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Chemical profiling and anti-biofilm efficacy of *Hyssopus officinalis* L. essential oil against bovine mastitis pathogens: A promising natural alternative

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Abstract

The increasing prevalence of antibiotic-resistant pathogens and the economic burden of bovine mastitis necessitate the exploration of natural therapeutic alternatives. This study investigated the chemical composition, antimicrobial and anti-biofilm activities of *Hyssopus officinalis* L. (hyssop) essential oil (EO) against major bovine mastitis pathogens: *Salmonella enterica*, *Escherichia coli*, *Staphylococcus aureus* and *Enterococcus faecalis*. Gas chromatography–mass spectrometry (GC-MS) analysis identified 37 compounds, with bicycloheptane (20.75 %), 1,5,8-o-menthatriene (4.48 %), cyclohexanemethanol (3.79 %), spathulenol (3.70 %) and myrtenol (3.25 %) as the predominant constituents. Antimicrobial evaluation revealed that *E. faecalis* was the most susceptible bacterium (minimum inhibitory concentration (MIC)/minimum bactericidal concentration (MBC) = 0.0312 mg/mL), while *S. enterica* exhibited the highest resistance (MIC/MBC ≥5 mg/mL). The EO demonstrated strong anti-biofilm activity, with the most pronounced effect against *E. faecalis* (up to 81 % inhibition at 60 mg/mL) and the least against *S. enterica* (57 % inhibition at the same concentration). This study provides the first comprehensive evidence linking the specific chemical profile of hyssop EO to its potent anti-biofilm and antimicrobial action against bovine mastitis pathogens, suggesting its potential as a viable natural alternative to conventional antibiotic therapies.

Keywords: anti-biofilm; antimicrobial; bovine mastitis; essential oil; *Hyssopus officinalis*

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1. Introduction

Bovine mastitis remains one of the most economically significant diseases affecting the dairy industry worldwide, with annual losses estimated at approximately USD 2 billion in the United States alone (1). In Iran, similar economic concerns exist; a study in Tehran province estimated the average annual economic loss due to mastitis at approximately 491.86 USD per cow in industrial dairy herds (2). The emergence of multidrug-resistant bacterial strains has complicated treatment protocols and increased the risk of therapeutic failure. *Staphylococcus aureus* and *Escherichia coli* are among the most prevalent causative agents of bovine mastitis. *Staphylococcus aureus* causes inflammation in the mammary gland and chronic mastitis, with biofilm formation playing an important role in disease progression and contributing to persistent infections and reduced antimicrobial susceptibility (3). Biofilm formation confers up to 1000-fold higher resistance to antimicrobial agents compared to planktonic cells, making these infections more chronic and challenging to cure (4). The extracellular polymeric matrix acts as a physical barrier, limiting antibiotic penetration and facilitating horizontal gene transfer, thereby resulting in escalated resistance against drugs and chemical stresses (5). This phenomenon underscores the urgent need for alternative therapeutic strategies capable of disrupting biofilm architecture. Today, the employment of antibiotics in animal husbandry has led to increased bacterial resistance (6). Multidrug resistance has been reported in *Salmonella enterica* (7, 8) and other pathogens in Iran and worldwide (9–11). Biofilm formation ability is considered widespread in *Salmonella* spp. (12). Given these challenges associated with biofilm-mediated resistance and the limitations of conventional antibiotic therapy, there is a growing interest in exploring natural compounds with antimicrobial and anti-biofilm properties. Essential oils (EO) have garnered significant attention as promising alternatives to conventional antibiotics due to their broad-spectrum antimicrobial and anti-biofilm properties (13). Their antimicrobial efficacy is primarily attributed to their hydrophobic nature, enabling disruption of bacterial cell membranes and interference with energy metabolism (14). Monoterpenoid

compounds such as myrtenol have demonstrated synergistic effects with conventional antibiotics (15, 16) and significant inhibitory effects on biofilm formation in methicillin-resistant *S. aureus* (17). Recent studies have further confirmed the efficacy of plant-derived essential oils in disrupting preformed biofilms of bovine mastitis pathogens (18). Among the various forms of mastitis, coliform mastitis, caused by *E. coli*, *Klebsiella pneumoniae* and *Enterobacter aerogenes*, is a common fatal form of mastitis, particularly in dairy cows kept in closed spaces during winter (19). Bacterial infections in dairy cattle also endanger human health through zoonotic transmission via raw milk consumption or contact with infected cattle (20, 21). These challenges have prompted extensive research into antimicrobial mixtures found in EO as potential alternatives to conventional antibiotics (13). Among the myriads of medicinal plants with documented antimicrobial properties, *Hyssopus officinalis* L. (Lamiaceae), commonly known as hyssop, is distributed in the Caucasus, northwestern Iran, Turkey and other regions (22). Research has shown that EO from various hyssop subspecies possess antibacterial and antifungal effects (23). The chemical composition of *H. officinalis* EO exhibits considerable variability depending on geographical origin, harvesting season and extraction methods, reinforcing the importance of chemotypic characterisation for therapeutic applications (24, 25). Despite documented antimicrobial properties, comprehensive investigations linking the specific chemical composition of hyssop EO to anti-biofilm activity against bovine mastitis pathogens remain limited. This study addresses this gap by providing detailed chemical profiling and evaluating the antimicrobial and anti-biofilm efficacy of hyssop EO against four major mastitis-causing bacteria: *S. enterica*, *E. coli*, *S. aureus* and *Enterococcus faecalis*.

2. Materials and Methods

Plant material

We collected *H. officinalis* from the farm of Maragheh Islamic Azad University in Iran and then we tried to authenticate it. Maragheh is located in East Azerbaijan Province, northwestern Iran, at approximately 37.39 °N latitude and 46.24 °E longitude, with an altitude of about 1455 m above sea level. The region experiences a cold semi-arid climate with significant seasonal temperature variations (26). The plant was identified and authenticated by Dr. Marzieh Babashpour-Asl at the Herbarium of Islamic Azad University, Maragheh branch, where a specimen voucher no. MA-1402-01 was deposited for future reference. We dried the plant at room temperature until it reached a fixed weight, then we turned it into powder with a laboratory grinder.

Essential oil analysis

We followed the European Pharmacopoeia guidelines for the oil extraction (27). Hyssop powder (20 g) was hydrodistilled for 3 hr with a Clevenger apparatus. The extraction yield was calculated based on the dry weight of the plant material and found to be 0.7 % (w/w). The resulting EO was dried using anhydrous sodium sulfate (Na_2SO_4) and was kept in glass vials at 4 °C until further use in biological experiments.

Evaluation of chemical compounds by gas chromatography-mass spectrometry (GC-MS)

The gas chromatography (GC) apparatus (Shimadzu-QP2010, Japan) with a ZB- WAX column (length 20 m, inner diameter 0.18 mm, thickness 0.18 µm) was applied to locate the mixtures of the EO of Hyssop. The essential oil was diluted with *n*-hexane and 1 µL was injected into gas chromatography-mass spectroscopy (GC-MS). The initial temperature of the oven was 50 °C, which was kept at this temperature for 5 min (thermal gradient: 3 °C per min) and later increased to a temperature of 240 °C, which was kept at this temperature for 3 min (thermal gradient: 3 °C per min). The temperature of the injector was 300 °C and the injection mode was split/split less (1 : 50). We employed helium (99.9999 %) as the carrier gas at a flow rate of 1 mL/min. Then, mass spectrometry (length 20 m, inner diameter 0.25 µm, thickness 0.25 mm) was applied. The temperature of the ionisation chamber was 150 °C, the temperature of the detector was 230 °C, the ionisation energy was 70 eV and the mass analyser was a quadrupole. The scan mass range was from 40–550 m/z. The mass spectrometry was used to locate the mixtures of the EO of Hyssop. The spectral values were compared with Kovats index values (retention indices) based on the standard tables and the compounds of the EO of Hyssop were identified on the basis of data and information available in the GC-MS library. To confirm identification, retention indices (RI) were calculated using a homologous series of *n*-alkanes (C_8–C_{20}) under the same chromatographic conditions and compared with reported values from the literature.

