The Journal of Medicinal Plants and By-products

Original Article

Comparative Antibacterial Activity of *Chelidonium majus* L. Extracts from Different Plant Organs and Geographical Origins

Maryam Mohammadian, Azim Ghasemnezhad*, Kamran Rahnama and Mostafa Khoshhalsarmast

Department of Horticultural Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

*Corresponding author: Email: ghasemnezhad@gau.ac.com

Article History: Received 25 February 2026/Accepted in revised form 22 August 2026

ABSTRACT

Growing concerns about food safety and the limitations of chemical preservatives have increased interest in natural antimicrobial sources. This study evaluated the antibacterial activity of root and leaf extracts from *Chelidonium majus* L. (greater celandine), collected from various regions in northern Iran, against three major foodborne pathogens: *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Methanol was used for extraction, and antibacterial activity was assessed by the agar well diffusion method within a three-factor factorial design (geographical origin × plant organ × bacterial species). Data were analyzed using three-way ANOVA and Tukey's post hoc test. Results showed significant main effects of plant organ, geographical source, and bacterial species on inhibition-zone diameters. Specifically, root extracts were more effective than leaf extracts, exhibiting the strongest inhibition against Gram-positive bacteria (*L. monocytogenes* and *S. aureus*). *S. aureus* consistently far more sensitive than *L. monocytogenes*, phytochemical analysis revealed a significant influence of geographical origin (genotype) on total alkaloid and total phenol contents of the extracts. A strong positive correlation was observed between total alkaloid content (notably in samples from the Kordkuy and Noor regions) and inhibitory potency against Gram-positive bacteria, whereas the correlation with total phenols was weaker. These findings suggest that alkaloids play a primary role in the antibacterial activity of celandine extracts, with phenolic compounds contributing through potential synergistic effects. Furthermore, the relative resistance of *E. coli* underscores the role of outer membrane structure in Gram-negative bacteria. Overall, the results highlight the impact of genetic and geographical diversity on the phytochemical profile and, consequently, the antibacterial efficacy of plant extracts as potential natural agents in food safety applications.

Keywords: Alkaloids, Antibacterial Selectivity, *Escherichia Coli*, *Listeria Monocytogenes*, *Staphylococcus Aureus*

INTRODUCTION

The global rise in foodborne pathogens, coupled with increasing restrictions on synthetic preservatives and antibiotics, poses a significant challenge for food safety and public health [1,2]. Regarding the emergence of microbial resistant strains to chemical drugs, it was important to make efforts for finding new antimicrobial factors with fewer side effects for substituting chemical drugs [3]. Bacteria such as *Escherichia coli*, *Listeria monocytogenes,* and *Staphylococcus aureus* are not only major causes of food poisoning but also rapidly adapt to chemical stress, reducing the effectiveness of many conventional control agents. This trend has spurred interest in natural, sustainable, and multi-mechanism approaches, which are at the forefront of contemporary research in food science and technology [2,4-6].

Plant secondary metabolites are widely recognized as rich reservoirs of bioactive molecules with diverse structures and cellular targets that can limit microbial growth and survival. They can disrupt cell membranes, inhibit enzymatic pathways, and interfere with nucleic acid synthesis [7,8]. Consequently, plant extracts have attracted attention as potential natural antimicrobial agents for integration into food processing and preservation systems [9]. In a study, crude ethanolic extract of *Cuminum cyminum* L had antimicrobial properties [10]. The Papaveraceae family holds a prominent place in pharmacognosy and applied microbiology, particularly due to the production of isoquinoline alkaloids with potent biological activity. *C majus L.* is well known as an indicator species for compounds such as sanguinarine, chelidonine, and berberine, alkaloids that have demonstrated potent and selective antibacterial effects against a range of Gram-positive and Gram-negative bacteria [11-13].

Nevertheless, existing evidence has largely focused on the overall biological activity of *C. majus* extracts rather than on a systematic understanding of the factors that determine the intensity, selectivity, and duration of antibacterial effects. From a chemical-ecology perspective, geographical origin and environmental conditions (soil type, moisture, light intensity, biotic stresses) can markedly influence biosynthetic pathways and, consequently, the phytochemical profiles of different plant organs. This chemical heterogeneity can lead to substantial variation in antibacterial efficacy against foodborne pathogens [14-16].

