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PHYSICOCHEMICAL, ANTIOXIDANT ACTIVITY, ANTIBACTERIAL ACTIVITY, AND MELISSOPALYNOLOGY STUDY OF FENNEL, ANISE, AND CORIANDER HONEY

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ABSTRACT

The specific composition of honey can impact its quality. So, the study aimed to evaluate the characterization and differentiation of physicochemical, biological, and melissopalynology analysis of three types of honey (fennel, anise, and coriander honey) from upper Egypt. The physicochemical properties (E.C, specific gravity, T.S.S, moisture, pH, free acidity, lactone, total acidity, (HMF), and total protein) were determined. The EC values ranged from (0.24-0.67 ms/cm), (0.28±0.02 ms/cm), and (0.21-0.46 ms/cm) at Fennel, Anise and Coriander honey respectively. The HMF content of honey samples varied from (0.96-38.78 ppm), (5.76-40.32 ppm) at Fennel and Anise honey, but Coriander honey ranged (1.54 to 33.02 ppm). In addition, total flavonoid content, total phenolic content, antibacterial, and antioxidant activity, and melissopalynology analysis were also evaluated in the samples. The physicochemical parameters of honey types are generally similar. As such, it isn't easy to differentiate between three types of honey based on the physicochemical analysis. Still, there's a significant difference in phenolic content between coriander honey and fennel or anise honey. In comparison, no significant differences were recorded in total flavonoid content or antioxidant activity among the three types of honey and flavonoid showed a highly significant positive correlation with antioxidant activity, in addition, the data of antibacterial were generally similar in the three types of honey under study, melissopalynology analysis showed that there's a slight difference in the shape of fennel, anise, coriander pollen grain. The difficulty of differentiating these honey types may be due to the similarity in plant chemical composition, which belongs to the same plant family (Apiaceae). This study will help the researcher and honey producer identify pure honey and verify its authenticity.

INTRODUCTION

The physical and chemical characteristics of honey vary mostly depending on the plant and geographic origins of the honey. Because honey is produced from many different plant flora substances, there's a variation in flavor, taste, aroma, and color of honey (Omoya and Akharaiyi, 2010). Since botanical authenticity has a direct impact on honey's marketing, many countries throughout the world carry out investigations into the authenticity of honey. Pollen analysis was one of the earliest techniques used to identify the botanical and geographic origin of honey (El Sohaimy *et al*., 2015). However, this method takes a lot of time and requires for specialized staff (Popek *et al.*, 2017). As a result, efforts are made to incorporate additional analytical techniques into the process of determining the honeybee honey's origin. Therefore, the most popular technique for identifying the source of honey is the physicochemical criteria used in the regular evaluation of honey (Svyatnenko *et al.*, 2023).

The quality of honey depends on the source of the nectar which includes bioactive substances such as polyphenols, proteins, organic acids, amino acids, minerals, vitamins and aroma compounds (Ferreira *et al.*, 2009 and Stelmakienė *et al.,* 2012). Acidity concentrations, apparent reducing sugar (which is determined as inverting sugar), apparent sucrose, moisture, HMF, mineral content and water-insoluble particles are among the compositional standards outlined in the current honey directive (Belay *et al.*, 2013). HMF is formed in an acidic media from reducing sugars in honey and used to determinate the honey quality because of a strongly correlated to the aging and overheating of honey (Yap *et al.*, 2019). 40 mg/kg is the HMF maximum limit in honey, while in tropical honey the limit is 80 mg/kg (Uzunca *et al*., 2023). Egyptian fennel honey chemical properties are compatible with most of the international standard specifications (Esmaeil *et al.*, 2020).

Honey typically has a protein level of less than 0.5% and a trace amount of enzymes. Its non-volatile components, such as minerals, sugar, amino acids, and phenolic compounds, contribute to its overall quality, including its taste, color, and other physical characteristics, whereas its volatile components mostly contribute to its aroma (Tarapoulouzi *et al.*, 2023).

Flavonoids and phenolic acids present in honey can also be used as markers of the honey's botanical origin. Because they are antioxidants, the phenolic components in honey help to make it healthy. The honey chemical compositions interact to influence the activity of honey bee product mixtures, which in turn affects their antioxidant activity (Kamel *et al.*, 2023). Antibacterial, antiviral, antimicrobial, anti-inflammatory, anti-diabetic, antioxidant, and wound and sunburn healing, antiparasitic, anti-mutagenic, and antitumoral actions are only a few of the biological capabilities of honey (Liu *et al.*, 2022).

The study aimed to characterize three types of honey, Fennel, Anise, and Coriander honey, based on their physicochemical, biological, and melissopalynology properties to identify the botanical origin and authenticity of honey, in progress to develop practical substitute methods and markers for assessment of the botanical and geographical origin of honey.

MATERIALS AND METHODS

The present investigation was carried out after identifying the honey samples' plant sources and ensuring that honey was collected from these plants. during the years 2022-2023. The physicochemical, and biological properties and microscopic examination were evaluated in the Bee Research Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. Thirty-three honey samples were collected directly from the primary producers at apiaries in Upper Egypt, without any thermal treatment. Immediately after harvesting, the samples were subjected to analysis: 17 samples of fennel honey, 11 samples of anise honey, and 5 samples of coriander honey.

To determine the physicochemical parameters. Every sample was made three times. A Hanna multimeter was used to measure the electrical resistance of honey aqueous solutions containing 20% dry matter at 25°C to calculate electrical conductivity (EC) (International Honey Commission Methods (2009)). The results were expressed as mille Siemens per centimeter (mS/cm). specific gravity was determined gravimetrically (Chen *et al*., 2014) and the results were expressed as (g/ml). The sample's moisture content and total soluble solid content were determined by Abbe Refractometer ATAGO RX-5000) (Saxena *et al*., 2010). The percent of total solids was determined using the equation (total solids (%) = 100 - moisture content), and the results were expressed as %.

pH value was measured using a solution of 10 g honey in 75 ml of distilled water using a potentiometric pH meter (Hanna Instruments). The titrimetric method was used to determine the free acidity, lactone and total acidity of honey (Bogdanov, 1997). Free acidity, lactone, and total acidity were expressed as (mille equivalent/kg). The method that determines the concentration of 5-hydroxymethylfural (HMF) in honey was using *p*-toluidine and barbituric acid solutions which added to the honey solution and the color intensity is measured at 550 nm (libra spectrophotometer) (Winkler, 1955)., and the results were expressed as ppm. The total soluble protein content in honey samples was determined by the colorimetric method at 550nm (Yatzidis, 1987)., and the results were expressed as mg/100g.

