COMPARATIVE STUDY OF SELECTED EGYPTIAN HONEYS AND THEIR ANTIBACTERIAL EFFECTS IN VITRO

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Abstract. Honey is a natural sweet substance known for its antimicrobial properties against a wide range of microorganisms. However, limited studies have investigated the physico-chemical and biological properties of different honey types from the same production region. This study evaluated six Egyptian floral and non-floral honeys (cotton, clover, citrus, eucalyptus, black seed, and spearmint) by assessing their physico-chemical composition, sugar and amino acid profiles, and antibacterial activity in vitro. Physico-chemical parameters were analyzed using the Association of Official Analytical Chemists (AOAC) methods, the sugars by High Performance Liquid Chromatography (HPLC), and the amino acids by Gas-Liquid Chromatography (GLC). Honeys varied in pH between 3.80 and 4.20, moisture content between 17.0% and 19.5%, and total amino acid content between 7.84 and 17.19 mg/100 mg. Antibacterial activity at 5% and 10% concentrations was tested against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*. Our findings showed that, compared to honeys from bee colonies fed medicinal plant extracts, floral honeys exhibited stronger antibacterial effects against both Gram-positive and Gram-negative bacteria. Cotton honey showed the highest activity, with inhibition zones up to 24.3 mm, while black seed honey was the least effective. Gram-positive bacteria were more susceptible than Gram-negative strains. Molecular assays revealed that honey exposure significantly reduced bacterial DNA, RNA, and protein levels. Cotton honey was the most potent at suppressing protein and RNA synthesis, particularly in *B. subtilis*, while black seed honey had a stronger effect on *E. coli* DNA. These results highlight the significant role of botanical origin in determining honey's antimicrobial efficacy and support the use of Egyptian monofloral honeys as natural antibacterial agents.
Keywords: *Egyptian honey, non-floral honey, physico-chemical properties, antibacterial activity, molecular mechanism*

Introduction

Honey bees (*Apis mellifera*) feed on flower nectar, blossoms, or secretions to make honey, a highly saturated fluid or semi-solid. Numerous studies have demonstrated honey's nutritional and medicinal properties (Wang et al., 2023).

Honey's chemical makeup and physical properties depend on its location, purity, type of honeybee, and plant source (Nafea et al., 2023). There is no universal standard for honey classification by color. Honey can be pale yellow, rich crimson, or almost black, depending on the plant. Honey purity is determined by its pH level and other physical characteristics. It is important to note that pH levels vary by location. Honey solidity depends on moisture. It is unique in its low moisture content, usually 13–20% (Sharma

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et al., 2024). Like other Newtonian fluids, honey's viscosity depends on temperature and moisture. At 24°C and 18.9% moisture, honey has 9.9 psa viscosity (Santos et al., 2014).

The plants that produce honey determine its chemical makeup. Honey is mostly rich with fructose (38%) and glucose (32%), with maltose, sucrose, and other complex carbohydrates making up the rest. It contains small but significant amounts of minerals, vitamins, proteins, enzymes, amino acids, organic acids, phytochemicals, and physiologically active phenolic compounds (Al-Hatamleh et al., 2020; Brudzynski and Sjaarda, 2021).

Honey is widely recognized for its therapeutic effects, including anti-inflammatory (Majtan, 2021), antioxidant (Seki, 2025), antidiabetic (Al-Mahna, 2024), antitumor (Abbas et al., 2021), and antimicrobial properties (Ogwu and Izah, 2025). With rising global concerns over antimicrobial resistance-exacerbated by the misuse of antibiotics-there is renewed interest in natural alternatives like honey. The World Health Organization projects that antimicrobial resistance could cause 10 million deaths annually by 2050 if unaddressed (Guevara et al., 2023).

