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Physicochemical Properties, Chemical Composition, Antioxidant Properties, and Antibacterial Effects of Four Palestinian Honey Varieties

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Abstract

The present study evaluates the physicochemical attributes, antibacterial efficacy, and antioxidant capacities of four distinct varieties of honey from the West Bank region of Palestine: Assal Barsem (*Medicago sativa*) AB, Assal Morar (*Centaurea dumulosa* Boiss) AM, Assal Horfesh (*Silybum*) AH, and Assal Sader (*Ziziphus spina-christi*) AS. The analysis encompassed parameters such as pH, electrical conductivity, Total Flavonoid Content (TFC), and Total Phenolic Content (TPC). Furthermore, the antioxidant potential was gauged through Total Antioxidant Capacity (TAC) determination and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In addition, the antibacterial effectiveness of the honeys was measured against a spectrum of bacterial strains including *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae,* and *Bacillus subtilis*, utilizing minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentrations (MBC). The outcomes of the physicochemical analysis adhered to the quality benchmarks outlined by the European Union Commission and the Codex Alimentarius Commission. The MIC and MBC values exhibited notable variance across the tested honey varieties, with MIC values ranging from 0.024% w/w to 1.56% w/w, and MBC values ranging from 0.048% w/w to 3.15% w/w. Particularly, AH demonstrated superior efficacy against all seven bacterial strains, with MIC values spanning from 0.1 to 0.6% w/w, and MBC values ranging from 0.3% w/w to 0.8% w/w. *Staphylococcus aureus* and *Escherichia coli* were notably susceptible to all honey samples. Collectively, our findings underscore the therapeutic potential of Palestinian honey varieties, highlighting their multifaceted health-promoting attributes. Further exploration is warranted to elucidate the mechanistic underpinnings of bioactive constituents and explore their potential applications in healthcare.

Keywords: Antibacterial, Antioxidant, *Medicago sativa, Ziziphus spina-christi, Silybum, Centaurea dumulosa*

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INTRODUCTION

The rise of antibiotic resistance has emerged as a worldwide health issue, prompting the need to investigate alternative antimicrobial agents.[1] Honey, a natural product with potent antibacterial properties, has garnered significant attention due to its broad-spectrum activity against various bacterial pathogens.[2] In this study, we investigate the antibacterial effects of different honey samples against a panel of clinically relevant bacterial strains, including *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae,* and *Bacillus subtilis.*

Pseudomonas aeruginosa, a widespread Gram-negative bacterium, is renowned for its innate resistance to numerous antibiotics and its propensity to induce severe nosocomial infections, especially in immunocompromised individuals.[3] *Staphylococcus aureus*, a Gram-positive bacteria, is commonly associated with skin and soft tissue infections, and can also lead to severe illnesses like bloodstream infections and heart valve inflammation.[4] *Escherichia coli,* a Gram-negative bacterium typically present in the gastrointestinal tract, can lead to various infections, spanning from urinary tract infections to severe sepsis.[5] *Streptococcus pneumoniae*, also known as pneumococcus, is a major cause of pneumonia, meningitis, and otitis media, particularly in young children and the elderly. Healthcare-associated infections, including pneumonia and bloodstream infections, are commonly linked with *Klebsiella pneumoniae*.[6,7] *Haemophilus influenzae*, a Gram-negative bacterium, is a significant respiratory pathogen responsible for a range of infections, including otitis media, sinusitis, and pneumonia.[8] *Bacillus subtilis*, a Gram-positive bacterium commonly found in soil, is widely studied for its probiotic properties but can also cause opportunistic infections in immunocompromised individuals.[9]

Honey, a delicious natural substance crafted by bees from flower nectar, has been celebrated for its health-promoting qualities throughout history. Honey is a complex substance with a diverse nutritional and chemical profile that contributes to its various health benefits. It is primarily composed of sugars, with fructose and glucose being the predominant types. These sugars provide a quick source of energy and contribute to honey's natural sweetness. In addition to carbohydrates, honey contains trace amounts of proteins, amino acids, vitamins (such as B vitamins and vitamin C), and minerals (including calcium, iron, magnesium, and potassium). The presence of antioxidants, such as flavonoids and phenolic acids, further enhances its nutritional value. These antioxidants play a crucial role in neutralizing free radicals, thereby protecting cells from oxidative stress and inflammation. Furthermore, honey's antimicrobial properties, attributed to its low pH and hydrogen peroxide content, make it effective in combating certain bacterial and fungal infections. Its prebiotic effect supports gut health by promoting beneficial gut bacteria. The combination of these factors not only makes honey a valuable addition to a balanced diet but also supports various medicinal uses, including wound healing, soothing sore throats, and enhancing digestive health.[10,11]

