Research Square

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High synergistic antimicrobial and antioxidant activities of Algerian honey-honey and honey-plant extracts mixtures

Nacira Siad
Amar Telidji University
Chahrazed Hamia
Amar Telidji University
Amar Djeridane
a.djeridane@lagh-univ.dz

Amar Telidji University
Mohamed Benalia
Amar Telidji University
Reguia Mahfoudi
Amar Telidji University
Mustafa Abdullah Yilmaz
Dicle University
Oguz Cakir
Dicle University
Abbas Tarha
Dicle University
Mohamed Yousfi
Amar Telidji University

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Abstract

This work aimed to investigate the binary combination effect between ten Algerian honey samples and honey-plant mixtures (*Atriplex halimus* L. and *Marrubium deserti* De Noé) on the antioxidant and antimicrobial properties. Twenty-two phenolic compounds were identified and quantified using a UHPLC-MS/MS method. The Antioxidant activity was assessed using FRAP, ABTS, and DPPH tests, which showed that all single honeys presented significant antioxidant capacities, especially the sample M_3 (*Bunium mauritanium* honey). Moreover, the binary combinations of almost honeys exhibited significant antioxidant synergistic effects ranging from 1% to 40%. furthermore, the plant extracts significantly enhanced the antioxidant activity of honeys from 5% to 80% in the three assays. The antibacterial activity against *Escherichia coli* (EC), *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) was evaluated using the agar well diffusion method and expressed as µg of gentamicin (CN) equivalent anti-strain capacity (GEAstrain) per g of honey or mixture. The results showed strong significant synergistic properties against the three tested bacterial strains, with an increase in effectiveness from 10% to 80%. The antibacterial activity values were: 0.322- 38312.64 µg CN/g, 0.143–2154.012 µg CN/g and 0.383–15659.174 µg CN/g, for SA, PA and EC, respectively. These results prove that the strong antimicrobial effect of honey binary combinations can serve as a natural alternative for treating illnesses caused by pathogenic microbes.

1. Introduction

Natural plants and animal products have a long history in medicine, with many traditional remedies and modern pharmaceuticals originating from these sources [1]. Honey is a sweet food with therapeutic qualities, including antioxidant, antimicrobial, anti-inflammatory and anti-cancer properties [2]. Its components, principally flavonoids and phenolic acids, play a significant role in contributing to its pharmacological properties.[3] Darker honey has higher concentrations of these compounds, resulting in stronger biological properties [4]. Honey contains antioxidants that help prevent the chain oxidation reactions associated with various diseases [5], while its antibacterial activity inhibits about 60 Gram-positive and Gram-negative bacterial types [6]. The primary antibacterial components in honey are non-peroxide inhibitors, such as phenolic acids and flavonoids [7].

Furthermore, Indigenous plants for medicinal use have gained more attention from the international health community in recent years.[8]. The Saharan medicinal plants *Atriplex halimus L.* and *Marrubium deserti De* Noé, are renowned for their significant biological activities, making them valuable in both traditional and modern medicine [9].

Atriplex halimus L., commonly known as (Elgttaf), due to its abundant phytochemical content, which includes flavonoids, tannins, and saponins. These compounds enhance its ability to fight oxidative stress and bacterial infections.[10].

Marrubium deserti De Noé, commonly identified as (Eldjaida), a native species of the Algerian Sahara, is celebrated for its anti-inflammatory and antibacterial properties. Its bioactive compounds, such as diterpenoids and phenolic acids, play a critical role in its therapeutic applications, particularly in addressing respiratory and inflammatory conditions. Together, these plants underscore the immense pharmacological potential of Saharan flora, inspiring further research into their bioactive compounds and medicinal applications [11].

The combination of honey with medicinal plants creates a potent synergy that amplifies their therapeutic effects [12]. Honey's antibacterial effects are well known, serving as a natural preservative and a carrier for the active compounds found in herbs [13]. This mixture not only enhances potency but also improves flavor, making remedies more enjoyable and easier to consume. In essence, the combining of honey and medicinal plants provides a holistic approach to health, harnessing the best of nature for wellness and healing [14]. The increasing problem of microbial resistance to antibiotics has prompted the search for new therapeutic agents [15]. Combinations of natural products are frequently utilized in traditional medicine and demonstrate synergistic effects, which enhance their efficacy in treating various diseases[16].

This study and for the first time, aims to evaluate the antimicrobial and antioxidant activities of a binary combination of ten samples of honey from ten various regions in Algeria. Additionally, we will investigate the effect of mixing these Algerian honeys with extracts from two medicinal plants: *Atriplex halimus* L. and *Marrubium deserti* De Noé. Furthermore, to significantly reduce our reliance on antibiotics, we will calculate the amount of Gentamicin (in micrograms) needed to match the antimicrobial capacity of one gram of honey, one gram of the binary combinations of honey, or one gram of the honey-plant mixtures. Moreover, the antioxidant capacity was evaluated using three in vitro assays (FRAP, DPPH, and ABTS) and expressed as micrograms of ascorbic acid equivalent per gram of honey or honey mixture (µg AAEC/g).

2. Materials And Methods

2.1. Biological and Chemical Reagents

For the biological experiments, the following items were used: Petri dishes, a sterile wire loop, sterile normal saline solution, sterile swabs, a sterile cork borer (6mm diameter), gentamicin antibiotic (80mg/2ml), sterile distilled water, nutrient agar, Mueller Hinton agar, test tubes, a micropipette, and Pasteur pipettes.

The chemical substances and solvents employed in these experiments were of analytical purity and sourced from Sigma-Aldrich (USA) and Honeywell (Germany). These included: methanol (CH_3OH), ethanol (C_2H_6O), dimethyl sulfoxide (DMSO), hydrochloric acid (37%), ammonium hydroxide (H_5NO), dichloromethane (CH_2Cl_2), acetic acid (CH_3COOH), sodium carbonate (Na_2CO_3), gallic acid ($C_6H_2(OH)_3COOH$), Folin–Ciocalteu reagent, sodium sulfate (Na_2SO_4), 2,2-azinobis-(3-ethyl-6-

benzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate ($K_2S_2O_8$), sodium phosphate monobasic ($NaH_2PO_4 \cdot 2H_2O$), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), aluminum trichloride ($AlCl_3$), sodium chloride (NaCl), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), sodium acetate trihydrate ($C_2H_3NaO_2 \cdot 3H_2O$), and ascorbic acid.

2.2. Honey Samples

Ten distinct honey samples were collected from beekeepers across various regions in Algeria. These samples were subsequently deposited in the laboratory at 4°C in darkness until analysis was conducted. Table 1 provides comprehensive details regarding the origin, harvest year, color, and botanical source of each sample.

Table 1: Honey samples

Sample	Aspect	Botanical origin	Crop Year	Region
M_1		*Urtica dioica*	June 2022	M'ssila
M_2		*Ziziphus lotus - Euphobia guyoniana Boiss.*	June 2022	Oued Mzi-Laghouat
M_3		*Bunium mauritanium*	June 2022	Sougger -Tiaret
M_4		*Ziziphus lotus*	June 2022	Oued djedai-Laghouat
M_5		*Euphorbia guyoniana Boiss- Retama raetam*	June 2022	Oued Twil-Laghouat
M_6		*Ziziphus lotus- Atractylis serraluloide*	July 202:2	Naama
M_7		*Thapsia garganica - Peganum harmala*	May 2022	Ain Elibel
M_8		*Euphorbia guyoniana Boiss*	July 2022	Aflou-Laghouat
M_9		*Eruca vesicaria ssp.*	January 2022	Illizi
M_{10}		*Eruca vesicaria ssp.*	July 2022	Elhadjeb-Laghouat

2.3. Plant material collection

In October 2022, aerial portions of *Atriplex halimus* L. and *Marrubium deserti* De Noé were gathered from the Sidi Makhlouf community (34.133333°N, 3.016667°E), located 40 kilometres from Laghouat, Algeria. Professor Amar Djeridane of Amar Telidji University of Laghouat authenticated these plant specimens. The authenticated samples, identified as *AH-SM-22* and *MD-SM-22*, were subsequently placed in the research herbarium of the Fundamental Science Laboratory at the University of Laghouat. Following collection, the samples were air-dried in the shade at ambient laboratory temperature. Fresh plant material was placed in a dry, well-ventilated, and light-protected area for a period of one month to ensure complete drying.

2.4. Plant extraction procedure

The collected plant samples underwent grinding and were then sieved through a 200 µm mesh. Subsequently, five grams (5 g) of the sieved material were subjected to extraction with 100 mL of methanol (Me) in an ultrasonic bath for 30 minutes at 37°C. The resulting mixtures were filtered using an 8 µm filter paper, and the filtrates were concentrated to dryness under vacuum at 45°C utilizing a rotary evaporator. The remaining residues were then dissolved in 30 mL of 1M acidic water and subjected to liquid-liquid partitioning with 30 mL of dichloromethane. The organic phase was treated with sodium sulfate, filtered, and evaporated to dryness using a rotary evaporator. For the aqueous phase, 7 mL of ammonium hydroxide was added until a pH of 10 was achieved, followed by partitioning with 37 mL of dichloromethane. The organic phase from this step was also treated with sodium sulfate, filtered, and evaporated to dryness, while the aqueous phase was similarly evaporated to dryness using a rotary evaporator. Finally, the residues obtained from both the organic and aqueous phases were redissolved in minimal volumes of DMSO and kept at 4°C for later use.

2.5. Preparation of mixtures

The binary combinations of honey are prepared by mixing equal mass ratios (g/g) of honey to get a total of 55 mixtures. On the other hand, a homogeneous mixture was produced by combining 1 gram of honey with 200 µL of a plant extract at a concentration of 10 mg/L.

For each experimental trial, extracts were prepared by dissolving 1 gram of either a single honey sample or one of the binary honey mixtures in 10 mL of distilled water. After that, ten minutes were spent extensively mixing the resultant solution using a vortex mixer.

2.6. Total phenolic contents (TPC)

The total phenolic content (TPC) was quantified using a modified spectrophotometric method, as previously described by Singleton & Rossi [17], which employs the Folin-Ciocalteu reagent. In this procedure, 100 µL of the sample solutions were combined with 500 µL of freshly prepared 10% Folin-Ciocalteu reagent. Following a 2-minute incubation period, 2 mL of a 2% sodium carbonate solution was added to the mixture. The solution was then incubated for an additional 30 minutes, and its absorbance

was measured at 760 nm. A calibration curve was constructed using gallic acid as the standard, and the total phenolic content was expressed in milligrams of gallic acid equivalent per 100 grams of either single honey or honey mixtures (mg GAE/100g).

2.7. Estimation of antioxidant capacity

The determination of antioxidant activity was assessed using DPPH, ABTS and FRAP assays. It was expressed as microgram ascorbic acid equivalent antioxidant capacity (AAEAC) per gram of honey or honey mixtures, using the calibration curve of ascorbic acid.

the DPPH radical-scavenging activity was measured using the method described by Ani et al. [18]. A mixture of 1 mL of a 125 µM ethanolic solution of DPPH and 1 mL of each sample or standard was prepared. The absorbance at 517 nm was measured 30 minutes later.

The FRAP test was carried out using the procedure outlined by Benzie and Strain [19], with some modifications. Each extract or varying concentrations of ascorbic acid (1 mL) was mixed with 1 mL of the FRAP reagent. For seven minutes, the mixture was left to react at room temperature in the dark. At 593 nm, the ferrous tripyridyl-triazine (TPTZ-Fe^{2+}) complex's absorbance was measured.

The ABTS radical scavenging capacity was assessed using a modified method by Rice-Evans et al. [20]. 100 µL of the extract or the standard was mixed with 900 µL of recently diluted ABTS solution. The absorbance at 734 nm was measured following a 15-minute incubation period at 37°C.

2.8. Estimation of Antibacterial Capacity

Three reference bacterial strains were used in vitro to assess the bactericidal effects of honey and honey mixes. These included one Gram-positive bacterium, *Staphylococcus aureus (ATCC 25923)*, and two Gram-negative bacteria, *Pseudomonas aeruginosa (ATCC 27853)* and *Escherichia coli (ATCC 259221).* The Pasteur Institute in Algeria provided the bacterial strains.

Pure honey and its mixes were tested for antibacterial activity using the agar well diffusion method. adapted from Alexander Fleming's 1929 methodology [21]. Mueller-Hinton agar plates were coated with standard inoculums (0.5 MacFarland), which were evenly distributed using a sterile glass spreader. and sterile cork borer was used to form three wells, and 100 µL of pure honey samples or combinations were placed into each well. After a 24-hour incubation period at 37°C, the plates were inspected for zones of inhibition. In this in vitro experiment, the Gentamicin antibiotic was used for calibration. Then, micrograms of Gentamicin equivalent (GEA) per gram of honey or honey mixture were used to express the antibacterial activity. This study represents the first application of this methodology.

2.9. UHPLC MS/MS Analysis

A Shimadzu Nexera UHPLC coupled with a tandem mass spectrometer was used to evaluate phenolic compounds quantitatively [22]. The optimal chromatographic separation was achieved using a reversed-phase Agilent Poroshell 120 EC-C18 column at 40°C, with a gradient elution method employing water,

methanol, ammonium formate, and formic acid as mobile phases. The method was optimized by systematically testing various column types, mobile phase compositions, and temperatures to minimize suppression effects and achieve optimal separation. Mass spectrometric detection was performed in both positive and negative ionization modes using multiple reaction monitoring (MRM) to quantify specific precursor-to-fragment ion transitions. Collision energies were optimized, and specific MS operating parameters (e.g., drying gas flow, interface temperature) were also set to ensure efficient fragmentation and detection of the target phytochemicals. The method employed a Shimadzu UHPLC system and an LCMS-8040 tandem mass spectrometer. Data analysis was conducted using LabSolutions software [23, 24].

2.10. Statistical Analysis

The Test results were described as mean ± standard deviation (SD) from three trials and analysed with Excel 2018. Descriptive analysis utilized XLSTAT 2018.5.03, employing a 95% confidence interval for the Pearson correlation coefficient and principal component analysis (PCA). One-way ANOVA and Tukey's test were used for group mean assessments.

3. Results And Discussion

3.1. Total phenolic content

The total phenolic content differs significantly $p < 0.0001$, depending on the Honey type (botanical origin) and type of combination (honey-honey and honey-extracts mixture) (Table 2). Among the 68 samples studied, the TPC varied from 16.764 mg/100 g in M_{10}-MD_{aq} to 161.846 mg/100 g in M_3.