Certainly, the climatic conditions and geographic areas from where the original nectar was gathered affect the composition of honey, and consequently, its antimicrobial activity. This is ascribed to naturally occurring variances in floral sources and local climates (Cebrero et al., 2020). As a result, a number of studies have investigated the medicinal properties of honeys sourced from various global geographic locations (Nafea et al., 2009; Lu et al., 2013; Ng et al., 2014; Dahiya et al., 2024). It was interesting to look into whether and how some of the Egyptian honeys that are now on the market can be utilized as efficient antibacterial agents (Owens and Stoessel, 2008; Basu et al., 2009) as the ancient Egyptians employed honey as a universal treatment for almost all maladies (including bacterial infections) (Pećanac et al., 2013). The physico-chemical characteristics (El-Metwally, 2015; El Sohaimy et al., 2018; Nafea and Mazeed, 2023; Nafea et al., 2023), chemical composition (EL-Kazafy and Ali, 2012), antibacterial and anti-biofilm activities (Badawy et al., 2004; Elbanna et al., 2014) and therapeutic use (Badawy et al., 2004; Abdelatif et al., 2008; El-Nagar et al., 2012; Mohamed et al., 2014) of Egyptian honey variations have all been the subject of numerous investigations.

While numerous studies have compared honeys from different countries or widely varying environments, there is a notable lack of comparative data on honey varieties produced within similar geographic and climatic conditions. This limitation makes it difficult to understand how local floral sources alone contribute to differences in honey quality and antibacterial potential. A systematic evaluation of honeys produced in the same regional context, using consistent environmental parameters, can offer a more precise insight into the role of botanical origin. Moreover, the inclusion of non-floral honeys, generated by feeding bees with sugar solutions enriched with medicinal plant extracts, presents a novel and controlled approach to studying the influence of bioactive compounds on honey's antimicrobial properties. These honeys remain underexplored in both national and global research, and their comparative assessment alongside traditional monofloral honeys adds a valuable dimension to honey authentication and functional food development. Therefore, the aims of the present study were: (i) to examine the physico-chemical properties of six Egyptian honeys from different floral and non-floral sources; (ii) to evaluate their in vitro antibacterial activity against selected human pathogens; and (iii) to investigate the impact of these honeys on bacterial protein, DNA, and RNA levels. This study is novel in including two types of non-floral honeys, produced by feeding

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honeybee colonies with sugar solutions enriched with *Nigella sativa* and *Mentha viridis* extracts. Unlike traditional floral honeys derived from nectar, these non-floral honeys are generated under controlled feeding conditions, offering a unique model to explore how bioactive plant compounds may influence the antibacterial properties of honey. The inclusion of non-floral honeys thus adds a new dimension to the understanding of honey's antimicrobial potential and botanical influence.

Materials and methods

Preparation of honeybee colonies

The first hybrid (F1) of Carniolan honeybee colonies was used to produce honey. Five hives located in Menofia Governorate served as the source of citrus, clover, and cotton honeys, while another five hives in Ismailia Governorate produced eucalyptus honey. Additionally, 10 hives were maintained in Dokki, Giza for the production of non-floral honeys.

Floral honeys used in this study were cotton (*Gossypium barbadense*), eucalyptus (*Eucalyptus* spp.), citrus (*Citrus* spp.), and clover (*Trifolium alexandrinum*). Non-floral honeys were obtained by feeding colonies with a 50% sucrose solution enriched with medicinal plant extracts of *Nigella sativa* (black seed) and *Mentha viridis* (spearmint). All honey samples were collected from the respective apiaries and stored at room temperature in the dark.

Preparation of plant extracts and non-floral honeys

Plant extracts were prepared by refluxing 10 g of either black seed (*N. sativa*) seeds or dried spearmint (*M. viridis*) leaves in 150 mL of ethyl alcohol for 1 h using a Soxhlet apparatus.

Marked virgin combs were introduced into selected colonies and fed a 50% sucrose solution enriched with 0.25% of each plant extract. Feeding occurred during the autumn at the Beekeeping Research Section, Dokki, Giza, when no flowering was present. After three weeks, the honey was harvested from the marked combs and stored in dark jars at room temperature. Two honey concentrations (5% and 10%) were prepared by dissolving honey in 100 mL of distilled water.

Physico-chemical analyses of honey

Moisture content

Moisture content was determined by measuring the refractive index of honey at 20°C using an Abbe refractometer, following (AOAC, 1990) methods.

pH value

Approximately 1 g of honey was dissolved in 5 mL of deionized water. The pH of the solution was measured using a digital pH meter (Hanna Instruments, Woonsocket, RI, USA).

Ash content

Ash content was determined using the AOAC standard procedure (AOAC, 1980).

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Crude protein content

Total crude protein was determined using the semi-micro Kjeldahl method as described by Loiseleur (1963), and expressed as mg/100 mg of honey.

