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Antimicrobial and Anti-Biofilm Potential of Selected Plant Essential Oils against *Salmonella enterica* Serovar Typhimurium

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ABSTRACT

Background: *Salmonella enterica serovar* Typhimurium is a major foodborne pathogen with growing resistance to antibiotics. Plant-derived essential oils (EOs) have emerged as potential alternatives due to their antimicrobial and anti-virulence properties. This study aimed to investigate the antibacterial, anti-biofilm, and quorum sensing (QS) inhibitory effects of EOs derived from *Thymus daenensis* and *Satureja hortensis*.

Materials & Methods: The antibacterial activity of the EOs was evaluated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using standard microbiological assays. Biofilm inhibition and disruption were assessed via crystal violet staining. Gas chromatography-mass spectrometry (GC-MS) was used to analyze the chemical composition of the EOs. Real-time PCR was performed to measure the expression of QS-related genes.

Findings: Both EOs exhibited antibacterial activity against *S. Typhimurium*, with MICs of 6.25 µg/mL (*T. daenensis*) and 12.5 µg/mL (*S. hortensis*) and MBCs of 25 µg/mL for both. GC-MS analysis revealed carvacrol, thymol, γ-terpinene, *p*-cymene, and α-terpinene as major constituents. At sub-MIC concentrations, *T. daenensis* EO inhibited biofilm formation by 68% and disrupted mature biofilms by 54%, while *S. hortensis* EO showed 45 and 37% inhibition, respectively. Both EOs significantly downregulated QS-related genes, indicating strong anti-QS activity.

Conclusion: The EOs derived from *T. daenensis* and *S. hortensis* exhibited strong antibacterial, anti-biofilm, and anti-QS properties against *S. Typhimurium*. These findings support their potential as natural therapeutic agents for combating resistant *Salmonella* infections.

Keywords: Alternative to antibiotics, Antimicrobial resistance, Biofilm formation, Quorum sensing, *Salmonella*

CITATION LINKS

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Introduction

Salmonella enterica serovar Typhimurium is a major foodborne pathogen responsible for numerous outbreaks associated with contaminated food products. Its ability to survive under diverse environmental conditions and tolerate multiple stresses makes it a persistent threat to food safety and public health. This bacterium is a leading cause of gastroenteritis worldwide, producing symptoms ranging from mild diarrhea to severe systemic infections, particularly in children, the elderly, and immunocompromised individuals [1, 2].

The pathogenicity of *S.* Typhimurium is closely linked to its efficient host colonization and immune evasion strategies. After ingestion, the bacterium survives gastric acidity and reaches the intestine, where it adheres to and invades epithelial cells. Inside the host, *Salmonella* manipulates cellular signaling pathways to support intracellular survival and replication. The secretion of virulence factors, including toxins and effector proteins, disrupts host cell functions and induces inflammatory responses that contribute to disease progression [3, 4].

Beyond acute infection, the persistence of *S.* Typhimurium is strongly associated with its ability to form biofilms [5]. Biofilms are structured bacterial communities embedded within a self-produced extracellular matrix that enhances adhesion to biotic and abiotic surfaces. Biofilm formation increases resistance to environmental stresses and antimicrobial agents and facilitates long-term survival in food processing environments. Biofilms act as reservoirs for microbial contamination and transmission within food distribution systems [6, 7].

Quorum sensing is a bacterial cell-to-cell communication system that regulates collective behaviors, including biofilm formation and virulence factor expression [8]. Through the production and detection of signaling molecules, bacteria coordinate gene expression according to population density. In *S.* Typhimurium, QS contributes to the regulation of virulence determinants and biofilm-associated gene expression, thereby influencing pathogenicity and adaptation to host and environmental conditions [9, 10].

Considering the close relationship between biofilm formation, QS, and *Salmonella* pathogenicity, targeting these mechanisms represents a promising strategy for controlling bacterial infections and reducing foodborne disease risks. Approaches aimed at interfering with bacterial colonization, persistence, and virulence may support the development of alternative antimicrobial and preventive strategies [11, 12].