Table 2
Total phenolic contents and the antioxidant activities of different honey samples and their combinations

Samples	Phenolic contents	Antioxidant activity (µg AAE/g Honey)		
	(mg GAE/100g)	DPPH	ABTS	FRAP
M_1	97.42 ± 0.52^{op}	46.406 ± 0.63^{b}	40.766 ± 0.17^{ef}	13.634 ± 0.04^{a}
M_2	94.315 ± 0.34^{no}	52.196 ± 0.28^{de}	45.651 ± 0.27^{hijk}	22.975 ± 0.2^{lm}
M_3	161.846 ± 0.59^{w}	246.389 ± 0.75^{aa}	126.043 ± 0.9^{ad}	81.619 ± 0.17^{aa}
M_4	111.403 ± 0.68^{r}	64.306 ± 0.86^{mnopq}	54.805 ± 0.13^{tu}	31.603 ± 0.48^{t}
M_5	89.847 ± 0.19l^{m}	56.905 ± 0.64^{ghij}	59.736 ± 0.34^{vw}	31.699 ± 0.1^{t}
M_6	78.502 ± 0.19^{ghi}	58.892 ± 0.97^{jk}	47.688 ± 0.32^{klmn}	18.1 ± 0.22^{cde}
M_7	61.584 ± 0.71^{a}	47.976 ± 0.34^{bc}	40.96 ± 0.25^{ef}	17.862 ± 0.15^{bcd}
M_8	86.953 ± 0.19^{kl}	58.065 ± 0.17^{ijk}	61.503 ± 0.09^{wx}	27.365 ± 0.44^{r}
M_9	61.049 ± 0.52^{a}	53.109 ± 0.11^{a}	37.092 ± 0.34^{c}	20.885 ± 0.16^{hijk}
M_{10}	73.592 ± 0.52^{ef}	55.69 ± 0.15^{efghij}	33.979 ± 0.31^{b}	16.492 ± 0.14^{b}
M_1-M_2	85.038 ± 0.59^{k}	55.087 ± 0.5^{efghi}	50.526 ± 0.39^{opqr}	23.209 ± 0.13^{lm}
M_1-M_3	116.016 ± 0.86^{s}	214.998 ± 0.25^{z}	101.489 ± 0.35^{ac}	49.861 ± 0.33^{wx}
M_1-M_4	92.763 ± 0.71^{mn}	68.284 ± 0.4^{rstuv}	48.656 ± 0.57^{mnop}	20.996 ± 0.15^{hijk}
M_1-M_5	81.292 ± 0.68^{ij}	71.592 ± 0.27^{tuvw}	54.517 ± 0.8^{tu}	24.892 ± 0.03^{nopq}
M_1-M_6	73.975 ± 0.34^{ef}	62.697 ± 0.95^{lmn}	41.962 ± 0.05^{efg}	19.412 ± 0.14^{efg}
M_1-M_7	69.785 ± 0.91^{bcd}	53.986 ± 0.19^{defgh}	43.545 ± 0.67^{ghi}	18.801 ± 0.03^{def}
M_1-M_8	91.725 ± 0.9^{mn}	67.396 ± 0.38^{pqrs}	55.138 ± 0.46^{u}	22.818 ± 0.35^{lm}
M_1-M_9	75.355 ± 0.52^{efg}	34.765 ± 0.83^{a}	39.705 ± 0.18^{de}	17.874 ± 0.06^{bcd}
M_1-M_{10}	79.599 ± 0.39^{hi}	50.67 ± 0.34^{cd}	60.342 ± 0.26^{vw}	19.076 ± 0.02^{defg}

AH_{Dc}: dichloromethane fraction of *Atriplex halimus*; AH_{aq}: aqueous fraction of *Atriplex halimus;* MD_{Dc}: dichloromethane fraction of *Marrubium deserti* De Noé,; MD_{aq}: aqueous fraction of *Marrubium deserti* De Noé, ; The results of the tests carried out are expressed as mean ± standard deviation, n = 3. Mean values followed by a different lowercase letter are significantly different ($p < 0.00001$).

Samples	Phenolic contents	Antioxidant activity (µg AAE/g Honey)		
	(mg GAE/100g)	DPPH	ABTS	FRAP
M_2-M_3	128.805 ± 0.52^{u}	256.707 ± 1.74^{ab}	93.27 ± 0.71^{ab}	42.488 ± 0.74^{u}
M_2-M_4	105.235 ± 0.59^{q}	71.881 ± 0.23^{uvw}	48.776 ± 0.43^{mnop}	26.022 ± 0.14^{qr}
M_2-M_5	92.349 ± 0.39^{mn}	76.155 ± 0.28^{y}	58.468 ± 0.08^{v}	25.689 ± 0.06^{pq}
M_2-M_6	72.705 ± 0.79^{cdef}	53.12 ± 0.52^{def}	49.504 ± 0.29^{nopq}	24.232 ± 0.11^{mnop}
M_2-M_7	97.958 ± 0.52^{op}	54.499 ± 0.37^{efghi}	46.553 ± 0.09^{jklm}	22.025 ± 0.09^{kl}
M_2-M_8	105.214 ± 0.68^{q}	78.424 ± 0.35^{y}	58.434 ± 0.32^{v}	23.984 ± 0.01^{mno}
M_2-M_9	112.906 ± 0.52^{rs}	59.235 ± 0.11^{jkl}	50.765 ± 0.22^{pqrs}	21.227 ± 0.08^{ijk}
M_2-M_{10}	130.589 ± 0.9^{u}	67.077 ± 0.31^{opqrs}	54.268 ± 0.28^{tu}	19.399 ± 0.03^{efg}
M_3-M_4	127.655 ± 0.71^{u}	282.084 ± 1.32^{ad}	73.391 ± 0.73^{z}	54.158 ± 0.36^{y}
M_3-M_5	113.587 ± 0.19^{rs}	293.184 ± 0.49^{ae}	73.467 ± 0.4^{z}	49.136 ± 0.17^{w}
M_3-M_6	114.992 ± 0.52^{rs}	291.637 ± 1.75^{ae}	59.488 ± 0.53^{vw}	54.707 ± 0.83^{y}
M_3-M_7	122.648 ± 0.68^{t}	280.594 ± 1.09^{ad}	83.161 ± 0.34^{aa}	46.583 ± 0.1^{v}
M_3-M_8	134.438 ± 0.19^{v}	273.772 ± 2.05^{ac}	81.992 ± 0.52^{aa}	58.401 ± 0.14^{z}
M_3-M_9	116.063 ± 0.52^{s}	305.19 ± 0.5^{af}	238.33 ± 0.87^{ae}	49.34 ± 0.36^{w}
M_3-M_{10}	128.34 ± 0.91^{u}	325.239 ± 0.76^{ag}	250.766 ± 0.87^{af}	51.013 ± 0.4^{x}
M_4-M_5	79.662 ± 0.52^{hi}	78.106 ± 0.21^{y}	48.361 ± 0.17^{lmnop}	22.89 ± 0.1^{lm}
M_4-M_6	92.276 ± 0.71^{mn}	68.145 ± 0.33^{rstu}	51.593 ± 0.47^{qrs}	18.262 ± 0.05^{cdef}
M_4-M_7	75.123 ± 0.34^{efg}	56.724 ± 0.33^{fghij}	58.884 ± 0.39^{v}	21.832 ± 0.21^{jkl}
M_4-M_8	84.755 ± 0.71^{jk}	68.11 ± 0.33^{rstu}	47.757 ± 0.29^{klmn}	23.646 ± 0.09^{mn}
M_4-M_9	80.303 ± 0.52^{i}	71.956 ± 0.1^{vw}	45.589 ± 0.17^{hijk}	19.659 ± 0.06^{fgh}

AH_{Dc}: dichloromethane fraction of *Atriplex halimus*; AH_{aq}: aqueous fraction of *Atriplex halimus;* MD_{Dc}: dichloromethane fraction of *Marrubium deserti* De Noé,; MD_{aq}: aqueous fraction of *Marrubium deserti* De Noé, ; The results of the tests carried out are expressed as mean ± standard deviation, n = 3. Mean values followed by a different lowercase letter are significantly different ($p < 0.00001$).

Samples	Phenolic contents	Antioxidant activity (µg AAE/g Honey)		
	(mg GAE/100g)	DPPH	ABTS	FRAP
M_4-M_{10}	76.009 ± 0.71^{fgh}	76.561 ± 0.13^{y}	48.046 ± 0.21^{klmno}	21.152 ± 0.02^{ijk}
M_5-M_6	69.42 ± 0.71^{bc}	72.345 ± 0.29^{wx}	52.265 ± 0.21^{rst}	25.992 ± 0.15^{qr}
M_5-M_7	69.618 ± 0.9^{bc}	61.126 ± 0.35^{klm}	53.058 ± 0.42^{rstu}	25.187 ± 0.24^{opq}
M_5-M_8	79.829 ± 0.9^{i}	75.936 ± 0.27^{xy}	60.922 ± 0.44^{vw}	29.305 ± 0.15^{s}
M_5-M_9	72.07 ± 0.9^{cde}	63.206 ± 0.22^{mn}	$48.287 \pm 0.44l^{mnop}$	25.967 ± 0.33^{qr}
M_5-M_{10}	75.048 ± 0.59^{efg}	63.603 ± 0.25^{mno}	53.291 ± 0.27^{stu}	25.842 ± 0.02^{q}
M_6-M_7	66.986 ± 0.19^{b}	52.083 ± 0.06^{de}	37.393 ± 0.64^{cd}	18.289 ± 0.14^{cdef}
M_6-M_8	60.627 ± 0.71^{a}	68.018 ± 0.33^{qrst}	45.887 ± 0.26^{ijkl}	20.337 ± 0.17^{ghi}
M_6-M_9	75.011 ± 0.19^{efg}	56.135 ± 0.12^{fghij}	37.238 ± 0.7^{cd}	17.245 ± 0.25^{bc}
M_6-M_{10}	85.043 ± 0.52^{k}	64.011 ± 0.25^{mnop}	47.795 ± 0.18^{klmn}	19.68 ± 0.03^{fgh}
M_7-M_8	72.893 ± 0.86^{cdef}	53.362 ± 0.16^{defg}	43.943 ± 0.54^{ghi}	20.435 ± 0.09^{ghij}
M_7-M_9	73.336 ± 0.71^{def}	57.181 ± 0.23^{hij}	30.665 ± 0.3^{a}	17.624 ± 0.31^{bcd}
M_7-M_{10}	80.318 ± 0.67^{i}	64.827 ± 0.48^{mnopqr}	44.307 ± 0.28^{ghij}	18.165 ± 0.05^{cde}
M_8-M_9	98.101 ± 0.68^{p}	65.129 ± 0.27^{nopqrs}	43.183 ± 0.08^{fgh}	23.17 ± 0.4^{lm}
M_8-M_{10}	111.308 ± 0.52^{r}	68.704 ± 0.35^{stuvw}	63.824 ± 0.22^{x}	23.691 ± 0.03^{mn}
M_9-M_{10}	92.755 ± 0.71^{mn}	71.487 ± 0.44^{tuvw}	67.056 ± 0.53^{y}	26.248 ± 0.04^{qr}
M_1-AH_{Dc}	73.71 ± 0.84^{lm}	55.955 ± 0.4^{f}	63.22 ± 0.08^{g}	230.273 ± 0.52^{e}
M_1-AH_{aq}	66.987 ± 0.51^{ij}	37.86 ± 0.11^{a}	69.157 ± 0.4^{h}	245.356 ± 0.86^{f}
M_1-MD_{Dc}	69.607 ± 0.51^{ijk}	65.186 ± 0.53^{ij}	86.725 ± 0.36^{p}	245.837 ± 0.78^{f}

AH_{Dc}: dichloromethane fraction of *Atriplex halimus*; AH_{aq}: aqueous fraction of *Atriplex halimus;* MD_{Dc}: dichloromethane fraction of *Marrubium deserti* De Noé,; MD_{aq}: aqueous fraction of *Marrubium deserti* De Noé, ; The results of the tests carried out are expressed as mean ± standard deviation, n = 3. Mean values followed by a different lowercase letter are significantly different ($p < 0.00001$).

Samples	Phenolic contents	Antioxidant activity (µg AAE/g Honey)		
	(mg GAE/100g)	DPPH	ABTS	FRAP
M_1-MD_{aq}	47.489 ± 0.69^{c}	52.87 ± 0.41^{e}	70.751 ± 0.13^{h}	245.598 ± 0.65^{f}
M_2-AH_{Dc}	83.744 ± 0.83^{o}	65.726 ± 0.15^{j}	80.505 ± 0.31^{lm}	375.558 ± 0.92^{t}
M_2-AH_{aq}	130.955 ± 0.85^{u}	68.662 ± 0.46^{klm}	33.486 ± 0.31^{ab}	383.666 ± 0.94^{uv}
M_2-MD_{Dc}	93.101 ± 0.7^{q}	67.651 ± 0.43^{jkl}	85.182 ± 0.34^{nop}	367.226 ± 0.55^{rs}
M_2-MD_{aq}	83.302 ± 0.33^{o}	67.28 ± 0.37^{jk}	96.292 ± 0.34^{q}	377.516 ± 1.21^{tu}
M_3-AH_{Dc}	120.041 ± 0.51^{s}	218.848 ± 1.06^{z}	165.81 ± 2.1^{s}	950.593 ± 3.44^{y}
M_3-AH_{aq}	58.824 ± 0.67^{ef}	225.3 ± 0.23^{aa}	149.673 ± 1.48^{r}	340.26 ± 1.41^{p}
M_3-MD_{Dc}	88.63 ± 0.51^{p}	213.009 ± 0.83^{y}	219.885 ± 1.49^{t}	1042.273 ± 0.39^{z}
M_3-MD_{aq}	126.159 ± 0.88^{t}	255.719 ± 1.98^{ab}	240.524 ± 0.74^{u}	1082.574 ± 1.04^{aa}
M_4-AH_{Dc}	61.959 ± 0.51^{fg}	94.169 ± 0.3^{w}	82.165 ± 0.42^{mn}	329.84 ± 0.52^{o}
M_4-AH_{aq}	124.457 ± 0.67^{t}	83.505 ± 0.17^{rs}	86.034 ± 0.31^{op}	179.607 ± 0.86^{a}
M_4-MD_{Dc}	83.726 ± 0.51^{o}	58.398 ± 0.34^{fg}	77.559 ± 0.36^{kl}	389.497 ± 1.62^{vw}
M_4-MD_{aq}	71.741 ± 0.69^{klm}	74.295 ± 0.34^{pq}	46.207 ± 0.08^{de}	350.454 ± 0.69^{q}
M_5-AH_{Dc}	93.213 ± 0.67^{q}	82.244 ± 0.2^{r}	77.883 ± 0.09^{kl}	315.901 ± 0.55^{n}
M_5-AH_{aq}	75.13 ± 0.51^{mn}	82.443 ± 0.26^{r}	31.104 ± 0.35^{ab}	371.542 ± 0.94st
M_5-MD_{Dc}	116.273 ± 0.85^{r}	87.666 ± 0.36^{tu}	74.172 ± 0.18^{ij}	399.509 ± 0.55^{x}
M_5-MD_{aq}	82.409 ± 0.88^{o}	73.176 ± 0.25^{op}	48.716 ± 0.41^{e}	394.566 ± 1.29^{wx}
M_6-AH_{Dc}	94.877 ± 0.67^{q}	88.718 ± 0.25^{uv}	87.732 ± 0.22^{p}	278.167 ± 0.55^{k}
M_6-AH_{aq}	70.839 ± 0.51^{kl}	58.041 ± 0.3^{fg}	76.781 ± 0.22^{jk}	258.816 ± 0.94^{gh}
M_6-MD_{Dc}	89.02 ± 0.51^{p}	71.212 ± 0.36^{mno}	47.609 ± 0.27^{e}	294.454 ± 1.95^{l}
M_6-MD_{aq}	78.076 ± 0.84^{n}	60.213 ± 0.25^{gh}	43.07 ± 0.17^{cd}	281.933 ± 0.78^{k}

AH_{Dc}: dichloromethane fraction of *Atriplex halimus*; AH_{aq}: aqueous fraction of *Atriplex halimus;* MD_{Dc}: dichloromethane fraction of *Marrubium deserti* De Noé,; MD_{aq}: aqueous fraction of *Marrubium deserti* De Noé, ; The results of the tests carried out are expressed as mean ± standard deviation, n = 3. Mean values followed by a different lowercase letter are significantly different ($p < 0.00001$).

