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Antibacterial Activity of Rose Geranium (*Pelargonium graveolens*) Essential Oil Against Clinical Isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* with Different Antimicrobial Resistance Profiles

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ABSTRACT

This research evaluated the antibacterial activity (ABA) of rose geranium (*Pelargonium graveolens* (L.) L'Hér.) essential oil (EO) against antibiotic-resistant clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and assessed its chemical composition. Hydrodistillation was employed to extract EO, and it was analyzed through gas chromatography-mass spectrometry (GC–MS). The agar disc diffusion technique was employed to assess the ABA of the *Pelargonium graveolens* EO at 50, 100, and 200 mg/mL against the clinical isolates with varying antimicrobial susceptibility profiles. The results of the GC–MS indicated phenylethyl alcohol (74.13%) as the most significant component, then geraniol (10.16), citronellol (7.67), and eugenol (~2). A concentration-dependent ABA of the EO was observed. Against *S. aureus*, inhibition zones increased from 20.00 ± 0.87 mm (50 mg/mL) to 30.00 ± 1.06 mm (200 mg/mL), while *P. aeruginosa* showed 24.00 ± 1.25 mm to 30.00 ± 1.61 mm. Geraniol showed the highest activity against *S. aureus* (up to 50.00 ± 2.76 mm), whereas *P. aeruginosa* was less susceptible to isolated components. *Pelargonium graveolens* EO demonstrated a promising potential for ABA against resistant clinical isolates, especially *S. aureus*. Its potential as a natural antimicrobial agent can be further developed because it has been shown to be possibly associated with the combined contribution of multiple oil constituents.

1. INTRODUCTION

The issue of antimicrobial resistance (AMR) has become a major and pressing challenge to worldwide health in the 21st century, which can jeopardize the successful control and management of infectious diseases. The World Health Organization [1] argues that AMR is mainly fuelled by excess and misuse of antibiotics in human health and agriculture, which have resulted in the rapid development of resistant bacterial strains. Among them, opportunistic pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa* are of particular interest, as these types are often involved in hospital- and community-acquired infections and are characterized by their impressive capacity to develop resistance to various classes of antibiotics [2, 3]. These organisms are linked to various types of infections, such as wound infections, respiratory tract infections, and bloodstream infections, which frequently lead to prolonged hospitalization, greater healthcare expenses, and higher mortality rates [4]. The number of antibiotic resistance cases is constantly growing, and the Centers for Disease Control and Prevention [5] categorizes multidrug-resistant bacteria as a serious issue to the health of the population. Pathogens that can resist antibiotics through adaptive resistance mechanisms like biofilm formation, efflux pumps, and enzymatic degradation are *Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), and multidrug-resistant *Pseudomonas aeruginosa*. These represent the pathogens of greatest clinical relevance. The mechanisms considerably restrict the number of therapeutic options and require the search for other antimicrobial agents.

During recent years, there has been heightened interest in essential oils (EOs) obtained by the extraction of plants as possible sources of new antimicrobial compounds. EOs are complicated blends of volatile secondary metabolites, mainly terpenoids and phenolic compounds, which show a wide-spectrum antimicrobial activity [6]. Their antimicrobial action is usually attributed to the fact that they cause disorganization of bacterial cell membranes, raise the permeability of membranes, and disrupt intracellular metabolic activities. As opposed to traditional antibiotics, EOs tend to have more than one bioactive constituent, which will limit the chances of resistance formation. Rose geranium (*Pelargonium graveolens* (L.) L'Hér.) is an aromatic medicinal plant that is widely dispersed over the temperate and subtropical areas and is extensively grown to extract EO. The oil contains a lot of monoterpenes and oxygenated derivatives, including citronellol, geraniol, and linalool, which have been stated to

have anti-microbial and anti-inflammatory properties [7, 8]. A number of works have shown that *P. graveolens* EO exerts an inhibitory influence on a variety of bacterial pathogens, such as Gram-positive or Gram-negative organisms, but the activity against antibiotic-resistant strains of clinical importance is still under active research [9, 10]. Although these are positive results, additional testing of the antibacterial activity (ABA) of *P. graveolens* EO against clinically isolated, antibiotic-resistant pathogens, and especially *S. aureus* and *P. aeruginosa*, would be beneficial. The growing prevalence of multidrug-resistant strains and the constraints of the existing antibiotic treatment methods are creating an acute need for alternative methods based on medicinal plants.

