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# Physicochemical properties, antibacterial activity, and antioxidant capacity of mixed Sidr honey from Saudi Arabia

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**Introduction:** Sidr honey has been used for centuries for its taste, odor, nutritive value, and therapeutic characteristics, including antibacterial and antioxidant activity. Sidr honey is high cost, and limited supply leads to frequent tampering. Honey's quality is established by its botanical origin and chemical composition, which, depending on whether it is categorized as organic, monofloral, or multifloral, are also utilized to increase sales. This study evaluated the quality parameters and antioxidant capacity of mixed Sidr honey (Sidr Al-tmayoz).

**Methodology:** Physicochemical, antibacterial properties, and antioxidant capacity of mixed Sidr honey (Sidr Al-tamayoz) from Saudi Arabia were performed. The Sidr Al-tamayoz was designed using three types of Sidr honey, i.e., Peshawar Sidr, Al-Barri Sidr, and Balady Sidr (1:1:1; w/w). physicochemical including pH level, total insoluble solids, hydroxymethylfurfural, hydroxymethylfurfural, sugars, diastase enzyme activity were carried out. Bioactive compounds that is, flavonoids and phenolics, also antioxidant activity (DPPH) were done. Additionally, antibacterial capacity was conducted using agar well diffusion assay.

**Results and discussion:** The physicochemical properties of Sidr Al-tamayoz honey were moisture (16.7%), pH (5.07), acidity (20 meq/kg), electrical conductivity (0.85 mS/cm), hydroxymethylfurfural (5.1 mg/kg), and total sugars (83 g/100 g) in accordance with Codex Alimentarius. Sidr Al-tamayoz honey exhibited high phenolic (45.76 mg GA/g), flavonoid (40.66 mg R/g), and antioxidant activity (62.68%). Moreover, Sidr Al-tamayoz displayed the greatest antibacterial activity (inhibitory zones and dehydrogenase activity) out of all the pathogenic bacteria: *Staphylococcus aureus*, *Salmonella Typhimurium*, *Pseudomonas aeruginosa*, and *Escherichia coli* O157:H7.

**Conclusion:** The findings demonstrated that Sidr Al-tamayoz honey is a promising natural product rich in bioactive compounds and antioxidants, as well as enhancing quality parameters.

## KEYWORDS

Sidr honey, mixed honey, antibacterial activity, physicochemical, antioxidant activity

## Introduction

Bee honey is a genuine gift from nature because of its unique flavor and amazing medicinal qualities; it is the most well-known and significant product of the honeybee (*Apis mellifera*) (Kumar, 2024). For millennia, honey has been employed as a natural sweetener or flavoring, and it is highly valued and frequently consumed (Predanócyová and Šedík, 2024). Its complex composition includes ~200 compounds, such as carbohydrates, enzymes, proteins, minerals, vitamins, phenolic compounds, and organic acids (Wilczyńska and Žak, 2024). Honey seems to possess antimicrobial, antidiabetic, anti-inflammatory, anti-obesity, and wound-healing properties (Mashal et al., 2023; El-Seedi et al., 2024). Global honey production reached 1.88 million tons in 2018. With 44% of the world's production, Asia is the continent that produces the most, followed by Europe (24%), the Americas (19%), Africa (11%), and Oceania (2%) (FAO, 2024; Marchese, 2024). The value of honey exports is around \$2 billion USD (FAO, 2024).

Sidr honey, a monofloral honey produced from *Ziziphus* species, is becoming more well-known after Manuka honey (Bouddine et al., 2024). Sidr, also called Jujuba, is primarily distributed in the arid regions of Yemen, Saudi Arabia, Egypt, Libya, and Pakistan (Ibrahim et al., 2024). Sidr honey is a high cost product with a limited supply leads to frequent tampering (Dar et al., 2024). Honey's quality is established by its botanical origin and chemical composition, which, depending on whether it is categorized as organic, monofloral, or multifloral, are also utilized to increase sales (Rosa et al., 2024).

Numerous studies have demonstrated the broad antibacterial activity of honey from different geographical locations; nonetheless, the honey's source significantly affects how effective it is (Ogwu and Izah, 2025). Additionally, studies have demonstrated that the antibacterial qualities of honey vary depending on the type of bloom and may even vary within the same floral source (Luca et al., 2025). Nectar, pollen, and propolis are among the basic ingredients that bees are known to gather to produce honey. Certain constituents of these raw ingredients possess significant antimicrobial qualities that can enhance honey's overall antimicrobial efficacy (Betta et al., 2024). Honey's antibacterial activity have been studied in relation to a number of diseases, including viruses (Luca et al., 2024), fungi and yeasts, (Hao et al., 2022), Gram-positive and Gram-negative bacteria (Faleiros-Quevedo et al., 2025), and multidrug-resistant bacteria (Ogwu and Izah, 2025).

Honey naturally contains hydrogen peroxide, as bees collect glucose oxidase from nectar, which converts glucose into gluconic acid and hydrogen peroxide. Hydrogen peroxide acts as an antimicrobial agent through several mechanisms, including disrupting bacterial membranes and generating reactive oxygen species (ROS), which lead to protein oxidation and DNA strand breakage (Xie and Coghi, 2025). However, the antimicrobial effect of hydrogen peroxide may be suppressed or enhanced by the presence of other honey compounds.

On the other hand, honey is a highly concentrated (hyperosmotic) sugar solution with low water content and a low water activity (aw) of ~0.5–0.6. Microbes cannot divide or grow in such dry conditions, as most bacteria and fungi

require an (aw) of >0.9. As well, its high sugar content makes it hypertonic compared to microbial cells, resulting in a strong osmotic effect that draws water from the microbial cells, leading to their dehydration and death. Furthermore, the osmotic pressure of honey works in conjunction with its acidity, hydrogen peroxide, bee-derived peptides, phytochemicals (phenols and flavonoids), and enzymes to kill or inhibit microbes (Khataybeh et al., 2023). In one study, Hegazi et al. (2017) collected 10 types of monofloral honey from Saudi Arabia. They examined them against 5 Gram-negative (*Klebsiella pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus* and *Streptococcus mutans*) bacteria. The antibacterial properties of various honey varieties varied depending on the type of bacteria they were tested against. Alqurashi et al. (2013) assessed the effectiveness of the antimicrobial properties of regional Sidr and mountain honey from Saudi Arabia against a number of human diseases and discovered that Sidr honey was more effective against Gram-negative bacteria (*E. coli*). Honey has been shown to reduce inflammation and oxidative stress while promoting the healing process due to its biochemical composition, which includes a wide range of antioxidants (Wilczyńska and Žak, 2024).

A unique method for creating new honey products is mixed Sidr honey (Sidr Al-tamayoz). Different types of honey contain distinct antibacterial compounds, and blending honey combines their active components, creating a broader, more potent antimicrobial effect. This helps target multiple microbial pathways, such as osmotic stress, oxidative damage, membrane disruption, and enzyme inhibition. Mixing honey may mimic multidrug therapy, reduce the chance of microbial resistance, and enhance efficacy. Furthermore, the honey mixture ensures that minor compounds, which may be negligible in one honey, contribute significantly when pooled. Furthermore, some honey may lack critical antibacterial agents (low peroxide activity or flavonoid). A mixture fills these gaps, making the final product more robust and effective. Sidr Al-tamayoz will improve the honey's quality attributes. Additionally, Sidr Al-tamayoz will compile distinct descriptions of Sidr honey from different botanical origins and geographical locations. Sidr Al-tamayoz will also provide a number of nutritional and physiological advantages. Honey from various botanical sources differs significantly in chemical composition, physical properties, sensory features, and medicinal benefits.

Previous studies did their best to identify and monitor the quality of Sidr honey, but there are no studies focused on mixed Sidr honey (Sidr Al-tamayoz). Therefore, the study aimed to (i) determine the physicochemical properties of mixed Sidr honey and (ii) evaluate the impact of mixed Sidr honey on antimicrobial activity and antioxidant capacity.

