

Research Article

In Vitro Evaluation of Palestinian Propolis as a Natural Product with Antioxidant Properties and Antimicrobial Activity against Multidrug-Resistant Clinical Isolates

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The present work reveals, for the first time, the antioxidant and antibacterial properties of propolis samples collected from different regions of Palestine. The content on bioactive compounds has been estimated by total phenolic and flavone and flavonol content, while their antioxidant activity has been determined by radical scavenging methods of 1,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical (ABTS), and ferric reducing power assay (FRAP). The disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) methods were carried out to evaluate the antibacterial activity of Palestinian propolis against multidrug-resistant clinical isolates, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus faecalis*. The results showed that the total phenolic content ranged from 9.62 mg to 124.94 mg gallic acid equivalent GAE/g of propolis, and the flavone and flavonol content ranged from 1.06 to 75.31 mg quercetin equivalent QE/g of propolis. The samples S6 from Al-Khalil presented the strongest radical scavenging activity toward DPPH, ABTS free radicals, and FRAP assay with IC₅₀ values of 0.02, 0.03, and 0.05 mg/mL, respectively. The results of antibacterial activity indicated that the propolis samples inhibit the growth of Gram-positive strains better than Gram-negative ones. In addition, a strong correlation was observed between the pH, resin, balsam, total phenolic, flavones and flavonol, and total antioxidant capacity (TAC) from one side and the antibacterial activity of propolis samples except on *Pseudomonas aeruginosa*.

1. Introduction

Over the past 50 years, antibiotics have played a crucial role in the fight against many diseases and infections, and their development has revolutionized the treatment of these diseases [1, 2]. However, with the increasing and sometimes unjustified use of these molecules, some bacterial strains could develop resistance to antibiotics. This situation appears to be particularly worrying in hospitals, and the number of resistant bacteria is constantly increasing [3, 4]. There is an urgent need to explore different potential strategies that could lead to the discovery of new classes of antibiotics. In this sense, the study of bee products constitutes an interesting strategy since this source has so far

been little exploited, despite the intensive use of honey and propolis to treat infections in traditional medicine in many regions of the world [5, 6].

Propolis is a resinous material that bees collect from exudates and plant buds and is one of the most widely used bee products in food, medicine, and cosmetics due to the therapeutic potentials [7]. In recent years, numbers of therapeutic properties of propolis have been noted including analgesic-anesthetic, antifungal, anti-inflammatory, immunomodulatory, antiallergic, hepatoprotective, antidiabetic, hypoglycemic, antihypertensive, antimicrobial, and antioxidant properties [7, 8]. These properties are the results of propolis-derived compounds, such as phenolic compounds, flavonoids, trace elements, and amino acids [9, 10]. In

propolis, over than 300 chemical compounds were identified, and the quantity of these compounds is highly different from an area to another and from season to season, depending on the origin of plants, climatic and environmental conditions, production methods, processing, and storage conditions [9, 11].

The antioxidant activity of propolis samples has been evaluated worldwide, using several antioxidant methodologies including capacity for scavenging the 1,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and nitric oxide (NO) free radicals, superoxide anion radical, hydrogen peroxide, ferric reducing power (FRAP), chelating activity, and oxygen radical antioxidant capacity (ORAC) [11, 12]. In addition, the *in vitro* antimicrobial activity of propolis against Gram-positive and Gram-negative strains has been reported [13–15]. However, and to the best of our knowledge, there is no previous detailed study on the activities of Palestinian propolis.

The aim of the present work is to evaluate the antioxidant and antibacterial activities of propolis samples collected from different areas in Palestine and to illustrate the quality of samples based on their physicochemical properties. In addition, the results of the evaluated parameters were used to study the correlations between these parameters and for the discrimination between different propolis samples employing the principal component analysis (PCA).

2. Materials and Methods

2.1. Propolis Samples. Ten Palestinian propolis samples were purchased from beekeepers in the month of June and July of the year 2018. The samples are labeled as shown in Table 1 with biotope of the region of collection. The propolis samples were stored at room temperature (22–24°C) in airtight plastic containers until analysis. According to the information of beekeepers, all samples were collected by the propolis grids method. This method is characterized by high quality compared to other methods that also need a few materials to collect propolis.

2.2. Chemicals, Reagents, and Equipment

2.2.1. Chemicals

(1) *Chemicals for Antioxidant and Physicochemical Properties.* 2,2'-diphenyl-1-picrylhydrazyl (DPPH), potassium ferricyanide ($K_3(Fe(CN)_6)$), sodium carbonate, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), sulfuric acid, quercetin, and gallic acid, were purchased from Sigma-Aldrich, Germany. Trisodium phosphate (Na_3PO_4), trichloroacetic acid, potassium dihydrogen phosphate (KH_2PO_4), and dipotassium hydrogen phosphate anhydrous (K_2HPO_4) were purchased from VWR, Leuven, Belgium. Iron (III) chloride was purchased from Buchs, Switzerland. Ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24}$) was purchased from Pronalab, Lisbon, Portugal. Folin–Ciocalteu's phenol reagent and $AlCl_3$ were purchased from Panreac

TABLE 1: Propolis samples, region of collection, and the biotope of these regions.