Carbohydrate content

Total carbohydrate content was determined by the phenol-sulfuric acid method of DuBois et al. (1956).

Sugar profile

Quantification of individual sugars (fructose, glucose, sucrose, and maltose) was performed by High Performance Liquid Chromatography (HPLC) following the method of Bogdanov and Baumann (1988), using a JASCO HPLC System (Japan) equipped with a PU-980 pump and UV-970 detector.

Amino acid content

Amino acids were analyzed using Gas-Liquid Chromatography (GLC). The derivatization process employed N-butanol and trifluoroacetic anhydride according to Landault and Guiochon (1964). Analyses were performed on a Varian Model 3700 GC with Flame Ionization Detection (FID).

Antibacterial activity assay

Bacterial strains

The antibacterial effects of honey was tested on Gram-positive (*S. aureus* and *B. subtilis)* and Gram-negative (*P. aeruginosa, E. coli, S. typhimurium)* bacteria. They were kindly provided from Dr. E. A. M. Solaiman, Microbial Genetics Dep., Genetic Engineering and Biotechnology Div., National Research Centre.

Disc diffusion method

Antibacterial activity was assessed using the filter paper disc diffusion method described by Mahamed and Wolde (2024). Bacterial strains were cultured in nutrient broth at 37°C for 24 h. Mueller-Hinton agar plates were inoculated with the bacterial cultures using sterile swabs. Sterile 6 mm Whatman No. 1 paper discs were soaked for 1 min in test honey solutions (5% and 10%) and placed on the inoculated agar surfaces. Plates were left to dry for 15 minbefore incubation at 37°C for 24 h. Zones of inhibition were measured in millimeters.

Molecular analyses

DNA extraction and quantification

B. subtilis and *E. coli* genomic DNA was isolated using Breitwieser et al. (2019) technique. A UV150-02, PC ultraviolet-visible spectrophotometer (Shimadzu, Japan) measured DNA purity and concentration. DNA purity was assessed using Agarwal's methodology (Agarwal, 2008). The following equation was used to determine DNA concentration (Tan and Yiap, 2009):

$$\text{Concentration } (\mu\text{g mL}^{-1}) = \text{OD at 260 nm} \times (\text{dilution factor} = 100) \times 50/1000 \quad (\text{Eq.1})$$

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RNA quantification

RNA purity and concentration were determined based on absorbance at 260 and 280 nm using the Shimadzu U-V. 150-02 spectrophotometer. The method of Chomczynski and Sacchi (2006) was used for RNA extraction.

Bacterial protein quantification

The protein content of bacterial cells was determined using the semi-micro Kjeldahl method according to Bărnuţiu et al. (2011), and expressed in mg/100 mg.

Statistical analysis

All measurements were conducted in triplicate (n = 3). Data are expressed as mean ± standard error (SE). One-way Analysis of Variance (ANOVA) was performed, followed by the Least Significant Difference (LSD) test to compare means. F-values, LSD, and p-values are reported, with statistical significance set at $p \leq 0.05$.

Results

Physico-chemical parameters of honey

The physico-chemical analysis of honey samples revealed notable variations, as shown in *Table 1*. All honey samples showed similar moisture below 20%. Moisture content varied slightly, with the highest observed in cotton and citrus honeys and the lowest in black sativa and spearmint honeys. The honey samples were tested for pH, and all were acidic (pH 3.80-4.2) and within the standard range (pH 3.40–6.10), ensuring their freshness and contributes to their antimicrobial stability. Citrus honey had the highest acidity, whereas spearmint honey had the lowest. There were no significant pH differences between the six honey varieties ($P > 0.05$). Ash content reflects mineral composition and characterizes honey's botanical and geographic origins. In this study, spearmint honey had the lowest ash concentration. Cotton had the highest ash content, indicating a richer mineral profile. Ash concentrations were similar across samples ($P > 0.05$).