Civilizations including the Greeks, Romans, and Arab-Islamic civilizations have integrated honey into their medicinal regimens, guided by cultural doctrines, theoretical frameworks, and historical chronicles.[10] Within the Arab-Islamic medical tradition, honey is revered as a salubrious beverage and is commonly employed for wound care, as evidenced in the works of Avicenna and Razes.[10] Various types of honey, some of which have received scientific validation, are routinely used as natural remedies for preserving health and preventing and treating diverse ailments.[11] Honey contains a diverse array of over 150 different chemicals, such as polyphenolic compounds, water, proteins, carbohydrates, vitamins, and minerals.[12,13] The makeup and levels of these active compounds in nectar are notably affected by the geographical location and climate conditions.[14] Honey, as well as its various constituents, has been demonstrated to possess antibacterial, anti-inflammatory, antioxidant, antiproliferative, antimetastatic, and anticancer properties.[10,11,15] Beyond its traditional culinary uses, honey has been extensively studied for its diverse biological activities, including antibacterial and antioxidant effects.[11] The composition of honey varies significantly depending on floral sources,

Table 1. Predominant botanical source, local honey name, collection year, and collection site in the West Bank of honey samples

Honey sample	Predominant botanical source	Local honey name	Collection year	Collection site in the West Bank
AB	*Medicago sativa*	Assal Barsem	2021	Jenin
AM	*Centaurea dumulosa Boiss*	Assal Morar	2021	Jordan Valleys
AH	*Silybum*	Assal Horfesh	2022	Tulkarm
AS	*Ziziphusspina-christi*	Assal Sader	2021	Jordan Valleys

geographical origin, and processing methods, leading to variations in its bioactive properties.[11,15]

Honey's ability to fight bacteria is attributed to multiple factors, including its concentrated composition, acidity, and the abundance of bioactive elements like hydrogen peroxide, phenolic acids, flavonoids, and peptides.[16] These elements collectively combat a broad spectrum of bacteria, spanning both Gram-positive and Gram-negative pathogens. Moreover, honey's acidic pH creates an inhospitable environment for bacterial proliferation, further augmenting its antibacterial prowess. These constituents vary in concentration depending on factors such as nectar source, bee species, and storage conditions. Working synergistically, they equip honey to effectively combat diverse microorganisms, including multidrug-resistant strains, while also influencing their susceptibility to antimicrobial agents.[11,15,16]

Additionally, honey showcases strong antioxidant capabilities credited to its abundant array of phenolic compounds, enzymes like glucose oxidase, catalase, and peroxidase, along with vitamins and minerals. These antioxidants actively neutralize free radicals, safeguarding cells against oxidative harm and lessening the likelihood of chronic ailments like cancer, cardiovascular diseases, and neurodegenerative disorders.[17] Honey's antioxidant properties are crucial in reducing the concentration of reactive oxygen species (ROS) produced during inflammation.[17,18] High ROS levels can cause severe diseases like cardiovascular, muscular, metabolic, neurodegenerative, and cancerous conditions. [17,18] Honey's antioxidant potential is mainly due to phenolic compounds, particularly flavonoids, which can neutralize ROS and bind with metals. Additionally, flavonoids can regulate enzymes, enhancing their antioxidant abilities and affecting other biological functions.[17,18]

This study sought to assess the antibacterial and antioxidant properties of honey samples gathered from various geographical regions of the West Bank-Palestine. The selected honey samples, including Assal Albarsem (*Medicago sativa*), Assal Almorar (*Centaurea dumulosa Boiss*), Assal Alhorfesh (*Silybum*), and Assal Alsader (*Ziziphus spina-christi*), represent diverse floral sources and environmental conditions, which may influence their chemical composition and biological properties. The variety of bacterial strains chosen mirrors the complexity of infectious diseases, highlighting the demand for innovative treatments. The findings of this research hint at the potential of Palestinian honey to harbor natural compounds boasting antibacterial and antioxidant properties. These compounds could serve as promising candidates for incorporation into novel therapeutic drugs aimed at combating diseases triggered by pathogenic bacteria and oxidative stress.

MATERIALS AND METHODS

The honey samples (Table 1) were collected from the West Bank in 2021 was obtained from the respected provider known as "Honey Spring," situated in Tulkarem in the Northern West Bank (Altitude: Approximately 200 meters above sea level and Latitude: Approximately 32.3° N). Sorting the honey into different types was based on factors such as where it came from, its pollen content, and when it was harvested. After being acquired, these samples were sealed in containers to maintain their quality and were kept

in a dry environment at room temperature. They stayed sealed until they were needed for further experiments.

Protein contents, total sugar content, pH, Brix index, Refractive index and electrical conductivity

Protein content, total sugar content, pH, Brix index, refractive index, and electrical conductivity were determined following the standardized protocols outlined in the Harmonized Methods of the International Honey Commission (IHC).[19]

Protein content

The Bradford protein assay is employed due to its sensitivity and ease of use for quantifying water-soluble proteins in honey.[12,19] To begin, one gram of honey is dissolved in 10 milliliters of distilled water. The resulting solution is then centrifuged at 10,000 rpm for 10 minutes to extract the water-soluble proteins, and the supernatant is collected. In parallel, a series of bovine serum albumin (BSA) standard solutions with known concentrations (0, 5, 10, 20, 40, and 80 µg/mL) are prepared by diluting a stock BSA solution with distilled water. Bradford reagent is added to each of these BSA standards and to the honey supernatant in a 1:1 ratio. The solutions are incubated at room temperature for five minutes to allow the dye to bind to the proteins. The absorbance of each solution is then measured using a spectrophotometer at 595 nm. A standard curve is generated using the absorbance values of the BSA standards, and this curve is used to determine the protein concentration in the honey sample. This method provides a reliable measurement of the water-soluble protein content in honey.

