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Comparative antibacterial activity of nettle (*Urtica dioica*) and mallow (*Malva spp.*) essential oils

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Abstract

Background: Essential oils derived from medicinal plants have attracted increasing attention as natural alternatives to synthetic antimicrobial agents, especially in food safety and preservation. *Urtica dioica* (Nettle) and *Malva spp*. (Mallow) are traditionally known for their medicinal properties, yet their combined antibacterial effects remain underexplored. This study investigated the in vitro antibacterial activity of nettle and mallow essential oils, both individually and in combination (1:1 ratio), against ten common foodborne pathogens responsible for spoilage and contamination.

Methods: Essential oils were extracted from *U. dioica* and *Malva* spp., then tested alone and in a 1:1 (v/v) combination for their antibacterial efficacy using agar disk diffusion and broth microdilution assays. The tested bacterial strains included *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Alcaligenes faecalis*, *Serratia marcescens*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, and *Escherichia coli*. Gentamicin (10 µg/disk) was used as a positive control. Data were analyzed to calculate the inhibition zone diameter (DIZ), minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC). All experiments were performed in triplicate (n = 3), and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way ANOVA followed by Tukey's post hoc test to compare differences among treatment groups.

Results: All essential oil formulations showed antibacterial activity, with MIC values for nettle essential oil (NEO) ranging from 1,250 to 5,000 µg/mL and for mallow essential oil (MEO) from 2,500 to 10,000 µg/mL. The 1:1 combination of NEO and MEO retained NEO's favorable MIC and delivered a lower MBC for *K. pneumoniae* compared to MEO alone. Across strains, MIC differences were not uniformly significant. For *L. monocytogenes*, NEO showed equal MIC and MBC (1,250 µg/mL), indicating its bactericidal activity.

Conclusion: Nettle and mallow essential oils possess significant antibacterial activity against key foodborne pathogens. Their simultaneous application yielded additive effects against some pathogens. These findings support the potential of these essential oils as natural antimicrobial agents to be used in food preservation systems, including for antimicrobial packaging and as edible coatings or surface sanitizers.

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Highlights

What is current knowledge?

- Plant essential oils are natural alternatives to synthetic antimicrobial preservatives.
- Nettle and mallow are medicinal plants with reported antimicrobial properties.
- Prior studies focused mainly on extracts, not essential oils.
- Comparative antibacterial effects of nettle and mallow essential oils remain unclear.

What is new here?

- First systematic comparison of nettle and mallow essential oils against foodborne pathogens.
- Nettle essential oil showed stronger antibacterial activity than mallow essential oil.
- Nettle oil exhibited bactericidal effect against Listeria monocytogenes at low concentration.
- A 1:1 nettle-mallow blend enhanced mallow oil's antibacterial performance.
- Essential oils show potential for use in food preservation applications.

Introduction

The increasing prevalence of microbial contamination in food products has become a critical concern in both developed and developing countries, driving global efforts to identify natural and effective alternatives to synthetic preservatives. While conventional antimicrobial agents are widely employed in food systems, their use is often associated with health risks, environmental concerns, and the emergence of resistant microbial strains, challenges that have prompted the food industry to explore more sustainable and consumer-friendly solutions (1,2). In this context, plant-derived antimicrobials have attracted significant attention due to their broad-spectrum activity, low toxicity, biodegradability, and alignment with consumer demand for "clean-label" products that minimize artificial additives (3). Higher plants are recognized as rich sources of bioactive secondary metabolites, many of which exhibit antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties (4). Among these phytochemicals, compounds such as phenolic acids, flavonoids, alkaloids, and essential oils (EOs) are known to exert antimicrobial effects through mechanisms including membrane disruption, enzyme inhibition, interference with nucleic acid synthesis, and quorum sensing modulation (5). Two botanicals of particular interest in this regard are *Malva* spp. (Mallow) and *Urtica dioica* (Stinging nettle). *Malva* has traditionally been used