Moreover, structural differences between gram-positive and Gram-negative bacteria, particularly the lipopolysaccharide-rich outer membrane and active efflux systems in Gram-negatives, act as important physicochemical barriers to the penetration of plant compounds

and shape antibacterial selectivity patterns [17-19]. Therefore, a simultaneous analysis of plant characteristics (organ and genotype) and microbial target characteristics is essential for designing effective natural pathogen-control strategies in food systems [11,20].
In this context, the present study employs a factorial, multi-regional approach to evaluate the antibacterial activity of *C. majus* L. root and leaf extracts collected from different ecotypes in northern Iran against three foodborne pathogens: *E. coli*, *L. monocytogenes*, and *S. aureus*. The innovation of this study lies in integrating a three-factor statistical design with ecological–phytochemical interpretation, enabling quantitative elucidation of the relative roles and interactions of each factor in determining the intensity and selectivity of microbial inhibition.

MATERIALS AND METHODS

Collection and Preparation of Plant Samples

Seeds of *C majus* L. were collected from multiple geographical locations in northern Iran, including Nowshahr, Gorgan, Shastakola, Kordkuy, Tonekabon, Langarud, Kabudwal, Noor, Aliabad, Babolsar, Azadshahr, and Behshahr. Botanical identification was verified by a specialist. To minimize the effects of environmental variation, seeds from all locations were cultivated under uniform climatic and soil conditions in Gorgan. Following maturation, root and leaf tissues were harvested separately, thoroughly washed, and shade-dried at room temperature.

Extraction Procedure and Yield

For extraction, 1 g of dried plant powder was mixed with 5 mL of methanol and incubated for 48 h under controlled temperature with constant agitation. The mixture was then filtered, and the solvent was evaporated using a rotary evaporator at 40 °C. The resulting dry extract was weighed to determine the extraction yield, expressed as milligrams of dry extract per gram of dry plant material (mg/g DW). Extracts were stored at 4 °C until antibacterial analysis. In preliminary solvent compatibility tests, both distilled water and dimethyl sulfoxide (DMSO) were assessed as reconstitution solvents; distilled water was chosen for subsequent antibacterial assays [21].

Bacterial Strains and Culture Conditions

Three foodborne pathogenic bacteria *E. coli*, *L. monocytogenes*, and *S. aureus* were obtained from the microbial culture collection of the Food Industry Group, Gorgan University of Agricultural Sciences and Natural Resources. Bacterial strains were activated in Nutrient Broth and subsequently cultured on Mueller–Hinton agar for antibacterial assays. Bacterial suspensions were standardized to 0.5 McFarland to ensure uniform inoculum density.

Evaluation of Antibacterial Activity

Antibacterial activity was assessed using the agar well diffusion method. After evenly spreading the bacterial suspension on Mueller–Hinton agar plates, wells of uniform diameter were created, and 70 µL of plant extract was added to each well. Plates were incubated at 37 °C for 24 h, after which inhibition zones were evaluated [22].

Measurement of Inhibition Zones using Image Processing

Quantitative measurement of inhibition zone diameters was performed using digital image analysis. Images of Petri dishes containing bacterial cultures and inhibition zones were captured using a 48-megapixel smartphone camera under uniform lighting conditions at an approximate distance of 15 cm from the Petri dish surface. A reference object with a known length was included in each image for scale calibration.
Image processing and analysis were conducted using Python (version 3.11) and the OpenCV library. Images were converted to grayscale and preprocessed using adaptive thresholding and noise-reduction filters. Edge detection was applied to accurately segment inhibition zones from the culture medium, and inhibition zone diameters were calculated quantitatively and expressed in millimeters. This image-based measurement approach reduces operator bias and improves reproducibility of inhibition zone assessment [23].

Phytochemical Analysis

Total alkaloid content of *C. majus* L. extracts was determined using a spectrophotometric method. Briefly, 0.1 g of dried plant material was extracted with 10 mL of 80% methanol, and the absorbance of the resulting solution was measured at 470 nm using a UV–Vis spectrophotometer. Atropine was used as the reference standard, and total alkaloid content was expressed as milligrams of atropine equivalents per gram of dry weight (mg AE/g DW) [24].

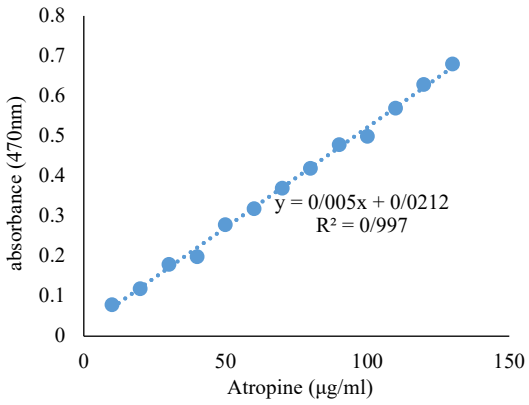

Fig. 1 Standard calibration curve of atropine

Total phenolic content was determined using the Folin–Ciocalteu colorimetric method with minor modifications. Absorbance was measured at 760 nm using a spectrophotometer. Total phenolic content was calculated based on a gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW) [25].

