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MICROWAVE-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM *Pelargonium* spp.: POLYPHENOL CONTENT, ANTIOXIDANT CAPACITY, AND ANTIMICROBIAL ACTIVITY

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Abstract

This study evaluated microwave-assisted hydromethanolic extracts from ten Pelargonium species as potential sources of bioactive compounds. Significant differences among extracts were observed ($p < 0.05$). Pelargonium 'Lizzy' and 'Cola Bottles' exhibited the highest total phenolic contents (126.09 and 122.16 mg GAE/g extract, respectively). Antioxidant activity ranged from 144.84 to 260.40 mg QE·g^{-1} extract (DPPH assay) and from 760.50 to 2940.90 µmol Fe^{2+}·g^{-1} extract (FRAP assay). Antimicrobial activity varied according to both the extract and the bacterial strain tested. P. odoratissimum showed the strongest inhibition against Escherichia coli and Salmonella Typhimurium, while Pelargonium 'Nova', 'Lizzy', and 'Cola Bottles' exhibited the most pronounced activity against Staphylococcus aureus, Enterococcus faecalis, and Proteus vulgaris using the agar well diffusion method. MIC and MBC results confirmed antibacterial potential, with lower inhibitory and bactericidal concentrations corresponding to larger inhibition zones, particularly for Pelargonium 'Cola Bottles', 'Nova', 'Lizzy', and P. roseum. These findings highlight the potential of selected Pelargonium species as sources of natural bioactive compounds.

Key words*: Pelargonium, plant extracts, polyphenols, antioxidant activity, antimicrobial activity.*

INTRODUCTION

The growing interest in natural antimicrobial agents has stimulated extensive research on medicinal plants and agro-industrial by-products (Diguta et al., 2014; Purcaru et al., 2017; Grosu et al., 2024, Megdar et al., 2025).

The genus *Pelargonium* L'Hér. ex Aiton, belonging to the family *Geraniaceae*, comprises more than 280 species predominantly native to Southern Africa and widely cultivated worldwide as ornamental, aromatic, and medicinal plants (Meyers et al., 2006; Saraswathi et al., 2011). In recent years, interest for *Pelargonium* has expanded beyond horticultural applications due to the remarkable diversity of secondary metabolites and their associated biological properties. Several studies have reported that *Pelargonium* species contain phenolic acids, flavonoids, tannins, coumarins, and terpenoids that contribute to a wide range of pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, and cytotoxic activities (Hamidpour et al., 2017; Roman et al., 2023; Asker & Al Haidar, 2024; Balabanova et al., 2025; Celi et al., 2025). Furthermore, *Pelargonium* extracts and essential oils have demonstrated insecticidal activities, extending their potential applications beyond pharmaceutical and food-related fields (M'hamdi et al., 2024).

Natural antioxidants derived from plant sources have attracted considerable attention as safer alternatives to synthetic compounds used in food, cosmetic, and pharmaceutical industries. Oxidative stress, resulting from the excessive accumulation of reactive oxygen species, plays a crucial role in the development of numerous chronic diseases.

Plant polyphenols are recognized as major contributors to antioxidant activity through their ability to donate electrons or hydrogen atoms and neutralize free radicals (Roman et al., 2023; Gevrenova et al., 2024). Previous investigations have demonstrated that *Pelargonium* species are rich sources of phenolic compounds, including gallic acid, protocatechuic acid, caffeic acid derivatives,

kaempferol, myricetin, and isorhamnetin (Dimitrova et al., 2015; El Aanachi et al., 2020; Gevrenova et al., 2024). Positive correlations between total phenolic content and antioxidant activity have been reported for various *Pelargonium* extracts (Ali et al., 2020; Ennaifer et al., 2020; Gevrenova et al., 2024).