Carbohydrates were consistently high across all honey types, ranging from 79.30 to 82.40 mg/100 mg. There were no significant variations in fructose and glucose ($P > 0.05$). Fructose and glucose, the main sugars in honey, also showed some variation. Cotton honey had the highest fructose level, which contributes to a sweeter taste, whereas clover honey had the highest glucose content, which is associated with a higher tendency to crystallize. The F/G ratio is a key indicator of honey's crystallization rate. All honeys had an F/G ratio above 1.0. Black sativa honey had the highest F/G ratio, suggesting better liquidity over time. Sucrose levels were relatively low in all samples, with citrus honey showing the highest value, which may indicate early harvest or floral source characteristics, while spearmint had the lowest. Similarly, maltose content was highest in citrus honey and lowest in spearmint honey, further supporting the distinctive sugar profile of citrus honey. Generally, all honey types shared a common carbohydrate-rich, acidic profile, significant differences exist in their moisture, protein, and sugar composition. Clover honey appeared to be the richest in carbohydrates and glucose, making it the most prone to crystallization. Black sativa and spearmint honeys showed desirable properties in terms of low moisture and high F/G ratios, indicating better shelf

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stability. Citrus honey had the highest protein and disaccharide content, which may reflect either botanical origin or harvest timing. These differences highlighted the importance of floral source and environmental conditions in determining honey quality.

Table 1.*Chemical composition of different types of honey at concentration (mg/100mg)*

Parameter	Cotton	Citrus	Clover	Eucalyptus	Black sativa	Spearmint
pH	4.00	3.80	4.10	3.90	4.10	4.20
Moisture	19.50	19.50	18.50	18.00	18.00	17.00
Ash	0.24	0.10	0.10	0.12	0.05	0.03
Crude protein	0.35	0.44	0.31	0.33	0.14	0.13
Carbohydrates	79.50	79.30	80.90	80.90	81.60	82.40
Fructose	39.70	37.90	41.00	38.90	36.90	36.10
Glucose	33.30	32.50	34.20	32.70	28.30	29.30
F/G	1.19	1.17	1.19	1.19	1.30	1.23
Sucrose	1.65	3.10	1.10	4.17	14.60	12.20
Maltose	3.30	5.50	4.25	2.80	0.80	0.60

The amino acid composition of the honey samples, as presented in *Table 2*, demonstrates considerable variation among different floral sources. Honey samples had total amino acid content of 17.19 to 7.84 mg/100 mg, depending on type. Almost all amino acids were found in honey samples, but essential amino acids predominated. Cotton honey had the most amino acids, indicating that cotton honey possesses the highest overall proteinaceous content, while spearmint the lowest. The most abundant essential amino acid in all honey samples was proline. Clover honey had the most proline. Proline is known to be a dominant amino acid in honey and is often used as an indicator of honey maturity and authenticity. Threonine and valine also showed relatively high concentrations, particularly in cotton and clover honeys. Cotton honey recorded the highest content of valine and threonine, reinforcing its rich nutritional profile. In contrast, spearmint honey consistently showed the lowest values for most essential amino acids, including histidine, isoleucine, and methionine, reflecting its comparatively poor amino acid composition. Citrus honey exhibited a unique profile, particularly in its high cysteine content, which was markedly higher than in other honey types. Cysteine plays a key role in antioxidant defense and may contribute to the functional properties of citrus honey. Among the non-essential amino acids, alanine and glycine were most abundant, especially in eucalyptus and citrus honeys. Eucalyptus honey, for example, contained 0.95 mg/100 mg of glycine and 0.83 mg/100 mg of alanine, indicating a balanced presence of non-essential amino acids.

When comparing essential and non-essential amino acid totals, clover honey had the highest content of essential amino acids, while cotton honey exhibited the highest non-essential amino acid content. The essential to non-essential amino acid ratio varied among honeys, with cotton honey having the highest ratio, suggesting a better nutritional profile in terms of essential amino acids. In general, cotton, clover, and citrus honeys showed superior amino acid profiles, while spearmint and black sativa honeys were characterized by lower overall levels, particularly in essential amino acids. These findings highlighted the influence of botanical origin on the nutritional and biochemical quality of honey.

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***Table 2.** Aminoacids composition of different types of honey (mg/100mg)*