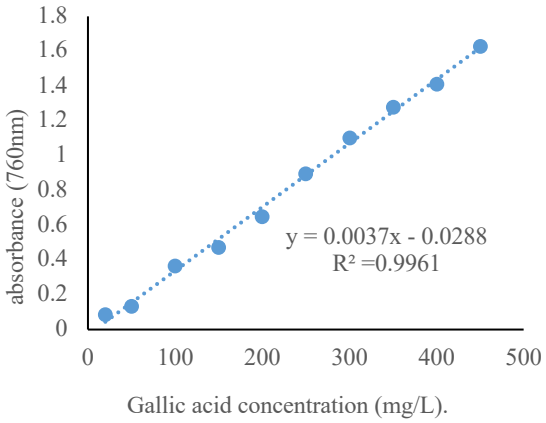

Fig. 2 Standard calibration curve of gallic acid

Experimental Design and Statistical Analysis

The experiment was conducted as a three-factor factorial design (geographical origin × plant organ × bacterial species) in a completely randomized design with three replications. Data were analyzed using three-way analysis of variance (ANOVA), and mean comparisons were performed using Tukey's HSD test. Statistical analyses were carried out using SAS software version 9.3.

RESULTS AND DISCUSSION

Three-way factorial ANOVA revealed significant main effects of treatment (geographical origin), plant organ, and bacterial species on the diameter of the inhibition zone ($p < 0.05$). Significant two-way and three-way interactions were also observed, indicating that antibacterial activity depended on the combined effects of plant source, organ, and bacterial species (Table 1).

Table 1 Three-way factorial ANOVA indicated significant main and interaction effects of geographical origin, plant organ, and bacterial species on inhibition zone diameter ($p < 0.05$).

S.O.V	Df	SS	MS	F-value	p- value
Geographical origin	11	24.49	2.22	18.33	<0/0001
Plant organ	1	561	561	4625	<0/0001
Bacteria	2	128	64	530	<0/0001
Geographical origin × Plant organ	11	28.23	2.56	21.13	<0/0001
Geographical origin × Bacteria	22	23	1.06	8.75	<0/0001
Plant organ × Bacteria	2	134	67.03	554	<0/0001
Geographical origin × Plant organ ×Bacteria	22	20	0.94	7.75	<0/0001
Error	144	17	0.12	-	-

The extremely large effect size of the plant organ reflects the near-zero inhibition observed in leaf extracts compared with consistently high inhibition by root extracts. Significant two-way and three-way interactions further indicate that antibacterial activity depends on the combined influence of geographical origin, organ type, and bacterial target (Table 2).

Table 2 partial eta squared (η^2) values represent the proportion of variance explained by each effect after controlling for other factors. Interpretation follows Cohen (1988): small (≥0.01), moderate (≥0.06), and large (≥0.14

Effect	Partial η^2	Effect size
Geographical origin	0/583	Large
Plant organ	0/970	Large
Bacteria	0/880	Large
Geographical origin * Plant organ	0/617	Large
Geographical origin * Bacteria	0/572	Large
Plant organ * Bacteria	0/885	Large
Geographical origin * Plant organ*Bacteria	0/542	Large

Tukey's HSD test ($\alpha = 0.05$) demonstrated that the antibacterial activity of *C. majus* extracts was significantly influenced by the interaction between geographical origin, plant organ, and bacterial species (Table 3). Overall, leaf extracts exhibited negligible antibacterial activity across almost all geographical origins. Detectable inhibition was observed only against *E. coli* in extracts from Nowshahr (0.21 mm), Shastkola (0.31 mm), and Kaboodwal (0.26 mm), whereas no inhibitory effect was detected against *L. monocytogenes* or *S. aureus* in any leaf extract.

In contrast, root extracts exhibited antibacterial activity against all three bacterial species across all geographical origins, although the magnitude of inhibition varied significantly among origins. Against *S. aureus*, the largest inhibition zones were recorded for Babolsar (6.81 mm), Behshahr (6.55 mm), and Tonekabon (6.41 mm), whereas the smallest was observed for Nowshahr (2.99 mm). For *L. monocytogenes*, the strongest activity was obtained from Noor (3.17 mm), Behshahr (3.06 mm), and Babolsar (3.05 mm). No detectable inhibition was observed for the Shastkola root extract, while Kaboodwal showed the lowest measurable activity (0.84 mm). Against *E. coli*, the Noor root extract produced the largest inhibition zone (3.53 mm), whereas Aliabad (0.75 mm) and Kordkuy (0.78 mm) exhibited the weakest antibacterial activity.

