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# Non-Traditional Egyptian Honeys: A Comprehensive Analysis of Chemical Composition, Bioactive Compounds, and Biological Activities

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## ABSTRACT

This study was conducted to evaluate the physicochemical, polyphenolic and antimicrobial properties of five Egyptian honeys: anise, caraway, fennel, sidr, and Egyptian clover. Significant variations were observed in sugar profiles, diastase activity, Hydroxy methyl furfural (HMF), pH, and free acidity, reflecting the influence of floral origin and processing methods. Notably, caraway honey exhibited a high fructose-to-glucose ratio suggestive of slow crystallization, while sidr honey showed high diastase activity and low HMF. Furthermore, each honey exhibited a unique polyphenolic profile, with anise honey being particularly rich in hesperidin and ferulic acid. Antimicrobial analysis revealed variations in efficacy against *Pseudomonas aeruginosa* ATCC 35023 and *Bacillus subtilis* ATCC 6633 which were the most affected strains by all types of honey as the inhibition zone by well diffusion assay was around 4 cm, the effect was bactericidal at concentrations ranging from 50 to 100% and bacteriostatic at concentrations lower than 50%. Minimum inhibitory concentration (MIC) results of honey diluted up to 1.95% can inhibit the growth of most strains except *Staphylococcus aureus* and minimum bactericidal concentration (MBC)results cleared that 50% honey concentrations was considered a bactericidal.. Notably, Caraway and Clover honeys showed strong activity against *Staphylococcus aureus*, highlighting their potential for therapeutic development, especially against antibiotic-resistant strains. Future investigations should delve into the impact of seasonal variations, geographical origins, and sensory attributes on these honey varieties to harness their full potential for human health. These results highlight the unique properties of certain untraditional Egyptian honeys and their potential health benefits

***Keywords:*** *Honey, Chemical composition, Polyphenols, antibacterial activity, MIC, MBC*

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## 1. INTRODUCTION

Egypt, with its ancient beekeeping traditions and diverse flora, boasts a variety of honey types that are known for their unique flavors and potential health benefits. The country's rich history with bees dates back thousands of years, evident in hieroglyphics and ancient scriptures where bees and honey are frequently mentioned. Egyptian beekeepers leverage the country's varied ecosystems, from the lush Nile Delta to the arid deserts, to produce honey from different floral sources, including clover, acacia, citrus, and wildflowers. These diverse environments contribute to the distinctive taste profiles and nutrient compositions of Egyptian honey (El-Seedi *et al.*, 2022).

Honey from Egypt is often prized for its therapeutic properties, used in traditional medicine to treat ailments such as coughs, wounds, and digestive issues. Modern scientific studies have confirmed many of these health benefits, noting honey's antibacterial, anti-inflammatory, and antioxidant properties. The unique combination of Egypt's flora and time-honored beekeeping methods ensures that each batch of honey carries not just nutritional value, but also a piece of the country's natural and cultural heritage (Yupanqui Mieles *et al.*, 2022).

Honeybees (*Apis mellifera* L.) are the ingenious makers of honey, a remarkable substance that goes beyond its role as a sweetener. Honey contains amino and organic acids, minerals, sugars, vitamins, and a wide range of antioxidants, including flavonoids, phenolics, and glycosides, earning it the label of a "nutraceutical food" (Fernández-Estellé *et al.*, 2023). Traditionally, honey has been used medicinally to treat various illnesses (Miguel *et al.*, 2017), and current research indicates that it may have antimicrobial, diuretic, and other health-enhancing properties.

The specific plants that honeybees visit impart unique characteristics to different types of honey, affecting their health benefits. The composition of honey varies based on the flowers visited by the bees, a factor that captivates both researchers and consumers (Pătruică *et al.*, 2022).

The quality and differentiation of local and imported honey samples can be determined by examining their botanical origin and physicochemical characteristics, including moisture content, color, electrical conductivity, free acidity, pH, HMF, sugar content, and diastase activity, which are important indicators of honey quality (Ismaila *et al.*, 2021; El Sohaimy *et al.*, 2015).

According to Roby *et al.* (2020) and El-Borai (2018), it is believed that the varying levels of antioxidant activity in different Egyptian honey types can be attributed to their phenolic compounds, which are known to be responsible for their antioxidant properties. Similarly, García-Seval *et al.* (2022) identified chrysin, galangin, hesperetin, and other flavonoids as key markers for distinguishing between lemon and orange honey.

Honey's chemical composition is influenced by several factors, most significantly the nectar's botanical origin (Chakir *et al.*, 2016; Solayman *et al.*, 2016; El Sohaimy *et al.*, 2015). Geographical location (Taha *et al.*, 2017; Draiaia *et al.*, 2015), storage time (Al-Ghamdi *et al.*, 2019; Badei & Shawer, 1986), comb age (Taha *et al.*, 2010; Taha and El-Sanat, 2007).

Moreover, according to Taha *et al.*, (2021), honey's physicochemical properties reflect the honey bee species producing it, allowing differentiation between honeys from different species or subspecies. Analysis of honey samples revealed that sidr honey from *Apis florea* colonies showed high levels of fructose, isomaltose, trehalose, ash, protein, free acidity, and invertase activity. In contrast, *Apis mellifera jemenitica* honey exhibited high specific gravity, turanose, maltose, and glucose oxidase activity. All Ziziphus honeys met Codex Alimentarius (2001) standards for good quality.

In the same trend Zyglis and Langland (2022) suggested that honey may possess potential antibacterial properties that could be beneficial against various bacterial strains. Further research is needed to explore the specific polyphenol profiles of different Egyptian honeys and their potential health implications.