The extraction method employed represents a critical factor influencing the yield and quality of bioactive compounds recovered from plant material (El Maaiden et al., 2022; Bitwell et al., 2023; Zhou et al., 2023; Pogorzelska-Nowicka et al., 2024). Conventional extraction techniques, such as Soxhlet extraction and maceration, often require prolonged processing times, elevated temperatures, and large solvent volumes, which may lead to degradation of thermolabile compounds (Zhang et al., 2018; Bitwell et al., 2023). In response to these limitations, microwave-assisted extraction (MAE) has emerged as a promising green alternative, offering advantages including reduced extraction time, lower solvent consumption, and enhanced extraction efficiency (El Maaiden et al., 2022; Boztaş, et al., 2024; Pogorzelska-Nowicka et al., 2024). The mechanism underlying MAE involves the direct interaction of microwave energy with polar molecules in the solvent and plant matrix, generating rapid and uniform heating that disrupts cell walls and facilitates the release of intracellular compounds (Zhang et al., 2018; Zhou et al., 2023). Recent studies have demonstrated the effectiveness of MAE for the recovery of polyphenols from various plant matrices, with optimized conditions yielding significantly higher extraction efficiencies compared to conventional methods (El Maaiden et al., 2022; Bitwell et al., 2023; Zhou et al., 2023; Pogorzelska-Nowicka et al., 2024). Furthermore, microwave-assisted processing has been successfully applied to *Pelargonium graveolens*, improving the recovery of volatile and non-volatile phytochemicals while reducing extraction time and energy consumption (Boztaş et al., 2024).

The adoption of green extraction technologies is increasingly encouraged by the food and pharmaceutical industries, as these approaches improve extraction efficiency while reducing environmental impact and solvent consumption, thereby supporting the sustainable valorisation of plant-derived bioactive compounds (El Maaiden et al., 2022; Pogorzelska-Nowicka et al., 2024).

Methanolic extracts of *Pelargonium graveolens* obtained through conventional extraction methods have shown pronounced antibacterial, antifungal, antioxidant, and enzyme-inhibitory activities (Ben Hsouna & Hamdi, 2012; Dimitrova et al., 2015; Ali et al., 2020; Ennaifer et al., 2020).

Additional studies have highlighted the therapeutic potential of *Pelargonium* species in the management of inflammatory and metabolic disorders (Martins et al., 2017; Neagu et al., 2018; Amel et al., 2022).

Despite the increasing number of studies focused on individual *Pelargonium* species, comparative investigations involving multiple species cultivated under similar environmental conditions remain relatively scarce. This aspect is particularly important because phytochemical composition and biological activity may vary considerably among species, cultivars, and genotypes. Such variability is influenced by genetic background, cultivation practices, environmental conditions, and nutrient management strategies (Mazeed et al., 2022; Siddiqui et al., 2024). Therefore, screening diverse *Pelargonium* species may facilitate the identification of promising sources of natural antioxidants and antimicrobial compounds for future breeding programs, food applications, and biotechnological exploitation (Roman et al., 2023; Celi et al., 2025).

The present study aimed to comparatively evaluate the total phenolic content, antioxidant activity, and antimicrobial potential of microwave-assisted methanolic extracts obtained from ten *Pelargonium* species maintained in a commercial breeding and propagation collection.

MATERIALS AND METHODS

Ten *Pelargonium* genotypes/cultivars were obtained from a specialized horticultural collection located in the Netherlands, where plants are maintained for propagation and breeding purposes (Table 1). Healthy aerial parts were collected and processed for extract preparation.

Table 1. *Pelargonium* cultivars used in this study

Code	Species
P1	*Pelargonium* 'Chocolate'
P2	*Pelargonium* 'Watermelon'
P3	*Pelargonium tomentosum*
P4	*Pelargonium odoratissimum*
P5	*Pelargonium* 'Orange Fizz'
P6	*Pelargonium* 'Cola Bottles'
P7	*Pelargonium* 'Nova'
P8	*Pelargonium* 'Lizzy'
P9	*Pelargonium roseum*
P10	*Pelargonium graveolens*

Preparation of plant extracts

The aerial parts of the investigated *Pelargonium* species were freeze-dried (FreeZone 6, Labconco, USA) at −50°C under a vacuum pressure of 0.04 mbar for 22 h. Following lyophilization, the plant material was milled to a particle size of <1 mm and used for subsequent extraction.

The extracts were obtained using 70% (v/v) methanol as extraction solvent at a plant material-to-solvent ratio of 1:30 (w/v). Microwave-assisted extraction (MAE) (Ethos Easy - Advanced Microwave Digestion SystemMilestone™ SpA, Italy) was performed using an extraction power of 500 W at 120°C. The total extraction time was 80 min and consisted of three stages: 10 min of sample preheating to the selected temperature, 60 min of extraction under continuous microwave irradiation at 70°C, and 10 min of cooling.

The extracts were centrifuged for 10 min at 6000 rpm (Universal 320 R, Hettich, France) and the supernatants filtered through 0.45 µm sterile membranes (Sartorius, Germany). The solvent was removed under reduced pressure at 40 °C using a rotary evaporator (Büchi R-210, Switzerland), followed by freeze-drying.