Mean comparisons further revealed significant differences among the bacterial species. *S. aureus* exhibited significantly larger inhibition zones than both *L. monocytogenes* and *E. coli*, while *L. monocytogenes* was significantly more susceptible than *E. coli* ($P < 0.05$). These findings indicate a clear susceptibility gradient, with *S. aureus* being the most sensitive species, followed by *L. monocytogenes*, whereas *E. coli* was consistently the most resistant. Although *E. coli* displayed greater variability in inhibition zone diameter among geographical origins, its inhibition zones remained consistently smaller than those of the two Gram-positive bacteria. Overall, root extracts consistently demonstrated substantially greater antibacterial activity than leaf extracts, confirming that the antibacterial efficacy of *C. majus* depends strongly on plant organ as well as geographical origin. The pronounced variation among geographical origins further suggests that genetic and environmental factors associated with plant provenance contribute significantly to the antibacterial potential of *C. majus* root extracts.

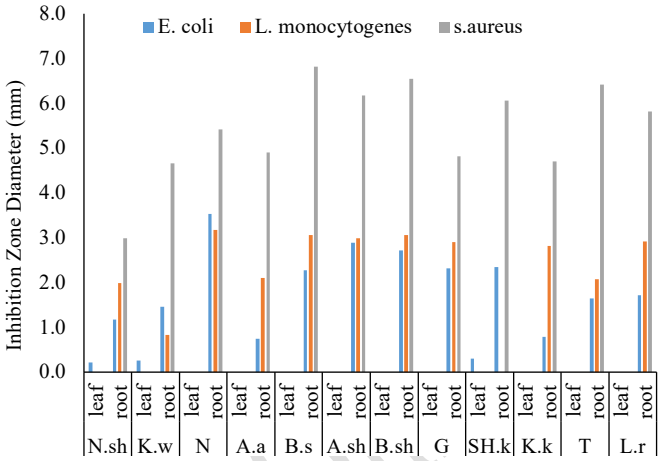

Fig. 3 Comparison of antibacterial effects of roots and leaves on three bacterial strains: *E. coli, L. monocytogenes, and S. aureus.* (K.k (Kordkuy), N sh (Nowshahr), N (Noor), A.a (Aliabad), L.r (Langarud), T (Tonekabon), B.s (Babolsar), B.sh (Behshahr), Sh k (Shast kola), A.sh (Azadshahr), K.w (Kaboodwal), G(Gorgan).)

Table 3 effect of geographical origin on antibacterial activity of plant extract

Geographical origin	*E. coli* (Leaf)	*E. coli* (Root)	*L. monocytogenes* (Leaf)	*L. monocytogenes* (Root)	*S. aureus* (Leaf)	*S. aureus* (Root)
Nowshahr	0.210 c	1.173 dc	0.0 a	1.993 ab	0.0 a	2.993 c
Gorgan	0.0 d	2.320 ab	0.0 a	2.9 a	0.0 a	4.817 b
Shastkola	0.307 a	2.353 ab	0.0 a	0.0 c	0.0 a	6.067 ab
Kordkuy	0.0 d	0.783 c	0.0 a	2.813 a	0.0 a	4.703 b
Tonekabon	0.0 d	1.647 bc	0.0 a	2.070 ab	0.0 a	6.413 a
Langarud	0.0 d	1.723 bc	0.0 a	2.910 a	0.0 a	5.820 ab
Kaboodwal	0.257 b	1.460 bc	0.0 a	0.837 bc	0.0 a	4.663 b
Noor	0.0 d	3.527 a	0.0 a	3.173 a	0.0 a	5.420 ab
Aliabad	0.0 d	0.747 c	0.0 a	2.103 ab	0.0 a	4.907 b
Babolsar	0.0 d	2.267 ab	0.0 a	3.053 a	0.0 a	6.813 a
Azadshahr	0.0 d	2.890 a	0.0 a	2.997 a	0.0 a	6.180 ab
Behshahr	0.0 d	2.723 a	0.0 a	3.057 a	0.0 a	6.553 a

Means followed by different superscript letters within each column are significantly different according to tukeys HSD test at $p<0.05$.

Geographical variation in antibacterial efficacy was significant, as demonstrated by the interaction between geographical origin and bacterial species. Mean inhibition zone diameters ranged from approximately 0.3 in the least effective treatments (e.g., Kabudwal and Aliabad) to values greater than 1.7 in the most effective treatments (e.g., Noor).