pH and free acidity

The pH was measured in a 10% solution of honey in ultra-pure decarbonized water, using a pH meter.[19]

Total soluble solids (Brix index)

Total soluble solids, representing total soluble sugars, were quantified using the Brix index. The Brix value, which indicates the percentage of sugar at 20°C, was determined by referencing a correspondence table between the refractive index at 20°C and Brix degrees.[19]

Electrical conductivity

Electrical conductivity was measured for a 20% honey solution (based on dry matter) using the MULTI 3320 multiparameter meter. The solution was prepared with ultrapure water from the Barnstead EASY PURE II system, and conductivity was reported in µS cm^{-1}.[19]

Refractive Index, Moisture, and Solid Substances

Refractive index measurements of raw honey were conducted using an ABBÉ AR 2008 refractometer, calibrated with distilled water. The temperature of the samples was recorded with an EKT Hei-Con temperature sensor, and the refractive index was corrected by adding 0.00023 for each degree Celsius above 20°C. Moisture content was determined using a correspondence table that relates water content to refractive index values at 20°C, and was expressed as a percentage.[19]

Determination of total antioxidant capacity (TAC)

The overall antioxidant capacity was determined using the phosphomolybdenum method described by Prieto, Pineda, and Aguilar.[20] This experimental process was conducted three times independently, and the outcomes were expressed in milligrams per gram of dry weight (DW), representing ascorbic acid equivalents.

Total polyphenols content (TPC)

The total polyphenolic content was determined using the Folin-Ciocalteu method, following the protocol outlined in the referenced literature.[21] In summary, a solution containing 15 µL of Folin-Ciocalteu reagent and 60 µl of Na_2CO_3 (75 g/L) was added to 5 µl of DW. After an incubation period of 5 minutes at 60°C, the resulting blue color intensity was measured at 760 nm using a UV/Vis spectrophotometer (Synergy HT, BioTek Instruments, Inc., U.S.A.). Gallic acid was used as the standard for creating the calibration curve (ranging from 2500-100 mg/L) and the results were expressed in milligrams of gallic acid equivalent (GAE) per gram of honey samples (mg GAE/100 g).

Total flavonoids content (TFC)

To determine the total flavonoid content, 100 µl of honey samples were combined with 5% sodium nitrite and 150 µl of 10% $AlCl_3$. After a 5 minutes interval, 200 µl of 1 M NaOH (1%) was added following a 1 hour incubation period in darkness. The resulting mixture's color intensity was measured at 510 nm. A calibration curve was constructed using quercetin (ranging from 500-0 mg/L, with R2 = 0.998), and the flavonoid content was expressed as milligrams of quercetin equivalent per gram of honey samples (mg QE/100g).[21]

Free radical scavenging activity (DPPH assay)

In this study, we assessed the radical scavenging ability of DPPH (1,1-diphenyl-2-picrylhydrazyl, SIGMA, St. Louis, MO, USA). In brief, a 0.1 mM DPPH solution was prepared, and 825 µL of this solution was mixed with 150 µL of honey solutions that had been diluted in a series. The mixture was then vigorously shaken and incubated at room temperature for 1 hour in the dark. After incubation, the absorbance was measured at 517 nm. The DPPH radical scavenging activity was expressed as a percentage of inhibition. Butylated hydroxytoluene (BHT) was used as a positive control. The concentration of honey required to achieve 50% radical inhibition (IC_{50}) was determined from a graph plotting inhibition percentage against honey concentrations.[19,20]

Antibacterial activities

Bacterial strains

The microbial strains utilized in this study, including *Pseudomonas aeruginosa* strain 27853, *Staphylococcus aureus* strain BAA-1026, *Escherichia coli* strain 25922, *Streptococcus pneumoniae* strain 49619, *Klebsiella quasipneumoniae* strain 700603, *Haemophilus influenzae* strain 49247, and *Bacillus subtilis* strain 6633, were procured from the American Type Culture Collection (ATCC), Manassas, VA, USA.

Preparation of suspension bacterial

A cell suspension was prepared in BHI/LB broth, with an optical density equivalent to the 0.5 McFarland standard, and then diluted 1:100 in the appropriate broth to achieve a final concentration of 5 x 10^5 CFU/mL.

Preparation of honey samples

Honey samples were prepared by serial dilution in BHI broth across the 96 well plate.