In addition, polyphenols play a significant role in cardiovascular health, exhibit anti-cancer properties and preventing and managing chronic inflammatory conditions (Behl *et al.,* 2020; Duda-Chodak and Tarko, 2023).

Harbane *et al.* (2024) and Dimakopoulou-Papazoglou *et al.* (2024) effectively employed this approach to discriminate between unifloral honeys, such as those derived from rape, clover, and caraway, in addition to polyfloral honeys. Additionally, the research conducted by Drivelos *et al.* (2021) identified that within the same honey variety the polyphenolic compound profiles can vary significantly depending on the geographical region of production. These findings underscore the intricate relationship between the floral origin and the distinct chemical composition of honey. By analyzing these "fingerprints," researchers can accurately trace the geographical and botanical origin of honey.

Honey, a renowned natural product with potent antimicrobial properties, exhibits a diverse range of inhibitory effects against various microorganisms. Its efficacy is influenced by a complex interplay of factors, including botanical origin, acidity, hydrogen peroxide content, and other bioactive components. Honey has a long history of use in traditional medicine for wound healing and treating infections (Almasaudi, 2020).

This study aimed to examine the general properties of Egyptian honeys and their potential as a natural alternative to conventional antibiotics. Investigating these characteristics may advance the identification of unique "fingerprints" of Egyptian honeys and contribute to the expanding body of knowledge on the health benefits associated with various Egyptian honey types.

## 2. MATERIALS AND METHODS

### 2.1 Honey Sample Collection and Storage

Twenty-five Egyptian honey samples were collected from local beekeepers across various regions of upper Egypt. The honey types included: Egyptian clover (*Trifolium alexandrinum*), caraway (*Carum carvi*), and anise (*Pimpinella anisum*) (El Minia region), sidr (*Ziziphus spina*) (Sohag region), fennel (*Foeniculum vulgare*) (Assiut region), and. Each honey type was represented by five individual samples. Upon collection, the samples were immediately stored at 4°C to prevent degradation and maintain their original characteristics. This storage condition was maintained until further analysis.

### 2.2. Chemical Composition Analysis

The chemical composition of the honey samples was determined according to the Harmonized methods of the International Honey Commission (International Honey Commission, 2009).

* Sugar Analysis: The sugar content (fructose, glucose, and sucrose) was analyzed using High-Performance Liquid Chromatography (HPLC). A Phenomenex® Luna NH2 250 x 4.6 mm column was used with a constant temperature of 30°C. The mobile phase consisted of acetonitrile: HPLC grade water (80:20 v/v). Refractive Index (RI) detection was employed, and data integration was performed using Claritychrom® software.
* Hydroxymethylfurfural (HMF) Determination: HMF content was determined by HPLC using the White method (White Jr & Siciliano, 1980).
* Diastase Activity: Diastase activity was determined using the Shade method to quantify the amount of reducing sugars released from starch. The results were expressed in D.N (Gothe units).
* Free Acidity and pH: Free acidity was measured using an equivalence point titration method. The pH of the honey samples was measured using a calibrated pH meter, with buffers used for calibration at pH 4, 7, and 10.

### 2.3. Polyphenols Analysis

Polyphenols were analyzed using an Agilent 1260 infinity HPLC series (Agilent, USA). The column used was a Kinetex® 1.7 µm EVO C15 0 mm x 4.6 mm (Phenomenex, USA) operated at 30°C. The separation was achieved using a ternary linear elution gradient with the following mobile phases: (A) HPLC grade water with 0.1% Trifluoroacetic acid (TFA), (B) acetonitrile, and (C) methanol. The flow rate was 1 ml/min, and an injection volume of 20 µL was used. The detection was performed using a Variable Wavelength Detector (VWD) set at 280 nm. The environmental conditions during analysis were maintained at 20°C with 38% relative humidity.

### 2.4 Antimicrobial Activity

The antibacterial potential of five distinct Egyptian honey varieties (sidr, caraway, fennel, clover, and anise) against a panel of clinically relevant bacterial pathogens were taken in consideration. These include six human pathogens - *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 35023, *Bacillus cereus* ATCC 33018, *Escherichia coli* O:157 wild type strain 93111, *Listeria monocytogenes* ATCC33090, and *Salmonella typhimurium* ATCC 14028 - and one food-spoiling bacterium: *Bacillus subtilis* ATCC

6633. By investigating the activity of these honeys against this diverse range of bacteria, the study seeks to contribute valuable insights into their potential as natural antibacterial agents.

This research also chimes with the growing global concern regarding antibiotic resistance and the urgent need for natural alternatives to combat infectious diseases.

### 2.4.1. Well diffusion assay

The antimicrobial activity of honey samples was evaluated against the selected bacterial pathogens using the well diffusion method as described by Holder and Boyce (1994). Briefly, bacterial strains were individually cultured in trypticase soy broth (TSB) at 37°C for 24 hours, with the exception of *Bacillus subtilis*, which was incubated at 30°C for 24 hours. Following incubation, liquefied trypticase soy agar (TSA) was inoculated with each bacterial culture separately and poured into Petri dishes. After solidification, wells of 0.8 cm diameter were aseptically punched into the agar, and each well was loaded with a specific honey sample. The plates were then incubated at their respective optimal growth temperatures (37°C or 30°C) for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zone surrounding each well in centimeter (cm). All experiments were conducted in triplicate, and the average inhibition zone diameter was calculated for each honey-pathogen combination.

### 2.4.2. Minimum inhibitory concentration (MIC)

Based on the results of the well diffusion assay, honey types exhibiting the largest zones of inhibition against the tested bacterial strains were selected for further quantitative analysis. The minimum inhibitory concentration (MIC), representing the lowest concentration of honey capable of inhibiting bacterial growth, was determined using a two-fold broth microdilution method in a 96-well microtiter plate. Honey dilutions were prepared serially with a 50% (w/v) reduction in concentration at each step, following the protocol outlined by Saubolle (2023). This quantitative assessment provided a more precise measure of the antibacterial potency of the selected honeys.