The current investigation thus examined the antibacterial influence of *Pelargonium graveolens* EO as an antimicrobial agent against clinical isolates with varying antimicrobial susceptibility profiles of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to contribute to the search for novel plant-based antimicrobial agents that could assist in the future development of therapeutics. The originality of this research is the ability to show the antibacterial influence of *Pelargonium graveolens* EO on clinically isolated, antibiotic-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which is supported by gas chromatography-mass spectrometry (GC–MS) profiling. It demonstrates concentration dependency and phytochemical synergy as a novel finding for its potential as an alternative to use against multidrug-resistant pathogens in clinical practice.

2. MATERIALS AND METHODS

2.1 Study population

A group of clinical bacterial isolates of patients who visited Al-Imamain Al-Kadhimain Medical City, Baghdad, Iraq, was employed as the study population. There were 60 non-duplicate isolates comprising 30 *Staphylococcus aureus* and 30 *Pseudomonas aeruginosa*. The isolates were obtained from various clinical specimens in the hospital microbiology laboratory and were reflective of both male and female infections in diverse age groups. The study included only confirmed, pure, and viable cultures as determined by standard microbiological and biochemical techniques. Duplicates of an isolate of a single patient were eliminated to prevent sampling bias. The organisms collected were maintained in the right laboratory conditions and utilized in antimicrobial susceptibility tests and assessment of the antimicrobial influence of *Pelargonium graveolens* EO.

2.2 Ethical approval and sample source description

This investigation received ethical approval from the Health Research Ethics Committee of Al-Nahrain University, Baghdad, Iraq (approval number: 20241141). The management of Al-Imamain Al-Kadhimain Medical City was also contacted to give consent to the collection and use of the clinical bacterial isolates. All the experiments with the clinical samples were carried out according to the ethical principles of the institutional and national research committees and the principles of the Declaration of Helsinki. There was no access or recording of patient identifiers or other isolates. The specimens were collected from wound isolates, blood isolates, throat swabs, urine, burn swab isolates, and fluid isolates. All isolates were treated anonymously to guarantee confidentiality and biosafety regulations.

2.3 Source of plant materials

Pelargonium graveolens plants were collected from the AL-Razi Center for Medicinal Herbs, Baghdad, Iraq. The plants were identified and verified by Prof. Ibrahim Saleh AI-Jubouri in the Department of Pharmacy, University of Al-Mustansiriya, Baghdad, Iraq. Plant identification was confirmed by an official certificate (Letter No. 437, dated June 3, 2025).

The new aerial sections, such as leaves and stems of *Pelargonium graveolens*, were cut and cleansed thoroughly with distilled water to remove any surface contaminants.

2.4 Source of microbial isolates

The microbiology laboratories of Al-Imamain Al-Kadhimain Medical City were sources of the clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Species identification was confirmed using VITEK 2 automated identification systems following preliminary biochemical characterization [11]. Upon identification, pure isolates were cultured and kept in proper media at 4 ℃ until the time they were needed to undergo further analyses.

2.5 Extraction of essential oil

Hydrodistillation was employed to extract EOs by means of a Clevenger-type apparatus. The method was applied to the plant materials (approximately 100 g) in distilled water (500 mL) for 3 hours. The acquired EO was then distilled off the aqueous layer and dried with anhydrous sodium sulphate to remove the remaining moisture. The oil was then put in sterile amber glass vials and kept at 4 ℃ until further examination. Hydrodistillation is also a common standard method of extracting EOs of aromatic plant materials [12]. The EO yield was calculated as the percentage volume per dry weight of plant material employing the following equation:

$$\text{Oil yield (\%)} = (\text{volume of extracted oil/weight of dried plant material}) \times 100$$

2.6 Gas chromatography-mass spectrometry

The chemical characterization of *Pelargonium graveolens* EO was done through the gas chromatograph method coupled with a mass selective detector by the use of GC–MS. The separation was done in a capillary column (30 m × 0.25 mm × 0.25 μm film thickness) in the presence of helium as the carrier gas at a flow rate of 1 mL/min. One microliter was injected in split mode (1:20). The temperature of the oven was kept at 50 ℃ at the beginning, with the increase of the temperature to 200 ℃ at 5 ℃/min, then to 250 ℃ at 10 ℃/min, and sustained at 250 ℃ at 10 ℃/min. The injector and detectors were heated to 250 ℃ and 280 ℃, respectively. Mass spectrometric analysis was performed in electron ionization (EI) at 70 eV with a scan range of 40-500 m/z and a scan rate of 1 scan/sec. Compounds were identified by comparing mass spectra with the NIST library and other literature. Normalization of peak areas was employed to calculate relative percentages of identified constituents. The GC–MS is a well-known method of identifying volatile compounds in EOs [13].

