Hindawi
Journal of Food Quality
Volume 2021, Article ID 5560182, 11 pages
https://doi.org/10.1155/2021/5560182

Research Article

Antioxidant and Antibacterial Effects of Pollen Extracts on Human Multidrug-Resistant Pathogenic Bacteria

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Received 25 January 2021; Revised 10 May 2021; Accepted 22 June 2021; Published 1 July 2021

Academic Editor: Flora V. Romeo

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The present work was designed to search the possible antibacterial effect of the ethanolic extract of pollens from six botanical origins, *Punica granatum, Quercus ilex, Centaurium erythraea, Coriandrum sativum*, *Ruta graveolens*, and *Citrus aurantium*, against multidrug pathogenic bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Acinetobacter baumannii, Klebsiella pneumoniae*, and *Staphylococcus aureus*. The content of phenolic compounds, flavones, and flavonols was measured. The antioxidant activities were evaluated using four assays: total antioxidant capacity, DPPH, ABTS, and reducing power. Antibacterial activity was studied using the agar disk diffusion method, and the MIC and MBC were determined. Results obtained showed a positive correlation between the antioxidant content of pollen extracts and the antibacterial capacity, *Punica granatum* and *Quercus ilex* pollen extracts were the most efficient against Gram-positive and Gram-negative bacteria, and *Centaurium erythraea, Coriandrum sativum,* and *Ruta graveolens* had a moderate effect, while *Citrus aurantium* had no antibacterial effect. It is concluded that pollens can be a good source of bioactive molecules that exhibit potent antioxidant effects and strong antibacterial activities.

1. Introduction

The biggest challenge of public health in recent years is antibiotic resistance. This phenomenon is caused by the unreasonable use of antibacterial drugs; it aggravates the impact of microbial infections on public health. The search for alternative therapeutic strategies by discovering other antibacterial agents has attracted many scientists [1].

Recently, research studies on beehive products, especially honey and bee pollen, have attracted much attention due to their richness in bioactive molecules and their pharmacological effects [2, 3]. On the contrary, pollens are essential for honey bees. They are the main source of proteins, fats, minerals, and vitamins necessary for their nutrition. The honey composition depends strongly on the type of flowers foraged by bees [4].

Pollens contain a mixture of essential nutrients used by the plant to boost their growth and development [5]. Hence, the chemical composition of pollen is mainly determined by its plant species. Their chemical compounds may have different biological activities such as antitumoral, antidiabetic, and antimicrobial effects [6–8].

Many studies indicate that polyphenol compounds and, especially, flavonoids are among secondary metabolites that have many biological activities, such as antioxidant [9], anticancer [10], and antibacterial activities [11].

During the present investigation, pollens from six plants were selected: *Punica granatum, Quercus ilex*, *Centaurium erythraea*, *Coriandrum sativum*, *Ruta graveolens*, and *Citrus aurantium*. Based on the literature, these plants are widely used in traditional medicine. They have significant biological activities such as the antimicrobial effect showed by *Punica*

granatum [12], the control of the gastrointestinal disorders and dietary uptake of glucose at the intestinal tract found by *Quercus ilex* [13], the protective effect against oxidative stress of *Centaurium erythraea* [14], the antinociceptive and antiedema properties of *Coriandrum sativum* [15], the protective effect of *Ruta graveolens* against gastric ulcer [16], and hepatoprotective effect of *Citrus aurantium* [17].

The main goal of this paper would help to highlight the antioxidant potential and antibacterial effect of pollens obtained from five plant families: Fagaceae, Gentianaceae, Punicaceae, Rutaceae, and Apiaceae. Polyphenol, flavone, and flavonol contents were determined, and the antioxidant activities were performed by four tests: total antioxidant capacity, DPPH, ABTS, and reducing power. In contrast, the antibacterial test was evaluated against six bacterial strains: *Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, Acinetobacter baumannii, Klebsiella pneumoniae,* and *Staphylococcus aureus.*

This is the first report on the antioxidant content and antibacterial activity of pollen of these selected plants.

2. Materials and Methods

2.1. Pollen Samples. Pollens of *Quercus ilex* (Fagaceae), *Centaurium erythraea* (Gentianaceae), *Punica granatum* (Punicaceae), *Citrus aurantium* (Rutaceae), *Ruta graveolens* (Rutaceae), and *Coriandrum sativum* (Apiaceae) were collected from the city of Fez (latitude: 34°01′59″ N; longitude: 5°00′01″ W; altitude: 406 m), Morocco.

2.2. Pollen Extracts. 1 g of pollen was extracted using ethanol (50%) for one week, sonicated for 5 min, and centrifuged at 2000× g and 20°C; the obtained supernatants were filtered through a 0.45 μm filter paper. The extracts were kept at −20°C until use.

2.3. Total Phenolic Content. The quantification of phenolic compounds was estimated using the protocol of Mărghitaş et al. [18]. In brief, 100 μL of pollen extracts was mixed with 500 μL of Folin–Ciocalteu reagent (0.2 N) and 400 μL of sodium carbonate solution (7.5%). The mixture was kept in the dark for two hours, and then the absorbance was measured at 760 nm (PerkinElmer Lambda 40 UV/Vis spectrophotometer). Gallic acid was used as a standard to achieve the calibration curve ($R^2 = 0.996$). The results were expressed in mg equivalent of gallic acid per gram of pollen (mgGAE/g). Tests were carried out in triplicate.