### 2.4.3. Minimum bactericidal concentration (MBC)

To determine whether the observed antibacterial effects were bacteriostatic or bactericidal, a drop plate technique was employed (Herigstad *et al.*, 2001). Following the MIC determination via broth microdilution, 10 microliters of the bacterial suspension were taken from each well, representing different honey concentrations, and spotted onto nutrient agar plates. These plates were then incubated under optimal conditions to allow for bacterial growth. The presence or absence of colony formation was assessed visually, providing insights into the viability of bacterial cells exposed to varying MIC levels of honey. This method allowed for differentiation between growth inhibition (bacteriostatic effect) and complete bacterial killing (bactericidal effect).

### 2.4.4.Observation of Morphological Bacterial changes using Transmission Electron Microscopy (TEM)

To investigate the morphological changes induced in bacteria upon exposure to honey, *Bacillus subtilis* was treated with caraway honey (50% v/v) and incubated for 24 hours. Following incubation, bacterial cells were harvested by centrifugation at 3000 ×g for 10 minutes at 4°C. To visualize the ultra structural alterations, TEM analysis was performed using a JEOL (JEM-1400 TEM) instrument located at the Faculty of Agriculture, Cairo University Research Center. Sample preparation and processing for TEM analysis were carried out according to the established protocols of Hanker and Giammara (1993) and Bravman and Sinclair (1984). This approach allowed for a detailed examination of the honey's impact on bacterial cell morphology at the ultra structural level.

### 2.4.5 Statistical Analysis

All results were presented as the mean ± standard deviation (SD). One-way ANOVA was conducted for the antimicrobial activity of honey samples against each bacterial pathogen using IBM SPSS Statistics for Windows, version 24.0 (IBM Crop, 2020). Duncan's multiple-range test was used to compare the differences between the honey types. A *p*-value of less than 0.01 was considered statistically significant.

# 3. RESULTS

## 3.1. Chemical Composition

### 3.1.1 Sugar content

The sugar content in honey is typically quantified by measuring the percentages of key sugars: fructose, glucose, and sucrose. In this study, the fructose content was found to vary significantly across different honey types, ranging from 35.58% in sidr to 45.00% in caraway honeys, indicating a considerable variation in sweetness levels among the samples (Table 1). Glucose content also exhibited variation, with the lowest percentage observed in fennel honey at 24.11%, and the highest in Anis honey at 31.07%.

The fructose-to-glucose (F/G) ratio significantly influences the crystallization tendency of honey. In this study, clover honey demonstrated the lowest F/G ratio (1.33) while, caraway honey exhibited the highest F/G ratio (1.83). Sucrose content, which was typically present in smaller quantities in honey, also varied among the samples. Caraway honey was found to have the lowest sucrose content (2.00 g/100g). On the other hand, clover honey had the highest sucrose content.

### 3.1.2. Diastase activity, HMF, pH, and free acidity

Diastase activity varied considerably in the samples analyzed (Table 2), as sidr honey recorded the highest activity level at 76.90 followed by clover honey with a diastase activity reached 50.00.

Fennel honey surprisingly, had the highest HMF content at 78.30, meanwhile, sidr honey had the lowest HMF content at 4.00. Among the samples, pH values ranged from 3.85 in anise honey to 5.88 in sidr honey. The generally low pH of these honey samples underscores their natural acidity, with anise honey being the most acidic and sidr honey the least.

This study found that free acidity levels varied across the samples, with fennel honey having the highest free acidity at 34.50, on the other hand, sidr honey had the lowest free acidity at 16.50.

Among honey samples, anise honey had the lowest pH value (3.85), while sidr honey exhibited the highest value (5.88).

## 3.2 Polyphenols content

The analysis of polyphenols in different honey samples revealed that each type of honey possesses a unique polyphenolic composition. This uniqueness is particularly evident in the variation of flavonoid content among the samples.. Interestingly, the analysis showed that flavonoid components were present in varying concentrations across the honey samples, with some honeys lacking certain flavonoids altogether (Table 3).

Anise honey absolutely dominates in hesperidin content (80.493 mg/kg), dwarfing the levels found in other samples. It was also contained a moderate amount of rutin (0.534 mg/kg). While low in concentration, the presence of catechin, myricetin, and apigenin adds nuances to the overall antioxidant profile of anise honey.

**Table (1): Content of sugars ±SD in different honey types.**

| Honey type | Fructose (g/100g) ±SD | Glucose (g/100g) ±SD | F/G ratio | Sucrose (g/100g) ±SD |
|---|---|---|---|---|
| **Anise** | 42.39 ±2.12 | 31.07±2.17 | 1.36 | 2.50±0.38 |
| **Caraway** | 45.00±2.25 | 24.64±1.72 | 1.83 | 2.00±0.30 |
| **Fennel** | 39.25±1.96 | 24.11±1.69 | 1.63 | 2.50±0.38 |
| **Sidr** | 35.58±1.78 | 25.18±1.76 | 1.41 | 2.51±0.38 |
| **Clover** | 40.00±2.00 | 30.00±2.10 | 1.33 | 5.00±0.75 |