2.7 Culture media

The culture media employed in the study were mannitol. Salt agar to selectively isolate *Staphylococcus aureus*, Cetrimide agar to grow *Pseudomonas aeruginosa*, and Mueller-Hinton agar (MHA) to test antimicrobial susceptibility. MHA plates were inoculated according to the instructions provided on the plate and the Clinical and Laboratory Standards Institute (CLSI) guidelines. To achieve uniformity of test conditions, the agar depth was kept at about 4 mm, following the CLSI protocols [14].

2.8 Preparation of inoculum

Colonies that were freshly grown were harvested and suspended in sterile normal saline and taken 18-24 hours later. The resulting suspension was then diluted to correspond to the turbidity of a 0.5 McFarland standard. This standardization was in line with the approximate concentration of 1×10^8 CFU/mL of bacterial isolates. The inoculum density was critically adjusted in line with the CLSI to maintain uniformity and reproducibility of the antimicrobial susceptibility testing [14].

2.9 Antimicrobial susceptibility testing

The agar disc diffusion method was employed to conduct antimicrobial susceptibility testing following the recommendations of the CLSI [14]. Pelargonium graveolens EO antimicrobial activity was assessed on sterile MHA plates in which evenly spread, standardized suspensions of microbes were inoculated using sterile cotton swabs. The plates that were inoculated were then left to stand for about 10 minutes to allow the absorption and drying of the surfaces before the disc was applied. EO solutions were prepared in dimethyl sulfoxide (DMSO) at concentrations of 50, 100, and 200 mg/mL. A volume of 20 µL of each solution was applied to each disc, providing final doses of 1, 2, and 4 mg/disc, respectively. A fixed volume of 20 µL was applied to each disc. Therefore, the tested concentrations of 50, 100, and 200 mg/mL corresponded to EO loadings of 1, 2, and 4 mg/disc, respectively.

The discs of 6 mm diameter filter paper were impregnated with the EO to prepare the discs. The discs were then dried in sterile conditions to make sure that the test substance adhered properly. The impregnated discs were then placed on the agar surface using sterile forceps and pressed to make contact with the media. Gentamicin (10 µg/disc) was used as the positive control for 30 clinical isolates. Discs containing solvent only served as negative controls. The bacterial cultures were incubated at 37 ℃ for 18-24 hours under the recommended conditions, as indicated by CLSI [14]. After incubation, the areas of inhibition were measured in millimeters with a calibrated ruler. Each assay was repeated three times, and the average values were obtained. Diameters of the inhibition zone were interpreted according to CLSI standards [14]. Multidrug resistance was defined as resistance to at least one agent in three or more antimicrobial categories according to internationally accepted criteria.

2.10 Statistical analysis

Statistical Package for the Social Sciences (SPSS; version 24) software was employed to analyze data statistically. The mean and the standard deviation were employed to present the results. One-way analysis of variance (ANOVA) was employed to determine differences between the experimental groups. A p-value of more than 0.05 was deemed not to be statistically significant. Appropriate statistical analysis is a key to reliable interpretation and validation of antimicrobial activity results [15]. All 60 clinical isolates were included in the essential-oil antibacterial assay (30 Staphylococcus aureus and 30 Pseudomonas aeruginosa isolates). For each concentration (50, 100, and 200 mg/mL), the inhibition-zone diameter is presented as mean ± Standard Deviation (SD) calculated from the 30 clinical isolates of each bacterial species.

3. RESULTS

3.1 Gender distribution of clinical isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Table 1 shows the percentages of clinical isolates by gender. A greater percentage of isolates among the patients was a higher proportion of male patients than female patients. In the case of *Staphylococcus aureus*, males (60%) and females (40%) had 18 and 12 isolates, respectively. On the same note, *Pseudomonas aeruginosa* was more predominant among the males with 19 isolates (63.33%) than the females with 11 isolates (36.67%). In general, 37 (61.67%) of the isolates were from males, and 23 (38.33%) isolates were from females. The difference in distribution observed was statistically significant ($p = 0.0084$), as shown in Table 1.