Preparation of bacterial strains

Four bacterial strains of Gram-positive bacteria, involving *E. faecalis* and *S. aureus* and Gram-negative bacteria, including *E. coli* and *S. enterica*, isolated from bovine mastitis (previously, these were identified via specific biochemical tests and Gram staining) were used for the investigation of the antimicrobial and anti-biofilm effects of Hyssop EO. *Staphylococcus aureus* PTCC 1764, *E. faecalis* PTCC 1237, *E. coli* PTCC 1330 and *S. enterica* PTCC 1787 were applied as standard strains. All experiments were performed in three independent biological replicates, each with three technical replicates (n = 3).

The evaluation of antimicrobial activity

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were employed to identify the antimicrobial effect of Hyssop EO by the microdilution method. For this purpose, the investigated bacteria were cultured in sterile brain heart infusion (BHI) (Merck Company, Germany) for 24 hr at 37 °C. The serial dilution was performed using Müller Hinton

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Broth (MHB, Merck Company, Germany) medium from 20–0.39 mg/mL. The MIC test was performed using the microdilution method. A volume of 100 µL of MHB was added to each well. Then, 100 µL of EO with a concentration of 20 mg/mL was poured into the first well and 100 µL was transferred from the contents of the first well to the second well; this continued to the 10th well. Therefore, dilutions of 20–0.39 mg/mL of EO were prepared. 100 µL of a new bacterial culture (the equivalent of a concentration of 0.5 McFarland turbidity) was added to each well and they were incubated at 37 °C for 24 hr. After 24 hr, the presence of turbidity (compared to the control row) showed bacterial growth and clarity, demonstrating no growth of the bacteria. The lowest concentration at which no bacterial growth was observed (lack of turbidity caused by bacterial growth) was found to be the MIC. A higher and lower concentration than the MIC were cultured in BHI agar medium and incubated at 37 °C for 24 hr. The first plate related to the well not showing a bacterial colony was considered as the MBC of EO. The MBC was explained as the concentration at which no microorganism growth was observed (28). A positive control using gentamicin (Sigma-Aldrich, USA) at concentrations ranging from 0.5–512 µg/mL was included in all assays to validate the experimental procedure and ensure the reliability of the results. Sterile MHB medium was used as a negative control to confirm the absence of contamination.

The evaluation of anti-biofilm activity

To evaluate the effect of Hyssop EO against biofilm formation by the tested bacteria, a microtiter plate adhesion assay was applied (29). In a plate with 96 wells, the Hyssop EO was serially diluted by the use of cation- adjusted Mueller- Hinton II broth from 60–0.47 mg/mL in a two-fold manner. Then, we added 100 µL of diluted overnight bacterial suspension (1 : 100) to each well. The microtiter plate was incubated at 37 °C for 24 hr to let the bacteria form different levels of biofilm. After incubation, we added 200 µL of crystal violet (0.06 %, w/v) to each well and shook it three times to help the biofilms stain. After 15 min at 25 °C, each well was washed at least three times with sterile normal saline (200 µL) to remove planktonic cells and the free stain. The biofilm-bound crystal violet was further extracted with 200 µL of ethyl alcohol (95 %) and transferred to a 96-well plate. The absorbance (595 nm) was recorded through a microplate reader in order to identify the level of biofilm formation. Culture medium and also different concentrations of the extract without the bacteria were employed as the negative control. Additionally, bacterial cultures without EO treatment were used as the positive control (100 % biofilm formation). The wells containing only sterile culture medium (without bacteria) served as blank controls to subtract background absorbance. The percentage of anti-biofilm activity was calculated using the following formula:

Anti-biofilm activity (%) = [(OD_{595} of positive control − OD_{595} of sample) / (OD_{595} of positive control − OD_{595} of blank)] × 100

All experiments were performed in three independent biological replicates, each with three technical replicates (n = 3).

Statistical analysis

Quantitative data obtained from antimicrobial and anti-biofilm assays were statistically analysed using a completely randomised design (CRD). All experiments were performed in 3 replicates and the results are expressed as mean values ± standard deviation (SD). Differences between treatments were evaluated using one-way analysis of variance (ANOVA) and significant differences among the means were further determined using Duncan's multiple range test at a significance level of $p < 0.05$. All statistical analyses were conducted using SPSS software, version 21.0 (Fig. 1).

3. Results

Chemical composition of essential oil

Hydrodistillation of the aerial parts of *H. officinalis* yielded 0.7 % (w/w) EO based on the dry weight of the plant material. The GC/MS analysis led to the identification and quantification of 37 distinct compounds, which collectively accounted for 100 % of the total oil composition (Fig. 2, Table 1). The identified constituents were classified into three principal chemical groups: monoterpenes (26 compounds, comprising 65.3 % of the total oil), sesquiterpenes (8 compounds, 10.6 %) and other compounds (3 compounds, 24.1 %). The most abundant compound was bicycloheptane, which constituted 20.75 % of the total EO. The remaining major compounds, in descending order of their relative area percentages, were 1,5,8-o-menthatriene (4.48 %), cyclohexanemethanol (3.79 %), spathulenol (3.70 %) and myrtenol (3.25 %). Together, these five compounds accounted for 35.97 % of the total oil composition. The complete list of all 37 identified compounds, along with their retention times and relative area percentages, is presented in Table 1. The mass spectra used for the definitive identification of spathulenol and myrtenol are displayed in Fig. 3 and 4 respectively.

Antimicrobial activity

The antimicrobial activity of *H. officinalis* EO was evaluated against four bovine mastitis pathogens, two Gram-positive species (*E. faecalis* and *S. aureus*) and two Gram-negative species (*E. coli* and *S. enterica*), using the broth microdilution method. Both clinical mastitis isolates and their corresponding standard reference strains (Persian type culture collection (PTCC)) were tested. The MICs and MBCs are presented in Table 2. *Enterococcus faecalis* was the most susceptible organism, with both the clinical isolate and the standard strain (PTCC 1237) exhibiting identical MIC and MBC values of 0.0312 mg/mL. The MBC/MIC ratio was 1 : 1, indicating a bactericidal effect at the inhibitory concentration. *Staphylococcus aureus* demonstrated the next highest susceptibility, with MIC

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and MBC values of 0.625 mg/mL and 1.25 mg/mL respectively, yielding an MBC/MIC ratio of 2 : 1 for both the standard strain (PTCC 1764) and the clinical isolate. The Gram-negative bacteria displayed markedly higher MIC and MBC values. *Escherichia coli* exhibited MIC and MBC values of 1.25 mg/mL and 2.50 mg/mL, respectively (MBC/MIC ratio of 2 : 1). *Salmonella enterica* was the most resistant organism, with neither the clinical isolate nor the standard strain (PTCC 1787) showing complete growth inhibition at the highest tested concentration of 5 mg/mL (MIC/MBC ≥5 mg/mL; Table 2). One-way ANOVA revealed that the differences in MIC values among the four bacterial species were statistically significant ($p < 0.05$). Duncan's multiple range test confirmed that the MIC and MBC values for *E. faecalis* were significantly lower than those for all other tested organisms ($p < 0.05$). No significant differences in MIC or MBC were observed between the clinical isolates and their corresponding standard strains for any of the four bacterial species ($p > 0.05$).

Anti-biofilm activity

The percentage of biofilm inhibition for each bacterial strain at each concentration is presented in Table 3 and illustrated graphically in Fig. 5. The EO exhibited a concentration-dependent anti-biofilm effect against all four bacterial species: as the concentration increased from 0.5–60 mg/mL, the percentage of biofilm inhibition progressively increased for each organism.