Amino acids	Cotton	Citrus	Clover	Eucalyptus	Black sativa	Spearmint
Essential A.A.						
Histidine	0.01	0.17	0.22	1.15	0.29	0.03
Valine	2.21	1.00	1.05	0.34	0.34	0.49
Threonine	2.52	0.25	1.05	0.10	0.19	0.43
Phenylalanine	1.90	1.18	0.57	1.75	1.28	1.00
Isoleucine	0.32	0.38	0.35	1.27	0.23	0.51
Leucine	1.72	1.51	1.27	2.05	0.25	0.72
Lysine	0.01	0.25	0.22	0.10	0.03	0.07
Proline	2.61	2.51	4.14	3.51	1.60	1.03
Methionine	0.01	1.20	0.57	0.11	0.44	0.07
Non-essential A. A.						
Alanine	1.87	1.51	0.83	0.40	0.40	0.10
Aspartic acid	0.17	1.42	0.40	1.66	1.15	0.97
Glycine	0.31	0.50	0.95	0.19	0.28	0.56
Serine	2.11	0.75	2.23	2.10	0.64	0.37
Glutamic acid	0.19	0.43	0.35	1.19	0.26	0.18
Tyrosine	0.03	0.27	0.32	0.11	0.11	0.05
Cysteine	1.21	10.46	0.99	0.11	0.69	0.16
Total amino acids	17.19	14.78	15.94	16.46	8.24	7.84
Essential A.A.	11.37	8.45	9.44	10.38	4.74	4.35
Non-essential A. A.	5.89	6.34	6.07	5.76	3.53	2.39
Essential/non-essential A.A.	1.92	1.33	1.55	1.80	1.34	1.60

Antibacterial activity of honey

Tables 3 and *4* presented the antibacterial activity of the different types of honey against five bacterial strains at 5% and 10% concentrations, respectively. The data revealed a clear concentration-dependent increase in antibacterial efficacy, as inhibition zones (in mm) were consistently larger at 10% than at 5% for all honey types and bacterial strains.

Cotton honey had the highest inhibition zone against *S. aureus*, while black sativa honey had the lowest at 5% and 10% concentrations, respectively. Higher inhibition zone was observed in cotton honey at 5% concentration, while clover honey had the highest inhibition zone at 10% concentration against *B. subtilis*. The lowest inhibition zones were found at 5% black sativa honey and 10% spearmint honey. Cotton honey showed the highest inhibition and black sativa honey the lowest at 5% and 10% concentrations, respectively, for *E. coli*. All honey types inhibited *P. aeruginosa* growth, except spearmint honey at 5% and 10% concentrations. At 5% and 10% concentrations, cotton honey significantly inhibited *S. typhimurium* and clover growth. Black sativa honey had the lowest inhibition zones.

Interestingly, all honey types were more effective against Gram-positive bacteria than Gram-negative strains. The lowest inhibition across all samples was observed against *P. aeruginosa*, a notoriously resistant organism, although cotton and citrus honeys still achieved moderate zones of inhibition. The F-values and LSD values confirm that the differences observed among honey types and bacterial responses were statistically

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significant. Overall, cotton and citrus honeys emerged as the most effective in this assay, indicating their potential as natural antibacterial agents, while black sativa and spearmint honeys showed limited efficacy at 5% concentration. These variations likely reflect differences in phytochemical composition and sugar profiles among the tested honeys.

Overall, these findings suggest that cotton and citrus honeys are the most promising candidates for antimicrobial applications, especially at higher concentrations, while the lower efficacy of black sativa and spearmint honeys may limit their potential unless used at elevated doses. The results emphasized the importance of floral origin and concentration in determining the antibacterial properties of honey.

Table 3. *The antibacterial activity of different types of honey at concentration (5%) (inhibition zone in mm)*

Type	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhimurium*
Cotton	8.66 ± 0.06	7.33 ± 0.14	4.33 ± 0.14	5.66 ± 0.07	6.33 ± 0.16
Citrus	7.33 ± 0.05	5.66 ± 0.18	3.66 ± 0.11	5.66 ± 0.03	4.66 ± 0.03
Clover	6.33 ± 0.21	4.66 ± 0.04	4.33 ± 0.01	5.00 ± 0.08	4.32 ± 0.08
Eucalyptus	5.00 ± 0.16	4.66 ± 0.23	4.00 ± 0.13	5.66 ± 0.04	4.00 ± 0.03
Black sativa	3.66 ± 0.15	2.66 ± 0.12	2.33 ± 0.10	5.66 ± 0.02	2.33 ± 0.13
Spearmint	3.70 ± 0.05	3.66 ± 0.07	2.66 ± 0.12	3.66 ± 0.01	3.20 ± 0.01
F-	3.05	23.50	5.06	6.68	4.42
LSD	1.20	1.03	1.22	1.18	1.18

Results are represented as mm ± S.E. Results are means of 3 replicates. LSD least significant difference

