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Decoding honey's signature: Pollen composition, physicochemical traits and antimicrobial activity of honey against *E. coli*

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Abstract
This study aims to elucidate the palynological composition, physicochemical characteristics, and antimicrobial efficacy of honey against *Escherichia coli*. Eight samples of honey collected from different areas of Junagadh & Jamnagar districts of Gujarat and Four Commercial honey samples were studied for sensory and physico-chemical properties. A comprehensive pollen analysis of the collected 8 honey samples revealed a diverse botanical origin, with a total of 20 distinct plant species identified. The honey samples were categorized as multifloral or unifloral based on pollen percentage, with predominant pollen types belonging to the following 18 families: Dominant (>80%): Apiaceae (99%), Malvaceae (97%), Lamiaceae (91%), Arecaceae (85%); High Abundance (50-80%): Sapindaceae (75%), Mimosaceae (60%), Fabaceae (58%), Myrtaceae (53%); Moderate Abundance (20-50%): Asteraceae (47%), Anacardiaceae (33%), Moringaceae (32%), Combretaceae (26%), Amaranthaceae (26%), Rutaceae (21%); Low Abundance (<20%): Pedaliaceae (16%), Brassicaceae (12%), Solanaceae (7%), Rhamnaceae (6%). The moisture and ash content of the samples exhibited mean values of 27.44% ± 0.34% and 16.05% ± 0.07%, respectively. The pH and acidity of the samples demonstrated mean values of 4.91 ± 0.014 and 14.65 ± 0.02 meq/kg, respectively. The HMF (hydroxymethylfurfural) and total solids of the samples exhibited mean values of 60.86 ± 1.01 mg/kg and 96.1 ± 0.14% respectively. In order to determine the *in vitro* antibacterial activity of honey samples, the agar well diffusion method was used. The ciprofloxacin antibiotic was used as a positive control in this method. The Ciprofloxacin (18mm) control suggests honey samples generally has comparable or better activity against *E. coli*.

Keywords: Honey, melissopalynology, multifloral, unifloral, physicochemical characteristics, Pollen, anti-microbial activity, *E. coli*.

1. Introduction
The distinct characteristics of honey are shaped by its botanical and geographical origins, which are reflected in its pollen composition, physicochemical properties, and bioactive compounds. Melissopalynology, the study of pollen and spores in honey, provides valuable insights into the plant species preferred by bees for nectar, pollen, or both (Mangi *et al*., 2018)[17]. By analyzing the pollen content of honey and bee-collected pollen loads, researchers can infer the local flora and vegetational assemblages of a particular region, thereby shedding light on the environmental context in which the honey is produced (Agwu *et al*., 2013; Campos *et al*.,1997) [1, 5]. This knowledge not only helps in understanding honey's quality and authenticity but also offers a window into the ecological dynamics of the region. The Codex Alimentarius Commission defines honey as, "the natural sweet substance produced by honeybees from the nectar of flowers or from secretions coming from living organisms feeding on plants, that bees gather, transform and combine with specific ingredients, store and leave to ripen in the combs of the hive" (Johnson *et al*.,2010) [10]. Beyond its sweetness, honey's complex composition and multifaceted properties have sparked scientific interest, particularly in its potential antimicrobial applications. The physicochemical properties of honey are an important indicator of the quality and origin of honey. Color is the primary physical attribute that consumers visually perceive and process upon initial product exposure. For honey, determining colour is a helpful categorization criterion. Honey in liquid form can be clear and colourless, yellow, amber-coloured and dark amber coloured (Bogdanov *et al*., 2004) [3]. Adulteration of honey is a matter of great concern to the people in respect of food safety, health benefits derived from the honey, fraudulent protection, maintaining the consumer trust and moreover to protect from hazardous effects of cheap added material in the honey.

Authenticity of honey is with respect to botanical or geographical origin, i.e. mislabelling of honey origin and adulteration with sugars and syrup (as adulterant). According to the Food Safety and Standards Act of India (FSSAI), definitions of adulterant are any substances that are used in food items to make it harmful or substandard or adding irrelevant material. In honey, it can be of two types, on the basis of way of manipulation, i.e. direct or indirect adulteration. Direct adulteration can be done by straight inclusion of sucrose syrups that are obtained from sugar beet, corn syrup that is rich in fructose, maltose syrup or by addition of industrial sugar, i.e. fructose and glucose, starch-derived syrups through heating and treatment of honey with acid or enzyme, whereas indirect adulteration done by excessive noshing of the bees present in the colonies by syrups in the course of prime nectar flow period (Tura and Seboka, 2019) [23]. There are branded and unbranded honeys available in the market. Significant differences may exist between honey brands in terms of nutritional as well as quality of honey. Most of the people are unaware about the quality of honey they consume. It is important to do the quality testing of commercial honey due to increasing problems related to adulteration and tampering with natural honeys sold in the market. Therefore, a study was designed to test the quality of commercial honey available in the market for comparison with International Honey Standards to get a useful data for the honey consumers (Lazarević *et al*.,2017) [16].