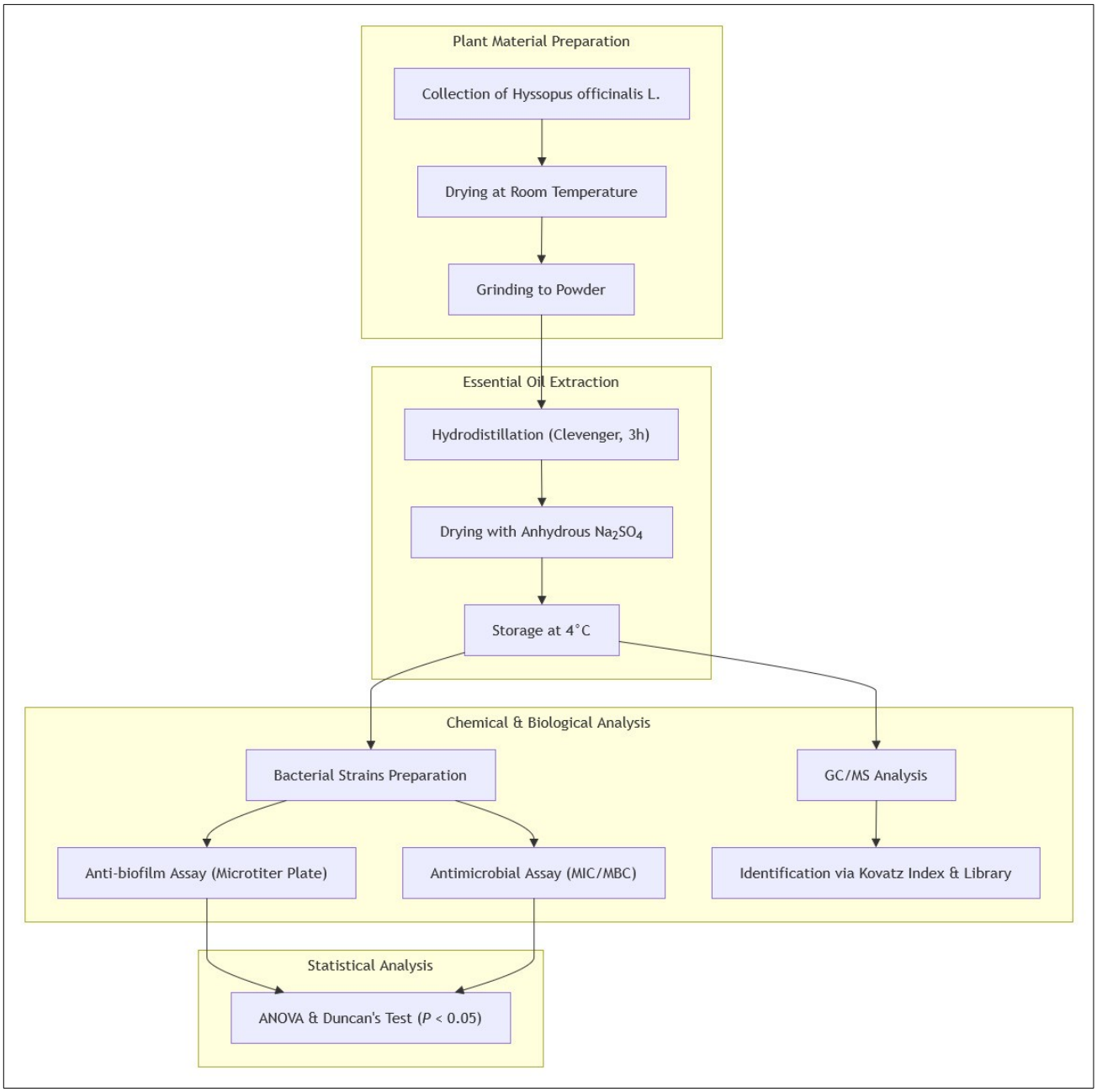


Fig. 1. Experimental workflow of the study.

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Table 1. Constituents of the essential oil of *Hyssopus officinalis*

No	Compounds	RT* (min) (min)	Area (%)	Class
1	α-Pinene	5.62	1.44	Monoterpene
2	Bicycloheptane	12.24	20.75	Monoterpene
3	β-Pinene	5.25	0.27	Monoterpene
4	Ticycloheptane	5.33	0.02	Monoterpene
5	Camphene	5.99	0.42	Monoterpene
6	α-Terpinene	7.87	0.38	Monoterpene
7	Thymyl methyl ether	14.56	0.03	Other
8	α-Humulene	20.04	0.52	Sesquiterpene
9	Trans-Sabinene	9.37	0.44	Monoterpene
10	Trans-Verbenol	11.77	0.41	Monoterpene
11	α-Terpinolene	9.99	0.25	Monoterpene
12	Isopulegol	13.65	0.07	Monoterpene
13	Myrtenyl acetate	16.73	1.31	Monoterpene
14	α-Phellandrene	5.44	0.81	Monoterpene
15	β -Phellandrene	9.77	0.03	Monoterpene
16	Neoalloocimene	20.23	1.84	Monoterpene
17	β-Myrcene	7.14	1.56	Monoterpene
18	β-Thujone	10.49	0.97	Monoterpene
19	Cis-Sabinene	10.33	0.08	Monoterpene
20	γ- Terpinene	9.08	0.83	Monoterpene
21	Terpineol	11.90	0.02	Monoterpene
22	Verbenene	6.15	0.09	Monoterpene
23	Oxirane	34.92	0.10	Other
24	1,5, 8-o-Menthatriene	12.06	4.48	Monoterpene
25	β-Bourbonene	18.30	2.41	Sesquiterpene
26	Germacrene-D	20.74	1.82	Sesquiterpene
27	Delta-3-carene	8.86	0.22	Monoterpene
28	γ-Cadinene	20.56	0.14	Sesquiterpene
29	Azulene	22.82	0.40	Sesquiterpene
30	Cyclohexanemethanol	22.45	3.79	Other
31	(-)(+)- Spathulenol	23.14	3.70	Sesquiterpene
32	Ledol	23.69	0.68	Sesquiterpene
33	Myrtenol	13.30	3.25	Monoterpene
34	Linalool	10.58	1.18	Monoterpene
35	Geranyl propionate	17.84	0.15	Monoterpene
36	Isospathulenol	24.49	1.17	Sesquiterpene
37	Hexadecanoic acid	32.82	0.40	Other
	Total identified (37 compounds)	—	100.00	—
	Monoterpenes (26 compounds)	—	65.3	—
	Sesquiterpenes (8 compounds)	—	10.6	—
	Other compounds (3 compounds)	—	24.1	—

RT: Retention time.

