Biological Properties and Cell Toxicity of Herbal Extracts Effective against Zoonotic Bacterial Pathogens: *Salmonella* spp. and *Listeria monocytogenes*

Mohammad Reza Hosseini Montazer[a], Naheed Mojgani[b], Fatemeh Bagheri[c], Mohammadreza Sanjabi[d], Solmaz Saremnejad[e]

[a] Department of Food Science and Technology, TeMS.C., Islamic Azad University, Tehran, Iran https://orcid.org/0009-0004-4568-4781

[b]Biotechnology Department, Razi Vaccine and Serum Research Institute-Agricultural Research, Education and Extension Organization, Karaj, Iran, https://orcid.org/ 0000-0002-3138-3433

[c]Department of Microbiology, TeMS.C. Islamic Azad University, Tehran, Iran https://orcid.org/0000-0002-1625-2232

[d]Department of Agriculture,, Iranian Research Organization for Science & Technology (IROST), Tehran, Iran .https://orcid.org/ 0000-0002-5364-8802

[e] Department of Food Science and Technology, TeMS.C., , Islamic Azad University, Tehran, Iran https://orcid.org/0000-00024784-9744

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Abstract

Introduction: The increasing prevalence of antimicrobial resistance among bacterial pathogens has intensified the search for safe and effective alternatives to conventional antibiotics in human and veterinary medicine.

Objective: This study aimed to evaluate the antibacterial activity, safety, and bioactive potential of selected natural herbal extracts against several foodborne pathogens, highlighting their potential as alternative or complementary strategies to conventional antibiotics.

Materials and Methods: Hydroalcoholic extracts of selected medicinal plants were screened for antibacterial activity against *S.* Enteritidis, *S.* Typhimurium and *Listeria monocytogenes* using agar well diffusion, followed by determination of their MIC and MBCs and Time–kill kinetics. Antioxidant activity was determined using the DPPH assay, while total phenolic and flavonoid contents were quantified by standard methods. Phytochemical profiles were analyzed by HPLC. *In vitro* cytocompatibility was evaluated using the MTT assay on Caco-2 human intestinal epithelial cells.

Results: Persian shallot (*Allium hirtifolium*), thyme (*Thymus* spp.), and rosemary (*Rosmarinus officinalis*) exhibited the highest antibacterial activity against both *Salmonella spp.*. Persian shallot showed rapid bactericidal action, achieving >4 log CFU/mL reduction of *S. Enteritidis* within 24 h. Rosemary demonstrated the strongest antioxidant capacity (>90% DPPH scavenging). The extracts showed limited cytotoxicity on CaCo-2 cell lines and maintained acceptable cytocompatibility.

Conclusion: The findings indicate that selected herbal extracts can be considered potential natural antibacterial agents with acceptable safety profiles, supporting their possible use as alternative or adjunct antimicrobial strategies in human and animal disease control.

Keywords: Natural antibacterial agents, Antioxidant capacity; flavonoids, Caco-2 cell line

1. Introduction

Bacterial diseases caused by foodborne and zoonotic pathogens remain a major challenge to public health, food safety, and animal production systems worldwide. Among these, *Salmonella Enteritidis* and *Salmonella Typhimurium* are leading causes of gastrointestinal infections in humans and animals and are frequently transmitted through contaminated food and feed [1]. In addition to *Salmonella* spp., *Listeria monocytogenes* is a major foodborne zoonotic pathogen of significant concern in food-producing animals and animal-derived food products.. *L. monocytogenes* is the causative agent of listeriosis, a severe invasive disease affecting humans and animals, characterized by septicemia, encephalitis, and reproductive disorders, particularly abortion in ruminants [2]. The extensive use of antibiotics in clinical therapy and livestock production has contributed to the emergence of antimicrobial-resistant strains, reducing treatment efficacy and raising serious concerns regarding the safety of the food chain and the environment. Control of pathogenic bacteria in food-producing animals is essential for safeguarding public health, minimizing economic losses in livestock production, and limiting the spread of antimicrobial resistance [3].

Natural antibacterial agents have gained increasing attention as potential complementary or alternative approaches to conventional antibiotics, particularly in food and feed systems where long-term safety is essential [4]. Plant-derived bioactive compounds are of particular interest due to their diverse phytochemical composition, including phenolics, flavonoids, terpenoids, and organosulfur compounds, which exhibit antimicrobial activity through mechanisms such as membrane disruption, enzyme inhibition, and interference with bacterial metabolism [5].