Total polyphenol content (TPC) is determined by the Folin-Ciocalteu method with a slightly modified. Gallic acid equivalent (GAE) in milligrams per 100 grams of honey was used to express the results. Total antioxidant activity was determent by diluted the sample to 40% of honey. The antioxidant activity was expressed as a percentage of 2,2 diphenyl-1-picrylhydrazyl solution (DPPH I%). Ascorbic acid and gallic acids were used as antioxidant standard chemicals to produce a calibration curve and the mean value was expressed as milligrams of ascorbic acid equivalent (mg Vit. C E) and milligrams of gallic acid equivalent (mg GA E) antioxidant per 100 g of honey (Pandey *et al.,* 2020).

Antimicrobial activity was conducted in the presence of *staphylococcus aureus* and *E. coli* isolates, which were transported in a brain-heart infusion broth media to the study samples. Two isolates were obtained from Animal Health Research Institute, Doki-Giza. The agar disc diffusion method was used to detect the antibacterial activities of the collected honey samples against the tested bacteria, The tested bacteria suspension (10^6 CFU/µl) was spread on Mueller-Hinton agar. The agar was cut into 5 mm discs diameter, and these wells were loaded with 100µL of honey solution sample (from 20% to 100% concentration) and incubated at 37°C for 24 h. The inhibition zone diameters were detected. The negative control was done by distilled water (Suhartatik *et al.,* 2023). The minimum inhibitory concentrations (MICs) for three studied honey types were evaluated by a microdilution technique.

Melissopalynological analysis were performed to confirm the botanical origin of the honey samples. The pollen grain slides for the honey sample under the study (fennel, anise, and coriander plant flower pollen grain) were prepared by putting it on a light microscope slide where it was mounted in glycerin jelly and covered as a key plant pollen grain. 10 g of honey sample was dissolved in 40 mL of distilled water and centrifuged (6000rpm/10min) in the sample solution, the sediment was collected and spread on a microscope slide. The different magnifications were used as follows: 40× and 100× for identification of honey's botanical origin by comparing with prepared slides of key plant pollen grains (Ketfi *et al.,* 2023).

Table (1): The type of plant identifying honey bee pollen grains

Common name	Scientific name	Order/Family	Flowering time
Fennel	*Foeniculum vulgare*	Order: Apiales Family: Apiaceae	Spring
Anise	*Pimpinella anisum*		Spring
Coriander	*Coriandrum sativum*		Spring/Summer

Statistical analysis

Each honey sample's measurements were taken in triplicate. Data analysis was performed using the SPSS for Windows Version 26 software (Escuredo *etal.,* 2023).

RESULTS AND DISCUSSION

Physicochemical analysis

The physicochemical characteristics of three types of Egyptian honey bees Fennel, Anise, and Coriander honey were reported in Table (2). The electrical conductivity (EC) of honey is an excellent indicator of its adulteration and can reflect its origin from nectar. The EC values in Table (2) ranged from (0.24-0.67 ms/cm), (0.28±0.02 ms/cm), and (0.21-0.46 ms/cm) at Fennel, Anise and Coriander honey respectively. The electrical conductivity showed no significant difference among the examined honey samples. The EC values in Fennel, Sidr, Nigella, Marjoram and Anise honey ranged from 0.008 to 0.039% (El-Dereny, 2023). honey collected from North Sinai Governorate, Egypt and obtained from different floral sources, The EC values showed no significant difference among the examined honey samples ranging from 0.007 to 0.0096% (Nafea *et al.,* 2023).

Density, expressed as specific gravity (Sg), is an important characteristic of honey. It is influenced by water content, temperature, and solids concentration. (Sg) values in Table (2) showed no significant difference among the examined honey samples in specific gravity. The density values for different Libyan honey types form different plant sources ranged from 1.39 to 1.43 g/ml (Nafea *et al.,* 2009). The specific gravity of the sider honey sample ranged from 1.415 ±0.018 g/ml to 1.417 ±0.073 g/ml (Zidan, 2019).

The data indicates that there is no significant difference in total soluble solids values among the tested honey samples, while the results ranged (from 82.0-83.5, 81.5-83.0, and 81.5-82.5%) at Fennel, Anise, and Coriander honey resp. the moisture content of honey is the quality property that evaluates the stability of honey and resists fermentation spoilage by yeast. The moisture % of fennel and anise honey ranged from 16.5-18.0% and 17.0-18.5%, while coriander honey ranged from 17.5-19.5%. The moisture content value was 17.2 ± 0.86 % in honey (Edo *et al.,* 2023).

Acidity participates in the honey flavor and also in its antimicrobial activity, statistical analysis showed that the pH, free acidity, lactone, and total acidity content were not significant difference values among the tested honey samples. The fennel honey pH values ranged from (3.7-4.4), while anise honey was (3.2-4.4) and coriander honey they were (3.9-4.1).

The pH of clover honey ranged from 3.7 to 4.15 (Essa *et al.*, 2010). The pH values of the fennel honey samples ranged from 4.1 to 4.8 (Esmaeil *et al.*, 2020).

The free acidity ranged between (11.5-42.5 meq./kg), (11.5-31.5 meq./kg), and (14.0-20.0 meq./kg) at the three types of honey. The lactone content ranged from 1.00-14.00 meq./kg, (0.50-6.00 meq./kg), and (1.00-4.50 meq./kg). while the fennel honey total acidity samples ranged from 17.5 to 45.0 meq./kg at the anise honey (15.00-32.0 meq./kg), while at coriander honey samples ranged from 16.50-21.0 meq./kg. these data is shown in Table (2). the pH values ranged from 3.28 – 5.33, with a mean value of 3.91, free acidity ranged from 19.5 to 31.5 meq/kg, the lactone content value ranged from 6.0 to 17.5 meq/kg and the total acidity ranged from 25.5 to 48.0 meq/kg of fennel honey samples (Edo *et al.*, 2023).

