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Research Article

Phytochemical Composition and Metabolic Health Benefits of Central Western Ghats Honey

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ABSTRACT

Background: Ayurvedic scriptures extensively chronicle the use of honey by individuals with metabolic dysfunctions, including *Madhumeha* (Diabetes mellitus) and *Ati-Sthulata* (Obesity). Oral administration of honey for weight loss, with hypoglycemic and hypolipidemic qualities which aid in lowering *Pitta, Kapha* and *Medas* is good in managing metabolic health and promoting longevity. **Aim/Objectives:** The present study is a small attempt to understand the biomedical properties of honey obtained from an indigenous bee, *Apis cerana indica*. **Materials and Methods:** The bio-activity of honey is evaluated using standard procedure through *in vitro* enzyme inhibition assays of carbohydrate degrading enzyme spectrophotometrically. **Results and Discussion:** The potency of honey to suppress the enzymes that breaks down carbohydrates such as α-amylase and α-glucosidase, for regulating postprandial glucose spikes. Additionally, honey's metabolic effect manages *Ati-Sthulata*, which is commonly associated with *Madhumeha*. This research finding supports the use of honey in moderation as a natural remedy for holistic diabetes care. **Conclusion:** Biomedical potential of honey depends on the bioavailability of the bioactive compounds and on their methods of absorption and metabolization.

Keywords: *Madhumeha*, *Ati-Sthulata*, *Apis cerana indica*, α-amylase, α-glucosidase.

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INTRODUCTION

In classical Vedic texts, honey is referred to as '*Madhu*', which means something sweet-bitter. It is venerable nutraceutical syrup that comes from a variety of flowers with different flavours, potencies, hues, etc., Honey is nature's miraculous golden liquid popularly known as cure-all, was an integral part of the therapeutic regimens of pre-ancient Egyptians, Assyrians, Persians, Greeks, Romans, traditional Chinese and Indian Ayurveda. It also received the religious endorsement by the sacred books of Hindus, Christians, Islamic communities and Buddhist monks. The history of honey as medicine is parallels that of men.[1,2] Honey packed with various nutrients can be simply taken orally to soothe sore throat and cold and also be applied externally as tropical ointment to promoting faster healing by rendering sterility, through promotion of repair and regeneration of affected, and providing instant relief from wounds, burns, ulcers and other skin infections.[3] Many Ayurvedic scholars have documented various health benefits of honey such as immune-modulator (LehanKarma and Susruta, 2010a; Vagbhata, 2007, 2012), *Yogavahi* i.e. carrier substance which enhances the properties and action of the substances with which it combines (Caraka, 2001a; Susruta, 2010c), drink taken with or after medicine (*Anupana*).[4] The health effect of honey is due to presence of various active ingredients. These active ingredients found in honey are secondary metabolites produced by plants and held in nectar, which are in various forms include flavonoids, phenolic acids, organic acids, terpenes, tannins, alkaloids, glycosides and other volatile organic compounds. This phytochemical composition of honey contributes to its special qualities like antimicrobial, antioxidant properties, anti-inflammatory effects, hepatoprotective, digestive health and cardiovascular health.[5]

Ayurvedic scriptures extensively chronicle the use of honey by individuals with metabolic dysfunctions, including diabetes mellitus (*Prameha*) and obesity (*Ati-Sthulata*). Oral administration of honey for weight loss, with hypoglycemic and hypolipidemic qualities which aid in lowering *Pitta, Kapha* and *Medas* is good in managing metabolic health and promoting longevity.[6] Ayurvedic writings provide a thorough explanation of the varieties, characteristics, and medicinal benefits of honey; nevertheless, this has not yet been investigated from a scientific standpoint, owing to its dense carbohydrate contents, primarily made up of sugars which include 40% levulose, 30% dextrose and less than 5% sucrose.[7] In recent decades many researchers now suggest that use of honey as a natural alternative in the management of diabetes related complications. The beneficial effects could be attributed to the bioavailability of the active

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phytochemicals and its rich nutritional composition including minerals, vitamins, proteins in the form of free amino acids and enzymes.[8]