Table 1. Distribution of bacterial isolates based on patient gender

Organism	Gender		Total
	Male	Female	
Staphylococcus aureus	18 (60.00%)	12 (40.00%)	30
Pseudomonas aeruginosa	19 (63.33%)	11 (36.67%)	30
Total	37 (61.67%)	23 (38.33%)	60
p-Value	0.0084		-
	$^{**}p \leq 0.01$		

3.2 Age and gender distribution of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates

The distribution of *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates in various age groups and genders is presented in Table 2. The isolates were found across all age groups, with the most frequent ones found in the 60–69 and 70–79 age groups, which had 10 cases (16.67%). This was followed by the 80–89 years group (13.33%) and the 20–29, 30–39 years group (11.67% each). The extreme age groups had fewer incidences, especially 1 to 9 and 90 to 100 (3.33 to 5.00%). In general, most of the age groups had a higher prevalence of isolates in males than in females. The observed differences were significant ($p = 0.0001$).

3.3 Gas chromatography-mass spectrometry profile of the *Pelargonium graveolens* essential oil

The EO from *Pelargonium graveolens* was analyzed by GC–MS and was found to consist mainly of phenylethyl alcohol (74.13%), geraniol (10.16%), and citronellol (7.67%), with a minor content of eugenol (2%), as shown in Table 3. These results suggest that the oil is mostly constituted of aromatic alcohols that have potential as fragrances and bioactive compounds for different uses.

Table 2. Age- and gender-based distribution of bacterial isolates

Age (year)	*Staphylococcus aureus*		*Pseudomonas aeruginosa*		Total
	Male	Female	Male	Female	
	N (%)	N (%)	N (%)	N (%)	N (%)
1-9	1 (1.67%)	0 (0.00%)	1 (1.67%)	1 (1.67%)	3 (5.00%)
10-19	2 (3.33%)	2 (3.33%)	1 (1.67%)	0 (0.00%)	5 (8.33%)
20-29	3 (5.00%)	2 (3.33%)	1 (1.67%)	1 (1.67%)	7 (11.67%)
30-39	3 (5.00%)	1 (1.67%)	2 (3.33%)	1 (1.67%)	7 (11.67%)
40-49	1 (1.67%)	0 (0.00%)	1 (1.67%)	0 (0.00%)	2 (3.33%)
50-59	2 (3.33%)	1 (1.67%)	2 (3.33%)	1 (1.67%)	6 (10.00%)
60-69	2 (3.33%)	3 (5.00%)	3 (5.00%)	2 (3.33%)	10 (16.67%)
70-79	3 (5.00%)	2 (3.33%)	3 (5.00%)	2 (3.33%)	10 (16.67%)
80-89	1 (1.67%)	1 (1.67%)	3 (5.00%)	3 (5.00%)	8 (13.33%)
90-100	0 (0.00%)	0 (0.00%)	2 (3.33%)	0 (0.00%)	2 (3.33%)
Total	30		30		60
p-Value	0.000**				

**$p \leq 0.01$

Table 3. Qualitative and quantitative assessment of *Pelargonium graveolens* essential oil (EO) using the gas chromatography-mass spectrometry (GC–MS) analysis

Phytochemical	Retention Time	Match Factor	Content (%)
Eugenol	11.188	98	~2
Geraniol	9.613	96	10.16
Phenylethyl alcohol	7.691	95	74.13
Citronellol	9.232	95	7.67
1-Hydroxy-2-butanone	4.757	25	0.09
2-Butanol, 3,3-oxybis	9.016	72	5.59
2,6-Octadien-1-ol, 3,7-dimethyl	10.037	50	0.15
4(1H)-pyrimidinone, 2-(ethylthio)	26.509	7	0.10

3.4 Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates using the VITEK 2 AST-GP67 card

The results of the antimicrobial susceptibility profile of *Staphylococcus aureus* isolates, which were tested by the use of the VITEK 2 AST-GP67 card, showed that nitrofurantoin and linezolid had complete sensitivity (100%) (Table 4). This observation indicates that all the isolates were highly sensitive to these agents. There was also high susceptibility to teicoplanin (90.00%), tigecycline and rifampicin (83.33%), and trimethoprim/sulfamethoxazole (80.00%). Tobramycin and moxifloxacin (56.67% each), gentamicin and tetracycline (53.33% each), and fusidic acid (50.00%) were found to have moderate sensitivity. Clindamycin (43.33%), inducible clindamycin resistance phenotype (40.00%), and levofloxacin (33.33%) were found to have lower sensitivities. Full resistance (100%), as well as high resistance, was found against cefoxitin screen, benzylpenicillin, and oxacillin, indicating a phenotypic MRSA profile according to the VITEK 2 AST-GP67 system.