for its soothing, emollient, and anti-inflammatory properties, and its phytochemical profile includes abundant polysaccharides, flavonoids, and phenolic compounds with demonstrated antimicrobial potential. Previous studies have reported inhibitory effects of *Malva* extracts, especially against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis*, supporting its possible use as a natural preservative in food systems (6). However, extracts and essential oils differ substantially in their chemical composition extracts are rich in non-volatile compounds such as polysaccharides, whereas essential oils contain volatile constituents with distinct antimicrobial mechanisms. This novelty underscores the importance of evaluating Malva essential oil specifically, as its biological activity may differ considerably from that of solvent-based extracts. Likewise, *Urtica dioica* has been widely studied for its nutritional and medicinal applications, and contains several bioactive constituents, such as caffeic acid, chlorogenic acid, quercetin derivatives, and lectins, with antioxidant, anti-inflammatory, and antimicrobial activities (7). Nevertheless, these compounds are generally associated with extracts rather than essential oils, as nettle is not traditionally considered an aromatic plant with high essential oil yield. Therefore, assessing Urtica essential oil represents a less-explored and potentially novel approach, albeit requiring careful justification of its expected activity. Despite these promising findings from extracts, the antimicrobial activity of Malva and Urtica essential oils has not been systematically compared under standardized in vitro conditions, particularly against a broad range of foodborne bacteria. Moreover, inconsistencies in plant origin, extraction techniques, solvent systems, and assay protocols across previous studies have contributed to variable results and limited reproducibility. Importantly, while individual evaluations are necessary, the rationale for testing their 1:1 (v/v) combination lies in the potential for synergistic or additive interactions between their volatile constituents, a hypothesis supported by previous reports of enhanced efficacy in combined essential oils. The present study aims to address this need by systematically evaluating the in vitro antibacterial properties of essential oils derived from *Malva* spp. and *Urtica dioica*, as well as their 1:1 (v/v) combination, against a panel of ten foodborne bacterial species: *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Alcaligenes faecalis*, *Serratia marcescens*, *Salmonella enteritidis*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Listeria monocytogenes*, *Klebsiella pneumonia* and *Escherichia coli*. Antimicrobial efficacy was assessed using standardized methods, including agar disk diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. The outcomes aim to support the development of natural, plant-based antimicrobial agents for use in food preservation applications, and to contribute to the broader understanding of phytochemicals as functional ingredients in food safety management (8).

Methods

Plant material and extract preparation

Mallow and Nettle plants were sourced and taxonomically verified by agricultural experts, with herbarium reference numbers recorded. Essential oils (EOs) were obtained by hydrodistillation using a Clevenger-type apparatus for 3 h, a method widely applied for extracting volatile fractions. Oils were dried over anhydrous sodium sulfate, stored in sterile, amber vials at 4 °C, and used within 14 days. Prior to assays, each EO was dissolved in 10% (v/v) DMSO and filter-sterilized (0.22 µm) (9).

Bacterial strains and preparation

Bacterial strains relevant to food spoilage and human infection were obtained from accredited repositories (ATCC and the Iranian Research Organization for Science and Technology, IROST). The panel comprised *Pseudomonas aeruginosa* (ATCC 27853), *Streptococcus pyogenes* (ATCC 19615), *Alcaligenes faecalis* (ATCC 8750), *Serratia marcescens* (ATCC 13880), *Salmonella enteritidis* (ATCC 13076), *Staphylococcus aureus* (ATCC 25923), *Shigella dysenteriae* (ATCC 13313), *Listeria monocytogenes* (ATCC 19115), *Klebsiella pneumoniae* (ATCC 13883) and *Escherichia coli* (ATCC 25922). Each strain was streak-cultured on its recommended agar medium and incubated for 24 h at 37 °C. A single colony was transferred to 10 mL of the corresponding broth and grown for 18 h (37 °C, 120 rpm). Bacterial suspensions were standardized to an approximate concentration of 10^6 CFU/mL by measuring optical density at 600 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). This uniform inoculum was employed in all subsequent experimental procedures.

Antibacterial assays

Antibacterial activity was assessed using two complementary methods: broth microdilution (To determine MIC and MBC) and the agar disk diffusion assay.

Microdilution method (MIC and MBC)

Serial two-fold dilutions of each extract (10,000 to 312.5 µg mL^{-1} in BHI broth; final DMSO ≤ 1 %) were prepared in 96-well microplates (160 µL broth + 20 µL inoculum + 20 µL extract). Plates were shaken for 30 s, incubated 24 h at 37 °C, and viability assessed with 0.01 % (w/v) resazurin. The MIC was defined as the lowest concentration preventing the resazurin color change. For MBC, 10 µL from each clear well were streaked on nutrient agar and incubated 24 h; the MBC corresponded to the lowest extract concentration yielding no growth. A soluble gentamicin sulfate standard (0.5-64 µg/mL) was used as the positive control, tested in the same microdilution format to ensure quantitative comparability across bacterial species (10).