Hydroxymethyl furfural (HMF), is a good quality criterion for evaluating the honey's freshness. The HMF content of honey samples varied from (0.96-38.78 ppm), (5.76-40.32 ppm) at Fennel and Anise honey, but Coriander honey ranged (1.54 to 33.02 ppm). The fennel honey samples HMF content collected from Upper Egypt ranged from 1.92 to 7.68 ppm., with a mean value 4.96 ± 0.591 ppm (Esmaeil *et al.*, 2020).

Table (2): physicochemical characteristics of three types of Egyptian honey (fennel, anise and coriander honey).

Honey types	Sample No.	EC (ms/cm)	Specific Gravity (g/ml)	TSS (%)	Moisture (%)	pH	Free acidity (meq/Kg)	Lactone (meq/Kg)	Total acidity (meq/Kg)	HMF (ppm.)	Total Protein (mg/100g)
Fennel	F1	0.28	1.420	82.50	17.50	3.90	20.00	2.00	22.00	27.26	258.10
	F2	0.30	1.420	83.00	17.00	4.00	17.50	2.50	20.00	8.06	249.40
	F3	0.27	1.420	82.50	17.50	3.90	20.00	1.00	21.00	2.88	232.00
	F4	0.28	1.420	82.50	17.50	3.80	17.00	6.00	23.50	9.41	284.20
	F5	0.24	1.430	82.50	17.50	3.70	15.00	4.00	19.00	0.96	229.10
	F6	0.23	1.430	83.00	17.00	3.90	17.50	3.50	21.00	32.83	188.50
	F7	0.26	1.430	83.00	17.00	4.00	20.00	3.50	23.50	17.47	226.20
	F8	0.28	1.430	82.50	17.50	3.90	17.50	4.00	21.50	38.78	197.20
	F9	0.27	1.430	83.00	17.00	4.00	19.50	8.50	28.00	36.48	272.60
	F10	0.27	1.430	83.00	17.00	4.00	11.50	6.00	17.50	7.10	243.60
	F11	0.31	1.420	83.50	16.50	4.10	11.50	14.00	25.50	7.87	205.90
	F12	0.26	1.420	83.00	17.00	3.90	15.00	4.00	19.00	8.64	232.00
	F13	0.66	1.420	82.00	18.00	4.30	42.50	2.50	45.00	18.24	234.90
	F14	0.29	1.420	83.00	17.00	4.20	20.50	1.00	21.50	14.40	205.90
	F15	0.26	1.420	82.50	17.50	4.00	16.50	4.00	20.50	11.71	237.80
	F16	0.67	1.420	82.00	18.00	4.40	38.50	4.50	43.00	36.48	394.40
	F17	0.67	1.420	82.00	18.00	4.30	42.50	2.00	44.50	25.15	246.50
Mean± Se		0.34± 0.04	1.424± 0.001	82.68± 0.101	17.32± 0.101	4.02± 0.04	21.32± 2.26	4.29± 0.72	25.62± 2.16	17.87± 2.96	243.43± 10.91
Rang		0.24-0.67	1.42-1.43	82.0-83.5	16.5-18.0	3.7-4.4	11.5-42.5	1.00-4.00	17.5-45.0	0.96-38.78	197.2-394.4
Anise	A1	0.21	1.410	83.00	17.00	3.90	15.50	2.00	17.50	8.64	217.50
	A2	0.24	1.420	83.00	17.00	4.00	20.00	2.00	22.00	13.63	278.40

Honey types	Sample No.	EC (ms/ cm)	Specific Gravity (g/ml)	TSS (%)	Moisture (%)	pH	Free acidity (meq/ Kg)	Lactone (meq/ Kg)	Total acidity (meq/ Kg)	HMF (ppm.)	Total Protein (mg/ 100g)
	A3	0.26	1.420	83.00	17.00	4.00	20.00	2.00	22.00	36.10	211.70
	A4	0.24	1.430	83.00	17.00	3.80	17.50	6.00	23.50	19.20	153.70
	A5	0.23	1.420	83.00	17.00	3.90	16.50	2.50	19.00	7.49	275.50
	A6	0.42	1.420	82.00	18.00	3.20	27.50	4.00	31.50	5.76	220.40
	A7	0.28	1.430	82.00	18.00	3.90	22.50	4.00	26.50	32.83	203.00
	A8	0.27	1.420	83.00	17.00	4.00	17.50	3.00	20.00	33.80	272.60
	A9	0.26	1.420	81.50	18.50	4.00	11.50	3.50	15.00	14.60	249.40
	A10	0.46	1.420	82.00	18.00	4.10	31.50	0.50	32.00	15.36	234.90
	A11	0.26	1.420	83.00	17.00	4.10	22.00	1.00	23.00	40.32	304.50
Mean± se		0.29± 0.02	1.421± 002	82.59± 0.176	17.41± 0.176	3.90± 0.08	20.18± 1.69	2.77± 0.47	22.91± 1.62	20.70± 3.82	238. 33± 12.99
Range		0.21- 0.46	1.410- 1.43	81.5- 83.0	17.0- 18.5	3.2-4.1	11.5- 31.5	0.50- 6.00	15.00- 32.0	5.76- 40.32	153.0- 304.5
Coriander	C1	0.29	1.420	81.50	18.50	4.10	15.00	3.50	18.50	23.42	179.80
	C2	0.28	1.420	82.50	17.50	4.00	20.00	1.00	21.00	26.88	205.90
	C3	0.28	1.420	82.00	18.00	4.10	15.00	4.50	19.50	1.54	197.20
	C4	0.20	1.410	82.50	17.50	3.90	14.00	2.50	16.50	3.26	171.10
	C5	0.21	1.420	82.00	18.00	4.00	16.50	3.50	20.00	33.02	229.10
Mean± Se		0.25± 0.02	1.418± 0.002	82.100 ± 0.187	17.90± 0.187	4.02± 0.04	16.10± 1.05	3.00± 0.59	19.10± 0.76	17.62± 6.41	196.62± 10.19
Rang		0.21- 0.29	1.410- 1.42	81.5- 82.5	17.5- 18.5	3.9-4.1	14.0- 20.0	1.00- 4.50	16.50- 21.0	1.54- 33.02	171.1- 229.10
P value		0.653	0.0906	0.0798	0.105	0.445	0.105	0.265	0.195	0.831	0.074