The determination of MIC and MBC of honey samples

The determination of MIC and MBC of honey samples was conducted in 96-well, flat-bottomed microtiter plates, following the outlined methodology. In summary, the broth microdilution assay involved a twofold serial dilution in Brain Heart Infusion (BHI) broth. Bacterial suspensions were prepared in BHI broth with an optical density corresponding to a 0.5 McFarland standard, then diluted 1:100 in BHI broth to attain a final concentration of 10^6 colony-forming units per milliliter (CFU/mL). Each microplate well, including controls with only broth and broth with bacteria but without antibacterial agents, received 100 µL of an antibacterial agent (Table 1) or honey samples, followed by serial dilution in BHI broth across the plate. All wells were supplemented with an additional 100 µL, corresponding to 10^6 CFU/mL. The plates were then incubated at 37°C for 18 hours overnight. Tetracycline and kanamycin served as positive controls. MIC was determined as the lowest concentration inhibiting visible bacterial growth in triplicate wells. Following incubation, MIC was identified as the lowest concentration at which no growth was observed in triplicate wells. To assess MBC values, 20 µL of p-iodonitrotetrazolium violet (8 mg/mL in ethanol) was introduced into each well. After a 30 minutes incubation, the plate was visually examined for any color alteration from yellow to pink, signifying dye reduction owing to bacterial growth. The highest dilution (lowest concentration) that retained a yellow hue was identified as the MBC.[14]

Statistical analysis

The error limits depicted, along with the illustrated error bars, represent the basic standard deviations of the averages. Usually, numerical results are reported with precision up to the least significant digit. When comparing different

samples, statistical significance was established at $p < 0.05$ (utilizing Student's t-test for unpaired samples).

RESULTS

Protein contents, total sugar content, pH, Brix index, Refractive index and electrical conductivity

The investigation into the physicochemical properties of four Palestinian honey samples (AB, AM, AH, and AS) unveiled notable variations across multiple parameters. Each sample has its own unique profile across these parameters, indicating differences in composition, acidity, sweetness, and purity (Table 2). Hydrosoluble protein analysis revealed AS to possess the highest content at 597.77 ± 70.28 mg/kg, while AB exhibited the lowest at 497.50 ± 54.25 mg/kg. Total sugar content ranged from 16.14 ± 0.96 g/100 g in AH to 24.97 ± 4.14 g/100 g in AB. Acidity levels varied, with AM registering the highest acidity (pH 3.63 ± 0.03) and AH being the most alkaline (pH 4.37 ± 0.02). Brix index (Primarily reflects the concentration of dissolved sugars in honey samples). values ranged from 69.6 ± 2.02 in AS to 80.3 ± 1.92 in AM, while refractive indices ranged

