent honey foaming. Then, the samples were incinerated at high temperature (550 $^{\circ}C$) in a burning muffle for 5 h. After cooling at room temperature, the obtained ash was weighed.^[11]

4. P^H : The pH of the honey samples were measured using a 211 microprocessor pH meter.

5. Moisture content

Moisture content of different honey samples were determined using Karl Fischer titrator (Matic D, Veego).

6. Anti-inflammatory activity

a. HRBC membrane stabilization method

i) Collection of human erythrocyte suspension

The blood sample was collected in heparinized vacutainer from a healthy volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment. The collected blood was mixed with equal volume of 2% dextrose, 0.42% sodium chloride in water, 0.05% citric acid and 0.8% sodium citrate. The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) & isosaline was used to prepare 10% v/v suspension.

ii) Hypotonic solution-induced hemolysis

The anti-inflammatory activity of different types of honey was determined by HRBC membrane stabilization method. The assay mixture contained 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%), 0.5 ml of HRBC suspension and different concentrations of honey samples. Honey was omitted in the blood control, while honey control did not contain the erythrocyte suspension. All the assay mixtures were kept at $37 \pm 2^{\circ}C$ for 30 min and centrifuged. The hemoglobin content was estimated at 560 nm. Following formula was used to calculate percentage of HRBC membrane stabilization

$$\text{Percent protection} = 100 \times \frac{(1 - OD_t)}{OD_c}$$

Where, OD_t = absorbance of test sample, OD_c = absorbance of control.

b. Protein denaturation method

The reaction mixture (5 ml) consisted of 2.8 ml of phosphate buffered saline (PBS, pH 6.4), 0.2 ml of egg albumin (from fresh hen's egg) and 2 ml of honey sample of desired concentration. Double-distilled water in similar volume served as control. BOD incubator (Labline Technologies) was used for incubation of samples at $(37 \pm 2)^{\circ}C$ for 15 min and then heated for 5 min at $70^{\circ}C$. After cooling, absorbance was measured at 660 nm (SHIMADZU, UV 1800). The percentage inhibition of protein denaturation was calculated by using the following formula

$$\% \text{ inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of test sample, V_c = absorbance of control.

RESULTS AND DISCUSSION

Individual honey samples as listed above were evaluated for Moisture content, Total Flavonoid content, Total Phenolic content, Ash content p^H , and Viscosity. Results of these evaluations were depicted in table 1.

1. Total phenolic contents

A colorimetric assay using the *Folin-Ciocalteu* reagent is often referenced in the literature for the determination of phenolic compounds.^[12] The reaction between the *Folin-Ciocalteu* reagent and phenolic compounds results in the formation of a blue color complex that absorbs radiation

and allows quantification. The total phenol content (mg GAE/100gm of honey) of the different honey samples was investigated using a modified *Folin-Ciocalteu* assay. The phenolic concentration of honey in this study ranged from 260 to 307 mg GAE/100gm of honey (Table 1). The honey sample with the highest phenolic content was Kashmir honey with total phenolic content of 307 mg GAE/100gm indicating its superior antioxidant potential. The sample with the lowest content was Melghat honey (260 mg GAE/100gm)

2. Total Flavonoid Content

The total flavonoid Content for each honey sample was determined using the colorimetric assay developed by.^[10] Flavonoids are low molecular weight phenolic compounds. Antioxidant potential of honey also depends on flavonoids content. The total flavonoids content of honey in this study ranged from 21 to 31 mg CEQ/100gm (Table 1). The honey sample with the highest flavonoids Content was Kashmir honey with total

flavonoids content of 31 mg CEQ/100gm and the sample with the lowest content was Melghat honey (21 mg CEQ/100gm).

3. Ash content: Ash content was determined according to the methods of Association of Analytical Communities (AOAC), 1999. Since the beginning of this century measurements of the total ash content of honey have been used as a criterion of the origin of honey and its authenticity. Ash content of honey in this study ranged from 0.24 to 0.28 gm/100gm (Table 1). The honey sample with the highest Ash content value was Kashmir honey with ash content of 0.28 gm/100gm.

4. P^H: Another important parameter during the extraction and storage of honey is the pH value. It influences honey texture, stability and shelf life.^[6] In general, a low pH of honey inhibits the growth and proliferation of microorganisms. Kashmir honey shows the lowest pH value of 4.8 (Table 3.85).