Samples	Phenolic contents	Antioxidant activity (µg AAE/g Honey)		
	(mg GAE/100g)	DPPH	ABTS	FRAP
M_7-AH_{Dc}	85.768 ± 0.67^{op}	70.29 ± 0.3^{lmn}	83.258 ± 0.32^{mno}	244.08 ± 0.95^{f}
M_7-AH_{aq}	52.434 ± 0.51^{d}	42.266 ± 0.26^{b}	56.756 ± 0.09^{f}	201.647 ± 0.95^{c}
M_7-MD_{Dc}	70.589 ± 0.33^{jkl}	50.816 ± 0.15^{de}	71.257 ± 0.09^{hi}	263.799 ± 0.65^{hi}
M_7-MD_{aq}	66.603 ± 0.84^{hi}	47.375 ± 0.1^{c}	58.796 ± 0.18^{f}	242.618 ± 0.77^{f}
M_8-AH_{Dc}	87.8 ± 0.51^{p}	88.318 ± 0.38^{x}	62.345 ± 0.46^{g}	352.81 ± 1.42^{q}
M_8-AH_{aq}	66.667 ± 0.51^{hi}	73.488 ± 0.1^{op}	57.025 ± 0.37^{f}	317.57 ± 1.81^{n}
M_8-MD_{Dc}	84.107 ± 0.51^{q}	80.638 ± 0.42^{v}	58.888 ± 0.18^{f}	363.003 ± 1.42^{r}
M_8-MD_{aq}	63.314 ± 0.51^{gh}	83.153 ± 0.3^{rs}	56.582 ± 0.14^{f}	382.354 ± 1.31^{u}
M_9-AH_{Dc}	48.473 ± 0.51^{c}	71.683 ± 0.05^{nop}	42.577 ± 0.57^{c}	301.286 ± 1.04^{m}
M_9-AH_{aq}	57.882 ± 0.51^{e}	85.38 ± 0.58st	43.713 ± 0.23^{cd}	256.201 ± 1.42^{g}
M_9-MD_{Dc}	88.387 ± 0.51^{p}	60.381 ± 0.15^{gh}	44.118 ± 0.23^{cd}	271.23 ± 1.03^{j}
M_9-MD_{aq}	56.256 ± 0.84^{e}	76.767 ± 0.1^{q}	42.461 ± 0.18^{c}	266.752 ± 1.04^{ij}
M_{10}-AH_{Dc}	23.088 ± 0.51^{b}	62.846 ± 0.11^{hi}	30.785 ± 0.33^{a}	181.824 ± 1.02^{b}
M_{10}-AH_{aq}	23.874 ± 0.33^{b}	48.846 ± 0.4^{cd}	32.784 ± 0.18^{ab}	187.826 ± 0.58^{bc}
M_{10}-MD_{Dc}	18.026 ± 0.84^{a}	65.123 ± 0.3^{ij}	33.884 ± 0.14^{b}	215.413 ± 1.02^{d}
M_{10}-MD_{aq}	16.764 ± 0.33^{a}	58.525 ± 0.2^{g}	32.718 ± 0.24^{ab}	212.385 ± 0.08^{d}

AH_{Dc}: dichloromethane fraction of *Atriplex halimus*; AH_{aq}: aqueous fraction of *Atriplex halimus;* MD_{Dc}: dichloromethane fraction of *Marrubium deserti* De Noé,; MD_{aq}: aqueous fraction of *Marrubium deserti* De Noé, ; The results of the tests carried out are expressed as mean ± standard deviation, n = 3. Mean values followed by a different lowercase letter are significantly different ($p < 0.00001$).

Firstly, the TPC of the ten single honeys varies significantly ($p < 0.0001$) between 61.048 for the M_8 sample and 161.846 mg GAE/100 g for the M_3 sample, with a mean of 81.65 mg GAE/100 g honey, surpassing other Algerian honeys (24.17-122.15 mg GAE/100 g honey) [25] and Polish honeys (3.43–22.33 mg GAE/100 g honey) [26]. However, Vîjan et al. [27] observed slightly higher values of TPC (124.09- 167.59 mg GAE/100 g honey), Additionally, our results align with Michał Halagarda's findings [28], highlighting higher phenolic content in darker honey varieties (M_3, M_4, M_2, M_5, M_8) compared to lighter ones (M_6, M_7, M_9, M_{10}).

Secondly, the TPC of the binary combination of honey showed a value varied from (60.62 to 134.43/100g honey-honey). The results showed that combining M_3 honey with other samples significantly ($p < 0.0001$) increased the TPC by 16–55%. Also, M_2-M_4, M_2-M_7, M_2-M_8, M_2-M_{10}, M_6-M_{10}, M_7-M_{10}, M_8-M_9 and M_8-M_{10} combinations demonstrated enhancement of TPC values

On the other hand, honey-plant mixtures exhibited varying TPC levels based on plant species and their fractions (dichloromethane or aqueous fraction). When honeys were combined with *Atriplex halimus L.* extracts, the TPC ranged (from 23.088 to 120.041 mg GAE/100 g honey-plant) and (23.074 to 130.855 mg GAE/100 g honey-plant) for the dichloromethane and aqueous fractions, respectively, with notable augmentation in M_6-AH_{Dc}; M_7-AH_{Dc}; M_8-AH_{Dc}, M_2-AHaq and M_4-AH_{aq} mixtures compared to the single honeys. Additionally, honeys mixed with *Marrubium deserti* De Noé extracts showed TPC values ranging from 18.026 to 116.273 mg GAE/100 g honey-plant and 16.764 to 126.158 mg GAE/100 g honey-plant for the dichloromethane and aqueous fractions, respectively, with an increase in M_7-MD_{Dc}, M_7-MD_{aq} and M_8-MD_{Dc} mixture against single honeys.

3.2. Antioxidant activity

The antioxidant capacity of honey's bioactive components is evaluated using a variety of techniques [29]. However, no single method can fully capture the complexity of antioxidants due to the diverse phytochemicals present in Honey [30]. We utilised three assays: ABTS, FRAP, and DPPH to evaluate the antioxidant activity of honey and its combinations. The results are shown in Table 2.

All single honey samples exhibited antioxidant capacity across the three methods (Table 2). Statistical analysis indicated that M3 had the highest AAEAC values, which were significantly ($p < 0.0001$) distinct from the other samples in all tests; their AAEAC values were 246.388, 126.043 and 81.618 in DPPH, ABTS and FRAP assays, respectively. While the M1 honey sample showed the lowest antioxidant capacity for DPPH (46.405 µg AAE/g honey and the lowest reducing power in the FRAP test (13.63 µg AAE/g honey). The M10 sample exhibited the lowest antioxidant potential in the ABTS assay, with an AAEAC value of 33.878 mg AAE/g honey. On the other hand, the antioxidant activity of our honeys, measured by the FRAP assay, showed higher values compared to those found by Necib et al. [31](15.240 to 17.784 µg /100g honey) and Otmani et al. [32](40–150µgGAE /100g honey).

In contrast, the results of the honey binary combinations (Table 2) showed significantly higher ($p < 0.0001$) antioxidant activity compared to the single honeys in the three different assays. The AAEAC values for the 55 combinations in the DPPH assay ranged from 325.238 to 34.764 µg AAE/g honey-honey, while the results of the ABTS test varied from 250.765 to 30.664 µg AAE/g honey-honey. The highest values in these two assays were observed in the M_3-M_{10} combination. On the other hand, the FRAP assay values ranged from 58.40 µg AAE/g honey-honey to 17.244 µg AAE/g honey-honey in (M_2-M_5) and (M_6-M_8) samples, respectively. Moreover, the combinations of M3 sample with other honeys significantly increase the antioxidant abilities of the overall mixtures by a remarkable enhancement

(40%). Whereas, the other binary honey combinations showed an increase in the antioxidant capacity, ranging from 1% to 20%.

The forty mixtures of honey with *Atriplex halimus* L. and *Marrubium deserti* De Noé extracts showed significant ($p < 0.0001$) differences in DPPH antioxidant activity (Table 2). Their AAEAC values ranged from 37.858 to 255.718 µg AAE/ g honey-plant, where the highest activity was detected in the mixture M_3-MD_{aq}. Most mixtures showed an increase in the anti-DPPH activity by 5% to 10%. However, the combinations M_1-AH_{aq}, M_4-MD_{Dc}, and M_{10}-MD_{Dc} showed no detectable synergistic effects. In contrast, the ABTS assay revealed that only 28 combinations showed a significant ($p < 0.0001$) increase in antiradical activity, ranging from 10% to 20%. Their AAEAC values varied from 30.785 µg AAE/ g for the M_{10}-AH_{DC} mixture to 240.524 µg AAE/g honey-plant for the M_3-MDaq mixture. While in the FRAP assay, the AAEAC values ranged from 178.606 to 1082.573 µg AAE/g honey-plant, where the lowest and the highest values correspond to M_4-AH_{aq} and M_3-MD_{aq}, respectively. Also, it is notable that all honey samples reduction activity was increased by over 80% when mixed with plant extracts. These results align with other published results by Dimitriu al.[33] who discovered a synergistic effect between phenolic compounds found in various plants, such as sea buckthorn, raspberries, tansy, and others, with honey. Additionally, Lella et a[34]l. revealed a synergistic antioxidant property between *Psidium guajava* extract and raw honey. Furthermore, research conducted by Padmanabhan and Doss[35] highlighted the synergistic antioxidant benefits of stingless bee honey and Cinnamomum verum.

3.3. Antibacterial activity

The antibacterial effectiveness of honey and the tested combinations was calculated from the calibration curves of gentamicin (Figures. 1–3) and expressed in terms of gentamicin equivalent anti-strain capacity and defined as the number of micrograms of gentamicin (CN) required to match the inhibitory power of one gram of honey or binary combinations of honey or honey-plant mixtures (µg CN/g). Table 3 shows the results of the antibacterial capacity of honey and its combinations against *S. aureus*, *P. aeruginosa*, and *E. coli.*

Table 3
Antibacterial activities of different honey samples and their combinations

Samples	Antibacterial activity (µg CN/g honey or honey-plant mixture)		
	Staphylococcus aureus (ATCC 25823)	*Pseudomonas aeruginosa* (ATCC 27853)	*Escherichia coli* (ATCC258221)
	GEASA	*GEAPA*	*GAEEC*
M_1	47.475 ± 1.84^{d}	2.308 ± 0.36^{bc}	1.188 ± 0.04^{b}
M_2	106.028 ± 3.24^{c}	1.487 ± 0.23^{cd}	3.774 ± 0.08^{a}
M_3	22.268 ± 1.6^{e}	3.564 ± 0.55^{ab}	1.268 ± 0.04^{b}
M_4	10.853 ± 1.3^{ef}	0.814 ± 0.14^{cd}	1.134 ± 0.08^{b}
M_5	131.781 ± 4.68^{b}	4.088 ± 0.64^{a}	5.384 ± 0.88^{a}
M_6	3.884 ± 0.76^{f}	0.787 ± 0.18^{cd}	1.228 ± 0.03^{b}
M_7	0.568 ± 0.08^{f}	0.714 ± 0.08^{d}	0.771 ± 0.02^{b}
M_8	176.468 ± 3.65^{a}	0.748 ± 0.1^{cd}	1.453 ± 0^{b}
M_9	0.678 ± 0.01^{f}	0.153 ± 0.02^{d}	1.538 ± 0.23^{b}
M_{10}	1.538 ± 0.05^{f}	0.887 ± 0.14^{cd}	0.385 ± 0.03^{b}
M_1-M_2	318.214 ± 6.75^{a}	300.078 ± 8.72^{j}	40.86 ± 0^{ab}
M_1-M_3	$3.612 \pm 0.07^{ef*}$	685.185 ± 22.52^{l}	813.885 ± 30.78^{h}
M_1-M_4	120.518 ± 0^{a}	30.188 ± 0.66^{abcd}	7.712 ± 0^{a}
M_1-M_5	$2.085 \pm 0.06^{cd*}$	177.785 ± 0^{i}	263.825 ± 8.88^{bcdef}
M_1-M_6	162.137 ± 18.75^{a}	18.087 ± 0.61^{abc}	36.327 ± 0.82^{ab}
M_1-M_7	142.548 ± 18.44^{a}	3.441 ± 0.86^{a}	20.348 ± 0.51^{a}
M_1-M_8	$1.842 \pm 0.05^{bcd*}$	87.331 ± 3.15^{fg}	371.834 ± 14.07^{efg}
M_1-M_9	73.252 ± 2.24^{a}	3.164 ± 0.11^{a}	11.812 ± 0.44^{a}

GEASA: Gentamicin Equivalent Anti-*Staphylococcus Aureus*, GEAPA: Gentamicin Equivalent Anti-*Pseudomonas Aeruginosa*, GAEEC: Gentamicin Equivalent Anti-*Escherichia Coli.* * The antibacterial activity is expressed in mg CN/g Honey or Honey-plant mixture.

Samples	Antibacterial activity (µg CN/g honey or honey-plant mixture)		
	Staphylococcus aureus (ATCC 25823)	*Pseudomonas aeruginosa* (ATCC 27853)	*Escherichia coli* (ATCC258221)
	GEASA	*GEAPA*	*GAEEC*
M_1-M_{10}	182.81 ± 5.8^{a}	188.083 ± 4.34^{i}	111.318 ± 2.84^{abcd}
M_2-M_3	8.273 ± 00^{g*}	432.758 ± 14.01^{k}	2.378 ± 00^{j*}
M_2-M_4	2.850 ± 0.06^{de*}	87.405 ± 2.13^{fg}	47.201 ± 1.2^{ab}
M_2-M_5	18.888 ± 00^{i*}	706.008 ± 0^{l}	1.365 ± 0.03^{s*}
M_2-M_6	86.484 ± 4.07^{a}	4.781 ± 0.64^{a}	88.78 ± 3.77^{abcd}
M_2-M_7	1.053 ± 0.05^{abc*}	14.163 ± 0.45^{ab}	318.252 ± 12.07^{defg}
M_2-M_8	35.371 ± 0.07^{k*}	2.154 ± 00^{n*}	7.771 ± 0.28^{k*}
M_2-M_9	186.011 ± 4.06^{a}	5.028 ± 0.68^{a}	485.113 ± 18.73^{fg}
M_2-M_{10}	20.203 ± 0.04^{j*}	317.181 ± 0^{j}	66.686 ± 1.7^{abc}
M_3-M_4	1.048 ± 0.03^{abc*}	78.823 ± 2.58^{efg}	184.708 ± 0^{abcde}
M_3-M_5	12.532 ± 0.03^{h*}	51.376 ± 1.12^{bcde}	516.553 ± 13.18^{g}
M_3-M_6	1.023 ± 0.03^{abc*}	74.806 ± 4.33^{efg}	508.684 ± 13.33^{g}
M_3-M_7	282.728 ± 6.07^{a}	12.323 ± 0.71^{ab}	180.088 ± 4.6^{abcde}
M_3-M_8	38.312 ± 1.17^{l*}	1.462 ± 0.04^{m*}	15.658 ± 0.01^{l*}
M_3-M_9	2.815 ± 0.08^{de*}	158.668 ± 0i	28.767 ± 0.73ab
M_3-M_{10}	817.716 ± 23.26^{ab}	60.652 ± 1.86^{cdef}	128.243 ± 4.85^{abcd}
M_4-M_5	1.838 ± 0.04^{bcd*}	25.734 ± 0.56^{abcd}	26.858 ± 1.01^{ab}
M_4-M_6	33.058 ± 1.01^{a}	11.826 ± 0.38^{ab}	6.164 ± 0.85^{a}
M_4-M_7	45.358 ± 0^{a}	24.735 ± 0.8^{abcd}	48.253 ± 0^{ab}

GEASA: Gentamicin Equivalent Anti-*Staphylococcus Aureus*, GEAPA: Gentamicin Equivalent Anti-*Pseudomonas Aeruginosa*, GAEEC: Gentamicin Equivalent Anti-*Escherichia Coli.* * The antibacterial activity is expressed in mg CN/g Honey or Honey-plant mixture.

Samples	Antibacterial activity (µg CN/g honey or honey-plant mixture)		
	Staphylococcus aureus (ATCC 25823)	*Pseudomonas aeruginosa* (ATCC 27853)	*Escherichia coli* (ATCC258221)
	GEASA	*GEAPA*	*GAEEC*
M_4-M_8	$4.380 \pm 0.01^{f*}$	153.76 ± 3.37^{hi}	128.071 ± 4.88^{abcd}
M_4-M_9	36.17 ± 0.75^{a}	7.206 ± 0.8^{ab}	13.588 ± 0.34^{a}
M_4-M_{10}	203.268 ± 0^{a}	13.287 ± 0.43^{ab}	40.081 ± 0.34^{ab}
M_5-M_6	546.284 ± 11.32^{a}	25.48 ± 0.55^{abcd}	28.571 ± 0^{ab}
M_5-M_7	127.187 ± 3.88^{a}	8.886 ± 0.18^{ab}	23.407 ± 0.53^{a}
M_5-M_8	$18.882 \pm 00^{i*}$	113.484 ± 3.67^{gh}	287.775 ± 6.52^{cdefg}
M_5-M_9	267.818 ± 0^{a}	67.853 ± 2.2^{def}	56.812 ± 1.28^{abc}
M_5-M_{10}	403.163 ± 12.33^{a}	8.183 ± 0^{ab}	$483.468 \pm 4.15f^{g}$
M_6-M_7	66.248 ± 0^{a}	2.68 ± 0.05^{a}	8.72 ± 0.08^{a}
M_6-M_8	846.122 ± 28.85^{abc}	8.868 ± 2.21^{ab}	104.672 ± 0^{abcd}
M_6-M_9	48.086 ± 1.5^{a}	3.068 ± 0.17^{a}	12.558 ± 0.32^{a}
M_6-M_{10}	10.82 ± 1.81^{a}	2.073 ± 0.04^{a}	7.385 ± 0.18^{a}
M_7-M_8	81.873 ± 1.8^{a}	14.203 ± 0.46^{ab}	28.264 ± 2.53^{ab}
M_7-M_9	53.128 ± 0^{a}	5.334 ± 0^{a}	7.348 ± 0.27^{a}
M_7-M_{10}	16.358 ± 1.17^{a}	2.083 ± 0.06^{a}	22.253 ± 0^{a}
M_8-M_9	620.827 ± 4.35^{a}	7.631 ± 0.16^{ab}	56.552 ± 1.28^{abc}
M_8-M_{10}	308.036 ± 2.15^{a}	8.848 ± 0^{ab}	23.24 ± 0.58^{a}
M_9-M_{10}	8.482 ± 0^{a}	1.881 ± 0.06^{a}	7.262 ± 0.16^{a}
M_1-AH_{Dc}	3.338 ± 0.62^{a}	1.728 ± 0.27^{a}	12.237 ± 0.27^{ab}

GEASA: Gentamicin Equivalent Anti-*Staphylococcus Aureus*, GEAPA: Gentamicin Equivalent Anti-*Pseudomonas Aeruginosa*, GAEEC: Gentamicin Equivalent Anti-*Escherichia Coli.* * The antibacterial activity is expressed in mg CN/g Honey or Honey-plant mixture.