Physicochemical parameters in honey can point to fermentation, sugar adding, heating, honey adulteration attempts, inappropriate storage, and so forth. Honey is primarily a saturated sugar solution, mainly composed of fructose (38%) and glucose (31%), and also contains a variety of minor components such as phenolic compounds (Gorjanović, S. Ž. *et al.*,2013) [8]. The water content in honey ranges from 15%to 23%. Honey which is placed on the market must not have water content higher than 20%. Permitted value of free acids in honey is <50 meq of acid/1000 g of honey. Their presence tells us whether the honey is older. Electrical conductivity (µS/cm) is a physical property which greatly depends on the content of mineral substances and acids in honey. Electrical conductivity of floral and blended honey needs to be lower than 0.8 mS/cm. The sum of glucose and fructose or reducing sugars (g/100 g) in honey must be at least 60 g/100 g. Carbohydrates are the main ingredient of honey and their proportion is 73%-83%. Determining the amount of saccharose (≤10 g/100 g) is important for confirming possible adulteration of honey, feeding the bees sugar (saccharose), or directly adding sugar in honey. Diastase activity (DN) represents one of the main parameters in determining the intensity of heating the honey during its processing and storage (Uršulin *et al*., 2017) [24].

The antioxidant and antimicrobial activity of honey strongly depends on the types and concentration of these minor components. The type and amount of these minor ingredients vary depending on the source of nectar (flora) and the geographic origin of honey. Studies have shown in general that dark coloured honeys have more phenolic components and hence better antioxidant activity (Bertoncelj *et al*., 2007; Keskin *et al*., 2021, Broadhurst, 1999) [2, 11, 4]. The nutritional and health promoting value of honey has been known since ancient times but it has been stated recently that there is a correlation between antioxidant activity and the health-supporting effect of honey. Honeys having higher antioxidant activity are reported as better for the prevention of aging, in degenerative heart and nervous system diseases and in wound healing property. (Kolayli *et al.*, 2020) [13].

The presence of microorganism in honey can also affect the quality of honey. The microorganisms present in honey and in the combs of hives include yeast, bacteria and moulds. The possible sources of the microbial contamination are winds, dust, pollens, intestine of honeybee, human, equipment and containers. The microorganism can't flourish in the honey, it is because of its viscous behaviour, low water level and as a result of its antimicrobial activity properties (Snowdon and Cliver, 1996) [22]. But there are reports of microbial presence in honeys that might be due to recent contamination. Usually, bacteria which come in the honey collected aseptically lose their potentiality within 8-24 days if storage is done at 20°C (Olaitan *et al.*, 2007) [19].

2. Materials and Methods

2.1 Sample collection

Samples have been collected from different villages of Junagadh and Jamnagar districts of Gujarat (Table: 1)

Table 1: Collection sites of honey samples from Junagadh & Jamnagar districts

Sample no.	Locality	Latitude	Longitude	Month of collection	Source of Honey	Type of Honey	Honeybee Species
SP1	Thanapipli	21.42º N	70.5º E	Sep-24	N/A	Multi-floral	*Apis mellifera*
SP2	Vanthli	21.42º N	70.5º E	Oct-24	N/A	Multi-floral	*Apis florea*
SP3	Mahobatpur	21.43º N	70.43º E	Jan-25	N/A	Multi-floral	*Apis florea*
SP4	Jodiya	22.69º N	70.31º E	Jan-25	*Foeniculum vulgare*	Uni-floral	*Apis mellifera*
SP5	Jodiya	22.69º N	70.31º E	Jan-25	*Ocinum tenuiflorum*	Uni-floral	*Apis mellifera*
SP6	Jodiya	22.69º N	70.31º E	Jan-25	*Hibiscus rosa-sinensis*	Uni-floral	*Apis mellifera*
SP7	Jodiya	22.69º N	70.31º E	Jan-25	*Trachyspermum ammi*	Uni-floral	*Apis mellifera*
SP8	Jodiya	22.69º N	70.31º E	Jan-25	*Litchi chinensis*	Uni-floral	*Apis mellifera*

(Note: The samples SP9, SP10, SP11, and SP12 comprised commercially available honey products)

