

Indian Science Technology and Engineering facilities Map (I-STEM)

Invites application for

1. **Partial financial support for using R&D facilities in other institutions through the I-STEM web portal to Users from Academic Institutions and Start-ups (Format-1)**

Format for requesting support for accessing and using facilities through the I-STEM web portal.

(i) Details of the Personal Information

- a. Name of the Researcher/Faculty Member: Dr. Hemlata K. Bagla
- b. Name of the Institution/~~Start-up~~: Kishinchand Chellaram College
- c. Name of the Department: Chemistry
- d. ~~Degree registered for~~/Product being developed: Honey testing kit
- e. Mob. No. +919821420698
- f. Email ID: hemlata.bagla@kccollege.edu.in

(ii) Project/Research topic Details:

- a. Project Title/Research Title: Comparison of quality parameters of Indian honeys from different botanical origins
- b. Sub area: Bioresources – Agriculture and Food
- c. Area Description: Secondary Agriculture & Food Processing, Medicinal Plants & Phytopharma
- d. Aims & Objective (in Bullets):
 1. To prepare a bench mark for the quality of honey employing quantitative specifications to enhance their commercial values. This work includes an effort to improve Indian honeys as compared to international brands.
 2. Enhancing shelf-life, antioxidant property, and removal/neutralization of pathogen.
 3. To determine the physical and biochemical properties of different honeys and to correlate them with fingerprinting techniques for differentiation.
 4. To identify possible marker compounds essential for tracing the geographical and botanical origin of honey.

e. Duration (MM/YYYY): 06/2022 to 05/2025

f. Project Summary (1000 words):

Origin of the proposal:

Honey composition is not fixed and differs not only in chemical properties, physical properties and taste, but also in different biological activities. The quality of honey is highly dependent on their floral source and geographical origin that influences the physicochemical properties, bioactive compounds patterns, pollen distribution, aroma compounds and special marker compounds. Therefore, these constituents of honey are proposed as the potential markers for authenticating the geographical and botanical origin of honey. Hence, there is a need to develop a uniform protocol for evaluating the quality assurance parameters of honey including expiry date, diminishing nutritional values with time, and adulterations, if any.

Rationale of the study:

In order to reduce the microbial contamination of honey, irradiation is often carried out. This may affect the color, texture and properties like antioxidant activity, phenolic content, etc. Thus, it is important to study the effects of irradiation on the physico-chemical properties of honey. Marketing of pure honey has become increasingly challenging due to widespread adulteration with corn sugar syrups like High Fructose Corn Syrups (HFCS), wheat and maize syrups, etc. in national and international markets. Such adulterated products do not have nutritional values; thus, consumer confidence is lost.

Current status of research and development in the subject (both international and national status)

Profiling of honey has been carried out by several researchers in different geographical locations like Argentina [3-5], Oman [6,7], Spain [8], Saudi Arabia [9], Romania [10], Ethiopia [11], Nigeria [12], South Africa, Zambia and Slovakia [13]. However, there have been limited studies on natural and commercial samples of honey produced in the Indian sub-continent [14-19]. Most of these studies focus on physico-chemical properties and mineral content with very few articles on marker compounds.

Physical properties of honey such as color, pH and ash content were evaluated by various researchers. The differences in color correlate with honey origin and composition. Another important indicator of honey quality is the ash percentage, which also gives the mineral content of honey [10]. Sterilization of honey is carried out to combat microbial growth, owing to the presence of *Saccharomyces* yeast, aerobic *Bacillus*, and anaerobic *Clostridium* spores [20]. Pasteurization is a common technique for sterilization, however, higher temperatures decrease the nutritional value of honey. At room temperature, Co-60 gamma irradiation can be used for sterilization of honey. Literature studies reveal that most researchers required 10 to 25 kGy of radiation dose [21-29] to completely sterilize honey. Effect of radiation dose on physico-chemical properties of honey was analyzed by researchers, however, very little data on irradiation of Indian honey was available [25][30]. Review studies on the chemical analysis of honey indicate that it is composed of ~80% carbohydrates, ~15% water, 0.3% protein, 0.2% ash, and minor quantities of amino-acids and vitamins along with lower concentrations of other components [5]. Analysis of protein content is useful for tracing the floral origin of honeys and to detect adulteration. Amino acid content is also used for characterization and differentiation of honey sources. Proline is the most abundant amino acid in honey and its concentration ratio helps in differentiating between natural and adulterated honey [31] and correlates with the antioxidant activity. Polyphenols are also associated with functional properties of honey, like antioxidant activity and are important indicators of floral origin [32].