Sample	Region of collection	Year	Biotope of state of Palestine
S1	Al-Khalil	2018	Eastern highland
S2	Jaricho	2018	Jordan valley
S3	Jenin	2018	Semicoastal
S4	Nablus	2018	Eastern highland
S5	Tulkarm	2018	Semicoastal
S6	Al-Khalil	2018	Eastern highland
S7	Salfeet	2018	Eastern highland
S8	Ramallah	2018	Eastern highland
S9	Qalqilya	2018	Semicoastal
S10	Bethlehem	2018	Eastern highland

Química, Montcada i Reixac, Barcelona, Spain. Ascorbic acid was purchased from Sigma-Aldrich, USA.

(2) *Chemicals for Antibacterial Activity.* Mueller–Hinton broth and Mueller–Hinton agar were purchased from Biokar Diagnostics (Beauvais, France), while triphenyltetrazolium chloride (TTC) was purchased from Sigma-Aldrich, USA.

2.2.2. Equipment. Jenway 720501 UV/visible scanning spectrophotometer, pH meter (WTW Inolab pH 720), and microplate reader (Tecan Infinite M200; Tecan, Austria) were used.

2.3. Extraction of Propolis Samples. The extracts of propolis samples in the present work are similar to the previous method performed by Touzani et al. [8]. 3g of each propolis was extracted by maceration using 30 mL of 70% ethanol and maintained for 7 days at 37°C under agitation. After that, the solution was filtered using Whatman filter paper, and the final extract was centrifuged at 4000 rpm for 10 min, and the supernatants were collected.

2.4. Physicochemical Characterization of Propolis Samples

2.4.1. Wax, Resin, and Balsam Contents. These parameters were determined according to the method described by Papotti et al. [16]. The results were expressed as a percentage (w/w), representing the rate of wax in each propolis sample.

2.4.2. Ash Content. The ash content was carried out according to the method described by Imtara et al. [17], with slight modification. Briefly, 2 g of each sample was weighted into a crucible and heated in a muffle furnace at 105°C overnight, and then, the crucibles were moved to 500°C for 4 hours. After cooling in a desiccator, they were weighed again. The proportion of ash was calculated according to the formula

$$\text{Ash} = \left(\frac{(W1 - W2)}{W0} \right) * 100, \quad (1)$$

where $W0$ is the weight of propolis; $W1$ is the weight of the crucible plus the ash; and $W2$ is the weight of the crucible.

2.4.3. Determination of pH. The pH of propolis samples was measured by a pH meter based on the method described by Dias et al. [18].

2.4.4. Determination of Moisture Content. The moisture content of samples was determined by gravimetric analysis at 105°C according to the AOAC method [19].

2.4.5. Mineral Contents. The mineral components in all samples were determined by atomic absorption spectrometry. Before that, a 5 mL of nitric acid 0.1 M was added to the ashes obtained above, and the mixture was stirred on a heating plate to almost complete dryness. Then, 10 mL of the same acid was added for the solubilization and made up to 25 mL with distilled water [17, 20].

2.5. Antioxidant Properties of Propolis Samples

2.5.1. Total Phenolic. The total phenolic was determined according to the Folin–Ciocalteu colorimetric method [21]. Briefly, 25 μ L of each sample dilution was mixed with 125 μ L of Folin–Ciocalteu reagent (0.2 N) and 100 μ L of sodium carbonate (7.5%). Then, all were incubated for 2 hours in the obscurest, and the absorbance of each solution was measured at 760 nm in a microplate reader. The results in this experiment were expressed as mg gallic acid equivalent (GAE)/g of propolis.

2.5.2. Total Flavone and Flavonol Content. The flavone and flavonol content was quantified according to the method described by Imtara et al. [22]. Briefly, sample dilution (100 μ L) was mixed with 100 μ L of AlCl_3 (5%) and incubated for 1 hour in dark at room temperature. The absorbance of the each solution was measured at 420 nm in a microplate reader. The results in this experiment were expressed as mg quercetin equivalent (QE)/g of propolis.

2.5.3. Total Antioxidant Capacity (TAC). The estimation of TAC was carried out according to the method reported by Prieto et al. [23]. Briefly, 25 μ L of propolis dilution was combined with 1 mL of phosphomolybdenum reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Then, the solution of each sample was incubated in a boiling water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm by a Jenway 720501 UV/visible scanning spectrophotometer. The results of TAC were expressed as mg of ascorbic acid equivalent AAE/g of propolis.

2.5.4. Capacity for Scavenging 1,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical. The ability of sample to scavenging the 2,2-diphenyl-1-picrylhydrazyl radical was estimated according to the method described by Brand-Williams et al. [24], with some modifications. Briefly, 25 μ L of various propolis dilutions was mixed with 175 μ L of DPPH solution. The

mixtures were shaken and then incubated at room temperature for 1 hour in the dark. The absorbance at 517 nm was measured in a microplate reader, and the scavenging activity of the DPPH radical was expressed as inhibition percentage using the following equation: % inhibition = ((control absorbance – sample absorbance)/control absorbance) \times 100. The results are expressed by IC_{50} (mg/mL), which means the concentration of a sample required to inhibit 50% of a radical.