Samples	Antibacterial activity (µg CN/g honey or honey-plant mixture)		
	Staphylococcus aureus (ATCC 25823)	*Pseudomonas aeruginosa* (ATCC 27853)	*Escherichia coli* (ATCC258221)
	GEASA	*GEAPA*	*GAEEC*
M_1-AH_{aq}	2.875 ± 0.44^{a}	10.756 ± 0.2^{ab}	74.577 ± 1.68^{d}
M_1-MD_{Dc}	2.641 ± 0.18^{a}	11.023 ± 1.38^{ab}	163.556 ± 3.71^{g}
M_1-MD_{aq}	8.42 ± 0.88^{abc}	12.641 ± 0.08^{ab}	154.881 ± 3.51^{g}
M_2-AH_{Dc}	34.358 ± 0.63^{abcd}	30.817 ± 0.22^{abc}	158.472 ± 1.25^{g}
M_2-AH_{aq}	$2.684 \pm 0.018^{m*}$	$131.885 \pm 2.56^{abcdef}$	301.838 ± 6.84^{h}
M_2-MD_{Dc}	52.58 ± 1.66^{abcd}	74.688 ± 1.45^{abcd}	768.324 ± 6.6^{l}
M_2-MD_{aq}	281.113 ± 1.85^{fg}	$3.812 \pm 0.07^{o*}$	743.11 ± 6.38^{k}
M_3-AH_{Dc}	30.256 ± 0.86^{abcd}	252.458 ± 1.85^{fg}	50.487 ± 0.43^{c}
M_3-AH_{aq}	120.623 ± 2.21^{de}	$1.070 \pm 0.01^{jk*}$	444.746 ± 3.85^{j}
M_3-MD_{Dc}	103.688 ± 0.72^{cde}	62.804 ± 0.46^{abc}	134.111 ± 1.15^{f}
M_3-MD_{aq}	3.286 ± 0.12^{a}	231.162 ± 1.7^{fg}	357.511 ± 8.11^{i}
M_4-AH_{Dc}	34.764 ± 1.06^{abcd}	4.818 ± 0.65^{ab}	7.427 ± 0.06^{ab}
M_4-AH_{aq}	$2.114 \pm 0.03^{k*}$	17.887 ± 0.35^{ab}	86.181 ± 1.85^{de}
M_4-MD_{Dc}	3.578 ± 0.12^{a}	2.857 ± 0.02^{a}	2.188 ± 0.01^{ab}
M_4-MD_{aq}	100.278 ± 1.71^{bcd}	358.487 ± 5.53^{g}	46.545 ± 0.4^{c}
M_5-AH_{Dc}	477.858 ± 3.32^{i}	$1.124 \pm 0.01^{k*}$	$1.837 \pm 00^{n*}$
M_5-AH_{aq}	$10.338 \pm 0.07^{o*}$	524.552 ± 10.2^{h}	168.528 ± 0^{g}
M_5-MD_{Dc}	$8.786 \pm 0.06^{n*}$	77.802 ± 0^{abcde}	10.771 ± 0^{ab}
M_5-MD_{aq}	$2.263 \pm 0.01^{l*}$	263.4 ± 1.78^{fg}	16.223 ± 0.13^{b}

GEASA: Gentamicin Equivalent Anti-*Staphylococcus Aureus*, GEAPA: Gentamicin Equivalent Anti-*Pseudomonas Aeruginosa*, GAEEC: Gentamicin Equivalent Anti-*Escherichia Coli.* * The antibacterial activity is expressed in mg CN/g Honey or Honey-plant mixture.

Samples	Antibacterial activity (µg CN/g honey or honey-plant mixture)		
	Staphylococcus aureus (ATCC 25823)	*Pseudomonas aeruginosa* (ATCC 27853)	*Escherichia coli* (ATCC258221)
	GEASA	*GEAPA*	*GAEEC*
M_6-AH_{Dc}	0.565 ± 0.03^{a}	228.606 ± 1.68^{fg}	1.286 ± 0.02^{ab}
M_6-AH_{aq}	0.615 ± 0^{a}	538 ± 00^{h}	$1.058 \pm 0.01^{m*}$
M_6-MD_{Dc}	28.815 ± 0.2^{abcd}	153.844 ± 2.37^{cdef}	0.478 ± 0.01^{ab}
M_6-MD_{aq}	17.158 ± 0.12^{abc}	217.488 ± 1.6^{f}	8.808 ± 0.08^{ab}
M_7-AH_{Dc}	0.323 ± 0.04^{a}	211.77 ± 4.12^{ef}	0.384 ± 0^{a}
M_7-AH_{aq}	1.576 ± 0.36^{a}	687.071 ± 22.83^{i}	4.225 ± 0.16^{ab}
M_7-MD_{Dc}	2.185 ± 0.01^{a}	138.301 ± 0^{bcdef}	0.807 ± 0.08^{ab}
M_7-MD_{aq}	1.381 ± 0.17^{a}	836.5 ± 18.22^{j}	0.811 ± 0^{ab}
M_8-AH_{Dc}	187.702 ± 0^{ef}	20.682 ± 0.15^{abc}	88.81 ± 0.78^{e}
M_8-AH_{aq}	381.206 ± 2.65^{h}	$2.238 \pm 0.04^{m*}$	8.077 ± 0.77^{ab}
M_8-MD_{Dc}	306.21 ± 5.63^{gh}	188.155 ± 1.46^{def}	3.436 ± 0.63^{ab}
M_8-MD_{aq}	840.048 ± 5.84^{j}	$8.180 \pm 0.06^{q*}$	11.115 ± 0.88^{ab}
M_9-AH_{Dc}	1.443 ± 0^{a}	0.64 ± 0.04^{a}	5.488 ± 0.04^{ab}
M_9-AH_{aq}	4.402 ± 0.24^{ab}	2.476 ± 0^{a}	14.744 ± 0.12^{ab}
M_9-MD_{Dc}	3.241 ± 0.2^{a}	0.144 ± 0.02^{a}	2.88 ± 0.46^{ab}
M_9-MD_{aq}	13.817 ± 0^{abc}	4.727 ± 0.03^{ab}	3.388 ± 0.25^{ab}
M_{10}-AH_{Dc}	1.268 ± 0.3^{a}	$1.584 \pm 0.03^{l*}$	0.52 ± 0^{ab}
M_{10}-AH_{aq}	1.746 ± 0^{a}	$2.422 \pm 0.01^{n*}$	8420.32 ± 0^{o}
M_{10}-MD_{Dc}	8.282 ± 0.17^{abc}	574.842 ± 4.26^{hi}	0.662 ± 0.1^{ab}

GEASA: Gentamicin Equivalent Anti-*Staphylococcus Aureus*, GEAPA: Gentamicin Equivalent Anti-*Pseudomonas Aeruginosa*, GAEEC: Gentamicin Equivalent Anti-*Escherichia Coli.* * The antibacterial activity is expressed in mg CN/g Honey or Honey-plant mixture.

Samples	Antibacterial activity (µg CN/g honey or honey-plant mixture)		
	Staphylococcus aureus (ATCC 25823)	*Pseudomonas aeruginosa* (ATCC 27853)	*Escherichia coli* (ATCC258221)
	GEASA	*GEAPA*	*GAEEC*
M_{10}-MD_{aq}	25.674 ± 0.88^{abcd}	$4.717 \pm 0.08^{p*}$	0.683 ± 0^{ab}

GEASA: Gentamicin Equivalent Anti-*Staphylococcus Aureus*, GEAPA: Gentamicin Equivalent Anti-*Pseudomonas Aeruginosa*, GAEEC: Gentamicin Equivalent Anti-*Escherichia Coli.* * The antibacterial activity is expressed in mg CN/g Honey or Honey-plant mixture.

The results of the antibacterial capacity vary significantly ($p < 0.0001$) based on the botanical origin of the honey, whether the binary combination of different honeys or honey-plant mixtures, also showed significant differences in their effectiveness ($p < 0.0001$).

The ten individual samples of honey showed a significant activity ($p < 0.0001$) against Staphylococcus aureus, with GEASA values ranging between 0.569 and 176.469 µg CN/g honey, where the M_8 sample exhibited the highest antibacterial activity compared to the other samples. On the other hand, the highest antibacterial activity against *Pseudomonas Aeruginosa* and *Escherichia coli* was found in the M_5 sample with a GEAPA value equal to 4.098 and 5.384 µg CN/g honey, respectively. However, the lowest antibacterial activity was detected in the M_9 sample. This finding revealed that honey's antibacterial properties against *Staphylococcus aureus* were higher than *Pseudomonas aeruginosa* and *Escherichia coli*, which is consistent with those observed by Wadi [36] and Usanga et al. [37]. However, the antibacterial activity of all the tested binary honey combinations had a much stronger antibacterial capacity against the three bacterial strains than single honeys, which may be related to the synergistic effect between bioactive compounds in the different honeys [38]. The GEASA values ranged from 9.492 to 38312.64 µg CN/g honey-honey, while GEAPA values varied from 1.981 to 2154.012 µg CN/g honey-honey, but GEAEC values ranged between 6.164 and 15659.174 µg CN/g honey-honey, where the M_3-M_8 combination exhibited the highest GEASA (38.312 mg CN/g) and GEAEC (15.659 mg CN/g honey-honey) values, and the M_2-M_8 combination had the highest GEAPA (2.154 mg CN/g honey-honey) value.

The binary combinations of M_8, M_5, and M_2 samples with the other honeys showed significant enhancement in their antibacterial activities, with an increase of 10–40%, 50–70% and 80% by M_2, M_5 and M_8, respectively. These three types of honey (M_2, M_5, M_8) were part of the same plant (*Euphorbia guyoniana* Boiss*)*, which proves that the antibacterial power of these honeys is due to the strong antibacterial properties of the plant constituents [39].

The results of the honey-plant mixtures demonstrated a positive effect against all the tested bacterial strains, with GEASA values ranging from 0.323 to 10338.998 µg CN/g honey-plant, where M_5-MD_{Dc} mixture exhibited the highest value, while M_7-AH_{aq} had the lowest activity. Additionally, the M_8-MD_{aq}

mixture showed the greatest capacity against Pseudomonas aeruginosa, with a GEAPA value of 8.190 mg CN/g honey-plant. Furthermore, the GEAEC values ranged from 0.384 to 9420.32 µg CN/g honey-plant for M_7-AH_{aq} and M_{10}-AH_{aq}, respectively. These findings are in agreement with the results published by Spoială et al.[40] where they found a synergistic effect in honeys combined with other natural products.

3.4. UHPLC-MS/MS analysis of honeys

The results of UHPLC-MS/MS revealed unique phenolic profiles for the ten honey samples, showing differences in the number, types, and concentrations of compounds based on their source. The phenolic composition and quantification of the honey extracts are detailed in Table 4.

Table 4
Results of UHPLC-MS/MS analysis of honeys

µg Analyte / g Honey										
Analytes	**M_1**	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**	**M_7**	**M_8**	**M_9**	**M_{10}**
Quinic acid	43	77	307	68	157	44	104	145	ND	233
Fumaric aid	ND	ND	311	ND	ND	ND	ND	ND	ND	ND
Gallic acid	ND	6	86	5	ND	21	30	ND	ND	7
Protocatechuic acid	ND	ND	90	ND	ND	ND	ND	ND	ND	ND
Chlorogenic acid	ND	ND	20	ND	ND	ND	5	ND	ND	ND
Protocatechuic aldehyde	ND	ND	13	ND	ND	ND	4	ND	ND	ND
Tannic acid	ND	ND	28	ND	ND	11	15	ND	ND	ND
Cynarine	ND	ND	17	ND	ND	ND	ND	ND	ND	ND
Caffeic acid	ND	ND	19	ND	ND	ND	ND	ND	ND	ND
Daidzine	ND	ND	4	ND	ND	ND	ND	ND	ND	ND
Cyranoside	ND	ND	11	ND	ND	ND	8	ND	ND	ND
isoquercitrin	ND	ND	ND	ND	ND	ND	17	ND	ND	ND
Genistin	ND	ND	25	ND	ND	ND	12	ND	ND	ND
Cosmosiin	ND	ND	26	ND	ND	ND	ND	ND	ND	ND
Quercitrin	ND	ND	34	ND	ND	ND	ND	ND	ND	ND
Astragalin	ND	ND	24	ND	ND	ND	12	ND	ND	ND
Daidzein	ND	ND	15	ND	ND	ND	ND	ND	ND	ND
Naringenin	ND	ND	14	ND	ND	ND	4	ND	ND	2
Hesperetin	ND	ND	5	ND	ND	ND	ND	ND	ND	ND
Luteolin	ND	ND	4	ND	ND	ND	ND	ND	ND	ND
Genistein	ND	ND	3	ND	ND	ND	ND	ND	ND	ND
Acacetin	ND	3	4	ND	ND	ND	ND	ND	ND	4

Twenty-two compounds were noticed in the ten honey samples. the concentration and diversity of these bioactive substances differ based on the honey's botanical and geographical sources [41] The sample M_{10} contain four composites were: quinic acid, gallic acid, naringenin and acacetin. M_1, M_5 and M_8 samples comprise only quinic acid. While nothing was detected in M_9 honey. In contrast, three

compounds were detected in M_2 and M_6 samples: quinic acid and gallic acid present in both honeys and Acacetin in M_2 and Tannic acid in M_6. Meanwhile, Quinic acid, one of the discovered phenolic acids, is well-known for its powerful inhibitory effects against both Gram-positive and Gram-negative bacteria as well as its significant antioxidant qualities.[42] was found with the highest concentration in all honey samples except M_9, which indicates the Hight antioxidant and antibacterial results in honeys and their mixtures. Similarly large amounts of gallic acid, witch known for having antioxidant properties, have been shown in M_2, M_3, M_4, M_6, M_7 and M_{10}. However, Quinic acid, Gallic acid, Chlorogenic acid, Protocatechuic aldehyde, Tannic acid, Cyranoside, Isoquercitrin, Genistin, Astragalin and Naringenin were quantified in the sample $M_{7,}$ and the isoquercitrin was found only in this honey. The isoquercitrin exhibits strong antioxidant effects especially in electron-transfer and metal-chelation pathways, and it contributes to the antibacterial impacts [43]. This composite may be the reason for the synergistic antioxidant and antibacterial effect in the mixtures of this honey.

However, in comparison to other samples, quinic acid, gallic acid, protocatechuic acid and fumaric acid as known for their highest antioxidant power[44–46] were found in M3 with the greatest concentration. On the other hand, fumaric acid, cosmosiin, quercitrin, cynarin, caffeic acid, hesperetin, luteolin, genistein, daidzin, and daidzein were found only in M_3 honey, which has strong antioxidant and antibacterial properties. These molecules, primarily Flavonoids and phenolic acids, are acknowledged for their antioxidant properties, as they scavenge reactive oxygen species and chelate metal ions to protect cells from oxidative stress. They also exhibit antibacterial activity by disrupting microbial membranes and interfering with bacterial communication[47] These results explain the highest TPC and antioxidant capacity of this honey and its mixtures and how it enhances the TPC and the antioxidant properties of the other honeys. The synergy and additive effects revealed by the Honey-Honey or Honey-extract interactions in the present work could be attributed to the different compounds in the individual Honeys.

