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Evaluation of the content of polyphenols, flavonoids, tannins, antioxidant capacity and antibacterial activity of *Pelargonium graveolens* flower extracts

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Abstract

Pelargonium graveolens has garnered attention for its therapeutic properties, especially in treating dermatological conditions. The aim of this study was to investigate the phytochemicals, antioxidant action and antibacterial effect of its flower extracts obtained through maceration, sonication and infusion. The findings show that the highest total phenolic content was obtained from the hydroethanolic sonication extract (280.39 mg GAE/g), followed by the hydroethanolic maceration extracts (272.87 mg GAE/g). The total flavonoid content varied from 21.51 mg EQ/g (hydroethanolic maceration) to 12.32 mg EQ/g (aqueous infusion). Condensed tannins were highest in the hydroethanolic maceration extracts (8.40 mg/mL). Additionally, the antioxidant action was assessed using the DPPH, FRAP, TAC and ABTS methods. The extracts from hydroethanolic sonication showed a high free radical scavenging capacity (IC_{50} = 0.31 mg/mL) compared with extracts from aqueous infusion and hydroethanolic maceration. However, antibacterial tests revealed that all extracts had better activity against Gram-positive bacteria. The hydroethanolic maceration extracts showed pronounced sensitivity, exhibiting zones of inhibition ranging from 13 to 24 mm for Gram-negative bacteria and 19 to 24 mm for Gram-positive bacteria. These extracts demonstrated significant efficacy against methicillin-resistant *Staphylococcus aureus* and *Panton-Valentine Leukocidin-Positive Staphylococcus aureus*. These findings suggest potential applications in the pharmaceutical and dermatological fields, particularly for developing natural antioxidant and antibacterial agents.

Keywords

Pelargonium graveolens; polyphenols; flavonoids; tannins; antioxidant capacity; antibacterial activity

Introduction

Bacterial skin infections such as impetigo, folliculitis, abscesses and necrotising fasciitis are prevalent in dermatological consultations (1). These infections can range from benign to serious and potentially fatal (2). They are mainly caused by *Streptococcus pyogenes* (group A) and *Staphylococcus aureus*, including methicillin-resistant strains (MRSA) and *Panton-Valentine leucocidin*-producing strains (*PVL-SA*). The persistence of these pathogens tableis often linked to a compromised host defense system and the

expression of virulence factors, notably surface proteins and toxins. However, the limited epidemiological data on these infections in Morocco represents a major challenge for the development of effective treatments, particularly against antibiotic-resistant strains (3).

In Morocco, traditional medicine holds significant importance, leveraging a rich botanical heritage that is supported by diverse bioclimatic conditions (4). The country is home to over 4200 species of flora, establishing it as a major global supplier of aromatic and medicinal plants (5). Furthermore, from a socioeconomic perspective, the cultivation and development of medicinal and aromatic plants (MAP) offer promising opportunities for diversifying agricultural production and enhancing income generation for local communities (6). In recent years, traditional medicine has gained renewed interest, significantly enhancing drug discovery, as a significant proportion of modern pharmaceuticals are derived from plant sources, underscoring its importance in meeting global health challenges and promoting therapeutic innovation (7). However, the main reason behind this interest lies primarily in the declining efficacy of synthetic drugs and awareness of their adverse effects (8, 9).

Pelargonium species, native to South Africa, were introduced into Europe in the 17th century and have since been hybridized throughout the world. Belonging to the *Geraniaceae* family (10), within the order Geraniales, it comprises of 5-7 genera and approximately 830 species of dicotyledonous flowering plants, distributed across temperate, subtropical and tropical regions. Their flowers are typically bisexual and actinomorphic, with 4 to 5 sepals that are free or slightly connate and persistent (11). *Pelargonium graveolens*, commonly known as Rose Geranium, is one such *Pelargonium* species that has garnered attention for its therapeutic properties with particular emphasis on dermatological conditions (44 %) (12). Numerous studies have demonstrated its pharmacological properties of arial part, particularly its antibacterial, antifungal and antioxidant activities (13). To date, however, no studies have been carried out on the effect of its flower against pathogenic bacteria implicated in skin infections. Further comprehensive phytochemical and pharmacological investigations of the flower are necessary to fully elucidate its therapeutic potential.