2.5.5. Capacity for Scavenging 2,2'-Azino-bis (3-Ethylbenzothiazoline-6-sulphonic Acid) Radical (ABTS). This assay was used to determine the ability of sample to scavenging the ABTS^+ radical in 6 minutes and carried out according to the method described by Migul et al. [25], with some modifications. Briefly, 25 μ L of various propolis dilutions was mixed with 275 μ L of ABTS^+ reagent. After 6 min of incubation, the absorbance at 734 nm was measured in a microplate reader. The IC_{50} of each sample was determined in the same way as in DPPH assay.

2.5.6. The Ferric Reducing Power Assay (FRAP). The ferric reducing power was determined according to the method described of Oyaizu [26], with some modifications. Briefly, 25 μ L of various propolis dilutions was mixed with 200 μ L of potassium buffer (0.2 M, pH 6.6) and 200 μ L of potassium hexacyanoferrate (1%). The mixture was incubated at 50°C for 20 min, before the addition of 200 μ L of trichloroacetic acid (10%), 600 μ L of distilled water, and 120 μ L of ferric chloride (0.1%). The mixture was stirred well, and the absorbance was measured at 700 nm. Results are expressed as IC_{50} (mg/mL).

2.6. Antibacterial Activity of Propolis Samples

2.6.1. Bacterial Strains and Inoculums Standardization. All bacteria strains used in this study were obtained from the Microbiology Laboratory of the FMP and University Hospital Hassan II, Fez, four strains of Gram-negative (*E. coli* BLSE (ATB: 87) BGN, *E. coli* (ATB: 57) B6N, *E. coli* (ATB: 97) BGM, and *Pseudomonas aeruginosa*) and two strains of Gram-positive (*Streptococcus faecalis* and *Staphylococcus aureus*).

Antibiotic susceptibility analysis was performed for the strains of bacteria used, and through it, the bacterial resistance to antibiotics of each strain was determined as follows: *E. coli* BLSE (ATB:87) BGN resistant to cefuroxime, ceftriaxone, cefaclor, amoxicillin, ceftazidime, cefotaxime, cephalothin, and ciprofloxacin; *E. coli* (ATB: 57) B6N resistant to cefuroxime, amoxicillin, cefotaxime, cephalothin, ciprofloxacin, and trimethoprim-sulphamethoxazole; *E. coli* (ATB: 97) BGM resistant to amoxicillin; *Pseudomonas aeruginosa* resistant to amoxicillin and trimethoprim-sulphamethoxazole; *Streptococcus faecalis* resistant to tetracycline, vancomycin, erythromycin, penicillin, oxacillin, and trimethoprim-sulphamethoxazole; and *Staphylococcus aureus* resistant to vancomycin.

2.6.2. Agar Well Diffusion (AWD) Assay. The AWD depends on the measured diameters of the inhibition zones by samples and was performed according to method described by Kirby-Bauer [27].

2.6.3. Determination of the Minimum Inhibitory Concentration (MIC) and Determination of the Minimal Bactericidal Concentration (MBC). The MIC and MBC were determined according to NCCLS standards methods in 96-well plates, which were explained in detail by Imtara et al. and Touzani et al. [8, 20].

2.7. Statistical Analysis. The statistical analyses were performed by Pearson correlation coefficient (r) at a significance level of 99% ($p < 0.01$). The data preprocessing and the PCA were accomplished using MultBiplot64 running in MATLAB R2017a.

3. Results and Discussion

3.1. Physicochemical Characterization of Propolis Samples. The results of physicochemical properties of propolis samples are summarized in Table 2. The wax content in samples was ranging from 22.28% to 63.67% and with an average of 45.91 ± 13.35 . The resin content of the analyzed samples was between 22.75% and 57.13%. The resin and wax are the main compounds in propolis with about ~80% of the weight [16, 28]. In general, propolis samples with high resin content had low wax content [29]. This claim is consistent with our results, as a strong negative correlation was obtained between resin and wax, with r values of -0.928 ($p < 0.01$) (Table 3). Regarding balsam content, the values ranged from 0.57% to 1.58%. pH values comprised between 4.05 and 5.2 are one of the factors inhibiting the growth of microorganisms in propolis, and the values were similar with what was reported elsewhere [29–31]. Regarding moisture, which is an indication of the quality of propolis, all propolis samples showed values within the limit established by the Brazilian legislation (not more than 8%) [32]. High water content in propolis is indicative of inadequate storage and manipulation conditions [33]. Another parameter that indicates the quality of propolis is the ash content, and the analysis of this parameter can identify a possible adulteration of the material through the presence of impurities [34]. The values of ash content in the propolis samples were ranging between 0.97% and 4.17% and with an average of $2.64 \pm 1.10\%$. These values are within the range accepted for propolis [32].