**Table (2): Content of Diastase activity, HMF, pH and free acidity ±SD in different honey types.**

| Honey type | Diastase (DN) ±SD | HMF (mg/kg) ±SD | pH±SD | Free Acidity (meq/kg) ±SD |
|---|---|---|---|---|
| **Anise** | 6.40±0.77 | 28.70±5.74 | 3.85±0.12 | 22±2.20 |
| **Caraway** | 37.50±4.50 | 23.70±4.74 | 5.09±0.15 | 23.5±2.35 |
| **Fennel** | 20.00±2.40 | 78.30±15.66 | 4.44±0.13 | 34.5±3.45 |
| **Sidr** | 76.90±9.23 | 4.00±0.80 | 5.88±0.18 | 16.5±1.65 |
| **Clover** | 50.00±6.00 | 15.00±3.00 | 4.76±0.14 | 24±2.40 |

**Table (3): Content of flavonoids (mg/kg) ±SD in different honey types.**

| Honey type | Catechin | Myricetin | Apigenin | Kaempferol | Quercetin | Hesperidin | Rutin |
|---|---|---|---|---|---|---|---|
| **Anise** | 0.207 ± 0.037 | 0.161 ± 0.029 | 0.176± 0.032 | 0.000 ± 0.000 | 0.000 ± 0.000 | 80.493 ± 12.074 | 0.534± 0.080 |
| **Caraway** | 0.000 ± 0.000 | 0.214 ± 0.039 | 0.000± 0.000 | 0.000 ± 0.000 | 0.314± 0.057 | 16.478 ± 2.472 | 0.102± 0.015 |
| **Fennel** | 1.020 ± 0.184 | 0.192 ± 0.035 | 0.000± 0.000 | 0.000 ± 0.000 | 0.315 ± 0.057 | 6.138 ± 0.921 | 0.150 ± 0.023 |
| **Sidr** | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.062 ± 0.011 | 0.114 ± 0.021 | 0.000± 0.000 | 1.660 ± 0.249 | 0.492± 0.074 |
| **Clover** | 0.000 ± 0.000 | 0.000 ± 0.000 | 0.071± 0.013 | 0.000 ± 0.000 | 0.287± 0.052 | 12.480 ± 1.872 | 0.354± 0.053 |

Caraway Honey had notable hesperidin concentration. While, significantly lower than anise honey, caraway honey still boasts a considerable amount of hesperidin (16.478 mg/kg). Caraway honey appeared out for containing quercetin (0.314 mg/kg), this honey contained a small amount of myricetin, adding to its diverse flavonoid profile.

Clover honey followed a pattern similar to caraway honey, with a moderate hesperidin concentration (12.48 mg/kg). Similar to caraway, clover honey contained a detectable amount of quercetin (0.287 mg/kg), and a small amount of rutin (0.354 mg/kg).

Fennel honey distinguished itself by being the only honey in this set with a measurable quantity of catechin (1.02 mg/kg).

While lower than anise, caraway and clover honeys, fennel honey still contained a moderate level of hesperidin (6.138 mg/kg). This honey contained a small amount of quercetin, similar to caraway and clover honey.

Sidr honey stands out with a distinct flavonoid signature. It lacks detectable levels of the major flavonoids found in other samples but contains small amounts of apigenin, kaempferol, hesperidin and rutin.

Table (4) highlights the diverse phenolic profiles of different honey types, each with a unique composition of bioactive compounds. Anise honey stands out with its high concentrations of hesperidin and ferulic acid, indicating strong antioxidant potential. caraway honey is notable for its high levels of p-coumaric acid, while sidr honey, although lower in many compounds, has the highest vanillic acid content. clover honey, with generally lower concentrations of these compounds, shows minimal levels of these antioxidants.

The defining feature of caraway honey was its exceptionally high p-coumaric acid content (9.238 mg/kg), far exceeding levels in the other samples. It also had a detectable level of p-hydroxybenzoic acid (1.44 mg/kg), while moderate levels of vanillic acid, ferulic acid, and syringic acid were observed, contributing to its overall antioxidant capacity.