Plant-derived compounds, including extracts and essential oils (EOs), have attracted increasing research interest as natural antimicrobial agents [13]. EOs are particularly valued due to their biodegradability, availability, and comparatively lower toxicity than synthetic antimicrobials [14]. Their antimicrobial activity is largely attributed to diverse bioactive constituents, which enable multiple modes of antibacterial action [15]. Importantly, plant-based compounds may reduce the likelihood of developing multidrug resistance by exerting antimicrobial effects through multiple targets rather than strong selective pressure [16, 17].

Objectives: Accordingly, the present study aimed to investigate the antibacterial, anti-biofilm, and quorum sensing inhibitory (QSI) activities of *T. daenensis* and *S. hortensis* EOs against *S.* Typhimurium. Positive outcomes may support the potential application of these plant-derived compounds as candidates for future antimicrobial development and infection control strategies.

Materials and Methods

Bacterial strain and culture conditions:

S. Typhimurium strain ATCC 14028 was

purchased from the Persian Type Culture Collection and applied as the standard strain (the strain that could produce biofilms). The culture media in the current study, including tryptic soy broth (TSB), were sourced from Merck (Germany). The standard bacteria were cultured aerobically in TSB at 37 °C for 24 hours.

Gas chromatography-mass spectrometry (GC-MS) analysis of EOs: The EOs of *T. daenensis* and *S. hortensis* were obtained from the Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Iran. The aerial parts of the plants, including leaves, stems, and flowers, were collected during the flowering stage in July 2023. To prevent degradation of volatile compounds, the extracted oils were stored in amber glass vials at 4 °C in the dark until analysis. The determination of EOs main components was performed according to the procedures described in our previous research [17].

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC): The antibacterial efficacy of the EOs was assessed against the planktonic form of *S.* Typhimurium using the microdilution technique on 96-well polystyrene plates, as previously described [18]. In this experiment, a solution of each EO was diluted in culture medium containing 0.1% dimethyl sulfoxide (DMSO) to create a series of concentrations ranging from 0.19 to 25 µg/mL. Subsequently, 100 µL of bacterial culture was added, reaching an approximate concentration of 5×10^5 CFU/mL. A negative control, consisting of bacteria in TSB medium with 0.1% DMSO, was included. Following 24-hour incubation at 37 °C, the MIC was determined as the lowest EO concentration that visually inhibited microbial growth.

The MBC was evaluated by transferring 10 µL of samples from MIC assay test wells that showed no macroscopic bacterial growth. This aliquot was then inoculated onto a TSA medium plate and incubated at 37 °C for 24 hours in triplicate. Bacterial growth was examined following the incubation period.

Anti-biofilm activity: The anti-biofilm activity of the EOs was evaluated using the microtiter plate method (MTP) as previously described [18, 19]. Biofilm formation was assessed in untreated, flat-bottom, polystyrene 96-well plates, without any pre-coating or surface roughening. This standard setup allows for reproducible adhesion and biofilm development under controlled conditions.

The EOs were tested at concentrations of MIC/2 (1.56 µg/mL for *T. daenensis*; 3.12 µg/mL for *S. hortensis*), MIC/4 (0.78 µg/mL for *T. daenensis*; 1.56 µg/mL for *S. hortensis*), and MIC/8 (0.39 µg/mL for *T. daenensis*; 0.78 µg/mL for *S. hortensis*), prepared in TSB-DMSO and added to individual wells. Following this, 100 µL of freshly cultured bacteria, at an approximate concentration of 5×10^5 CFU/mL, were added to the wells, along with appropriate negative and positive controls. Following a 24-hour incubation at 37 °C, non-adherent cells were removed by three washes with 200 µL of sterile saline. The adhering cells were then fixed in methanol for 15 min. Following the fixation, the methanol was removed, and 200 µL of 0.1% Safranin dye was added for 20 min. After re-washing and air-drying, 100 µL of 96% ethanol was added to each well and mixed for 5 minutes. The optical density (OD) was measured at 490 nm using a microplate reader (ELx808; BioTek; USA). Each experiment was performed in triplicate, and the results were reported as mean ± standard deviation (SD). The efficacy of the EOs was determined by comparing the mean OD values of treated wells with the negative control (without EO).