Species belonging to the *Allium* and Lamiaceae families have been widely investigated for their antibacterial potential. Persian shallot (*Allium hirtifolium*), traditionally consumed in Middle Eastern diets, remains relatively underexplored despite its richness in sulfur-containing compounds and flavonoids associated with antimicrobial and antioxidant activities [6]. In contrast, thyme (*Thymus* spp.) and rosemary (*Rosmarinus officinalis*) are well-established sources of phenolic terpenes and have demonstrated notable antibacterial effects against a

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number of foodborne zoonotic pathogens [7]. Their use has also been linked to reduced food waste and improved sustainability within food systems [8].

Despite growing interest, comparative studies integrating phytochemical characterization, antibacterial kinetics, and intestinal cytocompatibility remain limited. Moreover, the antibacterial potential of Persian shallot has not been adequately evaluated alongside commonly used Lamiaceae herbs. Therefore, the present study aimed to comparatively assess selected hydroalcoholic extracts for their antibacterial activity against *Salmonella* spp., antioxidant capacity, phytochemical composition, and in vitro cytocompatibility using Caco-2 intestinal epithelial cells, with emphasis on their relevance as safe antibacterial alternatives in human and animal health.

2. Materials and Methods

2.1 Bacterial cultures and growth conditions

Pure cultures of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* O157:H7 (ATCC 43895), *Salmonella enterica* serovar Typhimurium (ATCC 14028), *Salmonella enterica* serovar Enteritidis (ATCC 13076), and *Listeria monocytogenes* (ATCC 7644) were cultured in Brain Heart Infusion (BHI; Merck, Germany) or Tryptic Soy Broth (TSB; HiMedia, India) at 37 °C for 24 h. Short-term storage was performed on nutrient agar slants at 4 °C, while long-term preservation was carried out at −70 °C in 20% (v/v) glycerol.

2.2 Herb collection and extract preparations

Dried herbs—including thyme (*Thymus vulgaris* L.), Persian shallot (*Allium hirtifolium* Boiss.), rosemary (*Rosmarinus officinalis* L.), turmeric (*Curcuma longa* L.), cinnamon (*Cinnamomum verum* / *C. zeylanicum*), chamomile (*Matricaria chamomilla* L.), peppermint (*Mentha piperita* L.), and ginger (*Zingiber officinale* Roscoe) were obtained from a licensed supplier in Tehran, Iran, based on reported antimicrobial and antioxidant properties. Hydroalcoholic extracts were prepared by macerating powdered aerial parts in ethanol–water (70:30, v/v) at a 1:10 (w/v) ratio with agitation for 48 h, followed by filtration (Whatman No. 1) and concentration under reduced pressure at 40 °C using a rotary evaporator. Dried extracts were stored at −20 °C, and stock solutions were freshly prepared in DMSO and diluted to working concentrations, keeping final DMSO ≤0.5% (v/v) [9].

2.3 Antimicrobial activity

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Antibacterial screening was conducted using the agar well diffusion method [10] with 0.5 McFarland bacterial suspensions inoculated onto Mueller–Hinton agar. Wells were filled with 100 µL of extract and incubated at 37 °C for 24 h prior to measuring inhibition zones.

Rosemary, thyme, and Persian shallot extracts were further evaluated for MIC and MBC using the broth microdilution method according to CLSI (2020). Assays were performed in 96-well plates (200 µL; ≈1 × 10^5 CFU/mL). MIC was defined as the lowest concentration inhibiting visible growth, and MBC as the lowest concentration causing ≥99.9% bacterial reduction. All assays were performed in triplicate.

2.4 Time-kill kinetics assay

Time-kill kinetics of Persian shallot, thyme, and rosemary extracts against *S. enterica* serovar Enteritidis (ATCC 13076) were evaluated as described by Donkor et al. [11], with minor modifications. Extracts were tested at MIC levels (Persian shallot and thyme: 12.25 mg/ mL; rosemary: 50.0 mg/ mL). Cultures (10^6 CFU/mL) without extract served as growth controls, and solvent controls contained ≤0.5% DMSO. Samples were incubated at 37 °C with shaking (150 rpm). Viable counts were determined at 0, 2, 4, 8, and 24 h and expressed as log CFU mL^{-1}. A ≥3 log CFU/mL reduction was considered bactericidal.