The dried extracts were resuspended in sterile distilled water (100 mg·mL^{-1}), filter-sterilized through 0.22 µm membranes, and stored at +4°C until analysis.

Determination of total polyphenol content

The total polyphenol content (TPC) of the *Pelargonium* extracts was determined using the Folin-Ciocalteu colorimetric method. Briefly, 20 µL of plant extract was mixed with 1580 µL of distilled water, 100 µL of Folin-Ciocalteu reagent, and 300 µL of 20% (w/v) sodium carbonate solution. The reaction mixtures were thoroughly homogenized and incubated in the dark for 2 h at room temperature.

After incubation, the absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Thermo Scientific Helios, England). A calibration curve was prepared using gallic acid standard solutions, and the TPC was expressed as milligrams of gallic acid equivalents per g of extracts (mg GAE·g^{-1} extract). The polyphenol content was calculated according to the following equation:

$$\text{TPC (mg GAE}\cdot\text{g}^{-1}\text{ extract)} = \frac{A_{765}\text{-}0.0082}{0.001}$$

where:

- A_{765} is the absorbance of the sample measured at 765 nm;
- GAE represents gallic acid equivalents.

The calibration curve obtained for gallic acid was $y = 0.001x + 0.0082$ ($R^2 = 0.9991$).

Determination of antioxidant activity by the DPPH Assay

The antioxidant activity of the *Pelargonium* extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. This method is based on the ability of antioxidant compounds to neutralize the stable DPPH free radical, resulting in a colour change of the solution from deep violet to yellow.

For the analysis, 50 µL of plant extract was mixed with 1950 µL of DPPH solution prepared in ethanol. The reaction mixtures were incubated in the dark at room temperature for 30 min to ensure complete reaction between the antioxidant compounds and the DPPH radical.

After incubation, the absorbance was measured at 515 nm using a UV–Vis spectrophotometer (Thermo Scientific Helios, England). Absolute ethanol was used as the blank. The percentage of radical scavenging activity was calculated based on the decrease in absorbance relative to the control.

Antioxidant activity was expressed as quercetin equivalents (QE) and calculated using the calibration equation:

$$\text{AAE (QE)} = \frac{\%\Delta A_{515}\text{-}3.4954}{0.0811}$$

where:

- AAE (QE) represents the antioxidant activity expressed as quercetin equivalents;

- % ΔA_{515} represents the percentage inhibition determined at 515 nm.
The resulting linear regression equation was: y = 0.0811x + 3.4954.

Determination of antioxidant activity by the FRAP assay

The antioxidant activity of the *Pelargonium* extracts was evaluated using the Ferric Reducing Antioxidant Power (FRAP) assay. This method is based on the reduction of the ferric-tripyridyltriazine complex (Fe^{3+}–TPTZ) to the ferrous form (Fe^{2+}–TPTZ) by antioxidant compounds present in the extracts, resulting in the formation of an intense blue-coloured complex.
The FRAP reagent was freshly prepared by mixing acetate buffer (pH 3.6), 10 mM TPTZ solution prepared in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution in a ratio of 10:1:1 (v/v/v).
For the analysis, 100 µL of appropriately diluted plant extract was mixed with 3.0 mL of freshly prepared FRAP reagent. The reaction mixtures were incubated at 37°C for 4 min. After incubation, the absorbance was measured at 593 nm using a UV–Vis spectrophotometer (Thermo Scientific Helios, England). A reagent blank consisting of 3.0 mL FRAP reagent and 100 µL distilled water was used for baseline correction.
The antioxidant activity was expressed as Fe^{2+} equivalents and calculated using a calibration curve prepared with ferrous sulphate ($FeSO_4$). The concentration of Fe^{2+} equivalents was determined according to the calibration equation:

$$\text{FRAP } (Fe^{2+} \text{ equivalents}) = \frac{A_{593} - 0.0038}{1.4953}$$

where:
- FRAP (Fe^{2+} equivalents) represents the antioxidant activity expressed as ferrous ion equivalents;
- A_{593} represents the absorbance measured at 593 nm.
The resulting linear regression equation was: y = 1.4953x + 0.0038.