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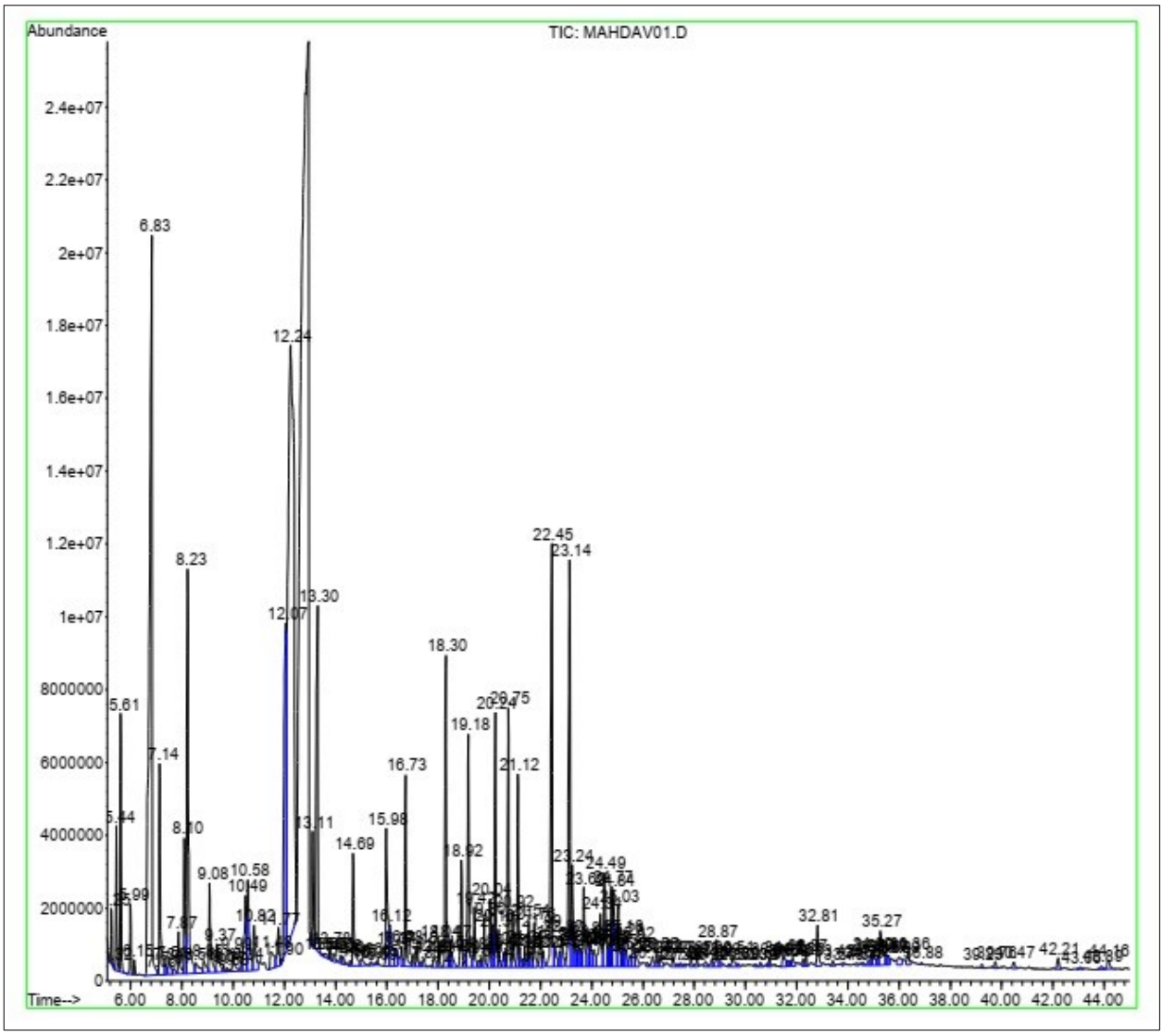


Fig. 2. Gas chromatography–mass spectrometry (GC-MS) total ion chromatogram (TIC) of the essential oil from *Hyssopus officinalis*.

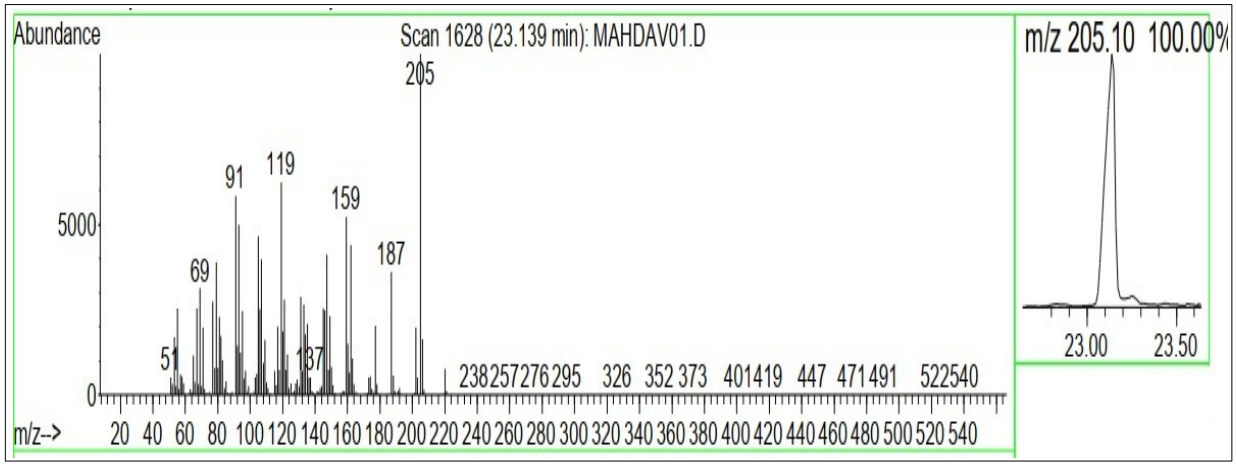


Fig. 3. Mass spectrum of spathulenol ($C_{15}H_{26}O$) from the *Hyssopus officinalis* essential oil (EO), identified at retention time of 23.14 min.

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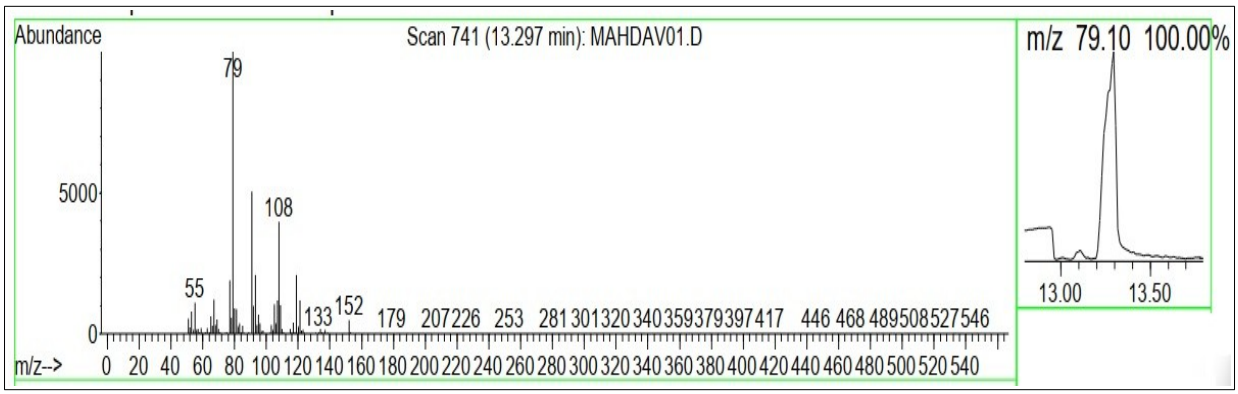


Fig. 4. Mass spectrum of myrtenol ($C_{10}H_{16}O$) from the *Hyssopus officinalis* essential oil (EO), identified at retention time of 13.30 min.

Table 2. The antimicrobial effect of *Hyssopus officinalis* essential oil (EO) on the tested bacterial strains

Bacteria	Gram classifiction	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC ratio
Escherichia coli PTCC 1330	Negative	1.25	2.50	2 : 1
Escherichia coli	Negative	1.25	2.50	2 : 1
Salmonella enterica PTCC 1787	Negative	≥5.00	≥5.00	-
Salmonella enterica	Negative	≥5.00	≥5.00	-
Enterococcus faecalis PTCC 1237	Positive	0.0312	0.0312	1 : 1
Enterococcus faecalis	Positive	0.0312	0.0312	1 : 1
Staphylococcus aureus PTCC 1764	Positive	0.625	1.25	2 : 1
Staphylococcus aureus	Positive	0.625	1.25	2 : 1

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration. All experiments were performed in three independent biological replicates (n = 3) with identical results.