## Materials and methods

### Honey samples

Three kinds of Sidr honey, i.e., Peshawar Sidr, Al-Barri Sidr, and Balady Sidr, were collected in 2024 from the local market in Riyadh, Saudi Arabia. The samples were transported to the laboratory in a carton box within 30 min to the Nahal association. The honey

samples were kept in glass jars in the cold-dark condition until used for preparation and analysis.

## Bacterial strains

Four bacterial strains, *Staphylococcus aureus* (ATCC 20231), *Salmonella* Typhimurium (ATCC 14028), *Pseudomonas aeruginosa* (ATCC 9027), and *Escherichia coli* O157:H7 (ATCC 6933), were obtained from the Agricultural Microbiology Department, Benha University, Cairo, Egypt. Tryptic soy broth (TSB; Biolife, Italy) was used to activate the bacteria, and they were then grown in the same medium for 24 h at  $37 \pm 1^\circ\text{C}$ . By counting on tryptic soy agar, the bacterial population of  $\sim 6 \log_{10}$  CFU  $\text{mL}^{-1}$  (CFU) was verified.

## Preparation of mixed Sidr honey (SidrAl-tamayoz)

Mixed Sidr honey (Sidr Al-tamayoz) was prepared by mixing three types of honey, i.e., Peshawar Sidr, Al-Barri Sidr, and Balady Sidr (1:1:1, w/w), using a homogenizer at  $25^\circ\text{C}$  in the Nahal association, Saudi Arabia. The samples were placed in glass jars (250 g) and dark conditions until analysis (Figure 1).

## Physicochemical properties

The pH level was determined by a pH meter (M21, Boeco, Germany), and electrical conductivity (EC; mS/cm) was measured in 20% (w/v) honey solution tested using an Ino lab conductometer (M21, Boeco, Germany) according to AOAC (2016). Total insoluble solids were estimated by an oven at  $105^\circ\text{C}$ , and free lactone and acidity were determined by a titrimetric method according to AOAC (2016). Hydroxymethylfurfural (HMF) of aqueous honey solution was analyzed using HPLC (Smart line, Knauer, Germany) (Shapla et al., 2018). The total and reduced sugars were estimated according to AOAC (2016). Glucose, fructose, and sucrose contents were determined using HPLC (Phenomenex® Luna NH<sub>2</sub> 250X4.6 mm) according to the standard methods (Zhou et al., 2014).

## Diastase enzyme activity (DEA)

Diastase activity was measured using spectrophotometric (UV/Vis spectrophotometry, Jenway, England) at 620 nm according to the Phadebas method (Schade et al., 1958). The findings are given in Schade units as diastase number (DN). The enzyme capacity of 1 g of honey is equivalent to one unit, which can hydrolyze 0.01 g of starch in 1 h at  $40^\circ\text{C}$  and pH 5.2. DN was calculated using Equation 1:

$$\text{DN} = 28.2 \times \Delta A_{620} + 2.64 \quad (1)$$

## Phenolic and flavonoid compounds

According to Bertonec et al. (2007), the Folin-Ciocalteu method was used to measure the total phenolic (TP) of honey at 750 nm. In brief, 2 mL of Na<sub>2</sub>CO<sub>3</sub> (20%; w/v) was added to 3 mL of distilled water (DW) after 200 mL of honey (1 mg  $\text{mL}^{-1}$ ) had been gently mixed with Folin reagent (0.5 mL/3 min), then the mixture was measured at 750 nm. Gallic acid equivalent (GAE) of TP was expressed as 1 milligram per 100 grams of honey. As elucidated by Shraim et al. (2021), the colorimetric measurement of honey's total flavonoid (TF) was conducted at 510 nm. Briefly, 50 mL of honey (1 mg/mL) was added to 1 mL of methanol, followed by 4 mL of DW, 0.3 mL of 5% (w/v) NaNO<sub>2</sub>, and 0.3 mL of 10% (w/v) AlCl<sub>3</sub> after 5 min of incubation. The mixture was then left for 6 min, and then 2 mL of NaOH (1 mol/L) was added. Double-DW was used to reduce the mixture's final volume to 10 mL. The absorbance at 510 nm was measured after the mixture was left for 15 min. The amount of rutin equivalents (RE) per gram of honey was used to calculate the TF. Three separate analyses were carried out.

## Antioxidant activity (AA)

Honey's capacity to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals was evaluated using the methodology described by Chua et al. (2013). The 200  $\mu\text{L}$  of honey and 3.8 mL of DPPH solution were combined, and the mixture was left to sit at ambient temperature in the dark for 60 min. The mixture was measured at 517 nm, and the positive control, or BHT, was conducted. AA was calculated as the following Equation 2:

$$\text{DPPHscavengingactivity (\%)} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100 \quad (2)$$

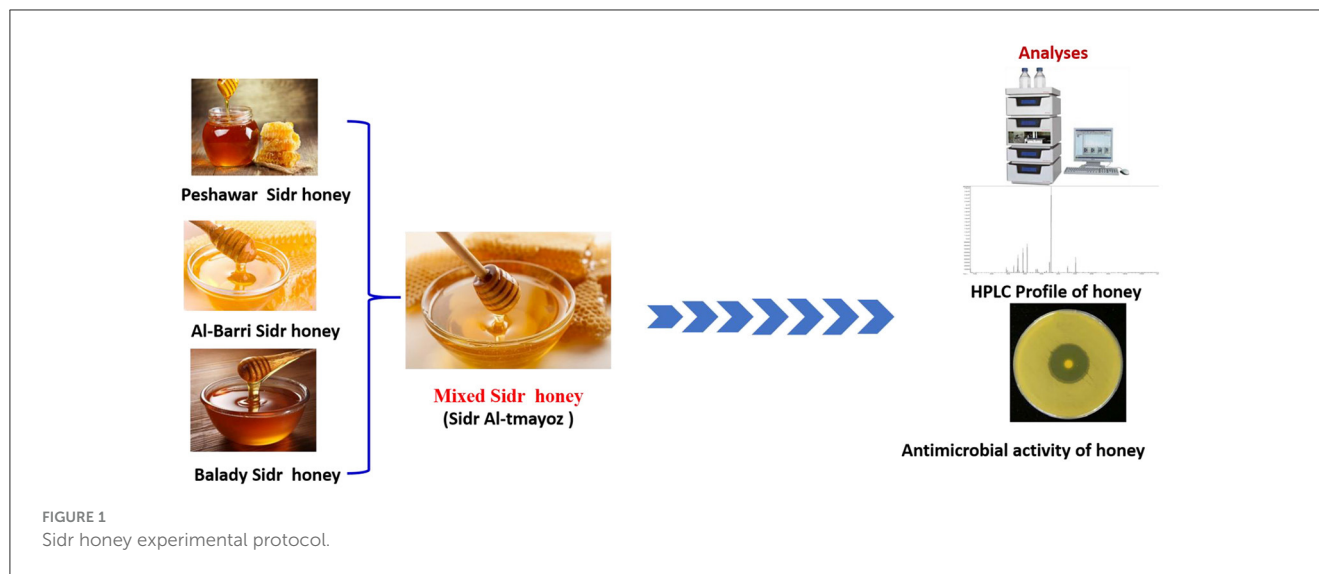
## Honey's antimicrobial properties

### Agar well-diffusion assay

On sterile N-agar plates, 1 milliliter of each pathogenic *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *E. coli* O157:H7 bacterial inoculum was applied. A measure of 50  $\mu\text{L}$  of honey was added to each well after 7-mm diameter wells were cut out of the middle of the plates using a sterile cork-borer. The plates were incubated for 1 h at ambient temperature and then for 24 h at  $37^\circ\text{C}$ . Three duplicates of each honey concentration —25, 50, and 100%— were used in this experiment. For the negative control, sterilized distilled water was used (Jaradat et al., 2022).

### Microbial growth

To achieve different concentrations (15, 25, 50, and 75%), honey were serially diluted twice with the nutrient broth in tubes. A 1 mL of bacterial inoculum that was 24 h old ( $10^6$  CFU/mL) was added to 9 mL of each concentration (Doughari, 2006). Then the tubes were incubated for 24 h at  $37^\circ\text{C}$ . The following activities were estimated.