2.5 Antioxidant activity

2.5.1 *Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity*

DPPH radical scavenging activity was determined as described earlier. [12]. Briefly, 100 µL of extract was mixed with 0.4 mM DPPH solution and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer (Shimadzu UV-1800, Japan). Radical scavenging activity (%) was calculated using the formula:

$$\text{Inhibition } \% = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100,$$

2.5.2 *Hydroxyl Radical (˙OH) Scavenging Activity*

Hydroxyl radical scavenging activity was evaluated using a commercial assay kit (Solarbio, Beijing, China) following the manufacturer's instructions. Absorbance was recorded at 536 nm.

2.6 Total phenolic and flavonoid content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method [13], and expressed as mg Gallic acid equivalents (GAE) /g extract. Total flavonoid content (TFC) was

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measured using the aluminum chloride colorimetric method with quercetin (5–250 µg/ mL) as the standard, and results were expressed as mg quercetin equivalents (QE) per 100 g extract.

2.7 Quantitative determination of active ingredients

Quantitative analysis of active compounds in Persian shallot, thyme, and rosemary extracts was performed using HPLC–DAD (Dionex, Idstein, Germany) with a C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile (B), applied under gradient elution at a flow rate of 1.0 mL/min. Detection was carried out at 280 and 320 nm. Identification and quantification were based on external calibration curves of authentic standards, and results were expressed as mg/ g extract

2.8 Cytotoxicity and safety assessment

Cytotoxicity was evaluated using Caco-2 human intestinal epithelial cells cultured in DMEM supplemented with 10% (v/v) fetal bovine serum and 1% penicillin–streptomycin at 37 °C under 5% CO_2. Cell viability was determined using the MTT assay [14]. Cells were exposed to extract concentrations ranging from 100 to 500 µg/ mL for 24 h, and absorbance was measured at 570 nm. Viability was expressed relative to untreated controls, and extracts were considered non-cytotoxic when cell viability exceeded 80%, in accordance with ISO 10993-5:2009.

2.9 Statistical analysis

All experiments were performed in triplicate. Data were analyzed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc tests using SPSS software (version 20). Results are presented as mean ± SD, with statistical significance set at $p < 0.05$.

3. Results

3.1 Antibacterial effects of herbal extracts

Agar well diffusion screening revealed marked differences in antibacterial activity among the eight herbal extracts (Figure 1). Persian shallot, thyme, and rosemary produced the largest inhibition zones and were therefore selected for further analysis. Cinnamon and peppermint showed no detectable antibacterial activity against any tested pathogen.

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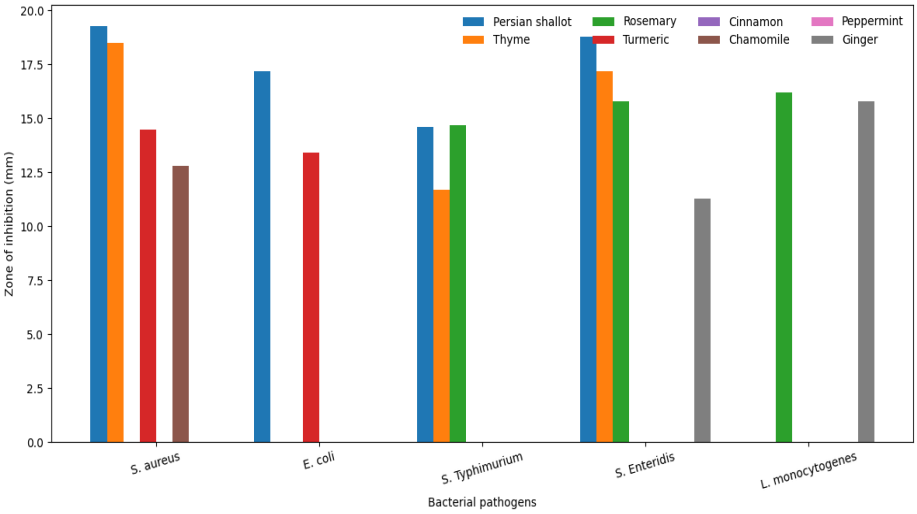

Figure 1: Antibacterial effects of hydroalcoholic herbal extracts against bacterial pathogens by agar well diffusion assay (zone of inhibition expressed in millimeters).
Values represent mean inhibition zones; bars with zero height indicate no detectable antibacterial activity under the tested conditions. Results are mean of three experiments

Persian shallot exhibited the broadest antibacterial spectrum, with strong inhibition of *S. aureus* and *S. Enteritidis*, moderate activity against *E. coli* and *S. Typhimurium*, and no activity against *L. monocytogenes*. Thyme inhibited *S. aureus*, *S. Enteritidis*, and *S. Typhimurium*, but was inactive against *E. coli* and *L. monocytogenes*. Its activity against *S. Enteritidis* was comparable to Persian shallot ($p > 0.05$). Rosemary showed significant activity against *L. monocytogenes*, *S. Enteritidis*, and *S. Typhimurium*, with no significant differences among inhibition zones ($p > 0.05$). Ginger inhibited only *L. monocytogenes*, with activity comparable to rosemary ($p > 0.05$). Turmeric and chamomile were active exclusively against *S. aureus*.