Similar to caraway honey, fennel honey

**Table (4): Content of phenolic acids components (mg/kg) ±SD in different honey types.**

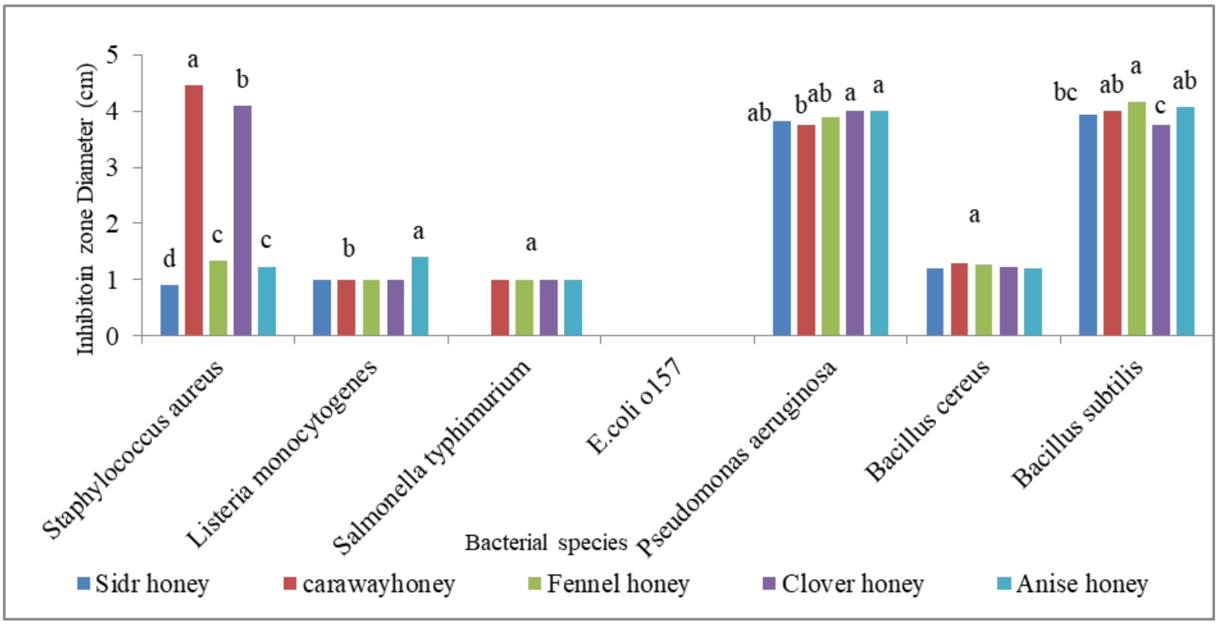
| Honey type | Anise | Caraway | Fennel | Sidr | Clover |
|---|---|---|---|---|---|
| **Vanillic Acid** | 1.052 ± 0.158 | 0.777±0.117 | 0.617±0.093 | 1.553±0.233 | 1.029±0.154 |
| **Chlorogenic Acid** | 1.829 ± 0.274 | 0.249±0.037 | 0.332±0.050 | 0.272±0.041 | 0.338±0.051 |
| **P Coumaric** | 0.203 ± 0.030 | 9.238±1.386 | 4.438±0.666 | 1.082±0.162 | 0.214±0.032 |
| **Ferulic** | 4.515 ± 0.677 | 0.817±0.123 | 0.475±0.071 | 0.296±0.044 | 0.13±0.020 |
| **O Coumaric** | 0.015±0.002 | 0.000±0.000 | 0.173±0.026 | 0.041±0.006 | 0.000±0.000 |
| **Rosemarinic Acid** | 1.431±0.215 | 1.257±0.189 | 0.662±0.099 | 0.192±0.029 | 0.506±0.076 |
| **Gallic Acid** | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 |
| **syringic acid** | 0.000±0.000 | 0.178±0.027 | 0.110±0.017 | 0.125±0.019 | 0.063±0.009 |
| **Caffeic Acid** | 0.519±0.078 | 0.595±0.089 | 0.481±0.072 | 0.087±0.013 | 0.000±0.000 |
| **Catechol** | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 | 0.000±0.000 |
| **P hydroxybenzoic** | 0.000±0.000 | 1.440±0.072 | 1.130±0.0565 | 0.000±0.000 | 0.060±0.009 |

boasted a notable amount of p-coumaric acid (4.438 mg/kg), though lower than caraway. Fennel honey was unique in this dataset for containing a measurable quantity of o-coumaric acid. Though present in a small amount (0.173 mg/kg), this phenolic acid could contribute to the specific aroma profile of fennel honey. Similar to caraway, fennel honey exhibited moderate levels of vanillic acid and ferulic acid, contributing to its overall phenolic profile. Anise honey distinguished itself with the highest concentration of ferulic acid (4.515 mg/kg), Chlorogenic acid (1.829 mg/kg), and Rosemarinic acid (1.431 mg/kg) among the tested samples. This honey contained moderate amounts of vanillic acid and chlorogenic acid, contributing to its overall phenolic profile. Unlike the previous honeys with one or two dominant compounds, sidr honey displayed a more balanced phenolic profile. While it doesn't have exceptionally high concentrations of any single phenolic acid, vanillic acid (1.553 mg/kg), it contained detectable amounts of several, including p-coumaric acid, chlorogenic acid, syringic acid and ferulic acid. Clover honey exhibited low overall phenolic content among the five analyzed honeys. While vanillic acid (1.029 mg/kg) was the most abundant phenolic acid in this honey.

## 3.3 Antimicrobial activity

### 3.3.1 Well diffusion assay

The antibacterial efficacy of five different honey types was evaluated against six human pathogens and one soil bacterium (*Bacillus subtilis*) using the well diffusion assay. As illustrated in Fig. 1, honey exhibited varying degrees of inhibitory activity depending on the bacterial species. Notably, *Pseudomonas aeruginosa* and *B. subtilis* were highly susceptible to honey, displaying larger inhibition zones compared to other tested pathogens. caraway and clover honeys demonstrated the most significant potent activity against *S. aureus*, significantly inhibiting its growth. All honey types exhibited significant substantial inhibitory effects against *P. aeruginosa* and *B. subtilis*, with inhibition zones reaching approximately 4 cm in diameter (Fig. 1 and exemplified in Fig 2).




**Columns of the same Bacterial species followed by the different letters are significantly different according to Duncan Multiple Range Test at 0.01 probability.**

**Fig.(1): Inhibition zone diameter (cm) of different honey types using well diffusion assay.**

(a) (b)