2.2 Pollen analysis

Honey extraction was performed by applying mechanical pressure to the entire honeycomb, preserving the intact pollen cells. Following extraction, the honey was stored at ambient temperature (20-25°C). Each sample was filtered through funnel shaped strainer to eliminate debris. From each sample of filtered honey, 10 gm were carefully weighed using a weighing balance. Following Agwu *et al.* (2013)[1], The sample was then diluted with 35 ml of warm (40-50°c) dilute sulphuric acid solution (3 ml in 1000 ml of water). After a through shaking of the honey acid solution, it was centrifuged for 5 minutes at 2000 rpm & supernatant was decanted. The recovered sediments were treated with 10 ml glacial acetic acid to remove water before acetolysis.

2.3 Physicochemical analysis

The pH was determined using the method of Iftikhar *et al.* (2014) [9], Free acidity was determined by potentiometric titration (Faleye *et al*., 2021) [6] and results were expressed as meq/kg, the color was determined by spectrophotometric measurement of the absorbance of honey solution at 635 nm. The honey was classified based on color using Pfund scale after conversion of the absorbance values: mm Pfund = -38.70 + 371.39 × Abs (Krell *et al*., 1996; White, 1979) [14, 25]. The ash content of honey was determined using the method of Silva *et al*. (2004) [21]. The moisture content was determined using gravimetric method. The electrical conductivity of the honey samples, was determined using the method of (Snowdon and Cliver, 1996) [22], and the results were expressed in µS/cm. HMF (Hydroxymethylfurfural) values were determined following the method of Ghorab *et al.* (2021) [7]. Layne (1975) [15] and Plummer (1990) [20] methods were referred to analyse the values of total carbohydrates.

2.4 Antimicrobial activity of honey against Escherichia coli

- **Preparation of honey samples:** The extraction of raw honey was performed using water. Ten grams (g) of honey was mixed with 25 mL of deionized water and centrifuged for 10 minutes at 3000 rpm at 25 °C. The supernatant was collected from the centrifuged tube into a 50 mL round-bottom flask by filtration and then dried at 50 °C using a rotary evaporator. To prepare the require honey concentrations of 25% (v/v), 50% (v/v), 75% (v/v), and 100% (v/v), we weighed the resulting product and then dissolved it in sterile deionized water before use, as described in an earlier study. (Mudenda *et al*., 2023) [18].

- **Sub-culturing and inoculation of bacteria:** The bacterial strains of *E. coli* (ATCC 25922) were obtained and cultured on nutrient agar in the Laboratory at the University. The colonies of *E. coli* were then counted. After that, a sterile swab was used to pick the pure colonies of *E. coli* and from the nutrient agar plates and then emulsified in 2 mL of normal saline. Further, to attain the required standard of 0.5 McFarland, we compared the turbidity of the inoculated normal saline to that of the standardized 0.5 Remel TM McFarland Turbidity (12076 Santa Fe Drive, Lenexa, KS 66215, USA). We used a sterile swab to inoculate the bacterial suspensions onto the Mueller-Hinton agar plates (Oxoid, Basingstoke, UK).

- **Antibacterial agar well diffusion assay:** The prepared honey concentrations (25% (v/v), 50% (v/v), 75% (v/v), and 100% v/v) were screened for antibacterial activity as reported by (Khalil *et al.,* 2013) [12]. Briefly, wells measuring 6 mm in diameter and 3.2 mm in height were made in the Mueller-Hinton agar plates that contained inoculated bacteria. This was followed by adding 100 µL of a test dilution to each well. Thereafter, the plates were incubated at 37 °C for 24 hours as described in previous studies. A standard ciprofloxacin (5 µg) was used as a positive control while sterile deionized water was used as a negative control. The antibacterial activities of honey were evaluated by measuring the diameter of zones of inhibition (in millimetres) on the wells using a ruler, as was done in another study.

3. Results and Discussion

A total of 22 kinds of pollen grains were discovered from 8 different honey samples belonging to 18 different plant families such as Apiaceae (99%), Malvaceae (97%), Lamiaceae (91%), Arecaceae (85%), Sapindaceae (75%), Mimosaceae (60%), Fabaceae (58%), Myrtaceae (53%), Asteraceae (47%), Anacardiaceae (33%), Moringaceae (32%), Combretaceae (26%), Amaranthaceae (26%), Rutaceae (21%), Pedaliaceae (16%), Brassicaceae (12%), Solanaceae (7%), and Rhamnaceae (6%). (Table: 2; Figure: 1 & 3).