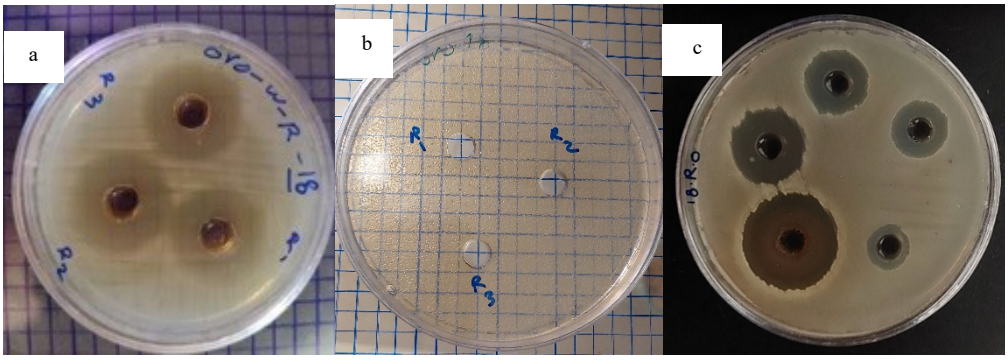

Fig. 4 (a): Root growth inhibition zone of *C. majus* L. Behshahr region on *S. aureus*. (b): Leaf growth inhibition zone of *C. majus* L. Behshahr region on *S. aureus* (c): IC50 test of five concentrations of *C. majus* L. root extract on *S. aureus* bacteria

Regions such as Kordkuy, Tonekabon, Langarud, and Azadshahr showed significantly larger inhibition zones against *L. monocytogenes* and *S. aureus* compared to *E. coli*. The difference between *E. coli* and Gram-positive bacteria was particularly pronounced in these regions, with inhibition zone diameters exceeding 1 mc in many cases.

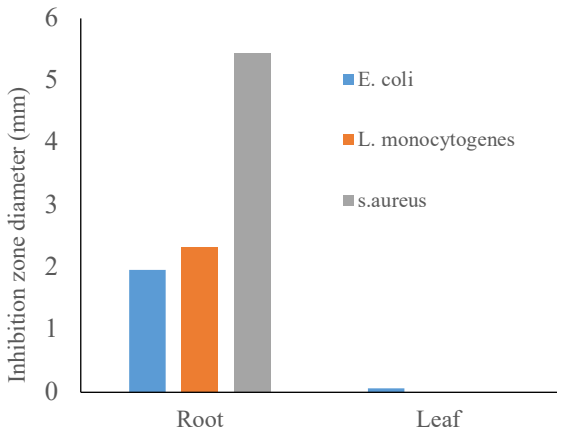

Fig. 5 Comparison of the control rate of foodborne pathogens in leaf and root extracts.

ANOVA revealed significant effects of plant geographical origin on both total alkaloid and total phenolic content at the 1% probability level. This indicates substantial intraspecific genetic variation in the concentration of bioactive compounds among the populations studied (Table 4).

The highest total alkaloid contents were observed in samples from the Kordkuy, Nowshahr, Noor, and Aliabad regions, while the lowest values were measured in Gorgan, Kabudwal, and Azadshahr samples. For total phenolic content, Noor exhibited the highest levels, followed by Shastakola, Kordkuy, and Aliabad, while Kabudwal and Azadshahr showed the lowest concentrations.

Table 4 One- way Analysis of variance was performed separately for phytochemical traits.

SOV	DF	MS	
		Alkaloids	Phenols
Treatment	11	0.002060 **	13.9
Experimental error	24	0.000014	0.2230
CV		7.9	9.61

Significance levels: $p < 0.05$ (*), $p < 0.01$ (**)