As part of the growing interest in natural remedies, this study assesses the polyphenol, flavonoid and tannin content of *P. graveolens* flower extracts. In addition, the *in vitro* antioxidant capacity was assessed using 4 complementary methods: DPPH, ABTS, TAC and FRAP. The antibacterial activity of these extracts was evaluated using the agar well diffusion method and the microdilution test on pathogenic and resistant clinical strains involved in skin diseases.

Materials and Methods

Plant materials

The flowers of *P. graveolens* were harvested (Fig. 1) from Sahel Boutaher, Taounate, located in the Fes-Meknes region of Morocco, in May 2022. Subsequently, the flowers underwent a 2 weeks drying process at room temperature, after which they were finely powdered using an electric grinder (Fritsch, Industriestrasse 8, 55743 Idar-Oberstein, Germany). The powdered material was then carefully stored for subsequent extraction procedures.

Fig. 1. *Pelargonium graveolens* flower collected from the field.

Preparation of the extracts

Extraction procedures represent a fundamental aspect in the isolation of specific chemical compounds from plant materials. Employing both polar and non-polar solvents is imperative to achieve effective extraction of the desired components. In the present study, we employed 3 distinct extraction methodologies: Maceration, sonication and infusion.

The infusion method involved introducing 2.5 g of plant material into a recipient containing 75 mL of boiling distilled water. Following a 40 min infusion period, the mixture was allowed to cool. Subsequently, the maceration technique was employed, wherein 10 g of plant material were introduced into an Erlenmeyer flask containing 100 mL of ethanol, or alternatively, a mixture of ethanol and water (70 % ethanol - 30 % water). Continuous magnetic stirring for 24 h at ambient temperature ensured comprehensive extraction of the target compounds. Lastly, the sonication method was employed, whereby 2.5 g of plant material were subjected to extraction using 50 mL of ethanol, followed by another extraction utilizing a mixture of 70 % ethanol and 30 % water. The application of ultrasonic waves for 45 min facilitated the breakdown of plant cell walls, thereby enhancing extraction efficiency.

Following each extraction procedure, the resultant solutions underwent filtration using Whatman no. 1 paper to eliminate solid residues. Subsequent centrifugation at 3000 rpm for 10 min facilitated the separation of any particulate matter. A fraction of the extract was maintained in its unaltered state, while another fraction underwent vacuum evaporation using a rotary evaporator to remove the

extraction solvent. All resulting extracts were meticulously stored at 4 °C for subsequent analysis and application.

Phenolic compounds quantification

Total polyphenol content

The total polyphenol content (TPC) of all extracts was determined (14) and as described in a previous study with some modifications (15), using the Folin-Ciocalteu reagent. Briefly, 450 µL of Folin-Ciocalteu (10-fold diluted) and 450 µL of sodium carbonate (7.5 %) were added successively to 50 µL of extracts. After 2 h of incubation in obscurity, absorbance was measured at 765 nm by a UV-VIS spectrometer (Rohs, UV-1800pc ultraviolet spectroscopy) and results were expressed in terms of gallic acid equivalents per g of plant material (µg GAE/g), (y = 4.081x + 0.2432, R^2 = 0.9965).

Total flavonoid content

Total flavonoid content (TFC) of crude extracts was quantified according to the aluminum trichloride method with some modifications (16). Briefly, 500 µL of aluminum chloride solution (10 %) was added to 500 µL of diluted extract, followed by incubation in the dark for 1 h. Absorbance was measured at 420 nm using a UV-VIS spectrometer (Rohs, UV-1800pc ultraviolet spectroscopy, Macy, China) and concentration was determined using a standard curve of quercetin prepared using the same conditions (y = 14.424 x + 0.206 and R^2 = 0.998).

