

CHARACTERIZATION OF INDIAN HONEY AND ESTIMATION OF ITS ANTI-PROLIFERATIVE ACTIVITY

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ABSTRACT

Objectives: Phytochemical and biochemical characterization along with estimation of anti-proliferation potential (*in vitro*) of four unifloral Indian honeys i.e. Jambhul, Rubber, Litchi and Drumstick honey. **Methods:** Presence of phytochemicals in honey samples were detected by HPTLC. The glucose and fructose concentrations of these four honey samples were quantified by using HPLC. Anti-proliferative potential of honeys were detected by MTT assay. **Results:** All four phytochemicals i.e. Quercetin, Chrysin, Apigenin and Caffeic Acid were detected in honey samples. Highest concentration of glucose was found in Litchi whereas that of fructose was found in Rubber honey. All four honey samples showed anti-proliferative activity on both cervical (HeLa) and breast (MCF-7) cancer cell lines. **Conclusion:** All four Indian honey samples contain potent phytochemical and biochemical constituents i.e. phenols and sugars. Jambhul honey showed the lowest IC₅₀ value amongst all four Indian honey samples.

Keywords: Honey, Phytochemicals, Sugars, MTT assay, Anti-proliferation.

INTRODUCTION

Honey is a natural product that has been widely used for its therapeutic effects. It is also one of the nature's wonders. Honey has been a common sweetener for food and a powerful medicinal tool for centuries.^(1,2) Apitherapy is a new branch of science which has emerged as a therapy which has honey and other bee products to be used as medicine. Depending on its origin, honey can be classified in different categories

among which, monofloral honey seems to be the most promising and interesting as a natural remedy. The composition of honey is rather variable and depends primarily on its floral source; seasonal and environmental factors can also influence its composition and thus its biological effects.⁽³⁾ Honey has been tested and approved scientifically for its functional and biological properties like antioxidant, anti-inflammatory, an-

tibacterial, antiviral and anticancer.^(4, 5) These activities are due to the phytochemical composition and thus varies among honeys, depending on the factors mentioned above. Honey contains more than 181 substances. It is a supersaturated solution of sugars, mainly composed of fructose (38%) and glucose (31%) & also contains minerals, proteins, free amino acids, enzymes and vitamins. A wide range of minor constituents is also present in honey, many of which are known to have antioxidant properties. These include phenolic acids and flavonoids, certain enzymes (glucose oxidase, catalase) and amino acids. Composition of honey depends greatly on its botanical origin, Pollen source, climate, environmental conditions, in fact that has seldom been considered in nutritional and physiological studies.⁽⁶⁾ Many different types of honeys are available, all differing in their colour, flavour, aroma, physical and chemical properties. This difference is primarily due to the flower from which the nectar of honey is obtained.⁽⁷⁾ Honey obtained predominantly from one flower is known as monofloral or unifloral honey. Since the nectar has been specifically collected from the region containing one specific flower, these unifloral honeys are specific representative of the floral origin with respect to their chemical composition and thus medicinal properties too.

Phytochemical is a wide class of nutraceuticals found in plants which are extensively researched by scientists for their health promoting potential. Different medicinal properties of honey are because of phytochemicals present in it. Honey has a wide range of phytochemicals including polyphenols. Polyphenols and phenolics acids found in the honey vary according to the geographical and climatic conditions. Among these phytochemicals, polyphenols available in the honey has been reported to have anti-proliferative effect on various cancer cell lines.⁽⁸⁾ Several research studies have shown that honey exerts anticancer effect through several mechanisms. Investigations have indicated that honey has anticancer property through its interference with multiple cell-signaling pathways, including inducing apoptosis, antimutagenic, antiproliferative and anti-inflammatory pathways.⁽⁹⁾ Honey has been indicated to prevent cell proliferation, induce

apoptosis, modify cell cycle progression and cause mitochondrial membrane depolarization in several types of cancer such as skin cancer cells⁽¹⁰⁾, adenocarcinoma epithelial cells, cervical cancer cells.⁽¹¹⁾

Thus in this study, we have performed the phytochemical and biochemical characterization of the four Indian unifloral honey samples along with the estimation of anti-proliferative activity of these honey samples on cervical (HeLa) and breast (MCF-7) cancer cell lines (*in vitro*).