Table 2: Pollen analysis of multifloral and unifloral honey collected from Junagadh and Jamnagar district, Gujarat India

Sr. No.	Plant and family	Common Name	Morphology of pollen grains	Flowering period (months)	Forage source	Life span	Classification of honey (Multifloral/Unifloral)
1	*Acacia auriculiformis* (Fabaceae)	Australian Babul	Polyad type, square in centre, polar outline circular, 12-celled	9-12	Tree	25-30 years	M
2	*Chenopodium album* (Amaranthaceae)	Bathua-chilani bhaji	Spheroidal, medium sized, with a circular outline and numerous, small, circular pores on the surface	1-3	Annual herb	Several decades	M
3	*Cocos nucifera* (Arecaceae)	Coconut-Nariyal	Spherical in shape, after shedding, they shrink and become ellipsoidal with a longitudinal suture	6-12	Tree	60-80 years	M
4	*Coriandrum sativum* (Apiaceae)	Coriander-dhaniya	3 colporate, prolate, per-prolate, bilateral symmetry	6-8	Herb	3-6 months	M
5	*Psidium guajava* (Myrtaceae)	Guava-jamrukh	Pollen grain in monad, triangular in polar view, oblate spheroid in equatorial view, psilate pollen, bilateral symmetry	4-9	Tree	40 years	M
6	*Terminalia* sp. (combretaceae)	Baheda, Sadad, Arjun	Pollen grain in monad,Equatorial outline elliptic,Pollar outline triangular	5-6	Tree	4-5 years	M
7	*Solanum lycopersicum* (Solanaceae)	Tomato	Monad, spheroidal, Tricolporate	3-4	Herb	3-5 months	M
8	*Citrus limon* (Rutuceae)	Lemon-limbu	Radially symmetrical,isopolar & typically 4-5 colporate,sub-prolate to prolate Spheroidal shape with a medium size.	9	Evergreen tree	50 years	M
9	*Brassica campenstris* (Brassicaceae)	Canola-sarasav	Typically prolate or sub- prolate, tricolporate (3- grooved), radially symmetrical, reticulate exine	4-6	annual herb	4-5	M

			ornamentation				
10	*Sesamum indicum* (Pedaliaceae)	sesame-til	A prolate shape, a psilate(smooth), radially symmetrical, iso-polar, isodiametric appearance in equatorial view	6-8	Herb	3-6 months	M
11	*Syzygium cumini* (Myrtaceae)	Black plum-jamun	Small, monads, isopolar,oblate to suboblate in shape, with a triangular outline in polar view, 3 colporate	3-4	Tree	More than 100 years	M
12	*Tridax procumbens* (Asteraceae)	Coatbuttons-ghaburi	Oblate - spheroidal, tricolporate & have a tectum monad	11-3	Herb	perennial	M
13	*Hibiscus rosa-sinensis* (Malvaceae)	China rose Jasud	Spheroidal shape, surface covered with long, sharp & symmetrically distributed spines	8-9	Shrub	5-10 years	U
14.	*Ziziphus jujuba* (Rhamnaceae)	Red date-bor	Yellow, spheroidal - prolate, isopolar, tricolporate & have a bicomponent exine surface with small perforate - undulate or wrinkled weakly perforate patterns	6-7	Tree	More than 100 years	M
15.	*Mimosa pudica* (Mimosaceae)	Lajamani-touch-me-not	Pollen grains in tetrads, spherical, polar outline circular, equatorial outline is quadragular obtuse plane ecto-exine psilate	5-9	Shrub	More than 100 years	M
16.	*Mimosa invisa* (Mimosaceae)	Giant sensitive plant-lajamani	Arranged in polyads, oblate to spheroidal shape, pollen grains tetrads, equatorial outline, psilate pollen	7-2	Scram-bling shrub	1-2 years	M
17.	*Moringa oleifera* (Moringaceae)	Drumstick tree-sargavo	Spheroidal, pollen grains are oily or sticky, 3 colporate	1-3	Tree	20 years	M
18.	*Mangifera indica* (Anacardiaceae)	Mango-aam	Pollen grains monad, tricolporate, reticulate exine ornamentation, shape sub- prolate to prolate spheroidal	2-3	Tree	30-40 year	M
19.	*Foeniculum vulgare* (Apiaceae)	Fennel-variaali	Pollen grains monad, isopolar, tricolporate, exhibit a rugulate striate ornamentation, per-prolate	6-8	Herb	Few ye ars	U
20.	*Trachyspermum ammi* (Apiaceae)	Carom seed-ajwain	Radially symmetrical, isopolar, and tricolporate with prolate to per-prolate shapes, and exhibit various exine ornamentation patterns	10-11	Annual herb	2 years	U
21	*Litchi chinensis* (Sapindaceae)	‘’Litchi”	Pollen grains are isopolar, radially symmetrical and tricolporate,colpi are granulate	2-4	Evergreen tree	50-100 years	U
22	*Unknown* (Lamiaceae)	Unknown	-	-	-	-	-