Se: standard error, *P* value: probability value (0.05), TSS: total soluble solids, ppm: part per million, meq: mille equivalent, HMF: hydroxymethyl furfural, ms: milli siemens, EC: electrical conductivity. F: fennel honey sample, A: anise honey sample, C: coriander honey sample

Data in Table (2) showed that the protein content levels of honey samples ranged from (197.2-394.4 mg/100 g), (153.0-304.5 mg/100 g), and (171.1-229.10 mg/100 g) at fennel, anise, and coriander honey, resp. the highest value was shown in the fennel honey sample, with insignificant difference values among the tested honey samples under the study.

Analysis of total phenolic content, total flavonoid content, and antioxidant activity

All the tested honey samples contained varied significant levels of phenolic compound content. The total phenolic content, flavonoid content and antioxidant activity of the fennel, anise, and coriander honey were reported in Table (3).

Fennel and anise honey were characterized by a significantly higher content of phenolic content (with mean of 15.57 and 14.34 mg GAE/100 g, resp.), and the lowest total phenolic content was recorded by coriander honey (7.47 mg GAE/100 g). There is no significant difference between fennel and anise honey in total phenolic content, but coriander honey differs slightly from fennel or anise honey in phenolic content. The obtained results are comparable with other authors' findings. The phenolic content of mustard and coriander honey ranged from 200 to 462 mg as gallic acid equivalent per kg of honey (GAE/kg). but, phenolic compound content in coriander honey was more than mustard honey, the mean value of coriander honey total phenolic content was 50.3 ± 0.4 mg/100 g. (Zhang *et al.*, 2021 and Vîjan *et al.*, 2023).

The phenolic content of the mono-floral honey collected from Anatolia ranged from 98 mg GAE/kg in the acacia honey to 1326 mg GAE/kg in the heather honey, 567 mg GAE/kg in the lavender honey, 44.8 mg GAE/kg in the acacia honey and 241.4 mg GAE/kg in honeydew. But the phenolic content ranged from 126.4 in acacia honey to 905.7 mg GAE/kg in forest honey) in Croatian honey (Kaygusuz *et al.*, 2016).

The honey samples phenolic compounds levels were varied according to the botanical organ species. It may be due to that phenolic compounds are transferred from plants to honey and each honey type sample has a different botanical profile, The total flavonoid content Table (3) showed a maximum average value level in the fennel and anise honey samples (3.51, 3.67 mg QE/100 g, respectively), followed by coriander honey (2.10 mg QE/100 g), and the three types of honey did not differ significantly in terms of total flavonoid content.

The total flavonoid concentration of honey samples varied between 1.7 and 4.5 mg of QE/100 g of honey and the highest total flavonoid concentration was found in ziziphus honey, which did not differ significantly from coriander honey. The lowest total flavonoid content was found in orange blossom honey, which did not differ significantly from alfalfa honey. The total flavonoid content of coriander and mustard honey varied from 43.39 to 54.92 mg QE/kg, while the total flavonoid concentration of fennel honey was 31.81±0.08 mg/100g QE. Initially, it appeared that all samples of coriander honey had more flavonoids than mustard honey (Zhang *et al.,* 2021).

The linden honey has 32.0 µg of rutin equivalents (RE)/g of flavonoids, which is nearly twice as much as rapeseed honey, which has 13.5 µg of RE/g. Up to 42% of the phenolic components in mono-floral honey are flavonoids. Furthermore, flavonoids, which are also expressed as quercetin equivalents, accounted for 2–10% of honey's total phenolic content (Mărgăoan *et al.*, 2021).

The propolis, pollen, and nectar are the primary sources of flavonoids in honey (Vîjan *et al.*, 2023 and Mărgăoan *et al.*, 2021).

Enzymes, products of the Maillard process, ascorbic acid, organic acids, phenolic acids, flavonoids, amino acids, peptides, phenolic acids and molecules that resemble carotenoid are some of the chemicals that give natural honey its antioxidant properties. The DPPH assay, which is commonly used to assess radical scavengers in natural foods and is one of the most stable free radicals, was employed to measure the antioxidant activity of the examined items. As a percentage of DPPH inhibition, the average antioxidant activity of the studied honey samples Table (3) for a 20% w/v honey solution varied from 1.03% for coriander honey to 2.21% for fennel honey and 3.27% for anise honey. However, there was no significant difference between the three types of honey. In addition, the antioxidant capacity of vitamin C or gallic acid equivalent is also reported.

Table (2): Total phenolic content, total flavonoid content, and antioxidant activity of fennel, anise and coriander honey samples (20% w/v)