MIC and MBC results (Table 1) confirmed Persian shallot as the most potent extract, particularly against *S. Enteritidis* and *S. aureus*. Rosemary showed moderate activity, with MIC values of 25.50–75.0 mg mL^{-1}, while thyme displayed selective activity against *S. aureus*, *S. Enteritidis*, and *S. Typhimurium*. Overall, Gram-positive bacteria were more susceptible than Gram-negative strains.

Table 1: Minimum inhibitory and bactericidal concentrations of aqueous herbal extracts against respective pathogens

	MIC mg/mL			**MBC mg/ mL**		
Bacterial Pathogens	***Thyme***	***Rosemary***	**Persian shallot**	***Thyme***	***Rosemary***	**Persian shallot**
E. coli	ND	ND	12.25	ND	ND	25.50
S. aureus	12.25	ND	12.25	50.0	ND	12.25
L. monocytogenes	ND	25.50	ND	ND	50.0	ND
S. Enteridis	12.25	50.0	12.25	12.25	75.0	12.25
S. Typhimurium	50.0	50.50	25.50	100.0	75.0	50.0

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Values are mean of three experiments (mean ± SD, n = 3).

3.2 Time-kill kinetics

Time-kill assays against *S. enterica* serovar Enteritidis revealed distinct bactericidal behaviors among the extracts (Figure 2). Control cultures reached 7.6 log CFU/ mL after 24 h. Persian shallot at its MIC (12.25 mg/mL) caused rapid bacterial inactivation, with a significant reduction within 2 h ($p < 0.05$), exceeding 3 log CFU/mL by 4 h and >4 log CFU/ mL after 24 h.

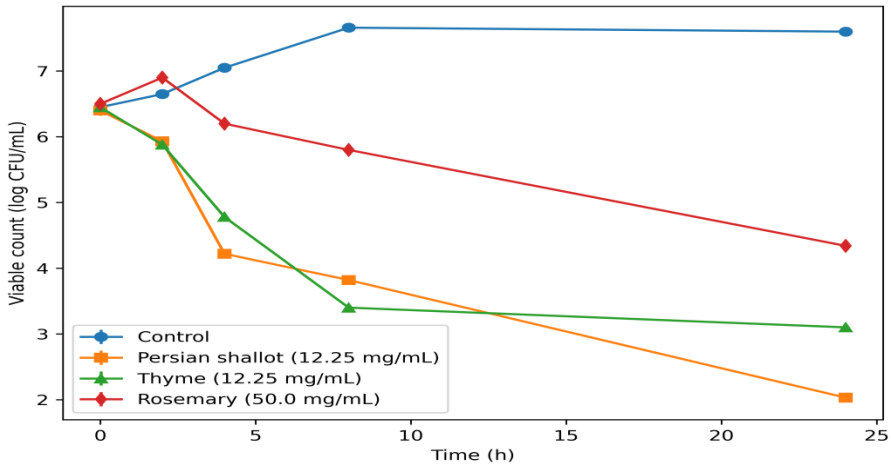

Figure 2: Time–kill kinetics of Persian shallot, thyme, and rosemary hydroalcoholic extracts against *Salmonella enterica* serovar *Enteritidis* at their respective MICs. Values are expressed as log CFU/mL (mean ± SD, n = 3).

Thyme showed intermediate kinetics, achieving bactericidal activity (>3 log CFU/ mL reduction) by 8 h and maintaining this effect through 24 h ($p < 0.05$). Rosemary exhibited slower, time-dependent killing, with no early reduction but a gradual decrease reaching 3.2 log CFU/ mL at 24 h. These results indicate that extracts with similar MIC values can differ substantially in killing rate and efficacy.