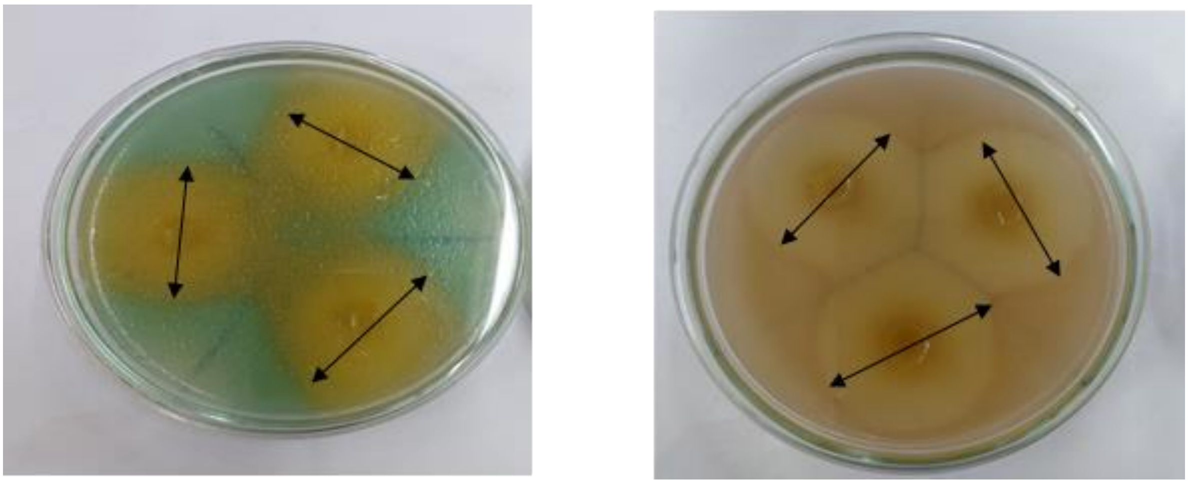


**Fig.(2):Inhibition zone of honey types against bacterial strains (a) Fennel honey against** *Pseudomonas aeruginosa* **(b) Caraway honey against** *Bacillus subtilis*

In contrast, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Bacillus cereus* showed moderate susceptibility to the tested honeys, exhibiting smaller inhibition zones of approximately 1 cm (Fig. 1). Unfortunately, *Escherichia coli* O157 appeared resistant to all tested honey types under the experimental conditions.

### 3.3.2. Minimum inhibitory concentration (MIC)

**Table 5** presents the MIC values for the tested honey-pathogen combinations. Remarkably, honey concentrations as low as 0.195% (v/v) were effective in inhibiting the growth of most bacteria. The exception was clover honey against *Staphylococcus aureus*, where inhibition was only observed at a concentration of 12.25% (v/v).

### 3.3.3 Minimum bactericidal concentration (MBC)

The MBC results, summarized in **Table 6,** reveal a concentration-dependent bactericidal activity for the tested honeys. A 50% (v/v) honey concentration consistently exhibited a bactericidal effect, effectively killing the bacteria, as evidenced by the absence of growth. However, further dilutions (25%, 12.5%, 6.25%, etc.) resulted in a shift towards a bacteriostatic effect, inhibiting bacterial growth without complete eradication.

**Table (5): Minimum inhibitory concentration(MIC) for *Staphylococcus aureus, Pseudomonas aeruginosa, and Bacillus subtilis* (2-fold dilutions in microtiter plate)**

| **Dilution** | | $2^{-1}$ | $2^{-2}$ | $2^{-3}$ | $2^{-4}$ | $2^{-5}$ | $2^{-6}$ | $2^{-7}$ | $2^{-8}$ | $2^{-9}$ | $2^{-10}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Honey conc. (v/v%)** | | **50** | **25** | **12.5** | **6.25** | **3.13** | **1.56** | **0.78** | **0.39** | **0.195** | **0.097** |
| ***Staphylococcus aureus*** | caraway | - | - | - | - | - | - | - | - | - | - |
| | clover | - | - | - | + | + | + | + | + | + | + |
| ***Pseudomonas aeruginosa*** | sidr | - | - | - | - | - | - | - | - | - | + |
| | caraway | - | - | - | - | - | - | - | - | - | + |
| | fennel | - | - | - | - | - | - | - | - | - | + |
| | clover | - | - | - | - | - | - | - | - | - | + |
| | anise | - | - | - | - | - | - | - | - | - | - |
| ***Bacillus subtilis*** | sidr | - | - | - | - | - | - | - | - | - | - |
| | caraway | - | - | - | - | - | - | - | - | - | - |
| | fennel | - | - | - | - | - | - | - | - | - | - |
| | clover | - | - | - | - | - | - | - | - | - | - |
| | anise | - | - | - | - | - | - | - | - | - | - |

- Growth Inhibition + Growth

**Table (6): Minimum bactericidal concentration (MBC) for *Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis***

| ***Dilution*** | | **$2^{-1}$** | **$2^{-2}$** | **$2^{-3}$** | **$2^{-4}$** | **$2^{-5}$** | **$2^{-6}$** | **$2^{-7}$** | **$2^{-8}$** | **$2^{-9}$** | **$2^{-10}$** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Honey conc. (v/v%)*** | | **50** | **25** | **12.5** | **6.25** | **3.13** | **1.56** | **0.78** | **0.39** | **0.195** | **0.097** |
| *Staphylococcus* | caraway | + | + | + | + | + | + | + | + | + | + |
| *aureus* | clover | + | + | + | + | + | + | + | + | + | + |
| *Pseudomonas* | sidr | + | + | + | + | + | + | + | + | + | + |
| *aeruginosa* | caraway | - | + | + | + | + | + | + | + | + | + |
| | fennel | - | + | + | + | + | + | + | + | + | + |
| | clover | - | + | + | + | + | + | + | + | + | + |
| | anise | - | + | + | + | + | + | + | + | + | + |
| *Bacillus subtilis* | sidr | - | + | + | + | + | + | + | + | + | + |
| | caraway | - | + | + | + | + | + | + | + | + | + |
| | fennel | - | + | + | + | + | + | + | + | + | + |
| | clover | + | + | + | + | + | + | + | + | + | + |
| | anise | - | + | + | + | + | + | + | + | + | + |

- Growth Inhibition + Growth

Interestingly, *S. aureus* displayed resistance to the bactericidal action of all honey types across all tested dilutions, with only bacteriostatic effects observed. Similarly, sidr honey exhibited solely a bacteriostatic effect against *Pseudomonas aeruginosa* meanwhile, clover honey showed only bacteriostatic activity against *Bacillus subtilis*. These findings highlight potential species-specific responses to different honey types.