**TABLE 1** Physicochemical parameters of Sidr honey samples (Mean  $\pm$  SD).

Parameters	Peshawar Sidr	Al-Barri Sidr	Balady Sidr	Sidr Al-tmayoz	Limit value (Codex)
Moisture content (%)	19.4 $\pm$ 0.25 <sup>a</sup>	17.0 $\pm$ 0.22 <sup>b</sup>	19.1 $\pm$ 0.3 <sup>a</sup>	16.7 $\pm$ 0.2 <sup>b</sup>	$\leq$ 20
pH	5.5 $\pm$ 0.11 <sup>a</sup>	5.14 $\pm$ 0.08 <sup>a</sup>	4.7 $\pm$ 0.12 <sup>b</sup>	5.07 $\pm$ 0.1 <sup>a</sup>	3.4–6.1
Free acidity (meq/kg) (FA)	10.0 $\pm$ 0.15 <sup>c</sup>	9.5 $\pm$ 0.18 <sup>c</sup>	11.0 $\pm$ 0.26 <sup>b</sup>	12.0 $\pm$ 0.22 <sup>a</sup>	Max. 50
Lactone acidity (meq/kg)	6.50 $\pm$ 0.1 <sup>b</sup>	5.5 $\pm$ 0.07 <sup>c</sup>	7.0 $\pm$ 0.12 <sup>b</sup>	8.0 $\pm$ 0.18 <sup>a</sup>	
Total acidity (meq/kg)	16.5 $\pm$ 0.15 <sup>c</sup>	15.0 $\pm$ 0.22 <sup>d</sup>	18.0 $\pm$ 0.32 <sup>b</sup>	20.0 $\pm$ 0.24 <sup>a</sup>	
Electrical conductivity EC (mS/cm)	0.577 $\pm$ 0.05 <sup>b</sup>	0.608 $\pm$ 0.03 <sup>a</sup>	0.579 $\pm$ 0.04 <sup>b</sup>	0.849 $\pm$ 0.09 <sup>a</sup>	0.8

<sup>a,b,c,d</sup>Significant differences are observed at  $p < 0.05$  for values with different letters in the same row.

### Bacterial growth curve

Nine milliliters per tube were used to split the dilutions into tubes. Each tube was then filled with 1.0 mL of the pathogenic bacteria culture, which was then incubated for 48 h at 37°C. At 0 time, 12, 24, 36, and 48 h, the optical density at 600 nm was measured. As a positive control, bacterial cultures devoid of honey were used (Burdock et al., 2011; Hamoda et al., 2021).

### Dehydrogenase activity (DHA)

After pipetting 1 milliliter from each prepared tube into four test tubes, each tube was filled with 1.0 milliliter of TTC-glucose solution (1.0 gram of glucose and 2.0 grams of TTC dissolved in 100 milliliters of distilled water) and 2.5 milliliters of 0.1 M Tris buffer (12.42 grams of hydroxyl methyl amino-methane in 373 milliliters of HCl 0.2 N and made up to 1 liter, pH 7.8). In place of the sample, distilled water was used as the control. The HCl solution (1 N) was added to get the final pH down to 7, and the tubes were then carefully Sidr Al-tamayoz. The tubes were centrifuged for 10 min at 5,000 rpm after being incubated for 1 h at 37°C (Burdock et al., 2011). DHA was calculated using the following Equation 3:

$$\text{DHA } (\mu\text{mol TF/mL}) = \text{Abs } 484\text{nm} \times 10.574 \quad (3)$$

### Statistical analysis

The analysis of variance (ANOVA) and *post-hoc* Tukey for mean comparison were used to identify significant differences between samples. The IBM SPSS statistical program was used for all statistical studies. A  $p$ -value of  $\leq 0.05$  was deemed significant.

## Results and discussion

### Physicochemical parameters

The physicochemical properties of honey are valuable indicators for both botanical origin identification and quality assessment. Table 1 presents the moisture, pH, free acidity, lactone, total acidity, and electrical conductivity (EC) of the honey samples. Since moisture content is important for fermentation, it can be used as a parameter to assess a food's vulnerability to deterioration. The moisture content of honey samples ranged between 16.7 and 19.4% for Sidr Al-tamayoz and Peshawar, respectively. According to the Codex Alimentarius, honey must have no more than 20% moisture (Codex, 2022). This indicates all samples are within the permissible limits. Previous studies found that the moisture content of Yemen Sidr honey ranged 13.4–16.0% (Roshan et al.,

TABLE 2 HMF, sugars profile, and diastase activity of Sidr honey samples (Mean  $\pm$  SD).

Parameters	Peshawar Sidr	Al-Barri Sidr	Balady Sidr	Sidr Al-tamayoz	Limit value* (Codex)
HMF (mg kg <sup>-1</sup> )	10.7 $\pm$ 0.12 <sup>a</sup>	6.5 $\pm$ 0.1 <sup>c</sup>	8.1 $\pm$ 0.15 <sup>b</sup>	5.10 $\pm$ 0.08 <sup>d</sup>	80
Total reducing sugars (g/100 g)	64.32 $\pm$ 0.23 <sup>c</sup>	64.0 $\pm$ 0.25 <sup>c</sup>	71.97 $\pm$ 0.18 <sup>a</sup>	67.17 $\pm$ 0.33 <sup>b</sup>	
Total non-reducing sugars (g/100 g)	15.78 $\pm$ 0.15 <sup>b</sup>	18.5 $\pm$ 0.2 <sup>a</sup>	8.33 $\pm$ 0.14 <sup>c</sup>	15.83 $\pm$ 0.21 <sup>b</sup>	
Total sugars (g/100 g)	80.1 $\pm$ 0.24 <sup>c</sup>	82.5 $\pm$ 0.22 <sup>b</sup>	80.3 $\pm$ 0.2 <sup>c</sup>	83.0 $\pm$ 0.25 <sup>a</sup>	$\geq$ 60
Fructose (g/100 g)	42.2 $\pm$ 0.08 <sup>b</sup>	42.2 $\pm$ 0.05 <sup>b</sup>	47.75 $\pm$ 0.11 <sup>a</sup>	43.86 $\pm$ 0.14 <sup>b</sup>	33–36
Glucose (g kg <sup>-1</sup> )	22.12 $\pm$ 0.04 <sup>b</sup>	21.8 $\pm$ 0.06 <sup>b</sup>	24.22 $\pm$ 0.08 <sup>a</sup>	23.31 $\pm$ 0.1 <sup>a</sup>	25–28
Sucrose (g/100 g)	ND	ND	ND	ND	10
Fructose/glucose ratio	1.91 $\pm$ 0.02 <sup>a</sup>	1.94 $\pm$ 0.05 <sup>a</sup>	1.97 $\pm$ 0.07 <sup>a</sup>	1.88 $\pm$ 0.04 <sup>b</sup>	0.9–1.35
Glucose/moisture ratio	1.14 $\pm$ 0.03 <sup>c</sup>	1.28 $\pm$ 0.06 <sup>b</sup>	1.27 $\pm$ 0.08 <sup>b</sup>	1.39 $\pm$ 0.05 <sup>a</sup>	
Diastase (g/100 g)	14.3 $\pm$ 0.1 <sup>a</sup>	14.3 $\pm$ 0.06 <sup>a</sup>	6.1 $\pm$ 0.02 <sup>c</sup>	10.1 $\pm$ 0.06 <sup>b</sup>	Up to 8

<sup>a,b,c,d</sup>Significant differences are observed at  $p < 0.05$  for values with different letters in the same row.

ND, Not detected.

2017), Saudi Arabia Sidr honey 18.0% (Hegazi et al., 2022), Omani Sidr honey ranged from 13.7 to 18.8% (Al-Farsi et al., 2018), and Egyptian Sidr honey was 19.0% (Omran et al., 2023). The variance in honey moisture content is due to honey's varieties, weather, and maturity level (Taha et al., 2021). The pH values of Balady honey and Al-Barri honey ranged from 4.7 to 5.5, respectively. Based on the Codex standard, the pH value ranged from 3.7 to 6.0 according to regional variation (Chakir et al., 2016), which revealed that the pH levels of Moroccan honey ranged between 3.2 and 5.0 (Guerzou et al., 2021). pH values in Algerian honey were between 3.5 and 5.1. Hegazi et al. (2022) investigated 794 Sidr honey samples imported into the Saudi market from 12 different nations and discovered that the pH values varied between 5.4 (Saudi Arabia), 3.6 (Egypt), and 7.4 (Yemen).