Determination of antibacterial activity of *Pelargonium* extracts

Antimicrobial activity of the extracts was evaluated against Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212, and *Listeria monocytogenes* ATCC 13932) and Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 13315, *Salmonella enterica* subsp. *enterica* Typhimurium ATCC 14028, and *Serratia marcescens* ATCC 14754). All strains were obtained from the American Type Culture Collection (ATCC). Briefly, bacterial inoculum was prepared from fresh overnight cultures grown on Tryptic Soy Agar (TSA; Scharlab S.L., Barcelona, Spain). The standardized cell suspensions were adjusted to the turbidity of a 0.5 McFarland standard, corresponding to approximately $1–2\times10^8$ CFU·mL^{-1}, prior to antimicrobial activity testing.

Agar well diffusion assay

Antimicrobial activity was evaluated using the agar well diffusion method with minor modifications based on Balouiri et al. (2016).
For the assay, 1 mL of each standardized cell suspension was inoculated into sterile 90-mm Petri dishes, followed by the addition of 25 mL of Tryptic Soy Agar at 42-45°C. Plates were gently homogenized and allowed to solidify.
Wells (6 mm diameter) were aseptically prepared, and 100 µL of each extract was added. Plates were incubated for 24 h under optimal growth conditions. Antimicrobial activity was determined by measuring inhibition zone diameters (mm).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined using the broth microdilution method in sterile 96-well microplates, according to the protocol described by Grosu et al. (2024), with minor modifications. Briefly, bacterial inoculum was prepared from fresh overnight cultures grown on Tryptic Soy Agar. The standardized cell suspensions were diluted in Mueller-Hinton broth (MHB, Accumix®, Microxpress, Verna, Goa, India) to obtain a final inoculum concentration of approximately 5×10^5 CFU·mL^{-1} in each well after addition to the microplate. The lyophilized extracts were

reconstituted in sterile distilled water and subsequently subjected to two-fold serial dilutions in sterile MHB using 96-well microtiter plates. The tested concentration range was 12.5-0.024 mg·mL^{-1}. Each well contained the appropriate concentration of extract and bacterial inoculum, resulting in a uniform final volume of 100 µL in each well. Positive controls consisted of inoculated medium without extract, while negative controls contained uninoculated medium. Following inoculation, the microplates were incubated at 37°C for 18-24 h under aerobic conditions. Subsequently, 20 µL of 0.01% (w/v) resazurin solution were added to each well, and the plates were further incubated for an additional 2 h at 37°C to allow colour development. Resazurin was used as a bacterial viability indicator, whereby metabolically active cells reduced the blue dye to pink resorufin. At the highest extract concentrations, a yellowish coloration was occasionally observed, likely due to interactions between phytochemical constituents and the resazurin reagent (Neufeld et al., 2018; Akter et al., 2019); therefore, MIC values were interpreted in conjunction with visible growth inhibition and subsequently confirmed by MBC determination. The MIC was defined as the lowest concentration showing no detectable bacterial growth, as indicated by the absence of resazurin reduction and confirmed by visual inspection of the wells. To determine the MBC, aliquots from wells corresponding to the MIC and higher concentrations were aseptically streaked onto TSA plates and incubated at 37°C for 24 h. The MBC was defined as the lowest concentration of the extract that completely prevented bacterial regrowth, as evidenced by the absence of visible colonies on the agar surface.

2.6. Statistical analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA). Significant differences among means were determined using Tukey's honestly significant difference (HSD) post hoc test at a significance level of $p < 0.05$. Results are expressed as mean ± standard deviation of three independent determinations. Different letters indicate statistically significant differences between samples.

RESULTS AND DISCUSSIONS

Total polyphenol content of *Pelargonium* extracts

To assess the phytochemical characteristics of the investigated samples, dry matter content and TPC were determined prior to the evaluation of their biological activities (Table 2).

Dry matter content varied between 8.55% and 15.50% among the investigated *Pelargonium* extracts; however, the observed variation was not directly associated with TPC, suggesting that phenolic accumulation is primarily genotype-dependent rather than related to biomass dry matter levels (Table 2).

Similar observations have been reported for *Pelargonium* species and other aromatic plants, where secondary metabolite production depends on genetic and physiological factors in addition to biomass composition (Gevrenova et al., 2024; Balabanova et al., 2025).

The TPC varied significantly among the investigated *Pelargonium* extracts ($p < 0.05$), indicating substantial differences in the accumulation of phenolic compounds among the studied genotypes. The highest TPCs were determined in the extracts obtained from *Pelargonium* 'Lizzy' (P8) and *Pelargonium* 'Cola Bottles' (P6), reaching 126.09 and 122.16 mg GAE/g extract, respectively (Table 2).