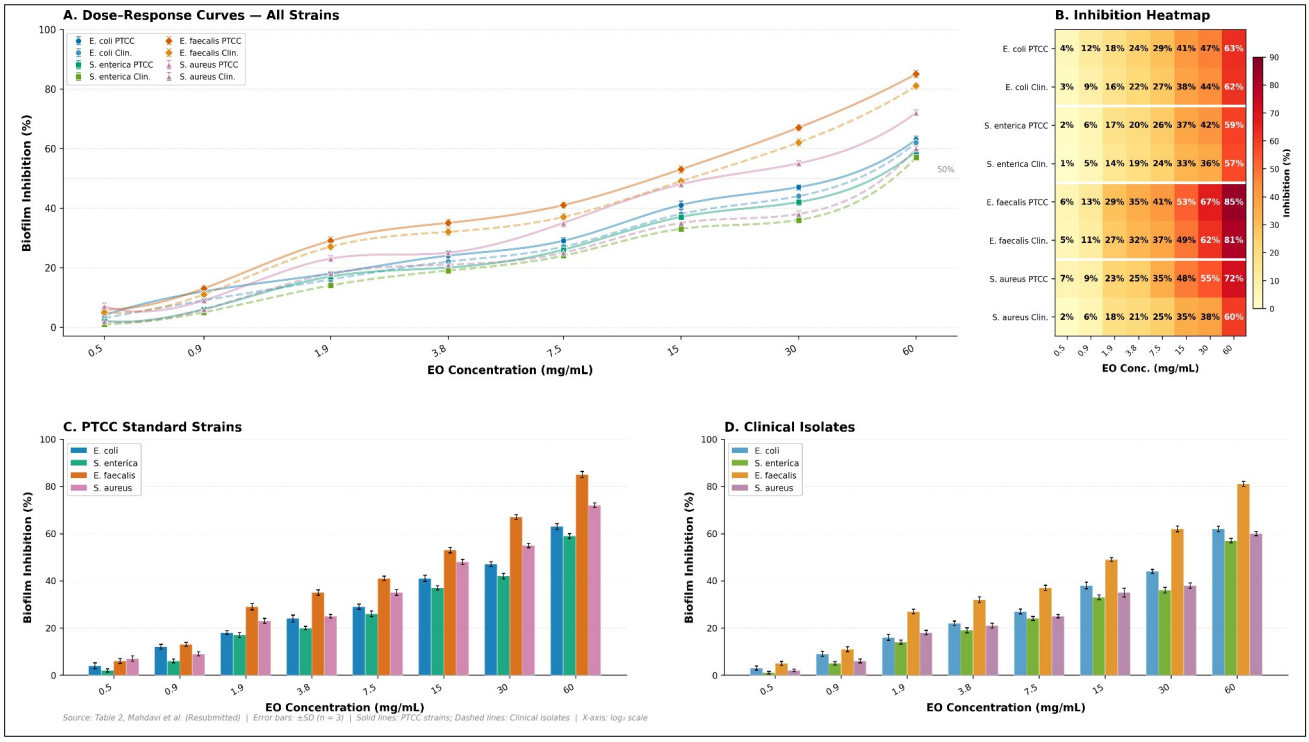


Fig. 5. Dose-response analysis of *Hyssopus officinalis* essential oil (EO) anti-biofilm activity against bovine mastitis pathogens.

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Table 3. Anti-biofilm effect of *Hyssopus officinalis* essential oil (EO) on the tested bacterial strains

Bacterial strain	Essential oil (EO) concentration (mg/mL)							
	0.5	0.9	1.9	3.8	7.5	15	30	60
Escherichia coli PTCC 1330	4 ± 1.2^{efg}	12 ±1.0 ^{def}	18 ± 0.8 ^{def}	24 ± 1.5^{def}	29 ± 1.1^{def}	41 ± 1.3^{de}	47 ± 1.0^{de}	63 ± 1.2^{de}
Escherichia coli	3 ± 0.9^{ef}	9 ± 1.1^{ef}	16 ± 1.2^{ef}	22 ± 0.9^{ef}	27± 1.0^{ef}	38 ± 1.4^{e}	44 ±0.8^{e}	62 ±1.1^{de}
Salmonella enterica PTCC 1787	2 ± 0.7^{f}	6 ± 0.8 ^{f}	17 ± 1.0 ^{def}	20 ± 0.7^{ef}	26 ± 1.2^{ef}	37 ± 0.9^{e}	42 ± 1.1^{e}	59 ± 1.0^{e}
Salmonella enterica	1 ± 0.5 ^{f}	5 ± 0.7 ^{f}	14 ± 0.9^{f}	19 ± 1.1^{f}	24 ± 0.8^{f}	33 ± 1.0^{e}	36 ± 1.2^{e}	57 ± 0.9^{e}
Enterococcus faecalis PTCC 1237	6 ± 1.0^{cde}	13 ± 0.9^{cde}	29 ± 1.3^{bc}	35 ± 1.1^{bc}	41 ± 0.9^{bc}	53 ± 1.2^{bc}	67 ± 1.0^{bc}	85 ±1.3^{a}
Enterococcus faecalis	5 ± 0.8 ^{def}	11 ± 1.0^{def}	27 ± 0.9 ^{cd}	32 ± 1.2^{cd}	37 ± 1.1^{cd}	49 ± 0.8^{cd}	62 ± 1.2^{cd}	81 ±1.0^{ab}
Staphylococcus aureus PTCC 1764	7 ± 1.1^{ef}	9 ± 0.9^{ef}	23 ± 1.0^{cde}	25 ± 0.8^{cde}	35 ± 1.2^{cd}	48 ± 1.1^{cd}	55 ± 0.9^{cd}	72 ± 1.0^{bc}
Staphylococcus aureus	2 ± 0.6^{f}	6 ± 0.8^{f}	18 ± 0.9 ^{def}	21 ± 1.0^{ef}	25 ± 0.7^{ef}	35 ± 1.9^{e}	38 ± 1.1^{e}	60 ± 0.9^{de}

Values represent mean percentage of biofilm inhibition ± standard deviation (n = 3). Different superscript letters indicated statistically significant differences among treatments at $p < 0.05$ as determined by Duncan's multiple range test.

At the maximum tested concentration of 60 mg/mL, the most pronounced inhibition was observed against *E. faecalis*, reaching 85 ± 1.3 % for the standard strain (PTCC 1237) and 81 ± 1.0 % for the clinical isolate. *Staphylococcus aureus* exhibited the second-highest degree of inhibition at 60 mg/mL, with 72 ± 1.0 % for the standard strain (PTCC 1764) and 60 ± 0.9 % for the clinical isolate. *Escherichia coli* demonstrated intermediate inhibition of 63 ± 1.2 % (PTCC 1330) and 62 ± 1.1 % (clinical isolate) at the same concentration. *Salmonella enterica* showed the least biofilm inhibition, reaching 59 ± 1.0 % (PTCC 1787) and 57 ± 0.9 % (clinical isolate) at 60 mg/mL. Even at the lowest tested concentration of 0.5 mg/mL, measurable biofilm inhibition was observed for all strains, ranging from 1 ± 0.5 % (*S. enterica* clinical isolate) to 7 ± 1.1 % (*S. aureus* PTCC 1764). Statistical analysis using one-way ANOVA followed by Duncan's multiple range test indicated that the anti-biofilm effect was significantly influenced by both the bacterial species and the EO concentration ($p < 0.05$). Notably, the standard strains of *S. aureus* and *E. faecalis* showed consistently higher biofilm inhibition percentages than their respective clinical isolates across all concentrations, while the differences between standard and clinical strains of *E. coli* were minimal.

4. Discussion

Chemotypic variability of *Hyssopus officinalis* essential oil

The EO yield of 0.7 % (w/w) obtained in the present study falls within the range previously documented for *H. officinalis* populations across different geographical origins. Essential oil yields in hyssop can range from 0.22–4.4 % depending on the plant part, phenological stage and environmental conditions, with the highest concentrations typically observed during full bloom (30). The yield from the Maragheh population is comparable to the 0.6 % (w/w) reported for *Hyssopus cuspidatus* from Xinjiang, China and the 0.65 % reported for *H. officinalis* cultivated in northwestern Poland (31). These comparisons suggest that the semi-arid climatic conditions of the Maragheh region, characterised by significant seasonal temperature variations and an altitude of approximately 1455 m above sea level, support moderate EO biosynthesis in this species. The chemical profile of the Maragheh chemotype, with bicycloheptane (20.75 %) as the predominant constituent, is notably distinct from those reported for other hyssop populations worldwide.