Resistance to levofloxacin was 66.67%, inducible clindamycin resistance was 60.00%, clindamycin resistance was 56.67%, resistance to fusidic acid was 50.00%, tetracycline, and gentamicin resistance rate was 46.67%. The sensitivity, intermediate response, and resistance of vancomycin were 73.33, 6.67, and 20.00, respectively. Significant differences in the pattern of susceptibility to most antibiotics were obtained through the statistical analysis ($p \leq 0.01$).

3.5 Anti-*Staphylococcus aureus* activity of *Pelargonium graveolens* essential oil and standard components

Table 5 demonstrates the inhibitory influences of EO constituents of *Pelargonium graveolens* on *Staphylococcus aureus*. The concentration-dependent ABA was observed in all tested compounds, with the size of the inhibition zone growing with the increase in concentration between 50 and 200 mg/mL. Geraniol demonstrated the greatest activity, with an inhibition zone of 30.00 ± 1.06 mm, 40.00 ± 2.17 mm, and 50.00 ± 2.76 mm at 50, 100, and 200 mg/mL, respectively, similar to that of the positive control. Citronellol showed moderate activity, ranging from 30.00 ± 1.06 mm to 40.00 ± 2.08 mm, while eugenol produced zones between 30.00 ± 1.06 mm and 35.00 ± 1.84 mm. The crude *P. graveolens* oil demonstrated the least activity (20.00 ± 0.87 mm to 30.00 ± 1.06 mm). The negative control did not show any inhibition. All the treatments were significant ($p \leq 0.01$).

3.6 Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* isolates using the VITEK 2 AST-N222 card

The results of the antimicrobial sensitivity pattern of *Pseudomonas aeruginosa* isolates that were tested using the VITEK 2 AST-N222 card are highlighted in Table 6. Cefepime and imipenem had the highest sensitivity rates with 12 isolates (40.00%) each, followed by ceftazidime, amikacin, and levofloxacin with 10 isolates (33.33%) each. Piperacillin/tazobactam was sensitive, with 30.00% sensitivity, with gentamicin and ciprofloxacin having lower sensitivities of 23.33% and 16.67%, respectively. Cefazolin and tigecycline (100%) were completely resistant. Ciprofloxacin (73.33%), levofloxacin (66.67%), piperacillin/tazobactam (63.33%), and imipenem/gentamicin (60.00 each) were also found to have high resistance rates. Cefepime (20.00%) occupied the intermediate position. Statistical evaluation revealed that there were considerable differences between the tested antibiotics ($p \leq 0.05$).

Table 4. Antimicrobial susceptibility profile of *Staphylococcus aureus* based on the VITEK 2 AST-GP67 card

Antimicrobial	Sensitive Number (%)	Intermediate Number (%)	Resistant Number (%)	Antimicrobial	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Cefoxitin Screen	0 (0%)	0 (0%)	30 (100%)	Teicoplanin	27 (90%)	0 (0%)	3 (10%)
Benzylpenicillin	0 (0%)	0 (0%)	30 (100%)	Vancomycin	22(73.33%)	2 (6.67%)	6 (20.00%)
Oxacillin	0 (0%)	0 (0%)	30 (100%)	Tetracycline	16 (53.33%)	0 (0%)	14 (46.67%)
Gentamicin	16 (53.33%)	0 (0%)	14 (46.67%)	Tigecycline	25 (83.33%)	0 (0%)	5 (16.67%)
Tobramycin	17 (56.67%)	0 (0%)	13 (43.33%)	Fosfomycin	-	-	-
Levofloxacin	10 (33.33%)	0 (0%)	20 (66.67%)	Nitrofurantoin	30 (100%)	0 (0%)	0 (0%)
Moxifloxacin	17 (56.67%)	1 (3.33%)	12 (40.00%)	Fusidic Acid	15 (50.00%)	0 (0%)	15 (50.00%)
Inducible Clindamycin Resistance	12 (40.00%)	0 (0%)	18 (60.00%)	Mupirocin	-	-	-
Erythromycin	20 (66.67%)	0 (0.00%)	10 (33.33%)	Rifampicin	25 (83.33%)	0 (0%)	5 (16.67%)
Clindamycin	13 (43.33%)	0 (0.00%)	17 (56.67%)	Trimethoprim/Sulfamethoxazole	24 (80.00%)	0 (0.00%)	6 (20.00%)
Linezolid	30 (100%)	0 (0.00%)	0 (0.00%)	-	-	-	-
p-Value	0.0001**	0.437 NS (0.9521)	0.0001**	-	0.0001**	0.0001**	0.0001**