Mineral elements are important parameters to determine the geographical origin of propolis and may reveal possible environmental pollution if toxic metals were detected [35, 36]. All analyzed samples showed a predominance of calcium with an average of 632.30 ± 287.99 mg/kg followed by potassium with an average of 305.10 ± 143.06 mg/kg (Table 2). The remaining minerals can be classified in descending order as follows: $Mg > Na > Fe$ with averages of 256.40, 250.30, and 51.60 mg/kg, respectively. The diversity in the mineral elements of the samples is influenced by the

TABLE 2: Physicochemical characterization and mineral contents of the analyzed propolis samples.

Assessed parameter (Unit)	Means	SD	Min	Max
Wax (%)	45.91	13.35	22.28	63.67
Resin (%)	35.31	10.73	22.75	57.13
Balsam (%)	1.07	0.29	0.57	1.58
Moisture (%)	2.07	0.48	1.17	2.99
Ash (%)	2.64	1.10	0.97	4.17
pH	4.63	0.40	4.05	5.2
Ca (mg/kg)	632.30	287.99	236	1180
Na (mg/kg)	250.30	145.10	62	434
K (mg/kg)	305.10	143.06	134	601
Mg (mg/kg)	256.40	193.02	74	764
Fe (mg/kg)	51.60	24.19	25	96

contents of these minerals in resinous material collected by honey bees and environmental factors that influence the formation of these minerals in plants such as soil pH, humidity, and mobility of trace elements [35, 37]. The results of mineral elements in the present work were similar to other studies [35, 37, 38].

3.2. Antioxidant Capacities of Propolis Samples. The samples were extracted with ethanol 70%. This ethanol to water ratio is more efficient and gives richer extracts in total phenolic compounds than other concentrations of ethanol [39]. Many studies dealing with the phenolic composition of propolis samples report that the most active compounds are caffeic acid, p-coumaric acid, 3,4-dimethoxycinnamic acid, quercetin, 5-methyl-pinobanksin ether, l-apegenine, kaempferol, cinnamylideneacetic acid, chrysin, pinocembrine, and galangin [40, 41]. The results of total phenolic contents are presented in Table 4. The lowest total phenolic content was obtained in sample S8 from Ramallah (9.62 ± 0.17 mg GAE/g), while the highest value was obtained in sample S6 from Al-Khalil (124.94 ± 2.64 mg GAE/g). In the same way as total phenolic content, the highest flavones and flavonol content was found in sample S6 with a value of 75.31 ± 1.39 mg QE/g of propolis, while a minimum content was seen in sample S10 from Bethlehem with a value of 1.06 ± 0.06 mg QE/g of propolis (Table 4). The quantity of total phenolic and flavones and flavonol found in propolis can be attributed to the vegetation where honey bees gather propolis [42]. Besides, the biological properties of propolis are related to its phenolic composition including flavonoids and/or other phenolic compounds [43]. The results of total phenolic and flavones and flavonol content in the present work are within the range reported by other studies [29, 44]. Some studies have shown that the total phenolic and flavonoids content in propolis samples depends mainly on botanical origin of the resins collected by the bees [45, 46]. From this point, the resin content correlated strongly positively with the total phenolic and flavones and flavonol content with r values of 0.953 and 0.943, respectively.

Regarding the total antioxidant activity (TAC), the results of propolis samples were ranging from 14.20 ± 0.47 mg of ascorbic acid equivalent AAE/g in sample S10 to 80.37 ± 1.77 mg AAE/g in sample S6. The antioxidant activity

TABLE 3: Pearson correlation coefficients between the analyzed parameters and antioxidant activity.

	Wax	Resin	Balsam	DPPH	ABTS	FRAP
Wax	1	−0.928**	−0.488	0.634*	0.829**	0.728*
Resin	−0.928**	1	0.561	−0.661*	−0.721*	−0.690*
Balsam	−0.488	0.561	1	−0.162	−0.335	−0.317
Total phenolic	−0.911**	0.953**	0.473	−0.744*	−0.811**	−0.769**
Flavone and flavonol content	−0.875**	0.943**	0.582	−0.589	−0.698*	−0.635*
TAC	−0.711*	0.903**	0.612	−0.711*	−0.602	−0.692*

*Correlation is significant at the level $p < 0.05$. **Correlation is significant at the level $p < 0.01$.

TABLE 4: Bioactive compounds estimations and antioxidant activities (DPPH, ABTS, and FRAP) of propolis samples.