Several studies were demonstrated the synergistic potential of the combinations of bioactive substances, such as the phenolic acids and flavonoids found in our results, as an example, in research published by Skroza et al.[48] examined the synergetic antioxidant properties of the combination of 10 phenolic acids (protocatechuic, gentisic, gallic, vanillic, syringic, *p*-coumaric, caffeic, ferulic, sinapic, and rosmarinic acid). Another study demonstrated that the antioxidants potential synergists of the mixtures of six phenolic acids were: 4-hydroxybenzoic acid, protocatechuic acid, gentisic acid, chlorogenic acid, caffeic acid, and syringic acid[49]. Moreover, Crina Vicol and Gheorghe Duca studied the antioxidant interactions between the combination of (catechin, quercetin, rutin, resveratrol, gallic acid and ascorbic acid) [50]. Additionally, Lalfakawmi et al. [51] determined the synergistic antioxidative potential of cosmosiin and vanillic acid and found that the mixture exhibited higher scavenging activities. therefore, Synergistic antimicrobial activities were found between gallic acid, acid, caffeic acid, vanillin, p-coumaric acid, ferulic acid, verbascoside, rutin, luteolin-7-glucoside, o-coumaric acid, apigenin-7-glucoside, oleuropein, pinoresinol, cinnamic acid, luteolin, apigenin, and the asterisk in aqueous olive pomace extract[52]. Also, Erick F. de Oliveira et al. [53] showed a synergistic antimicrobial activity of two phenolic acids: gallic acid and ferulic acid in apple juice. As well, Azaizeh et al. [54] studied the Synergistic

Antibacterial Effects of 9 Polyphenolic Compounds: hydroxytyrosol, 3, 4-dihydroxyphenylacetic acid, tyrosol, protocatechuic acid, verbascoside, vanillic acid, caffeic acid, ferulic acid and p-coumaric acid from Olive Mill Wastewater.

Our HPLC results are consistent with the findings reported by other researchers. Othmai et al. [32] reported that p-coumaric acid, caffeic acid, hesperetin, luteolin and daidzein were quantified in some Algerian honeys. on the other hand, the LC-MS/MS analyses of Different Honey Samples Collected in the pre-Saharan region in Algeria revealed the acid, p-coumaric acid and gallic acid [55]. Therefore, the chromatographic profiles of 35 samples of honey from many regions of Algeria showed the presence of apigenin, luteolin, caffeic, *p*-coumaric acids, syringic acids, gallic acids and daidzein [56], Viteri et al. [57] identified gallic, syringic, chlorogenic, caffeic acid, luteolin, and apigenin in monofloral Chilean honey from *Eucryphia cordifolia* (ulmo). However, gallic and caffeic acid were detected using the LC-MS method in Romanian oak and fir honeydew honeys [58]. In addition, p-coumaric acid, caffeic acid, salicylic acid, hesperetin, luteolin and acacetin were identified in Polish varietal honeys [59].

3.5. Correlation between TPC, antioxidant capacities and antimicrobial activities

Pearson's correlation coefficient was determined to assess the relationships between phenolic content and both antioxidant and antibacterial activities of honey samples and their mixtures, utilizing a significance level of $p < 0.05$.

Firstly, the Pearson’s correlation coefficient results presented in Table 5 for the single honeys and their combinations showed that the total phenolic contents (TPC) were positively and significantly correlated with antioxidant activities. The correlation coefficients were as follows: ($r = 0.7344$; $p < 0.0001$) for DPPH, ($r = 0.5601$; $p < 0.0001$) for ABTS, and ($r = 0.7815$; $p < 0.0001$) for FRAP. This means that 73.44% of anti-DPPH, 56.01% of anti-ABTS, and 78.15% of anti-FRAP activities in single honeys and their binary combinations are related to the content of phenolic compounds. These results align with other studies indicating that the phenolic content in honeys is responsible for scavenging free radicals [60]. Thereby, Zou et al. (2022), found significant Pearson’s correlation between TPC and DPPH ($r = 0.828$; $p \leq 0.01$) and between TPC and FRAP ($r = 0.702$; $p \leq 0.05$), in linden honeys. However, the antibacterial capacities and the total phenolic compounds were significantly positively correlated: GEASA-TPC ($r = 0.3501$; $p = 0.0088$), GEAPA-TPC ($r = 0.3112$; $p = 0.0207$) and GEAEC-TPC ($r = 0.3226$; $p = 0.0163$). This means more than 31% of the antibacterial activity is related to the phenolic contents. These findings are in agreement with those reported in previous studies [62]. Moreover, the results indicated strong positive significant correlations between the DPPH and FRAP assays ($r = 0.8932$; $p < 0.0001$) and between the DPPH and ABTS assays ($r = 0.7257$; $p < 0.0001$). This suggests that 89.32% and 72.57% of the proton-donating agents identified in the DPPH test are also reducing species in the FRAP and electron donors’ entities in the ABTS assays, respectively. Furthermore, a good significant correlation ($r = 0.6446$; $p < 0,0001$) was found between the antioxidant activities measured by the ABTS and FRAP assays. These results are in

accordance with previous reports indicating significant correlations between the DPPH, ABTS, and FRAP assesses in honeys [31, 32, 61].

Table 5
Pearson correlation coefficient (r) matrix between 7 different assays of single honeys and their binary combinations

Variables	Phenolics	DPPH	ABTS	FRAP	GEASA	GEAPA	GAEEC
Phenolics	**1**						
DPPH	**0.7344**	**1**					
ABTS	**0.5601**	**0.7257**	**1**				
FRAP	**0.7815**	**0.8832**	**0.6446**	**1**			
GEASA	**0.3501**	0.2375	0.0828	0.2323	**1**		
GEAPA	**0.3112**	0.2087	0.1112	0.2150	**0.8616**	**1**	
GAEEC	**0.3226**	**0.2811**	0.0806	**0.3037**	**0.8133**	**0.8183**	**1**
Bold values are different from 0 at a significance level alpha = 0.05							

On the other hand, weak positive significant correlations were observed between DPPH and GEAEC (r = 0.2911; p = 0.0311), and between FRAP and GEAEC (r = 0.3037; p = 0.0242). This suggests that about 30% of the antioxidant agents are responsible for the anti-*Escherichia coli* effect. Furthermore, the antibacterial capacities showed strong, significantly positive correlations between GEASA-GEAPA (r = 0.8616; p < 0.0001), GEASA-GEAEC (r = 0.81331; p < 0.0001) and GEAPA-GEAEC (r = 0.8193; p < 0.0001). These results suggest that honey and its binary combinations had a significant ability (more than 81%) to inhibit the growth of both Gram-positive and Gram-negative bacteria. This effectiveness may be attributed to mechanisms such as hydrogen peroxide production, high sugar content, and the presence of phenolic substances in honey [63].

Secondly, the results in Table 6 present the Pearson's correlation coefficients between TPC, antioxidant activities and antimicrobial activities of honey and honey plant mixtures. The TPC showed moderately significant positive correlations with antioxidant activities: TPC-DPPH (r = 0.3540; p = 0.0074), TPC-ABTS (r = 0.4055; p = 0.0019) and TPC-FRAP (r = 0.2995; p = 0.0250). However, there were very strong correlations between antioxidant activities: DPPH-ABTS (r = 0.9208; p < 0.0001) and DPPH-FRAP (r = 0.8789; p < 0.0001) and ABTS-FRAP (r = 0.9075; p < 0.0001). In contrast, the antibacterial capacities (GEASA and GEAPA) exhibited weakly significant positive and negative correlations with TPC (r = 0.2639; p = 0.0494), (r = -0.3321; p = 0.0128), respectively.

Table 6
Pearson correlation coefficient (r) matrix between 7 different assays of single honeys and their binary combinations with plant mixtures

Variables	Phenolics	DPPH	ABTS	FRAP	GEASA	GEAPA	GAEEC
Phenolics	**1**						
DPPH	**0.3540**	**1**					
ABTS	**0.4055**	**0.9208**	**1**				
FRAP	**0.2995**	**0.8789**	**0.9075**	**1**			
GEASA	**0.2639**	0.1507	0.1619	0.2607	**1**		
GEAPA	**-0.3321**	-0.0783	-0.0889	-0.0666	-0.0422	**1**	
GAEEC	0.0200	0.0953	0.1286	0.0414	-0.0023	0.1308	**1**
Bold values are different from 0 at a significance level alpha = 0.05							

To elucidate the relationships between the variables (TPC, antioxidant activities and antibacterial capacities) and the samples (honeys and their mixtures), a principal component analysis (PCA) has been established (Fig. 4, Fig. 5).

The PCA biplot of honey and its binary combinations Fig. 4 shows that all variables (TPC, DPPH, FRAP, ABTS, GEASA, GEAPA, and GEAEC) are mainly grouped on the right side of axis F1. Therefore, F1 was strongly correlated with TPC (81.76%), DPPH (82.79%), FRAP (82.66%), and ABTS (63.99%), which are significantly associated with the M_3 sample and almost all their combinations (blue circle). This observation confirms the previous results, which indicate that high levels of phenolics and antioxidant capacity characterize this honey sample and its combinations. On the other hand, F1 is positively correlated with GEASA (66.41%), GEAPA (65.12%) and GEAEC (67.86%), where the orange circle highlights that M_2-M_8 and M_3-M_8 combinations exhibit high antibacterial activities.

The PCA biplot of honeys and their honey-plant mixtures Fig. 5 demonstrates that the variables TPC, DPPH, FRAP and ABTS are assembled on the right side of F1, showing a high correlation (62.77% – 93.28%). The PCA also shows that M_2-AH_{aq}, M_5-MD_{DC}, and M_4-AH_{aq} are grouped in the yellow circle, highlighting similar responses in terms of TPC and GEASA. In the black circle, the four mixtures of M_3 with the extracts (AH_{DC}, AH_{aq}, MD_{DC}, MD_{aq}) were characterized by high levels of antioxidant capacity. Conversely, the mixtures of M_{10} honey with the four fractions and M_8-MD_{aq}, demonstrate a strong antibacterial capacity against *Pseudomonas aeruginosa* and *Escherichia coli*, as indicated in the green circle.

As shown in Table 7, the correlation between a particular number of compounds, including quinic acid, gallic acid, tannic acid, naringenin, acacetin and the antioxidant and antibacterial activities of single

honeys and their combinations, as well as the correlation between M_8 and M_3, which enhance the antibacterial and antioxidant capacities of other honeys.

Table 7
Pearson correlation coefficient (r) values between 7 different assays of single honeys and their combinations with principal phenolic constituents

	Variables	DPPH	ABTS	FRAP	GEASA	GEAPA	GAEEC
Single honeys	Quinic acid	**0,6761**	**0,7464**	**0,6660**	0,2599	**0,7631**	0,0797
	Gallic acid	**0,9121**	**0,8408**	**0,8301**	-0,3048	0,4106	-0,2331
	Tannic acid	**0,8086**	**0,7364**	**0,7153**	-0,3627	0,2996	-0,2790
	Naringenin	**0,9316**	**0,8598**	**0,8604**	-0,2314	0,4930	-0,2129
	Acacetin	0,5240	0,4672	0,4375	0,0435	0,5008	0,0481
Honey combinations	Quinic acid	**0,6619**	**0,3846**	**0,7121**	0,2659	**0,2958**	**0,2979**
	Gallic acid	**0,8927**	**0,5298**	**0,8441**	0,0785	0,1089	0,2227
	Tannic acid	**0,7910**	**0,4417**	**0,7322**	0,0051	0,0226	0,1684
	Naringenin	**0,9178**	**0,5874**	**0,8833**	0,1194	0,1520	0,2502
	Acacetin	**0,5029**	**0,3479**	**0,5146**	0,1940	**0,3335**	0,2073
Honey combinations with M3	Quinic acid	**0,6360**	0,3423	**0,7348**	0,3188	0,3721	0,2444
	Gallic acid	**0,8355**	**0,4807**	**0,8732**	0,2245	0,2635	0,1713
	Tannic acid	**0,7720**	0,4239	**0,7887**	0,1881	0,2115	0,1461
	Naringenin	**0,8468**	**0,5212**	**0,8927**	0,2542	0,3004	0,1917
	Acacetin	**0,5054**	0,3064	**0,5220**	0,1591	0,2939	0,1133
Honey combinations with M8	Quinic acid	**0,7240**	**0,7171**	**0,7471**	0,5337	0,3260	**0,6292**
	Gallic acid	**0,8979**	0,6050	**0,8479**	0,5233	0,4056	**0,8047**
	Tannic acid	**0,7774**	0,4521	**0,7202**	0,3798	0,2690	**0,6678**
	Naringenin	**0,9251**	**0,6929**	**0,9012**	0,5438	0,4013	**0,8178**
	Acacetin	0,5856	0,6010	0,5437	**0,6566**	**0,6615**	**0,7087**

However, quinic acid (QA), gallic acid (GA), tannic acid (TA), naringenin (NG), and acacetin (AC) showed a significant positive correlation with antioxidant activities. A good to strong correlation was observed

between DPPH and these five compounds, both in single honey samples and in combinations. The correlation coefficients and their corresponding p-values are as follows: In single honey: DPPH-QA ($r = 0.6761$, $p < 0.0105$), DPPH-GA ($r = 0.9121$, $p < 0.0212$, DPPH-TA ($r = 0.8086$, $p < 0.1084$), DPPH-NG ($r = 0.9316$, $p < 0.0125$). In honey combinations: DPPH-QA ($r = 0.6619$, $p < 0.0001$), DPPH-GA ($r = 0.8927$, $p < 0.0001$), DPPH-TA ($r = 0.7910$, $p < 0.0001$), DPPH-NG ($r = 0.9178$, $p < 0.0001$), DPPH-AC ($r = 0.5029$, $p < 0.0004$), In honey combinations with M3: DPPH-QA ($r = 0.6360$, $p < 0.0034$), DPPH-GA ($r = 0.8355$, $p < 0.0001$), DPPH-TA ($r = 0.7720$, $p < 0.0001$), DPPH-NG ($r = 0.8468$, $p < 0.0001$), DPPH-AC ($r = 0.5054$, $p < 0.0273$). In honey combinations with M8: DPPH-QA ($r = 0.7240$, $p < 0.0274$), DPPH-GA ($r = 0.8979$, $p < 0.001$), DPPH-TA ($r = 0.7774$, $p < 0.0137$), DPPH-NG ($r = 0.9251$, $p < 0.0004$). On the other hand, a strong correlation was recorded between ABTS and the five composites in single honey and Honey combinations with M_8, a moderate correlation was with honey combinations and honey combinations with M_3. The correlation coefficients were as follows: for ABTS-QA, $r = 0.7464$ ($p < 0.0319$) for single honey, $r = 0.3846$ ($p < 0.0091$) for honey combinations, and $r = 0.7171$ ($p < 0.0297$) for honey combinations with M8. For ABTS-GA, the coefficients were $r = 0.8408$ ($p < 0.0023$) for single honey, $r = 0.5298$ ($p < 0.0002$) for honey combinations, and $r = 0.4807$ ($p < 0.0372$) for honey combinations with M_3. Additionally, ABTS-TA showed a strong correlation in single honey with $r = 0.7364$ ($p < 0.0046$) and a modest correlation in honey combinations with $r = 0.4417$ ($p < 0.0024$). A strong correlation was also present between ABTS-NG in single honey ($r = 0.8598$, $p < 0.0001$) and a good correlation in honey combinations, as well as those with M_3 and M_8, with coefficients of $r = 0.5874$ ($p < 0.0001$), $r = 0.5212$ ($p < 0.0221$), and $r = 0.6929$ ($p < 0.0.85$), respectively. In contrast, a weak correlation for ABTS-AC was found in honey combinations, with $r = 0.3479$ ($p < 0.0192$).