2.4. Flavone/Flavonol Content. Flavone/flavonol content was determined according to the method of Miguel et al. [19]. A mixture of 300 μL of Al_2Cl_3 (20%) and 300 μL of each ethanolic pollen extract was incubated for 1 h. Then, the absorbance was measured at 420 nm with a PerkinElmer Lambda 40 UV/Vis spectrophotometer. Quercetin (0.261 to 1.423 mg/mL) was used as a standard to achieve the calibration curve ($R^2 = 0.997$). The results were expressed in milligram of quercetin equivalent (QE) per gram of pollen (mg QE/g). The tests were carried out in triplicate.

2.5. Total Antioxidant Capacity by the Phosphomolybdenum Assay. The phosphomolybdenum assay was used for the determination of the total antioxidant capacity (TAC) according to the method of Zengin et al. [20]. About 25 μL of each ethanolic pollen extract or ascorbic acid (used as the standard) was mixed with 1 mL of reagent solution (6 M of sulfuric acid, 28 mM of sodium phosphate, and 4 mM of ammonium molybdate). Then, after 90 min of incubation in a water bath at 95°C, the absorbance of the solution was measured at 695 nm against the control with a PerkinElmer Lambda 40 UV/Vis spectrophotometer. The results were expressed in milligrams of ascorbic acid equivalent (AAE) per gram of pollen (mg AAE/g). The tests were carried out in triplicate.

2.6. Free Radical Scavenging Activity (DPPH). Free radical scavenging activity (DPPH assay) was measured according to the method described by Miguel et al. [19]. 75 μL of different dilutions of each pollen extract or BHT (used as the positive control) was added to 825 μL of DPPH solution (63.4 μM, prepared in ethanol), the reaction was carried out in the dark for one hour, and the absorbance was taken at 517 nm with a PerkinElmer Lambda 40 UV/Vis spectrophotometer. The tests were carried out in triplicate. The percentage of the DPPH radical scavenging inhibition was estimated using the following equation:

$$\%\ \text{inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100. \quad (1)$$

The concentration of each pollen sample required to scavenge 50% of DPPH (IC_{50}) was determined graphically using the curve plotted by the percentage of DPPH inhibition as a function of the sample concentration. The IC_{50} values were expressed in μg/mL.

2.7. Azino-bis(3-ethylbenzothiazoline-6-sulphonic Acid) (ABTS) Free Radical Scavenging Activity. The ABTS assay was determined following the method of Silva et al. [21]. In brief, 75 μL of different dilutions of each ethanolic pollen extract or BHT (used as the positive control) was added to 825 μL of ABTS radical cation solution. The resulting solutions were incubated in the dark for 6 min at room temperature, and the intensity of the produced color was measured at 734 nm by UV/Vis spectrophotometer. The percentage of ABTS radical cation inhibition was calculated using equation (1). The IC_{50} values were determined graphically and expressed in μg/mL.

2.8. Reducing Power (RP). The reducing power was carried out according to the method of Padmanabhan and Jangle [22]. A mixture of 50 μL of the ethanolic pollen extract, 200 μL of 0.2 M sodium phosphate buffer (pH 6.6), and 200 μL of 1% potassium ferricyanide was incubated at 50°C for 20 min, and then 200 μL of 10% trichloroacetic acid was

added and immediately mixed with 600 μL of distilled water and 120 μL of 0.1% ferric chloride. The mixture's absorbance was measured at 700 nm with a PerkinElmer Lambda 40 UV/Vis spectrophotometer. Ascorbic acid was used as a positive control. A graph was plotted using the absorbance at 700 nm against the sample concentration. The results were represented as the concentration of the pollen extract providing 0.5 of absorbance (EC_{50}). The EC_{50} values were expressed in μg/mL.

2.9. Antibacterial Activity

2.9.1. Bacterial Strains. The study was performed on Gram-positive bacteria, *Staphylococcus aureus* (20s1), and Gram-negative bacteria, *Acinetobacter baumannii* (118e1), *Enterobacter cloacae* (57e2/n), *Escherichia coli* (7), *Klebsiella pneumoniae* (6), and *Pseudomonas aeruginosa* (8e1); all bacterial strains were collected from human specimens and obtained from the Laboratory of Microbiology, Faculty of Medicine and Pharmacy Fez. Bacterial strains were selected based on their resistance to antibiotics.

2.9.2. Sensitivity Test. (1) *Agar Disk Diffusion Method.* The antibacterial activity of pollen extracts was studied using the disk diffusion method [23]; 20 ml of the Mueller–Hinton agar (MHA) medium was poured into Petri dishes. Each Petri dish was inoculated with a bacterial inoculum consisting of 0.5 McFarland (1-2) $*$ 10^8 CFU/ml. Sterile filter paper discs (6 mm diameter) were impregnated with 10 μl of the ethanolic pollen extract and then deposited into the medium. Negative control was made using a filter paper disk saturated with 50% ethanol to check the possible activity of this solvent against the bacteria tested, and standard discs containing antibiotics (6 mm) were used as a reference control. All Petri dishes were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured in mm. Each experiment was carried out in triplicate.

2.9.3. Activity Measurement. (2) *Minimum Inhibitory Concentration (MIC).* The broth microdilution method was used to determine the minimum inhibitory concentration [24]. It was performed by a dilution series of pollen extracts (0.097 to 50 mg/ml). 10 μl of each concentration of this dilution series was mixed in microplate wells with 180 μl of Mueller–Hinton broth and 10 μl of bacterial inocula at a final microbial concentration of 5×10^5 CFU/ml. The final volume was 200 μl, and the concentration of ethanol in each well does not exceed 2.5%. The same percentage of ethanol was used as a negative control; the microplates were cultivated at 37°C for 20 h. The bacterial growth was determined after adding 20 μl of 0.5% TTC (2,3,5-triphenyltetrazolium chloride) aqueous solution and incubated for 30 min at 37°C and 120 rpm. MIC was defined as the lowest concentration of the extract that inhibited visible growth (showed by the absence of red color after the addition of TTC) [25].