MATERIALS AND METHODS

1. Collection of honey samples:

Four Different types of unifloral Indian honey i.e. Jambhul, Rubber, Litchi and Drumstick honey were procured from standard apiaries of India. They were stored at room temperature in dry and sterile conditions.

2. Extraction of honey samples⁽¹²⁾

Liquid – liquid extraction of these four honey samples were carried out by using organic solvents of different polarities i.e. Ethyl acetate, Diethyl ether and Chloroform. Yield of all these extracts of each solvents for all four honey samples were recorded.

3. Phytochemical analysis⁽¹³⁾

Phytochemical components of these four honey samples were detected by High Performance Thin-Layer Chromatography (HPTLC). It was performed at Anchrom Test Labs Private Limited, Mumbai, India. Coated uniform silica gel 60 F254 TLC plates of thickness 0.2mm and size 20 x 10cm were used on which honey extracts were loaded along with standards from sigma i.e. Quercetin, Chrysin, Apigenin and Caffeic Acid by Linomat 5 semi-automatic sampler. Solvent systems used for Chrysin, Apigenin and Caffeic Acid was Toluene: Ethyl acetate: Formic acid (6:4: 0.3) and for Quercetin it was Toluene: Methanol: Ethyl acetate: Formic acid (5:1:4:0.3). Visual imprints of the TLC plates were taken using Camag TLC visualizer.

4. Biochemical characterization⁽¹⁴⁾

Sugar composition of all four honey samples were detected by High Performance Liquid Chromatog-

raphy (HPLC). Column used for the detection of sugars was Aminex 87P column (Agilent Technologies). The sugar peaks were detected by refractive index (RI) detector in comparison with the standards i.e. glucose & fructose (sigma). The corresponding peaks were analyzed using Chemstation software provided by Agilent Technologies.

5. Anti-proliferative activity^(5,15,16)

To study *in vitro* anti-proliferative activity of all four honey samples, 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay was carried out using two cell lines – HeLa and MCF-7. MCF-7 cells were seeded in 96 well plate at a concentration of 5000 cells/well and HeLa cells were seeded at a concentration of 10,000 cells/well. Both cell lines were treated with all four honey samples (1-32 %) for 48 Hrs. in triplicates. After 48 Hrs., 10 µl of MTT reagent (5mg/ml) per well was added and incubated for 4 Hrs. at 37°C,

5% CO₂. After incubation, well content was replaced by 100 µl of DMSO. After 30 min of incubation at 37°C, plate was gently shaken and O.D. was taken at 540 nm. Percentage Inhibition was calculated by using following formula:

$$\% \text{ Inhibition} = 100 - \left[\left(\frac{\text{O.D. of test}}{\text{O.D. of control}} \right) \times 100 \right]$$

IC₅₀ i.e. concentration inhibiting 50% cell growth was calculated for all honey samples by using Graph pad software.

RESULTS

1. Extraction of honey samples

Yield of extracts of each solvent for all four honey samples were recorded. Weight of the extract obtained from the liquid - liquid extraction of all four Indian unifloral honey samples is mentioned in Table no. 1.

Table 1: Yield of extracts from Indian honey samples

Sr. No.	Solvent	Jambhul honey (mg)	Rubber honey (mg)	Litchi honey (mg)	Drumstick honey (mg)
1	Ethyl acetate	79.1	24.8	56.9	21.4
2	Diethyl ether	26.9	22.1	4.5	53.5
3	Chloroform	20.8	12.5	11.0	27.8

2. Phytochemical analysis

It was observed from TLC plate prints that out of four phytoconstituents two i.e. Apigenin and Caffeic Acid were detected in all extracts of all four

honey samples. Thin band of Quercetin was seen only in one i.e. chloroform extract of Jambhul honey (Figure 1).