Acharya Charaka in his book Caraka Samhita divided Madhu into four categories based on the bees that originated from, namely: (a) *Maksika* (from *Maksika*) has gingerly oil-like color, (b) *Bhramara* (from *Bhramara*) is white, (c) *Kshaudra* (from *Ksaudra*) is brownish, and (d) *Pauttika* (from *Puttika*) has a color similar to ghee.[9,10] *Maksika* is honey produced by the Indian bee, *Apis cerana indica;* it is considered supreme, and has qualities such as *Laghu* (lighter than Kshaudra) and *Ruksha* (dry), which are useful for illness such as asthma.[11,12] The use of honey for various medical conditions has not yet been validated. As a result, the current study is a small attempt to understand the effect of honey's phytochemical makeup on metabolic dysfunctions such as diabetes and obesity.

MATERIALS AND METHODS

Sample Collection

For the present study, three different honey varieties produced by the local bee *Apis cerana* were purchased from beekeepers in the Central Western Ghats region of Karnataka. The honey samples thus collected were filtered through cheesecloth to remove any unwanted dirt and each honey sample was coded viz., HS1, HS2, and HS3 then stored in hygiene conditions at 4°C until analysis.

Phytochemical Screening

The preliminary qualitative phytochemical screening of honey samples for the detection of presence or absence of phytochemicals like alkaloids, amino acids, carbohydrates, phenols, flavonoids, tannins, saponins, triterpenoids, Phlobatannins and cardio glycosides by following the official methods recommended by the AOAC.[13,14]

Bioactivity of Honey

α- amylase inhibitory activity

The inhibitory activity of α-amylase was evaluated based on the standard protocol with some minor adjustments.[15] After dissolving a 100 µL solution of procaine pancreatic α- amylase (1 U/mL) in 0.1M phosphate-buffered saline (pH 6.9), the test samples (0-100 µg/mL) were pre-incubated at 37°C for 10 min then100 µL of 1% soluble starch (made in 0.1 M phosphate buffer, pH 6.9) was added to start the reaction. 250 µL of dinitrosalicylic acid reagent (containing 1% 3, 5-dinitrosalicylic acid, 0.2% phenol, 005% Na_2SO_3, and 1% NaOH) was added to terminate the reaction. After 10 min of boiling and cooling to room temperature, 250 µL of a 40% potassium sodium tartrate solution was added to the reaction mixture. A microplate reader was used to measure the absorbance at 540 nm. The percentage inhibition of was determined using the equation given below.

$$\%\ of\ inhibition = \frac{Mean\ OD\ of\ blank - Mean\ OD\ of\ test\ samples}{Mean\ OD\ of\ blank} \times 100$$

α- glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the honey samples was assessed using a method adapted from Poovitha *et al.*, (2016).[15] After preparing 50 µL of α-glucosidase enzyme (1 U/mL) in 50 mM phosphate buffer (pH 6.9), the test samples at different concentrations (0-50 µg/mL) were pre-incubated for 10 min at 37°C 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside substrate was added to phosphate buffer to start the reaction, and the reaction mixture was then incubated at 37°C for an half an hour. A microplate reader was used to measure absorbance at 405 nm after 100 µL of 1 M sodium carbonate was added to terminate the reaction. The percentage inhibition was determined using the equation given below.

$$\%\ of\ inhibition = \frac{Mean\ OD\ of\ blank - Mean\ OD\ of\ test\ samples}{Mean\ OD\ of\ blank} \times 100$$

Pancreatic lipase inhibitory assay

The lipase inhibitory assay was carried as per the Zheng *et al.*, modified protocol.[16] Freshly prepared lipase enzyme stock solution (1 mg/mL) and honey samples of different concentrations ranging 0-100 µg/mL were combined in Tri-HCl buffer (pH 7.4) to make up the final volume of 1 mL. For 15 min, the reaction mixture was incubated at 25°C. The incubation was then continued after adding 100 µL of PNPB to each tube. The reaction mixture was incubated at 25°C for 15 min. Subsequently, 100 µL of PNPB (p-nitrophenyl butyrate solution) was added to each tube, and the incubation was continued at 37°C for 30 min. Lipase activity was determined by measuring the hydrolysis of p-nitrophenyl to p-nitrophenol at 405 nm using a spectrophotometer.