(3) *Minimum Bactericidal Concentration (MBC).* To determine MBC, a portion from each well (>or = MIC) was subcultured on Muller–Hinton agar (MHA) and incubated at 37°C for 24 h. MBC was defined as the lowest concentration of the extracts at which the inoculated bacteria were 99.9% killed. The experiments were done in triplicate.

The antibacterial effect was considered bactericidal or bacteriostatic according to the report MBC/MIC: if MBC/MIC = 1-2, the effect is bactericidal and if MBC/MIC = 4 to 16, the effect is bacteriostatic [26].

2.10. Statistical Analyses. GraphPad Prism 5 software was used for statistical comparisons using one-way ANOVA, followed by Tukey's multiple comparisons. The level of significance was set at $p < 0.05$. Correlations between the parameters studied were achieved by the Pearson correlation coefficient (r). XLSTAT software was used to create heatmaps.

3. Results and Discussion

3.1. Antioxidant Content and Activities of Pollen Extracts. The chemical composition and pollen antioxidant activities are enormously varied worldwide and related to plant species and their environmental conditions [27]. Results illustrated in Figure 1 showed wide variability of the polyphenol content between samples. *Punica granatum* pollen extract had the highest value (246.07 ± 2.62 mg GAE/g), followed by *Quercus ilex* pollen extract (146.37 ± 1.95 mg GAE/g). However, the lowest polyphenol content was recorded in the *Centaurium erythraea* pollen extract (21.39 ± 0.31 mg GAE/g), which is higher than that reported by Cosmulescu et al. [28] for three Romanian walnut pollen samples ranging between 10.8 and 17.64 mg GAE/g. Avşar et al. found that the phenolic content of *Castanea sativa* pollens ranged between 64.02 ± 0.26 and 103.8 ± 6.72 mg GAE/g; these results were higher than those obtained by *Centaurium erythraea, Coriandrum sativum, Ruta graveolens*, and *Citrus aurantium* pollen extracts and lower than those found in *Quercus ilex* and *Punica granatum* pollen extracts [8].

For flavones/flavonols, *Quercus ilex* pollen extract presented the highest content (40 ± 0.26 mg QE/g) followed by the *Punica granatum* pollen extract (32 ± 0.9 mgQE/g) and *Ruta graveolens* pollen extract (9.78 ± 1.17 mg QE/g), whereas *Citrus aurantium, Centaurium erythraea*, and *Coriandrum sativum* pollen extracts expressed the lowest contents (6.11 ± 0.03 mg QE/g, 5.56 ± 0.13 mgQE/g, and 5.18 ± 0.26 mgQE/g, respectively). The flavone/flavonol content average of analyzed samples (86.42 ± 83.05 mg QE/g) was higher than that found in three pollen samples harvested from different areas of Romania (7.98 ± 0.26 mg QE/g) [28].

Regarding antioxidant ability, it is recommended to be concluded based on multiple antioxidant test models [29–31]. In the present study, the examined extracts were evaluated by four different and complementary methods (TAC, DPPH, ABTS, and RP).

Total antioxidant assay (TAC) is a test carried out at acidic pH. It is based on the reduction of Mo(VI) to Mo(V)