Agar disk-diffusion method

Following the Clinical and Laboratory Standards Institute (CLSI) guidelines, bacterial suspensions were uniformly spread on Mueller-Hinton agar plates using sterile swabs. Sterile 6 mm paper disks (Oxoid, UK) were impregnated with 10 µL EO at 200 mg/mL (A concentration selected based on preliminary trials that ensured measurable yet non-saturating inhibition zones) and placed onto the inoculated agar surface. As positive control, a soluble gentamicin solution (10 µg/mL) was applied to sterile disks, ensuring methodological consistency with broth assays. Disks 10% DMSO functioned as negative controls. Plates were incubated at 37 °C for 24 h, and the diameters of the inhibition zones (In mm) were measured using digital calipers (Mitutoyo, Japan) (11). The cutoff values for interpreting inhibition zones were guided by CLSI standards and previous EO studies to ensure reproducibility.

Statistical

Data obtained from antibacterial assays were statistically evaluated with SPSS (IBM Corp., USA) to investigate the significance of the essential oils' antibacterial activity against various strains. Normal distribution of the data was verified using the Kolmogorov-Smirnov test, and homogeneity of variance was assessed through Levene's test. When these assumptions were satisfied, statistical differences among groups were identified by one-way ANOVA with Tukey's post hoc test. If assumptions were violated, the non-parametric Kruskal-Wallis test with Dunn's post hoc correction was applied. Statistical significance level was 0.05. The findings represented as mean ± SD from three replicates (n = 3).

Results

MIC and MBC

The MIC and MBC values of Urtica dioica essential oil (NEO), Malva spp. essential oil (MEO), and their 1:1 (v/v) combination were determined against ten bacterial strains using the broth microdilution method. The results are summarized in Table 1. Overall, NEO exhibited the strongest antibacterial activity, with MIC values as low as 1,250 µg/mL against A. faecalis, S. aureus, and L. monocytogenes, and corresponding MBC values of 2,500, 2,500, and 1,250 µg/mL, respectively. The identical MIC and MBC values for L. monocytogenes indicate a strong bactericidal effect. In contrast, MEO required higher concentrations (2,500-10,000 µg/mL) to inhibit growth, though it retained bactericidal potential against P. aeruginosa and S. aureus (MBC/MIC ≤ 2). The 1:1 combination displayed MIC values comparable to NEO against highly susceptible strains. In some cases, such as K. pneumoniae, the mixture reduced the MBC compared to MEO alone. Statistical analysis revealed significant differences (P-Value < 0.05) between NEO and MEO for S. aureus and L. monocytogenes. The combination was significantly different from MEO but not from NEO. However, across the full bacterial panel, no consistent trend of significant differences was observed. These findings suggest that the antibacterial effects are both descriptive and statistically robust. Importantly, some MIC values approached the activity level of gentamicin, indicating potential applications of these oils in food preservation as natural antimicrobial agents.

Table 1. Antibacterial activity of nettle essential oil (NEO), mallow essential oil (MEO), and their 1:1 combination based on MIC and MBC values

Bacterial strain	MIC (NEO)	MBC (NEO)	MIC (MEO)	MBC (MEO)	MIC (NEO+MEO)	MBC (NEO+MEO)
S. enteritidis	5000	10000	10000	10000	5000	10000
S. dysenteriae	5000	5000	5000	10000	5000	5000
P. aeruginosa	2500	5000	5000	5000	2500	5000
E. coli	5000	10000	10000	10000	5000	10000
S. pyogenes	2500	2500	5000	5000	2500	5000
S. marcescens	2500	2500	5000	5000	2500	2500
K. pneumoniae	2500	5000	5000	10000	5000	5000
A. faecalis	1250	2500	2500	5000	1250	2500
S. aureus	1250	2500	2500	5000	2500	2500
L. monocytogenes	1250	1250	2500	5000	1250	2500

Table 2. Antibacterial activity of nettle essential oil (NEO), mallow essential oil (MEO), and their 1:1 combination in agar disk diffusion assay (Mean ± SD)