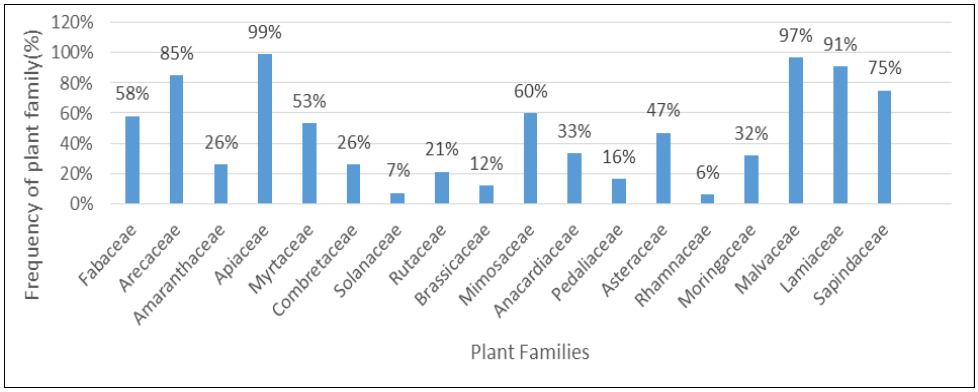


Fig 1: Frequency of occurrence of pollen types recovered from different honey samples.

All the 12 honey samples were tested for Moisture, pH, Free acidity, Total sugar content, Electrical conductivity (EC), Hydroxymethylfurfural (HMF), Total solids (TS) & Ash content. The pH (means) of three honey samples that is SP1, SP2 & SP3, are 4.21, 4.91 and 4.35 respectively. Five honey samples SP4, SP5, SP6, SP7, & SP8 have pH means of 4.54, 4.47, 4.86, 4.11 and 4.53. Then four honey samples which were collected from local marketed honey samples SP9, SP10, SP11 & SP12 have pH mean of 3.87, 5.00, 4.71, 4.57; which are significantly different from the above samples determined by the p-value of a hypothesis test at 95% confidence level. In comparison to the others, SP3 exhibited the highest acidity. It entails extending the shelf life of honey by inhibiting microbial growth.

Table 3: Physicochemical properties of multi-floral honey samples collected from Junagadh and Jamnagar district, Gujarat, India (mean ± standard deviation)

Sample no.	pH	Free Acidity (meq/kg)	Moisture Content (%)	Ash Content (%)	Electrical conductivity (µs/cm)	HMF (mg/kg)	Total Solids (%)	Total Sugar Content (mg/ml)
SP 1	4.21 ± 0.05	10.35 ± 0.35	12.33 ± 0.05	0.45 ± 0.07	1.112 ± 0.017	12.06±0.084	87.65±0.03	5.16±0.169
SP 2	4.91±0.014	3.95±0.07	4.17±0.03	0.5±0.0	155.5±7.77	4.8±0.02	95.80±0.007	2.795±0.106
SP 3	4.35±0.06	14.65±0.02	3.9±0.14	0.65±0.014	2.16±0.24	13.54±0.05	96.1±0.14	3.325±0.106
SP 4	4.54±0.07	4.9±0.14	16.73±0.014	0.35±0.070	492.25±3.18	17.85±0.15	85.77±3.52	0.71±0.127
SP 5	4.47±0.02	2.26±0.007	14.4±0.07	0.155±0.0070	355.7±8.06	41.16±0.014	87.1±2.05	0.375±0.0070
SP 6	4.86±0.014	4.2±0.14	13.25±0.35	0.165±0.000707	313.25±16.61	13.51±0.69	86.75±0.35	2.69±0.042
SP 7	4.11±0.014	4.1±0.14	18.2±0.02	0.35±0.070	675.35±6.57	5.37±0.24	81.98±0.03	1.163±0.024
SP 8	4.53±0.014	4.35±0.014	15.68±0.04	0.1	275.85±5.87	10.52±0.58	84.32±0.04	3.39±0.056

Table 4: Physicochemical properties of commercial honey samples (mean ± standard deviation)