Honey type		Total phenolic content. (mgGAE/100g)	Total flavonoid content. (mgQE/100g)	Antioxidant activity		
				DPPH I%	As mgVit.C E/100g	As mgGAE/100g
Fennel	F1	13.31	2.38	1.81	19.79	10.90
	F2	9.96	2.73	0.93	11.00	5.07
	F3	12.43	2.61	1.72	18.90	10.31
	F4	12.87	3.72	0.28	4.50	0.77
	F5	9.35	2.59	0.89	10.60	4.81
	F6	14.58	2.73	2.54	27.09	15.79
	F7	11.55	2.48	1.02	11.90	5.67
	F8	13.26	2.32	2.49	26.59	15.41
	F9	12.71	2.54	1.92	20.89	11.63
	F10	13.97	2.98	1.72	18.90	10.31
	F11	11.99	2.25	1.55	17.20	9.18
	F12	11.55	2.61	1.90	20.69	11.50
	F13	25.52	7.91	4.93	4.93	31.54
	F14	12.43	3.44	2.35	25.19	14.48
	F15	11.99	2.77	2.08	22.49	12.69
	F16	33.22	7.97	5.92	60.89	38.14
	F17	33.94	5.67	3.47	36.39	21.90
mean± se		15.57[a]±1.79	3.51±0.44	2.21±0.35	21.06±3.18	13.54±2.24
Rang		9.35-33.94	2.25-7.97	0.28-5.92	4.50-60.89	0.77-38.14
Anise	A1	8.14	2.73	0.58	7.50	2.75
	A2	8.75	2.26	1.30	14.70	7.52
	A3	12.16	2.71	2.08	22.49	12.69
	A4	10.40	2.61	2.18	23.49	13.35
	A5	10.84	2.77	2.20	23.69	13.49
	A6	32.62	9.91	9.60	97.69	62.52
	A7	16.67	2.30	2.58	27.49	16.01
	A8	18.98	3.62	0.84	10.10	4.48
	A9	11.99	2.23	1.98	21.49	12.03
	A10	9.90	4.37	9.88	100.49	64.37
	A11	17.33	4.84	2.83	29.99	17.66
Mean± se		14.96 [a]±2.14	3.67±0.68	3.27±0.99	34.47±9.86	20.62±6.53
Range		8.14-32.62	2.23-9.91	0.58-9.88	7.50-100.49	2.75-64.37
Coriander	C1	7.81	2.30	1.64	18.06	9.75
	C2	13.48	1.22	1.85	20.19	11.17
	C3	6.66	2.52	0.71	8.80	3.61
	C4	4.79	1.97	0.71	8.80	3.61
	C5	4.62	2.48	0.26	4.30	0.63
mean± se		7.47 [b]±1.62	2.10±0.24	1.03±0.30	12.03±3.03	5.75±2.01
Rang		4.62-13.48	1.22-2.48	0.26-1.85	4.30-20.19	0.63-11.17
P value		0.020	0.054	0.145	0.207	0.130
LSD		2.692	ns	ns	ns	ns

Se: standard error, ns: not significant, LSD: low significant difference at 5%, (0.05), GAE: gallic acid equivalent, QE: quercetin equivalent, DPPH I%: a percentage of inhibition 2,2- di phenyl picrylhydrazyl, Vit. C: vitamin C equivalent, F: fennel honey sample, A: anise honey sample, C: coriander honey sample.

Fennel honey recorded 2601.84 ±51.23 (µg/100 g, rutin) antioxidant capacity (Zhang *et al.,* 2021).

The honey samples capacity to scavenge the DPPH radical, as measured by their inhibition concentration (IC50), varied between 4.58 and 5.54 mg/ml. The maximum antioxidant activity was found in coriander-processed honey (4.58 mg/ml), which was comparable to raw honey (4.59 mg/ml). The findings were explained by variations in the antioxidants, flavonoids, and phenols that depended on the honey's floral source. Ziziphus and coriander honey have significant levels of antioxidant activity, which is likely due to high levels of phenolic chemicals (Khalafi *et al.*, 2016). the percentage of DPPH inhibition for 20% w/v honey solution ranged from 23.8% in polish honeydew to 100% in polish heather and buckwheat honey (Wilczynska, 2010). The Italian honey radical scavenging activity ranged from 55.06% for citrus honey to 75.37% for chestnut honey when assessed for 3-60% w/v honey solution (Perna *et al.,* 2013). Geography has a significant impact on the scavenging of free radicals. Pollen and nectar are the primary sources of phenolic chemicals, which give honey its antioxidant properties (Kamel *et al.,* 2023).

Data in Fig. (1) showed that the correlation matrix between the total phenol and flavonoid content of each honey type and their respective anti-oxidation capacity is significant at the 0.01 level (2-tailed). This work focused on evaluating the relationship of bioactive content (phenolic or flavonoid) in honey samples under study with their antioxidant activity. The correlation matrix data (Fig. 1) indicates that flavonoids exhibit a strong significant positive correlation in studied honey samples with antioxidant activity (r=0.746**), and a positive correlation between phenolic compound content and flavonoid content (r=0.842**), in addition, the correlation was slightly medium in the relation between antioxidant and phenolic content (r=594**) and correlation matrix relation was in a high significant level ($p < 0.001$).

The obtained results confirmed the significant influence of the phenolic content and flavonoid content of honey on their antioxidant activity and flavonoid content was more affected than phenolic content on antioxidant activity. Data indicates that antioxidants, phenolic, and flavonoid content could be useful parameters for identify the botanical origin of honey.

In reference to the relationship between the biochemical quality measures of honey, there’s a strong positive correlation between the total flavonoid content and DPPH I% in Algerian honey and several Malaysian samples (Ailli *et al.,* 2024).
The phenolic components and the exceptional antioxidant and antiradical properties of the coriander and mustard flower honey from Rajasthan State, India. A noteworthy positive correlation between DPPH (1%), and total phenolic content (Khan *et al.,* 2017).

According to earlier studies, honey's flavonoid and total phenolic acid contents are important bioactive ingredients that have an antioxidant function; the higher the flavonoid content, the stronger the honey's antioxidant qualities (Cianciosi *et al.,* 2018 and Cheung *et al.,* 2019). The findings were explained by variations in the antioxidants, flavonoids, and phenols that rely on the honey's floral source. it is challenging to identify reliable indicators that can be used to determine the origin of honey, particularly since these indicators differ significantly depending on the honey's botanical origin. Therefore, it is advised to use more sensitive techniques to identify honey, such as infrared and spectroscopy, as well as chromatographic methods like HPLC or GC-MS (Mădaş *et al.*, 2019).