Sample	Phenols (mg GAE/g)	Flavone and flavonol (mg QE/g)	TAC (mg AAE/g)	DPPH (IC ₅₀ = mg/mL)	ABTS (IC ₅₀ = mg/mL)	FRAP (IC ₅₀ = mg/mL)
S1	68.90 ± 0.36	26.19 ± 0.51	46.46 ± 0.39	0.15 ± 0.007	0.09 ± 0.001	0.16 ± 0.003
S2	80.79 ± 1.71	36.30 ± 0.92	74.39 ± 1.54	0.04 ± 0.001	0.20 ± 0.007	0.14 ± 0.005
S3	66.95 ± 0.59	31.20 ± 0.56	46.07 ± 0.39	0.07 ± 0.001	0.09 ± 0.022	0.10 ± 0.002
S4	24.72 ± 0.2	6.29 ± 0.41	52.63 ± 2.30	0.35 ± 0.022	0.63 ± 0.028	0.49 ± 0.006
S5	31.43 ± 1.2	7.23 ± 0.98	36.87 ± 0.33	0.14 ± 0.009	0.38 ± 0.023	0.17 ± 0.001
S6	124.94 ± 2.64	75.31 ± 1.39	80.37 ± 1.77	0.02 ± 0.001	0.03 ± 0.001	0.05 ± 0.001
S7	39.62 ± 0.38	5.85 ± 0.41	31.20 ± 0.98	0.10 ± 0.038	0.22 ± 0.015	0.21 ± 0.003
S8	9.62 ± 0.17	2.16 ± 0.37	15.21 ± 0.66	1.13 ± 0.054	0.92 ± 0.035	1.02 ± 0.02
S9	19.27 ± 0.34	8.83 ± 0.12	45.30 ± 0.97	0.77 ± 0.007	0.50 ± 0.029	0.53 ± 0.017
S10	10.56 ± 0.12	1.06 ± 0.06	14.20 ± 0.47	1.02 ± 0.071	0.47 ± 0.007	0.67 ± 0.009

and the ability of samples to scavenge free radicals were evaluated by three methods, and the results are expressed as IC₅₀ mg/mL (Table 4). Large differences were found in their antioxidant activity, which are related to their chemical composition, especially, the total phenolic and flavonoids contents [17, 48, 49]. The results showed a strong free radical scavenging by DPPH assay with IC₅₀ ranging between 0.02 ± 0.001 mg/mL in sample S6 from Al-Khalil and 1.13 ± 0.054 mg/mL in sample S8 from Ramallah. In addition, the IC₅₀ of DPPH assay correlated negatively with the total phenolic and TAC ($r = -0.744$ and $r = -0.711$, respectively) (Table 3). The same correlation was mentioned in other studies [20, 43, 49]. Antioxidant activities were also assessed by the ABTS and FRAP assays (Table 3). The sample S6 and S8 had the best and lowest activities, respectively. In Table 3, A strong negative correlation was shown between the IC₅₀ of samples for both assays with the total phenolic ($r = -0.811$; $r = -0.769$, respectively) and flavones and flavonol content ($r = -0.698$; $r = -0.635$, respectively). This is similar with a study conducted previously by Imtara et al. [17].

3.3. Antibacterial Activity of Propolis Samples. Numerous studies have demonstrated the mechanism of propolis action on bacteria, such as, inhibition of cell division, collapsing microbial cytoplasm cell membranes and cell walls, inhibition of bacterial motility, enzyme inactivation, bacteriolysis, and protein synthesis inhibition [15, 50]. In the present work, the zone inhibitions of bacterial growth inhibited by the disc agar diffusion method of the propolis samples are shown in Table 5. This method was used for primary screening to evaluate the antibacterial activity [51]. Propolis

samples inhibited *E. coli* growth, with zone inhibition ranging from 10 ± 1 mm in sample S7 to 20.67 ± 2.51 mm in sample S6. The weakest antibacterial activity of propolis samples was on *Pseudomonas aeruginosa* with zone inhibition ranging from 8.5 ± 0.58 mm in sample S5 to 11.3 ± 0.6 mm in sample S6. Concerning the Gram-positive strains, the results showed that the sample S6 had the best activity with a zone inhibition of 29.6 ± 0.78 mm on *Staphylococcus aureus* and 28.25 ± 0.57 mm on *Streptococcus faecalis*, while the sample S10 presented the lowest zone inhibition with values of 14 ± 0.45 mm on *Staphylococcus aureus* and 10 ± 0.13 mm on *Streptococcus faecalis*.

The results of the disc agar diffusion method indicated that the propolis samples studied in the present work inhibits the growth of Gram-positive strains better than Gram-negative strains. Our results are in agreement with the findings of other authors [13, 15]. In addition, pH, resin, balsam, total phenolic, flavone and flavonol content, and TAC content correlated positively with the zone inhibition of propolis on all strains tested except on *Pseudomonas aeruginosa*, while wax content and flavone and flavonol content correlated negatively with the zone inhibition of propolis on all studied strains (Table 6). This means that the more wax content in the sample, the less activity it will have against bacteria.

The MIC values for the propolis samples and MBC values are shown in Table 5. All propolis evaluated in the present study showed the antibacterial effect against Gram-positive strains pathogens with MIC ranging from 0.01 mg/mL to 5 mg/mL. The sample S6 showed the remarkable bactericidal effect against Gram-positive microorganisms followed by samples S2 and S9. *Staphylococcus aureus* strain

TABLE 5: The diameter of inhibition zone (mm), the minimum inhibitory concentration (mg/mL), and minimal bactericidal concentration (mg/mL) of Palestinian propolis samples.