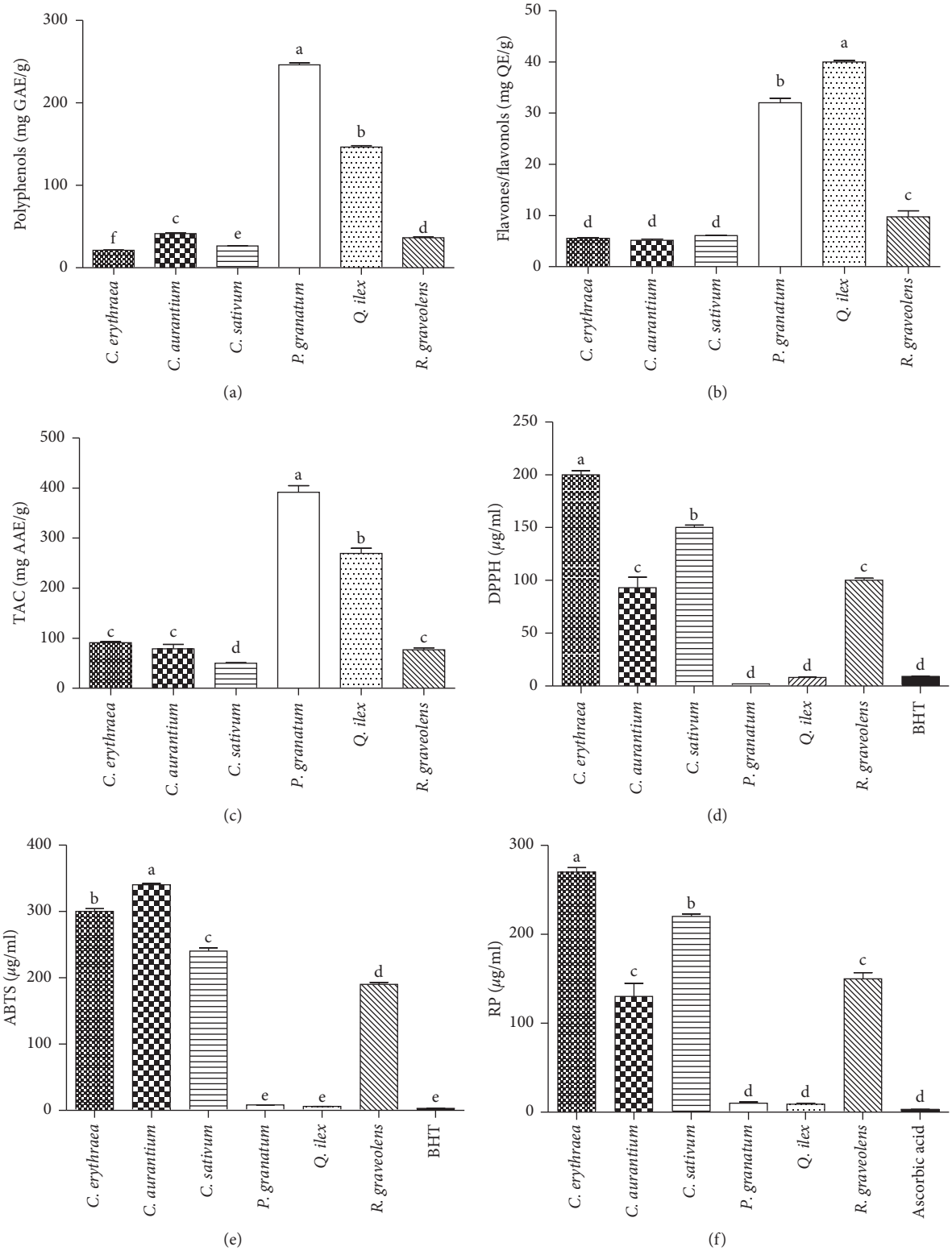


Figure 1: Antioxidant content and antioxidant activities of pollens: (a) the content of polyphenols; (b) the content of flavones/flavonols; (c) the content of total antioxidant capacity (TAC); (d) IC_{50} of the DPPH test; (e) IC_{50} of the ABTS test; (f) EC_{50} of reducing power. The results with the same letter in the same test are not significantly different by Tukey's multiple range test ($p < 0.05$); data are the means of three replicates.

by the analyzed extract and the subsequent formation of a green phosphate/Mo(V) complex [32].

Free radical scavenging DPPH is a rapid, easy, economical, and widely used method. It is based on the reduction of purple 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH•) radical to nonradical yellow-colored diphenylpicrylhydrazine (DPPH-H) in the presence of the hydrogen-donating antioxidant [31, 33].

The ABTS cation radical assay ($ABTS^{\bullet+}$) is based on the loss of an electron by the nitrogen atom of ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid)) following an interaction with the hydrogen-donating antioxidant. In the presence of antioxidant compounds, the nitrogen atom quenches the hydrogen atom, producing solution decolorization [33].

The reducing power method is based on the absorbance increase of the reaction mixture. The antioxidant compounds which have a reducing power react firstly with potassium ferricyanide (Fe^{3+}), then with trichloroacetic acid, and finally with ferric chloride to form a colored complex that has an absorption maximum at 700 nm (ferric-ferrous complex) [29].

The highest total antioxidant capacity was registered in the *Punica granatum* pollen extract (391.47 ± 13.39 mg AAE/g) followed by the *Quercus ilex* pollen extract (269.97 ± 9.69 mg AAE/g). In contrast, the *Coriandrum sativum* pollen extract presented the lowest antioxidant capacity (50.35 ± 0.63 mg AAE/g), the same has been observed concerning DPPH, ABTS, and RP tests, and *Quercus ilex* and *Punica granatum* pollen extracts had the highest antioxidant capacities (DPPH, $IC_{50} = 8 \pm 0.19$ and 2 ± 0.03 μg/ml, ABTS, $IC_{50} = 6 \pm 0.3$ and 8 ± 0.1 μg/ml, and RP, $EC_{50} = 9 \pm 1$ and 10 ± 1.5 μg/ml, respectively). These antioxidant capacities were close to those expressed by BHT and ascorbic acid (Figure 1), while the *Centaurium erythraea* pollen extract expressed the lowest antioxidant activities (DPPH, $IC_{50} = 200 \pm 3.91$ μg/ml, ABTS, $IC_{50} = 300 \pm 4$ μg/ml, and (RP, $EC_{50} = 270 \pm 5$ μg/ml); these results were better than those reported by Araújo et al. for nine poly- and monofloral bee pollen samples from different localities of Brazil, where the IC_{50} values of DPPH, ABTS, and RP were ranging from 1.94 ± 0.17 to 7.99 ± 0.21 mg/ml, the IC_{50} values of ABTS were ranging between 0.91 ± 0.05 and 5.73 ± 0.16 mg/ml, and the EC_{50} values of RP were ranging from 1.82 ± 0.14 to 8.77 ± 0.23 mg/ml [27]. Despite the significant correlation found between the four antioxidant tests (Table 1), it is necessary to highlight that the examined pollen extracts react differently toward each antioxidant assay [34]. For instance, using DPPH and TAC tests, *Punica granatum* was categorized as the pollen extract with the highest antioxidant activity followed by the *Quercus ilex* pollen extract, while by ABTS and RP tests, we observed the opposite. Similarly, the pollen extracts identified as having the lowest antioxidant activity were classified depending on the chosen test. For DPPH and RP assays, the lowest activity was displayed by the *Centaurium erythraea* pollen extract. For the ABTS test, *Citrus aurantium* pollen extract was categorized as the lowest scavenging activity, while with the TAC test, it was found that the lowest antioxidant activity was registered by *Coriandrum sativum*. Thus, the hierarchization of pollen extracts depends on the sensitivity of the applied method to discriminate the antioxidant activities of the studied samples [35].

