Pharmacological and Melissopalynological Study of Honey

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***Abstract*—This study explored the pharmacological and melissopalynological characteristics of honey obtained from different floral and geographical regions across Karnataka and Kerala, India. Five honey samples were analyzed for their physicochemical and biochemical profiles, including pH, moisture, ash, color, electrical conductivity, and the levels of protein, total sugars, reducing sugars, phenolics, and flavonoids. The antioxidant activity was evaluated through the DPPH radical scavenging assay, while antimicrobial efficiency was tested against Escherichia coli, Staphylococcus aureus, and Candida albicans using the agar well diffusion technique. Pollen analysis was performed to determine the floral origin of each sample. The physicochemical parameters varied notably among the samples, with color ranging from extra light amber to dark amber, pH values between 3.5 and 5.0, and moisture content from 10.02% to 18.05%. The Palakkad sample (T5) exhibited the strongest antioxidant capacity (81.57%) and the highest protein concentration (0.8 mg/g), whereas the Yellapura sample (T3) recorded the greatest phenolic content (0.08 mg/g). Antibacterial activity was most prominent in T2 and T3, while T3 also showed considerable antifungal potential. Melissopalynological analysis identified forty-one pollen taxa dominated by the Solanaceae, Brassicaceae, and Asphodelaceae families, indicating a multifloral nature. Overall, the findings emphasize the rich botanical diversity and significant therapeutic potential of regional honeys, supporting their use as safe, natural alternatives to synthetic antimicrobial agents.**

***Index Terms*—Honey, Melissopalynology, Antibacterial activity, Antifungal activity, Antioxidant, Physicochemical analysis, Bioactive compounds.**

I. INTRODUCTION

Honey is a naturally occurring sweet substance that honey bees make from the nectar of flowers, the secretions of living plant parts, or the excretions of plant-sucking insects on plant parts. The honey bees then gather, process, and combine these substances with other substances of their own, store, and leave in the honey comb to mature and ripen. According to reports, honey includes over 25 distinct oligosaccharides in addition to carbs, primarily fructose and glucose. Honey has strong antibacterial qualities that can be used safely and affordably. Honey's antibacterial properties have been documented in a number of research (Gauthami and Bhat, 2023).

Honey is a natural product containing about 600 different constituents. It consists mainly of carbohydrates and water, and traces of other components, such as vitamins, minerals, and aromatic substances. Honey is also rich in enzymatic (e.g., glucose oxidase and catalase) and non-enzymatic antioxidants, such as flavonoids (chrysin, pinocembrin, pinobanksin, quercetin, kaempferol, luteolin, galangin, apigenin, hesperetin and myricetin), phenolic acids (caffeic, coumaric, ferulic, ellagic, and chlorogenic), organic acids, ascorbic acid, amino acids, proteins, Maillard reaction products, α-tocopherol and carotenoids. Darker honeys have higher total polyphenol and flavonoid values and the polyphenol level in honey is directly associated with flower nectar, propolis, and pollen. High correlations between antioxidant activity and total polyphenol and flavonoid contents have been found in several studies. Honey properties and compositions depend, above all, on the chemical content of the nectar of the plant that the honey is derived from, as well as on the geographic area, as soil and weather determine melliferous flora, bee species, and even storage mode (Kivima et al 2021).

Honey (Apis mellifera; Family: Apidae), a golden elixir created by nature's crafty pollinators, has piqued

human curiosity for approximately 5500 years. In addition to its delicious sweetness, honey has long been appreciated for its possible therapeutic benefits. Ancient Egyptians praised honey as a cure-all, and Ayurveda and traditional Chinese medicine both used it as part of their therapeutic regimens. It has recently been discovered that this natural material contains a complex tapestry of pharmacological and therapeutic properties as a result of the fusion of conventional wisdom and contemporary scientific study. (Riaz et al 2023).

II. MATERIALS AND METHODS

Five honey samples were collected from different regions of Karnataka and Palakkad, Kerala. Each sample was labelled and maintained in room temperature.

- Sample (T1): Sringeri, Chikamagalur
- Sample (T2): Sagara, Shimoga
- Sample (T3): Yellapura, Uttara Kannada
- Sample (T4): Karwar, Uttara Kannada
- Sample (T5): Palakkad, Kerala

Methods

1. Color measurement

Colour measurement of honey samples was performed using the honey coloration chart based on the Pfund scale. This method involves visual comparison of the honey sample against a standardized colour chart to determine its colour grade. The honey samples were placed in transparent tubes and visually compared with reference colour grades ranging from water white (0-8 mm) to dark amber (>114 mm). Based on their visual appearance, the samples were classified into respective colour categories.

2. pH

The pH of honey samples was determined by preparing a 10% (w/v) honey solution in distilled water and measuring it using a calibrated digital pH meter, calibrated with standard buffer solutions (pH 4.0 and 7.0) before analysis.

3. Moisture Content

The moisture content of honey was estimated using the hot air oven method. A known weight of honey sample was taken in a pre-weighed dry dish and placed in hot air oven at 105°C for 3 hours. After drying, the dish was cooled in a desiccator and weighed again and calculated using formula: Moisture % = Weight of sample after drying (g) ×100 Original weight of sample (g)

4. Ash content

The ash content in honey was determined by igniting pre-weighed crucible with 5g of honey in muffle furnace. The ash content is calculated using formula, Ash % = Weight of Ash× 100 Original weight of the sample

5. Electrical conductivity

Electrical conductivity was determined by suspending 20% (w/v) of honey samples with distilled water using electrical conductivity meter.