$$\%\ of\ inhibition = \frac{Mean\ OD\ of\ blank - Mean\ OD\ of\ test\ samples}{Mean\ OD\ of\ blank} \times 100$$

Statistical analysis

Each test was carried out in triplicate and the findings were shown as mean % inhibition ± standard deviation calculated in Microsoft Excel spreadsheet version 2010.

RESULTS

Phytochemical Screening: The presence (+ve) and absence (-ve) of various plant derived compounds in honey samples were recorded in the Table1.

Bioactivity of honey: The bioactivity of honey is measured through inhibition of key metabolic enzymes including, α-amylase, α-glucosidase and pancreatic lipase. The results exhibited concentration-dependent increase in inhibitory activity, with HS3 has the highest bioactivity followed by HS1 & HS2.

α-amylase inhibition assay: The α-amylase inhibition data was recorded in the Table 2 revealed that HS3 is the most

effective inhibitor, with 40.44±2.12% inhibition at the highest concentration (100 µg/mL). HS1 shows moderate activity, while HS2 exhibits the least inhibition (24.77±1.08 at 100 µg/mL). The low variability among replicates, indicated by small standard deviations, underscores the reliability of these results.

α-glucosidase inhibition assay: For α-glucosidase, HS3 demonstrated highest percentage of inhibition (67.44±5.67% at 100 µg/mL) followed by HS1 showed moderate inhibitory potential, and HS2 with the lowest activity (33.06±2.46% at 100 µg/mL) is illustrated in Table 3.

Pancreatic lipase inhibition: The observations of anti-obesity activity of honey measured through inhibition of pancreatic lipase in Table 4 shows a similar trend as of anti-diabetic activity, with HS3 has the highest inhibition (67.62±2.01% at 100 µg/mL), followed by HS1 and HS2. HS2 consistently exhibits the least inhibition across all concentrations, with 39.71±1.65% inhibition at 100 µg/mL.

DISCUSSION

Honey is a complex mixture, primarily made up of carbohydrates and water, with other bioactive ingredients such as phenols, flavonoids, proteins, steroids, tannins, alkaloids, cardio glycosides saponins and amino acids. Phenolic acids are observed to be prominent, followed by flavonoids. Amino acids (roughly 0.5%) are present either in the form of free amino acids such as proline, arginine, glutamic acid, cysteine and aspartic acid or as parts of proteins.[17] In the present work, three *Apis cerana* honey varieties were analysed, which indicates the presence of carbohydrates, flavonoids, tannins, amino acids, phenols, saponins, cardio glycosides and protein. None of the honey samples contain triterpenoids. Although honey samples showed consistence results, alkaloids were detected only in HS2 and phlobatannins only in HS1. These bioactive compounds existence in honey is directly linked with its beneficial nutraceutical properties.

The most frequent phenolic acids present in honey mainly caffeic acid, chlorogenic acid, ellagic acid, gallic acid, *p*-coumaric acid, syringic acid and vanillic acid.[18] These dietary phenolic acids in honey offer a promising role in the management of obesity and its associated risk factors. For example, the gallic acid, has been found to be useful in controlling energy homeostasis,[19] which helps reduce excess body fat and maintain a healthy, optimal body mass index. Similarly, the different flavonoids found in honey include chrysin, galangin, myricetin, kaempferol and quercetin was significant in reductions of excess weight gain and adiposity index.[20] Thus bioactive molecules in honey contribute to improvement of lipid metabolism.

Variations in origin of honey and its composition results in different α-amylase, α-glucosidase and pancreatic lipase inhibitory abilities as there was a positive co-relation between bioactive molecules and inhibitory effects.

Table 1: Phytochemical Screening of Central Western Ghat Honey Samples.