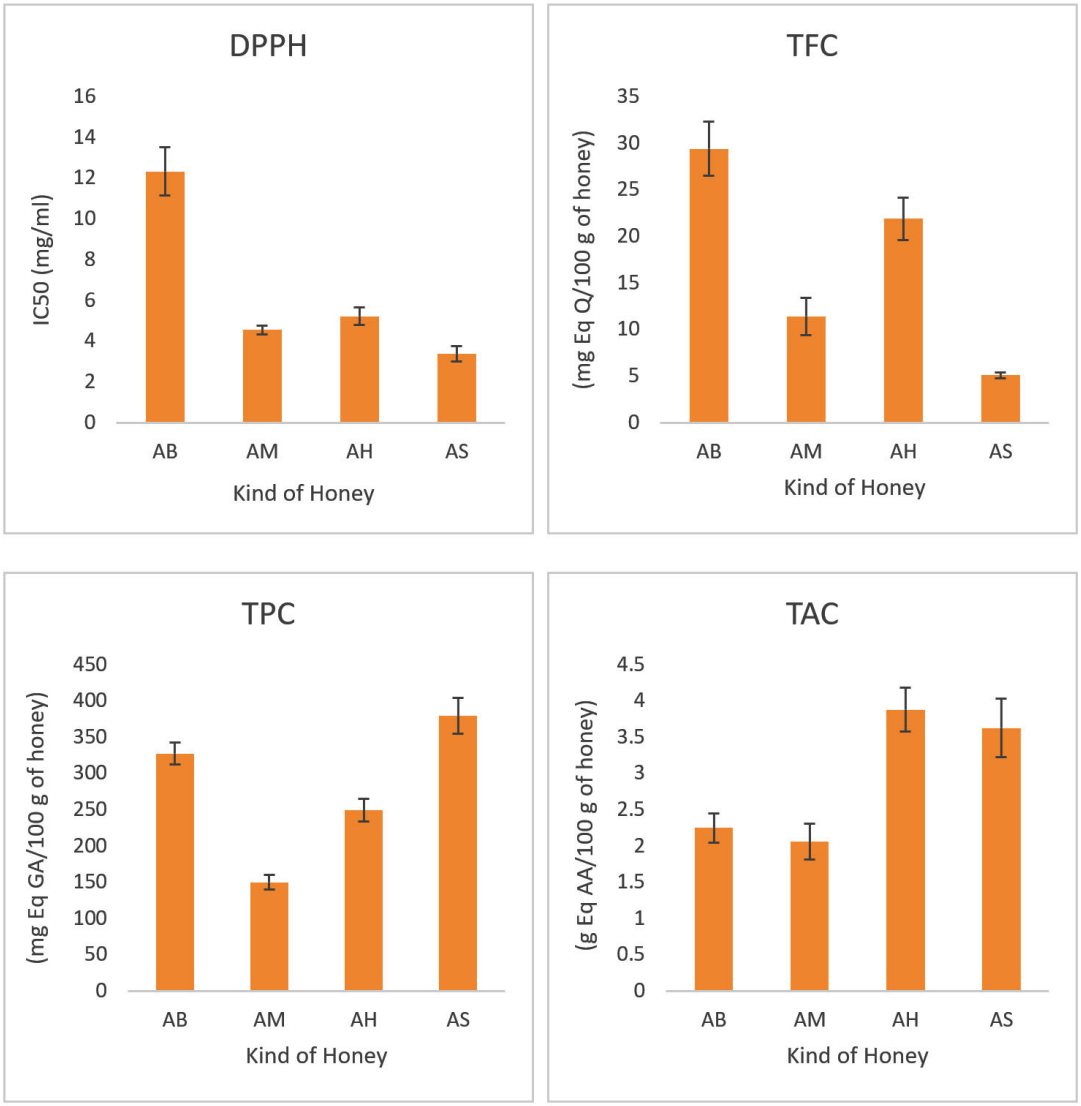

Figure 1. Antioxidant properties and phytochemical composition of honeys

from 16.5 in AS to 18 in AB. Conductivity displayed significant discrepancies, with AH exhibiting markedly higher values (140.7 ± 1.67 µS/cm) compared to the other samples. These findings underscore the diverse physicochemical profiles of Palestinian honey samples, suggesting potential variations in floral sources, geographical origins, and processing methods, which could influence their quality and nutritional attributes.

Total polyphenols, flavonoids, and antioxidant capacity

The antioxidant properties and phytochemical composition of the four different honeys (AB, AM, AH, and AS) were evaluated in this study. The results of the DPPH assay, TFC, TPC, and TAC are summarized in Figure 1. Noticeable differences in antioxidant properties and phytochemical composition were observed among the various honey samples, likely due to disparities in floral sources and geographical origins. The DPPH IC_{50} values ranged from 3.37 to 12.33 mg/mL across the honey samples. AS exhibited the lowest DPPH IC_{50} value (3.37 ± 0.28 mg/mL), indicating the highest antioxidant activity among the samples. Conversely, AB showed the highest DPPH IC_{50} value (12.33 ± 0.68 mg/mL), indicating relatively lower antioxidant activity compared to the other honeys.

Regarding phytochemical composition, AS had the highest TFC (5.08 ± 0.14 mg Eq Q/100 g of honey) and TPC (379.59 ± 46.78 mg Eq GA/100 g of honey) values, suggesting the highest concentration of flavonoids and phenolic compounds among the samples. AM exhibited the lowest TFC and TPC values among the honeys studied. In terms of Total Antioxidant Capacity (TAC), AH showed the highest value (3.88 ± 0.07 g Eq AA/100 g of honey), indicating the highest overall antioxidant capacity, while AM exhibited the lowest TAC value (2.06 ± 0.00 g Eq AA/100 g of honey).

DPPH, TFC, TPC, and TAC. Data represent the mean ± SD from three independent experiments conducted in triplicate. The phenol concentrations in AB, AM, AH, AND AS are quantified as TPC (Total Phenolic Content) in mg of gallic acid equivalent per 100 g of honey. Similarly, the total flavonoids content (TFC) is determined in mg of quercetin equivalent per 100 g of DH or in g equivalent to ascorbic acid per 100 g of honey. Total Antioxidant Capacity (TAC) is assessed in g equivalent to

Table 2. Protein contents, total sugar content, pH, Brix index, Refractive index and electrical conductivity

Honey sample	Hydrosoluble proteins	Total sugars	pH	Brix index	Refractive index	Conductivity
AB	497,50 ± 54,25	24,97 ± 4,14	3,28 ± 0,02	72,6 ± 3,2	18	55,6 ± 2,05
AM	562,02 ± 7,40	18,84 ± 2,54	3,63 ± 0,03	80,3 ± 1,92	17,5	51,9 ± 1,29
AH	560,33 ± 203,45	16,14 ± 0,96	4,37 ± 0,02	78,2 ± 1,24	17,5	140,7 ± 1,67
AS	597,77 ± 70,28	16,88 ± 1,94	3,91 ± 0,03	69,6 ± 2,02	16,5	73,3 ± 2,29

Hydrosoluble Proteins (mg Eq BSA/100 g of honey), Total Sugars (mg Eq Glu/100 g of honey), and Conductivity (µs)

Table 3. The MIC and MBC values for tetracycline (TC) and kanamycin (KM) antimicrobial agents were determined. These data are the averages obtained from three separate experiments, each conducted with four replications