Conversely, A strong and positive correlation was found between FRAP and the phenolic analytes in all types of studies. In the analysis of single honey, the correlation coefficients were as follows: for Quinic acid ($r = 0.6660$, $p < 0.0132$), Gallic acid ($r = 0.8301$, $p < 0.0023$), Tannic acid ($r = 0.7153$, $p < 0.0151$), and Naringenin ($r = 0.8604$, $p < 0.0014$). In honey combinations, the corresponding FRAP values were recorded as follows: FRAP-QA ($r = 0.7121$, $p < 0.0001$), FRAP-GA ($r = 0.8441$, $p < 0.0001$), FRAP-TA ($r = 0.7322$, $p < 0.0001$), and FRAP-NG ($r = 0.8833$, $p < 0.0001$). Additionally, when analysing honey combinations with M3, the correlations were with QA ($r = 0.7348$, $p < 0.0003$), GA ($r = 0.8732$, $p < 0.0001$), TA ($r = 0.7887$, $p < 0.0001$), NG ($r = 0.8927$, $p < 0.0001$), and AC ($r = 0.5220$, $p < 0.0219$). Furthermore, in honey combinations with M8, the correlations with FRAP were: Quinic acid ($r = 0.7471$, $p < 0.0207$), Gallic acid ($r = 0.8479$, $p < 0.0039$), Tannic acid ($r = 0.7202$, $p < 0.0287$), and Naringenin ($r = 0.9012$, $p < 0.0009$).

However, the antibacterial activity analysis showed that GEASA exhibited a strong correlation with Acacetin ($r = 0.6566$, $p < 0.0476$) in honey combinations with M8. Additionally, GEAPA demonstrated good correlations with QA ($r = 0.7631$, $p < 0.4683$) and AC ($r = 0.6615$, $p < 0.0476$) in both single honey and honey combinations with M8. However, there was a weak correlation between GEAPA and QA ($r = 0.2958$, $p < 0.0485$) as well as between GEAPA and AC ($r = 0.3335$, $p < 0.0252$) in honey combinations. In honey combinations with M8, GEAEC showed strong correlations with Quinic acid, Gallic acid, Tannic acid, Naringenin, and Acacetin, with correlation coefficients of $r = 0.6292$, $p < 0.046$; $r = 0.8047$, $p <$

0.0098; r = 0.6678, p < 0.0493; r = 0.8178, p < 0.0017; and r = 0.7087, p < 0.0326, respectively. These strong positive correlations indicate that these combinations exhibit significant antibacterial activity against *E. coli*. Conversely, GEAEC and Quinic acid were weakly correlated in honey combinations, with a correlation coefficient of r = 0.2979, p < 0.0001.

On the other hand, we can observe the contributions of honey combinations with M_3 and M_8 to antioxidant and antibacterial activities, respectively. Notably, there is a strong correlation between Acacetin found in the M_3 sample, which shows a greater correlation value in the combination of M_3 compared to single honey samples and other combinations. This is particularly significant regarding antioxidant efficacy, especially when measured by FRAP. The antioxidant power of honey mixtures with M_3 is attributed to their richness in phenolic compounds, which are known to exhibit strong anti-oxidising properties, unlike other samples. Conversely, honey mixtures with M_8 demonstrate notable antibacterial power, as seen in Table 7. There is a strong significant correlation between Acacetin and these mixtures. This strength is evident with the three bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Furthermore, there is a strong correlation regarding the anti-*E. coli* effectiveness and Quinic acid, Gallic acid, Tannic acid, Naringenin and Acacetin in these honey combinations. This efficacy may result from the high concentration of Quinic acid and its interaction with other compounds present in the other honey samples, which together contribute to a potent antibacterial activity.

Through these results, which indicate strong antioxidant power of these honeys and their combinations, our findings were more favorable when compared to other studies on honeys. For example, a significant positive correlation was found between gallic acid and the DPPH assay (r > 0.5, p < 0.05) in samples of Chinese honey[4], although no statistically significant correlation was observed with the FRAP and ABTS assays. In contrast, Hernanz et al. (2023) reported significant relationships (r = 0.288, p < 0.05) between gallic acid and the ABTS assay in Spanish oak (Quercus) honeydew honey. In Croatian honey, FRAP and gallic acid were strongly correlated (r = 0.71, p < 0.05) [65]. Additionally, gallic acid showed strong correlations (p < 0.01) with ABTS, FRAP, and DPPH, with Pearson correlation values of 0.740, 0.803, and 0.711, respectively [66]. However, a significant negative correlation was observed between FRAP and gallic acid with r = -0.925, p < 0.01 [67]. Regarding the correlation between phenolic compounds and antibacterial activity, no correlation was found between gallic acid and the antibacterial activity against *P. aeruginosa*, *S. aureus*, and *E. coli* in four Palestinian honey samples [68]. Meanwhile, a study found that acacetin is significantly negatively correlated with DPPH (r = -0.495, p < 0.001) in honeydew honeys [69]. Moreover, another study reported that quinic acid and naringenin, identified through the LC-MS method, showed strong correlations with ABTS, DPPH, and FRAP with Pearson correlation values of 0.809, 0.823, and 0.745, all with p < 0.01[70]. Likewise, Liao et al. (2022) found no correlation between quinic acid, gallic acid, and DPPH in coffee beans Additionally, no correlation was observed between the antibacterial activity against P. aeruginosa, S. aureus, and E. coli and naringenin in various propolis samples collected from African and Asian regions [72]. Lastly, a moderate positive correlation between *S. aureus* and tannic acid (r = 0.566, p < 0.05) in Rhizophoraceae extracts [73]. One notable study found a strong negative Pearson correlation coefficient (r = -0.856, p < 0.05) between quinic acid content and the

IC_{50} values of pine bark polar extracts in a reducing power assay, but no correlation was found with the ABTS and DPPH [74].

4. Conclusions

The *in vitro* evaluation results indicate that all honey samples and their combinations exhibit significant antibacterial and antioxidant activities, in particular M_3 and M_8 honey samples which demonstrated the highest antioxidant and antibacterial activities, respectively. Furthermore, the combination of these two honeys with other honeys and plant extracts revealed a strong synergistic effect in both activities. These findings establish a strong basis for further *in vivo* research on antiviral and antiparasitic effects of the analyzed combinations in this study.

Declarations

Competing interests

All authors declare no conflict of interest.

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Author Contribution

Author Contribution Nacira SIAD, Chahrazed HAMIA, Amar DJERIDANE and Mohamed BENALIA: Experimental acquisition, Writing original draft, Investigation, Formal analysis, Methodology and Data curation. Reguia MAHFOUDHI, Mustafa Abdullah YILMAZ, Oguz CAKIR, Abbas TARHAN: LC-MS-MS analysis. Mohamed YOUSFI: Resources and Funding acquisition.

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References

1. S. Ravichandran, E.A. Ahandani, B. Ozdemir, S. Hajipour, Z. Selamoglu, A. Riaz, H.Y. Issa, K.M. Bhargavi, A. Rai, R.M. Madhumitha Sri, Use of natural products in preventive medicine and healthy life. J. Prev. Med. Holist. Health. **9**, 5–9 (2023). https://doi.org/10.18231/j.jpmhh.2023.003

2. M.W. Ahmed, M. Asif, R. Ahmed, A.S. Khan, R. Raza, Pharmacology, nutrition value and therapeutic potential of honey: A review. J. Pharmacogn Phytochem. **13**, 40–47 (2024). https://doi.org/10.22271/phyto.2024.v13.i2a.14868
3. O.R. Johnson-Ajinwo, C.A. Obi, *A review of the pharmacological activities of honey* (JDDR, 2024)
4. N. Qi, W. Zhao, C. Xue, L. Zhang, H. Hu, Y. Jin, X. Xue, R. Chen, J. Zhang, Phenolic Acid and Flavonoid Content Analysis with Antioxidant Activity Assessment in Chinese C. pi. Shen Honey. Molecules. **30** (2025). https://doi.org/10.3390/molecules30020370
5. D. Kumar, K. Hazra, P.V.V. Prasad, R. Bulleddu, Honey: an important nutrient and adjuvant for maintenance of health and management of diseases. J. Ethnic Foods. (2024). https://doi.org/10.1186/s42779-024-00229-3. 11:
6. F. Coppola, M. Abdalrazeq, F. Fratianni, M.N. Ombra, B. Testa, G. Zengin, J.F. Ayala Zavala, F. Nazzaro, (2025) Rosaceae Honey: Antimicrobial Activity and Prebiotic Properties. Antibiotics 14:. https://doi.org/10.3390/antibiotics14030298
7. D. Grabek-Lejko, T. Hyrchel, (2023) The Antibacterial Properties of Polish Honey against Streptococcus mutans—A Causative Agent of Dental Caries. Antibiotics 12:. https://doi.org/10.3390/antibiotics12111640
8. P. Niazi, A.W. Monib, The role of plants in traditional and modern medicine. J. Pharmacogn Phytochem. **13**, 643–647 (2024). https://doi.org/10.22271/phyto.2024.v13.i2d.14905
9. A.S. Ould Kaddour, R. Kouadria, M. Bouzouina, B. Lotmani, Phytochemical identification and antioxidant activities of wild Algerian plant Atriplex halimus L. Int. Food Res. J. **31**, 1494–1506 (2024). https://doi.org/10.47836/ifrj.31.6.10
10. M. Roubi, A. Elbouzidi, M. Dalli, S. Azizi, M. eddine, Aherkou, M. Taibi, B. Guerrouj, M. El, Addi, N. Gseyra, Phytochemical, antioxidant, and anticancer assessments of Atriplex halimus extracts: In silico and in vitro studies. Sci. Afr. **22** (2023). https://doi.org/10.1016/j.sciaf.2023.e01959
11. S. Saad, S. Ouafi, S. Karoune, H. Meguellati, N. Djemouai, L. Harchaoui, *Algerian Journal of Arid Regions Journal Algérien des Régions Arides JARA Marrubium deserti De Noé* (a Spontaneous Medicinal Plantfrom Algerian Sahara, An Ethnobotanical Survey, 2022)
12. L. Ait Abderrahim, K. Taïbi, N. Ait Abderrahim, M. Boussaid, C. Rios-Navarro, A. Ruiz-Saurí, Euphorbia honey and garlic: Biological activity and burn wound recovery. Burns. **45**, 1695–1706 (2019). https://doi.org/10.1016/j.burns.2019.05.002
13. Himanshu, I. Khangwal, (2024) Honey as a Potential Preservative in the Food Industry. *Kumar Rajesh and Hajam YA and BDS and GA (ed) Honey in Food Science and Physiology*. Springer Nature Singapore, Singapore, 181–195
14. A.S. Gadge, D.V. Shirsat, P.S. Soumia, C.L. Pote, M. Pushpalatha, T.R. Pandit, R. Dutta, S. Kumar, S.V. Ramesh, V. Mahajan, V. Karuppaiah, (2023) Physiochemical, biological, and therapeutic uses of stingless bee honey. Front. Sustain. Food Syst. **7**
15. A. Karnwal, A.Y. Jassim, A.A. Mohammed, A.R.M.S. Al-Tawaha, M. Selvaraj, T. Malik, (2025) Addressing the global challenge of bacterial drug resistance: insights, strategies, and future

directions. Front. Microbiol. **16**

16. S. Kumar, M. Verma, Y.A. Hajam, R. Kumar, (2024) Honey infused with herbs: A boon to cure pathological diseases. Heliyon 10
17. V.L. Singleton, J.A. Rossi, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am. J. Enol. Vitic. **16**, 144 (1965). https://doi.org/10.5344/ajev.1965.16.3.144
18. V. Ani, M.C. Varadaraj, K.A. Naidu, Antioxidant and antibacterial activities of polyphenolic compounds from bitter cumin (Cuminum nigrum L). Eur. Food Res. Technol. **224**, 109–115 (2006). https://doi.org/10.1007/s00217-006-0295-z
19. I.F.F. Benzie, J.J. Strain, The Ferric Reducing Ability of Plasma (FRAP) as a Measure of Antioxidant Power: The FRAP Assay. Anal. Biochem. **239**, 70–76 (1996). https://doi.org/https://doi.org/10.1006/abio.1996.0292
20. C.A. Rice-Evans, N.J. Miller, G. Paganga, Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol. Med. **20**, 933–956 (1996). https://doi.org/10.1016/0891-5849(95)02227-9
21. A. Fleming, A. Fleming, On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to Their Use in the Isolation of B. Influenzae. Br. J. Exp. Pathol. **10**, 226 (1929)
22. M.A. Yilmaz, Simultaneous quantitative screening of 53 phytochemicals in 33 species of medicinal and aromatic plants: A detailed, robust and comprehensive LC–MS/MS method validation. Ind. Crops Prod. **149**, 112347 (2020). https://doi.org/10.1016/J.INDCROP.2020.112347
23. R. Ceylan, J. Katanić, G. Zengin, S. Matić, A. Aktumsek, T. Boroja, S. Stanić, V. Mihailović, G.O. Guler, M. Boga, M.A. Yilmaz, Chemical and biological fingerprints of two Fabaceae species (Cytisopsis dorycniifolia and Ebenus hirsuta): Are they novel sources of natural agents for pharmaceutical and food formulations? Ind. Crops Prod. **84**, 254–262 (2016). https://doi.org/10.1016/J.INDCROP.2016.02.019
24. H. Bouakkaz, Djridane, M. Yilmaz, M.A. Oguz, C. TARHAN, A. lassakeur, Z. Yousfı, M (2024) Xanthine oxidase inhibitory activity and uric acid dissolution power of some local plant extracts: in vitro therapeutical approach in the treatment of gout. **34**:1340–1352
25. D. Bereksi-Reguig, H. Allali, N. Taib, N. Aissaoui, M. Wlodarczyk-Stasiak, R. Kowalski, Bioactive Compounds, Antioxidant Properties, and Antimicrobial Profiling of a Range of West Algerian Honeys: In Vitro Comparative Screening Prior to Therapeutic Purpose. Foods. **13** (2024). https://doi.org/10.3390/foods13244120
26. E. Majewska, B. Drużyńska, D. Derewiaka, M. Ciecierska, P. Pakosz, Comparison of Antioxidant Properties and Color of Selected Polish Honeys and Manuka Honey. Foods. **13** (2024). https://doi.org/10.3390/foods13172666
27. L.E. Vîjan, I.C. Mazilu, C. Enache, S. Enache, C.M. Topală, (2023) Botanical Origin Influence on Some Honey Physicochemical Characteristics and Antioxidant Properties. Foods 12:. https://doi.org/10.3390/foods12112134