Sl. No.	Phytochemical compounds	HS1	HS2	HS3
1	Carbohydrate	+ve	+ve	+ve
2	Alkaloids	-ve	+ve	–ve
3	Triterpenoids	–ve	–ve	–ve
4	Flavonoids	+ve	+ve	+ve
5	Tannins	+ve	+ve	+ve
6	Amino Acids	+ve	+ve	+ve
7	Phenols	+ve	+ve	+ve
8	Saponins	+ve	+ve	+ve
9	Phlobatannins	+ve	–ve	–ve
10	Cardio glycosides	+ve	+ve	+ve
11	Protein	+ve	+ve	+ve

Table 2: α-amylase inhibition of Central Western Ghat Honey Samples.

Honey Samples	% of inhibition at different concentrations				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
HS1	6.68±0.96	12.88±1.42	20.37±0.27	26.74±0.66	36.61±1.07
HS2	2.79±0.41	8.91±2.12	14.50±1.63	19.02±1.56	24.77±1.08
HS3	5.79±1.11	15.79±1.21	23.69±2.05	31.06±1.01	40.44±2.12
Mean±S.D	5.08±2.03	12.52±3.45	19.52±4.65	25.61±6.09	33.94±8.16

Table 3: α-glucosidase inhibition of Central Western Ghat Honey Samples.

Honey Samples	% of inhibition at different concentrations				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
HS1	5.62±1.72	16.17±5.16	29.23±2.78	40.07±2.38	50.75±2.22
HS2	2.60±0.89	10.67±0.49	18.67±0.49	25.75±0.32	33.06±2.46
HS3	11.71±4.10	29.69±2.46	42.46±2.29	53.94±3.28	67.44±5.67
Mean±S.D	6.65±4.63	18.85±9.78	30.12±11.91	39.92±14.09	50.41±17.19

Table 4: Pancreatic lipase inhibition of Central Western Ghat Honey Samples.

Honey Samples	% of inhibition at different concentrations				
	20 µg/mL	40 µg/mL	60 µg/mL	80 µg/mL	100 µg/mL
HS1	12.18±3.66	21.82±1.52	32.06±1.47	46.14±2.19	56.35±0.97
HS2	13.17±2.75	18.27±2.25	25.45±2.38	32.49±2.44	39.71±1.65
HS3	16.46±3.60	32.45±4.58	41.39±3.06	52.89±3.54	67.62±2.01
Mean±S.D	13.93±2.23	24.18±7.37	32.96±8.01	43.84±10.39	54.56±14.04

CONCLUSION

In conclusion, therapeutic efficiency of honey depends on the bioavailability of the bioactive compounds and on their methods of absorption and metabolization. Phytochemicals in honey derived from its bee pasture possess a positive effect on enzyme inhibition (i.e. α-amylase, α-glucosidase and pancreatic lipase) activities. The findings of the present study underscore that diverse phytochemical composition of honey, which varies across the samples HS1, HS2 and HS3 contributes for betterment of metabolic dysfunctions such as diabetes and obesity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

HS1: Honey sample 1; **HS2:** Honey sample 2; **HS3:** Honey sample 3.

ETHICAL APPROVAL

In this research article there is no involvement of animals and human subjects and hence there is no need of ethics approval.

SUMMARY

The present study the phytochemical composition and metabolic health benefits of honey produced by domesticated *Apis cerana indica* colonies in the Central Western Ghats of Karnataka, known for its rich diversified bee pasturage. The honey samples were screened to determine the presence of various plant derived secondary metabolites. Furthermore, the honeys potential benefits on metabolic health were measure by *in vitro* enzyme inhibitory assays targeting α-amylase, α-glucosidase and pancreatic lipase. The results displayed the rich phytochemical profile contributing to its potent bioactivity. To summarize the honey from Central Western Ghat origin can be used as a natural dietary supplement with enzyme inhibitory activities, suggesting its beneficial role in managing diabetes and obesity. This research also underscores the value of indigenous honey varieties and emphasizes the need for extensive clinical studies to validate its efficacy.

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