Moderate phenolic concentrations were observed in extracts of *Pelargonium* 'Orange Fizz' (P5), *Pelargonium* 'Nova' (P7) and *Pelargonium roseum* (P9), which formed the same statistical group. Extracts of *Pelargonium tomentosum* (P3) and *Pelargonium* 'Chocolate' (P1) exhibited intermediate values, whereas the extract of *Pelargonium odoratissimum* (P4) showed a significantly lower phenolic content (Table 2).

The lowest TPCs were found in extracts of *Pelargonium graveolens* (P10) and *Pelargonium* 'Watermelon' (P2), which did not differ significantly from each other. The approximately 2.5-fold variation between the highest and lowest values highlights the strong influence of genotype on the biosynthesis and accumulation of phenolic compounds within the *Pelargonium* genus.

Tabel 2. Total polyphenol content of *Pelargonium* extracts

Sample	Dry matter (%)	TPC (mg GAE·g⁻¹ extract)
P1	14.15	79.02 ± 2.65[c]
P2	13.30	49.99 ± 2.30[e]
P3	12.94	80.47 ± 0.11[c]
P4	8.55	66.63 ± 3.05[d]
P5	11.13	105.26 ± 2.60[b]
P6	12.92	122.18 ± 6.02[a]
P7	9.24	103.67 ± 9.88[b]
P8	15.50	126.09 ± 11.79[a]
P9	14.60	102.75 ± 5.20[b]
P10	14.04	50.71 ± 3.20[e]

Considering the well-documented role of phenolic compounds as antioxidants and antimicrobial agents, the elevated phenolic contents observed in *Pelargonium* 'Lizzy' and *Pelargonium* 'Cola Bottles' extracts may contribute to their enhanced biological activity. The results indicate that the selected *Pelargonium* extracts constitute promising sources of natural bioactive compounds for food and pharmaceutical applications. Similar observations were reported for *Pelargonium graveolens* extracts, in which phenolic compounds represented major contributors to the biological activity of the plant material (Ali et al., 2020; Ennaifer et al., 2020; Gevrenova et al., 2024).

The significant differences observed in TPC among the investigated *Pelargonium* extracts are consistent with previous reports demonstrating considerable phytochemical variability within the genus. Dimitrova et al. (2015), El Aanachi et al. (2020) and Gevrenova et al. (2024) reported that *Pelargonium* species are rich sources of phenolic acids and flavonoids, although their concentrations strongly depend on genotype, extraction conditions and environmental factors.

Antioxidant activity determined by DPPH assay

The antioxidant capacity of the investigated *Pelargonium* extracts was evaluated using DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays to assess their ability to neutralize free radicals and act as reducing agents (Table 3).

The DPPH assay revealed notable radical-scavenging activity in all *Pelargonium* extracts. The highest antioxidant activity was observed for P1 (260.40 mg QE·g⁻¹ extract), followed by P5 (242.19 mg QE·g⁻¹ extract) and P4 (196.50 mg QE·g⁻¹ extract). Lower values were recorded for P9, P7, P10, and P8. Interestingly, the DPPH results did not fully follow the same trend as the TPC, suggesting that radical-scavenging activity may depend not only on the total amount of phenolics, but also on the specific structure and reactivity of individual antioxidant compounds present in each extract.

Tabel 3. Antioxidant activity of *Pelargonium* extracts

Sample	Antioxidant activity (mg QE·g⁻¹ extract)	Antioxidant activity (μmol Fe^{2+}·g⁻¹ extract)
P1	260.40 ± 0.34[a]	1005.30 ± 2.40[h]
P2	168.45 ± 0.11[e]	1150.20 ± 5.10[g]
P3	160.77 ± 0.11[f]	1729.26 ± 11.35[e]
P4	196.50 ± 0.26[c]	1268.10 ± 1.80[f]
P5	242.19 ± 0.16[b]	1916.10 ± 1.50[d]
P6	169.19 ± 0.11[d]	2595.90 ± 40.80[b]
P7	154.62 ± 0.20[h]	2256.00 ± 154.80[c]
P8	159.61 ± 0.21[g]	2940.90 ± 3.30[a]
P9	144.84 ± 0.57[i]	1232.10 ± 5.10[f]
P10	154.92 ± 0.10[h]	760.50 ± 5.70[i]

The FRAP assay showed substantial differences in ferric reducing antioxidant power among the investigated extracts. The highest FRAP values were obtained for P8 (2940.90 μmol Fe^{2+}·g⁻¹ extract), P6 (2595.90 μmol Fe^{2+}·g⁻¹ extract), and P7 (2256.00 μmol Fe^{2+}·g⁻¹ extract), indicating a strong reducing capacity. The lowest value was recorded for P10 (760.50 μmol Fe^{2+}·g⁻¹ extract), followed by P1 and P2 extracts.