The most frequently reported major compounds of *H. officinalis* EO are pinocamphone isomers (cis- and trans-pinocamphone), which have been identified as the dominant constituents in samples from Turkey (isopinocamphone: 57.27 %) (32), Lithuania (pinocamphone isomers as principal components) (33), Egypt (cis-pinocamphone: 26.85 %, β-pinene: 20.43 %) (34) and Romania (30). In fact, the pinocamphone + isopinocamphone + β-pinene chemotype is the most widely recognised and is the basis of the ISO 9841 standard for hyssop EO quality (35). The absence of pinocamphone as a major constituent in the present sample and the preponderance of bicycloheptane instead, indicates that the Maragheh population may represent a distinct, previously uncharacterised chemotype of *H. officinalis*. The observed chemotypic variability is consistent with the well-documented influence of geographical origin, climatic conditions, soil composition, harvest time and genetic factors on the secondary metabolite profile of aromatic plants (22, 24, 30). Previous studies demonstrated that even within the same growing season, the EO composition of *H. officinalis* shifts dramatically between the vegetative growth phase (dominated by elemol, germacrene D and

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bicyclogermacrene) and the flowering phase (dominated by pinocamphone and isopinocamphone) (30). These findings reinforce the necessity for comprehensive chemotypic characterisation of locally sourced plant material before any therapeutic application, as the biological activity of an EO is directly determined by its chemical composition.

Differential antimicrobial susceptibility among bacterial species

The results of the present study demonstrated a clear hierarchy of susceptibility among the four tested bovine mastitis pathogens, with Gram-positive bacteria being significantly more susceptible to the hyssop EO than Gram-negative bacteria. This pattern is consistent with a large body of evidence in the EO literature indicating that Gram-positive bacteria generally exhibit greater sensitivity to EO than their Gram-negative counterparts (24, 36, 37). The higher susceptibility of *E. faecalis* (MIC = 0.0312 mg/mL) and *S. aureus* (MIC = 0.625 mg/mL) compared to *E. coli* (MIC = 1.25 mg/mL) and *S. enterica* (MIC ≥ 5 mg/mL) can be attributed to fundamental differences in cell envelope architecture. Gram-positive bacteria possess a relatively simple cell wall structure consisting of a thick peptidoglycan layer (20–80 nm) external to the cytoplasmic membrane, but lacking the outer membrane that is characteristic of Gram-negative organisms (36, 38). The hydrophobic components of EOs can readily penetrate this peptidoglycan layer and reach the cytoplasmic membrane, where they disrupt lipid packing, increase membrane permeability and cause leakage of intracellular constituents (24, 37). In contrast, Gram-negative bacteria are protected by an additional outer membrane containing lipopolysaccharide (LPS), which presents a formidable barrier to the diffusion of hydrophobic molecules (36). The outer membrane displays a low degree of permeability to hydrophobic compounds, thereby limiting the access of EO constituents to their intracellular targets (37).

Furthermore, Gram-negative bacteria possess efflux pumps and periplasmic enzymes that may actively extrude or degrade EO components, adding another layer of resistance (36, 39). The remarkable susceptibility of *E. faecalis* to the hyssop EO, with an MBC/MIC ratio of 1 : 1, is of particular interest. This ratio indicates that the EO is bactericidal rather than merely bacteriostatic at its inhibitory concentration, leaving no viable cells in the transition from growth inhibition to cell death (40). The observed MIC of 0.0312 mg/mL (31.2 µg/mL) for *E. faecalis* is substantially lower than the MIC of 128 µg/mL reported for purified myrtenol against *S. aureus* (41), suggesting that the complex mixture of compounds in the whole EO may exert synergistic effects that enhance overall antimicrobial potency beyond the activity of any individual constituent (42). The finding that *S. enterica* was the most resistant organism (MIC/MBC ≥ 5 mg/mL) is consistent with the well-documented intrinsic resistance of *Salmonella* spp. to EOs and other antimicrobial agents. This resistance has been attributed to the robust outer membrane barrier, the presence of multiple efflux systems (e.g., AcrAB-TolC) and the organism's capacity for biofilm formation, which further limits antimicrobial penetration (7, 12). It is noteworthy that no significant differences were found between the clinical mastitis isolates and the standard reference strains for any of the four bacterial species, suggesting that *in vitro* susceptibility to the EO is not substantially altered by phenotypic adaptations that may occur during the infection process in the host animal.

Anti-biofilm activity and its relationship to direct antimicrobial effects

The anti-biofilm activity of the hyssop EO demonstrated a clear concentration-dependent pattern across all four bacterial species, which is a characteristic feature of EO activity that has been widely reported in the literature (Fig. 5, Table 3) (36, 43, 44). A particularly noteworthy finding was that the degree of biofilm inhibition generally exceeded what would be predicted from the direct bactericidal activity alone, especially for the Gram-negative organisms. For instance, at 60 mg/mL, biofilm inhibition for *S. enterica* reached 57–59 %, whereas the MIC exceeded 5 mg/mL. This discrepancy strongly suggests that the EO may disrupt biofilm formation through mechanisms that are, at least partially, independent of direct microbial killing. Several mechanisms have been proposed to explain the anti-biofilm activity of EOs at sub-bactericidal concentrations. These include interference with quorum-sensing pathways that regulate biofilm gene expression, inhibition of initial bacterial adhesion to abiotic surfaces, suppression of extracellular polymeric substance production and disruption of the preformed biofilm matrix architecture (36). In a comprehensive review, concluded that EOs and their components can target multiple genes involved in quorum sensing, motility, adhesion and virulence, thereby impairing the ability of bacteria to form and maintain mature biofilms. The observation in the present study that measurable biofilm inhibition (1–7 %) occurred even at the lowest tested concentration of 0.5 mg/mL, which is far below the MIC for all organisms except *E. faecalis*, lends support to the hypothesis that the anti-biofilm activity operates, at least in part, through non-lethal mechanisms (43). The pronounced anti-biofilm activity against *E. faecalis* (up to 85 % inhibition at 60 mg/mL) is of particular clinical significance. *Enterococcus faecalis* is recognised as a formidable biofilm-forming pathogen that contributes to persistent and recurrent infections, including bovine mastitis and endodontic infections (45). The ability of the hyssop EO to substantially inhibit biofilm formation by this organism suggests its potential utility as an adjunctive therapeutic agent for the management of biofilm-associated mastitis, particularly in cases involving multidrug-resistant strains. An interesting observation was the consistently lower anti-biofilm activity against the clinical isolates of *S. aureus* compared to the standard strain (PTCC 1764). At 60 mg/mL, the clinical isolate showed 60 % inhibition versus 72 % for the standard strain (Table 3). This difference may reflect enhanced biofilm matrix

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production or altered surface protein expression patterns acquired during chronic intramammary infection, as clinical isolates of *S. aureus* have been reported to develop thicker and more robust biofilms under *in vivo* conditions (46). Such phenotypic adaptations may confer additional resistance to anti-biofilm agents and should be considered when translating *in vitro* findings to clinical applications.

Potential contribution of individual essential oil components

The biological activities observed in the present study may be attributed, individually or synergistically, to several of the major constituents identified in the hyssop EO. Myrtenol (3.25 %), a monoterpene alcohol, has been the subject of considerable research attention. Previous studies demonstrated that myrtenol attenuated methicillin-resistant *Staphylococcus aureus* (MRSA) biofilm formation and virulence by suppressing the expression of *sarA* and *agr*, two key regulatory genes that control biofilm-associated gene expression in *S. aureus*. Importantly, myrtenol exhibited this anti-biofilm activity without causing any harmful effect on cell growth and viability, indicating a specific anti-virulence mechanism rather than a general bactericidal effect (47). Myrtenol acts by interfering with bacterial cell wall synthesis, with MIC and MBC values of 128 µg/mL against *S. aureus* (41). Additionally, the efficacy of myrtenol in increasing the treatment efficacy against *S. aureus–C. albicans* mixed infections, highlighting its potential in polymicrobial infection contexts (16). Spathulenol (3.70 %), a sesquiterpene alcohol, has also been recognised for its antimicrobial properties.