3.3 Antioxidant activity

Significant differences in antioxidant activity were observed among the extracts (Table 2). Rosemary showed the highest DPPH scavenging activity (93.25%), followed by Persian shallot (87.42%), while thyme exhibited markedly lower activity (28.52%) ($p < 0.05$). Rosemary and Persian shallot did not differ significantly from each other ($p > 0.05$).

A similar trend was observed for hydroxyl radical scavenging, with rosemary (71.04%) and Persian shallot (68.26%) showing significantly greater activity than thyme (16.43%) ($p < 0.05$), and no significant difference between rosemary and Persian shallot.

Table 2: Antioxidant activity (DPPH % and HRS %), phenolic (TPC) and flavonoid (TFC) contents of the aqueous herbal extracts

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Herbal extracts	DPPH %	HRS% inhibition	TPC mg GAE/g	TFC mg Q/ 100 g
Thyme	28.52[a]	16.43[a]	1511 ± 1.12[a]	1245 ± 0.92[a]
Rosemary	93.25[b]	71.04[b]	2831 ± 0.78[b]	1966 ± 0.71[b]
Persian shallot	87.42[b]	68.26[b]	2167±1.14[b]	1611 ± 0.55[c]

DPPH: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging assay; HRS: Hydroxyl radical scavenging; TPC: total phenolic contents; TFC: total flavonoid contents. Means within a column followed by different lowercase letters are significantly different (Tukey's test: $P < 0.05$).

3.4 Total phenolic and flavonoid contents

Total phenolic content was highest in rosemary (2831 mg GAE/ g), followed by Persian shallot (2167 mg GAE/ g) and thyme (1511 mg GAE/ g) (Table 3). Differences among all extracts were statistically significant ($p < 0.05$).

Total flavonoid content followed a similar pattern, with rosemary showing the highest value (1966 mg QE/ 100 g), followed by Persian shallot (1611 mg QE /100 g) and thyme (1245 mg QE/ 100 g), with significant differences among all groups ($p < 0.05$).

3.5 Phytochemical composition

HPLC analysis revealed distinct phytochemical profiles among the extracts (Table 3). Persian shallot was rich in flavonoids and phenolic acids, with quercetin as the dominant compound, and contained shallomin exclusively. Thyme was dominated by monoterpenoid phenols, primarily thymol and carvacrol, along with rosmarinic acid. Rosemary was characterized by high rosmarinic acid content and a diverse phenolic acid profile, including quercetin and ferulic acid, as well as low levels of allicin and apigenin.

Table 3: Major phenolic and bioactive compounds in hydroalcoholic extracts of Persian shallot, thyme, and rosemary quantified by HPLC

No.	Compound	Detection wavelength (nm)	Persian shallot (mg/ g)	Thyme (mg/ g)	Rosemary (mg/ g)
1	Gallic acid	280	1.85 ± 0.06	0.92 ± 0.04	1.12 ± 0.05
2	Protocatechuic acid	280	2.14 ± 0.08	1.37 ± 0.05	1.54 ± 0.06
3	*p*-Coumaric acid	310	0.76 ± 0.03	1.89 ± 0.07	1.22 ± 0.05
4	Ferulic acid	320	1.23 ± 0.05	2.41 ± 0.09	2.10 ± 0.08
5	Rosmarinic acid	330	ND	6.78 ± 0.21	8.25 ± 0.28
6	Quercetin	370	3.56 ± 0.11	1.44 ± 0.06	2.18 ± 0.09
7	Kaempferol	365	1.98 ± 0.07	0.88 ± 0.03	1.12 ± 0.04
8	Thymol	275	ND	9.32 ± 0.34	0.75 ± 0.03
9	Carvacrol	275	ND	7.61 ± 0.29	0.63 ± 0.02
10	Allicin	210	ND	ND	1.10 ± 0.04
11	Shallomin	210	2.75 ± 0.10	ND	ND
12	Apigenin	340	ND	ND	1.05 ± 0.04
13	Cinnamic acid	275	ND	ND	0.68 ± 0.03

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ND = Not Detected. Values are expressed as mean ± SD (n = 3)..

3.6 Safety assessment by MTT assay

All extracts showed concentration-dependent effects on Caco-2 cell viability (Figure 3). At 100–200 µg/ mL, viability remained above 94% for all extracts. Moderate reductions were observed at 300–400 µg mL^{-1}. At 500 µg/ mL, viability decreased significantly ($p < 0.05$) to 86.4 ± 5.1% (Persian shallot), 79.8 ± 5.7% (thyme), and 85.4 ± 3.6% (rosemary), with thyme showing slightly higher cytotoxicity, though not significantly different ($p > 0.05$).