6. Determination of total protein content

Total protein content was estimated by Lowry`s method. BSA was used as standard. In series of test tube with protein standard (0.2, 0.4, 0.6, 0.8, 1ml) and 1ml of test sample (0.5g of honey samples were dissolved in 10ml if distilled water) were added with 1ml of 0.01 N sodium hydroxide. 5ml of alkaline copper tartrate added to each of the test tube. Allow it to stand for 10min and then add 0.5ml of 1N FC reagent mix well incubate at laboratory temperature for 30 min. The blue color was developed and the absorbance was taken at 660nm using colorimeter. The concentration of protein in honey samples was determined and calculated.

7. Estimation of total sugar content

Total sugar content was estimated by phenol-sulphuric acid method. Aliquots of working standards glucose was transferred to series of test tube (S1-S5) volume was made up to 1ml using distilled water. 0.5g of honey samples were diluted to 10ml distilled water 1ml from each was taken as test sample. 0.8 ml of 5% phenol, 5ml of concentrated sulphuric 15 acid were added to all test tubes, mixed and incubated at lab temperature for 30min absorbance was read at 490nm using colorimeter.

8. Total reducing sugar

Total reducing sugar was estimated by DNS method. Dextrose was used as standard. 0.5g of honey samples was dissolved in 10ml of distilled water. 1ml of test samples were taken for each sample. 2ml of DNS

reagent was added to all test tubes and incubated in boiling water bath for 10min. 1ml of Rochelle`s solution was added to all the test tubes. The absorbance was measured at 520nm using a colorimeter.

9. Total phenolics estimation

Phenol estimation of honey was done by using Folin Ciocalteau reagent. Aliquots of working standard (Gallic acid) were transferred to series of test tube marked S1 to S5 and volume was made up to 3ml. 0.5g of each honey samples were dissolved in distilled water. 0.5ml of FC reagent was added to all test tubes including 3ml control without test sample. After 3 min, 2ml of 20% Na2CO3 was added, mixed and placed in boiling water bath for 1min. Test tubes were cooled and absorbance was measured at 650nm using colorimeter.

10. Total flavonoid content

Total flavonoid estimation was done using Aluminum chloride method. Rutin was used as standard. 0.5 g of honey samples were dissolved in distilled water. Aliquots of standard was transferred into series of test tube and volume was made up to 1ml using distilled water. 0.3 ml of 5% of NaNO2 is added to all the test tubes. Leave it for 5 min then add 0.3ml of 10% of AlCl3 to all test tube. After 5min add 2ml of 1M NaOH to each of the test tubes. The absorbance was measured at 510nm.

11. Antioxidant activity of honey

DPPH radical scavenging activity

The honey samples were prepared by dissolving 1g of honey in 10ml of water. Ascorbic acid was taken as standard. The 400, 600, 800, 1000 concentration of samples and standard was taken in series of test tubes and volume was made up to 0.5ml by adding methanol. To each test tube 5ml of 0.1M methanolic solution of 2,2-diphenyl-1 picryihydrazyl (DPPH) was added and shaken vigorously. A control was maintained without test samples. Absorbance was measured at 517nm and free radical scavenging activity was calculated using formula, DPPH scavenging activity (%) = Absorbance of control Absorbance of sample × 100 Absorbance of control

12. Determination of antibacterial activity of honey

The antibacterial activity of honey is determined using well diffusion method. Fresh bacterial cultures of were spread evenly on nutrient agar plates, and 6mm diameter wells were made in the agar using a sterile corkborer. 500, 1000, 1500, 2000 concentrations of the honey samples were carefully added to these wells, and the plates were then incubated at 37°C for 24 h. The diameter of zones of inhibitions was measured.

13. Determination of antifungal activity of honey

The antifungal activity of honey was determined using the agar well diffusion method. Fungal strains - Candida albicans was used as test organisms for the study. Fresh fungal cultures were evenly spread over agar plates, and wells were made using a sterile 6mm corkborer. Different concentrations of the honey samples were added into these wells, and the plates were incubated at 28°C for 48 to 72 h to allow fungal growth. After incubation, the diameter of zones of inhibition were measured.

14. Melissopalynological study of honey

The melissopalynological study of honey was carried out to identify the botanical origin of the honey samples by analysing the pollen grains present in them. For this, 0.5g of honey was dissolved in warm distilled water, and the mixture was centrifuged to collect the pollen sediment. The sediment was then mounted on a microscope slide using glycerine jelly and examined under a microscope. The pollen grains were identified based on their shape, size, and surface features using Pollen Atlas as reference.

15. Statistical analysis

All the experiments and assays were performed in three replicates. The results of the work analyzed and expressed as mean ± standard deviation.