Recent studies have reported that spathulenol and related sesquiterpenes exhibit antimicrobial activity and can enhance the effects of monoterpenes through synergistic interactions (48). The antimicrobial potential of *H. officinalis* EO confirmed and highlighted the contributions of both monoterpene and sesquiterpene constituents to its overall efficacy (17). The concept of synergistic interactions among EO components is well-established and may partially explain why the whole EO demonstrated more potent activity against *E. faecalis* (MIC = 0.0312 mg/mL) than would be predicted from the activity of myrtenol alone (MIC = 128 µg/mL for purified myrtenol) (41). Bassole and Juliani proposed that the complex mixture of compounds in EOs can target multiple cellular structures and metabolic pathways simultaneously, resulting in an overall antimicrobial effect that exceeds the sum of the individual components. This multi-target mechanism also has important implications for the development of resistance, as simultaneous mutation at multiple targets is statistically far less probable than mutation at a single target (42).

5. Conclusion

The present study provides the first comprehensive characterisation of the chemical composition, antimicrobial activity and anti-biofilm efficacy of *Hyssopus officinalis* essential oil (EO) against four major bovine mastitis pathogens. Gas chromatography–mass spectrometry (GC-MS) analysis revealed 37 compounds, with the Maragheh, Iran population representing a previously uncharacterised chemotype dominated by bicycloheptane, 1,5,8-o-menthatriene, cyclohexanemethanol, spathulenol and myrtenol. This chemotypic profile is clearly distinct from the pinocamphone-dominant chemotypes reported for European, Turkish and Egyptian populations, highlighting the critical influence of geographical origin on the secondary metabolite composition of this species. The EO demonstrated potent antimicrobial activity, with *Enterococcus faecalis* being the most susceptible organism and *Salmonella enterica* the most resistant. The Gram-positive bacteria were consistently more susceptible than the Gram-negative organisms, a finding consistent with the known protective role of the outer lipopolysaccharide membrane in Gram-negative bacteria. Importantly, no significant differences in susceptibility were observed between clinical mastitis isolates and standard reference strains, supporting the reliability of standard strain data for predicting efficacy against field isolates. The anti-biofilm assays revealed a pronounced concentration-dependent inhibition of biofilm formation across all four bacterial species. Notably, the anti-biofilm activity was observed at concentrations below the minimum inhibitory concentration (MIC) for Gram-negative organisms, suggesting the involvement of mechanisms beyond direct microbial killing, such as interference with quorum-sensing pathways and inhibition of extracellular polymeric substance production. These findings collectively suggest that *H. officinalis* EO possesses promising potential as a natural therapeutic alternative for the management of bovine mastitis, particularly against Gram-positive biofilm-forming pathogens. Further investigations, including *in vivo* efficacy studies, bioassay-guided fractionation and safety evaluations, are warranted to advance the translational application of these findings.

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Authors' contributions

SM carried out the conceptualisation, investigation, methodology, data curation, formal analysis and drafted the manuscript. MBA participated in the research, provided resources (plant material) and contributed to the investigation, methodology and writing-review and editing. SG performed the investigation, methodology

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and formal analysis. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Gemini Pro in order to edit and improve the language, formatting, and reference style of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

1. Rollin E, Dhuyvetter K, Overton M. The cost of clinical mastitis in the first 30 days of lactation: an economic modeling tool. Prev Vet Med. 2015;122(3):257–64. http://doi.org/10.1016/j.prevetmed.2015.11.006
2. Sharifi H, Adeli Sardooei M, Bodagh Abadi M, Babaei H. Economic impact of mastitis in dairy cows: case study of Tehran Province, Iran. Iran J Vet Surg. 2014;9(2):39–44. http://doi.org/20.1001.1.20083033.2014.09.2.5.0
3. Kumar CG, Anand SK. Significance of microbial biofilms in food industry: a review. Int J Food Microbiol. 1998;42(1-2):9–27. http://doi.org/10.1016/S0168-1605(98)00060-9
4. Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001;9(1):34–9. http://doi.org/10.1016/S0966-842X(00)01913-2
5. Peng D. Biofilm formation of Salmonella. IntechOpen; 2016.
6. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277–83.
7. DiMarzio M, Shariat N, Kariyawasam S, Barrangou R, Dudley EG. Antibiotic resistance in *Salmonella enterica* serovar Typhimurium associates with CRISPR sequence type. Antimicrob Agents Chemother. 2013;57(9):4282–9. http://doi.org/10.1128/aac.00913-13
8. Wiesner M, Calva JJ, Bustamante VH, Pérez-Morales D, Fernández-Mora M, Calva E, et al. A multi-drug resistant *Salmonella typhimurium* ST213 human-invasive strain (33676) containing the bla CMY-2 gene on an IncF plasmid is attenuated for virulence in BALB/c mice. BMC Microbiol. 2016;16(1):18. http://doi.org/10.1186/s12866-016-0633-7
9. Dallal MMS, Doyle MP, Rezadehbashi M, Dabiri H, Sanaei M, Modarresi S, et al. Prevalence and antimicrobial resistance profiles of *Salmonella* serotypes, Campylobacter and Yersinia spp. isolated from retail chicken and beef, Tehran, Iran. Food Control. 2010;21(4):388–92. http://doi.org/10.1016/j.foodcont.2009.06.001
10. Stevenson JE, Gay K, Barrett TJ, Medalla F, Chiller TM, Angulo FJ. Increase in nalidixic acid resistance among non-Typhi *Salmonella enterica* isolates in the United States from 1996 to 2003. Antimicrob Agents Chemother. 2007;51(1):195–7. http://doi.org/10.1128/aac.00222-06
11. Su L-H, Chiu C-H, Chu C, Ou JT. Antimicrobial resistance in nontyphoid *Salmonella serotypes*: a global challenge. Clin Infect Dis. 2004;39(4):546–51. http://doi.org/10.1086/422726
12. Nair A, Rawool DB, Doijad S, Poharkar K, Mohan V, Barbuddhe SB, et al. Biofilm formation and genetic diversity of *Salmonella* isolates recovered from clinical, food, poultry and environmental sources. Infect Genet Evol. 2015;36:424–33. http://doi.org/10.1016/j.meegid.2015.08.012
13. Panáček A, Kvitek L, Prucek R, Kolář M, Večeřová R, Pizúrová N, et al. Silver colloid nanoparticles: synthesis, characterization and their antibacterial activity. J Phys Chem B. 2006;110(33):16248–53. http://doi.org/10.1021/jp063826h
14. Iscan G, Kırımer N, Kurkcuoglu M, Baser KHC, Demirci F. Antimicrobial screening of *Mentha piperita* essential oils. J Agric Food Chem. 2002;50(14):3943–6. http://doi.org/10.1021/jf011476k
15. Abdulkareem ZA. Zataria multiflora essential oil: a potent agent with antibacterial, anti-NorA efflux pump and anti-biofilm activity against ciprofloxacin-resistant *Staphylococcus aureus* isolates from bovine mastitis. Braz J Microbiol.2025;56(3):2181–92. http://doi.org/10.1007/s42770-025-01731-9
16. Mahmoud RY, Trizna EY, Sulaiman RK, Pavelyev RS, Gilfanov IR, Lisovskaya SA, et al. Increasing the efficacy of treatment of *Staphylococcus aureus-Candida albicans* mixed infections with myrtenol. Antibiotics. 2022;11(12):1743. http://doi.org/10.3390/antibiotics11121743
17. Nistor AL, Pop CR, Mitrea L, Cătunescu GM, Vârban R, Lipșa FD, et al. Antimicrobial potential of *Hyssopus officinalis* L. and *Agastache foeniculum* (Pursh) Kuntze essential oils for food applications: a review of their chemical compositions and antimicrobial efficacy. Appl Sci. 2025;15(9):4772. http://doi.org/10.3390/app15094772
18. Dhaouadi S, Khamessi O, Dassi RB, Boulila A, Elandoulsi RB, Chaouachi RD. Leveraging the antibacterial and antibiofilm activities of *Cymbopogon fle*xuosus essential oil against multidrug-resistant bacteria recovered from avian colibacillosis and bovine mastitis: *in vitro* and molecular docking insights. Vet Res Commun. 2026;50(5):366. http://doi.org/10.1007/s11259-026-11311-3
19. Tabatabayi A, Firouzi R. Diseases of animals due to Bacteria. 2001. p. 20–4.
20. Maity S, Ambatipudi K. Mammary microbial dysbiosis leads to the zoonosis of bovine mastitis: a One-Health perspective. FEMS Microbiol Ecol. 2021;97(1). http://doi.org/10.1093/femsec/fiaa241
21. Sugrue I, Tobin C, Ross RP, Stanton C, Hill C. Foodborne pathogens and zoonotic diseases. In: Raw milk. Elsevier; 2019. p. 259–72.
22. Kazazi H, Rezaei K, Ghotb-Sharif SJ, Emam-Djomeh Z, Yamini Y. Supercritical fluid extraction of flavors and fragrances from Hyssopus officinalis L. cultivated in Iran. Food Chem. 2007;105(2):805–11. http://doi.org/10.1016/j.foodchem.2007.01.059
23. Nasirpour M, Yavarmanesh M, Mohhamadi Sani A, Mohamdzade Moghadam M. Antibacterial effect of aqueous extract of *Artemisia aucheri*, *Artemisia sieberi* and *Hyssopus officinalis* L. on the food borne pathogenic bacteria. J Food Sci Technol. 2014;12(46)73–84.