A positive correlation was observed between TPC and antioxidant activity measured by the FRAP assay. Extracts with high TPC values, particularly *Pelargonium* 'Lizzy' (P8) and *Pelargonium* 'Cola Bottles' (P6), exhibited correspondingly elevated FRAP values, indicating that phenolic compounds are major contributors to the reducing capacity of these extracts.

These findings indicate that phenolic compounds contribute substantially to the reducing capacity of *Pelargonium* extracts. Similar relationships have been previously documented in *Pelargonium* species and other phenolic-rich plant matrices (Ali et al., 2020; El Aanachi et al., 2020; Gevrenova et al., 2024; Grosu et al., 2024).

In contrast, the correlation between TPC and DPPH radical-scavenging activity was less pronounced. Although *Pelargonium* 'Chocolate' (P1) extract exhibited the highest DPPH activity (260.40 mg QE·g^{-1} extract), its TPC was lower than that of *Pelargonium* 'Cola Bottles' (P6) and *Pelargonium* 'Lizzy' (P8) extracts. This observation suggests that antioxidant activity is influenced not only by the total concentration of phenolic compounds but also by their qualitative composition, molecular structure, and specific reaction mechanisms involved in each assay. Comparable antioxidant activities have been reported for *Pelargonium graveolens* extracts rich in polyphenols and flavonoids (Hamidpour et al., 2017; Ennaifer et al., 2020; Gevrenova et al., 2024).

Our results demonstrate that *Pelargonium* extracts represent valuable sources of natural antioxidants and highlight significant phytochemical variability among the investigated genotypes.

The strong reducing capacity observed for extracts of *Pelargonium* 'Cola Bottles' (P6), *Pelargonium* 'Nova' (P7), and *Pelargonium* 'Lizzy' (P8) indicates their potential as promising candidates for future studies focused on the isolation and characterization of bioactive compounds with antioxidant properties.

Evaluation of antibacterial activity of *Pelargonium* extracts

The antibacterial potential of the investigated *Pelargonium* extracts was assessed using agar well diffusion (Table 4), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays (Tables 5 and 6).

Table 4. Antimicrobial activity of the investigated extracts against pathogenic bacteria

Pathogens	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
S. aureus ATCC 6538	8.50 ± 0.41^{c}	10.50 ± 0.41^{b}	8.33 ± 0.24^{c}	8.33 ± 0.24^{c}	10.17 ± 0.24^{b}	12.50 ± 0.00^{a}	12.17 ± 0.29^{a}	12.50 ± 0.87^{a}	12.00 ± 0.50^{a}	8.17 ± 0.76^{c}
Ent. faecalis ATCC 29212	8.33 ± 0.24^{d}	8.33 ± 0.24^{d}	9.50 ± 0.41^{c}	9.00 ± 0.41^{d}	10.33 ± 0.24^{c}	11.50 ± 0.50^{b}	14.17 ± 0.29^{a}	14.17 ± 0.29^{a}	12.17 ± 0.29^{b}	7.33 ± 0.29^{e}
L. monocytogenes ATCC 13932	8.17 ± 0.24^{a}	7.33 ± 0.24^{b}	8.33 ± 0.24^{a}	6.33 ± 0.24^{b}	6.33 ± 0.24^{b}	9.00 ± 0.50^{a}	8.17 ± 0.29^{a}	8.00 ± 0.50^{a}	8.83 ± 1.04^{a}	6.50 ± 0.50^{b}
E. coli ATCC 8739	2.67 ± 0.24^{f}	4.17 ± 0.29^{e}	4.33 ± 0.24^{e}	19.17 ± 0.62^{a}	18.33 ± 0.24^{a}	5.67 ± 0.76^{d}	8.33 ± 0.29^{c}	8.33 ± 0.29^{c}	12.50 ± 0.00^{b}	4.33 ± 0.29^{e}
P. aeruginosa ATCC 27853	7.33 ± 0.24^{e}	14.33 ± 0.24^{c}	17.17 ± 0.24^{b}	17.33 ± 0.24^{b}	14.17 ± 0.24^{c}	18.33 ± 0.29^{a}	17.00 ± 0.50^{b}	9.83 ± 0.29^{d}	10.17 ± 0.29^{d}	10.50 ± 0.50^{d}
P. vulgaris ATCC 13315	8.33 ± 0.24^{d}	8.33 ± 0.24^{d}	7.17 ± 0.47^{d}	12.33 ± 0.24^{b}	12.33 ± 0.24^{b}	10.17 ± 0.29^{c}	15.30 ± 0.52^{a}	15.17 ± 0.76^{a}	10.67 ± 0.76^{c}	8.33 ± 0.29^{d}
S. Typhimurium ATCC 14028	8.00 ± 0.00^{f}	6.50 ± 0.00^{g}	6.50 ± 0.00^{g}	22.17 ± 0.24^{a}	17.50 ± 0.00^{c}	17.33 ± 0.29^{c}	20.17 ± 0.29^{b}	15.17 ± 0.29^{d}	11.67 ± 0.76^{e}	8.33 ± 0.29^{f}
S. marcescens ATCC 14754	2.30 ± 0.22^{d}	2.50 ± 0.00^{d}	2.33 ± 0.24^{d}	11.33 ± 0.24^{a}	7.33 ± 0.24^{b}	5.67 ± 0.76^{c}	7.33 ± 0.76^{b}	7.00 ± 0.50^{b}	2.50 ± 0.00^{d}	2.50 ± 0.00^{d}