Impact of EOs on the disruption of pre-formed biofilms: The effects of the EOs

derived from *T. daenensis* and *S. hortensis* on the disruption of pre-formed biofilms were examined using the methodology described by Asadi et al. (2023) [18].

Biofilm formation was initiated for 24 hrs in a 96-well microtiter plate, with each well containing 100 µL of TSB and 100 µL of bacterial suspension, as described for the MIC assay. Incubation was then statically performed at 37 °C for another 24 hrs. Following the incubation, the supernatants were removed and replaced with 100 µL of TSB-DMSO containing the EOs at concentrations of MIC/2, MIC/4, and MIC/8. Each concentration was designed for four wells. Negative control wells contained 100 µL of TSB-DMSO; then an additional incubation occurred at 37 °C for 24 hrs. Afterwards the supernatants were carefully discarded, and the wells were rinsed three times with physiological saline and stained with 0.1% Safranin. The OD was measured at 490 nm using a microplate reader (ELx808; BioTek; USA). The results were interpreted by comparing the mean OD values of each well with those of the negative control wells.

Impact of EOs on *S.* Typhimurium QS:

As previously described [18], the swarming and swimming motility of *S.* Typhimurium were assessed following treatment with the EOs to evaluate their QS inhibitory effects. For the swimming assay, bacterial samples were inoculated into the center of agar plates containing 1% tryptone, 0.5% NaCl, and 5% agar, supplemented with varying concentrations of *T. daenensis* and *S. hortensis* EOs (MIC/2, MIC/4, and MIC/8). Similarly, the swarming assay was performed using a medium composed of 1% peptone, 0.5% NaCl, 0.5% filter-sterilized D-glucose, and 5% agar, enriched with different EO concentrations.

Due to the high agar concentration, bacterial motility was significantly restricted, allowing for the observation of EO effects under limited mobility conditions. The inhibition of QS was evaluated by measuring the diameter of migration zones around the inoculation point using a transparent millimeter ruler under sterile conditions.

Real-time RT-qPCR analysis of *luxS* and *pfs* gene expression: The anti-QS inhibitory efficacy of *T. daenensis* and *S. hortensis* EOs was investigated by analyzing the expression of genes involved in the QS system of *S.* Typhimurium. Specifically, expression levels of *luxS* and *pfs* genes in the treated bacteria were assessed using real-time RT-qPCR (quantitative reverse transcription polymerase chain reaction).

RNA extraction and cDNA synthesis: To evaluate the effects of *T. daenensis* and *S. hortensis* EOs on the expression of *luxS* and *pfs* during the biofilm phase, biofilms treated with MIC/2 values of the EOs were compared to control biofilms grown without EOs, utilizing real-time RT-qPCR. Bacteria were cultured on 6-well polystyrene tissue culture plates containing TSB-DMSO in the absence and presence of the EOs and incubated at 37 °C for 24 hours. Subsequently, non-adherent cells were removed by washing with deionized water, and adherent cells were mechanically scraped from the surface and rinsed with deionized water. Next, the collected cells were processed for RNA extraction using a commercial RNA isolation and purification kit (SinaClon; Iran). The quality and concentration of the extracted RNA were evaluated through agarose gel electrophoresis and absorbance measurements at 260/280 nm using a Nanodrop spectrophotometer (ND-1000; Thermo Fisher Scientific; USA), respectively. The isolated RNA was stored at -70 °C for subsequent analyses. Reverse transcription of the purified RNA into cDNA was performed using a commercial cDNA synthesis kit (Takara, Japan) following the manufacturer's protocol. The synthesized

Table 1) Oligonucleotides used for real-time RT-qPCR assay.

Genes	Sequence (5'-3')	Annealing Temperature	Reference
luxS	TCACGGAGTGGCCAAAATTT	53°C	[19]
	GACGCGCATTTGTTATCATCA		
pfs	GGAAGAAGAAGTTACGCTGC	53°C	
	GATTTCAGCAACGCCACTTC		
16S rRNA	CGGGGAGGAAGGTGTTGTG	53°C	
	GAGCCCGGGGATTTCACATC		

cDNA was then stored at -70 °C for subsequent use as a template in real-time RT-qPCR.