### 3.3.4. Transmission Electron Microscopic imaging (TEM)

TEM revealed a pronounced reduction in the internal content of *Bacillus subtilis* cells treated with honey (Fig. 3), evident in both longitudinal and cross-sectional views. Notably, while the honey treatment induced significant cytoplasmic shrinkage, the integrity of the outer cell membrane and spore coat remained intact.

## 4. DISCUSSION

The results of this study highlight the unique physicochemical, polyphenolic, and antimicrobial properties of five non-traditional Egyptian honey types: anise, caraway, fennel, sidr, and clover who met Codex Alimentarius Commission (2022) standards except for anise honey, which had lower than expected diastase activity. Significant variations were observed across the honey samples, emphasizing the influence of botanical origin on honey quality and composition.

### 4.1. Chemical Composition

The observed variations in sugar profiles, diastase activity, hydroxymethylfurfural (HMF) content, pH, and free acidity across the five honey types provide compelling evidence of the intricate relationship between honey

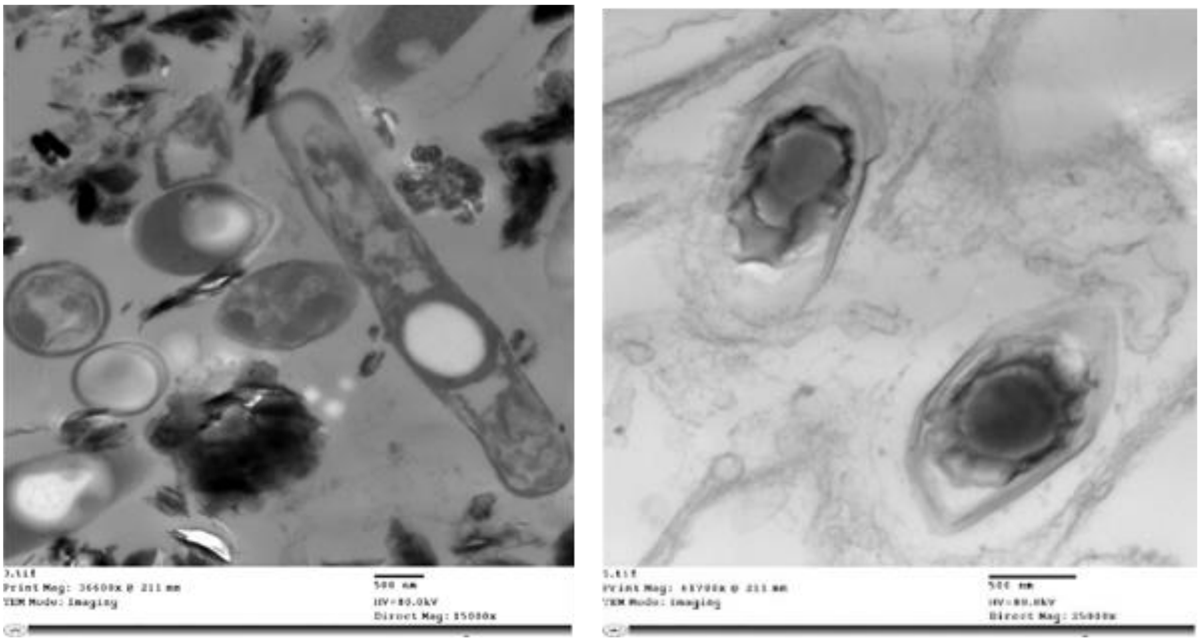


**Fig.(3):Morphological alterations in *Bacillus subtilis* cells following treatment with 50% (v/v) Caraway honey Left fig. provides a longitudinal view of the treated cells, while the right one offers. a cross**-sectional (vertical) perspective. Both images are presented with a scale bar of 500 nm for reference.

composition, floral origin, and processing methods. These findings align with the broader understanding of honey as a complex natural product whose physicochemical properties are intricately linked to its botanical and geographical origins (Bogdanov *et al.,* 2008).

It is possible to use sugar type as a marker to determine the physicochemical characteristics and nutritional worth of honey. The maturity and freshness of honey can be assessed based on its sugar content. Honey is considered well-matured when sugar content is reduced (Pereira *et al.*, 2020; Aljohar *et al.* 2018).

Honey collected from cultivated blooming plants may have higher glucose values, according to Pereira *et al.* (2020). The high fructose-to-glucose (F/G) ratio observed in caraway honey, for instance, suggests a slower crystallization process, a characteristic often favored by consumers (Ouchemoukh *et al.*, 2010). These findings highlight the diversity in sugar composition across different types of honey and provide valuable insights into their unique properties and potential uses (Al-Kafaween *et al.,* 2023). Furthermore, the varying levels of diastase activity and HMF content offer insights into the freshness and processing of each honey type. The high diastase activity and low HMF content in sidr honey, indicate careful handling and minimal heat treatment, contributing to its reputation as a premium-quality honey.

The variations in pH and free acidity, further reinforces the role of organic acids in honey's inherent antimicrobial properties, a characteristic central to its traditional use as a natural preservative (Bogdanov *et al.,* 2008). This natural acidity, coupled with other bioactive components, contributes to the remarkable stability and longevity of honey.

## 4.2. Polyphenols components

Polyphenols are a large and diverse group of naturally occurring plant compounds (Scalbert & Manach, 2005; Spencer *et al.,* 2008). They are known for their potent antioxidant properties, which have been associated with numerous health benefits (Khan *et al.,* 2021). The identification of rutin in all tested honeys at varying concentrations highlights the value of honey as a source of antioxidants (Ganeshpurkar and Saluja, 2017). These findings highlight the distinct polyphenolic profiles of different honey types, which could be influenced by factors such as the botanical origin, geographical location, and environmental conditions (Mongi, 2024). Consequently, the analysis of polyphenols in honey not only provides insights into the honey's nutritional and therapeutic potential but also underscores the importance of understanding the complex chemical composition of honey in relation to its health-promoting properties.