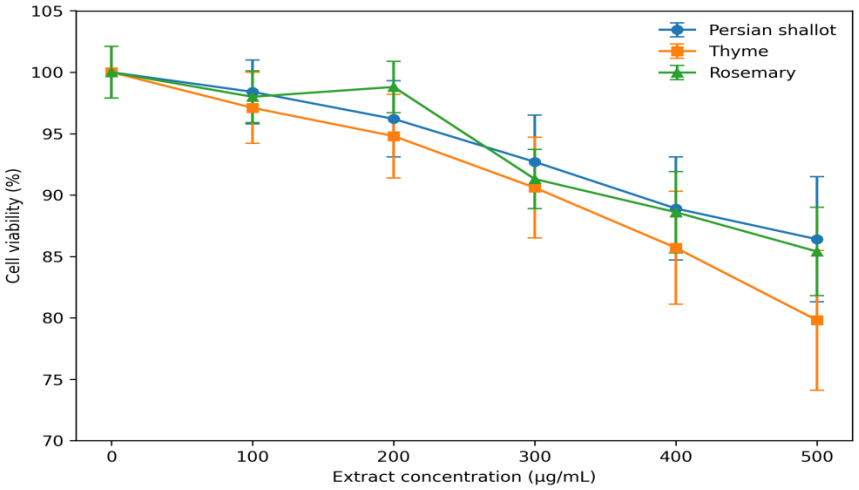

Figure 3: Effect of Persian shallot, thyme, and rosemary extracts on cell viability of Caco-2 cell lines at different concentrations (0–500 µg/ mL). Data are expressed as mean ± standard deviation (n = 3).

4. Discussion

The global increase in foodborne and zoonotic bacterial infections, together with the rapid spread of antimicrobial resistance, has intensified efforts to identify effective non-antibiotic strategies for controlling major pathogens in food-producing animals and along the food chain [3, 4]. *Salmonella* spp. and *Listeria monocytogenes* remain among the most important zoonotic bacteria responsible for disease transmission from animals to humans, and their control is a priority in veterinary public health [2]. In this context, the present study demonstrates the antibacterial and biological efficacy of different herbal extracts, emphasizing the importance of bioactive composition in targeting these pathogens.

Persian shallot, thyme, and rosemary exhibited the strongest inhibitory activity against *Salmonella Enteritidis*, *Salmonella Typhimurium*, and *L. monocytogenes*, supporting their potential role as complementary or alternative antimicrobial agents to conventional antibiotics

[15, 16].The higher susceptibility of *L. monocytogenes* compared with *Salmonella* spp. is consistent with known structural differences between Gram-positive and Gram-negative bacteria, particularly the protective outer membrane of *Salmonella*, which limits the penetration of many antibacterial compounds [17]. Quantitative MIC, MBC, and time–kill analyses provided mechanistic insight beyond agar diffusion assays, revealing pathogen-specific antibacterial kinetics. Persian shallot demonstrated rapid bactericidal activity against *S. Enteritidis*, suggesting the presence of fast-acting sulfur-containing compounds capable of disrupting bacterial membranes or essential metabolic pathways. In contrast, rosemary exhibited slower, time-dependent antibacterial effects, indicative of cumulative interference with cellular functions rather than immediate cell lysis [18, 19].

Phytochemical profiling by HPLC supported these observations by revealing distinct bioactive signatures among the extracts. Rosemary was characterized by a high content of phenolic acids, thyme by monoterpenoid phenols such as thymol, and Persian shallot by sulfur-based compounds. These phytochemicals have been previously associated with antimicrobial mechanisms relevant to zoonotic bacteria, including membrane destabilization, enzyme inhibition, and modulation of oxidative stress, which may reduce bacterial survival and virulence [16, 20].

Beyond antibacterial activity, antioxidant capacity represents an added advantage for controlling bacterial persistence in food and feed systems. Rosemary displayed the strongest radical scavenging activity, while Persian shallot and thyme showed moderate but significant antioxidant effects. This dual antibacterial–antioxidant functionality is particularly relevant for integrated disease control strategies aimed at reducing bacterial load and limiting oxidative stress that may favor pathogen survival during processing and storage [21, 22].