Real-time RT-qPCR: Real-time RT-qPCR assays were performed using a commercial SYBR Green master mix (Amplicon; Denmark) along with specific primer pairs detailed in Table 1 (BioNEER; Korea). Amplification was carried out on a Corbett Life Science Rotor-Gene 6000 Cycler (Qiagen; Germany). The expression levels of *luxS* and *pfs* genes were normalized against the ribosomal protein-encoding gene rrSD. PCR amplification conditions included an initial denaturation step at 95 °C for 10 min, followed by 40 cycles consisting of denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 30 s. A negative control was included for all responses. Melt curve analysis was conducted at the end of each run to verify the specificity of the amplified products. All samples were analyzed in triplicate, and relative gene expression was calculated using the 2-ΔΔCT method [19].

Statistical analysis: Statistical analyses were performed using GraphPad Prism software (version 6). All experiments were conducted in triplicate, and the results were presented as mean ± SD. Data normality was evaluated using the Shapiro–Wilk test, and homogeneity of variances was assessed using Levene's test. For comparisons between two groups, Student's *t*-test was applied. For multiple group comparisons, including different EO concentrations (e.g., MIC/2 and MIC/4), one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was performed. A *p*-value of less than 0.05 was considered statistically significant.

Findings

GC-MS analysis: Based on GC-MS analysis, *T. daenensis* EO primarily comprised carvacrol (40.69%), γ-terpinene (30.28%), and α-terpinene (5.52%). In contrast, *S. hortensis* EO was characterized by its thymol content (41.28%), along with significant amounts of γ-terpinene (37.63%), p-cymene (12.2%), and α-terpinene (3.52%).

MIC and MBC: The MIC values of the EOs extracted from *T. daenensis* and *S. hortensis* against *S.* Typhimurium were 6.25 and 12.5 µg/mL, respectively. The MBC value of both EOs was 25 µg/mL. These findings indicate the susceptibility of *S.* Typhimurium to the EOs extracted from *T. daenensis* and *S. hortensis*.

Anti-biofilm activity: The findings showed that *T. daenensis* and *S. hortensis* EOs at concentrations of MIC/2 and MIC/4 notably inhibited biofilm formation by *S.* Typhimurium. Conversely, both EOs at MIC/8 concentration exhibited no inhibitory effect on biofilm formation (Figure 1). Also, *T. daenensis* at MIC/2 and MIC/4 levels and *S. hortensis* at MIC/2 level had significant effects on the biofilm disruption of *S.* Typhimurium strain. This suggests that the potential of *T. daenensis* EO is greater than

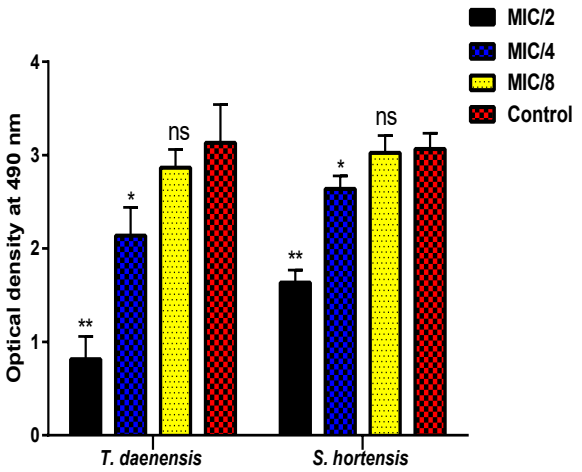

Figure 1) Biofilm inhibition properties of EOs at various concentrations against *S.* Typhimurium. Error bars demonstrate the SD of three replicates (ns: not significant; *: p<0.05; **: p<0.001).