Electron transfer-based assays used in this study are consistent, and conventional methods are used to examine the antioxidant properties of plant extracts and functional foods. However, the acidic condition may affect the effectiveness of the antioxidant activity in pollen; thus, this condition is addressed with a specific assay, namely, CUPRAC (cupric ion reducing antioxidant capacity) method [36]. This assay was also recommended to describe the hydroxyl-based antioxidative capacity for dietary polyphenols [36, 37].

3.2. Antibacterial Activity. The antibacterial susceptibility testing was carried out using twenty-eight standard antibiotics; the results obtained were interpreted according to Clinical and Laboratory Standards Institute (CLSI) as susceptible (if the inhibition zone was ≥20 mm), intermediate (if the inhibition zone was between 15 and 19 mm), and resistant (if the inhibition zone was ≤14 mm) [38]. Following these criteria, it has been concluded from the results presented in Table 2 that *Enterobacter cloacae* was resistant to amoxicillin + clavulanic acid (20 + 10 μg/disc) (12 ± 0.11 mm), ampicillin (10 μg/disc) (6 ± 0 mm), ceftazidime (30 μg/disc) (6 ± 0 mm), cefotaxime (30 μg/disc) (9 ± 0 mm), cefoxitin (30 μg/disc) (11 ± 0.12 mm), cephalothin (30 μg/disc) (6 ± 0 mm), gentamicin (10 μg/disc) (10 ± 0.54 mm), pefloxacin (5 μg/disc) (6 ± 0 mm), ciprofloxacin (5 μg/disc) (6 ± 0 mm), nalidixic acid (30 μg/disc) (6 ± 0 mm), and trimethoprim + sulfamethoxazole (1.25 + 23.75 μg/disc) (6 ± 0 mm). *Escherichia coli* was resistant to amoxicillin + clavulanic acid (20 + 10 μg/disc) (6 ± 0 mm) and ampicillin (10 μg/disc) (6 ± 0 mm), while *Klebsiella pneumonia*e was resistant to amoxicillin + clavulanic acid (20 + 10 μg/disc) (11 ± 0 mm) and ampicillin (10 μg/disc) (6 ± 0 mm), *Staphylococcus aureus* was resistant to fusidic acid (10 μg/disc) (8 ± 0 mm), and *Pseudomonas aeruginosa* was resistant to ticarcillin (75 μg/disc) (11 ± 0.21 mm), piperacillin (100 μg/disc) (10 ± 0 mm), and ceftazidime (30 μg/disc) (10 ± 0 mm).

Over the last years, antibiotic resistance has become one of the severe threats to human health. Nowadays, the incurable infections caused by multidrug-resistant bacteria remain among the leading causes of mortality in both developed and developing countries. For this, it is essential to find solutions and to develop new strategies to overcome this phenomenon [39, 40]. In this context, pollens could represent an appropriate matrix to search for new, safer, and effective antibacterial molecules that can replace or boost the efficiency of conventional antibacterial drugs.

In the present study, ethanolic extracts of pollens were evaluated as natural antibacterial agents against multidrug-resistant clinical isolates: Gram-positive (*Staphylococcus aureus*) and Gram-negative bacteria (*Acinetobacter baumannii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*). As shown in Table 3, the used

Table 1: Pearson correlation coefficients.

	Polyphenols	Flavones/flavonols	TAC	DPPH	ABTS	RP
Polyphenols		0.862*	0.989***	−0.857*	−0.870*	−0.861*
Flavones/flavonols	0.862*		0.891*	−0.868*	−0.950**	−0.884*
TAC	0.989***	0.891*		−0.827*	−0.878*	−0.840*
DPPH	−0.857*	−0.868*	−0.827*		0.835*	0.997***
ABTS	−0.870*	−0.950**	−0.878*	0.835*		0.833*
RP	−0.861*	−0.884*	−0.840*	0.997***	0.833*	
Acinetobacter baumannii	0.765	0.911*	0.768	−0.803	−0.979***	−0.793
Enterobacter cloacae	0.723	0.823*	0.766	−0.504	−0.830*	−0.518
Escherichia coli	0.703	0.870*	0.717	−0.793	−0.934**	−0.788
Klebsiella pneumoniae	0.865*	0.936**	0.870*	−0.797	−0.920**	−0.802
Pseudomonas aeruginosa	0.908*	0.989***	0.934**	−0.864*	−0.932**	−0.882*
Staphylococcus aureus	0.759	0.907*	0.750	−0.834*	−0.963**	−0.822*