28. M. Halagarda, S. Groth, S. Popek, S. Rohn, V. Pedan, Antioxidant activity and phenolic profile of selected organic and conventional honeys from Poland. Antioxidants. **9** (2020). https://doi.org/10.3390/antiox9010044
29. E. Kurtdede, S. Sevin, Evaluation of Antioxidant Properties and Total Phenolic and Flavonoid Contents of Honey Bee Hive Products Collected from the Ankara Region. Kocatepe Veterinary J. (2022). https://doi.org/10.30607/kvj.1138870
30. C. Stefanis, E. Stavropoulou, E. Giorgi, C. Voidarou, T.C. Constantinidis, G. Vrioni, A. Tsakris, (2023) Honey's Antioxidant and Antimicrobial Properties: A Bibliometric Study. Antioxidants 12
31. A. Necib, L. Kefti, R. Draiaia, N. Mohamadi, S. Rezig, Evaluation of Polyphenols, Vitamin C Contents and Antioxidant Activity of Two Types of Algerian Honey. Turkish J. Agric. - Food Sci. Technol. **10**, 2539–2544 (2022). https://doi.org/10.24925/turjaf.v10i12.2539-2544.5475
32. A. Otmani, N. Amessis-Ouchemoukh, C. Birinci, S. Yahiaoui, S. Kolayli, M.S. Rodríguez-Flores, O. Escuredo, M.C. Seijo, S. Ouchemoukh, Phenolic compounds and antioxidant and antibacterial activities of Algerian honeys. Food Biosci. **42** (2021). https://doi.org/10.1016/j.fbio.2021.101070
33. L. Dimitriu, D. Preda, A. Nichițean, D. Constantinescu-Aruxandei, F. Oancea, N. Băbeanu, (2020) Synergistic Antioxidant Activity between Honey and Phenolic Compounds. MDPI AG, p 11
34. T. Lella, G. Vishnupriya, S. Brigida, A.A. Elizabeth, G. Soujania Singh, Evaluation of Synergistic Antioxidant Activity of Psidium Guajava and Raw Honey-An Invitro study. Texila Int. J. Public. Health. **12** (2024). https://doi.org/10.21522/TIJPH.2013.12.03.Art020
35. A.M. Padmanabhan, V.A. Doss, Synergistic Antioxidant Effect of Cinnamomum verum and Stingless Bee Honey. Biointerface Res. Appl. Chem. **13** (2023). https://doi.org/10.33263/BRIAC131.003
36. M.A. Wadi, In Vitro Antibacterial Activity of Different Honey Samples against Clinical Isolates. Biomed. Res. Int. 2022. (2022). https://doi.org/10.1155/2022/1560050
37. V.U. Usanga, M.O. Elom, N.O. Umoh, A. Nworie, B.N. Ukwah, M.E. Kalu, S.O. Azi, Antibacterial Activity of Honey on Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes Isolated from Wounds. J. Adv. Microbiol. 51–57 (2020). https://doi.org/10.9734/jamb/2020/v20i430236
38. S. Almasaudi, The antibacterial activities of honey. Saudi J. Biol. Sci. **28**, 2188–2196 (2021)
39. Z. Shakoori, E. Salaseh, A.R. Mehrabian, D.M. Tehrani, N.F. Dardashti, F. Salmanpour, The amount of antioxidants in honey has a strong relationship with the plants selected by honey bees. Sci. Rep. **14** (2024). https://doi.org/10.1038/s41598-023-51099-9
40. A. Spoială, C.I. Ilie, D. Ficai, A. Ficai, E. Andronescu, (2023) Synergic Effect of Honey with Other Natural Agents in Developing Efficient Wound Dressings. Antioxidants 12
41. M.H.A. Suleiman, W.M.A. ALaerjani, M.E.A. Mohammed, Influence of altitudinal variation on the total phenolic and flavonoid content of Acacia and Ziziphus honey. Int. J. Food Prop. **23**, 2077–2086 (2020). https://doi.org/10.1080/10942912.2020.1842445
42. L. ERCAN, M. DOĞRU, Antioxidant and Antimicrobial Capacity of Quinic Acid. Bitlis Eren Üniversitesi Fen Bilimleri Dergisi. **11**, 1018–1025 (2022). https://doi.org/10.17798/bitlisfen.1167047

43. X. Li, Q. Jiang, T. Wang, J. Liu, D. Chen, Comparison of the antioxidant effects of quercitrin and isoquercitrin: Understanding the role of the 6"-OH group. Molecules. **21** (2016). https://doi.org/10.3390/molecules21091246
44. M. Hadidi, R. Liñán-Atero, M. Tarahi, M.C. Christodoulou, F. Aghababaei, (2024) The Potential Health Benefits of Gallic Acid: Therapeutic and Food Applications. Antioxidants 13
45. R.N. Jadeja, F.L. Powell, P.M. Martin, *Repurposing Fumaric Acid Esters to Treat Conditions of Oxidative Stress and Inflammation*: A Promising Emerging Approach with Broad Potential
46. K. Krzysztoforska, D. Mirowska-Guzel, E. Widy-Tyszkiewicz, Pharmacological effects of protocatechuic acid and its therapeutic potential in neurodegenerative diseases: Review on the basis of in vitro and in vivo studies in rodents and humans. Nutr. Neurosci. **22**, 72–82 (2019)
47. M.C. Dias, D.C.G.A. Pinto, A.M.S. Silva, (2021) Plant flavonoids: Chemical characteristics and biological activity. Molecules **26**
48. D. Skroza, V. Šimat, L. Vrdoljak, N. Jolić, A. Skelin, M. Čagalj, R. Frleta, I. Generalić Mekinić, (2022) Investigation of Antioxidant Synergisms and Antagonisms among Phenolic Acids in the Model Matrices Using FRAP and ORAC Methods. Antioxidants 11:. https://doi.org/10.3390/antiox11091784
49. P. Švestková, J. Balík, I. Soural, Synergistic effect of selected carboxylic acids and phenolic compounds detected by the FRAP method. Food Chem. X. **23** (2024). https://doi.org/10.1016/j.fochx.2024.101573
50. C. Vicol, G. Duca, Synergistic, Additive and Antagonistic Interactions of Some Phenolic Compounds and Organic Acids Found in Grapes. Acta Chim. Slov. 588–600 (2023). https://doi.org/10.17344/acsi.2023.8214
51. L. Lalfakawmi, F. Lalsangpuii, L. Tochhawng, S.T. Lalzarzovi, P. Lalnuntluanga, Z. Zothansiama, Synergistic Antioxidative Potential of Cosmosiin and Vanillic Acid in vitro and ex vivo. Indian J. Sci. Technol. **16**, 48–56 (2023). https://doi.org/10.17485/IJST/v16sp1.msc7
52. Y. Kim, H. Zhao, R.J. Avena-Bustillos, S.C. Wang, N. Nitin, Synergistic antimicrobial activities of aqueous extract derived from olive byproduct and their modes of action. Chem. Biol. Technol. Agric. (2024). https://doi.org/10.1186/s40538-024-00634-5. 11:
53. de E.F. Oliveira, C.H. Nguyen, K. Stepanian, A. Cossu, N. Nitin, Enhanced bacterial inactivation in apple juice by synergistic interactions between phenolic acids and mild food processing technologies. Innovative Food Sci. Emerg. Technol. (2019). https://doi.org/10.1016/j.ifset.2019.102186. 56:
54. H. Azaizeh, A. Tafesh, N. Najami, J. Jadoun, F. Halahlih, H. Riepl, Synergistic antibacterial effects of polyphenolic compounds from olive mill wastewater. Evidence-based Complement. Altern. Med. **2011** (2011). https://doi.org/10.1155/2011/431021
55. S. Amor, S. Ben, Mekious, L.A. Benfekih, M.H. Abdellattif, W. Boussebaa, F.A. Almalki, T. Hadda, S.M.A. Ben, Kawsar, (2022) Phytochemical Characterization and Bioactivity of Different Honey

Samples Collected in the Pre-Saharan Region in Algeria. Life 12:. https://doi.org/10.3390/life12070927

56. S. Ouchemoukh, N. Amessis-Ouchemoukh, M. Gómez-Romero, F. Aboud, A. Giuseppe, A. Fernández-Gutiérrez, A. Segura-Carretero, Characterisation of phenolic compounds in Algerian honeys by RP-HPLC coupled to electrospray time-of-flight mass spectrometry. LWT - Food Sci. Technol. **85**, 460–469 (2017). https://doi.org/10.1016/J.LWT.2016.11.084
57. R. Viteri, A. Giordano, G. Montenegro, M.J. Simirgiotis, F.C. Zacconi, (2025) Metabolomic Profiling and Antioxidant Properties of Chilean Eucryphia cordifolia Cav.: Insights from Leaves, Flowers, and Monofloral Honey. Antioxidants 14:. https://doi.org/10.3390/antiox14030292
58. C. Hulea, D. Obistioiu, A. Hulea, M.A. Suleiman, D. Floares, E. Alexa, I.M. Imbrea, A.G. Neacșu, M. Pentea, C.A. Popescu, F. Imbrea, (2025) Integrated Assessment of Antibacterial Activity, Polyphenol Composition, Molecular Docking, and ADME Properties of Romanian Oak and Fir Honeydew Honeys. Antibiotics 14:. https://doi.org/10.3390/antibiotics14060592
59. M. Kędzierska-Matysek, A. Teter, T. Daszkiewicz, M. Florek, (2023) Microbiological Quality of Polish Artisanal Varietal Honeys. Foods 12:. https://doi.org/10.3390/foods12183349
60. N. Zawawi, P.J. Chong, N.N.M. Tom, N.S.S. Anuar, S.M. Mohammad, N. Ismail, A.Z. Jusoh, Establishing relationship between vitamins, total Phenolic and total flavonoid content and antioxidant activities in various honey types. Molecules. **26** (2021). https://doi.org/10.3390/molecules26154399
61. S. Zou, H. Tao, Y.N. Chang, Characterization of antioxidant activity and analysis of phenolic acids and flavonoids in linden honey. Food Sci. Technol. (Brazil). **42** (2022). https://doi.org/10.1590/fst.76621
62. T. Chau, R. Owusu-Apenten, P. Nigam, Total Phenols, Antioxidant Capacity and Antibacterial Activity of Manuka Honey Extract. J. Adv. Biol. Biotechnol. **15**, 1–6 (2017). https://doi.org/10.9734/jabb/2017/37101
63. M. Kunat-Budzyńska, A. Rysiak, A. Wiater, M. Grąz, M. Andrejko, M. Budzyński, M.S. Bryś, M. Sudziński, M. Tomczyk, M. Gancarz, R. Rusinek, A.A. Ptaszyńska, Chemical Composition and Antimicrobial Activity of New Honey Varietals. Int. J. Environ. Res. Public. Health. **20** (2023). https://doi.org/10.3390/ijerph20032458
64. D. Hernanz, M.J. Jara-Palacios, J.L. Santos, A. Gómez Pajuelo, F.J. Heredia, A. Terrab, (2023) The profile of phenolic compounds by HPLC-MS in Spanish oak (Quercus) honeydew honey and their relationships with color and antioxidant activity. LWT 180:. https://doi.org/10.1016/j.lwt.2023.114724
65. I. Šola, V. Vujčić Bok, I. Fabijanić, J. Jablan, L. Borgese, A. Humski, M. Mikulić, K. Sanković, Z. Franić, G. Rusak, Influence of Season, Hive Position, Extraction Method and Storage Temperature on Polyphenols and Antioxidant Activity of Croatian Honey. Molecules. **30** (2025). https://doi.org/10.3390/molecules30040919

66. C. Gu, K. Howell, F.R. Dunshea, H.A.R. Suleria, LC-ESI-QTOF/MS characterisation of phenolic acids and flavonoids in polyphenol-rich fruits and vegetables and their potential antioxidant activities. Antioxidants. **8** (2019). https://doi.org/10.3390/antiox8090405
67. P. Wanyo, T. Chamsai, N. Toontom, L.K. Nghiep, K. Tudpor, (2025) Evaluation of In Vitro Digested Mulberry Leaf Tea Kombucha: A Functional Fermented Beverage with Antioxidant, Anti-Inflammatory, Antihyperglycemic, and Antihypertensive Potentials. Fermentation 11:. https://doi.org/10.3390/fermentation11050258
68. B. Abu-Farich, H. Hamarshi, M. Masalha, A. Aboulghazi, A. Kmail, M. Ouassete, H. El, Imtara, B. Lyoussi, B. Saad, Polyphenol Contents, Antibacterial and Antioxidant Effects of Four Palestinian Honey Samples, and their Anticancer Effects on Human Breast Cancer Cells. J. Pure Appl. Microbiol. **18**, 1372–1385 (2024). https://doi.org/10.22207/JPAM.18.2.60
69. K. Jaśkiewicz, T. Szczęsna, J. Jachuła, How Phenolic Compounds Profile and Antioxidant Activity Depend on Botanical Origin of Honey—A Case of Polish Varietal Honeys. Molecules. **30** (2025). https://doi.org/10.3390/molecules30020360
70. M. Irakli, A. Skendi, E. Bouloumpasi, P. Chatzopoulou, C.G. Biliaderis, Lc-ms identification and quantification of phenolic compounds in solid residues from the essential oil industry. Antioxidants. **10** (2021). https://doi.org/10.3390/antiox10122016
71. Y.C. Liao, T. Kim, J.L. Silva, W.Y. Hu, B.Y. Chen, (2022) Effects of roasting degrees on phenolic compounds and antioxidant activity in coffee beans from different geographic origins. LWT 168:. https://doi.org/10.1016/j.lwt.2022.113965
72. S. Touzani, H. Imtara, S. Katekhaye, H. Mechchate, H. Ouassou, A.S. Alqahtani, O.M. Noman, F.A. Nasr, H. Fearnley, J. Fearnley, A. Paradkar, I. Elarabi, B. Lyoussi, Determination of phenolic compounds in various propolis samples collected from an african and an asian region and their impact on antioxidant and antibacterial activities. Molecules. **26** (2021). https://doi.org/10.3390/molecules26154589
73. I. Indriaty, B. Ginting, K. Hasballah, D. Djufri, A Comparative Study of Total Tannin Contents and Antimicrobial Activities in Methanol Extracts of Rhizophoraceae Species. Heca J. Appl. Sci. **1**, 62–70 (2023). https://doi.org/10.60084/hjas.v1i2.89
74. P.A.B. Ramos, C. Pereira, A.P. Gomes, R.T. Neto, A. Almeida, S.A.O. Santos, A.M.S. Silva, A.J.D. Silvestre, Chemical Characterisation, Antioxidant and Antibacterial Activities of Pinus pinaster Ait. and Pinus pinea L. Bark Polar Extracts: Prospecting Forestry By-Products as Renewable Sources of Bioactive Compounds. Appl. Sci. (Switzerland). **12** (2022). https://doi.org/10.3390/app12020784

Figures

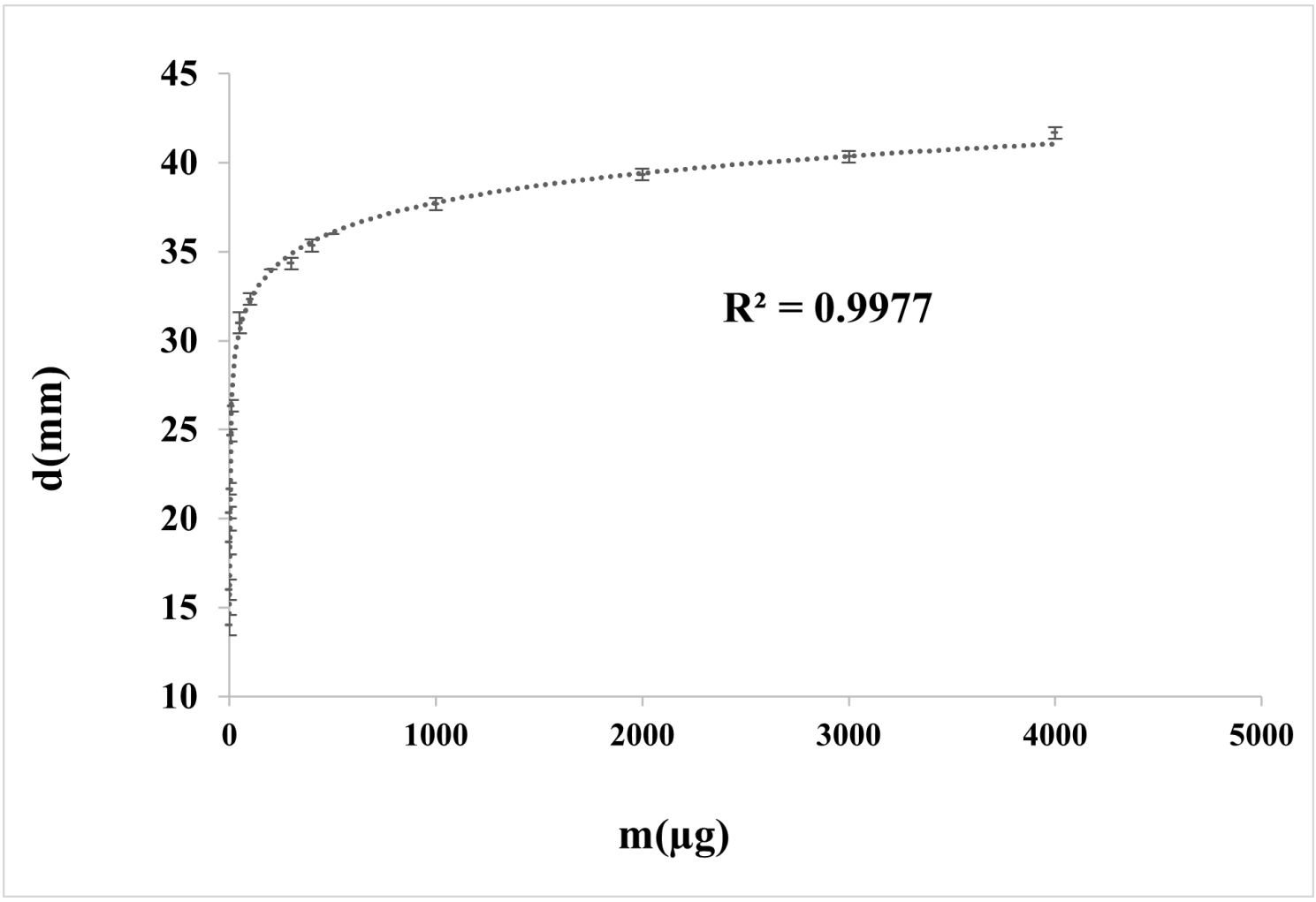


Figure 1

Calibration curve of antibiotic gentamicin (CN) against the *Staphylococcus aureus* strain