Floral origin of honey can be determined using melissopalynology, which is a manual pollen counting and identification technique that is time consuming and requires taxonomical expertise. Recently, researchers have started employing DNA barcoding technique, which is ideal for determination of floral origin of honeys [33-37]. In India, this technique has only been used in the state of Mizoram [38-40]. A recent research article on floral markers and biological activity of Saudi honey detected an alkaloid using GC-MS which can be used as a fingerprint marker for the botanical sources of certain honey samples in Saudi [41]. Another study on chemical and DNA marker analysis was

successfully carried out to authenticate the well-known manuka honey, from New Zealand [42].

The relevance of the proposed study:

Consumers use honey for health and medical benefits, and as a substitute for white sugar. As honey is one of the most adulterated foods in the world, it is crucial to have a large database of unbiased information to increase customer awareness and promote the sale of authentic honey, while benefitting beekeepers and the Indian food industry. Comprehensive research data on the botanical and geographical origin, chemical composition and marker compounds of Indian honey is not available. This research aims to study commercial and organically sourced Indian honey, available in supermarkets for their physico-chemical properties, antioxidant properties, sugar content, phenolic and flavonoid content, effects of sterilization using irradiation, marker compounds and studies on botanical and geographical origin to determine authenticity of honey. This will provide a uniform protocol for evaluating the quality assurance parameters of honey and boost confidence in the Indian food industry.

The Outcome of Proposed Study:

This project will provide better understanding of the nutritional and medicinal values of the honeys of different origins. The present work would help in preparing the specifications for better marketing. Thus, major beneficiaries of this work will be Indian food industries for having built the consumer confidence in their products.

Scope of the Application indicating anticipated product and processes:

This project will lead to the finger printing of different botanical origin honeys, and their nutritious values. This project will also help in developing a test kit and protocols for the evaluation of quality parameters of honey which will be beneficial for both seller and consumers not only nationally but also making the brand value in the international market.

g. Technical Details

- a. TRL: Level 3
- b. Methodology:
 1. Sample Collection: Commercially available honey samples from different geographical and floral origins will be collected. Additionally, samples will be collected from natural sites and apiaries. This will facilitate comparison between raw and processed honey.
 2. Physical parameters: Physical tests such as color intensity, viscosity, refractive index, pH, total ash content, and hygroscopic properties of all samples will be carried out.
 3. Chemical parameters: Chemical composition of honey samples like phenolic content, protein, free amino acids, and flavonoid content will be measured using different methods, for routine analysis and quality control. For total phenolic content, the Folin-Ciocalteu method, used by several researchers [6][35], will be applied.
 4. Antioxidant Activity: Antioxidant Activity of different samples of honey will be studied using Diphenylpicrylhydrazyl (DPPH) radical scavenging method.