III. RESULTS AND DISCUSSION

1. Color measurement

The five honey samples collected for present study were compared visually according to the honey coloration chart and showed different colors (Table 1). The T1, T3 (Honey collected from Sringeri forest area, Yellapura rared honey) are light amber and extra light amber in color. T2, T5 (Honey collected from Sagara, rared honey from Palakkad) are dark amber in color. T4 (Honey collected from Karwar) which is only one sample amber in color. Singh et al. (2019) reported similar color diversity in multifloral honeys from

different regions of India, linking darker shades to higher antioxidant activity and richer floral sources. Al-Farsi et al. (2018) and, Gül and Pehlivan (2020) highlighted that dark amber honeys often possess elevated levels of phenolic compounds and minerals. In the present study, samples T2 and T5 were categorized as Dark Amber, suggesting a possible correlation with richer phytochemical content, while T3, labelled Extra Light Amber, may represent a lighter floral contribution. Gauthami and Bhat (2023) reported that honey color varied significantly depending on botanical origin, with darker honeys generally showing higher antioxidant potential. Their study emphasized that darker amber-colored honeys tended to be richer in phenolic compounds, which aligns with our results where the darker samples (T2 and T5) were associated with enhanced biological activity.

Table 1: Color measurement of five different samples

Honey samples	Color measurement
T1	Light Amber
T2	Dark Amber
T3	Extra Light Amber
T4	Amber
T5	Dark Amber

2. pH measurement

The average pH of honey is 3.9, but can range from 3.4-6.1 The T4 (Rared honey, Karwar) has lowest pH of 3.5 and T2 (Rared honey, Sagara) has high pH of 5.01 compared to all the honey samples (Table 2). There are previous reports supporting the present results with a pH rang of 3.2 - 5.5 (Saxena et al., 2010; Gül and Pehlivan, 2020). The acidity range may be attributed to the presence of organic acids, such as gluconic acid, which is produced by glucose oxidase activity. Overall, the acidic nature of all samples supports their antimicrobial potential and confirms their freshness and authenticity. Gauthami and Bhat (2023) reported that the pH of ten honey samples from different locations typically falls within the acidic range of 3.2 to 5.5, influenced by factors such as floral origin and maturity. Their research emphasized that lower pH enhances honey's preservation potential.

Table 2: Physiochemical properties of honey samples

Honey Samples	pH	ontent (%)	Moisture content (%)	ic conductivity (mS/cm)
T1	4.13	0.04	15.11	0.352
T2	5.01	0.01	10.02	0.578
T3	4.40	0.06	16.89	0.641
T4	3.50	0.02	14.12	0.501
T5	3.60	0.09	18.05	0.823

3. Ash content

The average ash content of honey falls between 0.2% and 0.6%. The T5 (Palakkad) 0.09% has highest percentage of ash and T2 (Rared honey, Sagara) 0.01 and T4 (Rared honey, Karwar) 0.02 has lowest percentage of ash compared to all the honey samples (Table 2). As reported by Codex Alimentarius and supported by studies of Terrab et al. (2004), the ash content in honey serves as a key indicator of its mineral composition and typically falls within the range of 0.2% to 0.6%, depending on the floral and geographical origin. Presence of higher cash value in T5 suggests a richer floral or soil source contributing to its mineral load, possibly correlating with its classification as Dark Amber. In contrast, the lower ash levels in T2 and T4 may indicate nectar from lighter floralsources with lower mineral uptake. These findings further validate the natural origin and quality of the analyzed honey samples. Kamaruddin et al. (2021), who reported ash values between 0.02% and 0.13% in Malaysian honey.

4. Moisture content

Moisture content is a critical parameter in evaluating the quality, stability, and shelf life of honey. It ranges between 14% and 20% (Table 2). The highest moisture content was recorded in T5 (honey collected from Palakkad) 18.05 and the T2 (Rared honey, Sagara) 10.02 lowest percentage of moisture content. White et al. (1975) and Rizelio et al. (2011), also emphasized that moisture variation is influenced by environmental conditions, harvesting time, and floral origin. The observed range confirms that the samples are of good quality and safe for storage under appropriate conditions. These values are consistent with recent findings by Singh et al. (2022), who reported moisture content between 13% and 19% in Indian honey samples, and by Gul and Pehlivan (2020), who found values from 14% to 17% in Turkish honeys.

5. Determination of electric conductivity

The electrical conductivity of honey in the present study recorded within 0.10 to 0.80 mS/cm (Table 2). According to the results T5(Palakkad) 0.823 and T3(Yellapura) 0.641 has highest electric conductivity. The T1(Forest honey, Sringeri) 0.352 has lowest electric conductivity. Similar findings have been reported by Terrab et al. (2004) and Gomes et al. (2010), where darker honeys and those with higher ash content showed elevated conductivity. Notably, the trend observed in T5's higher conductivity aligns with its higher ash and darker color, reinforcing the correlation between electrical conductivity and mineral richness in honey. This parameter thus not only supports classification but also reflects potential nutritional and functional value. Silva et al. (2021), who reported conductivity values between 0.30 and 0.80 mS/cm in multifloral honeys. Escuredo et al. (2022) also found similar variations in European honeys.