Statistical significance, $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

solvent for pollen extraction (50% ethanol) did not exert any inhibition diameter on the examined strains as expected. This result was also observed in another study carried out by Akter et al. [41]. However, *Centaurium erythraea* pollen extract was efficient against *Enterobacter cloacae* with an inhibition zone of 12 ± 1 mm, *Citrus aurantium* pollen extract does not affect all bacterial strains, and *Coriandrum sativum* pollen extract was efficient against all bacteria except *Pseudomonas aeruginosa* and *Escherichia coli*. According to the classification of Ramalhosa et al. [42], *Punica granatum* and *Quercus ilex* pollen extracts exhibited a strong antibacterial potential with high inhibition diameter against all tested strains with a remarkable inhibition zone of 19.33 ± 0.33 mm and 22.33 ± 1.20 mm, respectively, against *Staphylococcus aureus*. However, *Ruta graveolens* pollen extract was effective against *Escherichia coli* (14 ± 0.57 mm), *Acinetobacter baumannii* (13 ± 0.88 mm), and *Staphylococcus aureus* (14 ± 0.57 mm). The results of the agar disk diffusion method revealed that *Staphylococcus aureus* (Gram-positive) was more sensitive to the examined pollen extracts than Gram-negative bacterial strains. These results are in line with the results of Velásquez et al. who showed that Gram-positive bacteria are more susceptible than Gram-negative bacteria [43]. This may be due to the difference in the composition of the bacterial wall [44, 45]. The cytoplasmic membrane of Gram-positive bacteria is particularly rich in anionic phospholipids than that of Gram-negative bacteria, which contributes to the intrinsic resistance of some Gram-negative bacteria to antibiotics. For instance, daptomycin, a lipopeptide antibiotic, is effective against Gram-positive bacteria but is not active against Gram-negative bacteria because of its inability to cross the outer membrane, which is a necessary step for its antibacterial activity [46].

As can be seen from Table 4, all pollen extracts that are effective against bacterial strains present minimum inhibitory concentration (MIC) values ranged between 0.31 and 2.5 mg/ml. The lowest MIC was referred to *Punica granatum* and *Quercus ilex* pollen extracts against *Acinetobacter baumannii* (0.31 mg/ml) and *Staphylococcus aureus* (0.62 mg/ml). At the same time, no antibacterial effect was registered against all bacterial strains treated with the *Citrus aurantium* pollen extract. Semeniuc et al. [44] showed that MIC values of ethanolic extracts of date palm pollen were ranged between 7.81 mg/ml for *Escherichia coli* and 15.6 mg/ml for *Bacillus cereus*. These values were higher than those carried out by our extracts.

The minimum bactericidal concentration (MBC) values were also ranged between 0.31 and 2.5 mg/ml (Table 4), with the lowest minimum bactericidal concentration being expressed by *Punica granatum* pollen on *Acinetobacter baumannii*. However, the highest MBC values were carried out by *Centaurium erythraea* on *Enterobacter cloacae* and *Coriandrum sativum* against all tested strains except *Escherichia coli* (no effect) and *Pseudomonas aeruginosa* (no effect), *Ruta graveolens* on *Escherichia coli,* and *Punica granatum* and *Quercus ilex* pollen extracts against all bacteria except *Acinetobacter baumannii* and *Staphylococcus aureus*. Most importantly, among all pollen samples, *Punica granatum* and *Quercus ilex* pollen extracts that presented the highest antioxidant potential (Figure 1) displayed better antibacterial activity against *Pseudomonas aeruginosa* that was found to be resistant against all types of synthetic and natural antibacterial agents [47]. The wide MIC and MBC differences between pollen samples could be attributed to the characteristic of the chemical composition of each pollen in addition to the membrane structure and variable cell wall of examined bacterial strains [44, 48].

It has been reported that polyphenols were used as a new strategy to fight bacterial resistance [49]. Several reports have shown that phenolic compounds could be used in combination with antibacterial drugs to potentiate their therapeutic effects or to minimize their side effects [50, 51]. Lin et al. showed that the combination of rifampicin, kaempferol, and quercetin (flavonol compounds) inhibit synergistically β-lactamase of clinical methicillin-resistant *Staphylococcus aureus* (MRSA) [52]. Similarly, Liu et al. studied the effect of kaempferol glycosides isolated from *Laurus nobilis* combined with fluoroquinolones against MRSA, and they found that kaempferol glycosides boost the anti-MRSA effect of fluoroquinolones [53]. Furthermore, Wu et al. demonstrated that flavonoids (baicalein, chrysin, galangin, kaempferol, luteolin, myricetin, quercetin, tangeritin, and nobiletin) exhibited good antibacterial activities against *Escherichia coli* via the inhibition of bacterial DNA gyrase [54]. Nawwar et al. showed that *Punica granatum*

Table 2: Antibacterial activities of standard antibiotics.

Acinetobacter baumannii		*Enterobacter cloacae*		*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*		*Staphylococcus aureus*	
Antibiotic used	Inhibition zone (mm)	Antibiotic used	Inhibition zone (mm)	Antibiotic used	Inhibition zone (mm)	Antibiotic used	Inhibition zone (mm)	Antibiotic used	Inhibition zone (mm)	Antibiotic used	Inhibition zone (mm)
TZP	27 ± 0.54 (S)	AMP	6 ± 0 (R)	AMC	6 ± 0 (R)	AMC	11 ± 0 (R)	TIC	11 ± 0.21 (R)	FOX	30 ± 0.54 (S)
TIC	27 ± 0.22 (S)	AMC	12 ± 0.11 (R)	CAZ	31 ± 0.52 (S)	CAZ	36 ± 0.78 (S)	PRL	10 ± 0 (R)	MY	30 ± 0.22(S)
CAZ	24 ± 0.56 (S)	CAZ	17 ± 0.63 (I)	CTX	35 ± 0.42 (S)	CTX	40 ± 0.65 (S)	CAZ	10 ± 0 (R)	FOS	46 ± 0.55 (S)
PRL	25 ± 0.62 (S)	CTX	9 ± 0 (R)	IPM	32 ± 0.54 (S)	IPM	38 ± 0.32 (S)	TOB	22 ± 0.26 (S)	FD	8 ± 0 (R)
IPM	33 ± 0.77 (S)	FOX	11 ± 0.12 (R)	AMP	6 ± 0 (R)	AMP	6 ± 0 (R)	CS	16 ± 0.87 (I)	P	18 ± 0.54 (I)
TOB	26 ± 0 (S)	KF	6 ± 0 (R)	NA	26 ± 0 (S)	NA	30 ± 0.69 (S)	CIP	34 ± 0.69 (S)	E	28 ± 0.25 (S)
AK	25 ± 0.15 (S)	ETP	31 ± 0.85 (S)	CN	20 ± 0.52 (S)	CN	19 ± 0 (I)	CN	18 ± 0.77 (I)	CN	30 ± 0.35 (S)
CN	25 ± 0.82 (S)	CN	10 ± 0.54 (R)	FOX	26 ± 0.12 (S)	FOX	31 ± 0.21(S)	AK	23 ± 0.25 (S)	RD	34 ± 0.85 (S)
CIP	30 ± 0.42 (S)	AK	23 ± 0.10 (S)	NOR	31 ± 0.45 (S)	NOR	33 ± 0.52 (S)	ETP	21 ± 0.36 (S)		
SXT	27 ± 0.14 (S)	PEF	6 ± 0 (R)	FOS	27 ± 0.98 (S)	FOS	37 ± 0.32(S)				
		CIP	6 ± 0 (R)								
		NA	6 ± 0 (R)								
		SXT	6 ± 0 (R)								
		FOS	28 ± 0.25 (S)								