		<i>Escherichia coli</i> 87	<i>Escherichia coli</i> 57	<i>Escherichia coli</i> 97	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus faecalis</i>
S1	DI (mm)	14.22 ± 0.74	16.39 ± 1.18	16.54 ± 0.57	9.83 ± 0.18	19.2 ± 0.5	21.3 ± 0.3
	MIC (mg/mL)	5	2.5	2.5	2.5	0.63	2.5
	MBC (mg/mL)	5	2.5	5	5	1.25	2.5
S2	DI (mm)	13.54 ± 1.21	16.67 ± 1.37	17 ± 1	10.45 ± 0.38	21.3 ± 1.01	20.5 ± 0.4
	MIC (mg/mL)	2.5	1.25	1.25	5	0.31	2.5
	MBC (mg/mL)	2.5	1.25	2.5	5	0.31	2.5
S3	DI (mm)	15.67 ± 0.57	16.62 ± 0.73	18.33 ± 0.89	9 ± 0.5	19.5 ± 1	21.65 ± 0.84
	MIC (mg/mL)	1.25	1.25	0.63	5	1.25	2.5
	MBC (mg/mL)	2.5	1.25	0.63	ND	1.25	5
S4	DI (mm)	9.66 ± 0.59	12.82 ± 1.12	12.33 ± 0.6	9.45 ± 0.5	16.95 ± 0.29	15.15 ± 0.56
	MIC (mg/mL)	ND	5	5	ND	2.5	2.5
	MBC (mg/mL)	ND	5	5	ND	2.5	2.5
S5	DI (mm)	14.22 ± 0.6	14.33 ± 1.33	17.67 ± 1.1	8.5 ± 0.58	18.5 ± 0.8	19.5 ± 0.8
	MIC (mg/mL)	2.5	2.5	1.25	5	0.63	2.5
	MBC (mg/mL)	5	2.5	1.25	ND	2.5	5
S6	DI (mm)	20.67 ± 2.51	18.83 ± 1.37	20.33 ± 0.57	11.3 ± 0.6	29.6 ± 0.78	28.25 ± 0.57
	MIC (mg/mL)	0.31	0.31	0.16	2.5	0.01	1.25
	MBC (mg/mL)	0.31	0.31	0.31	5	0.01	2.5
S7	DI (mm)	10 ± 1	12.67 ± 0.6	11.53 ± 1.37	9.05 ± 0.3	17.15 ± 1.02	15.5 ± 0.33
	MIC (mg/mL)	ND	5	5	5	1.25	2.5
	MBC (mg/mL)	ND	5	5	ND	1.25	5
S8	DI (mm)	ND	10.23 ± 0.37	12.22 ± 0.57	ND	10.3 ± 0.33	15.3 ± 0.6
	MIC (mg/mL)	ND	ND	2.5	ND	2.5	5
	MBC (mg/mL)	ND	ND	2.5	ND	2.5	5
S9	DI (mm)	15.33 ± 0.57	15.28 ± 0.80	13.33 ± 1.02	9 ± 0.33	18.85	19.8 ± 1.11
	MIC (mg/mL)	1.25	1.25	1.25	2.5	0.31	2.5
	MBC (mg/mL)	1.25	1.25	2.5	ND	0.31	2.5
S10	DI (mm)	ND	11.43 ± 0.89	12 ± 1.02	10.15 ± 0.31	14 ± 0.45	10 ± 0.13
	MIC (mg/mL)	ND	5	5	5	2.5	5
	MBC (mg/mL)	ND	5	ND	ND	2.5	5

DI, diameter of inhibition; ND, not determined.

showed resistance against vancomycin, while *Streptococcus faecalis* strain exhibited resistance against tetracycline, vancomycin, erythromycin, penicillin, oxacillin, and trimethoprim-sulphamethoxazole. However, propolis extract samples represented good activity against these strains.

