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SHORT COMMUNICATION

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# Anti-quorum sensing and antibacterial activities of *Satureja sahendica* hydroalcoholic extract against avian isolate of *Salmonella* Typhimurium

Razieh Sharchi, Jalal Shayegh, Somayyeh Hosseinzadeh

Department of Veterinary Medicine, Shabestar branch, Islamic Azad University, Shabestar, Iran

## ABSTRACT

Quorum sensing (QS) is a cell density dependent mechanism used by many pathogenic bacteria for regulating virulence gene expression. Inhibition or interruption of QS by herbal remedies has been suggested as a new strategy for fighting against antibiotic resistant bacteria. The aim of this study was to evaluate the antibacterial activity of *Satureja sahendica* hydroalcoholic extract (SSHE) against *Salmonella* Typhimurium (*S.* Typhimurium) isolates, and to assess the impact of sub-inhibitory concentrations of this extract on the QS-associated gene (*sdiA*) expression. Using the Soxhlet method, hydroalcoholic extract of *S. sahendica* leaves was prepared and antimicrobial effects of the SSHE were determined by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) method*S.* The reverse transcription quantitative PCR (RT-qPCR) assay was used to analyze the expression of *sdiA* in 20 *S.* Typhimurium isolates from poultry flocks in response to the treatment of sub-inhibitory concentrations of SSHE at 60-min time point. The MIC values of SSHE against *S.* Typhimurium isolates were ranged from 0.29-4.68 $mg/ml^{-1}$ and MBC values were ranging from 75-150 $mg/ml^{-1}$. The results also indicated that the expression of *sdiA* gene was reduced in *S.* Typhimurium isolates triggered by the treatment of SSHE comparatively with those from the control culture*S.* Findings suggest that SSHE possess the antibacterial and anti-QS activity and can be used to control the expression of virulence genes in pathogenic bacteria, such as *S.* Typhimurium.

**Keywords**

*Satureja sahendica, Hydroalcoholic extract, sdiA gene, gene expression, Salmonella* Typhimurium

**Abbreviations**

QS: Quorum sensing
SSHE: *Satureja sahendica* hydroalcoholic extract
*S.* Typhimurium: *Salmonella* Typhimurium
MIC: Minimum inhibitory concentration
MBC: Minimum bactericidal concentration
RT-qPCR: Reverse trasncription quantitative PCR



*Corresponding author:*
*Jalal Shayegh*
*Tell/Fax: +98 4133341260*
*Email: Jalalshayegh@iaushab.ac.ir*

*Salmonella enterica,* subspecies *enterica* serotype Typhimurium (*S.* Typhimurium) is one of the most important causes of bacterial food-borne outbreaks and systemic diseases in humans and animals (1). In *S.* Typhimurium, various secreted proteins and regulatory cascades of virulence genes are located within the *Salmonella* pathogenicity islands (SPIs) and the *Salmonella* plasmid virulence (spv) that are regulated by a microbial cell to cell communication process called quorum sensing (QS) (2, 3). It is widely accepted that QS is a form of bacterial communication that involves the production, secretion, detection, and response to extracellular signaling molecules called autoinducers (AIs) (4). There are three different QS autoinducer systems in *Salmonella* that include acyl-homoserine lactone (AHL), autoinducer-2 (AI-2), and autoinducer-3 (AI-3) signals (5). *S.* Typhimurium encodes a LuxR homolog, named SdiA (suppressor of cell division inhibition) that responds to AHLs which is produced by other bacterial species (6). Existence and expression of the *sdiA* and *sdiA*-regulated genes would be beneficial to *Salmonella* within the gastrointestinal tract, and the observed upregulation of this gene in the population brings the relevance of the differentiated state closer to the milieu of the host environment (7). Also, the potential roles of *sdiA* may be construed from the recognized functions of genes known to be regulated by SdiA. SdiA regulates seven genes located in virulence plasmid and chromosomes of *Salmonella* which are involved in *Salmonella*'s colonization of the intestine. These genes include *pefI*/*srgC* operon, *srgE* (SdiA-regulated gene E), and *sirA* (*Salmonella* invasion regulator) (7).

One of the common infection management strategies to treat, prevent, and control common salmonellosis in human and animals include the use of antibiotic therapy (8). However, increased occurrence of antimicrobial-resistant *S.* Typhimurium strains have been reported from different food animals around the world (9-11). For example, emergence of multidrug-resistant (MDR) *S.* Typhimurium definitive phage type 104 (DT104), is a particular concern for animal husbandry, and in human medicine (12).