Condensed tannins (CT)

Condensed tannins were analyzed using the Bate-Smith reaction (17). In short, 1 mL of each extract was combined with 0.5 mL of distilled water and 1.5 mL of 37 % hydrochloric acid. The mixture was heated for 30 min in a water bath at 100 °C, then absorbance was measured at 550 nm against a control containing tubes at room temperature. The difference in the absorbance between the hydrolyzed sample and the control was considered as the content of tannins contained in the extract using the following equation:

$$\text{Tannins (mg/mL)} = \left(A_{\text{Hydrolysed}} - A_{\text{Control}} \right) \times 19.33$$

Antioxidant activity

DPPH radical scavenging method

The DPPH° free radical scavenging activity of extracts was determined following the method (18). In brief, a 0.05 mL sample of the diluted extract was added to 1.95 mL of the freshly prepared methanolic solution of DPPH (2.5 mg dissolved in 100 mL methanol under stirring for 3 h) and then the mixtures were incubated for 30 min in the dark. The absorbance was then measured at 517 nm, and ascorbic acid was used as the standard antioxidant following the same operating conditions.

ABTS° + radical scavenging method

Extracts of *P. graveolens* flowers were also evaluated for their ability to trap the $ABTS^{\circ+}$ radical (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) according to standard procedure (19). The ABTS solution was prepared by mixing 50 % of 7 mM ABTS and 50 % of 2 mM potassium peroxide sulfate, followed by incubation at ambient temperature for 16 h. For further processing, this solution was diluted with H_2O until an absorbance of 0.700 ± 0.002 at 734 nm was obtained. For sample analysis, 500 µL of sample solution was homogenized with 1 mL of ABTS solution, then the mixture was incubated at room temperature for 7 min and its absorbance was recorded at 734 nm.

Ferric reducing antioxidant power

Ferric reducing antioxidant power (FRAP) test was performed according to the standard method (20). Briefly, 0.25 mL of each diluted extract (6 sets of dilutions) was added to 1.25 mL of the phosphate buffer solution and 1.25 mL of the aqueous solution of potassium ferricyanide (1 %) and the mixture was incubated for 20 min at 50 °C in a water bath. After cooling to ambient temperature, 1.25 mL of 10 % trichloroacetic acid was added to the reaction mixture, followed by centrifugation (Mikro 220R, Hettich, Westphalian, Germany) at 3000 rpm for 10 min, then 1.25 mL of the supernatant was added to 1.25 mL of distilled water and 0.25 mL of ferric chloride solution (0.01 %). Absorbance was measured after 10 min of incubation at a wavelength of 700 nm. Results are expressed in terms of ascorbic acid equivalents per mg of plant material (µg GAA/mg), Y = 3.2283 + 0.2706 and R^2 = 0.9999.

Total antioxidant capacity

The total antioxidant capacity (TAC) was estimated (21) with minor modifications. Briefly, 200 µL of each extract at different concentrations was added to 3000 µL of a reagent composed of sulfuric acid (60 M), disodium phosphate (280 mM) and ammonium molybdate (40 mM). The mixture was incubated at 95 °C for 90 min, then cooled down to room temperature for 20-30 min before measuring the absorbance at 695 nm. The control consisted of 200 µL of methanol with 3000 µL of the above reagent. Results are expressed in terms of ascorbic acid equivalents/mg of plant material (µg GAA/mg), y = 1.5683x + 0.3666, R^2 = 0.9688.

TAC and FRAP results were quantified as mg of ascorbic acid equivalent per g of plant material (mg GAA/g), while DPPH and ABTS tests were represented by IC_{50} (mg/mL). This parameter is defined as the concentration of the antioxidant that induces a 50 % reduction of DPPH or ABTS radical activity.

Antibacterial activity

P. graveolens flower extracts were tested against 8 clinical bacteria: *Pseudomonas aeruginosa*, *Streptococcus pyogenes, Staphylococcus aureus, Panton-Valentine Leukocidin Staphylococcus aureus (PVL-SA)*, methicillin-resistant *Staphylococcus aureus (MRSA), Escherichia coli*, *E. coli* producing extended spectrum β-lactamase *(E. coli - ESBL), Bacillus anthracis* and *Klebsiella pneumoniae.* These strains, selected for their pathogenicity and propensity to cause skin infections, were sourced from the University Hospital Center Hassan II.