Data are presented as mean ± SD. Values followed by different superscript letters differ significantly ($p < 0.05$). Antimicrobial activity is expressed as inhibition zone diameter (mm).

Extracts that produced larger inhibition zones also exhibited lower MIC and MBC values, confirming their stronger antibacterial efficacy. This relationship was particularly evident for extracts of *Pelargonium* 'Cola Bottles' (P6), *Pelargonium* 'Nova' (P7), *Pelargonium* 'Lizzy' (P8), *Pelargonium roseum* (P9) and *Pelargonium odoratissimum* (P4), which demonstrated both high inhibition zone diameters and low inhibitory or bactericidal concentrations.

Pelargonium odoratissimum (P4) extract showed the strongest activity against *E. coli* ATCC 8739 and *S. typhimurium* ATCC 14028 in the agar diffusion assay, producing inhibition zones of 19.17 mm and 22.17 mm, respectively. However, MIC and MBC values for these microorganisms remained relatively high (>12.5 mg·mL^{-1}), suggesting that although the extract contains compounds capable of diffusing efficiently through agar and inhibiting bacterial growth, higher concentrations are required to achieve complete growth inhibition and bacterial killing in liquid media. This discrepancy may reflect differences in the diffusion behavior and bioavailability of active compounds.

Table 5. MIC values (mg·mL⁻¹) of *Pelargonium* cultivar extracts against the tested bacterial strains

Pathogens	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
S. aureus ATCC 6538	3.13	3.13	1.56	3.13	1.56	0.78	0.78	0.78	0.78	3.13
Ent. faecalis ATCC 29212	6.25	6.25	3.13	6.25	3.13	1.56	1.56	1.56	1.56	3.13
L. monocytogenes ATCC 13932	6.25	6.25	3.13	6.25	3.13	3.13	1.56	3.13	3.13	6.25
E. coli ATCC 8739	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	6.25	6.25	>12.5	6.25
P. aeruginosa ATCC 27853	12.5	6.25	6.25	0.78	12.5	12.5	6.25	6.25	12.5	6.25
P. vulgaris ATCC 13315	1.56	1.56	0.78	1.56	0.78	0.78	0.78	0.78	0.78	6.25
S. Typhimurium ATCC 14028	>12.5	>12.5	3.13	>12.5	>12.5	>12.5	6.25	>12.5	>12.5	>12.5
S. marcescens ATCC 14754	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5

Data are presented as mean ± SD.