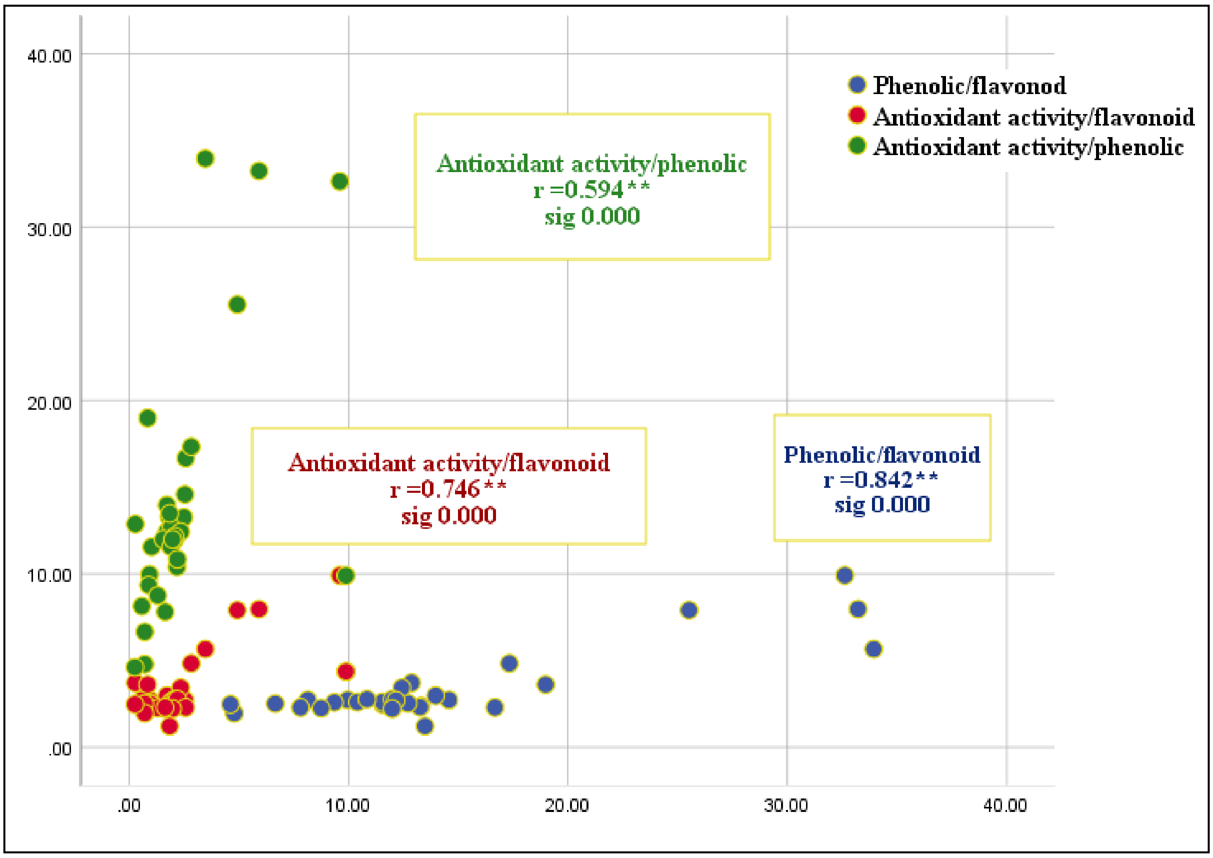


Figure (1): Pearson correlation coefficients between total phenolic content, flavonoid content, and antioxidant activity of fennel, anise, and coriander honey samples (r values are presented and the level of significance $p < 0.001$).

Antibacterial activities analysis

The investigated types of honey samples showed significant antibacterial activities against the tested bacteria at a concentration of 40% to 100% Table (4). At two isolates (*Escherichia coli*) and (*Staphylococcus aureus*) The findings revealed the honey's antibacterial effectiveness according to different concentrations of all honey samples sensitivity of gram-positive bacteria and gram-negative bacteria to honey types at the concentration of 40% to 100% except sample no. (A3 and A4) in anise, the honey type was sensitive at 60% concentration. The antimicrobial effectiveness of honey is influenced by various crucial elements. These include osmolarity, the amount of H_2O_2 present, a high concentration of sugars, a low pH level, the levels of phenolic acid, and the presence of flavonoids. Additionally, honey contains other phytochemical factors like peroxides, benzoic acid fatty acids, terpenes, phenols, ascorbic acid, benzyl alcohols and amylase. These factors contribute to honey's ability to combat pathogenic bacteria, resulting in either bacteriostatic or bactericidal effects. The Floral honey, cotton, citrus, and camphor, had a stronger antibacterial effect than non-floral honey. All types of honey were most effective on Gram-positive bacteria, including against *S. aureus*, and *B. subtilus*, compared to Gram-negative (Alvarez-Suarez *et al.*, 2010).

Melissopalynology Analysis

Melissopalynology deals with pollen analysis of honey. This analysis provides relevant information about the plants honeybees prefer as pollen and nectar sources. It also aids in determining the geographical and botanical origin of the honey (Ponnuchamy *et al.,* 2014). In this study, we conducted meliss palynological and physicochemical analyses of honey samples of fennel, anise, and coriander. A collection of reference pollen slides and photographic figures is very helpful for identifying pollen types and interpreting pollen spectra. The pollen grains in the honey samples were identified using the plant reference pollen slides of these kinds of honey (Fig. 2).

The pollen grains of fennel, anise, and coriander were elliptic in shape and monad (dispersal unit consisting of a single pollen grain) and slightly convex in shape. The pollen grain has a thicker wall surrounding it, and there is a circular protrusion in the middle of it. The three pollen grains are very similar with a slight difference; fennel and coriander pollen grains are more likely a bean shape, but anise pollen grains are more likely to have a kidney shape. In anise grain there is a contraction or a squeezing in the middle of it; otherwise, there are two protrusion curves in coriander pollen grain cell walls. (Arguelles *et al*., 2015).