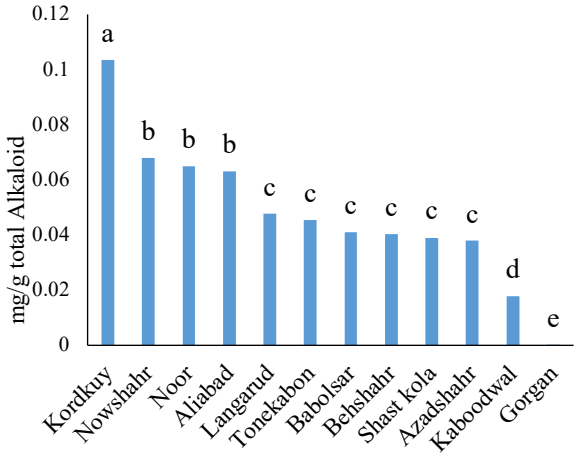

Fig. 6 Comparison of the average total Alkaloid of *C. majus* L. extract

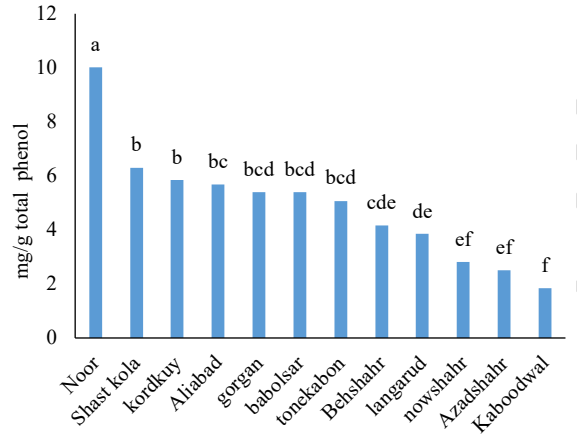

Fig. 7 Comparison of average total phenols of *C. majus* L. extract

The results of this study demonstrate that Gram-positive bacteria (*L. monocytogenes* and *S. aureus*) were more sensitive to the extracts than the Gram-negative bacterium (*E. coli*). This selective antibacterial effect is consistent with established knowledge on the structural differences between Gram-positive and Gram-negative bacteria. Gram-negative bacteria have an outer lipopolysaccharide membrane and efflux systems that act as barriers to the penetration of many plant-derived antimicrobial compounds, thereby reducing their susceptibility [17,26]. Similar patterns have been reported in other studies, where Gram-positive bacteria were found to be more susceptible to plant extracts and essential oils [4, 27], validating the current findings.

Root extracts consistently exhibited superior antibacterial effects over leaf extracts. This observation is likely due to the higher concentration of bioactive compounds such as alkaloids in the roots, which are specifically produced to combat soil-borne pathogens. Roots are in continuous contact with the rhizosphere and, as a result, accumulate more defensive secondary metabolites [11,28]. This finding is consistent with the findings of Fazeli Nasab (2023), who reported significant differences in the antibacterial activity of methanol extracts of different medicinal plants and highlighted the role of phytochemical composition in determining their antimicrobial potential. The near-zero inhibition zones observed for leaf extracts, despite occasional statistical significance, were biologically negligible [29]. This reinforces the notion that leaf extracts, while containing antimicrobial compounds, may not be as potent as root extracts.

The significant geographical variation in antibacterial activity observed in this study can primarily be attributed to genetic differences between populations. These differences could be shaped by local environmental factors, such as soil composition and climate, which influence the biosynthetic pathways of secondary metabolites [30,31]. The Noor and Kordkuy regions, in particular, displayed stronger antibacterial efficacy, likely due to the higher concentrations of bioactive alkaloids in these populations. In contrast, regions such as Kabudwal and Aliabad, which showed lower antibacterial activity, may harbor less productive genotypes or plants that produce fewer antimicrobial compounds.

A clear correlation was observed between total alkaloid content and antibacterial activity, especially against Gram-positive bacteria. Samples from the Noor and Kordkuy regions, which had higher levels of total alkaloids, also demonstrated wider inhibition zones, particularly against *S. aureus* and *L. monocytogenes*. Known isoquinoline alkaloids such as sanguinarine have been shown to possess potent antibacterial activity by disrupting cell membrane integrity and interfering with bacterial protein function [32,34]. In contrast, total phenolic content did not show a direct and consistent relationship with antibacterial potency. This suggests that phenolic compounds might act synergistically with alkaloids but are not the primary determinants of antibacterial activity [4,15].

The findings of this study suggest that root extracts, especially those from alkaloid-rich genotypes such as those from Noor and Kordkuy, hold considerable promise as natural antibacterial agents, particularly against Gram-positive foodborne pathogens. While leaf extracts also possess some antibacterial properties, they are more suitable for complementary or combined uses rather than as primary antimicrobial agents [33].

CONCLUSION

This study demonstrates that the antibacterial activity of *C. majus* L. extracts is strongly influenced by plant organ, geographical origin, and bacterial species. Root extracts showed significantly greater efficacy than leaf extracts. Among the tested pathogens, *S. aureus* was markedly more susceptible to the extracts than *L. monocytogenes* and *E. coli*, and *L. monocytogenes* was in turn more susceptible than *E. coli*, a Gram-negative species. A strong positive correlation was observed between total alkaloid content, particularly in extracts from the Kordkuy and Noor regions, and antibacterial potency, underscoring the primary role of alkaloids in mediating the observed effects. In contrast, total phenolic content exhibited a less direct relationship with antibacterial activity.