6. Determination of total protein content (TPC)

The amount of protein present in the sample was estimated by Lowry`s method with BSA as standard (Fig. 1), the results revealed that T5 exhibited the highest protein content of 0.8 mg/g, followed by T4 with 0.7 mg/g, indicating a richer nutritional profile in these two samples. T2 also showed a considerable amount at 0.6 mg/g, while T1 had a moderate value of 0.4 mg/g. (Table 3). In contrast, T3 possessed the least protein, 0.1 mg/g, suggesting it may have lower biological activity or come from different floral sources. This variation reflects the natural differences in protein content based on factors like botanical origin, geographic location, and environmental conditions. Aljadi and Kamaruddin (2004) investigated Malaysian honeys and reported protein concentrations between 0.1 and 0.6 mg/g, depending on the floral source. T1 (0.4 mg/g) and 2 (0.6 mg/g) in the current study fall within this range, suggesting similar botanical origins. However, T5 (0.8 mg/g) exceeds this range, indicating a potentially higher nutritional quality or protein-rich nectar source. Alshammari et al (2024) analyzed Acacia, Ziziphus, and polyfloral honeys from Saudi Arabia and found protein concentrations ranged from 0.416 to 1.260 mg/g with regional and botanical origin playing a significant role.

Table 3: Total protein, phenolics, flavonoids, sugar and reducing sugar contents* of five honey samples

Honey sample	Protein (mg/g)	Phenolics (mg/g)	avonoid (mg/g)	Total sugar content (mg/g)	Reducing sugar (mg/g)
T1	0.11±0.02	0.06±0.12	0.02±0.07	0.62±0.88	0.09±0.48
T2	0.49±0.17	0.03±0.6	0.04±0.14	0.48±0.75	0.04±0.56
T3	0.53±0.27	0.08±0.16	0.01±0.66	0.25±0.02	0.06±0.78
T4	0.05±0.12	0.04±0.4	0.06±0.59	0.39±0.6	0.08±0.41
T5	0.26±0.9	0.03±0.08	0.03±0.04	0.51±0.8	0.02±0.19

Mean ± SD; N=3

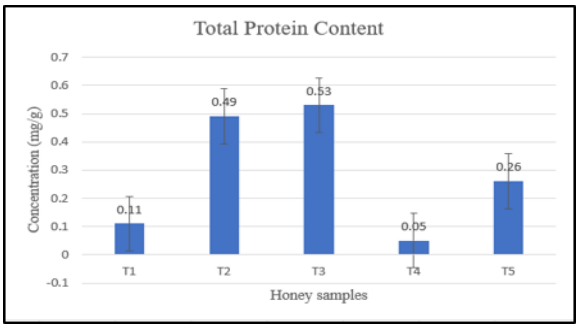

Fig. 1: Total protein content in five honey samples
Mean ± SD; N=3

7. Total phenolic estimation

The total phenolic content was determined using gallic acid as standard (Fig. 2) which varied in a different honey sample. Among them, T3 showed the highest phenol concentration at 0.08 mg/g, indicating it may possess stronger antioxidant potential compared to the others T1 followed with 0.06 mg/g, while T2 and T5 recorded the lowest phenol levels, both at 0.03 mg/g while T4 showed a moderate amount at 0.04 mg/g (Table 3). These variations in phenolic content suggest differences in the floral sources of nectar and environmental factors influencing the phenolic profile of each honey sample. Alvarez-Suarez et al., 2010) (Cuban Honey Study) reported total phenolic content in honeys ranging from 0.12 to 0.26 mg/g, which is higher than the values found in the current study. Their study highlighted that honeys derived from tropical plants tend to have higher phenolic content, enhancing

antioxidant activity. Akyol et al. (2023) reported phenolic content between 0.04 and 0.10 mg/g in various in Turkish honeys.

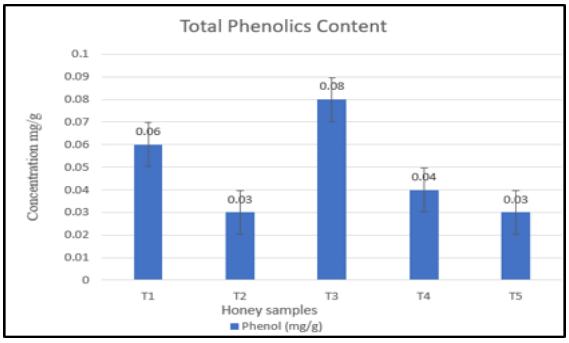

Fig. 2: Total phenolics content in honey
Mean ± SD; N=3

8. Total flavonoid estimation

The total flavonoid content was expressed as of quercetin equivalent per 100g of the sample (Fig.5.). Among the honey samples, T4 stood out with the highest flavonoid concentration with 0.06 mg/g, suggesting it may have stronger antioxidant properties. T2 followed with 0.04 mg/g, while T5 and T1 showed moderate values of 0.03 mg/g and 0.02 mg/g, respectively. T3 had the lowest flavonoid content 0.01 mg/g, indicating a relatively lower bioactive compound level (Table 3, Fig. 3). These results highlight the natural variation in flavonoid content among different honey types, likely influenced by floral source, region, and harvesting conditions. Moniruzzaman et al. (2013) documented flavonoid concentrations of 0.05 to 0.10 mgQE/g in Bangladeshi honeys, with T4 in this study comparable with their lower range. These variations in flavonoid content are likely due to differences in floral origin, geographical conditions, and environmental factors.