Sensitivity testing

A disc diffusion method as described by the Clinical and Laboratory Standards Institute (22), was employed to evaluate the sensitivity profile of the targeted bacterial strains. Clinical isolates were cultivated on Brain Heart Infusion (BHI) agar plates and incubated at 37 °C for 18 h. A single colony from each strain was then inoculated into 5 mL of Mueller-Hinton broth (MHB). Following a 6 h incubation at 37 °C, bacterial suspensions were adjusted to the 0.5 McFarland standard (1.5 $\times 10^8$ CFU/mL) using sterile saline (0.85 % NaCl). Mueller-Hinton Agar (MHA) plates were uniformly inoculated with bacterial suspensions using sterile swabs. After a 5 min drying period, antibacterial discs (amoxicillin 10 µg, ciprofloxacin 5 µg, gentamicin 10 µg, tetracycline 30 µg, amikacin 30 µg, ampicillin 10 µg, methicillin 5 µg, Oxacillin 10 µg, vancomycin 30 µg) were placed on the inoculated plates using sterile forceps, ensuring adequate spacing to prevent overlapping inhibition zones. The plates were then incubated at 37 °C for 18 h. Post-incubation, the diameters of the inhibition zones were measured in mm and are presented in Table 1 for further comparison with extracts inhibition capacity. The absence of an inhibition zone was indicated as a resistant strain.

Table 1. Antibiogram profile of the selected bacterial strains

Antibiotic (family)	Disc Potency	Inhibition zone diameter (mm) and resistance of clinical isolates							
		EC	BLSE	PA	SP	BA	SARM	KSP	SA
Gentamicin (Aminoglycosids)	10 µg	14	14	20	17	15	23	R	
Amikacin (Aminoglycosids)	30 µg	16	20	18	-	-	22	-	-
Ampicillin (Betalactamins)	10 µg	R	R	16	R	R	14	R	20
Methicilin (Betalactamins)	5 µg	-	-	-	-	-	R	-	17
Oxacillin (Betalactamins)	10 µg	R	R	14	R	-	14	-	-
Tetracyclin (Cyclins)	30 µg	R	R	22	R	R	R	-	24
Vancomycin (Glycopeptids)	30 µg	R	R	20	R	R	16	17	22

EC : Escherichia coli, **BLSE** : E.coli producing extended spectrum β-lactamase, PA: Pseudomonas aeruginosa, **SP**: Streptococcus pyogenes, **BA**: Bacillus anthracis, **SARM** : Methicillin-resistant Staphylococcus aureus, **KSP**: Klebsiella pneumoniae, **SA**: Staphylococcus aureus, **R**: no inhibition zone recorded as resistant strain, (**-**): not specified.

Inoculum's preparation

Bacterial regrowth was achieved by subculturing the agar plate on the Luria-Bertani (LB) agar surface and incubating at 37 °C for 18 to 24 h. The bacterial inoculum was prepared from fresh colonies using the direct colony suspension method. Briefly, 1-2 colonies were suspended in sterile saline (NaCl 0.9 %) and adjusted to the 0.5 McFarland standard to achieve standardized inoculum (10^8 CFU/mL).

Agar well diffusion method

The antibacterial activity of our extracts was qualitatively tested against the pathogenic strains as reported earlier (23) using the agar well diffusion method. Briefly, 1 mL of the bacterial inoculum was spread evenly across the entire surface of Luria-Bertani agar (LB). Following this, a sterile plug drill was used to aseptically create a hole with a diameter of 5 mm, into which 80 µL of the extract solution was added at a concentration of 50 mg/mL. The bacterial strains were then incubated for 24 h at 37 °C and the diameter of the inhibition zones was measured in mm.

Determination of the minimum inhibitory concentration

The determination of the minimum inhibitory concentration (MIC) of our extracts was conducted using a 96-well plate method (24). In brief, 100 µL of serial concentrations ranging from 16 to 0.12 mg/mL of the extracts were dispensed into each well. Subsequently, 50 µL of bacterial inoculum prepared in LB liquid medium was added to each well. The plates were then incubated at 37 °C for 18 h, after which 10 µL of resazurin (0.05 %) was added to each well to assess bacterial growth. The plates were re-incubated for an additional 2 h at 37 °C. The MIC values represent the concentration (mg/mL) observed in the well just prior to the initial occurrence of pink coloration.

Statistical analysis

The experiments were performed in triplicate, with results presented as mean ± SD. One-way ANOVA followed by Tukey's test was used for statistical analysis and comparison of means, considering differences significant at $p < 0.05$. The data were analyzed using Origin 2024 software.