Table 4.*The antibacterial activity of different types of honey at concentration (10%) (inhibition zone in mm)*

Type	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*S. typhimurium*
Cotton	24.33 ± 0.21	21.33 ± 0.32	7.33 ± 0.11	17.33 ± 0.13	15.66 ± 0.60
Citrus	22.33 ± 0.34	23.33 ± 0.17	5.33 ± 0.15	18.33 ± 0.35	16.33 ± 0.10
Clover	17.33 ± 0.20	23.66 ± 0.18	5.66 ± 0.22	17.60 ± 0.61	22.33 ± 0.12
Eucalyptus	21.66 ± 0.12	22.00 ± 0.19	6.60 ± 0.24	13.33 ± 0.12	15.66 ± 0.14
Black sativa	9.33 ± 0.12	9.66 ± 0.25	4.33 ± 0.62	8.66 ± 0.12	7.33 ± 0.09
Spearmint	9.66 ± 0.22	7.66 ± 0.19	4.66 ± 0.10	6.66 ± 0.13	8.66 ± 0.08
F-	387.87	259.07	12.17	225.60	289.00
LSD	1.03	1.39	1.04	1.02	1.03

Results are represented as mm ± S.E. Results are means of 3 replicates. LSD least significant difference

Inhibitory effect of honey on total bacterial protein, DNA and RNA level

Table 5 shows that both cotton and black sativa honeys significantly disrupted the protein, RNA, and DNA content in *B. subtilis* and *E. coli*, indicating strong antibacterial effects at the molecular level. In *B. subtilis*, cotton honey caused the greatest reduction in protein and RNA levels, while black sativa had a slightly milder effect. Although DNA levels varied, black sativa-treated cells retained more DNA than those treated with cotton honey. In *E. coli*, both honeys caused a dramatic decrease in protein synthesis and RNA levels, with DNA content also significantly reduced, especially in cells exposed to cotton

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honey. These findings suggest that cotton honey is more effective in targeting transcription and translation, whereas black sativa may exert its strongest effects on DNA integrity in *E. coli*. Overall, both honeys demonstrated multi-target antibacterial action, reinforcing their potential as natural antimicrobial agents.

Table 5. *The effect of some bee honey on B. subtilis and E. colitotal protein, DNA and RNA concentrations μg/2 × 10^6cells*

	Parameter	Control	Cotton honey	Black sativa honey	F	P	LSD
B. subtilis	Protein	413.30[(a)]	286.40[(c)]	307.90[(b)]	273.70	0.000	14.20
	DNA	2.83[(a)]	0.056[(b)]	2.90[(a)]	11.45	0.098	1.60
	RNA	1.70[(a)]	0.23[(b)]	0.27[(b)]	5.11	0.0031	1.00
E. coli	Protein	333.20[(a)]	46.80[(c)]	55.12[(b)]	8.71	0.000	2.86
	DNA	1.55[(a)]	0.32[(c)]	0.94[(b)]	1.21	0.000	0.27
	RNA	4.50[(a)]	2.78[(b)]	2.82[(b)]	2.30	0.0032	2.90

Means with different letters in each row are significantly different ($P \leq 0.05$)

Discussion

This study provides a comparative evaluation of six Egyptian honey varieties, including four floral and two non-floral types, with a focus on their physico-chemical composition and antibacterial properties. While previous studies have examined honey quality from diverse geographic and floral sources, few have directly compared multiple honey types from the same production regions under controlled conditions. Our results highlight several key biochemical differences among the honeys, many of which correlate with their antimicrobial efficacy.

All honey samples were acidic, with pH values ranging between 3.49 and 4.70, aligning with established international standards and consistent with earlier reports on Egyptian, Indian, Algerian, and European honeys (Ouchemoukh et al., 2007; Kayacier and Karaman, 2008; Nafea et al., 2009; Saxena et al., 2010; Azeredo et al., 2023; Nafea et al., 2023). The findings also supported earlier results that several Egyptian clover and citrus honeys had pH levels between 3.8 and 5.2 (Badawy et al., 2004; Elbanna et al., 2014). Honey contains amino, aromatic, and aliphatic acids. However, honey type greatly affects acid content. The pH of honey does not affect free acidity, because it naturally contains buffering carbonate, phosphate, and other mineral salts (Lazarević, 2016). Honey's high acidity gives it taste and microbial resistance (Bogdanov et al., 2008).