Sample no.	pH	Free Acidity (meq/kg)	Moisture Content (%)	Ash Content (%)	Electrical conductivity (µs/cm)	HMF (mg/kg)	Total Solids (%)	Total Sugar Content (mg/ml)
SP 9	3.87±0.03	13.33±0.014	27.44±0.34	1.05±0.07	381.2±1.6	26.01±0.05	72.56±0.34	1.915±0.176
SP 10	5.0±0.014	7±1.41	14.74±0.05	0.3±0.3	139.9±0.42	26.45±0.07	85.265±0.05	3.375±0.077
SP11	4.71±0.014	4.63±0.04	17.93±0.03	0.5	194.3±6.08	36.94±1.81	82.07±0.03	1.85±0.127
SP 12	4.57±0.03	4.65±0.014	18.04±0.08	0.7±0.35	130.25±1.34	60.865±1.01	81.745±0.15	4.33±0.042

The moisture content in honey is a critical quality parameter influencing its stability & shelf life. The lowest moisture content in SP3 suggests it can be stored for long time. Contradictory, the highest moisture content in SP9 leads to spoilage, reduced shelf life & increases fermentation risk due to yeast activity.

The Ash content in honey represents the total mineral content, which is a crucial parameter for assessing honey's botanical & geographical origin, quality & purity. Dark coloured honey tends to have higher mineral content. As shown in table 3 & 4, the dark coloured honey SP9 has higher ash content 1.05%, SP5 than SP8. Very low ash content might suggest dilution or processing.

Electrical conductivity (EC) is an important parameter for assessing honey quality and botanical origin. The higher EC value of SP7 (675.35 µS/cm) in honey samples indicates high mineral content & possibly stronger antimicrobial properties. The lower EC value of SP1 (1.112 µS/cm) suggests a pure nectar source with lower mineral content.

The HMF is an important indicator of honey freshness & quality. As shown in table 3 and 4 HMF level of SP12 (60.86 mg/kg) indicates artificial honey production, excessive heating or storage under improper condition. The low HMF level of 4.8 mg/kg in SP2 indicates that the honey is likely fresh and has undergone minimal heating or storage, preserving its quality. When the HMF value ranges between 10 - 40 mg/kg, it indicates slight aging or mild heating honey sample.

Total solids in honey represent the percentage of all dissolved & suspended substances, mainly sugars, minerals, proteins, organic acids & other minor compounds. As indicated in tables 3 and 4, SP3's high total solids (96.1%) suggest good honey quality and decreased moisture content. The relatively low total solids content of 72.56% in honey SP9 indicates a higher moisture level, which may compromise shelf life by increasing the risk of fermentation and spoilage, as honey with lower total solids tends to exhibit reduced viscosity and greater fluidity. The relatively low sugar content in honey SP5 may indicate potential adulteration, fermentation, or an elevated water content, warranting further investigation.

Table 5: Results of antimicrobial activity of honey against *Escherichia coli*

Test Sample	Sample No.	Zone of inhibition(mm)			
		Concentration of honey			
		25%	50%	75%	100%
Escherichia coli	SP1	20	27	20	21
	SP2	19	19	20	18
	SP3	20	18	17	19
	SP4	18	19	22	19
	SP5	17	20	24	23
	SP6	24	19	24	24
	SP7	20	18	17	19
	SP8	17	21	20	19
	SP9	22	22	19	20
	SP10	19	21	22	23
	SP11	19	21	20	18
	SP12	21	22	17	21
	Mean ± standard deviation	19.67±2.015	20.58±2.47	20.17±2.48	20.34±2.06
Antibiotic (As a control)	Ciprofloxacin (5µg)	18mm			

As shown in Table 5, the highest antibacterial activity was observed in SP6 (24mm at 25%, 75%, and 100%) and SP1 (27mm at 50%), indicating strong antibacterial effects potentially attributed to high phenolic content or unique floral sources. Conversely, the lowest antibacterial activity was found in SP8 and SP5 (17mm at 25%), SP12 (17mm at 75%),

and SP4 (18mm at 25%). These values are still comparable to ciprofloxacin, meaning the honey still possesses antimicrobial properties but to a lesser content.