Bacteria	MIC µg/mL		MBC µg/mL		MBC/MIC	
	TC	KM	TC	KM	TC	KM
P. aeruginosa	0.07 ± 0.017	0.85 ± 0.034	0.07 ± 0.0042	1.7 ± 0.051	1	2
S. aureus	0.02 ± 0.003	0.11 ± 0.0088	0.04 ± 0.0028	0.11 ± 0.0077	2	1
E. coli	0.03 ± 0.001	0.47 ± 0.014	0.06 ± 0.0018	0.94 ± 0.0564	2	2
B. subtilis	0.78 ± 0.047	0.39 ± 0.039	1.56 ± 0.0624	0.78 ± 0.0624	2	2
H. influenzae	0.024 ± 0.005	0.048 ± 0.003	0.024 ± 0.0022	0.048 ± 0.004	1	1
S. pneumoniae	1.56 ± 0.1248	0.78 ± 0.038	3.12 ± 0.1248	1.56 ± 0.140	2	2
K. pneumoniae	0.19 ± 0.026	0.048 ± 0.005	0.19 ± 0.0133	0.096 ± 0.008	1	2

ascorbic acid per 100 g of honey, and Antioxidant activity is measured using DPPH IC_{50} values, reported in mg/mL

The antibacterial properties

The antimicrobial activities of tetracycline and kanamycin against various pathogenic

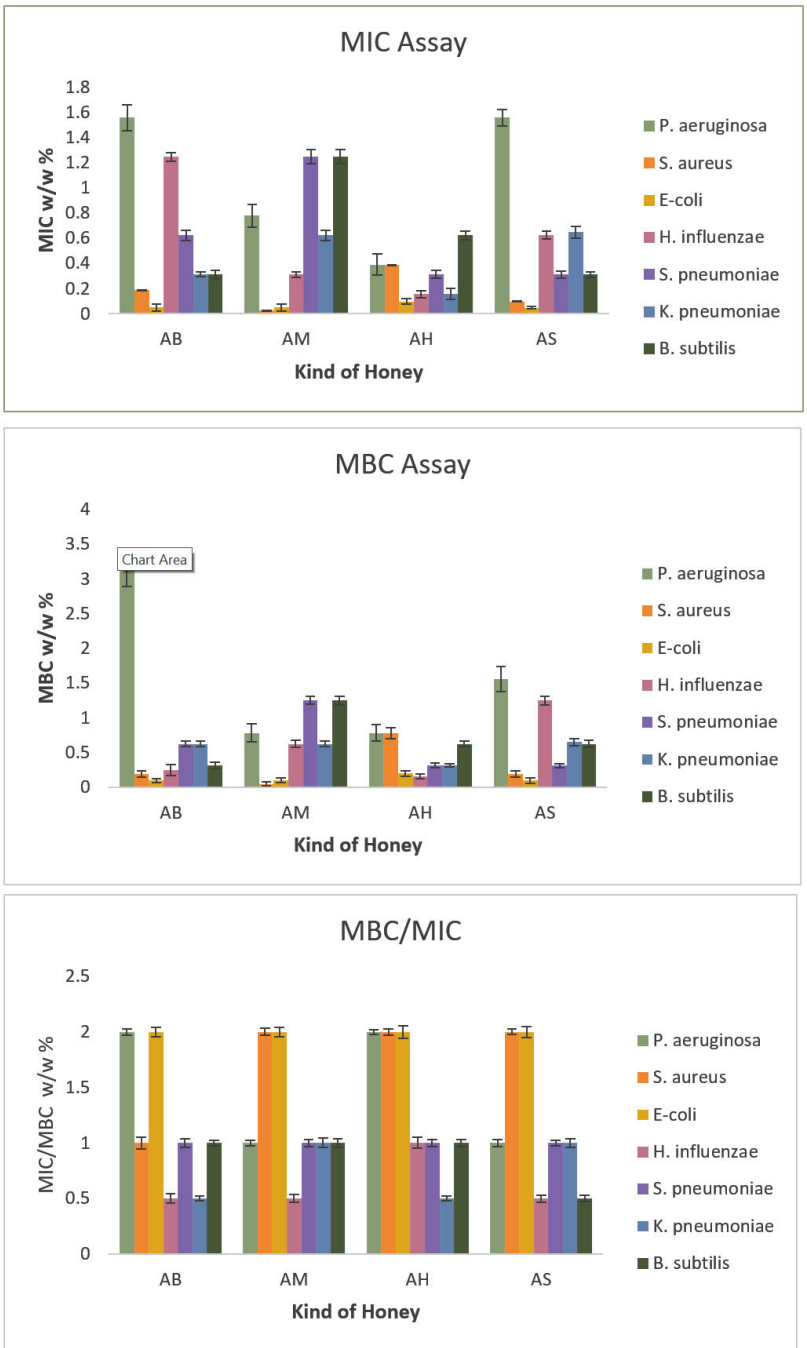

Figure 2. Illustrates the antibacterial effects of honey samples labeled AB, AM, AH, and AS. It displays the MIC and MBC values for these samples against various bacterial strains. The data presented are the averages derived from three independent experiments, each consisting of four replications

microorganisms were evaluated in this study as positive control. MIC, MBC, and the ratio of MBC to MIC were determined for each antibiotic against the seven tested pathogens. The results are summarized in Table 3. Tetracycline demonstrated potent antimicrobial activity against all tested pathogens, with MIC values ranging from 0.02 µg/mL to 1.56 µg/mL. For most pathogens, the MBC values were equal to or twice the MIC values, indicating bactericidal activity. However, for some pathogens such as *P. aeruginosa* and *H. influenzae,* the MBC was equal to the MIC, suggesting a bacteriostatic effect.