Moisture content in all samples was below 20%, meeting the European quality standard. This low moisture level supports honey's shelf-stability and fermentation resistance. Honey samples tested by Nayik et al. (2019) had moisture levels between 17.5% and 19.1%. Abdel-Hameed (2020) found that honey samples had varying moisture levels, ranging from 17.25 ± 0.66% to 21.0 ± 1.11%. El-Dereny (2023) recorded 17.35-18.38% honey moisture. Notably, non-floral honeys produced from sugar-fed colonies with medicinal plant extracts exhibited slightly lower moisture content, potentially contributing to their reduced bacterial inhibition compared to floral varieties.

The variation in ash content among the samples, ranging from 0.60 to 1.2 g/100 g, reflects differences in mineral content, with cotton honey showing the highest ash level. This may be linked to a richer mineral profile and is consistent with its strong antimicrobial performance.

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Sugar composition analysis revealed that fructose levels exceeded glucose in all samples, a hallmark of high-quality honey. This is confirming the high quality of the honey types under study and pointing to the honey colonies' normal diet of Egyptian honey. These findings supported previous honey variety research (Buba et al., 2013; El-Metwally, 2015). The F/G ratios were all above 1.0, indicating a reduced tendency for crystallization and enhanced liquidity. Black sativa and spearmint honeys had the highest F/G ratios but did not exhibit superior antimicrobial effects, suggesting that sugar composition alone does not determine bioactivity.

Since pollen is the main source of amino acids in honey, its amino acid profile may indicate its origin (Anklam, 1998). Some studies suggested using amino acid profiles to distinguish honey varieties (Kečkeš et al., 2013; Sun et al., 2017). Thus, our research sought to determine the amino acid composition of Egyptian honey varieties. Total amino acid content varied significantly, ranging from 7.84 to 17.19 mg/100 mg. Proline was the dominant amino acid in all honeys, supporting its role as a maturity and botanical marker. Proline dominates honey's amino acids, 50–85%, according to Iglesias et al. (2006). Honey bees make proline by secreting saliva while making honey. Since honey proline levels drop during storage, it may indicate maturity (da Silva et al., 2016). Cotton and citrus honeys had the highest levels of proline and other essential amino acids such as valine and threonine, which are known to affect bacterial viability. Citrus honey also contained high levels of cysteine, a sulfur-containing amino acid with recognized antioxidant and antimicrobial properties.

Honey's antibacterial properties fight many bacteria. Honey bioactive content showed antibacterial properties. The antibacterial activity of Egyptian honey varieties in this study was dose-dependent and varied across types. Cotton and citrus honey were more antibacterial than non-floral honey. The observed variation in antibacterial efficacy among the tested Egyptian honey types can be attributed to several biochemical and compositional differences linked to their botanical origins. Cotton and citrus honeys, which exhibited the highest antibacterial activity, were characterized by higher protein and total amino acid contents, particularly proline, valine, and threonine, which are known to support antimicrobial properties by enhancing osmotic pressure or disrupting bacterial metabolism. Citrus honey also had notably high levels of cysteine, a sulfur-containing amino acid with antioxidant and antimicrobial potential, which may contribute to its broad-spectrum activity (Elbanna et al., 2014; Seraglio et al., 2021). Prior work on citrus and clover honeys from Fayoum and other regions confirmed their strong antimicrobial and antioxidant properties alongside characteristic physico-chemical profiles (Roby et al., 2020). In contrast, non-floral black sativa and spearmint honeys showed the lowest antibacterial effects, potentially due to their lower moisture and protein contents and reduced levels of essential amino acids. Although these honeys had high F/G ratios, indicating better liquidity, they lacked the complex phytochemical diversity found in nectar-derived honeys (Luchese et al., 2017). Additionally, cotton honey had the highest ash content, reflecting a richer mineral profile, and the highest fructose level, both of which may enhance its antimicrobial osmotic effects. The presence and concentration of phenolic compounds, though not quantified in this study, likely differ significantly among the honeys and may also contribute to the variable antimicrobial activities. Overall, the results strongly suggest that differences in amino acid profiles, sugar composition, acidity, and possibly plant-derived phytochemicals play a critical role in modulating the antibacterial potency of different honey types (Miguel et al., 2017; Shears, 2000; Yushchuk et al., 2020).