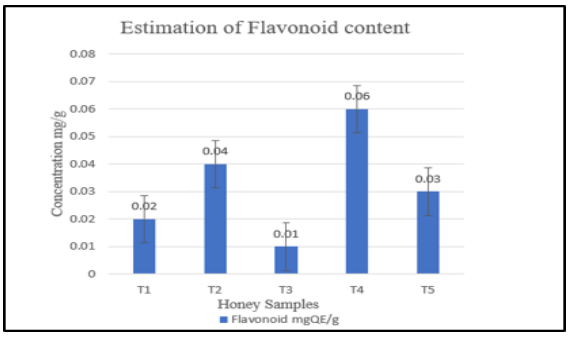

Fig. 3: Total flavonoid content in honey
Mean ± SD; N=3

9. Estimation of total sugar content

The total sugar content in five honey samples was measured using dextrose as standard (Fig.7), it revealed that in T1 had the highest sugar concentration of 0.62 mg/g, suggesting it may have a sweeter taste and denser consistency. T5 also showed a relatively high sugar level 0.51 mg/g, followed by T2 with 0.48 mg/g. On the otherhand, T4 recorded a moderate value of 0.39 mg/g, while T3 had the lowest sugar content at 0.25 mg/g (Table 3, Fig. 4.) These differences in sugar levels could be attributed to variations in floral sources, bee foraging behaviour, and geographical conditions, all of which influence the natural sugar profile of honey. Meda et al. (2005) conducted a study on honey from Burkina Faso, reporting sugar concentrations between 0.4-0.65 mg/g using spectrophotometric analysis. In comparison, T1 (0.6 mg/g) and T5 (0.5 mg/g) are within this range, while T3 (0.25mg/g) appears to have lower sugar content, which might indicate early harvest, over- processing, or floral source with low sugar concentration.

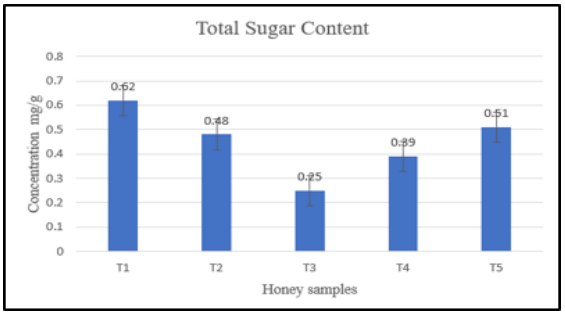

Fig. 4: Total sugar content in five honey samples
Mean ± SD; N=3

10. Total reducing sugar

Total reducing sugar content was estimated by DNS method with dextrose as standard (Fig. 9) Among the honey samples, T1 exhibited the highest reducing sugar content of 0.09 mg/g, followed by T4 at 0.08 mg/g, while the lowest reducing sugar content 0.02 mg/g in T3 (Table 3, Fig. 5). Ahmed et al. (2007) reported reducing sugar content in Pakistani honeys ranging from 59.7% to 76.1%. Gauthami and Bhat (2022) reported higher levels of reducing sugars in their analysis of Indian honeys, with values ranging between 60% and 75%. This difference can be attributed to variations in floral sources and climatic conditions.

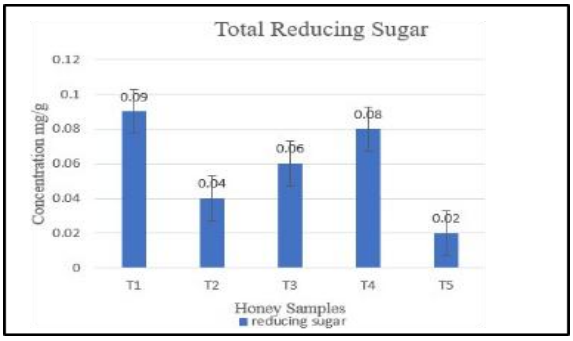

Fig. 5: Total reducing sugar in five honey samples
Mean ± SD; N=3

11. Antioxidant activity of honey

DPPH radical scavenging activity:

The DPPH radical scavenging activity of the tested honey samples indicates their antioxidant potential, which ranges from 71.83% to 81.57%. Among the five samples, T5 showed the highest antioxidant activity (81.57 ± 4.39%), followed closely by T4 (77.85 ± 5.68%) and T2 (76.27 ± 8.59%). T3 exhibited moderate activity at 74.25 ± 10.57%, while T1 showed the lowest scavenging percentage at 71.83 ± 3.39% (Table 4, Fig.6). The variation in antioxidant activity among the samples may be attributed to differences in their floral sources, phenolic content, and bioactive compound composition. The presence of standard deviation highlights the reliability of the data and natural variability within the samples. Beretta et al. (2005) analyzed antioxidant activities in various types of Italian honey and observed that darker honeys with higher phenolic content showed increased DPPH inhibition, often reaching 90% at concentrations of 500-1000 mg/g. The IC_{50} values of the tested honey samples revealed that T4 exhibited the highest antioxidant potential with the lowest IC_{50} value of 396.94 µg/ml, followed by T3 429.03 µg/ml (Table 5). T5 showed moderate antioxidant activity and T2 1279.02 µg/ml displayed comparatively weaker antioxidant effects.