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https://doi.org/10.14719/pst.14110

24. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol.2004;94(3):223–53. http://doi.org/10.1016/j.ijfoodmicro.2004.03.022

25. Reid ML, Sekhon JK, LaFramboise LM. Toxicity of monoterpene structure, diversity and concentration to mountain pine beetles, *Dendroctonus ponderosae*: beetle traits matter more. J Chem Ecol. 2017;43(4):351–61. http://doi.org/10.1007/s10886-017-0824-1

26. Kheiri M, Soufizadeh S, Ghaffari A, AghaAlikhani M, Eskandari A. Association between temperature and precipitation with dryland wheat yield in northwest of Iran. Climatic Change. 2017;141(4):703–17. http://doi.org/10.1007/s10584-017-1904-5

27. Wiggins JM, Albanese JA. A brief history of pharmacopoeias: a global perspective. 2019. p. 1–6.

28. Oroojalian F, Kasra-Kermanshahi R, Azizi M, Bassami M. Synergistic antibacterial activity of the essential oils from three medicinal plants against some important food-borne pathogens by microdilution method. Iran J Med Aromat Plants.2010;26(2):133–46. http://doi.org/10.22092/ijmapr.2010.6836

29. Kubota H, Senda S, Nomura N, Tokuda H, Uchiyama H. Biofilm formation by lactic acid bacteria and resistance to environmental stress. J Biosci Bioeng. 2008;106(4):381–6. http://doi.org/10.1263/jbb.106.381

30. Kotyuk L, Shvaika O. Seasonal rhythms of *Hyssopus officinalis* L. cultivated in Zhytomyr Polissya. Ukrainian Journal of Ecology. 2018;8(1):335–41. http://doi.org/10.15421/2018_219

31. Zhou X, Hai-Yan G, Tun-Hai X, Tian S. Physicochemical evaluation and essential oil composition analysis of Hyssopus cuspidatus Boriss from Xinjiang, China. Pharmacogn Mag. 2010;6(24):278–81. http://doi.org/10.4103/0973-1296.71790

32. Kizil S, Haşimi N, Tolan V, Kilinc E, Karataş H. Chemical composition, antimicrobial and antioxidant activities of hyssop (*Hyssopus officinalis* L.) essential oil. Not Bot Horti Agrobot.2010;38(3):99–103. http://doi.org/10.15835/nbha3834788

33. Bernotiene G, Butkiene R. Essential oils of *Hyssopus officinalis* L. cultivated in East Lithuania. Chemija. 2010;21(2-4):135–8.

34. Said-Al Ahl HA, Abbas ZK, Sabra AS, Tkachenko KG. Essential oil composition of *Hyssopus officinalis* L. cultivated in Egypt. Int J Plant Sci Ecol. 2015;1(2):49–53.

35. Sharifi-Rad J, Quispe C, Kumar M, Akram M, Amin M, Iqbal M, et al. Hyssopus essential oil: an update of its phytochemistry, biological activities and safety profile. Oxid Med Cell Longev. 2022;2022(1):8442734. http://doi.org/10.1155/2022/8442734

36. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals.2013;6(12):1451–74. http://doi.org/10.3390/ph6121451

37. Patterson JE, McElmeel L, Wiederhold NP, editors. *In vitro* activity of essential oils against gram-positive and gram-negative clinical isolates, including carbapenem-resistant Enterobacteriaceae. Open Forum Infectious Diseases. 2019. Oxford University Press US.

38. Xu F, Xie Y, Yu W, Wang Z. Breaking the outer membrane barrier: structure, targets and antimicrobial strategies for Gram-negative bacteria. Front Microbiol. 2026;17:1734749. http://doi.org/10.3389/fmicb.2026.1734749

39. El-Sakhawy MA, El Raheim Mohammed Donia A, Juraybi TN, El-Sherbiny Ateya AA, Osman W, Abd-Elghany AA, et al. Action mechanism of essential oils or their components against pathogenic bacteria: literature review. Int J Microbiol. 2026;2026(1):7468450.

40. Pankey GA, Sabath LD. Clinical relevance of bacteriostatic versus bactericidal mechanisms of action in the treatment of Gram-positive bacterial infections. Clin Infect Dis. 2004;38(6):864–70. http://doi.org/10.1086/381972

41. Cordeiro L, Figueiredo P, Souza H, Sousa A, Andrade-Júnior F, Barbosa-Filho J, et al. Antibacterial and antibiofilm activity of myrtenol against *Staphylococcus aureus*. Pharmaceuticals (Basel). 2020;13(6):133. http://doi.org/10.3390/ph13060133

42. Bassolé IHN, Juliani HR. Essential oils in combination and their antimicrobial properties. Molecules. 2012;17(4):3989–4006. http://doi.org/10.3390/molecules17043989

43. Maggio F, Rossi C, Serio A, Chaves-Lopez C, Casaccia M, Paparella A. Anti-biofilm mechanisms of action of essential oils by targeting genes involved in quorum sensing, motility, adhesion and virulence: a review. Int J Food Microbiol. 2025;426:110874. http://doi.org/10.1016/j.ijfoodmicro.2024.110874

44. Huang Y, Liu X, Chen L, Li M, Xia Y, Yang K. Research progress and antibacterial mechanisms of plant essential oils as alternative therapies for periodontitis. Front Microbiol. 2026;17:1802802. http://doi.org/10.3389/fmicb.2026.1802802

45. Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. Crit Rev Oral Biol Med. 2004;15(5):308–20. http://doi.org/10.1177/154411130401500506

46. Melchior M, Vaarkamp H, Fink-Gremmels J. Biofilms: a role in recurrent mastitis infections? Vet J. 2006;171(3):398–407. http://doi.org/10.1016/j.tvjl.2005.01.006

47. Selvaraj A, Jayasree T, Valliammai A, Pandian SK. Myrtenol attenuates MRSA biofilm and virulence by suppressing sarA expression dynamism. Front Microbiol. 2019;10:2027. http://doi.org/10.3389/fmicb.2019.02027

48. Shahid S, Dasgupta D, Pani B, Singh AK, Saji VS. Biofilm in food materials: a review. The Microbe. 2025:100518. http://doi.org/10.1016/j.microb.2025.100518

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