Kanamycin also exhibited notable antimicrobial activity against the tested pathogens, with MIC values ranging from 0.048 µg/mL to 0.85 µg/mL. The MBC values were generally equal to or twice the MIC values, indicating bactericidal activity. Similar to tetracycline, kanamycin showed a bacteriostatic effect against *P. aeruginosa* and *H. influenzae*.

The ratio of MBC to MIC provides insights into the bactericidal or bacteriostatic nature of the antibiotics. A ratio of 1 indicates bactericidal activity, while a ratio greater than 1 suggests bacteriostatic activity. Both tetracycline and kanamycin predominantly exhibited bactericidal activity against the tested pathogens, with some exceptions observed for specific pathogens.

The antimicrobial activity of AB, AM, AH, and AS was assessed against a panel of pathogenic microorganisms, including *H. influenzae, S. pneumoniae, K. pneumoniae, B. subtilis, P. aeruginosa, S. aureus,* and *E. coli*. MIC and MBC values were determined for each honey type against the tested pathogens. Figure 2 summarizes the MIC and MBC values of honeys against the tested strains. Across all honey types, MIC values ranged from 0.024% w/w to 1.56% w/w, while MBC values ranged from 0.048% w/w to 3.15% w/w. AH was the most effective against all seven bacterial strains with MIC values ranging from 0.1% w/w to 0.6% w/w and MBC values ranging from 0.3% w/w to 0.8% w/w. *S. aureus* and *E. coli* were the most affected strains by all four samples. The results highlight variations in the effectiveness of different honey types against these Gram-negative and Gram-positive bacteria. Further analysis of the MBC/MIC ratios reveals insights into the bactericidal or bacteriostatic nature of honey against the tested pathogens. All honey types demonstrate predominantly bactericidal activity (MBC/MIC ratio ≤1).

DISCUSSION

This study focuses on evaluating the physicochemical attributes, as well as the antibacterial and antioxidant capabilities of four distinct honey varieties from the West Bank region of Palestine, namely AB, AM, AH, and AS. Key physicochemical parameters including pH, moisture content, electrical conductivity (EC), and total acidity were analyzed. Antibacterial efficacy was assessed against various multidrug-resistant Gram-positive and Gram-negative bacterial strains including *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae,* and *Bacillus subtilis*, utilizing MIC and MBC measurements. Additionally, antioxidant potential was gauged through DPPH. TFC, TPC, and TAC were also measured.

Protein contents, total sugar content, pH, Brix index, Refractive index and electrical conductivity

Protein contents, total sugar content, pH, Brix index, Refractive index and electrical conductivity serve as key indicators for quality control. The pH of DH was measured at 3.28 ± 0.02 and 4.37 ± 0.02, falling within the accepted range for honey (pH 3.40-6.10)[19] and aligning with values observed in Palestinian, Moroccan, and Algerian varieties.[22-26] The acidity in honey stems from a variety of organic acids, primarily gluconic, alongside formic, tartaric, maleic, citric, succinic, butyric, lactic, and oxalic acids, as well as several aromatic acids. This acidity significantly contributes to the flavor profile and aids in maintaining honey's stability against microbial spoilage and inhibits microbial growth.[27] Electrical conductivity gauges the presence of ionizable organic and inorganic substances, with values ideally staying below 800 µS/cm for quality assurance.[22] The obtained values for the four tested varieties (51.9 ± 1.29 and 140.7 ± 1.67 ms) closely resembled that of other Palestinian honey samples.[22] While sugar and protein content can vary across different honey batches, the predominant protein profiles remain

similar across various botanical and geographical origins. In this study, the average total sugar and protein content of the honey were comparable to those reported in Palestinian, Algerian, and Moroccan varieties.[22-25,28]

Antioxidant properties of the honey samples

Honey serves as a robust source of natural antioxidants, which play a crucial role in protecting against the effects of oxidizing agents on both food preservation and human health. It contributes to the reduction of health risks such as heart disease and cancer, prevents the deterioration of the immune system, cataracts, and various inflammatory processes.[29,30] As presented in Figure 1, The DPPH IC_{50} values ranged from 3.37 to 12.33 mg/mL across the honey samples. AS exhibited the lowest DPPH IC_{50} value, indicating the highest antioxidant activity among the samples. Conversely, AB showed the highest DPPH IC_{50} value, indicating relatively lower antioxidant activity compared to the other honeys. The observed IC_{50} values were similar to the values previously reported for other Palestinian, Moroccan, and Algerian samples, indicating a correlation between the IC_{50} of DPPH free radicals and the values of polyphenolic compounds, as well as the TFC.[22-25,31]