Table 4: Radical scavenging activity of honey samples

Samples	DPPH radical scavenging activity*(%)
T1	71.83±3.39
T2	76.27±8.59
T3	74.25±10.57
T4	77.85±5.68
T5	81.57±4.39

* Mean ± SD; N=3

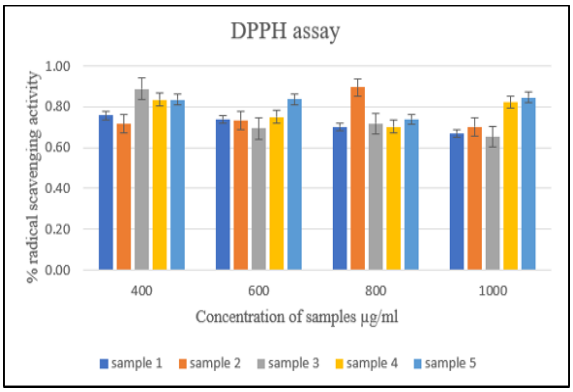

Fig. 6: DPPH radical scavenging activity of five honey samples
Mean ± SD; N=3

Table 5: IC_{50} values of DPPH radical scavenging activity

Honey samples	IC_{50} value
T1	804.98
T2	1279.02
T3	429.03
T4	369.94
T5	620.20

12. Determination of Antimicrobial activity of honey samples

The antibacterial activity of different honey samples (T1 to T5) was tested against Escherichia coli at 500-2000 concentrations to assess their effectiveness. Among all the samples, T2 and T3 showed higher zone of inhibition at 1000 µg/g 6.9 ± 0.3 mm and 5.7 ± 0.35 mm (Table 6), respectively. In contrast, the other samples (T1, T4, and T5) exhibited relatively steady and lower inhibition zones across all tested concentrations. These variations suggest that the effectiveness of honey can differ depending on its origin and composition, possibly due to varying levels of bioactive compounds like phenolics, flavonoids, and enzymes, which contribute to its antimicrobial properties (Plate 1). Irish et al. (2011) reported inhibition zones of around 4.0 cm for E. coli Manuka honey. T2 and T3 from our study exhibited inhibition zones even larger than that (up to 6.9 cm) suggesting that some of the local honey samples tested may possess antibacterial potential comparable to, or exceeding, specialized medicinal honeys.

The antibacterial activity of the honey samples (T1 to T5) was further assessed against Staphylococcus

aureus across concentrations ranging from 500 to 2000mg/g. Overall, the inhibition zones observed were modest, with slight increases at higher concentrations. Notably, T1 and T5 showed the highest activity at 2000 µg/g, each producing a zone of inhibition measuring 1.9 mm, (Plate 2) suggesting a concentration-dependent response. For Staphylococcus aureus, the inhibition zones ranged from 1.0 cm to 1.9 cm in our study. Albaridi (2019) observed inhibition zones ranging between 11-18 mm using 50% honey concentrations, while Ceksteryte et al. (2023) and Beretta et al. (2024) reported zones up to 24–27 mm with undiluted Latvian and German honeys. The IC50 values revealed differences in in their ability to inhibit microbial growth. T1 exhibited strongest antimicrobial effect with lowest IC50 value of 474.55 µg/ml (Table 7), followed by T3 483.28 µg/ml and T4 487.01 µg/ml indicating their high effectiveness against microbial strains.

Table 6: Antibacterial activity of five honey samples against bacterial strains

Bacterial strains	Concentration (mg/g)	Diameter of zone of inhibition*(mm)				
		T1	T2	T3	T4	T5
/Eqscherichia coli	500	1.5±0.025	1.60±0.2	1.4±0.3	1.7±0.05	1.6±0.05
	1000	1.6±0.15	6.9±0.3	5.7±0.35	1.6±0.3	1.5±0.25
	1500	1.5±0.05	1.4±0.15	6.2±0.35	1.6±0.15	1.4±0.35
	2000	1.6±0.1	1.4±0.3	1.4±0.2	1.7±0.25	1.8±0.45
hylococcus aureus	500	1.2±0.57	1.5±0.35	1.3±0.48	1.8±0.32	1.5±0.07
	1000	1.0±0.29	1.7±0.42	1.5±0.19	1.4±0.66	1.8±0.26
	1500	1.5±0.45	1.4±0.22	1.2±0.39	1.6±0.09	1.4±0.54
	2000	1.9±0.33	1.2±0.36	1.7±0.15	1.6±0.510	1.9±0.35
Enterobacter sp.	500-2000	-	-	-	-	-