Results and Discussion

Phenolic compounds

Total phenolic compounds (TPC) contents, flavonoids (TFC) and condensed tannins (CT) of *P. graveolens* flower extracts are presented in Table 2. The hydroethanolic soni-

Table 2. Phenolic compounds of *P. graveolens* flowers extracts

Methods	Sonication		Maceration		Infusion
	EtOH- H_2O	EtOH	EtOH- H_2O	EtOH	H_2O
TPC (mg GAE/g of plant material	280.39 ± 3.78^{a}	134.31 ± 1.57^{b}	272.87 ± 2.58^{c}	168.94 ± 0.19^{d}	159.69 ± 1.23^{e}
TFC (mg QE/g of plant material)	$19.93 \pm 1.61^{a,b}$	$18.16 \pm 2.59^{a,b}$	21.51 ± 2.16^{a}	$16.15 \pm 1.04^{b,c}$	12.32 ± 0.36^{c}
CT (mg /mL)	6.63 ± 0.44^{a}	3.50 ± 0.43^{b}	8.40 ± 0.59^{c}	$7.92 \pm 0.42^{a,c}$	4.83 ± 0.62^{b}

Values are expressed as means ± standard error (n = 3). Letters in the same row are statistically significant at $p < 0.05$. values were compared by using one way ANOVA followed by t-test. **TPC**: total phenolic content; **TFC**: total flavonoid content; **CT**: Condensed Tannin; mg GAE/ g plant material: mg gallic acid equivalent per g of plant material; mg QE/ g plant material: mg quercetin equivalent per g of plant material.

cation extract yielded the highest content of TPC (280.39 ± 3.78 mg GAE/g of plant material), followed by the hydroethanolic and ethanolic extracts via maceration (272.87 ± 2.58 and 168.94 ± 0.19 mg GAE/g plant material respectively), infusion (159.69 ± 1.23 mg GAE/g) and ethanolic extract via sonication (134.31 ± 1.57 mg GAE/g). In a recent study (25), there conducted in Meknes, Morocco, the aerial parts of *P. graveolens* have been reported to provide the highest total phenolic content in the methanol extract, reaching 381.25 ± 2.65 mg GAE/g. In the current investigation, *P. graveolens* flower phenolic content was extracted using solvents of different polarities and a selection of extraction techniques. The results underscore that polar and non-polar organic solvents are suitable for extracting phenolic compounds. Additionally, statistical analysis revealed a significant difference ($p > 0.05$) between maceration, sonication and infusion methods for TPC extraction using ethanol and water as eco-friendly solvents. The hydroethanolic extracts have been indicated to promote the extraction of specific polyphenols like catechin, epicatechin and epigallocatechin (26). Comprehensively, the addition of water to organic solvents increases the solubility of polyphenols because it weakens the hydrogen bonds in aqueous solutions, facilitating the extraction process (27). TFC varied from 21.51 ± 2.16 to 12.32 ± 0.36 mg QE/g of plant material, corresponding respectively to the hydroethanolic extracts by maceration and the water infusion. Moreover, the aqueous extract exhibited the lowest TFC, possibly attributable to the limited solubility of certain compounds such as quercetin and kaempferol in water solvent and extractable with organic solvents (28). However, flavonoids, prevalent in plant extracts, exist either as aglycones or glycosides, the latter being linked to a sugar moiety via a glycosidic bond (29). Glycosylated flavonoids exhibit enhanced water solubility (30). The findings reveal a significant difference ($p < 0.05$) in TFC between the infusion method and the sonication method. Notably, within the maceration method, a distinction emerged between absolute ethanol and a 70 % ethanol-water solution. In contrast, the sonication method exhibited no statistical difference ($p < 0.05$) when employing absolute ethanol or a hydroethanolic solution for TFC extraction.

Similarly, the highest overall CT was obtained by maceration extraction using hydroethanolic solution (8.40 ± 0.59 mg/mL), followed by ethanolic maceration (7.92 ± 0.42 mg/mL), hydroethanolic sonication (6.63 ± 0.44 mg/mL) and water infusion (3.50 ± 0.43 mg/mL). Statistically, within the sonication method, a significant difference was observed between CT values when using absolute ethanol and ethanol diluted with 30 % water as extract solution, whereas this was not the case for the maceration method ($p < 0.05$).