Total polyphenols, flavonoids, and antioxidant capacity

The TPC values were found to be in the range of 149.84 ± 5.79 and 379.59 ± 46.78 Eq GA/100 g of honey. These values surpass the total polyphenols content reported in ten Palestinian honey samples from diverse geographical regions,[22] which ranged between 26.96 ± 0.71 mg/100 g and 70.73 ± 0.71 mg/100 g. These findings are akin to those discovered in thyme honey from Morocco. [24] The Total Flavonoid Content (TFC) values were recorded as 5.08 ± 0.14 and 29.37 ± 0.24 mg Eq Q/100 g of honey, which aligns with the values reported for other Palestinian, Moroccan, and Algerian honey samples. TAC was found to be in same range as reported for other samples from the Mediterranean region.[22,24,25,28] Multiple scientific studies have indicated a correlation between total phenolic and flavonoid contents and antioxidant activities *in vitro*. The levels of phenolic compounds are influenced by several factors such as geographical location, botanical origin, types of phenolic compounds, storage duration, and processing methods.[32]

Antibacterial effects of the honey samples

Given the pressing global concern regarding antimicrobial resistance, there is an urgent need for alternatives to antibiotics. Honey, a natural product with a long history of medicinal use, is attracting attention for its broad-spectrum antibacterial properties, particularly in combating drug-resistant bacteria. Unlike antibiotics, bacteria are less likely to develop resistance against honey. Its effectiveness is attributed to factors such as viscosity, acidity, and the presence of hydrogen peroxide. Research indicates variations in antibacterial potency across different bacterial species and honey concentrations, underscoring its potential as a treatment for bacterial infections. The antibacterial efficacy of honeys was evaluated in the present study against a panel of clinically relevant bacterial strains, including *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Klebsiella pneumoniae, Haemophilus influenzae,* and *Bacillus subtilis*, using MIC and MBC. In this study, MIC values ranged from 0.024% w/w to 1.56% w/w, while MBC values ranged from 0.048% w/w to 3.15% w/w. *S. aureus* and *E. coli* were the most affected strains by all four samples, with MIC values of 0.097% w/w to 0.19% w/w and 0.096% w/w to 0.19% w/w, respectively. These values are lower than those reported by Imtara *et al*.,[33] Mandal *et al*.,[34] Boukraa *et al*.,[35] and Masalha *et al*.,[36] who found MICs of honey ranging from 0.52% to 1.0% for *E. coli.*

The main antimicrobial properties of honey primarily stem from hydrogen peroxide, in addition to non-peroxide components like phenolic acids and flavonoids, which collectively enhance both its antibacterial and antioxidant effectiveness.[37,38] Studies suggest that the efficacy of these antibacterial properties might fluctuate based on the phytogeographical region, impacting the synthesis of unique compounds. Recent research has revealed additional antimicrobial components, such as the antimicrobial peptide Bee defensin-1, 5-Hydroxymethylfurfural, and methylglyoxal, alongside phenolic compounds like flavonoids.[39,40]

Honey demonstrates broad-spectrum antibacterial activity against all tested Gram-positive and Gram-negative bacteria. Its effectiveness extends to drug-resistant isolates, encompassing MRSA, drug-resistant hemolytic *Streptococci*, and vancomycin-resistant *Enterococci*.[41,42] Moreover, honeys from various regions worldwide may possess similar or superior potency compared to Manuka honey. Despite this, there is limited research investigating the bioactive potential of traditional Palestinian honeys.

In a recent study, Abu-Farich *et al*.[43] utilized liquid-liquid HPLC analysis to extract phenolic compounds with antibacterial, antioxidant, and anticancer attributes from AB, AM, AH, and AS. They identified fifteen such compounds, which included Caffeic acid, carvacrol, chrysin, ellagic acid, galangin, gallic acid, kaempferol, p-coumaric acid, pinobanksin, pinocembrin, protocatechuic acid, quercetin, rutin, salicylic acid, and silydamin. Noteworthy is that ellagic acid, gallic acid, kaempferol, and p-coumaric acid were consistently found across all samples, albeit with varying concentrations. These compounds are recognized for their potential health benefits, encompassing anticancer, antioxidant, and antibacterial properties.

CONCLUSION

In this study, we thoroughly assessed the physicochemical attributes, antibacterial efficacy, and antioxidant potential of four distinct honey varieties sourced from the West Bank region of Palestine. Our analysis of key physicochemical parameters such as pH, moisture content, electrical conductivity, and total acidity revealed that the honey samples met established quality standards, with acidity levels conducive to microbial stability. Moreover, the protein and sugar contents were consistent with those reported in similar honey varieties from diverse geographical regions, indicating uniform composition and quality. Antioxidant assays demonstrated robust antioxidant activity across all samples, with one variety exhibiting particularly high potency. These findings align with previous studies and suggest a correlation between antioxidant activity and polyphenol content, highlighting the potential health benefits of these honey varieties. Our study demonstrated significant antibacterial efficacy against clinically relevant bacterial strains, including drug-resistant variants. The MIC and MBC values highlighted the strong antibacterial attributes of these honeys, especially against prevalent pathogens like *Staphylococcus aureus* and *Escherichia coli.*

Overall, our findings emphasize the therapeutic potential of Palestinian honey varieties, showcasing their multifaceted health-promoting properties. Further research is warranted to elucidate the underlying mechanisms of action of bioactive constituents and explore potential applications in healthcare and food industries. By leveraging the natural benefits of these honeys, we can potentially address pressing global health challenges, including antimicrobial resistance, while also promoting overall well-being and longevity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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