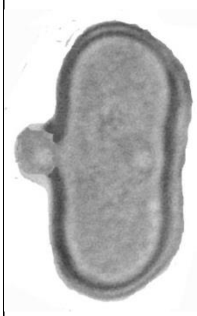
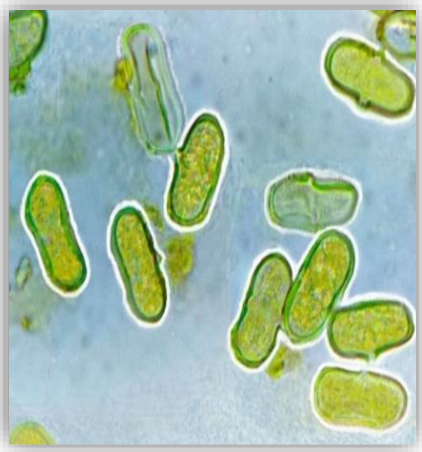
The pollen grains' shape varies from species to species. The pollen grains shape is found to be useful in pollen identification. the shape may vary considerably within one grain type or even within one species. The pollen grains of the Apiaceae species, presented a similar external morphology (D'Ávilaa *et al.*,2016). the three species (*Anethum graveolens* L., *Coriandrum sativum* L., *Foeniculum vulgare* Mill.) of Apiaceae's pollen grains could not be distinguished morphologically. as the form, ornamentation, as well as the dimensions of the aperture were all similar (Jones and Jones ,2001). For the identification types of pollen and the interpretation of pollen spectra, extensive experience, and specific training are required. A reference pollen slide collection and a photographic atlas are helpful (Campos et al., 2021). There are many disadvantages to the melissopalynological method. These include the fact that this method requires a comprehensive collection of pollen grains. Pollen grains of various species can vary in size and aspect: filamentous, disc, round or bean-shaped, and sometimes oval-shaped.

Table (3): The antibacterial activity of different types of honey Fennel, Anise and Coriander

Honey types \ Isolate of		*Escherichia coli*					*Staphylococcus aureus*				
		Honey concentration (%w/v)									
		20	40	60	80	100	20	40	60	80	100
Fennel	F1	-	+	+	+	+	+	+	+	+	+
	F2	-	+	+	+	+	+	+	+	+	+
	F3	-	+	+	+	+	+	+	+	+	+
	F4	-	+	+	+	+	+	+	+	+	+
	F5	-	+	+	+	+	+	+	+	+	+
	F6	-	+	+	+	+	+	+	+	+	+
	F7	-	+	+	+	+	+	+	+	+	+
	F8	-	+	+	+	+	+	+	+	+	+

Honey types	Isolate of	*Escherichia coli*					*Staphylococcus aureus*				
		Honey concentration (%w/v)									
		20	40	60	80	100	20	40	60	80	100
	F9	-	+	+	+	+	+	+	+	+	+
	F10	-	+	+	+	+	+	+	+	+	+
	F11	-	+	+	+	+	+	+	+	+	+
	F12	-	+	+	+	+	+	+	+	+	+
	F13	-	+	+	+	+	+	+	+	+	+
	F14	-	+	+	+	+	+	+	+	+	+
	F15	-	+	+	+	+	+	+	+	+	+
	F16	-	+	+	+	+	+	+	+	+	+
	F17	-	+	+	+	+	+	+	+	+	+
Anise	A1	-	+	+	+	+	+	+	+	+	+
	A2	-	+	+	+	+	+	+	+	+	+
	A3	-	-	+	+	+	+	+	+	+	+
	A4	-	-	+	+	+	+	+	+	+	+
	A5	-	+	+	+	+	+	+	+	+	+
	A6	-	+	+	+	+	+	+	+	+	+
	A7	-	+	+	+	+	+	+	+	+	+
	A8	-	+	+	+	+	+	+	+	+	+
	A9	-	+	+	+	+	+	+	+	+	+
	A10	-	+	+	+	+	+	+	+	+	+
	A11	-	+	+	+	+	+	+	+	+	+
Coriander	C1	-	+	+	+	+	+	+	+	+	+
	C2	-	+	+	+	+	+	+	+	+	+
	C3	-	+	+	+	+	+	+	+	+	+
	C4	-	+	+	+	+	+	+	+	+	+
	C5	-	+	+	+	+	+	+	+	+	+