Antioxidant activity

The antioxidant potential of *P. graveolens* flower extracts were evaluated using the DPPH, FRAP, TAC and ABTS methods, as detailed in Table 3. Across all extracts, a notable capacity for scavenging free radicals was observed. Vitamin C, serving as our positive control, exhibited the highest DPPH radical scavenging potential, with an IC_{50} value of 0.14 ± 0.03 mg/mL, followed by the hydroethanolic sonication extract (IC_{50} = 0.31 ± 0.02 mg/mL), while the lowest capacity was attributed to the ethanolic maceration extract (IC_{50} = 0.68 ± 0.04 mg/mL). There was no significant difference ($p < 0.05$) observed among the IC_{50} values of the hydroethanolic sonication and the water infusion extracts. Regarding the ABTS radical scavenging ability of *P. graveolens* flower, no significant difference ($p < 0.05$) was found between vitamin C and the hydroethanolic sonication, the hydroethanolic and the ethanolic maceration extracts. Following vitamin C, both hydroethanolic extracts from sonication and maceration demonstrated the highest activity in scavenging the ABTS radical, with the lowest IC_{50} values recorded as 0.025 ± 0.021 mg/mL and 0.180 ± 0.030 mg/mL respectively. Conversely, the water infusion extract exhibited the lowest potential in scavenging the ABTS radical (IC_{50} = 1.27 ± 0.264 mg/mL). However, it is noteworthy that the IC_{50} value of Vitamin C in scavenging the ABTS radical, which is equal to 0.004 mg/mL, was lower than that of all tested extracts.

Concerning the FRAP test, the hydroethanolic extract obtained by sonication and water infusion extract showed the highest activity (435.78 ± 1.95 and 418.19 ± 1.94 mg GAA/g of plant material respectively), followed by the hydroethanolic and the ethanolic maceration extracts (431.63 ± 1.39 and 403.15 ± 2.05 mg GAA/g respectively) and the ethanolic sonication extract (389.44 ± 5.02 mg GAA/g). Similarly, the hydroethanolic sonication and the water infusion extracts presented the highest total antioxidant capacity (TAC), (458.05 ± 1.92 and 340.21 ± 3.59 mg GAA/g respectively), whereas the ethanolic extracts in both maceration and sonication methods exerted the lowest activity. In the case of hydroethanolic maceration extract, a statistically significant correlation was found between the TPC and its corresponding antioxidant activity, as evaluated according to the DPPH and ABTS methods ($p < 0.05$, r = 0.99). Moreover, phenolic compounds such as gallic acid and vanillic acid have been shown to have significant

Table 3. Antioxidant activity of *P. graveolens* flower extracts

Methods	Vitamin C	Sonication		Maceration		Infusion
		EtOH- H_2O	EtOH	EtOH- H_2O	EtOH	H_2O
FRAP (mg GAA/g plant material)	-	435.78 ± 1.95^a	389.44 ± 5.02^b	431.63 ± 1.39^a	403.15 ± 2.05^c	418.19 ± 1.94^d
TAC (mg GAA/g plant material)	-	458.05 ± 1.92^a	334.86 ± 2.09^b	428.19 ± 3.03^c	325.28 ± 5.53^d	340.21 ± 3.59^b
DPPH (IC_{50} mg/mL)	0.14 ± 0.03^a	0.31 ± 0.02^b	0.58 ± 0.03$^{c, d}$	0.52 ± 0.11^d	0.68 ± 0.04^c	0.35 ± 0.05^b
ABTS (IC_{50} mg/mL)	0.004 ± 0.001^a	0.025 ± 0.021^a	0.610 ± 1.165^b	0.180 ± 0.030^a	0.170 ± 0.062^a	1.270 ± 0.264^b

Values are expressed as means ± standard error (n = 3). Letters in the same row are statistically significant at $p < 0.05$. **DPPH**: DPPH free radical scavenging activity; **FRAP**: Ferric reducing antioxidant power assay; **ABTS**: ABTS radical scavenging assay; **TAC**: Total antioxidant capacity. mg GAA/ g plant material t: mg gallic acid equivalent per g of plant material.

antioxidant properties (31, 32). Additionally, flavonoids including kaempferol, myricetin and eriodictyol glucoside pentoside, have been reported to contribute to the antioxidant effect, as previously elucidated by researchers (33-35). Phenolic compounds, valued for their antioxidant qualities, are notably influenced by specific structural features, such as the presence of an ortho-dihydroxybenzene group, the arrangement of hydroxyl groups and the existence of an enone unit (36). These structural factors play a pivotal role in determining the extent of their antioxidant activity (37).