Samples originating from the Kabudwal and Azadshahr regions displayed the lowest metabolite levels and the weakest antibacterial effects. The significant three-way interaction among plant genotype (geographical origin), plant organ, and bacterial species highlights that selecting an optimal extract for antimicrobial applications requires the concurrent consideration of these variables. In conclusion, the root of *C. majus* L., especially alkaloid-rich genotypes from specific regions such as Kordkuy and Noor, shows considerable promise as a natural antibacterial agent against Gram-positive foodborne pathogens.

Consent for Publication

Not applicable.

Data Availability

The data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

Funding

This research received no external financial support.

Authors' Contributions

M. M: Conceptualization, methodology, investigation, data collection, data analysis, and writing original draft, A. Gh: Supervision, Corresponding author, conceptualization, methodology, and writing review and editing, K. R: methodology, and review and editing.
M. Kh.S: methodology, and writing review and editing. All authors read and approved the final manuscript.

Acknowledgements

The authors gratefully acknowledge Dr. Khomeiri, Scientefic the Food Industries Group at the University of Agricultural Sciences and Natural Resources, Gorgan, Iran, for providing the bacterial strains used in this study.

REFERENCES

1. Fokas R., Giormezis N., Vantarakis A. Synergistic approaches to foodborne pathogen control: a narrative review of essential oils and bacteriophages. Foods. 2025;14(9):1508.
2. Farrukh M., Munawar A., Nawaz Z., Hussain N., Hafeez A.B., Szweda P. Antibiotic resistance and preventive strategies in foodborne pathogenic bacteria: a comprehensive review. Food Science and Biotechnology. 2025;34(10):2101.
3. Arab M., Arab N., Kahrizi D., Ebadi A.G. Evaluating antibacterial effects of alcoholic extracts and essential oil of *Althaea officinalis* against two types of gram-positive and gram-negative bacteria (*Bacillus cereus* and *Klebsiella pneumoniae*). Journal of Medicinal Plants and By-products. 2023;12(1):107-115.
4. Hyldgaard M., Mygind T., Meyer R.L. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology. 2012;3:12.
5. Elbehiry A., Marzouk E., Abalkhail A., Edrees H.M., Ellethy A.T., Almuzaini A.M., Abu-Okail A., *et al.* Microbial food safety and antimicrobial resistance in foods: a dual threat to public health. Microorganisms. 2025;13(7):1592.
6. Fokas R., Giormezis N., Vantarakis A. Synergistic approaches to foodborne pathogen control: a narrative review of essential oils and bacteriophages. Foods. 2025;14(9):1508.
7. Allemailem K.S. Antimicrobial potential of naturally occurring bioactive secondary metabolites. Journal of Pharmacy and Bioallied Sciences. 2021;13(2):155-162.
8. Soares A., Nocchi N., Díaz-Marrero A., Pereira R., Fernández J. Smart secondary metabolites in marine environments: the case of elatol. Pharmacognostic Potential of Seaweed Extracts and Metabolites. 2026;24(2):61.
9. Lemoni Z., Evangeliou K., Lymperopoulou T., Mamma D. Incorporation of edible plant extracts as natural food preservatives: green extraction methods, antibacterial mechanisms and applications. Foods. 2025;14(23):4000.
10. Osanloo E., Soleymani A.A., Taheri M., Hosseini S.S., Javadi F., Karimi-Yazdi M., Hakemi-Vala M., *et al.* Evaluation of antibacterial, antibiofilm and cytotoxicity effect of crude ethanolic extract of *Cuminum cyminum* against Streptococcus mutans. Journal of Medicinal Plants and By-products. 2023;12(2):191-196.
11. Stefanowski N., Tkachenko H., Kurhaluk N. Antibacterial activity of ethanolic extracts obtained from roots and stems of *Chelidonium majus* L. against Enterococcus faecalis strains. Agrobiodiversity for Improving Nutrition, Health and Life Quality. 2021;5(2).
12. Zielińska S., Wójciak-Kosior M., Dziągwa-Becker M., Gleńsk M., Sowa I., Fijałkowski K., Junka A., *et al*. The activity of isoquinoline alkaloids and extracts from *Chelidonium majus* L. against pathogenic bacteria and Candida sp. Toxins. 2019;11(7):406.
13. Dzięgielewska M., Zielińska S., Dudek B., Brożyna M., Kozłowska W., Matkowski A., Junka A., *et al.* Isoquinoline alkaloids from traditional medicinal plants of the Papaveraceae as a third line of defense against bacterial ocular infections. bioRxiv. 2025;2025-11.
14. Boutahiri S., Benrkia R., Tembeni B., Idowu O.E., Olatunji O.J. Effect of biostimulants on the chemical profile of food crops under normal and abiotic stress conditions. Current Plant Biology. 2024;40:100410.
15. Zouine N., El Ghachtouli N., El Abed S., Koraichi S.I. A comprehensive review on medicinal plant extracts as antibacterial agents: factors, mechanism insights, and prospects. Scientific African. 2024;26:e02395.
16. Ogwu M.C., Izah S.C., Joshua M.T. Ecological and environmental determinants of phytochemical variability in forest trees. Phytochemistry Reviews. 2025;24(6):5109-5137.
17. Zhang G.E., Meredith T.C., Kahne D. On the essentiality of lipopolysaccharide to Gram-negative bacteria. Current Opinion in Microbiology. 2013;16(6):779-785.