* Mean ± SD; N=3

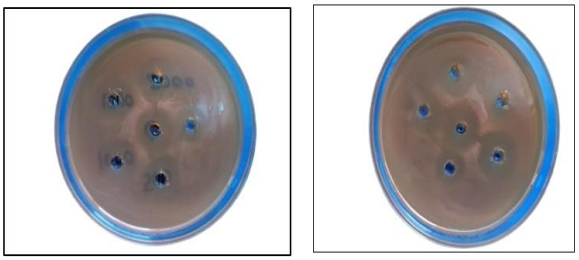

Plate 1: Antibacterial assay of honey samples against Escherichia coli

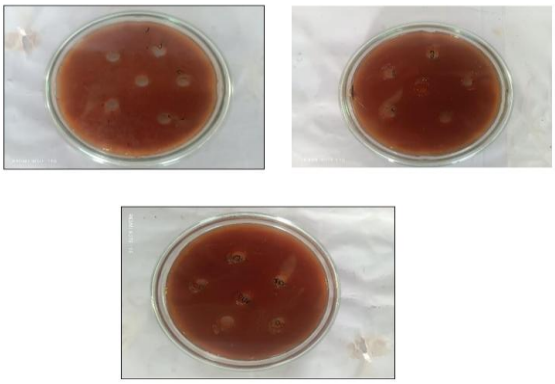

Plate 2: Antibacterial assay of Honey samples against Staphylococcus aureus

Table 7: IC_{50} value for antimicrobial activity of five honey samples

Honey sample	IC50 value
T1	474.55
T2	1316.85
T3	483.28
T4	487.01
T5	2051.17

13 Determination of antifungal activity of honey samples

The antifungal activity of honey samples (T1, T2, T3, T5, and T4) was evaluated at concentrations ranging from 2000 to 5000 µg/g. Among these, T3 exhibited the highest zone of inhibition at 2000 µg/g (3 ± 0.25 mm) (Table 8), suggesting strong antifungal potential at lower concentrations. T2 showed a gradual increase in activity with concentration, peaking at 4000 µg/g (3.4 ± 0.2 mm), indicating a concentration- dependent response. In contrast, T1 and T5 showed moderate and fairly consistent activity, with inhibition zones ranging between 1.4 mm and 2.2 mm (Plate 3). Notably, T4 did not exhibit any zone of inhibition at any concentration, indicating a lack of antifungal activity. These results

highlight the variability in antifungal potential among different honey samples, which may be attributed to differences in their bioactive components, such as phenolics, flavonoids, hydrogen peroxide content, and osmotic effects. Almasaudi et al. (2017) observed inhibition zones ranging from 2.5 to 5.0 mm when Saudi honey varieties were tested against C. albicans, supporting the present study where certain samples, particularly T2 and T3, showed enhanced antifungal activity at higher concentrations. El-Kased et al. (2020) observed that Egyptian honeys, especially those with high flavonoid and peroxide levels, showed strong antifungal activity in vitro, supporting the potential of certain honey types-such as T2 and T3 in this study-as effective natural antifungal agents.

Table 8: Antifungal activity of five honey against Candida albicans

Concentration (mg/g)	Zone of inhibition *(mm)				
	T1	T2	T3	T4	T5
2000	2.2±0.15	1.7±0.15	3±0.25	-	2.2±0.1
3000	1.4±0.35	2.8±0.1	2.5±0.75	-	1.6±0.1
4000	1.8±0.1	3.4±0.2	1.9±0.05	-	1.7±0.05
5000	1.8±0.2	1.8±0.1	1.2±0.1	-	1.7±0.25

* Mean ± SD; N=3

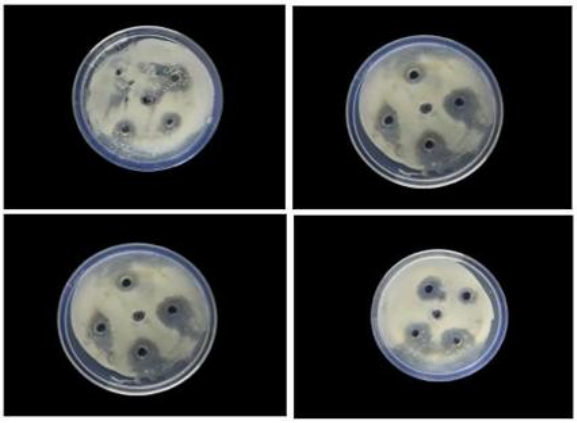

Plate 3: Antifungal activity of Honey against Candida albicans

14 Melissopalynological study of honey

The palynology study revealed that the pollen family distribution in a honey sample, revealing significant variability in floral origin. Among the identified families, Solanaceae was the most abundant, comprising 23.2% of the total pollen content, indicating its dominance as a nectar source for honeybees in the sampled area. This was followed by Brassicaceae (12.5%), Asphodelaceae (10.7%), and Poaceae and Fabaceae with equal contributions of 8.9% each. Several families such as Cyperaceae, Bromeliaceae, and Bignoniaceae were moderately represented at 5.4%. In contrast, Lamiaceae was the least represented family, contributing only 1.8%, suggesting its minor role in the floral landscape or limited foraging by bees on its flowers (Table 9). This distribution highlights the diverse but uneven pollen composition in honey, reflective of the foraging behavior of bees and surrounding floral biodiversity (Plate 4). Sharma et al. (2021) noted Fabaceae and Asteraceae as dominant families in North Indian honeys, while in studies from southern regions, Poaceae and Solanaceae were more prominent, reflecting regional floral differences. In comparison, our results show a higher prevalence of Solanaceae (23.2%), which aligns with the floral composition typical of semi-arid and tropical landscapes. Gauthami and Bhat (2023) examined ten honey samples from Dakshina Kannada and reported a broader diversity: 36 pollen types across 18 families, with

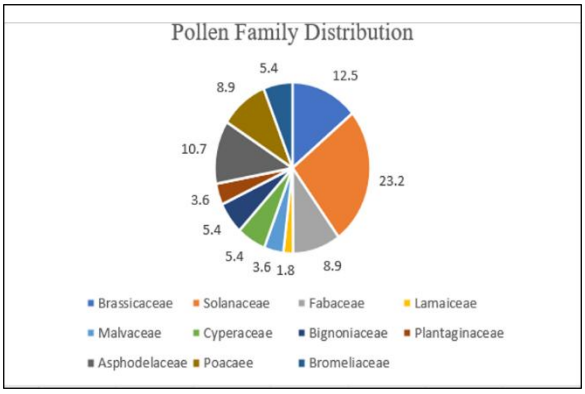

Fig. 7: Pollen abundant family in five honey samples

Asteraceae, Cistaceae, Fabaceae, Lamiaceae, Moraceae, Rosaceae and Saliceae frequently occurring. They observed physiochemical and bioactive variations linked to floral origin, but pollen from Lamiaceae was more

prominent in their multi-floral samples. In contrast, present reveals comparatively minor presence of Lamiaceae member (1.8%).