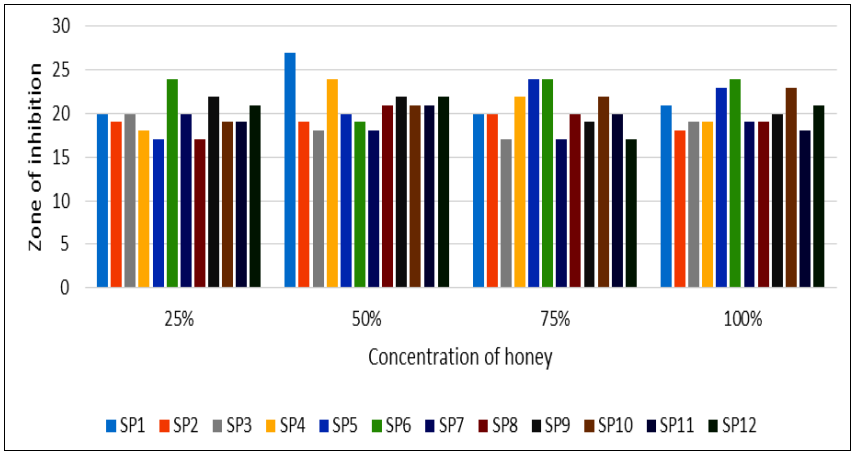


Fig 2: Antimicrobial activity of honey against *E. coli*

Honey activity at different Honey concentration (Figure: 2): At 25% concentration; ZOI ranges approximately 19.67mm, at 50% concentration; ZOI values approximately 20.58mm, at 75% concentration; ZOI values approximately 20.17 mm, at 100% concentration; ZOI ranges approximately 20.34 mm, showing strong antimicrobial activity. Several honey samples at different concentration exhibit inhibition zones equal to or larger than ciprofloxacin (eg.SP1, SP6 & SP9 at 50% show 27 mm, 24mm & 22mm respectively). This indicates potent antibacterial activity of these honey samples against *E. coli.*

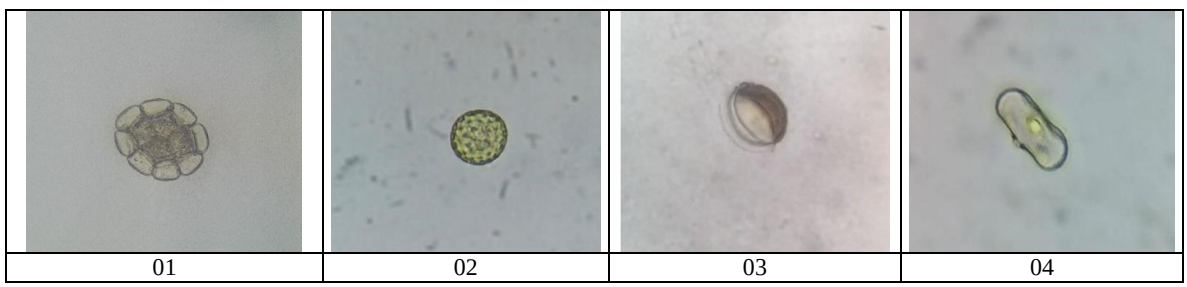
4. Conclusion

This study investigated the pollen composition, physicochemical properties, and antimicrobial activity of honey samples collected from different districts, with a focus on their activity against *Escherichia coli*. Pollen analysis revealed that bees predominantly foraged from Fabaceae and Apiaceae families, with Fabaceae pollen identified as tricolporate with elongated colpi and a prolate shape, while Amaranthaceae, Solanaceae, Rutaceae, Rhamnaceae, and Mimosaceae pollens were predominantly spheroidal with apertures. The observed morphological diversity, including variations in size, shape, aperture type, and polarity, contributes valuable taxonomic information for identifying plant species within their respective families.

Physicochemical analyses showed that all honey samples complied with international quality standards set by the Codex Alimentarius Commission and the International Honey Commission. Acidity levels varied across samples, with the highest recorded in sample SP3. Moisture content remained below 20%, indicating low susceptibility to fermentation, while total solids exceeded 80%, ensuring high stability during storage. Electrical conductivity values ranged between 0.15 and 0.18 mS/cm, aligning with European acacia honey standards, and hydroxymethylfurfural (HMF) levels were well below the maximum recommended threshold, confirming the freshness and high quality of the honey samples. Notably, honey from SP2 and SP3 demonstrated superior overall quality across these physicochemical parameters.

The antimicrobial activity assessment, conducted using the agar-well diffusion method, revealed strong inhibitory effects of the honey samples against *E. coli*, with some inhibition zones comparable to or exceeding those produced by ciprofloxacin. The antimicrobial effect was concentration-dependent, with the highest activity observed at 50% and 100% concentrations. In particular, samples SP1, SP6, and SP9 exhibited superior antimicrobial effects, suggesting a higher content of bioactive compounds.

Overall, these findings highlight the high quality and potent antimicrobial properties of honey from the studied regions, reinforcing its potential value not only as a nutritional product but also as a natural antimicrobial agent with possible preservative, cosmetic and therapeutic applications.