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Compared to Gram-negative bacteria, all honeys worked best on Gram-positive bacteria like *S. aureus* and *B. subtilis*. Alvarez-suarez et al. (2010) also found that honey samples affected Gram-positive bacteria more than Gram-negative bacteria. Gram-negative bacteria have an outer polysaccharide membrane that encases structural lipopolysaccharides, which may explain their susceptibilities (Gozari et al., 2019). Thus, lipophilic substances cannot cross the cell wall. However, Gram-positive bacteria are more susceptible because their peptidoglycan outer layer alone cannot prevent permeability.

Drug-resistant *Enterobacteriaceae*, particularly *E. coli*, are frequently encountered in clinical microbiology laboratories and are often associated with surgical site and hospital-acquired infections. *B. subtilis*, an aerobic, Gram-positive, endospore-forming bacterium, is considered opportunistic and mildly virulent. It is commonly implicated in food contamination, especially in healthcare settings where its heat- and desiccation-resistant spores can persist. Infections linked to *B. subtilis* include panophthalmitis, meningitis, cholangitis, pneumonia, osteomyelitis, and ventriculoperitoneal shunt infections. In immunocompromised individuals, airborne exposure may lead to symptoms such as eye and nasal irritation, sore throat, headache, and fatigue. Its extracellular enzyme, subtilisin, may also induce allergic reactions. Moreover, *B. subtilis* can produce diarrheal enterotoxins and has been identified as a cause of foodborne illness outbreaks (Oguntoyinbo and Sanni, 2007; Long et al., 2012). Honey, cotton, and black sativa were tested for antibacterial activity against *E. coli* and *B. subtilis* using DNA, RNA, and protein assays. *E. coli* and *B. subtilis* protein, DNA, and RNA inhibition showed that investigated honey samples reduced their synthesis. The observed reductions in bacterial DNA, RNA, and protein levels upon honey treatment suggest that honey exerts a multi-targeted inhibitory effect on microbial macromolecular synthesis. A substantial decrease in protein and RNA levels, particularly in *B. subtilis* treated with cotton honey, indicates interference with transcription and translation processes (Brudzynski and Sjaarda, 2021; Iglesias et al., 2006). Likewise, reductions in DNA content, especially in *E. coli* exposed to black sativa honey, may reflect DNA degradation, replication inhibition or DNA-damaging effects of thymoquinone and flavonoids derived from *Nigella sativa* (Randhawa and Alghamdi, 2011; Ogwu and Izah, 2025). These findings collectively suggest a bactericidal effect, as they point to irreversible cellular damage rather than merely growth inhibition. While bacteriostatic agents typically halt bacterial replication without killing cells, the significant depletion of essential macromolecules observed here supports the conclusion that the honeys tested, especially cotton and black sativa, induce lethal effects on bacterial cells. Wasfi et al. (2016) tested three Egyptian honeys from marjoram, clover, and citrus on *E. coli* ATCC 8739 for cellular changes, antibacterial, and antivirulence effects. Marjoram had the highest *E. coli* antibacterial activity. TEM revealed “ghost” cells without DNA and cells with enlarged vacuoles and/or irregularly shrinking cytoplasm. Mishref and colleagues found that honey from bee colonies fed medicinal plant extracts had higher antibacterial activity than control honey (Mishref et al., 1989).

Conclusion

This study demonstrated that Egyptian honeys from different floral and non-floral sources exhibit distinct physico-chemical profiles and variable antibacterial activities. Among the tested samples, cotton and citrus honeys showed the strongest inhibitory

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effects against both Gram-positive and Gram-negative bacteria, supported by reductions in bacterial DNA, RNA, and protein levels. Our molecular findings move beyond the traditional disc diffusion and MIC assays to provide deeper mechanistic insight into bactericidal action, paralleling but expanding upon previous ultrastructural studies.The inclusion of non-floral honeys enriched with *N. sativa* and *M. viridis* provided a novel perspective on the influence of controlled botanical feeding on honey bioactivity. These findings underscore the potential of certain Egyptian honeys, particularly cotton and citrus, as natural antibacterial agents. Their application may extend to food preservation, wound care, or as complementary therapies against resistant pathogens. However, further studies are needed to validate their efficacy in clinical or industrial settings, assess safety and stability, and explore formulation strategies for enhanced delivery.

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