Table 6. MBC values (mg·mL⁻¹) of *Pelargonium* cultivar extracts against the tested bacterial strains

Pathogens	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
S. aureus ATCC 6538	3.13	3.13	1.56	3.13	1.56	0.78	0.78	0.78	0.78	3.13
P. aeruginosa ATCC 27853	12.5	12.5	6.25	0.78	12.5	12.5	6.25	6.25	12.5	12.5
E. coli ATCC 8739	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
E. faecalis ATCC 29212	6.25	6.25	3.13	6.25	3.13	1.56	1.56	3.13	1.56	6.25
P. vulgaris ATCC 13315	1.56	1.56	1.56	1.56	0.78	0.78	0.78	0.78	0.78	6.25
L. monocytogenes ATCC 13932	6.25	6.25	3.13	6.25	3.13	3.13	3.13	3.13	3.13	6.25
S. Typhimurium ATCC 14028	>12.5	>12.5	3.13	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5
S. marcescens ATCC 14754	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5	>12.5

Data are presented as mean ± SD.

A strong correlation between agar well diffusion and microdilution methods was observed for *Proteus vulgaris* ATCC 13315. *Pelargonium* 'Nova' (P7) and *Pelargonium* 'Lizzy' (P8) extracts generated inhibition zones exceeding 15 mm and exhibited low MIC and MBC values (0.78 mg·mL⁻¹), indicating pronounced antibacterial and bactericidal activity. Similar findings were obtained for *Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212, where extracts with larger inhibition zones also displayed lower MIC and MBC values.

Listeria monocytogenes ATCC 13932 showed relatively small inhibition zones and comparatively higher MIC and MBC values, indicating reduced susceptibility to the investigated extracts. Likewise, *Serratia marcescens* ATCC 14754 exhibited the highest resistance, characterized by small inhibition zones and the maximum MBC value (>12.50 mg·mL⁻¹) for all extracts tested.

The combined evaluation of inhibition zone diameters, MIC and MBC values indicates that the extracts of *Pelargonium* 'Cola Bottles' (P6), *Pelargonium* 'Nova' (P7), *Pelargonium* 'Lizzy' (P8), and *Pelargonium roseum* (P9) possess the most promising antibacterial properties. Their low MIC and MBC values, together with their strong inhibition zones, suggest the presence of bioactive compounds capable of exerting both bacteriostatic and bactericidal effects. These findings are consistent with the higher phenolic content previously reported for these extracts, suggesting a role in their antimicrobial activity.

The higher susceptibility of Gram-positive bacteria observed in the present study is consistent with previous reports concerning *P. graveolens*. Dimitrova et al. (2015) reported antibacterial activity of *P. graveolens* leaf extracts primarily against *Listeria monocytogenes*, while little or no activity was observed against *E. coli* and *Salmonella* spp. Similarly, Ennaifer et al. (2020) demonstrated that aqueous preparations of *P. graveolens* (infusion and decoction) exhibited considerable antibacterial activity against *S. aureus*, supporting the high sensitivity of Gram-positive bacteria observed in the present study.

The lower susceptibility of Gram-negative bacteria may be attributed to the structural characteristics of their cell envelope. The

presence of an outer membrane containing lipopolysaccharides constitutes an additional permeability barrier that restricts the diffusion of phenolic compounds and other antimicrobial phytochemicals into the bacterial cell. This mechanism has frequently been proposed to explain the reduced effectiveness of plant-derived extracts against Gram-negative microorganisms. Furthermore, previous investigations on *P. graveolens* extracts have shown that their antimicrobial activity is largely associated with phenolic compounds, flavonoids, and volatile constituents such as citronellol, geraniol, and linalool, which may act individually or synergistically to disrupt bacterial membranes and interfere with essential cellular processes (Ben Hsouna & Hamdi, 2012).

CONCLUSIONS

The present study demonstrated that *Pelargonium* extracts represent valuable sources of bioactive compounds with significant antioxidant and antimicrobial properties. Considerable variability was observed among the investigated extracts, highlighting the influence of genotype on phenolic accumulation and biological activity. *Pelargonium* 'Lizzy' and *Pelargonium* 'Cola Bottles' extracts exhibited the highest total phenolic contents and antioxidant capacities, indicating a strong contribution of phenolic compounds to the antioxidant potential of the extracts.

The antimicrobial assays revealed activity against both Gram-positive and Gram-negative foodborne pathogens, with susceptibility varying among bacterial species. The strongest overall antimicrobial performance was recorded for *Pelargonium* 'Cola Bottles' (P6), *Pelargonium* 'Nova' (P7) and *Pelargonium* 'Lizzy' (P8), which consistently produced large inhibition zones and low MIC and MBC values. These findings indicate that selected *Pelargonium* species represent promising natural sources of antioxidant and antimicrobial compounds. Further research is required to identify the bioactive constituents responsible for these activities and to assess their potential applications in food preservation, nutraceutical formulations, and other value-added products.

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