01	02	03	04

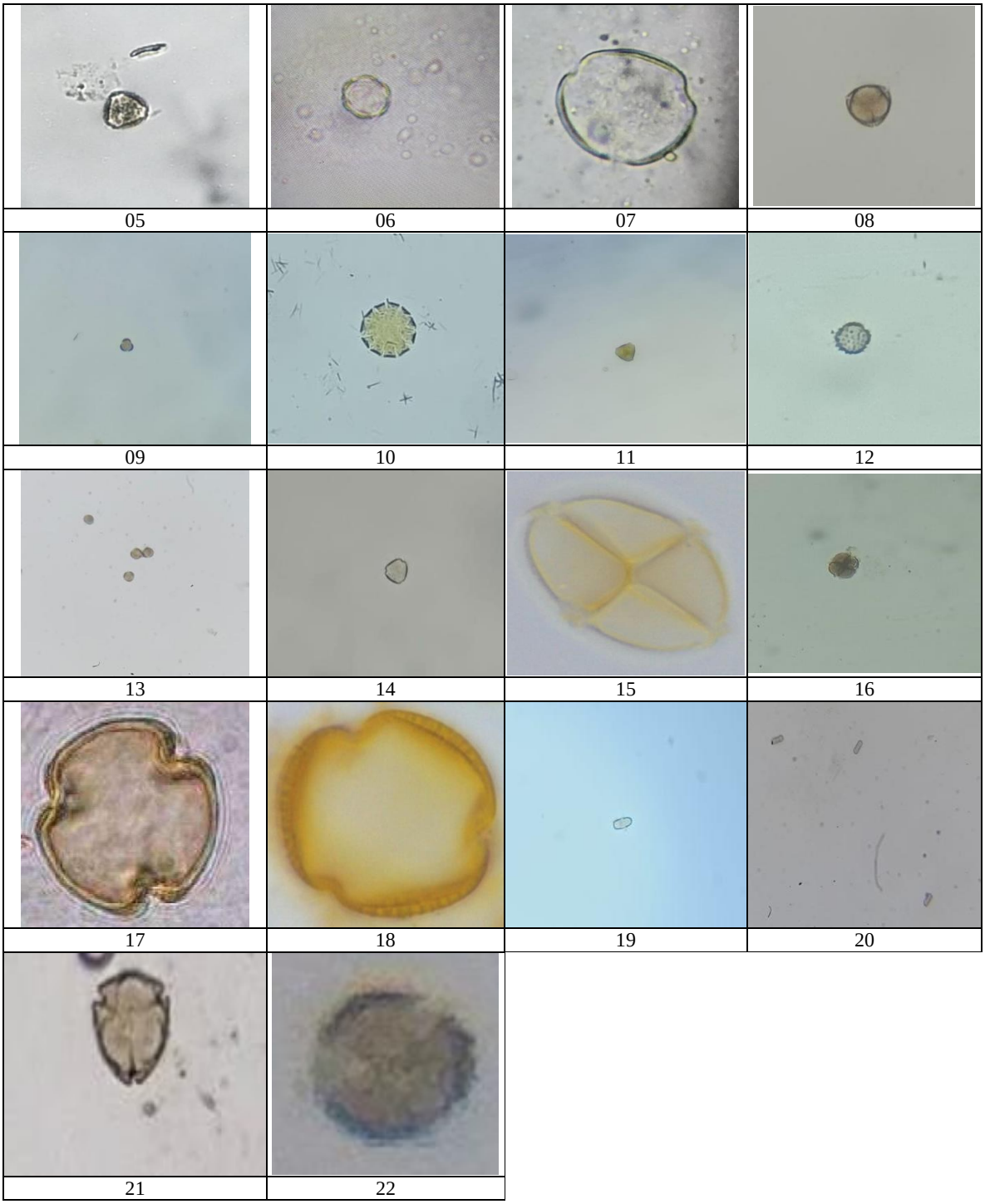


Fig 3: (01) *Acacia auriculiformis*, (02) *Chenopodium album* (03) *Cocos nucifera*, (04) *Coriandrum sativum*, (05) *Psidium guajava*, (06) *Terminalia sp.*, (07) *Solanum lycopersicum*, (08) *Citrus limon*, (09) *Brassica campenstris*, (10) *Sesamum indicum*, (11) *Syzygium cumini*, (12) *Tridax procumbens* (13) *Hibiscus rosa- sinensis*, (14) *Ziziphus jujuba*, (15) *Mimosa pudica*, (16) *Mimosa invisa*, (17) *Moringa oleifera*, (18) *Mangifera indica*, (19) *Foeniculum vulgare*, (20) *Trachyspermum ammi*, (21) *Litchi chinensis*.(22) *N/A*

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