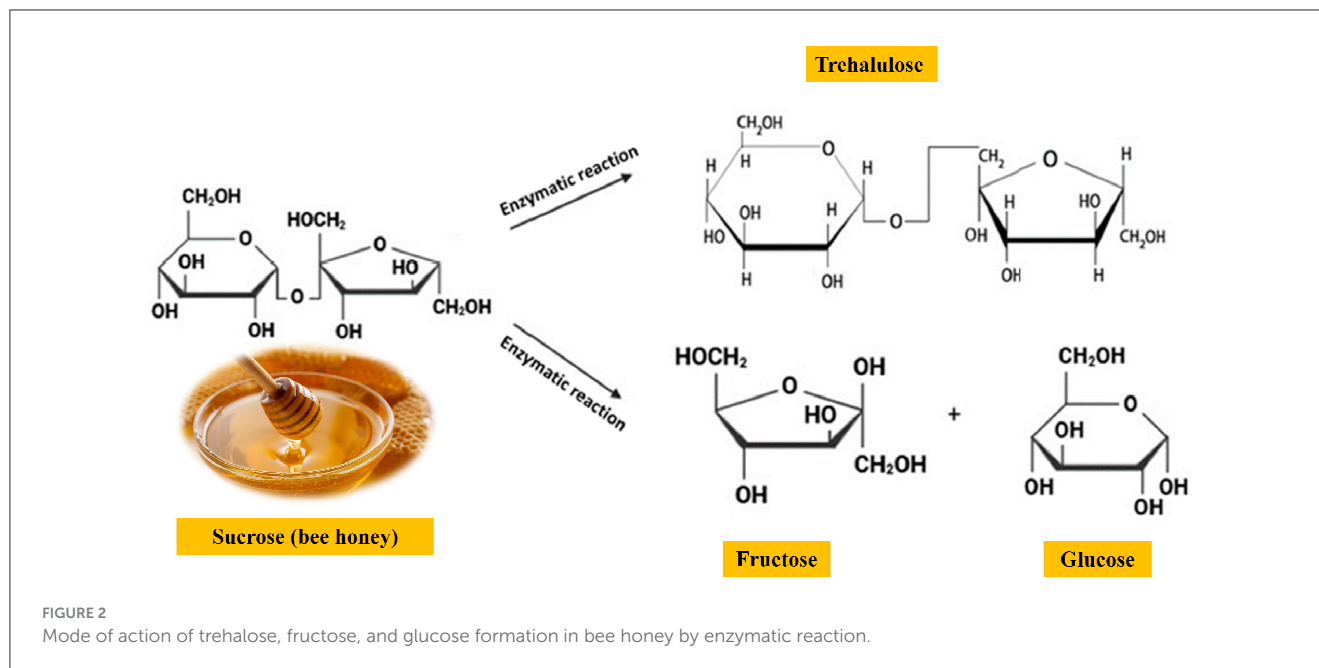
Honey becomes acidic due to the presence of organic acids, specifically citric, acetic, formic, succinic, gluconic, pyruvic, tartaric, lactic, and maleic acids (Suto et al., 2020). Gluconic acid provides the majority of the acid in honey. Honey's durability and antibacterial qualities are influenced by its acidity (Pauliuc et al., 2021). Table 1 shows that free acidity (FA) levels in Sidr honey samples were low ( $\leq 12$  meq/kg). FA is linked to the characteristics of the plant of origin and the circumstances of storage. FA is recognized to have no bearing whatsoever on the quality of honey and at preserving its freshness; it is strongly connected with relative humidity and electrical conductivity (EC), but not with pH (Al-Yahya et al., 2013). Lactones have a slightly sour undertone to their sweet scent. Lactones helped to explain some of honey's remarkable resistance to germs and greatly enhanced its overall scent (Moreira et al., 2002).

The strong link between EC and its ionic and organic acid content makes it a common substitute for ash content in regular quality control. The EC value of Sidr honey samples ranged between 0.57 and 0.84 mS/cm for Peshawar Sidr and Sidr Al-tamayoz, respectively. The Council of the European Union recommends a maximum acceptable value of 0.8 mS/cm (Hegazi et al., 2022).

## Hydroxymethylfurfural (HMF), sugar profile, and diastase activity

HMF is among the most significant markers of honey freshness and quality. HMF concentrations in Sidr samples were 5.1 to 10.7 mgkg<sup>-1</sup> for Sidr Al-tamayoz and Peshawar, respectively (Table 2). The maximum amount of HMF allowed in honey is 40 mg kg<sup>-1</sup> (Codex, 2022). Similarly, the GCC Standardization Organization (GSO) advises that honey from tropical regions have 80 mg kg<sup>-1</sup> (Bicudo de Almeida-Muradian et al., 2020). A HMF result  $> 40$  mgkg<sup>-1</sup> indicates that the sugar contents have been cooked and/or processed, and is linked to hot weather and extended harvest times (Yang et al., 2019). Conversely, HMF results below 40 mg kg<sup>-1</sup> are indicative of relative freshness (Sanz et al., 2003). Honey received straight from beekeepers had HMF that was almost five times lower than honey kept in markets.

Table 2 provides an overview of the sugar profiles, fructose (F)-to-glucose (G), and glucose (G)/moisture (M) ratios of the examined honey samples. The total sugar content of Sidr honey samples ranged from 80.1 to 83%. While the fructose content ranged from 42.20 to 47.75%, and glucose was 21.8 to 24.22% in the tested Sidr samples. The (F) to (G) ratio was 1.88–1.97 for Sidr Al-tamayoz and Balady, respectively. Since the (F)/(G) ratio and humidity are markers of honey's propensity to crystallize, the (F)/(G) ratios were  $< 1.0$ , which caused the honey to crystallize more quickly (Amariei et al., 2020). When the fructose/glucose ratio was 1.1, the crystal with the strongest van der Waals and electrostatic interactions was created (Naik et al., 2019). Additionally, the results demonstrated that all analyzed Sidr honey samples contained no detectable sucrose. The amount of sucrose in honey can provide insight into its botanical origin and adulteration (Islam et al., 2020). According to GSO, the maximum amount of sucrose was 10% (Bicudo de Almeida-Muradian et al., 2020). The sucrose level of Oman's Sidr honey ranged from 0.1 to 17.5% (Al-Farsi et al., 2018). None of the examined samples contained sucrose. The idea that trehalose is formed by an enzymatic process in which sucrose occupies the enzyme's active site and functions as a donor



to generate a  $\beta$ -D-glucosyl-enzyme intermediate is supported by the absence of sucrose (Zhang et al., 2022). This glucosyl-enzyme intermediate forms trehalose by binding to either the same or another free fructose moiety at 1-OH after releasing a fructose moiety (Figure 2).

Analyzing the sugar profile of honey is essential for determining its quality and spotting possible adulteration. The lack of measurable sucrose levels in the examined samples suggests that there was no adulteration, since a high sucrose concentration could indicate that commercial sugar was added (Al-Yahya et al., 2013; de Sousa et al., 2016). However, it is crucial to emphasize that the sugar profile of honey should not be the only factor used to identify adulteration. This is due to the possibility that bees may convert sucrose into trehalose when given it, producing honey with a sugar profile that is comparable to that of honey found naturally, but probably lacking other crucial elements that enhance honey's quality (Fletcher et al., 2021). Furthermore, the glucose level of premium honey usually decreases relative to the fructose content, a feature found in all bee honey samples examined (Julika et al., 2020).

One of the key markers is diastase activity, which measures honey freshness. The diastase activity of Sidr honey samples ranged from 6.1 to 14.3 g/100 g for Balady honey and Peshawar, respectively. The diastase levels of the samples in the current investigation were within an acceptable range. According to the Codex, honey has a minimum diastase activity of 8 diastase number (DN) or Gothe units, and 3 DN for honey with low natural enzyme content (Codex, 2022). Honey's storage conditions and heating process have an impact on diastase activity (Bako et al., 2019).