18. Huseby D.L., Cao S., Zamaratski E., Sooriyaarachchi S., Ahmad S., Bergfors T., Karlén A., *et al.* Antibiotic class with potent in vivo activity targeting lipopolysaccharide synthesis in Gram-negative bacteria. Proceedings of the National Academy of Sciences. 2024;121(15):e2317274121.
19. Mohammadi A., Arabhosseini A. Antioxidant and anti-microbial properties of *Euphorbia condylocarpa* roots under hot-air drying conditions. Journal of Medicinal Plants and By-products. 2024;13(2):405-415.
20. Diwan D., Rashid M.M., Vaishnav A. Current understanding of plant-microbe interaction through the lenses of multi-omics approaches and their benefits in sustainable agriculture. Microbiological Research. 2022;265:127180.
21. Balouiri M., Sadiki M., Ibnsouda S.K. Methods for in vitro evaluating antimicrobial activity: a review. Journal of Pharmaceutical Analysis. 2016;6(2):71-79.
22. Amankwah F.K.D., Gbedema S.Y., Boakye Y.D., Bayor M.T., Boamah V.E. Antimicrobial potential of extract from a Pseudomonas aeruginosa isolate. Scientifica. 2022;2022:4230397.
23. Saviano A.M., Lourenco F.R. Using image analysis to determine gentamicin potency by agar diffusion microbiological assay and its measurement uncertainty. Measurement. 2019;146:315-321.
24. Ghomushi R., Shamsa Fazel, Monsef Isfahani H.R. Presence of alkaloids in plant samples: method of alkaloid reagents in comparison with bromocresol green. Journal of School of Medicine, Tehran University of Medical Sciences. 2008;66(July 25):237-241.
25. Slinkard K., Singleton V.L. Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture. 1977;28(1):49-55.
26. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiology and Molecular Biology Reviews. 2003;67(4):593-656.
27. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology. 2004;94(3):223-253.
28. Cowan M.M. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999;12(4):564-582.
29. Fazeli-Nasab B., Ghafari M., Jahantigh M., Beigomi Z., Saeidi S. Evaluation of phenolic and flavonoid content, alkaloids, antioxidant capacity and antibacterial properties of methanolic extract of Zahak native medicinal plants against seven pathogens. Journal of Medicinal Plants and By-products. 2023;13(1):57-65.
30. Li Y., Jin J., Li P., Wang Q., Xu L., Wei G., Li Z. Regional variations and plant compartments shape the community structures of the endophytic microbiome and secondary metabolites of Astragalus mongholicus. Industrial Crops and Products. 2023;192:116037.
31. He Y., Junker R.R., Xiao J., Lasky J.R., Cao M., Asefa M., Sedio B.E., *et al.* Genetic and environmental drivers of intraspecific variation in foliar metabolites in a tropical tree community. New Phytologist. 2025;246(6):2551-2564.
32. Qi J., Zhang X., Guo X., Yang Y., Fan X., Han Y., Liu Y. Evaluation of the in vitro and in vivo antimicrobial activity of alkaloids prepared from *Chelidonium majus* L. using MRSA-infected C. elegans as a model host. Fitoterapia. 2024;175:105944.
33. França F., Souza J.C., O'Connor P.M., Matos A.P., Pimentel-Filho N.D.J. Plant antimicrobials: extraction, characterization, and activity against foodborne microorganisms. Folia Microbiologica. 2026;1-21.
34. Zielińska S., Jezierska-Domaradzka A., Wójciak-Kosior M., Sowa I., Junka A., Matkowski A.M. Greater celandine's ups and downs − 21 centuries of medicinal uses of *Chelidonium majus* from the viewpoint of today's pharmacology. Frontiers in Pharmacology. 2018;9:299.