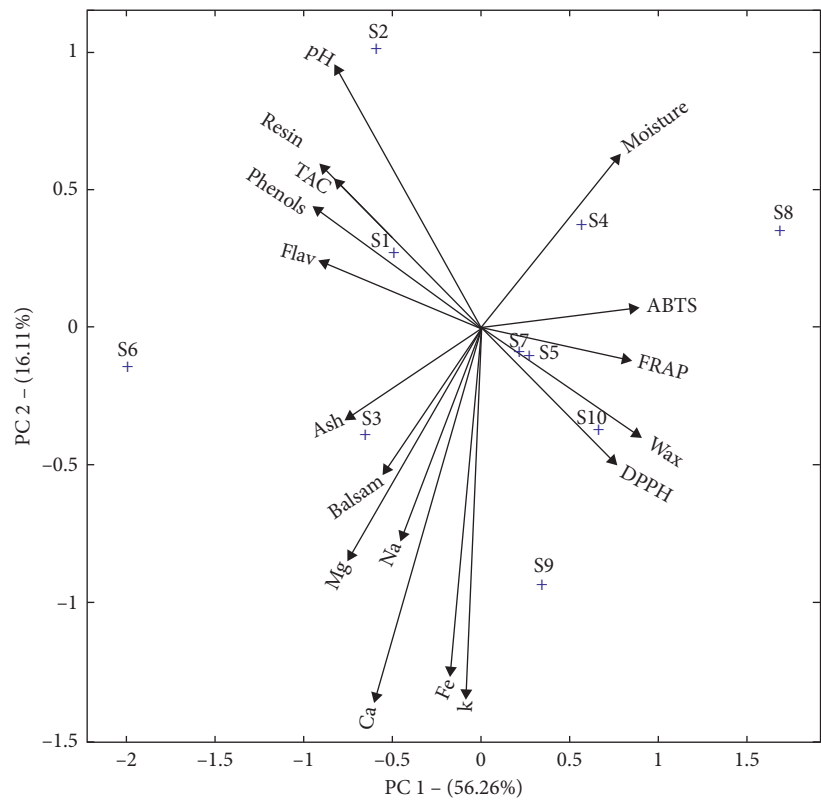
Lower MIC values of samples were observed for *P. aeruginosa* ranging from 2.5 to 5 mg/mL. An earlier study by Bhalchandra et al. reported that *P. aeruginosa* strains showed high resistance against aztreonam and ceftazidime [52]. In the present work, *P. aeruginosa* strain showed resistance against amoxicillin and trimethoprim-sulphamethoxazole.

Regarding the activity of propolis on *E. coli* strain (Gram-negative), in spite of the high resistance to antibiotics by this strain as indicated above, the propolis samples exhibited moderate efficacy with MIC values ranging between 0.16 mg/mL and 5 mg/mL. Besides, the changes in the results of the MBCs were similar to the MICs with a small increase in the concentrations of some samples.

The difference in activity of propolis on bacteria can be attributed to variable cell wall and membrane structure of the bacterial strains and to the nature of the bioactive compounds that are found in propolis samples [15, 53]. The bacterial cell wall ranges from 20 nm to 80 nm thick for Gram-positive and between 1.5 nm and 10 nm thick for

Gram-negative bacteria. The main component of the cell wall is peptidoglycan, which is found in almost all bacteria, and is responsible for preserving the integrity of the cell. Destruction of peptidoglycan either through mutations or external stresses (e.g., propolis) will lead to cell lysis [54]. Moreover, several studies have shown that the antibacterial activity of propolis is not directly related to concentration of the bioactive compounds, such as phenolics and flavonoids, but can be attributed to the synergistic effects of these compounds [55, 56]. In Table 6, the total phenolic content and flavones and flavonol content correlated negatively with the MIC values of propolis on *S. faecalis* and *S. aureus* strains (*S. aureus*: $r = -0.688$ and $r = -0.644$, respectively; *S. faecalis*: $r = -0.719$ and $r = -0.651$, respectively), while flavone and flavonol content correlated negatively only with the MIC values of propolis on *E. coli* 97 with r value is -0.651 .

3.4. Multivariate Analysis. The studied parameters were used to understand the homogeneity of the propolis samples by using the principal component analysis (PCA). The PCA is a statistical analysis used to convert multivariate matrices and represent it in a few components [17]. The results were divided into two main groups and represented as a figure. The results of first group are shown in Figure 1(a) and were formed by all parameters except the antibacterial activities. The first component (PC1) explained 56.26% of the given



(a)
FIGURE 1: Continued.