Antibacterial activity

The most common bacterial agents that cause skin and soft tissue infections are *S. aureus*, including methicillin-resistant forms (*MRSA*) and *Panton Valentine Leukocidin Staphylococcus aureus* (*PVL-SA*). Other implicated microorganisms include *P. aeruginosa, E. coli, B. anthracis* and *K. pneumoniae* (38-41).

In the present study, the inhibitory effect of *P. graveolens* flower extract against a range of pathogenic bacterial strains involved in skin infections was evaluated and the results are presented in Table 4. Inhibition zone (IZ) measurements are expressed in millimeters, ranging from sensitive (9-14 mm) to very sensitive (15-19 mm) based on the established criteria (42). Notably, all extracts exhibited greater activity against Gram-positive bacteria compared to Gram-negative counterparts. Inhibition zones ranged from 11.0 to 29.0 mm and MIC values for sensitive strains fell within the range of 0.25 to 2.67 mg/mL. Particularly the hydroethanolic maceration extract exhibited pronounced sensitivity, displaying inhibition zones ranging from 13.0 to 24.5 mm for gram-negative strains and 19.0 to 24.3 mm for gram-positive strains. The water infusion extract showcased a bacteriostatic effect against *P. aeruginosa,* evidenced by a substantial 29.0 mm inhibition zone and a MIC value of 0.50 mg/mL. This extract also exerted robust inhibitory effects on *B. anthracis, S. aureus* and *S. pyogenes*, with inhibition zones in order, measuring 20.0, 20.0 and 22.0 mm in diameter and MIC values of 0.75, 0.70 and 1.16 mg/mL. Conversely, *ESBL-EC, E. coli* and *K. pneumoniae* demonstrated heightened resistance against the extracts, with inhibition diameters ranging from 11.0 to 15.0 mm.

Table 1 lists the inhibition diameters of gentamicin, amikacin, ampicillin, oxacillin, tetracycline and vancomycin of 20, 18, 16, 14, 22 and 20 mm respectively, against *P. aeruginosa*. Conversely, *S. pyogenes* showed resistance to ampicillin, oxacillin, tetracycline and vancomycin but displayed sensitivity to the tested *P. graveolens* flower extracts. This discovery therefore adds considerable potential value regarding its pharmaceutical and dermatological applications. Furthermore, *MRSA* and *PVL.SA* showed lower MICs, ranging from 2.67 to 0.83 mg/mL and from 0.33 to 1.08 mg/mL respectively. Phenolic compounds such as kaempferol, quercetin, protocatechuic acid and caffeic acid, have been proven to be potent antibacterial agents against clinical strains of *S. aureus* and *MRSA* (43). These compounds, with their phenolic hydroxyl groups, show a strong affinity for protein binding, potentially inhibiting bacterial enzymes and increasing their interaction with cytoplasmic membranes.

Conclusion

The present study demonstrated that *P. graveolens* flower

Table 4. Antibacterial activity of *P. graveolens* flower extracts, expressed by inhibition zone in mm and minimum inhibitory concentration in mg/mL