$^{**}p \leq 0.01$

Table 5. Anti-*Staphylococcus aureus* activity of *Pelargonium graveolens* essential oils (EOs) and standard components

Conc.	Inhibition Zone per Millimeter (mm)							
	Geraniol	*p*-Value	Eugenol	*p*-Value	Citronellol	*p*-Value	*P. gravolens*	*p*-Value
50 mg/mL	30.00 ± 1.06 c	6.94** (0.0001)	30.00 ± 1.06b	7.26** (0.0001)	30.00 ± 1.06 c	7.15** (0.0001)	20.00 ± 0.87 c	6.95** (0.0001)
100 mg/mL	40.00 ± 2.17b		32.00 ± 1.27b		35.00 ± 1.84 bc		28.00 ± 1.47b	
200 mg/mL	50.00 ± 2.76a		35.00 ± 1.84b		40.00 ± 2.08b		30.00 ± 1.06a	
Positive Control	50.00 ± 2.76a		50.00 ± 2.76a		50.00 ± 2.67a		50.00 ± 2.76a	
Negative Control	0.00 ± 0.00 d		0.00 ± 0.00c		0.00 ± 0.00 d		0.00 ± 0.00 d	

Note: The results are expressed as mean ± standard deviation; means having different letters in the same column differed significantly. **: $p \leq 0.01$.

Table 6. Antimicrobial susceptibility profile of *Pseudomonas aeruginosa* based on the VITEK 2 AST-N222 card

Antimicrobial	Sensitive N (%)	Intermediate N (%)	Resistant N (%)	Antimicrobial	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Piperacillin/tazobactam	9 (30.00%)	2 (6.67%)	19 (63.33%)	Amikacin	10 (33.33%)	3 (10.00%)	17 (56.67%)
Cefazolin	0 (0%)	0 (0%)	30 (100%)	Gentamicin	7 (23.33%)	5 (16.67%)	18 (60.00%)
Ceftazidime	10 (33.33%)	5 (16.67%)	15 (50.00%)	Levofloxacin	10 (33.33%)	0 (0%)	20 (66.67%)
Cefepime	12 (40.00%)	6 (20.00%)	12 (40.00%)	Ciprofloxacin	5 (16.67%)	3 (10.00%)	22 (73.33%)
Imipenem	12 (40.00%)	0 (0%)	18 (60.00%)	Tigecycline	0 (0%)	0 (0%)	30 (100%)
p-Value	0.0001**	0.0245*	0.0045**	-	0.0024**	0.0374*	0.0394*

$^{*}p \leq 0.05$; $^{**}p \leq 0.01$

3.7 Anti-*Pseudomonas aeruginosa* activity of *Pelargonium graveolens* essential oil components and standard components

The inhibitory effects of the EO components of Pelargonium graveolens against *Pseudomonas aeruginosa* (Table 7) revealed no activity at 50 and 100 mg/mL. At 200 mg/mL, geraniol, citronellol, and eugenol each produced an inhibition zone of 7.00 ± 0.31 mm. However, the crude *P. graveolens* EO showed significant activity at all concentrations, rising between 24.00 ± 1.25 mm at 50 mg/mL and 30.00 ± 1.61 mm at 200 mg/mL. The negative control had no inhibition (0.00 mm), whereas the positive control demonstrated the maximum inhibition of 50.00 mm. The inhibition zone increased with increasing concentration; however, significant differences were observed among the tested concentrations of crude *P. graveolens* EO. However, significant differences were observed between the EO treatments and the positive and negative controls.