Importantly, the inclusion of intestinal cytocompatibility assessment strengthens the translational relevance of the findings. All extracts maintained acceptable viability of Caco-2 intestinal epithelial cells at antibacterial concentrations, indicating a favorable safety profile for potential oral exposure in humans and animals [23]. The slightly increased cytotoxicity observed for thyme at higher concentrations may be attributed to its high content of membrane-active phenolic compounds and underscores the importance of dose optimization [20].

The integration of the antibacterial efficacy of the herbal extracts against key zoonotic pathogens, phytochemical characterization, antioxidant activity, and cytocompatibility, this

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study supports the potential of selected natural antibacterial agents as adjunct or alternative strategies to antibiotics for the control of *Salmonella* spp. and *Listeria monocytogenes* in food-producing animals and animal-derived food systems [24, 25].

5. Conclusion

Hydroalcoholic extracts of Persian shallot, thyme, and rosemary demonstrated significant antibacterial activity against key zoonotic pathogens, including *Salmonella Enteritidis*, *Salmonella Typhimurium*, and *Listeria monocytogenes*, while maintaining acceptable intestinal cell compatibility. Persian shallot and thyme exhibited rapid and potent bactericidal effects, whereas rosemary provided strong antioxidant activity, suggesting complementary mechanisms that could enhance pathogen control and reduce bacterial persistence in food and feed systems. These findings highlight the potential of these natural bioactive agents as safe and effective alternatives or adjuncts to conventional antibiotics in controlling zoonotic bacterial infections in animal production and food safety contexts. Further evaluation in in vivo models and practical food and feed matrices will be critical to confirm their efficacy and inform integrated disease management strategies.

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Data availability: The authors confirm that the data supporting the findings of this study are available within the article.

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Authors' contributions Conceptualization and study design: MR Montazer, MR Sanjabi and N Mojgani; Data acquisition: MR Montazer, MR Sanjabi, N Mojgani, F Bagheri and S Saremnejad; Writing the original draft: MR Montazer; Review and editing: MR Montazer, and N Mojgani.

References

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1. Zizza A, Fallucca A, Guido M, Restivo V, Roveta M, Trucchi C. Foodborne infections and *Salmonella*: Current primary prevention tools and future perspectives. Vaccines (Basel). 2025;13(1):29. [DOI:10.3390/vaccines13010029].
2. Mantovam VB, Dos Santos DF, Giola Junior LC, Landgraf M, Pinto UM, Todorov SD. *Listeria monocytogenes*, *Salmonella* spp., and *Staphylococcus aureus*: Threats to the food industry and public health. Foodborne Pathog Dis. 2025;22(12):809–824. [DOI:10.1089/fpd.2024.0124].
3. O'Neill J. Tackling drug-resistant infections globally: Final report and recommendations. Review on Antimicrobial Resistance; 2016.
4. Kumar V, Singh A, Sharma N, Saini R, Kumar H, El-Shazly M, Dev K. Combating bacterial antibiotic resistance with phytocompounds: Current trends and future perspectives. Med Drug Discov. 2023;18:100182. [DOI:10.1016/j.medidd.2023.100182].
5. Angelini P. Plant-derived antimicrobials and their crucial role in combating antimicrobial resistance. Antibiotics (Basel). 2024;13(8):746. [DOI:10.3390/antibiotics13080746].
6. Ghafarifarsani H, Yousefi M, Hoseinifar SH, Paolucci M, Lumsangkul C, Jaturasitha S, Doan HV. Beneficial effects of Persian shallot (*Allium hirtifolium*) extract on growth performance, biochemical, immunological and antioxidant responses of rainbow trout (*Oncorhynchus mykiss*) fingerlings. Aquaculture. 2022;555:738162. [DOI:10.1016/j.aquaculture.2022.738162].
7. El Saadony MT, Saad AM, Mohammed DM, Alkafaas SS, Abd El Mageed TA, Fahmy MA, et al. Plant bioactive compounds: Extraction, biological activities, immunological, nutritional aspects, food application, and human health benefits—a comprehensive review. Front Nutr. 2025;12:1659743. [DOI:10.3389/fnut.2025.1659743].
8. Husain SA, Farheen SR, Husain M. Medicinal plant compounds as natural food preservatives: A review. J Postharvest Technol. 2022;10(4):213–219.
9. Hassanzadeh M, Mirzaie S, Rahimi Pirmahalle F, Yahyaraeyat R, Razmyar J. Effects of thyme (*Thymus vulgaris*) essential oil on bacterial growth and expression of some virulence genes in *Salmonella enterica* serovar Enteritidis. *Vet Med Sci.* 2024;10(6):e70088. [DOI:10.1002/vms3.70088]
10. Donkor MN, Mosobil R, Abugri J, Addai-Mensah Donkor A. Antibacterial activities and time-kill analysis of leaf extracts of *Combretum adenogonium* Steud. ex A. Rich in vitro. *Biomed Res Int*. 2025;3097612. [DOI:10.1155/2025/3097612]
11. Jalali S, Mojgani N, Haghighat S, Sanjabi MR, Sarem-Nezhad S. Investigation of antimicrobial and antioxidant properties of postbiotics produced by *Lactobacillus rhamnosus* and *Limosilactobacillus reuteri* and their potential application in surface decontamination of red meat. *LWT Food Sci Technol.* 2024;209:116758. [DOI:10.1016/j.lwt.2024.116758]
12. Vinholes J, Lemos G, Barbieri R, Franzon R, Vizzotto M. In vitro assessment of antioxidant properties of Brazilian fruits. *Food Biosci.* 2017;19:92–100. [DOI:10.1016/j.fbio.2017.06.005]
13. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols by Folin–Ciocalteu reagent. *Methods Enzymol.* 1999;299:152–178. [DOI:10.1016/S0076-6879(99)99017-1]
14. Dolghi A, Buzatu R, Dobrescu A, Olaru F, Popescu GA, Marcovici I, et al. Phytochemical analysis and in vitro cytotoxic activity against colorectal adenocarcinoma cells of *Hippophae rhamnoides* L., *Cymbopogon citratus* (D.C.) Stapf, and *Ocimum basilicum* L. essential oils. *Plants.* 2021;10(12):2752. [DOI:10.3390/plants10122752]