F: fennel honey sample, A: anise honey sample, C: coriander honey sample

		Plant reference pollen grain	Honey sample pollen grain
Fennel			

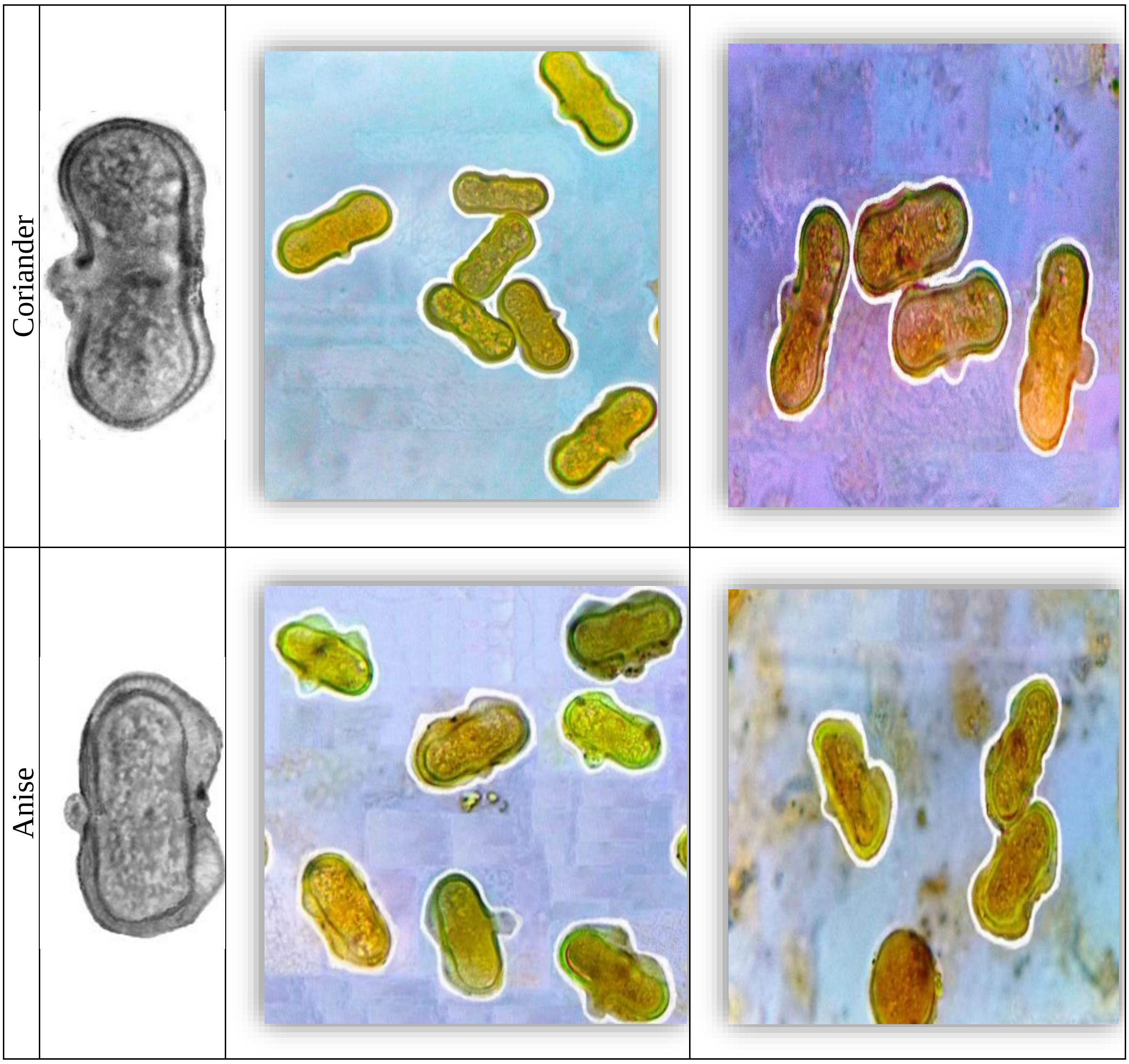


Figure (2): Photomicrographs showing the fennel, anise and coriander pollen grain in honey samples with plant reference pollen slides (40x).

The natural color is mostly yellow, cream, white, or orange color. The texture of the cell wall shows also great variations, from spiky to smooth. Techniques based on the analysis of honey composition as reference methods are typically time-consuming, require the use of expensive and environmentally harmful chemicals, and can only be carried out by skilled technicians to perform. As a result, spectroscopic techniques, combined with appropriate chemometric multivariate methods, have gained importance in honey analysis as a tool for accurately classifying, authenticating, and detecting adulterating honey (Abd El Dayem *et al.*, 2024).

CONCLUSIONS

The physicochemical parameters of honey are generally similar so, it is very difficult to differentiate between three honey types based on the physicochemical analysis, while phenolic content, flavonoid content and melissopalynology study may be slightly helpful. Under these conditions, many other non-destructive, fast, reliable, easy, and inexpensive analysis methods are needed like FTIR, UV-VIS spectroscopy, chromatography analysis, and chemometric analysis which may enable the authentication of the honey samples.

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CONFLICT OF INTEREST

There is not conflict of interest.

النشاط الفيزيائي الكيميائي والنشاط المضاد للأكسدة والنشاط المضاد للبكتيريا ودراسة حبوب اللقاح لعسل الشمر واليانسون والكزبرة

هبة الله سيد السايح [1]، وائل محمود مرزوق [1]، محمد رمضان عبد الدائم [1]، امنية فكري حسان [2]، احمد علي كامل [1]

قسم بحوث النحل / معهد بحوث وقاية النبات / مركز البحوث الزراعية / الجيزة / مصر [1]

قسم البكتيريا / معهد بحوث صحة الحيوان / مركز البحوث الزراعية / الجيزة / مصر [2]

الخلاصة

تم تحديد الخصائص الفيزيائية والكيميائية (التوصيل الكهربائي، الوزن النوعي، والمواد الصلبة الذائبة الكلية، ومحتوى الرطوبة، ودرجة الحموضة، والحموضة الحرة، واللاكتون، والحموضة الكلية، وهيدروكسي ميثيل فورفورال (HMF)، ومحتوى البروتين الكلي). بالإضافة إلى ذلك، تم تقييم محتوى الفلافونويد الكلي، ومحتوى الفينول الكلي، والخصائص المضادة للبكتيريا، ونشاط مضادات الأكسدة، وتحليل حبوب اللقاح في العينات. كانت المعايير الفيزيائية والكيميائية لأنواع العسل متشابهة بشكل عام. على هذا النحو، ليس من السهل التمييز بين ثلاثة أنواع من العسل بناءً على التحليل الفيزيائي والكيميائي. مع ذلك، هناك فرق كبير في محتوى الفينول بين عسل الكزبرة وعسل الشمر أو اليانسون. وبالمقارنة لم تسجل أي فروق معنوية في محتوى الفلافونويد الكلي أو النشاط المضاد للأكسدة بين الأنواع الثلاثة من العسل وأظهر الفلافونويد ارتباطًا إيجابيًا شديد الدلالة مع النشاط المضاد للأكسدة، بالإضافة إلى أن بيانات مضادات البكتيريا كانت متشابهة بشكل عام في الأنواع الثلاثة من العسل قيد الدراسة، وأظهر تحليل حبوب اللقاح بالعسل وجود اختلاف طفيف في شكل حبوب لقاح الشمر واليانسون والكزبرة. وقد تكون صعوبة التمييز بين أنواع العسل هذه بسبب التشابه الشديد في التركيب الكيميائي للنبات، والذي ينتمي إلى نفس الفصيلة النباتية. هذه الدراسة يمكن أن تساعد الباحث ومنتج العسل على تحديد العسل النقي والتحقق من أصله النباتي.

الكلمات المفتاحية: عسل الشمر، الينسون، الكزبرة، مصدر حبوب اللقاح الموجودة بالعسل، الخصائص الكيموفيزيائية، النشاط المضاد للأكسدة، البروتين الكلي.

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