AMC: amoxicillin + clavulanic acid (20 + 10 μg/disc); AK: amikacin (30 μg/disc); AMP: ampicillin (10 μg/disc); CAZ: ceftazidime (30 μg/disc); CIP: ciprofloxacin (5 μg/disc); CN: gentamicin (10 μg/disc); CS: colistin (30 μg/disc); CTX: cefotaxime (30 μg/disc); E: erythromycin (15 μg/disc); ETP: ertapenem (10 μg/disc); FD: fusidic acid (10 μg/disc); FOS: fosfomycin (50 μg/disc); FOX: cefoxitin (30 μg/disc); IPM: imipenem (10 μg/disc); KF: cephalothin (30 μg/disc); MY: lincomycin (15 μg/disc); NA: nalidixic acid (30 μg/disc); NOR: norfloxacin (10 μg/disc); P: penicillin G (10 μg/disc); PEF: pefloxacin (5 μg/disc); PRL: piperacillin (100 μg/disc); RD: rifampicin (30 μg/disc); SXT: trimethoprim + sulfamethoxazole (1.25 + 23.75 μg/disc); TEC: teicoplanin (30 μg/disc); TIC: ticarcillin (75 μg/disc); TOB: tobramycin (10 μg/disc); TZP: piperacillin + tazobactam (30 + 6 μg/disc); VA: vancomycin (30 μg/disc); (R): resistant; (S): susceptible; (I): intermediate.

Table 3: Antibacterial activities of pollen extracts and ethanol.

Pollen samples	Mean of the inhibition diameter zone (mm)					
	Acinetobacter baumannii	*Enterobacter cloacae*	*Escherichia coli*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Centaurium erythraea	7 ± 0.88^{e}	12 ± 1^{d}	7 ± 1^{e}	6 ± 0.01^{d}	6 ± 0.01^{c}	6 ± 0.01^{f}
Citrus aurantium	6 ± 0.01^{e}	6 ± 0.01^{f}	6 ± 0.01^{f}	6 ± 0.01^{d}	6 ± 0.01^{c}	7 ± 1^{e}
Coriandrum sativum	11 ± 0.18^{d}	13 ± 0.57^{c}	8 ± 0.2^{d}	11 ± 0.88^{c}	6 ± 0.01^{c}	13 ± 0.88^{d}
Punica granatum	17 ± 0.54^{b}	17 ± 0.88^{b}	15 ± 0.57^{b}	18.66 ± 0.88^{b}	17 ± 1^{b}	19.33 ± 0.33^{b}
Quercus ilex	19 ± 0.88^{a}	18 ± 0.33^{a}	17.33 ± 0.88^{a}	19.66 ± 0.88^{a}	18.33 ± 0.88^{a}	22.33 ± 1.20^{a}
Ruta graveolens	13 ± 0.88^{c}	8 ± 0.1^{e}	14 ± 0.57^{c}	6 ± 0.01^{d}	6 ± 0.01^{c}	14 ± 0.57^{c}
Ethanol (control)	6 ± 0.01^{e}	6 ± 0.01^{f}	6 ± 0.01^{f}	6 ± 0.01^{d}	6 ± 0.01^{c}	6 ± 0.01^{f}

Diameter of the inhibition zone produced around the disks by the addition of 10 μl of extracts. Diameter of the disc = 6 mm is included. All values are represented as the mean of the inhibition zone ± SD. Values in the same column followed by the same letter are not significantly different by Tukey's multiple range test ($p < 0.05$).

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration of pollen extracts.