Preprint

15. Grigore-Gurgu L, Dumitrașcu L, Aprodu I. Aromatic herbs as a source of bioactive compounds: An overview of their antioxidant capacity, antimicrobial activity, and major applications. *Molecules*. 2025;30(6):1304. [DOI:10.3390/molecules30061304]
16. Nieto G. Biological activities of three essential oils of the Lamiaceae family. *Medicines*. 2017;4(3):63. [DOI:10.3390/medicines4030063]
17. Sivaram S, Somanathan H, Kumaresan SM, Muthuraman MS. The beneficial role of plant based thymol in food packaging application: A comprehensive review. *Appl Food Res*. 2022;2(2):100214. [DOI:10.1016/j.afres.2022.100214]
18. Silva AM, Félix LM, Teixeira I, Martins Gomes C, Schäfer J, Souto EB, et al. Orange thyme: Phytochemical profiling and bioactivities. *Food Chem X*. 2021;12:100171. [DOI:10.1016/j.fochx.2021.100171]
19. Nieto G, Ros G, Castillo J. Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis* L.): A review. *Medicines*. 2018;5(3):98. [DOI:10.3390/medicines5030098]
20. Mokhtari R, Kazemi Fard M, Rezaei M, Moftakharzadeh SA, Mohseni A. Antioxidant, antimicrobial activities, and characterization of phenolic compounds of thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and thyme–sage mixture extracts. *J Food Qual*. 2023;2602454. [DOI:10.1155/2023/2602454]
21. Pandey P, Khan F, Alshammari N, Saeed A, Aqil F, Saeed M. Updates on the anticancer potential of garlic organ UAE organosulfur compounds and their nanoformulations. *Front Pharmacol*. 2023;14:1154034. [DOI:10.3389/fphar.2023.1154034]
22. De Rossi L, Rocchetti G, Lucini L. Antimicrobial potential of polyphenols: Mechanisms of action and microbial responses—a narrative review. *Antioxidants*. 2025;14:200. [DOI:10.3390/antiox14020200]
23. ISO 10993-5. Biological evaluation of medical devices—Part 5: Tests for in vitro cytotoxicity. Geneva: International Organization for Standardization; 2009.
24. Alijani Alijanvand L, Bonyadian M, Moshtaghi H. The effect of spearmint, oregano, and thyme extracts on biofilm formation by *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella* Typhimurium. *Arch Razi Inst*. 2025;80(1):201–208. [DOI:10.32592/ARI.2025.80.1.201]
25. Kiss A, Papp VA, Pál A, Prokisch J, Miran S, Toth BE. Comparative study on antioxidant capacity of diverse food matrices: Applicability, suitability and inter-correlation of multiple assays to assess polyphenol and antioxidant status. *Antioxidants*. 2025;14:317. [DOI:10.3390/antiox14030317]

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