Table 1. Evaluation of different types of honey samples (Mean $\pm$ S.D for n=3).

Honey Samples	Kashmir honey	Morena Honey	Melghat Honey	Vindhyachal Honey
Moisture content (gm/100gm)	13.8 $\pm$ 0.67	14.2 $\pm$ 0.87	14 $\pm$ 0.45	14.2 $\pm$ 0.38
Total Flavonoid content (mg CEQ/100gm)	31 $\pm$ 0.75	23 $\pm$ 0.87	21 $\pm$ 0.56	25 $\pm$ 0.69
Total phenolic content (mg GAE/100gm)	307 $\pm$ 1.32	278 $\pm$ 1.34	260 $\pm$ 1.66	289 $\pm$ 1.72
Ash content (gm/100gm)	0.28 $\pm$ 0.12	0.26 $\pm$ 0.15	0.24 $\pm$ 0.61	0.25 $\pm$ 0.37
pH	4.8 $\pm$ 0.43	5.1 $\pm$ 0.32	5.3 $\pm$ 0.17	5.0 $\pm$ 0.36

5. Moisture content: Quality of honey can be determined by evaluating its moisture content. The moisture content determination is important because it contributes to the honey's ability to resist crystallization and fermentation during storage. Low moisture content in honey suggests a protective effect against microbial attack, especially during long term storage.^[13,14] Kashmir honey showed lowest moisture content of 13.8 gm/100 gm amongst all other honey samples evaluated.

6. Anti-inflammatory activity: Anti-inflammatory activity of any agent can be correlated with its protective effect on heat and hypotonic saline-induced erythrocyte lysis. As human RBC membrane is analogous to the lysosome membrane, the detail idea about inflammatory process can be obtained by studying the inhibition of RBC hemolysis.^[15] Result of anti-inflammatory activity showed that Kashmir honey possess greater activity with both the methods used (Fig. 2-3). It showed 95% of inhibition using HRBC membrane stabilization at 50 mg/ml concentration of honey.

Well-documented cause of inflammatory and arthritic disease was denaturation of tissue proteins. In certain arthritic diseases production of autoantigens may be due to denaturation of proteins *in vivo*.^[16] Kashmir honey showed 93% of inhibition by protein denaturation method.

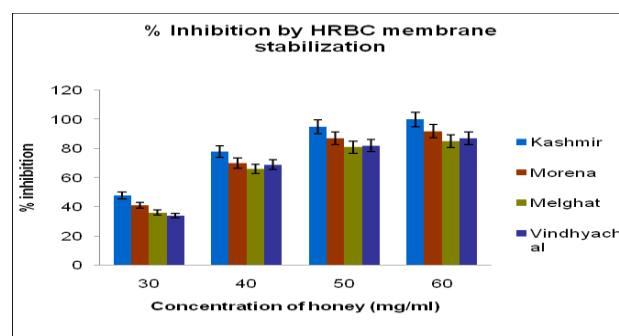


Fig. 2: Anti-inflammatory activity of different honey samples using HRBC membrane stabilization method.

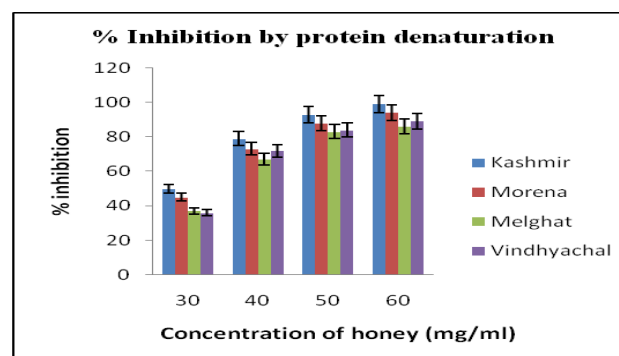


Fig. 3 Anti-inflammatory activity of different honey samples using protein denaturation method.

CONCLUSION

Honey possesses variety of pharmacological properties especially antioxidant, antimicrobial and anti-inflammatory activity properties. Due to presence of different phytochemical compounds honey exhibits multiple biological activities. From this study, it can be concluded that antioxidant and anti-inflammatory properties of honey is related to presence of phenolic acids and flavonoids. Anti-inflammatory potential of honey was clearly seen by the results of HRBC membrane stabilization and protein denaturation inhibition.

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CONFLICT OF INTEREST

Authors explicitly declare that there is no conflict of interest.

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