Bacterial strain	NEO (mm)	MEO (mm)	Blend (mm)	Gentamicin (mm)
S. enteritidis	8.60 ± 0.10 ^{Aa}	7.55 ± 0.17 ^{Aa}	8.60 ± 0.10 ^{Aa}	13.82 ± 0.17 ^{Ab}
E. coli	8.70 ± 0.15 ^{Aa}	7.73 ± 0.20 ^{Aa}	8.70 ± 0.15 ^{Aa}	14.10 ± 0.15 ^{Bb}
S. dysenteriae	8.82 ± 0.14 ^{Aa}	7.78 ± 0.10 ^{Aa}	8.82 ± 0.14 ^{Aa}	13.27 ± 0.27 ^{Cb}
P. aeruginosa	9.50 ± 0.20 ^{Ba}	8.25 ± 0.14 ^{Ba}	9.50 ± 0.20 ^{Ba}	13.40 ± 0.40 ^{Cb}
K. pneumoniae	9.53 ± 0.14 ^{Ba}	8.21 ± 0.15 ^{Ba}	9.53 ± 0.14 ^{Ba}	12.85 ± 0.10 ^{Db}
S. marcescens	9.60 ± 0.17 ^{Ba}	8.45 ± 0.10 ^{Ba}	9.60 ± 0.17 ^{Ba}	14.43 ± 0.15 ^{Eb}
S. pyogenes	10.60 ± 0.10 ^{Ca}	9.05 ± 0.14 ^{Ca}	10.60 ± 0.10 ^{Ca}	14.20 ± 0.20 ^{Bb}
S. aureus	11.42 ± 0.17 ^{Da}	10.05 ± 0.15 ^{Da}	11.42 ± 0.17 ^{Da}	12.42 ± 0.12 ^{Fb}
A. faecalis	11.60 ± 0.20 ^{Da}	9.91 ± 0.14 ^{Da}	11.60 ± 0.20 ^{Da}	12.33 ± 0.10 ^{Fb}
L. monocytogenes	11.68 ± 0.20 ^{Da}	8.85 ± 0.12 ^{Ca}	11.68 ± 0.20 ^{Da}	12.20 ± 0.12 ^{Fb}

Different capital letters in each column indicate a statistically significant difference (P-Value < 0.05).
Different small letters in each row indicate a statistically significant difference (P-Value < 0.05).

Agar disk diffusion assay

The antimicrobial activity was further evaluated using the agar disk diffusion method, with soluble gentamicin sulfate included as the standard reference antibiotic. NEO produced the widest inhibition zones, including 11.68 ± 0.20 mm for L. monocytogenes and 11.60 ± 0.20 mm for A. faecalis (Table 2). By comparison, gentamicin produced zones of 12.20 ± 0.12 mm and 12.33 ± 0.10 mm, respectively. MEO generated smaller zones overall, with its maximum effect against S. aureus (10.05 ± 0.15 mm, Table 2). The NEO-MEO blend achieved inhibition zones that were generally intermediate to or slightly larger than those of MEO alone, for example 11.42 ± 0.17 mm against S. aureus compared with 10.05 ± 0.15 mm for MEO (P-Value = 0.04, one-way ANOVA with Tukey's post hoc test). While the blend did not consistently outperform NEO, its activity was often enhanced relative to MEO. Statistical analysis (ANOVA with Tukey's post hoc test) confirmed significant differences among treatments (P-Value = 0.03), as indicated by superscript letters. Gentamicin remained the most potent agent in all comparisons, yet the oils demonstrated reproducible antibacterial activity across both Gram-positive and Gram-negative strains.

Discussion

Antibacterial efficacy

The present study demonstrated that *Urtica dioica* essential oil (NEO) exhibits superior antibacterial efficacy compared to *Malva* spp. essential oil (MEO) across most tested bacterial strains. This conclusion is supported by both the lower MIC/MBC values in the broth microdilution method and the reproducible results across triplicate experiments (n = 3). For example, the lowest MIC (1,250 µg/mL) recorded for *L. monocytogenes* with NEO corresponded exactly with the MBC (1,250 µg/mL), confirming a bactericidal effect against this high-risk foodborne pathogen. This finding has practical importance, as it demonstrates that nettle EO can achieve complete killing of a clinically relevant organism at relatively low concentrations (12). The blended NEO-MEO formulation resulted in comparable or enhanced inhibition zones relative to the individual oils and, in some cases, approached the efficacy of Gentamicin. For example, against *S. pyogenes* and *S. marcescens*, the inhibition zones for the blend (10.60 mm and 9.60 mm, respectively) were relatively close to those of Gentamicin (14.20 mm and 14.43 mm, respectively). The practical significance of the combination lies in its ability to reduce the required MBC for certain pathogens compared to MEO alone, suggesting that combining essential oils could lower the concentrations needed for effectiveness in food preservation contexts (13).

Effects of oil blending

The combined oil formulation demonstrated antibacterial activity that was in several cases greater than MEO alone, but generally comparable to NEO. For example, against *S. aureus*, the blend achieved an MIC of 2,500 µg/mL and an MBC of 2,500 µg/mL, which was significantly different from MEO (MIC 2,500 µg/mL; MBC 5,000 µg/mL, P-Value = 0.04), but not significantly different from NEO (MIC 1,250 µg/mL; MBC 2,500 µg/mL). A similar trend was observed for *K. pneumoniae*, where the combination reduced the MBC compared to MEO (5,000 vs. 10,000 µg/mL, P-Value = 0.03) (14,15). These results indicate that the

combination can enhance the antibacterial activity of the weaker oil (MEO), though its performance does not consistently exceed that of NEO (16). The observed effects are best described as additive or complementary rather than synergistic. The combination improved activity relative to MEO and, in selected cases, produced statistically significant differences, but it did not consistently outperform NEO.