## Antioxidant compounds and activity

### Total phenols (TP) and total flavonoids (TF)

As shown in Table 3, the Sidr honey's photochemical, including TP and TF components, were assessed. Findings showed that

the TP varied from 23.84 to 55.73 mg GAE/g. Balady honey was determined to have the highest TP, while Peshawar honey had the lowest value. However, TF was the lowest 25.42 mg R/g of Al-Barri honey, while it was highest 40.66 mg R/g of Sidr Al-tamayoz honey. One study by Zawawi et al. (2022) conducted earlier research on TP in stingless bee honey and discovered that the range of TP for Australian honey was 88.3–132.0 mg GAE/100 g, while the range for Malaysian honey was 22.3–54.2 mg GAE/100 g. An additional investigation by Carina Biluca et al. (2021), the TP values for Brazilian bee honey ranged from 11.0 to 38.9 mg GAE/100 g. The variance in TP may also be a result of the samples' varying botanical origins in addition to being produced by various bee species (Mello dos Santos et al., 2024). Regarding TF, the results were in agreement with those noted by Hegazi et al. (2022), who found that flavonoid contents in Peshawar, Yemen, Punjab, and Saudi Arabia natural Sidr honey were 41.3, 38.13, 35.3, and 81.5 mg RE/100 g, respectively.

### DPPH scavenging activity

DPPH scavenging activity is one of the popular, rapid, and simple ways to evaluate honey's antioxidant activity and the ability of its ingredients to donate hydrogen or scavenge free radicals. A high DPPH is indicative of inhibiting lipid peroxidation, which enhances honey's potency as a therapeutic food. Table 4 also displays the average radical scavenging activity of the examined honey samples at different levels, i.e., 1.0, 2.5, and 5.0%. The DPPH scavenging capacity of the honey samples increased with the concentration of honey extract. The DPPH activity was increased up to a range of 43.02 and 62.68% at 5% for Balady and Sidr Al-tamayoz honey, respectively.

The ability of various honey to scavenge DPPH was demonstrated by earlier studies. Milek et al. (2021) found that the DPPH capacity of 16 different nectar and honeydew honey ranged from 10.82 to 79.37%. Al Qahtani et al. (2022) discovered that the DPPH scavenging activity of natural Saudi Sidr honey ranged

**TABLE 3** Total phenolic (TP), total flavonoid (TF) contents and radical scavenging activity of Sidr honey samples (Mean  $\pm$  SD).

Parameters		Peshawar Sidr	Al-Barri Sidr	Balady Sidr	Sidr Al-tmayoz
Total phenols (mg GA/g)		23.84 $\pm$ 0.54 <sup>d</sup>	49.75 <sup>b</sup>	55.73 $\pm$ 0.62 <sup>a</sup>	45.76 $\pm$ 0.48 <sup>c</sup>
Total flavonoids (mg R/g)		25.99 $\pm$ 0.32 <sup>c</sup>	25.42 $\pm$ 0.58 <sup>c</sup>	31.70 $\pm$ 0.66 <sup>b</sup>	40.66 $\pm$ 0.64 <sup>a</sup>
DPPH (% Radical Inhibition)	1.0 (%)	23.36 $\pm$ 0.24 <sup>b</sup>	17.38 $\pm$ 0.36 <sup>c</sup>	14.53 $\pm$ 0.22 <sup>d</sup>	28.21 $\pm$ 0.38 <sup>a</sup>
	2.5 (%)	34.47 $\pm$ 0.24 <sup>c</sup>	43.02 $\pm$ 0.36 <sup>b</sup>	28.77 $\pm$ 0.28 <sup>d</sup>	47.58 $\pm$ 0.32 <sup>a</sup>
	5.0 (%)	56.70 $\pm$ 0.36 <sup>b</sup>	57.55 $\pm$ 0.4 <sup>b</sup>	43.02 $\pm$ 0.34 <sup>c</sup>	62.68 $\pm$ 0.42 <sup>a</sup>

<sup>a,b,c,d</sup> Significant differences are observed at  $p < 0.05$  for values with different letters in the same row.

**TABLE 4** Antibacterial efficacy of Sidr honey against food borne pathogens *in vitro* reported as inhibition zone (Mean  $\pm$  SE).

Sidr honey	<i>S. aureus</i>	<i>S. typhimurium</i>	<i>P. aeruginosa</i>	<i>E. coli</i> O157:H7
	Inhibition zone (mm)			
Peshawar Sidr	12.0 $\pm$ 0.22 <sup>c,D</sup>	15.1 $\pm$ 0.2 <sup>a,B</sup>	13.5 $\pm$ 0.28 <sup>b,B</sup>	15.8 $\pm$ 0.16 <sup>a,D</sup>
Al-Barri Sidr	18.0 $\pm$ 0.18 <sup>b,C</sup>	ND	11.2 $\pm$ 0.21 <sup>c,C</sup>	20.3 $\pm$ 0.24 <sup>a,C</sup>
Balady Sidr	21.1 $\pm$ 0.14 <sup>a,B</sup>	4.5 $\pm$ 0.1 <sup>c,C</sup>	11.3 $\pm$ 0.23 <sup>b,C</sup>	22.4 $\pm$ 0.26 <sup>a,B</sup>
Sidr Al-tmayoz	38.0 $\pm$ 0.24 <sup>a,A</sup>	33.8 $\pm$ 0.18 <sup>b,A</sup>	22.5 $\pm$ 0.2 <sup>c,A</sup>	34.0 $\pm$ 0.22 <sup>b,A</sup>

<sup>a,b,c</sup> Significant differences are observed at  $p < 0.05$  for values with different letters in the same row.

<sup>A,B,C,D</sup> Significant differences are observed at  $p < 0.05$  for values with different letters in the same column.

ND, Not detected.

from 1.28 to 1.59 Trolox equivalents/g ethanol and ethyl acetate extracts, respectively. However, [Hegazi et al. \(2022\)](#) calculated that the concentrated natural Sidr honey from Saudi Arabia, Yemen, Pakistan, and Peshawar had DPPH scavenging activities of 98.3, 95.0, 135.3, and 131.3 mg ascorbic acid/100 g honey, respectively. The differences in the honey samples' antioxidant activity could be caused by the changes in phenolic concentrations and distinct types of phenolic compounds present ([Shamsudin et al., 2019](#)). There are several factors that can influence honey's antioxidant activity, including the type and concentration of phenolic chemicals, geographical location, time of harvest, and environmental conditions ([Cianciosi et al., 2018](#); [Ávila et al., 2019](#)). Furthermore, additional variables, including storage conditions, processing methods, and handling procedures, could affect the antioxidant properties of the honey ([Abu et al., 2017](#)).

## Antibacterial efficacy of Sidr honey samples

The antibacterial efficacy of the Sidr samples was evaluated against various Gram-negative and Gram-positive pathogenic bacteria via the zone of inhibition, dehydrogenase activity, and the microbial growth curve.

### The zone of inhibition

[Table 4](#) and [Figure 3](#) show the Sidr honey's capacity to suppress the growth of *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *E. coli* O157:H7. At a 25% concentration, all honey samples demonstrated varying degrees of bacterial growth inhibition. The range of inhibition zones for *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *E. coli* O157:H7 was 12–38 mm, 4.5–33.8 mm, and 15.8–34.0 mm, respectively. Peshawar Sidr showed the lowest inhibition of both

*E. coli* and *S. aureus*. Al-Barri Sidr and Balady Sidr showed the least amount of inhibitory activity against *P. aeruginosa* and *S. typhimurium*, respectively. Furthermore, it was noted that *S. typhimurium* is not inhibited by Balady Sidr. Nevertheless, Sidr Al-tmayoz displayed the greatest inhibitory zones out of all the pathogen bacteria that were investigated. The outcomes concur with those published by [Almasaudi et al. \(2017\)](#) and [Aljaghwan et al. \(2021\)](#), who found that honey is more effective in inhibiting foodborne pathogens.