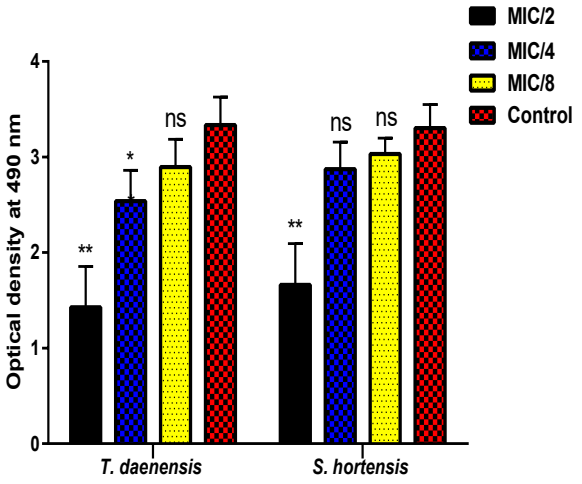

Figure 2) Effects of EOs (at various concentrations) from *T. daenensis* and *S. hortensis* on the disruption of preformed biofilms by *S.* Typhimurium. Error bars demonstrate the SD of three replicates (ns: not significant; *: p<0.05; **: p<0.001).

that of *S. hortensis* EO in disrupting the biofilm of *S.* Typhimurium strain (Figure 2).

Impact of EOs on swimming and swarming motility: The findings revealed that *T. daenensis* and *S. hortensis* EOs at a concentration of MIC/2 exhibited anti-motility properties against the bacteria tested (Figure 3).

Impact of EOs on QS-mediated genes: The results indicated that both *T. daenensis* and *S. hortensis* EOs at a concentration of MIC/2

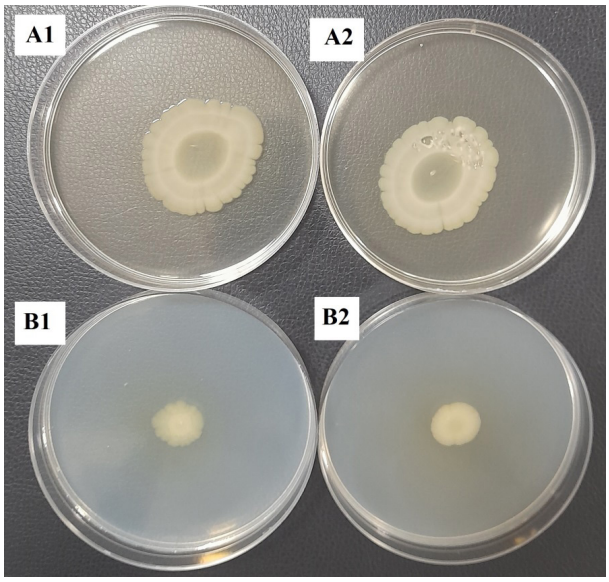

Figure 3) Effect of tested plant EOs at MIC/2 concentration on the bacterial motility (A1 and A2: controls, B1: treated with *T. daenensis EO, B2: treated with S. hortensis* EO).

significantly downregulated the expression levels of *lux* (-13.12 and -9.24-fold, respectively) and *pfs* (-2.9 and -8.33-fold, respectively) genes in the tested bacteria. This suggests that both EOs exhibit anti-quorum sensing activities against *S. Typhimurium* strain.

Discussion

EOs are intricate natural, aromatic compounds obtained from various plant parts through various methods. Phenolics, polyphenolics, flavonoids, and alkaloids represent the primary constituents of plant EOs, demonstrating significant antimicrobial, antioxidative, and cytotoxic activities [20]. The EO compositions of *T. daenensis* and *S. hortensis* were analyzed, and five primary constituents were identified. In *T. daenensis* EO, the main components were carvacrol, γ-terpinene, and α-terpinene. In contrast, *S. hortensis* EO comprised thymol, γ-terpinene, p-cymene, and α-terpinene. *T. daenensis* is recognized for its EOs, which prominently feature bioactive compounds like carvacrol, with certain sources reporting concentrations as high

as 51.89%. Compounds such as γ-terpinene and α-terpinene are also present, enhancing the oil's antimicrobial and antioxidant capabilities [21]. Consistent with this study findings, another study indicated that *S. hortensis*, or summer savory, comprised several significant components in its EO. Research has identified thymol, γ-terpinene, p-cymene, and α-terpinene as the primary constituents [22]. A previous study reported that *S. hortensis* essential oil contained significant amounts of thymol (37.2%), p-cymene (32.3%), and γ-terpinene (27.3%) [23]. Furthermore, thymol, p-cymene, and γ-terpinene were identified by Khajeh et al. (2004) as the primary constituents of *S. hortensis* EO [24]. In other studies, P-cymene has been identified as a prominent component of *S. hortensis* EO, while γ-terpinene has been noted as the second most prevalent constituent [23]. This study identified γ-terpinene as the second most prevalent compound in *T. daenensis* essential oil, corroborating the findings of Khajeh et al. (2004) [24].