3.8 Broad-spectrum antimicrobial activity of *Pelargonium graveolens* essential oil against tested microorganisms

Table 8 shows the antimicrobial properties of *Pelargonium graveolens* EO against the tested bacterial isolates. The oil was found to be concentration-dependent against most organisms, and inhibition zones in most cases increased between 50 and 200 mg/mL. Intermediate levels of inhibition were observed

against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The maximum inhibition (50.00 mm) was in positive controls, and no inhibition was observed in negative controls. The overall ANOVA indicated a significant treatment effect, although not all pairwise comparisons were significant.

Table 7. Anti-*Pseudomonas aeruginosa* activity of *Pelargonium graveolens* essential oils (EOs) and standard components

Conc.	Inhibition Zone per Millimeter (mm)							
	Geraniol	*p*-Value	Eugenol	*p*-Value	Citronellol	*p*-Value	*P. graveolens*	*p*-Value
50 mg/mL	0.00 ± 0.00c	6.71** (0.0001)	0.00 ± 0.00c	6.71** (0.0001)	0.00 ± 0.00c	6.71** (0.0001)	24.00 ± 1.25b	6.43** (0.0001)
100 mg/mL	0.00 ± 0.00c		0.00 ± 0.00c		0.00 ± 0.00c		28.00 ± 1.54b	
200 mg/mL	7.00 ± 0.31b		7.00 ± 0.31b		7.00 ± 0.31b		30.00 ± 1.61b	
Positive Control	50.00 ± 2.76a		50.00 ± 2.76a		50.00 ± 2.67a		50.00 ± 2.76a	
Negative Control	0.00 ± 0.00c		0.00 ± 0.00c		0.00 ± 0.00c		0.00 ± 0.00c	

Note: The results are expressed as mean ± standard deviation; means having different letters in the same column differed significantly. **: $p \leq 0.01$.

Table 8. Antimicrobial activity of *Pelargonium graveolens* essential oils (EOs)

Treatment	Tested Microorganism	
	Staphylococcus aureus	*Pseudomonas aeruginosa*
50 mg/mL	20.00 ± 0.87c	24.00 ±1.25b
100 mg/mL	28.00 ± 1.47b	28.00 ±1.54b
200 mg/mL	30.00 ± 1.06b	30.00 ±1.61b
Positive Control	50.00 ± 2.76a	50.00 ±2.76a
Negative Control	0.00 ± 0.00d	0.00 ±0.00c
LSD	6.95	6.43
p-Value	0.0001**	0.0001**

Note: The results are expressed as mean ± standard deviation; means having different letters in the same column differed significantly. **$p \leq 0.01$. LSD = Least Significant Difference.

4. DISCUSSION

The current study demonstrated that *Pelargonium graveolens* EO has a significant antibacterial influence on clinically isolated, antibiotic-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with an apparent concentration-dependent response observed in the tested organisms. The findings are consistent with the emerging literature that shows that plant-based EOs are promising alternative antimicrobial agents in the age of increasing rates of AMR [16, 17]. The growing clinical burden of multidrug-resistant pathogens, especially S. *aureus* and *P. aeruginosa*, has been extensively reported as a global public health issue with limited treatment options and high adaptability of the organisms [3, 5, 18].

The GC–MS analysis showed that the major component of the oil was phenylethyl alcohol, and the others were geraniol, citronellol, and eugenol. Through this chemical profile, it is possible to agree with the past literature on the compositions of *P. graveolens* EO, but differences in ratios of the constituents are usually dependent on geographical location, climate, and method of extraction. Phenylethyl alcohol was not separately evaluated because the study focused on commercially available major bioactive standards. The strong antimicrobial potential of oxygenated monoterpenes like geraniol and citronellol is well documented, especially because of the lipophilic property that enables them to interact with the cell membranes of the microbes. These compounds can disrupt lipid bilayers, raise membrane permeability, and eventually cause leakage of intracellular contents and loss of membrane integrity [19, 20]. The high activity of the EO toward *S. aureus* can be explained by its higher susceptibility to the EO since it is a Gram-positive bacterium. Gram-positive bacteria have a thick peptidoglycan layer but no outer membrane, which means that the outer membrane is permeable to hydrophobic compounds in EOs. Geraniol was found to exert the greatest inhibitory activity on *S. aureus*, then citronellol and eugenol, which suggests that monoterpene alcohols significantly contribute to ABA.