Honey's osmolarity is merely one of the several significant components that contribute to its antibacterial properties ([Carnwath et al., 2014](#)). These factors rely significantly on the nectar source for the bees, the flowers' location and associated weather, the storage period and circumstances, and the preservative treatment technique. Additionally, honey's chemical composition—which includes high sugar, low moisture, low water activity, low pH, and high phenolic content—prevents microbial growth ([Figure 4](#)).

### Microbial growth and dehydrogenase activity

This investigation evaluated the effect of Sidr honey samples on the growth response (bacterial count and dehydrogenase activity) of tested Gram-positive and Gram-negative bacteria. The number of cells was correlated with DAH as TF yield ( $\mu\text{mol/mL}$ ), which was determined by optical density (OD600) ([Burdock et al., 2011](#)). For the bacterial count and dehydrogenase activity, the honey samples were administered at varying quantities and compared to the control (no honey added) during a range of incubation times (0, 12, 24, 36, and 48 h). Each graph in [Figures 5–8](#) shows a honey sample at each of the four concentrations (15, 25, 50, and 75%) that were examined in conjunction with one pathogen and a control (no honey added). [Figure 5](#) demonstrates

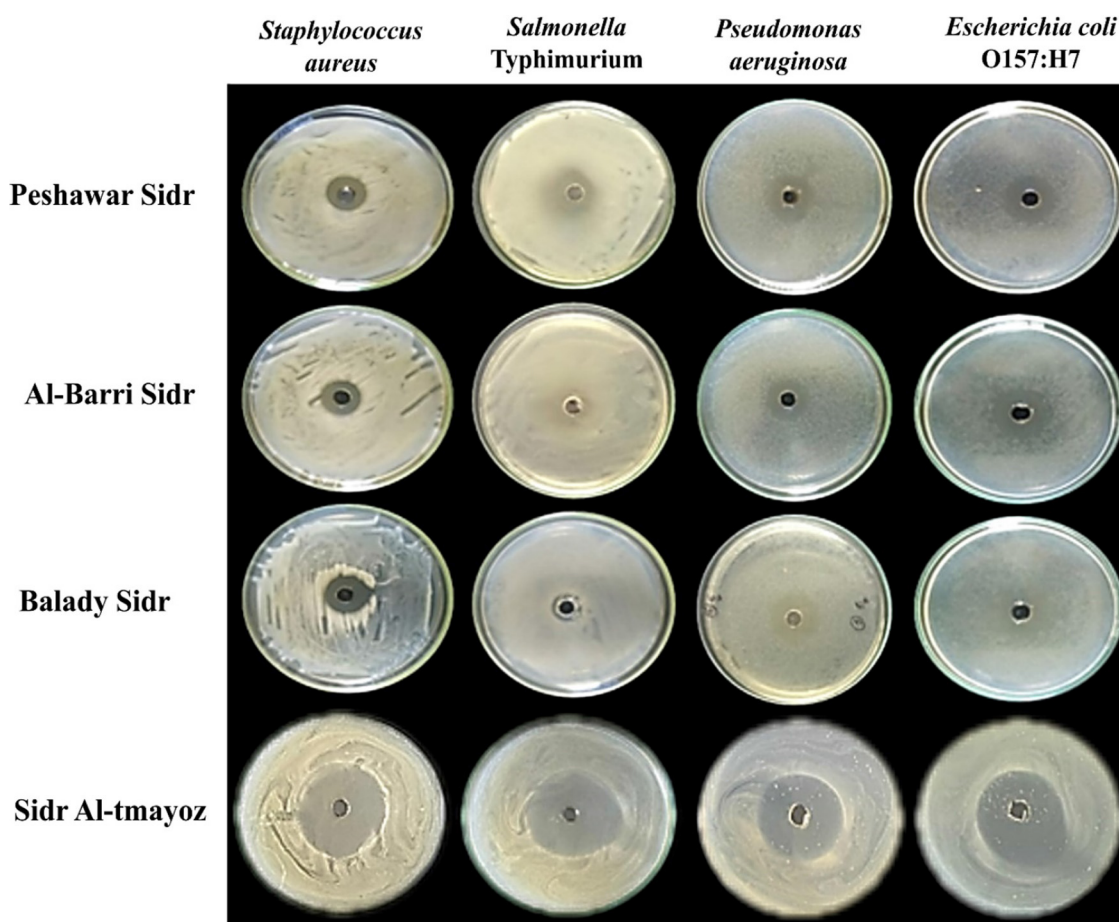


FIGURE 3  
Antibacterial capacity of Sidr samples against Gram-positive and Gram-negative bacteria.

that after 12 h of incubation, the number of *S. aureus* cells progressively declined and peaked after 48 h. Four samples of honey at different concentrations showed the same pattern of outcomes. At 25%, Sidr Al-tamayoz showed the largest decline, with a drop of ~99.9% (Figure 5d). Since the dilution of honey activates glucose oxidase, which oxidizes glucose to gluconic acid and  $H_2O_2$ , this result was consistent with the zone of inhibitory activity (Table 4) and may be the reason for the antibacterial activity (Mandal and Mandal, 2011). Furthermore, honey's antibacterial action is significantly influenced by its physicochemical characteristics.

Sidr Al-tamayoz was the most efficient sample against *S. typhimurium*, followed by Peshawar Sidr, as shown in Figure 6. In general, the cell count dropped steadily after 12 h of incubation and peaked after 48 h (Figures 6a–d). Furthermore, after 48 h with any honey samples present at varying concentrations, the lowest DHA values were noted (Figures 6e–i). The addition of any honey samples had an impact on *P. aeruginosa*'s cell count and DHA. Sidr Al-tamayoz was the honey sample that inhibited development the most out of all of them, and the most effective concentration was 25% (Figures 7a–i).

Furthermore, Figure 8 demonstrates that the growth behavior of *E. coli* O157:H7 was impacted by all honey samples, particularly

Sidr Al-tamayoz, which led to a notable reduction in both cell count and DHA (Figures 8a–i). In general, the presence of Sidr honey samples affected the cell count and DHA of all tested pathogens when compared with the control, when different amounts of honey were present, while the bacterial growth was increased logarithmically in the control (no honey added). The lowest growth rate (count and DHA) was observed after 48 h of incubating the bacteria with honey.

Since the antibacterial compounds in the Sidr honey are based on their physicochemical properties, their antimicrobial activity varies due to the fresh, real honey (Hegazi et al., 2022). As a result, honey's antibacterial qualities are greatly enhanced by its total phenols and flavonoids. These substances may be more effective against a range of diseases at larger concentrations. Additionally, honey's organic acids may enhance its antioxidant capacity by binding to minerals, so boosting antioxidant phenolics, which in turn indirectly contribute to its antagonistic effect (Chua et al., 2013).

Furthermore, honey's phenolics can effectively suppress a variety of harmful bacteria by blocking their quorum-sensing mechanism (Takó et al., 2020). Pathogenic bacteria are known to produce biofilms through several methods. The most important of which is quorum sensing, which promotes cell attachment. Toxins

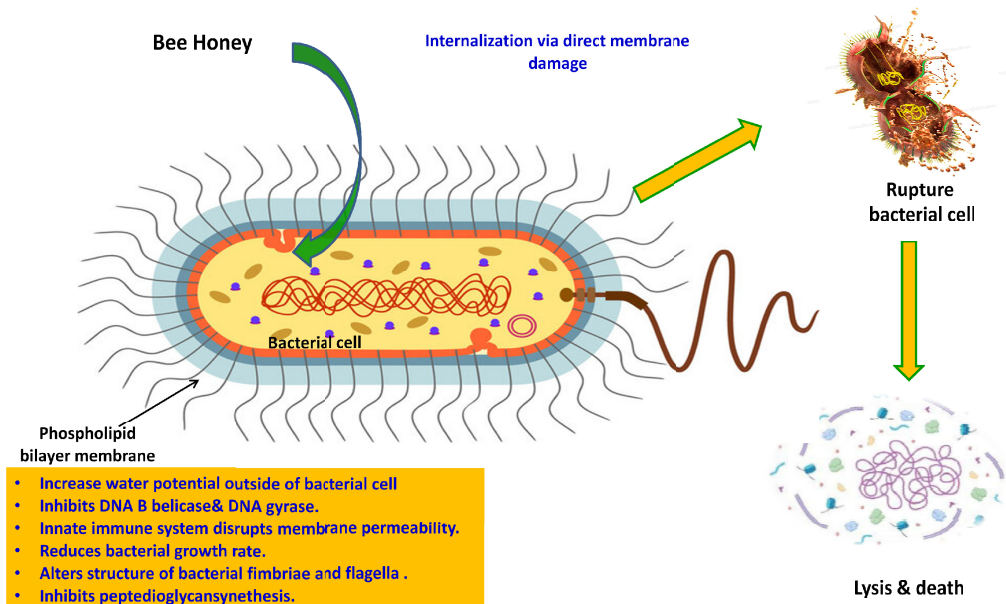


FIGURE 4

The mechanisms underlying the antibacterial efficacy of Sidr honey against microorganisms.