The variations in other polyphenols components emphasize the link between botanical origin and honey composition. For instance, the high hesperidin content observed in anise honey aligns with previous research highlighting the antioxidant properties of monofloral honeys (Gül and Pehlivan, 2018).

## 4.3. Antimicrobial Activity

The study's findings on the antimicrobial activity of Egyptian kinds of honey offer compelling evidence for their potential as therapeutic agents, particularly in the face of rising antibiotic resistance. While all honey types demonstrated inhibitory effects against specific bacterial strains, the variations in efficacy highlight the importance of considering both honey type and target organism when exploring therapeutic applications. The significant inhibitory effects observed with caraway and clover honeys against *Staphylococcus aureus* warrant further investigation (Foster, 2002), Identifying the specific components responsible for this activity could lead to the development of novel antimicrobial agents. The sensitivity of *Staphylococcus aureus* to honey has been attributed to its susceptible for hydrogen peroxide and non-peroxide inhibitory actions (Molan and Russell, 1988; Dustmann, 1979).

The consistent inhibitory effect of all honey types against *Pseudomonas aeruginosa* aligns with the long-standing tradition of using honey for wound healing and supports its continued use as a topical antimicrobial agent (Yi *et al.,* 2023; Estahbanati *et al*., 2002; Bendahbia *et al.*, 2020), suggesting its potential as a natural wound healing agent.

The variations in efficacy against other tested pathogens, including *Escherichia coli* O157, *Listeria monocytogenes*, and *Salmonella typhimurium*, highlight the complex and strain-specific nature of honey's antimicrobial activity (İstanbullugil *et al*., 2023; Zainol *et al.* (2013). Similarly, our study demonstrated the potent inhibitory effect of certain honey types against *S. aureus*.

Furthermore, our findings are consistent with the work of Mama *et al*. (2019), These comparable results highlight the consistent antibacterial potential of honey across different geographical origins and against various bacterial strains, including antibiotic-resistant

## Conclusion

This study provides a valuable contribution to the growing body of knowledge on the unique characteristics and health benefits of Egyptian honeys. The observed variations in chemical composition, antimicrobial activity, and polyphenolic content underscore the importance of understanding the specific properties of different honey types. Our antimicrobial assays revealed differential sensitivity of various pathogens to the tested honey types. This variability in antimicrobial efficacy appears to be closely linked to the honey's botanical origin. Notably, all tested honey varieties demonstrated both bacteriostatic and bactericidal activities, albeit to varying degrees. For instance, caraway and clover honeys exhibited particularly strong inhibitory effects against *Staphylococcus aureus*, suggesting their potential use in targeting this pathogen. Future studies could benefit from exploring seasonal variations, geographical influences, and the sensory characteristics associated with these compositional differences.

## Conflicts of interest

There are no conflicts to declare.

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# الأعسال المصرية غير التقليدية: تحليل شامل للتركيب الكيميائي والمركبات النشطة بيولوجيا والأنشطة البيولوجية

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**ملخص**

أجريت هذه الدراسة لتقييم الخواص الفيزيائية والكيميائية والمواد الفينولية والمضادة للميكروبات لخمسة أنواع من عسل النحل المصري: اليانسون والكراوية والشمر والسدر والبرسيم المصري. لوحظت اختلافات كبيرة في مستويات السكر، نشاط انزيم الدياستيز، هيدروكسي ميثيل فورفورال (HMF) ، درجة الحموضة والحموضة الحرة، مما يعكس تأثير الأصل الزهري وطرق التداول على تركيب العسل. والجدير بالذكر أن عسل الكراوية كانت به قيمة معدل الفركتوز إلى الجلوكوز عالية مما يشير إلى تأخر عملية التبلور، في حين أظهر عسل السدر نشاط مرتفع لأنزيم الدياستيز وقيمة منخفضة من الهيدروكسي ميثيل فورفورال. وعلاوة على ذلك، أظهر كل عسل خصائص فينولية فريدة ، حيث كان عسل اليانسون غنيًا بشكل خاص بالهسبيريدين وحمض الفيروليك. وقد كشف التحليل المضاد للميكروبات عن اختلافات في الفعالية ضد *Pseudomonas aeruginosa* و *Bacillus subtilis* والتي كانتا أكثر السلالات تأثرًا بجميع أنواع العسل حيث كانت منطقة التثبيط حوالي 4 سم، وكان التأثير قاتلا للجراثيم عند تركيزات تتراوح من 50 إلى 100٪ ومضادًا للبكتيريا عند تركيزات أقل من 50٪. طبقا لنتائج الحد الأدنى للتركيز المثبط (MIC) يمكن للعسل المخفف حتى 1.95٪ أن يثبط نمو معظم السلالات باستثناء *Staphylococcus aureus*وأظهرت نتائج الحد الأدنى للتركيز القاتل(MBC) أن تركيزات العسل بنسبة 50٪ تعتبر قاتلة للجراثيم. والجدير بالذكر أن عسل الكراوية والبرسيم أظهرا نشاطًا قويًا ضد *Staphylococcus aureus*، مما يسلط الضوء على إمكاناتهما لتطوير علاج ضد السلالات المقاومة للمضادات الحيوية. تسلط هذه النتائج الضوء على الخصائص الفريدة لبعض أنواع العسل المصري غير التقليدية وفوائدها الصحية المحتملة.

**المجلة المصرية للعلوم الزراعية – المجلد (75) العدد الثالث (أكتوبر 2024) :116-130.**