5. The fingerprinting of honey will be carried out by measuring carbon isotope ratio ($^{13}\text{C}/^{12}\text{C}$), FTIR, Gas chromatography, High-performance anion exchange chromatography, High-performance liquid chromatography, Nuclear magnetic resonance, and Raman spectroscopy.
- c. Design & development:
1. Analytical kit for honey testing will be developed
 2. Shelf-life and nutritious value of honey will be enhanced
 3. The properties of honey with different botanical origins will be characterized which will enhance their medicinal values.
 4. This project will help in building brand value of honeys in international market with authentication
- d. Facilities and Instrumentation required:
1. Microbial and Fungal Testing
 2. HPAEC/HPLC
 3. Gas Chromatography
 4. NMR
 5. Raman spectroscopy
 6. Densitometer
 7. Viscometer
- e. Consumables (Scientific materials)
1. Honey samples.
 2. Chemicals like DPPH, Folin Ciocalteu reagent, and other chemicals required for analysis of protein content, amino acids, etc.
 3. Sample analysis at institutes/industries.
 4. Analytical training for two persons.
 5. Travel required for sample collection and irradiation of honey.
- h. **Expected output and outcome**
- i. **Industry/Institute Collaboration:** No industry participants at present. Expression of interest will be obtained after initial characterization work. **If, yes**
- (i) Name of the Collaborating Industry/Institute
 - (ii) Collaboration Details
 - (iii) Additional Details if any

j. **Funding required from I-STEM (with justification):**

Details of Requirements & Mode of the Engagement: (Attach the quotation for the consumables)

(i) Short term Engagement:

a. Usage of testing/characterization facilities across the country (Characterization Mode)

i. Testing Charges: At IIT-SAIF (Bombay)

Sr. No.	Technique	Cost per sample (University)
1.	HPLC	1328/-
2.	NMR	944-1298/-
3.	GC-MS	1770/-
4.	Raman	708/-

BTRA (Bombay Textile Research Association):

Sr. No.	Technique	Cost per sample (University)
1.	Microbial and Fungal analysis	2000/-
2.	TGA	2500/-
3.	DSC	2500/-

ii. Consumable charges

Sr. No.	Consumable	Approx. cost per bottle
1.	Honey samples – Natural and Commercial	200-900/-
2.	DPPH reagent	7000/- for 250mg
3.	Folin-Ciocalteu reagent	8000/- for 100mL

iii. Travel Cost (Boarding and Lodging, if any)

b. Usage of fabrication and testing facilities across the country (Fabrication Mode)

- i. Fabrication and testing Charges
- ii. Consumable charges (if any)
- iii. Travel Cost (Boarding and Lodging, if any)

(ii) Medium term Engagement:

- a. Execution of project in any one of the Host Institution (Project Mode)
 - a. Fabrication and or testing Charges
 - b. Consumable charges (if any)
 - c. Travel Cost (Boarding and Lodging, if any)
- b. Execution of project in Multi Host Institutions (Project Mode)
 - a. Fabrication and or testing Charges
 - b. Consumable charges (if any)
 - c. Travel Cost (Boarding and Lodging, if any)

(Round-trip to the Host Institution by 2nd class (Non-AC) railway via the nearest route)

Details of User's Bank Account:

(Please send a copy of the cancelled cheque/copy of the passbook carrying all the information needed)

Beneficiary Name (User's name): Principal Kishinchand Chellaram College DST A/C

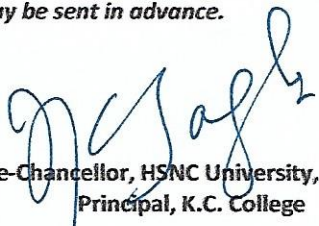
PAN: AAATH0064R

Bank Account No.: 001010110007877

Name of the Branch: Churchgate

IFSC Code: BKID0000010

Note: Proposed proposal must be executed within stipulated duration (max. 6 month) to avoid fund availability issues. Travel form will be provided by the I-STEM office. The users will submit the technical report, original invoice (duly signed by the Head of the Institution) raised by the Host Institution through I-STEM portal only along with a copy of the travel forms and original tickets through post to the Nodal Officer, I-STEM, Indian Institute of Science, Bangalore. A scan copy of all the documents may be sent in advance.


Vice-Chancellor, HSNL University, Mumbai
Principal, K.C. College