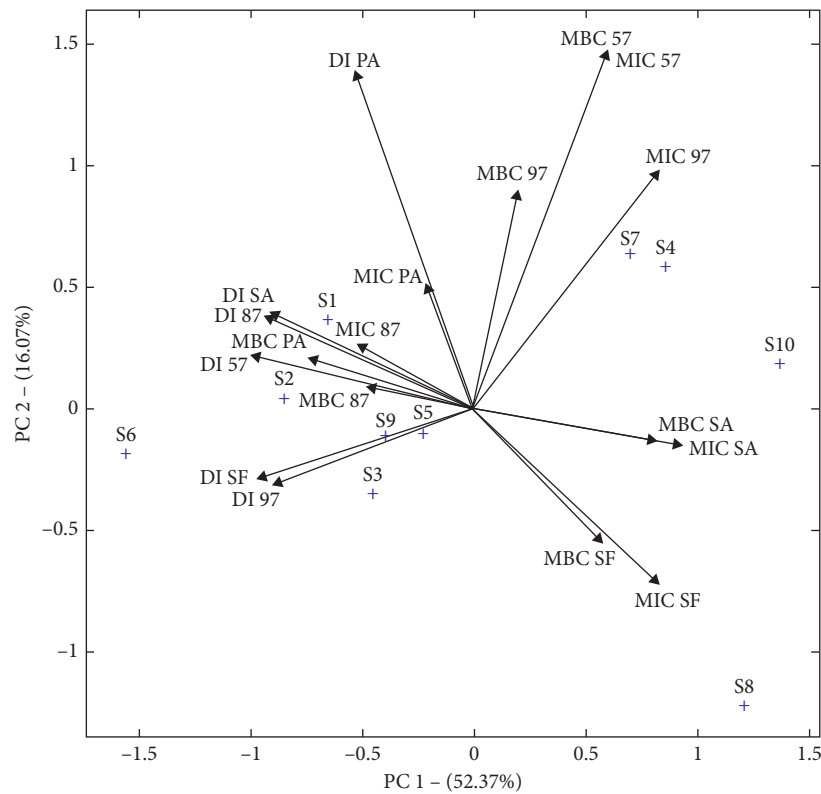


FIGURE 1: Principal component analysis of the assessed parameters. (a) PCA of the physicochemical parameters and the antioxidant activities. (b) PCA of the antibacterial activity. pH; resin; wax; ash; moisture; phenols, total phenolic; Flav, flavone and flavonol content; TAC, total antioxidant capacity; DPPH; ABTS; FRAP; K, potassium; Na, sodium; Ca, calcium; Mg, magnesium; Fe, iron; DI, diameter of inhibition zone; MIC, minimum inhibitory concentration; 57, *E.coli* (ATB: 57) B6N; 87, *E.coli* BLSE (ATB:87) BGN; 97, *E.coli* (ATB: 97) BGM; PA, *Pseudomonas aeruginosa*; SA, *Staphylococcus aureus*; SF, *Streptococcus faecalis*.

TABLE 6: Pearson correlation coefficients between the analyzed parameters and antibacterial activity.

	DI 57	DI 87	DI 97	DI PA	DI SA	DI SF	MIC 57	MIC 87	MIC 97	MIC PA	MIC SA	MIC SF
Wax	−0.853**	−0.635*	−0.837**	−0.596	−0.797**	−0.733*	0.326	−0.503	0.532	−0.277	0.615	0.548
Resin	0.886**	0.714*	0.803**	0.562	0.879**	0.813**	−0.405	0.359	−0.566	0.073	−0.642*	−0.677*
Balsam	0.745*	0.667*	0.631	0.324	0.635*	0.713*	−0.671*	0.318	−0.828**	0.045	−0.733*	−0.469
pH	0.788**	0.658*	0.694*	0.618	0.777**	0.672*	−0.158	0.501	−0.351	0.120	−0.581	−0.689*
Total phenolic	0.883	0.750*	0.832**	0.513	0.900**	0.860**	−0.421	0.293	−0.589	0.189	−0.688*	−0.719*
Flavone and flavonol content	0.862**	0.711*	0.828**	0.447	0.905**	0.872**	−0.524	0.164	−0.651*	0.076	−0.644*	−0.651*
TAC	0.863**	0.817**	0.696*	0.586	0.886**	0.800**	−0.351	0.256	−0.550	0.013	−0.696*	−0.831**

*Correlation is significant at the level $p < 0.05$. **Correlation is significant at the level $p < 0.01$. DI, diameter of inhibition zone; MIC, minimum inhibitory concentration; 57, *E. coli* (ATB: 57) B6N; 87, *E. coli* BLSE (ATB: 87) BGN; 97, *E. coli* (ATB: 97) BGM; PA, *Pseudomonas aeruginosa*; SA, *Staphylococcus aureus*; SF, *Streptococcus faecalis*.

data and represented in its negative part the total antioxidant capacity (TAC), total phenolic, flavones and flavonol content, mineral contents, and physicochemical parameters except the moisture and wax, while the IC₅₀ of DPPH, ABTS, and the reducing power (FRAP) were represented in the positive part. For the second component, 16.11% of the given data was retained and represented, mainly the pH, resin, moisture, TAC, total phenolic content, and flavones and flavonol content, in the positive part, and other parameters in the negative part. Considering the similarities of the samples, the samples S1, S2, S3, and S6 had high resin, total phenolic content, and flavone and flavonol content, and thus high antioxidant. Those samples were in the negative part of the plot. In contrast, other samples presented the opposite properties, and hence were plotted in the positive part of the plot.

The second group was formed by the results of antibacterial activity and is shown in Figure 1(b). The two principal components (PC) accounted for 52.37% and 16.07% successively of the given data. The samples S4, S7, S8, and S10 were characterized by low antibacterial activity and were loaded in the positive part of the first principal component (PC 1). The other samples were in the negative part of the plot and were characterized by high antibacterial activity.

4. Conclusion

Palestinian propolis exerted a marked inhibition against multidrug-resistant clinical isolate such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus faecalis* and showed high ability to radical scavenging. The sample S6 from Al-Khalil exhibited the highest antimicrobial and antioxidant activities with the highest total phenolic content and flavone and flavonol content. This may be to the fact that these regions contain a huge diversity of plants and trees. In addition, a strong correlation between resin, total phenolic content, and flavone and flavonol content with the antibacterial and antioxidant activities was observed. Palestinian propolis samples could be used as a natural alternative to other natural food additives and alternative therapy for resistant strain infection.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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