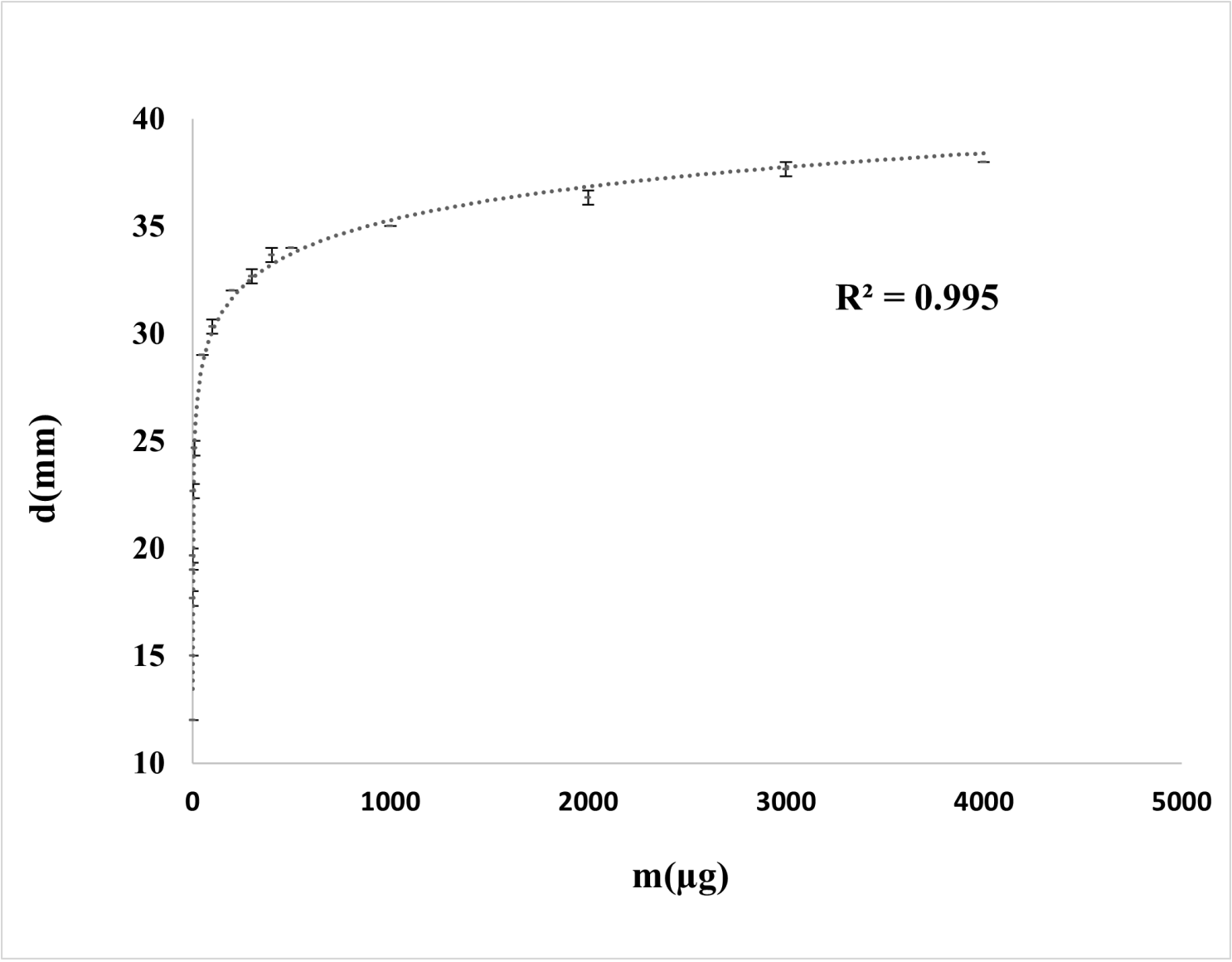


Figure 2

Calibration curve of antibiotic gentamicin (CN) against the *Pseudomonas aeruginosa* strain

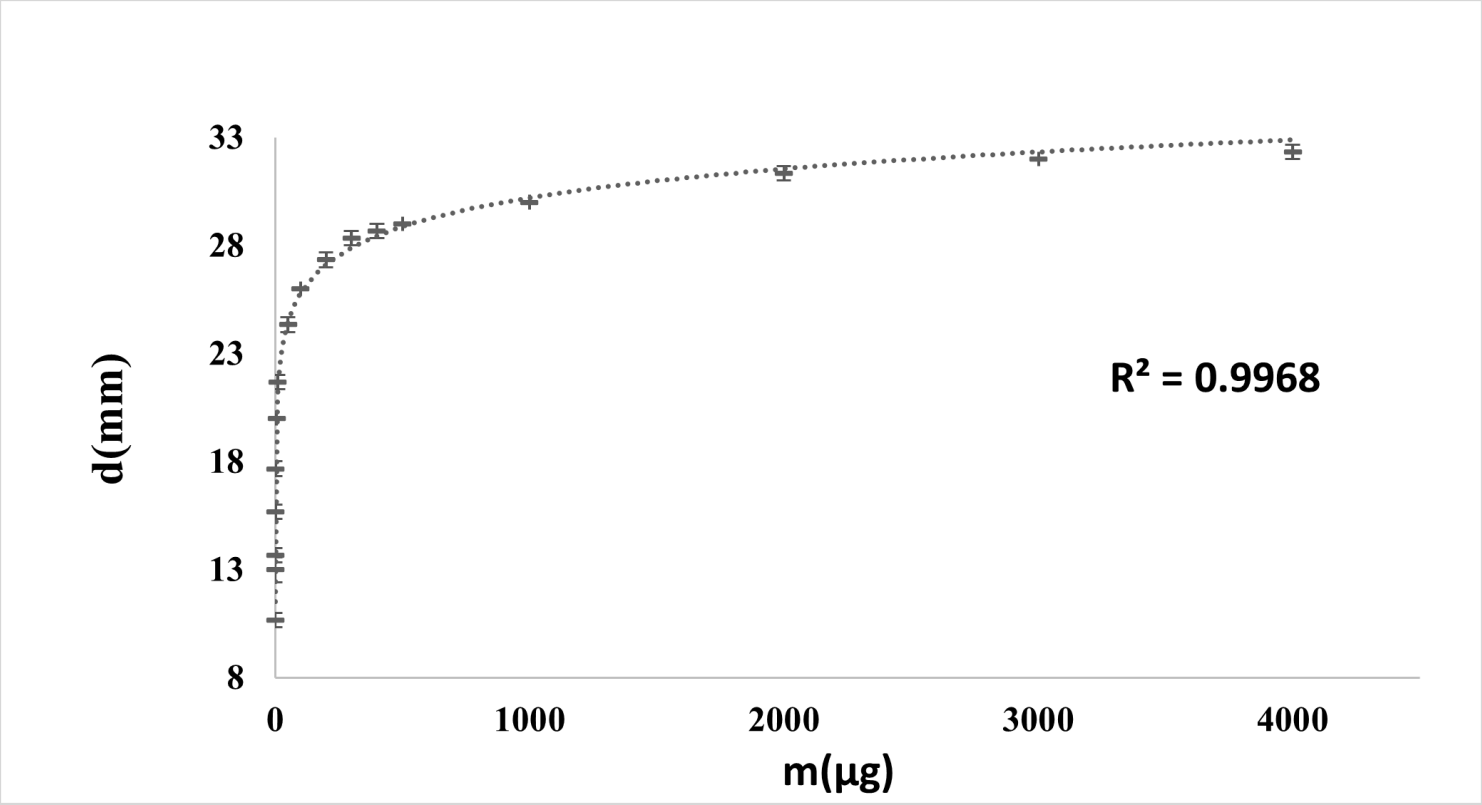


Figure 3

Calibration curve of antibiotic gentamicin (CN) against the *Escherichia coli* strain

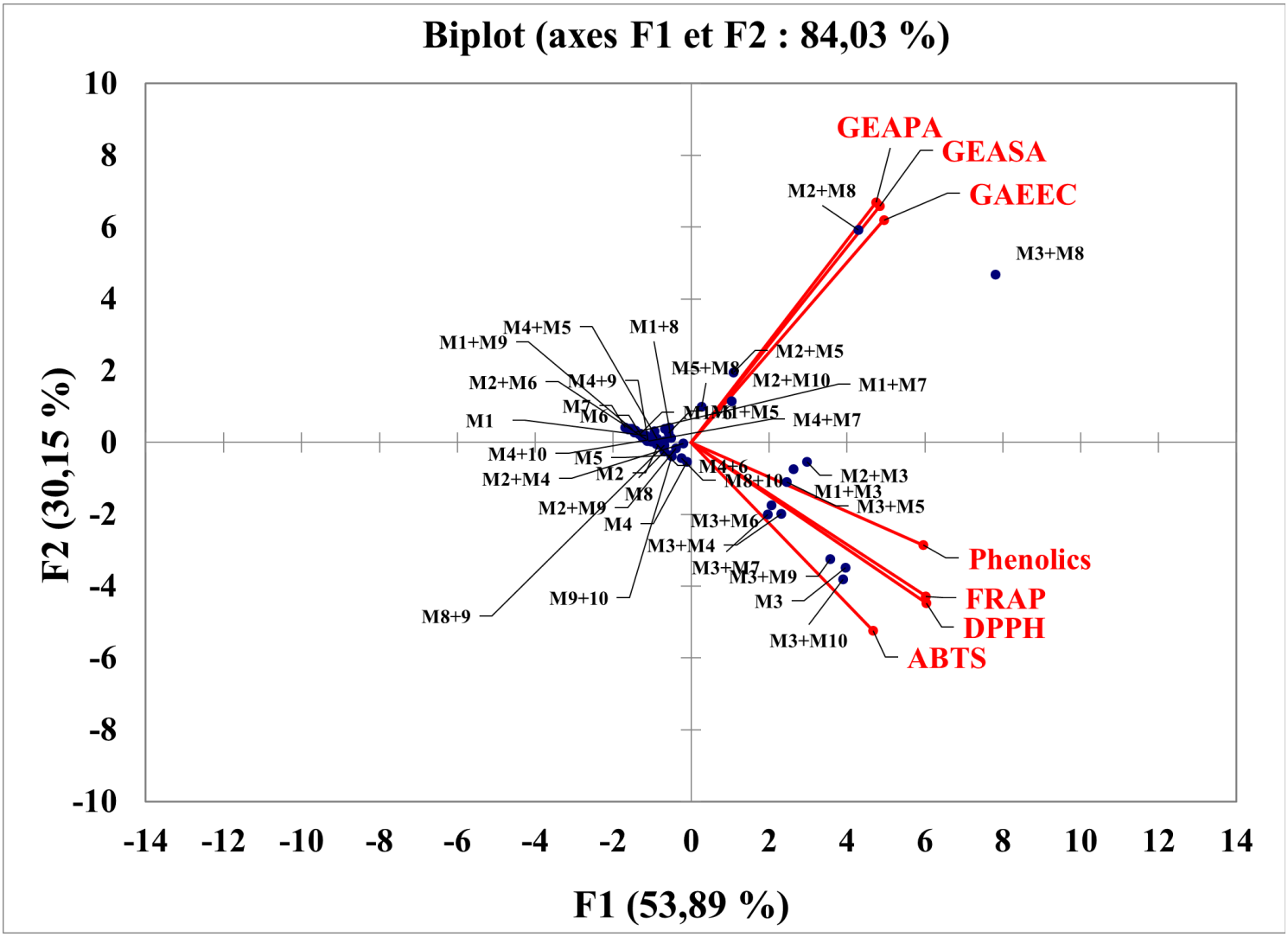


Figure 4

The PCA biplot showing the correlation between total phenolic contents and the biological activities of honey and binary combination of honey

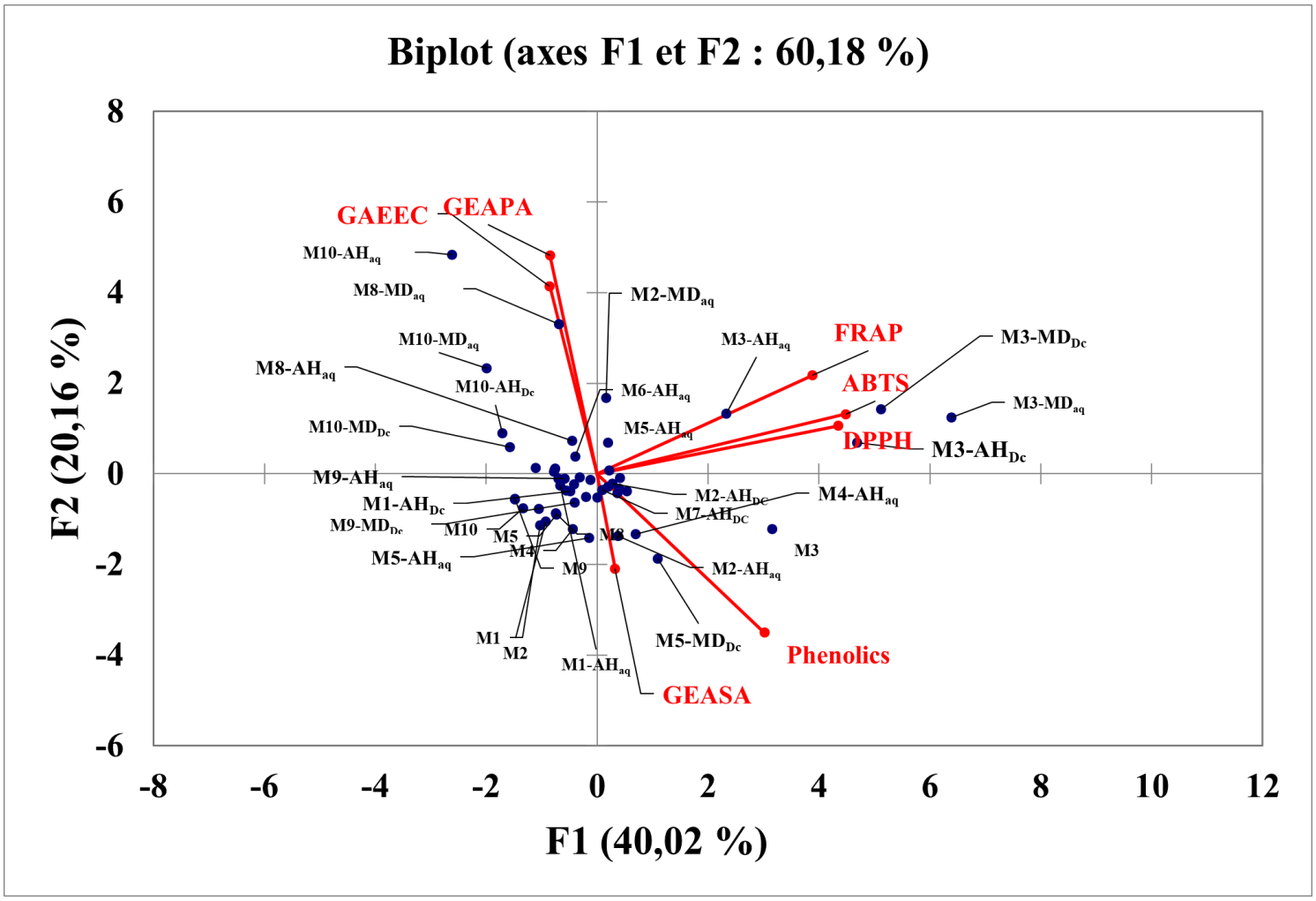


Figure 5

The PCA biplot showing the correlation between total phenolic contents and the biological activities of honey and honey plant mixtures