Sl. No	Plant Family	Pollen Shape	Common Name
1	Asteraceae	Spheroidal	Tridax Daisy (Coat Button)
2	Fabaceae	Oblate-Spheroidal	Babul Tree
3	Myrtaceae	Prolate	Jamun (Black Plum)
4	Malvaceae	Tricolpate (Triangular)	Hibiscus
5	Asteraceae	Oblate	Sunflower
6	Brassicaceae	Prolate-Spheroidal	Mustard Plant
7	Lamiaceae	Prolate	Tulsi (Holy Basil)
8	Apiaceae	Spheroidal	Coriander
9	Euphorbiaceae	Prolate-Spheroidal	Asthma Weed
10	Rubiaceae	Spheroidal	Ixora (Jungle Flame)
11	Combretaceae	Prolate-Spheroidal	Indian Almond
12	Sapindaceae	Spheroidal	Soap nut (Reetha)
13	Cucurbitaceae	Oblate-Spheroidal	Pumpkin
14	Lamiaceae	Spheroidal	Indian Heliotrope
15	Fabaceae	Oblate	Cajanus (Toor/Arhar Dal)
16	Apocynaceae	Prolate-Spheroidal	Nerium (Oleander)
17	Rutaceae	Spheroidal	Curry Leaf Tree
18	Solanaceae	Oblate-Spheroidal	Black Nightshade
19	Lamiaceae	Oblate	Mint
20	Lamiaceae	Prolate-Spheroidal	Lavender
21	Poaceae	Spheroidal	Sugarcane
22	Malvaceae	Spheroidal	Cotton
23	Amarantaceae	Oblate	Spiny Amaranth
24	Asphodelaceae	Prolate	Aloe vera
25	Poaceae	Oblate	Maize (Corn)
26	Poaceae	Prolate	Paddy (Rice)
27	Cyperaceae	Spherical	Grass
28	Fabaceae	Prolate-Spheroidal	White Clover
29	Asteraceae	Prolate	Tridax procumbens
30	Lamiaceae	Spheroidal	Sweet Basil
31	Poaceae	Spheroidal	Cynodon (Durva Grass)
32	Fabaceae	Prolate	Albizia (Siris Tree)
33	Combretaceae	Spheroidal	Bahera (Terminalia bellirica)
34	Meliaceae	Spheroidal	Neem
35	Brassicaceae	Oblate	Field Mustard
36	Fabaceae	Oblate	Red Clover
37	Rosaceae	Spheroidal	Blackberry
38	Sapindaceae	Spheroidal	Lychee Fruit
39	Brassicaceae	Spheroidal	Radish
40	Liliaceae	Oblate	Onion
41	Anacardiaceae	Oblate (Dyad Form)	Mango

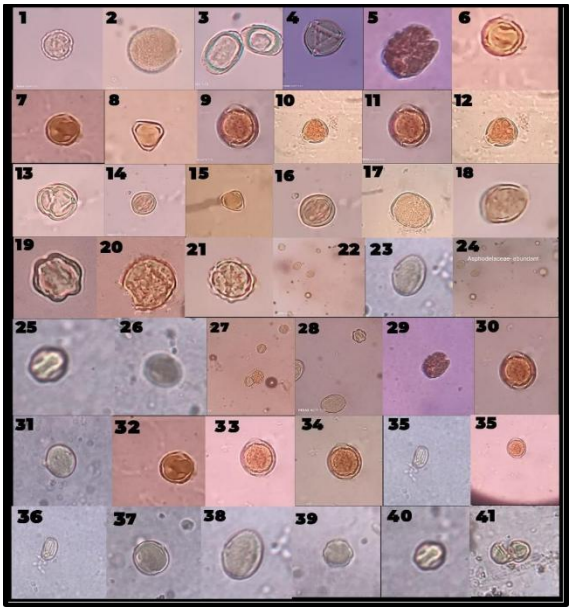

Plate 4: Pollen plates of five honey samples

IV. SUMMARY

The project analysed five different honey samples from different places. The findings from this study highlight the value of honey, extending beyond its nutritional properties to include significant medicinal potential. The melissopalynological profile confirmed a diverse floral origin, while physicochemical analyses demonstrated that the honey samples possessed desirable quality parameters. The results also show all the honey samples are effective against the selected Gram-positive and Gram-positive bacteria. These

results showed the importance of honey not only as a food product but also as a functional therapeutic agent. This study helped us understand both the healing power and botanical origins of honey.

- The pharmacological tests showed that the honey samples have strong antibacterial and antifungal effects.
- This supports the traditional belief that honey is good for treating wounds and infections.
- This study supports honey as a safe, natural alternative to synthetic drugs for minor infections.
- This study confirms that honey is not just a sweetener but also a natural medicine.

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