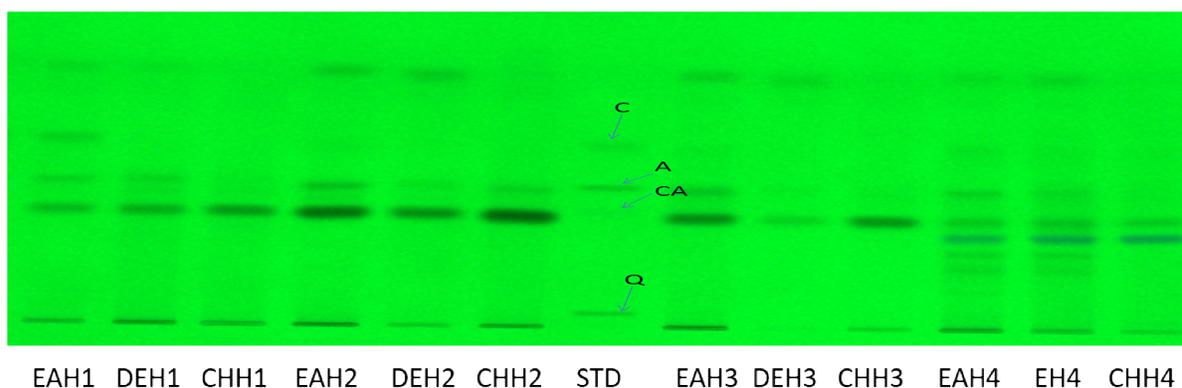


Figure 1: TLC fingerprint of honey samples.

[H1: Litchi, H2: Rubber, H3: Drumstick, H4: Jambhul; EA: Ethyl acetate, DE: Diethyl ether, CH: Chloroform; Q: Quercetin, C: Chrysin, A: Apigenin, CA: Caffeic Acid]

Table 2: Phytochemicals in Indian honey samples.

Honey	Extract	A	Q	CA	C
Litchi	EA	+	-	+	+
	DE	+	-	+	-
	CH	+	-	+	-
Rubber	EA	+	-	+	+
	DE	+	-	+	+
	CH	+	-	+	-
Drumstick	EA	+	-	+	+
	DE	+	-	+	+
	CH	+	-	+	-
Jambhul	EA	+	-	+	+
	DE	+	-	+	+
	CH	+	+	+	+

[EA: Ethyl acetate, DE: Diethyl ether, CH: Chloroform; Q: Quercetin, C: Chrysin, A: Apigenin, CA: Caffeic Acid]

3. Biochemical characterization

In all four honey samples, glucose and fructose were detected by High Performance Liquid Chromatography (HPLC). Concentrations of these sugars are given in Table 3.

Table 3: Glucose and fructose in honey samples

Honey	Glucose (%)	Fructose (%)
Jambhul	32	20
Rubber	27	33
Litchi	38	24
Drumstick	40	29

4. Anti-proliferative activity

All four honey samples inhibited the proliferation of both, HeLa and MCF-7 cancer cell lines. IC₅₀ values of samples are as follows (Table 4).

Table 4: IC₅₀ values of honey samples.