The findings align with previous studies that reported that *P. graveolens* oil exhibits strong inhibitory influences against *S. aureus* [21]. Conversely, *P. aeruginosa* was more resistant to each of the oil components, whereas the crude EO showed medium but stable activity. The intrinsic resistance mechanisms are also known to be intrinsic to this organism, such as low outer membrane permeability, overexpression of efflux pumps, and biofilm formation, which all restrict the efficacy and penetration of antimicrobial agents [3]. The comparatively low activity of the isolated components toward *P. aeruginosa* in this research supports the idea that Gram-negative bacteria demand increased concentrations or a combination of bioactive compounds to be inhibited. Nevertheless, the crude oil was measurably active, indicating that mixture effects between its compounds contribute to antimicrobial influences in the crude oil.

The hypothesis that the concentration of EOs affects the availability of active phytochemicals to promote membrane disruption and metabolic interference is further supported by the concentration-dependent antibacterial influence in this study. More concentration-dependent trends were noted in recent research on the assessment of geranium oil encapsulation and antimicrobial delivery systems, with higher dose levels significantly enhancing the zones of bacterial inhibition [19, 22-24]. Moreover, the moderate-to-high inhibition of *S. aureus* over *P. aeruginosa* is also a characteristic trend in EO research since Gram-positive organisms are generally more sensitive than Gram-negative organisms because of the structural variation of cell envelopes. The antimicrobial influence of *P. graveolens* EO is thought to act on a variety of targets, mechanistically. Phenolic and monoterpene components have the potential to cause disruption of cytoplasmic membranes, a block on adenosine triphosphate (ATP) synthesis, and enzyme activity. In addition, phenylethyl alcohol, which was found to be the dominant compound in this study, has been noted to display antimicrobial influences by destabilising membranes and disrupting quorum-sensing signalling pathways and thus may potentially reduce bacterial virulence and biofilm formation. The broad-spectrum activity is probably due to the mixture effects of geraniol, citronellol, eugenol, and phenylethyl alcohol [25, 26].

The findings of the present study are consistent with the

recent reviews, which highlighted the renewed interest in plant EOs as alternative antimicrobial agents. Schardong et al. [27] emphasized that mathematical and biological modelling of AMR is more and more contributing to the incorporation of phytochemicals into antimicrobial development pipelines because of their multi-target and reduced resistance development potential. On the same note, the WHO [1] has stressed the importance of using alternative and adjunct antimicrobial interventions, especially in the treatment of multidrug-resistant infections in clinical practice. Though the crude EO usually had a lower antibacterial efficacy than some of the individual compounds (e.g., geraniol) against *S. aureus*, the overall findings indicate that whole EO preparations could possess greater efficacy owing to the mixture effects of multiple bioactive compounds.

The greater activity observed with crude EO compared with individual tested constituents may suggest a possible mixture effect; however, this requires confirmation using dedicated synergy assays. This mixture effect could be the reason why crude *P. graveolens* oil showed significant inhibitory activity against both the pathogens tested, even though the activity of individual compounds varied.

The present study has its limitations, though, such as the use of *in vitro* disc diffusion only, which might not entirely recapitulate *in vivo* pharmacokinetics, host immune interactions, and bioavailability. Also, inconsistency in the EO composition and the absence of the minimum inhibitory concentration (MIC) can restrict the direct clinical translation. In spite of these shortcomings, the results give good preliminary data on the ABA of *P. graveolens* EO against resistant clinical isolates. MRSA classification in this study was based on the phenotypic antimicrobial susceptibility results obtained using the VITEK 2 AST-GP67 system. Molecular confirmation by mecA gene or PBP2a detection was not performed, which represents a limitation of this study.

5. CONCLUSION

This research has shown that *Pelargonium graveolens* EO has a significant antibacterial influence against antibiotic-resistant *Staphylococcus aureus* and a moderate influence against *Pseudomonas aeruginosa* with a definite concentration response. The crude oil showed inhibitory activity against both bacterial species, which indicates mixed impacts between its bioactive agents, such as geraniol, citronellol, eugenol, and phenylethyl alcohol. This evidence confirms the promise of the use of *P. graveolens* EO as a potential candidate for further investigation as a plant-derived antimicrobial source. Further studies involving MIC/minimum bactericidal concentration (MBC) determination, toxicity evaluation, formulation studies, and in vivo assessment are required.

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