Pollen samples		Bacterial strains					
		Acinetobacter baumannii	*Enterobacter cloacae*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Centaurium erythraea	MIC (MCB) (mg/ml)	No effect	2.5 (2.5)	No effect	No effect	No effect	No effect
	MBC/MIC	—	1	—	—	—	—
	Effect type	—	Bactericidal	—	—	—	—
Citrus aurantium	MIC (MCB) (mg/ml)	No effect	No effect	No effect	No effect	No effect	No effect
	MBC/MIC	—	—	—	—	—	—
	Effect type	—	—	—	—	—	—
Coriandrum sativum	MIC (MCB) (mg/ml)	2.5 (2.5)	2.5 (>2.5)	No effect	2.5 (>2.5)	No effect	2.5 (2.5)
	MBC/MIC	1	—	—	—	—	1
	Effect type	Bactericidal	—	—	—	—	Bactericidal
Punica granatum	MIC (MCB) (mg/ml)	0.31 (0.31)	2.5 (2.5)	2.5 (2.5)	2.5 (>2.5)	2.5 (2.5)	0.62 (0.62)
	MBC/MIC	1	1	1	—	1	1
	Effect type	Bactericidal	Bactericidal	Bactericidal	—	Bactericidal	Bactericidal
Quercus ilex	MIC (MCB) (mg/ml)	0.31 (0.31)	1.25 (1.25)	1.25 (2.5)	2.5 (2.5)	2.5 (2.5)	0.62 (0.62)
	MBC/MIC	1	1	1	1	1	1
	Effect type	Bactericidal	Bactericidal	Bactericidal	Bactericidal	Bactericidal	Bactericidal
Ruta graveolens	MIC (MCB) (mg/ml)	1.25 (1.25)	No effect	2.5 (2.5)	No effect	No effect	1.25 (1.25)
	MBC/MIC	1	—	1	—	—	1
	Effect type	Bactericidal	—	Bactericidal	—	—	Bactericidal

pollen contains several phenolic compounds such as rutin, kaempferol, luteolin, catechin, epicatechin, naringin, and quercetin, which possess potent antibacterial efficacy [55]. Similarly, Han et al. analyzed different parts of *Punica granatum* (juice, peel, and seeds), and they found 18 polyphenol compounds including cinnamic acid, caffeic acid, gallic acid, protocatechuic acid, ellagic acid, ferulic acid, chlorogenic acid, p-hydroxybenzoic acid, benzoic acid, phloridzin, phloretin, luteolin, punicalagin, catechin, taxifolin, epicatechin, vanillin, and quercetin in which punicalagin and protocatechuic acid were the major components [56]. Likewise, *Quercus ilex* contains a wide range of polyphenols, including tannin, flavonol, phenolic acid, flavanone, and coumarin compounds [57]. Furthermore, it was found that the main phenolic compounds present in *Centaurium erythraea* were sinapic acid, *p*-coumaric acid, ferulic acid, and kaempferol [58]. All these arguments support the hypothesis that our samples might contain these bioactive molecules and exert their antibacterial effect through their synergic interactions.

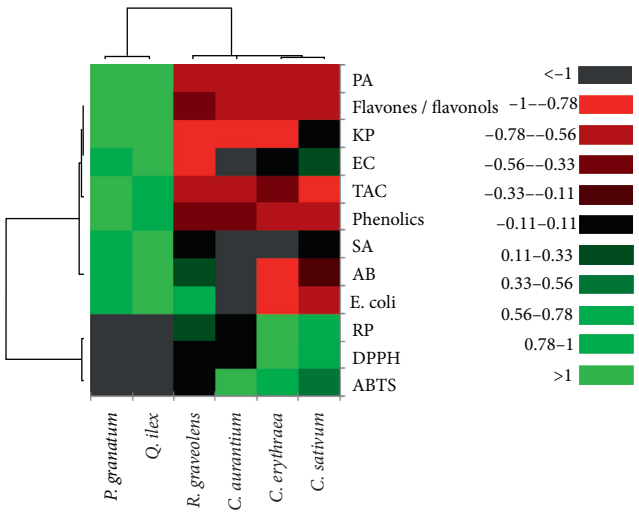


Figure 2: Heatmap representation of antioxidant and antibacterial correlations.

3.3. Correlation. The correlation test is considered as a good tool to reveal any relationship between different parameters studied. In the current work, by using the mean values of antioxidant capacity of all pollens tested, we studied the correlation between the antioxidant content and antioxidant activities and between the antioxidant content and antibacterial activities. The outcomes are represented in Table 1. A strong positive relationship between phenolics, flavone/flavonol contents, and the total antioxidant capacity (TAC) was observed. On the contrary, a strong negative correlation was established between bioactive content (phenolic compounds and flavones/flavonols) and antioxidant activity evaluated by TAC, DPPH, ABTS, and RP assays. Results were mainly explained by the high content of bioactive ingredients in pollens. Concerning the relationship between the antioxidant content and antibacterial activities against different strains studied, we found a strong positive correlation between the flavone/flavonol content and the antibacterial activities exerted by different pollen extracts against all bacterial strains. Our findings are in good agreement with previous studies, which showed the correlation relationship between the antioxidant content and antibacterial activity [59–61].

Figure 2 shows the analysis based on the Pearson correlation. Lower concentrations were displayed in black, while higher concentrations were displayed in red color. The heatmap facilitates the task to regroup different samples based on their similitudes. It is observed that the pollen samples have been divided into two groups. One group was composed by *Quercus ilex* and *Punica granatum*, while the other group was formed by *Centaurium erythraea*, *Coriandrum sativum*, *Ruta graveolens*, and *Citrus aurantium*. The heatmap also shows the correlation relationship between the antioxidant content and antioxidant/antibacterial activities.

4. Conclusion

This is the first research to report the antioxidant content and the effect of six pollen samples collected from different botanical sources on human multidrug-resistant bacterial strains. The current results indicated that the studied pollen extracts are promising sources of natural antioxidants with potent bactericidal action. The best activities were recorded by *Punica granatum* and *Quercus ilex* pollen extracts.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors would like to thank Laboratory (SNAMOPEQ), Faculty of Sciences, Dhar El Mehraz, for providing the facilities to carry out the research work. This study was funded by University Sidi Mohamed Ben Abdellah, P.O. Box 1796, Fez Atlas, Fez, Morocco (USMBA/PPSE/L08FSDM2018).

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