Honey	IC ₅₀ (%)	
	HeLa	MCF 7
Jambhul	2.4	2.0
Rubber	2.9	2.9
Litchi	4.0	5.4
Drumstick	4.2	4.1

DISCUSSION

Traditional Indian medicine as drugs against various human diseases has received considerable attention these days. The continuing search for new anticancer compounds in traditional medicine is realistic and promising strategy. At the same time though it is very true that traditional medicine has been recognized for

treating cancer but their mode of action in killing cancer cells is not well explored. Honey can act as less expensive alternative to expensive therapeutic modalities toward the treatment strategies for cancer either as separate entities or in synergism to slow down the progression of this disease.⁽¹⁷⁾ In order to understand the chemical composition of honey on which it's me-

dicinal properties are based, we explored the phytochemical and biochemical parameters of four different unifloral honey samples. In this study we have also performed the MTT assay on cervical and breast cancer cell lines i.e. HeLa and MCF-7 respectively in order to find out the anti-proliferative effect of these unifloral honey samples and to understand the correlation of this activity with the phytochemical constituents of Indian honey.

It was observed that, among three solvent, ethyl acetate was prominent with respect to extract yield in Jambhul, Litchi and Rubber honey whereas Drumstick honey gave highest yield in diethyl ether. Phytochemical constituents i.e. Caffeic Acid and Apigenin were detected in all extracts of all four honey samples whereas Quercetin was detected only in Jambhul honey. This indicates that the source of the honey i.e. nectar changes the phytoconstituents of honey. These variations have been reported to be related to the factors like flora and fauna, climatic and geographical conditions.^(18, 19, 20)

Honey is a naturally occurring sweetener that contains a mix of both simple and complex sugars.⁽²¹⁾ Surveys of floral honey composition have established that the three major components are fructose, glucose, and water, averaging 38.2, 31.3 and 17.2%, respectively. Glucose and fructose are the only monosaccharides in honey.⁽²²⁾ Though glucose and fructose are the prominent sugars of honey irrespective of their floral origin, the actual concentration of these may vary with respect to nectar. Hence, the concentrations of fructose and glucose have been advanced as useful indicators for the classification of unifloral honeys.⁽²³⁾ Unlike refined sugars, diabetic patients can safely and harmlessly eat this natural and sweetest sugar (fructose)-containing product, natural honey. In addition to that, it gives more energy than artificial sweeteners.⁽²⁴⁾ Among four honey samples, glucose was prominently detected in Drumstick honey whereas fructose was detected prominently in Rubber honey. MTT assay is a gold standard colorimetric assay for assessing the anti-proliferative effect of drugs on cell lines (*in vitro*). HeLa and MCF-7 cells were treated for 48 Hrs. with four honey samples. All four honey samples

showed inhibition of proliferation on both, HeLa and MCF-7 cell line. Various medicinal properties of honey are proposed to be derived from phytochemicals especially polyphenols present in them, which are found to be varying in proportions with respect to various factors specially flora of origin. Most of these polyphenols of honey have been evolved as promising pharmacological agents in treatment of cancer.⁽²⁵⁾ From the phytochemical fingerprints of all four unifloral honey samples, Quercetin was detected only in Jambhul honey which has been reported to be an anti-proliferative agent which seems to be responsible for the lowest IC₅₀ value of Jambhul honey in both HeLa as well as MCF-7 cells among all four honey samples. IC₅₀ value is inversely proportional to the anti-proliferative potential. Hence, from the data obtained we can conclude that among these four unifloral honey samples, Jambhul honey is the more potent with respect to its anti-proliferative activity. From the data obtained in this study, the said correlation between the phytochemical constituent and potent anti-proliferative activity⁽⁸⁾ can be seen predominantly in Jambhul honey due to the presence of all tested phytochemicals and lowest IC₅₀ value amongst all four Indian unifloral honey samples.

CONCLUSION

From the results obtained in this study it can be concluded that all four Indian honey samples contain potent phytochemical and biochemical constituents i.e. phenols and sugars. Phytochemical constituents have been proven to possess strong medicinal properties and hence these honey samples showed anti-proliferative activity on both cervical (HeLa) and breast (MCF-7) cancer cell lines. Further studies to estimate their potential to induce cell cycle arrest and apoptosis of all four honey samples along with its mechanistic aspects are being initiated.

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