S. enterica serovar Typhimurium is a notable enteric pathogen capable of infecting humans and animals, typically through the consumption of contaminated food or water. This bacterium is known to cause gastrointestinal diseases and may result in systemic infections, especially in rats, akin to human typhoid fever [25]. This research examined the antibacterial and anti-biofilm properties of *T. daenensis* and *S. hortensis* EOs against *S. enterica* serovar Typhimurium. The present investigation illustrated that treatment with *T. daenensis* and *S. hortensis* EOs led to a decrease in the proliferation of the standard strain of *S.* Typhimurium, with the MIC of *T. daenensis* EO (6.25 µg/mL) being twofold lower than that of *S. hortensis* EO (12.5 µg/mL). Elmi et al. (2020) reported that thyme EO inhibited the proliferation of *Salmonella* bacteria in stool culture colonies on the eighth day of the test [26]. The antibacterial properties of the EOs derived from *T. vulgaris* and *S. hortensis* were assessed against *S.* Typhimurium in a separate study, the results revealed that MIC values ranged from 0.05 to 5 µg/mL [27]. According to the research conducted by Seyedtaghiya and colleagues (2021) [28], the MIC values of *S. hortensis* against *Salmonella* varied between 0.31 and 0.62 µg/mL. The effectiveness of *T. daenensis* and *S. hortensis* EOs in the treatment of salmonellosis may be attributed to their potent phytochemicals, antioxidant properties, direct bactericidal activity against *Salmonella*, and their ability to enhance the immune system [29].

Biofilms are populations of bacteria that adhere to biological or non-biological surfaces and are encased in a matrix created by the bacteria themselves [30]. This structure enables bacteria to endure adverse circumstances, including ultraviolet (UV) radiation exposure, metal toxicity, acidic environments, dehydration, salt, phagocytosis, and many antibiotics and antimicrobial agents [31]. This study also assessed the efficacy of *T. daenensis* and *S. hortensis* EOs in disrupting biofilms, enhancing swimming motility, and modulating the expression of QS-mediated genes in *S.* Typhimurium. The observations indicated that MIC/4 and MIC/2 concentrations of both EOs may inhibit biofilm formation in certain bacteria; however, only MIC/2 doses exhibited anti-motility effects. The considerable downregulation of QS-related genes (*lux* and *pfs*) in *S.* Typhimurium bacteria after treatment with *T. daenensis* and *S. hortensis* EOs corroborated these results.

QS, a mechanism of intercellular communication in bacteria, utilizes signaling molecules known as autoinducers (AIs) that enable bacteria to react to their environment in

relation to their population density. Among several QS systems used by bacteria, the AI-2 system requires the *luxS* gene to produce its signaling molecules. Research has indicated that in *S.* Typhimurium, *luxS* regulates the *lsr* operon, facilitating AI-2 internalization. This method has been proposed as a way to regulate AI-2 concentration around a cell or inhibit AI-2 signaling from other bacterial species in its surroundings. Recent reports indicate that in *S.* Typhimurium, *luxS* is implicated in the production of virulence genes and polarization of flagellar phase variation [32]. Additionally, Pfs is an essential protein for AI-2 production, catalyzing two distinct processes in bacteria [33]: 1) the conversion of S-adenosylhomocysteine (SAH) to S-ribosylhomocysteine (SRH), releasing adenine, and 2) the transformation of 5′-methylthioadenosine (MTA) into 5′-methylthioribose (MTR), likewise releasing adenine [34]. Studies have indicated that in *S.* Typhimurium, the transcription of the *pfs* gene is closely linked to AI-2 synthesis, while *luxS* expression is constitutive [35]. The EOs used in this research effectively reduced biofilm development and disrupted existing biofilms by influencing the expression of QS-associated genes (*lux* and *pfs*). A potential method by which EOs affect biofilms is their capacity to permeate the extracellular polymeric substance (EPS) matrix, facilitating interaction with bacterial membrane proteins and reducing the adhesion of planktonic cells to surfaces. A further documented mechanism is a decrease in motility and disruption of the synthesis of adhesins or structures such as curli proteins and flagella [36].