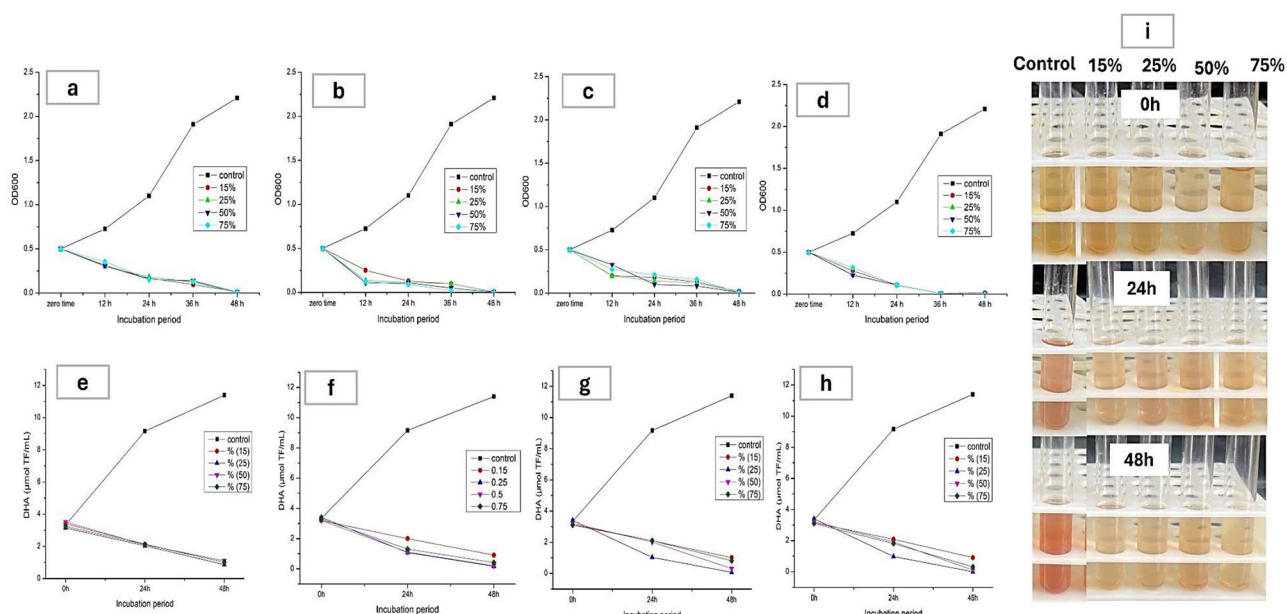


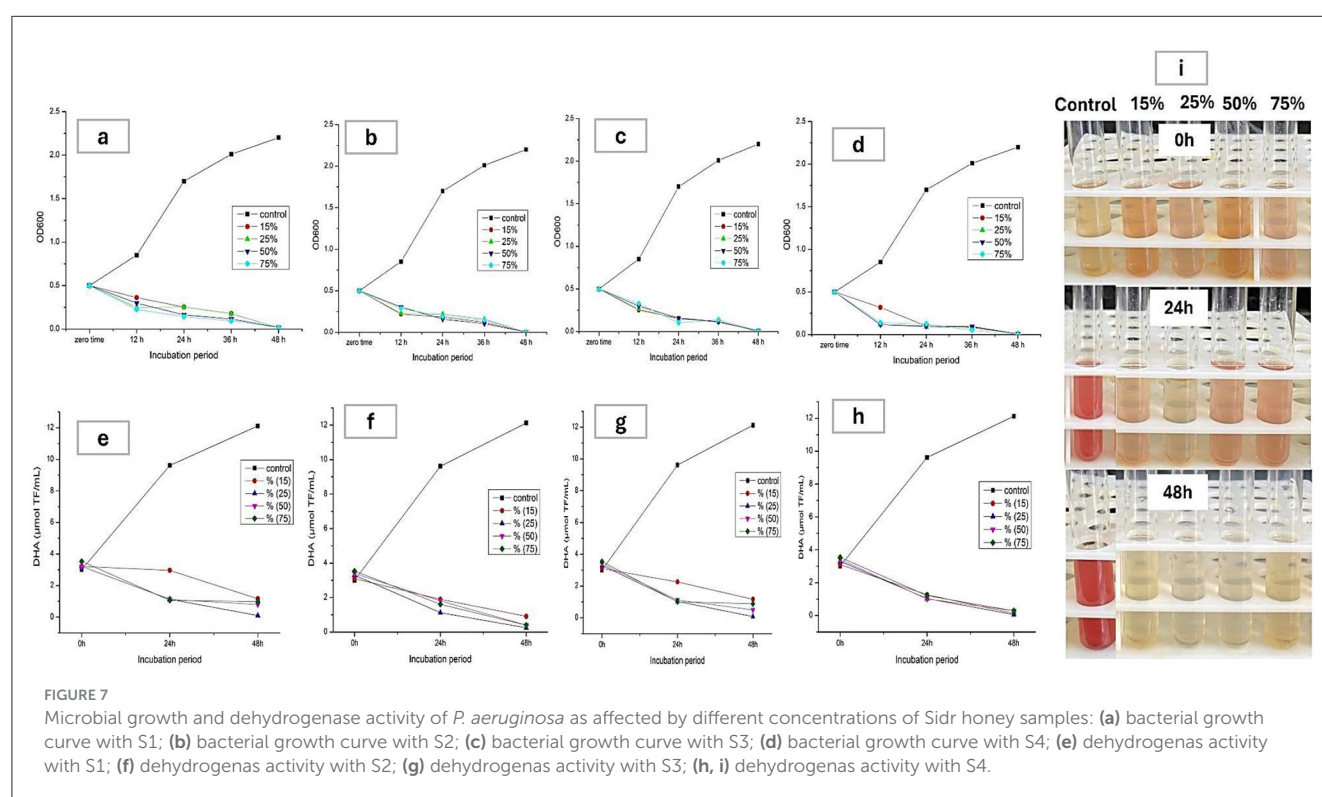
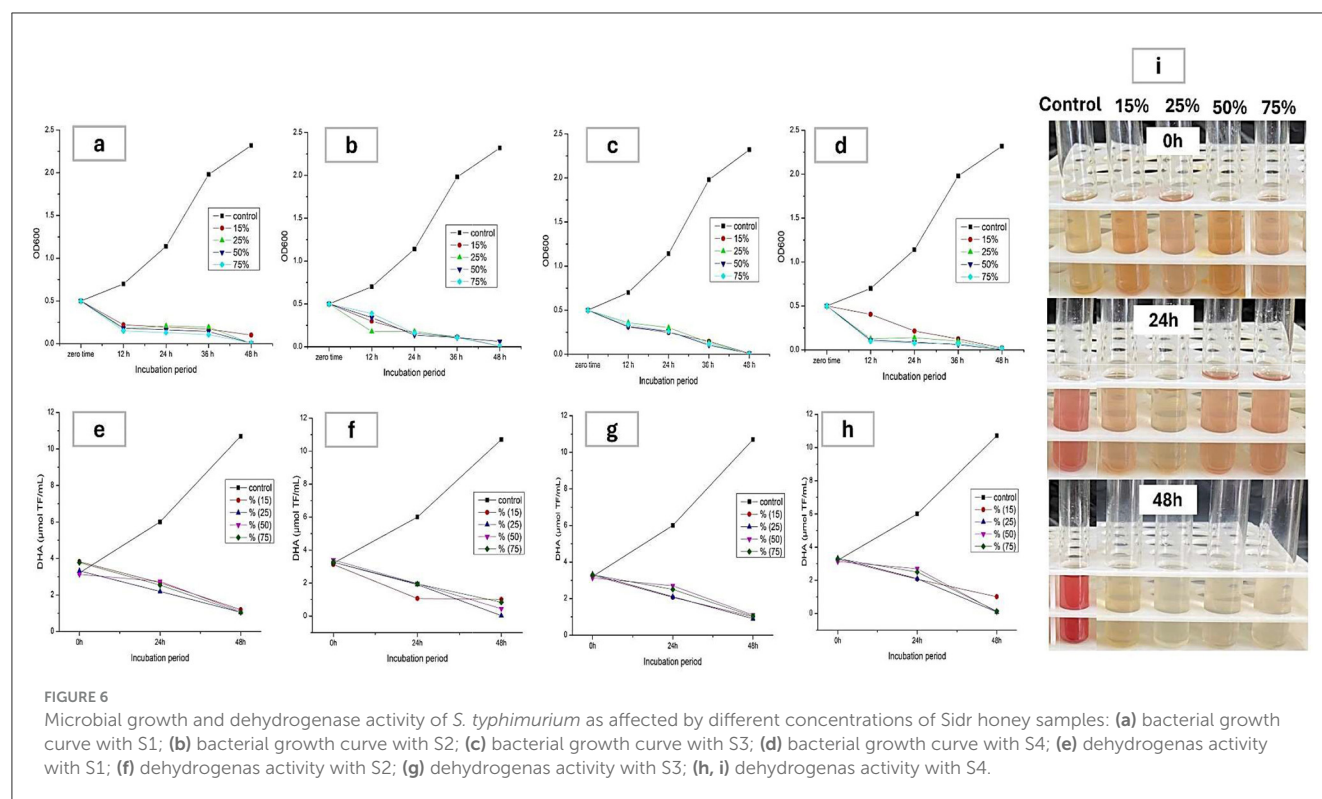
FIGURE 5