Strains	Methods	Sonication		Maceration		Infusion
		EtOH-H_2O	EtOH	EtOH-H_2O	EtOH	H_2O
P. aeruginosa	IZ	22.5 ± 0.1^{a}	24.5 ± 0.1^{ab}	24.5 ± 0.1^{ab}	26.0 ± 0.3^{ac}	29.0 ± 0.1^{c}
	MIC	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}	0.50 ± 0.00^{a}
S. aureus	IZ	20.5 ± 0.1^{a}	19.5 ± 0.1^{a}	19.0 ± 0.1^{a}	19.5 ± 0.1^{a}	20.0 ± 0.0^{a}
	MIC	0.37 ± 0.18^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}	0.70 ± 0.35^{a}
E. coli	IZ	13.5 ± 0.2^{a}	13.5 ± 0.3^{a}	14.0 ± 0.2^{a}	16.5 ± 0.1^{a}	15.0 ± 0.0^{a}
	MIC	> 16	> 16	> 16	> 16	> 16
B. anthracis	IZ	20.0 ± 0.1^{a}	23.5 ± 0.1^{a}	20.0 ± 0.2^{a}	20.0 ± 0.1^{a}	20.0 ± 0.1^{a}
	MIC	0.37 ± 0.17^{a}	0.50 ± 0.00^{a}	0.25 ± 0.00^{a}	0.25 ± 0.00^{a}	0.75 ± 0.35^{a}
K. pneumoniae	IZ	14.0 ± 0.5^{a}	11.0 ± 0.2^{a}	14.0 ± 0.5^{a}	13.0 ± 0.3^{a}	12.0 ± 0.0^{a}
	MIC	> 16	> 16	> 16	> 16	>16
BLSE-EC	IZ	15.0 ± 1.0^{a}	14.0 ± 0.1^{a}	13.0 ± 0.7^{a}	11.0 ± 0.0^{a}	15.0 ± 1.1^{a}
	MIC	> 16	> 16	> 16	>16	> 16
MRSA	IZ	11.0 ± 0.1^{ac}	13.0 ± 0.0^{a}	15.0 ± 0.2^{b}	11.0 ± 0.0^{ac}	10.0 ± 0.0^{c}
	MIC	2.67 ± 1.15^{a}	1.67 ± 0.58^{a}	0.83 ± 0.29^{a}	2.16 ± 1.75^{a}	2.33 ± 1.52^{a}
PVL-*SA*	IZ	21.3 ± 0.6^{ab}	21.3 ± 0.6^{ab}	24.3 ± 1.2^{a}	22.7 ± 1.5^{ab}	19.3 ± 0.6^{b}
	MIC	0.41 ± 0.14^{a}	0.41 ± 0.14^{a}	0.33 ± 1.14^{a}	1.08 ± 0.87^{a}	0.58 ± 0.38^{a}
S. pyogenes	IZ	22.3 ± 0.6^{a}	21.0 ± 0.0^{a}	24.0 ± 0.0^{a}	22.0 ± 0.0^{a}	22.0 ± 0.1^{a}
	MIC	0.50 ± 0.43^{a}	0.22 ± 0.28^{a}	0.29 ± 0.19^{ab}	1.67 ± 0.58^{b}	1.16 ± 0.77^{ab}

Values are expressed as means ± standard error (n = 3). Letters in the same row are statistically significant at $p < 0.05$. **MIC**: Minimum inhibitory concentration, **IZ**: Inhibition zone. *B. anthracis*: *Bacillus anthracis*, ***E. coli*** : *Escherichia coli*, **ESBL-EC**: *Escherichia coli* producing extended spectrum β-lactamase, **MRSA**: Methicillin-resistant *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*, **PVL-SA**: Panton-valentine leukocidin *Staphylococcus aureus*, *S. aureus*: *Staphylococcus*

extracts are abundant in phenolic compounds when extracted by sonication and hydroethanolic maceration. Furthermore, *P. graveolens* extracts show strong antioxidant activity, particularly in DPPH and FRAP assays and significant inhibition of Gram-positive bacteria, including resistant strains such as MRSA and PVL-SA. Resistant strains such as MRSA and S. pyogenes in particular showed greater sensitivity to the extracts as compared to antibiotics. The high content of phenolic compounds probably contributes to this antibacterial effect, underlining the potential of these extracts in pharmaceutical and dermatological applications against resistant bacterial infections. Future studies are required to identify and isolate the active compound behind the potent antibacterial activity.

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Authors' contributions

NE and AZ were responsible for the study design and manuscript preparation. NE, IZ and HB conducted the laboratory experiments and data analysis. All authors reviewed and approved the final version of the manuscript.

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Conflict of interest: The authors have no conflicts of interest to declare.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used GPT-3.5 in order to improve language and readability, with caution. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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