Consistent with this study results, Galgano et al. (2023) demonstrated that Thymus EO effectively inhibited the growth of *S.* Typhimurium at low dilutions, the MIC and MBC ranges for suppressing biofilm development were 0.039 and 0.156%, respectively [37]. Furthermore, it has been shown that some EOs with elevated levels of carvacrol and thymol exhibit the greatest percentage of biofilm inhibition (>65%) against *S.* Typhimurium by downregulating QS-related genes (*uxR*, *luxS*, *qseB*, and *sdiA*) [38]. Further investigation has demonstrated that *S. hortensis* essential oil at MIC/2 and MIC/4 levels significantly reduces biofilm development in *Salmonella* species [28]. The antimicrobial and anti-biofilm properties of *S. hortensis* EO against *S.* Typhimurium isolated from chicken were established by Mohammadi et al. (2021). In their study, a notable downregulation of *luxS*, *pfs*, and *hld* genes was observed following exposure to the extracted *A. dracunculus* essential oil at a MIC/2 concentration [39]. Our observations, in comparison with previous literature, indicate that both *T. daenensis* and *S. hortensis* EOs exhibit antibacterial, anti-biofilm, and anti-QS action against *S.* Typhimurium.

Despite these promising findings, several limitations should be acknowledged. The study was performed under *in vitro* conditions, which could not fully reproduce the complexity of food systems or host environments. Only a single *S.* Typhimurium reference strain was examined, limiting conclusions regarding strain variability and resistance diversity. Furthermore, essential oil compositions are influenced by environmental factors such as geographical origin, cultivation conditions, harvesting stage, and extraction methods, which may affect reproducibility and antimicrobial performance.

From an applied perspective, the demonstrated antibacterial and anti-QS activities of these EOs suggest their potential use as natural antimicrobial agents in food preservation or infection control strategies. Nevertheless, practical challenges remain, including stability during food processing, interactions with food matrices, sensory impacts, optimal dosage determination, and

safety evaluation. Standardization of EO formulations and regulatory considerations will be essential before industrial or clinical implementation.

It is recommended that future research focus on evaluating these EOs against multiple clinical and foodborne *Salmonella* isolates, validating efficacy in food model systems and animal studies, and investigating synergistic combinations with conventional antimicrobials. Advanced delivery approaches such as nanoencapsulation may enhance stability and bioavailability. In addition, comprehensive molecular studies, including transcriptomic and proteomic analyses, are needed to further elucidate the mechanisms underlying quorum sensing inhibition and biofilm disruption.

Despite the promising findings, this study was limited to *in vitro* conditions and a single strain of *S.* Typhimurium and did not account for strain variability or *in vivo* relevance. Future studies should evaluate multiple clinical and food-derived isolates, confirm efficacy in vivo, and explore formulation strategies to improve stability and applicability. Variability in essential oil composition also highlights the need for standardization. Overall, the present study expands current knowledge regarding plant-derived EOs as potential alternatives to conventional antimicrobials and provides a basis for future translational research aimed at controlling *S.* Typhimurium.

Conclusion

T. daenensis EO with higher carvacrol concentration, compared to *S. hortensis* EO, showed better antibacterial, anti-biofilm, and anti-QS activity against the standard strain of *S.* Typhimurium. Variations in antimicrobial activity could be ascribed to alterations in the expression of genes associated with biofilm formation, indicating that the EOs utilized in this study not only impede QS signaling, impacting biofilm production, but also change the mRNA expression of genes related to bacterial adhesion and motility. Therefore, these EOs may serve as a potential antibacterial adjunct to inhibit and manage biofilm development by *S.* Typhimurium isolates. Nevertheless, more in vivo research is necessary to ascertain the therapeutic applicability of these EOs.

Acknowledgments

None declared.

Ethical approval: This study was performed under *in vitro* conditions using a standard bacterial strain (*Salmonella* Typhimurium). No human participants, animal subjects, or clinical samples were involved; therefore, ethical approval was not required.

Conflicts of interests: The authors declare no conflict of interest.

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