Application in food safety and preservation

The inclusion of Gentamicin as a reference standard in the agar disk diffusion assay enabled a comparative benchmark to evaluate the performance of the essential oils. While Gentamicin produced consistently higher inhibition zones, the essential oils, especially NEO and the blended formulation, exhibited notable natural antimicrobial potential, with some inhibition zone diameters approaching those of the antibiotic standard. This is particularly important in the context of food safety, where natural antimicrobials are increasingly sought after as alternatives to synthetic preservatives. The oils demonstrated activity against key foodborne pathogens, including *S. enteritidis*, *L. monocytogenes*, and *E. coli*, which supports their potential for use in preservation strategies, especially for "clean-label" or organic food products (17). While Gentamicin remains more potent, the bactericidal nature of NEO and the synergistic enhancement seen in the blend offer practical benefits when antibiotic use is not permitted or must be minimized. Incorporating these essential oils into antimicrobial packaging, surface sanitizers, or edible coatings could contribute to improving food safety and extending shelf life, provided their sensory impacts and regulatory approvals are properly managed (18).

Mechanistic considerations

Although this study primarily focused on antimicrobial efficacy, the observed differential responses across bacterial strains suggest multiple potential mechanisms of action. The greater effectiveness of the oils against Gram-positive bacteria may be attributed to differences in cell wall structure and outer membrane permeability compared to Gram-negative species. Additionally, the range of MIC/MBC ratios suggests that the oils may exhibit bacteriostatic activity against some pathogens and bactericidal effects against others (19). The antimicrobial activity is likely driven by bioactive constituents such as phenolics, flavonoids, and terpenoids, which are known to disrupt bacterial membranes, inhibit critical enzymes, or generate reactive oxygen species that damage cellular components. The complex, multi-compound composition of essential oils may contribute to broad-spectrum activity and reduced risk of resistance development compared to single-agent antibiotics (20,21).

Study limitations and future directions

The in vitro design of this study does not fully capture the complexity of clinical infections or food systems, limiting the direct translatability of the findings. Although the extraction method preserved thermolabile compounds, it may not have optimized the recovery of all bioactive constituents. Moreover, the 48-hour maceration period and solvent ratios employed may not reflect the most effective conditions for maximizing antimicrobial potency. Future research should address these gaps. Isolation and characterization of individual active compounds would facilitate structure-activity relationship studies and may lead to the development of more potent synthetic analogs. In Vivo studies in appropriate animal models are essential to assess safety, pharmacokinetics, and therapeutic efficacy. Long-term studies evaluating resistance potential and the durability of antimicrobial effects are also needed to inform real-world applications. Mechanistic investigations using techniques such as electron microscopy, membrane integrity assays, and metabolomics could elucidate the specific cellular targets and pathways affected by these oils. Such insights would support the rational design of optimized formulations and may aid in identifying predictive biomarkers of antimicrobial responsiveness.

Conclusion

This study highlights the antibacterial potential of nettle (Urtica dioica) and mallow (Malva spp.) essential oils. Nettle oil exhibited the strongest overall activity, with particularly low MIC and MBC values against *L. monocytogenes*, *S. aureus*, and *A. faecalis*. Mallow oil was less potent but still showed measurable inhibitory effects. The combined formulation demonstrated additive or complementary effects, improving antibacterial performance relative to mallow oil alone and, in some cases, reducing MBC values. However, the combination did not consistently exceed the efficacy of nettle oil. These findings support the potential use of nettle and mallow essential oils as natural antimicrobial agents in food preservation strategies, including antimicrobial packaging, edible coatings, and surface sanitizers. Future work should include quantitative interaction analyses, such as checkerboard assays and FIC index calculations, to determine whether the observed effects represent true synergy or additive interactions, and to validate their applicability under real-world food system conditions.

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Not applicable.

Ethical statement

This study did not involve human participants, human data, or human tissue. Therefore, ethics approval and consent to participate were not required.

Conflicts of interest

All authors declare no conflict of interest.

Author contributions

M.R. contributed to the design of the study, data collection and drafting of the article. P.T. contributed to the data analysis, interpretation and drafting of the article. M.A.M. contributed to the overall project management, study design and drafting of the article. All authors were involved in the final approval of the version to be published.

Data availability statement

all data underlying the results are available as a part of the article and no additional source data are required.

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