Microbial growth and dehydrogenase activity of *S. aureus* as affected by different concentrations of Sidr honey samples: (a) bacterial growth curve with S1; (b) bacterial growth curve with S2; (c) bacterial growth curve with S3; (d) bacterial growth curve with S4; (e) dehydrogenase activity with S1; (f) dehydrogenase activity with S2; (g) dehydrogenase activity with S3; (h, i) dehydrogenase activity with S4.

from bacteria and pathogenic organisms that cause food poisoning and/or spoilage can be biologically controlled by inhibiting this signaling mechanism. Phenolics and oligosaccharides are important bioactive components of honey that possess antioxidant and antibacterial properties (Fyfe et al., 2017). Together with its nutritional advantages, Sidr honey's innate antibacterial qualities are enhanced by these qualities.

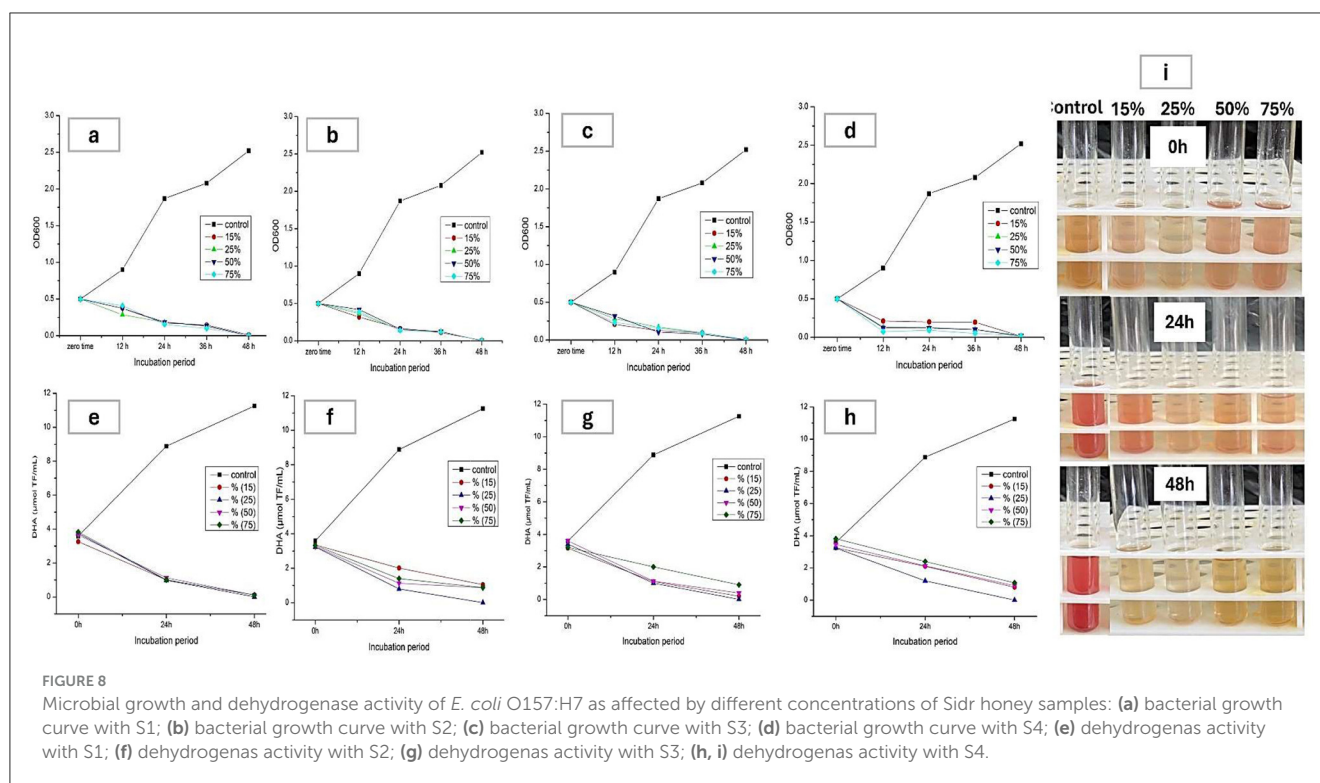
## Conclusion

The study evaluated several physicochemical characteristics of Sidr Al-tamayoz honey, including pH, moisture content, electrical conductivity (EC), and sugar concentration. Most of these characteristics were found to align with the latest Codex Alimentarius honey guidelines, indicating good quality.



Furthermore, the investigation into Sidr Al-tamayoz's bioactivity, demonstrated through its antioxidant activity and total phenolic content, suggests its potential as a readily accessible natural antioxidant. Notably, Sidr Al-tamayoz honey exhibited high levels

of phenolic and flavonoid compounds, correlating with its strong antioxidant activity. Crucially, Sidr Al-tamayoz demonstrated superior antibacterial activity against all tested pathogenic bacteria (*S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *E. coli* O157:H7),



as evidenced by its significant inhibitory zones and impact on dehydrogenase activity. In summary, the findings robustly indicate that Sidr Al-tamayoz honey is a promising natural product, rich in bioactive compounds and antioxidants, offering potential health benefits.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

RE: Conceptualization, Investigation, Methodology, Writing – original draft. MM: Conceptualization, Formal analysis, Methodology, Writing – original draft, Data curation, Software. SK: Project administration, Resources, Supervision, Validation, Writing – original draft, Funding acquisition. SA: Project administration, Resources, Software, Supervision, Writing – review & editing, Funding acquisition, Visualization. MA: Software, Visualization, Writing – review & editing, Validation. SS: Conceptualization, Data curation, Supervision, Writing – review & editing, Methodology. EN: Investigation, Methodology, Software, Writing – original draft, Formal analysis.

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## Conflict of interest

SK was employed by Api Pharm Company, Consultant Al-Asal Al-Barri Company, and Al-Asal Al-Barri Company. SA was employed by Al-Asal Al-Barri Company.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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