In recent years, strategies have been taken to combat bacterial resistance by new antimicrobial agents from natural sources (13-15). Among the new therapeutic strategies, anti-virulence strategies have emerged as promising alternatives, since instead of killing the pathogens, these strategies try to deprive the bacteria from their virulence factor*S.* One of the novel therapeutics that are currently being developed is focused on quench pathogen QS systems, because QS is a key regulatory system in the pathogenesis of various bacterial infections (16).

It has been demonstrated that plant-derived natural products contain abundant sources of anti-bacterial or anti-QS compounds (17, 18). The genus *Satureja* is known as "Marze" in Persian, belongs to the *Lamiaceae* family. This genus consists of 16 species in Iran, 10 species are endemic (19). *Satureja sahendica* (*S. sahendica*), one of these species, is a perennial and bushy aromatic herb with small white-viola colored flower*S.* It is a late flowering species, grows in the rock walls and mountains of northwestern and western Iran. The aerial parts of some *Satureja* species have been widely used as a flavoring compound in food and as a traditional plant for the treatment of gastrointestinal disorders (20).

Although, many studies reported that the essential oils and extracts of *Satureja* species have potential antimicrobial activities against different pathogenic bacteria (20-22), the antimicrobial and anti-QS effects of *S. sahendica* hydroalcoholic extracts (SSHE) against the *S.* Typhimurium are still poorly understood. Therefore, the objectives of this study were to observe the antimicrobial and anti-QS effects of SSHE on *S.* Typhimurium isolated from poultry flocks.

### *The Antimicrobial susceptibility testing*

In the assessment of the antimicrobial activities of SSHE, the MIC and MBC values against 20 *S.* Typhimurium isolates were ranged from 0.29-4.68 mg/ml$^{-1}$ and from 75-150 mg/ml$^{-1}$, respectively.

### *Confirmation of presence of 16S rRNA and sdiA genes by PCR*

Among 20 different *S.* Typhimurium isolates, all stains showed the presence of *16S rRNA* and *sdiA* genes (100%). PCR results confirming the presence of these genes are shown in Figure1.

### *Growth of S.* Typhimurium *in the presence of sub-inhibitory concentrations of SSHE*

In this study, the MIC value of *S.* Typhimurium RITCC1730 versus SSHE was 2.34 mg/ml$^{-1.}$ The growth curve of *S.* Typhimurium RITCC1730 is shown in Figure 2. The test revealed that after 30 min of SSHE treatment, there was no obvious difference in the OD600 value among all culture*S.* A steady increase in the optical density with 0.58, 1.17 mg/ml$^{-1}$ of SSHE treatment and control occurred after 45 min. The optical density increased at a slower rate than the lower concentration*S.* After 300 min, the OD value of the *S.* Typhimurium treated with 0.58, 1.17, 2.34, 4.68, and 9.37 mg/ml$^{-1}$ of SSHE was approximately 95.03%,

89.17%, 66.62%, 41.52% and 33.63% of the control cultures, respectively.

These results show that SSHE concentration of 1MIC, 2 MIC and 4 MIC strongly inhibited the growth of *S.* Typhimurium RITCC1730. To study the effects of a low SSHE concentration on the transcription of the *S.* Typhimurium, we reduced the inhibitory SSHE concentration to 1/2 MIC according to the growth curve. It was claimed that compound concentrations should be at concentrations that are just low enough not to affect the growth of the organism (23). We chose 60 min time point for SSHE treatment in the experiment based on the growth curve mentioned above and our preliminary experiment*S.* In the preliminary experiments, cultures were harvested for RNA preparation following 45, 60, and 120 min of SSHE treatment. Our results demonstrated that time point 45 min delivered the most meaningful results (data not shown).

### *Influence of subinhibitory concentrations of SSHE*

Expression level of *sdiA* gene in 20 treated *S.* Typhimurium strains with SSHE were examined using RT-qPCR and compared to that in non-treated ones of the same strain. The results indicated that *sdiA* gene expression in the SSHE-treated strains was significantly down regulated in comparison to that in the non-treated isolates ($p$ =0.0246) (Figure 3).

The widespread emergence of resistance to a large number of antimicrobial agents in pathogenic bacteria has become a significant global public health threat (24). Most plant extracts studied have various antimicrobial activities and have been used to cure many infections (25). The findings of our study show that SSHE has a good inhibitory effect on *S.* Typhimurium with a minimum inhibitory concentration in the range of 0.29-4.68 mg/ml$^{-1}$ and minimum bactericidal concentration of 75-150 mg/ml$^{-1}$. These results clearly indicated that SSHE has a strong antimicrobial activity against *S.* Typhimurium isolate*S.* To our knowledge this is the first report on the antimicrobial activity of the SSHE. Previous studies reported that the essential oils of the *S. sahendica* are known to possess antibacterial activity against both Gram positive and Gram negative bacteria (20-22). In a report of Yousefzadeh et al. the minimum inhibition concentration of *S. sa-*

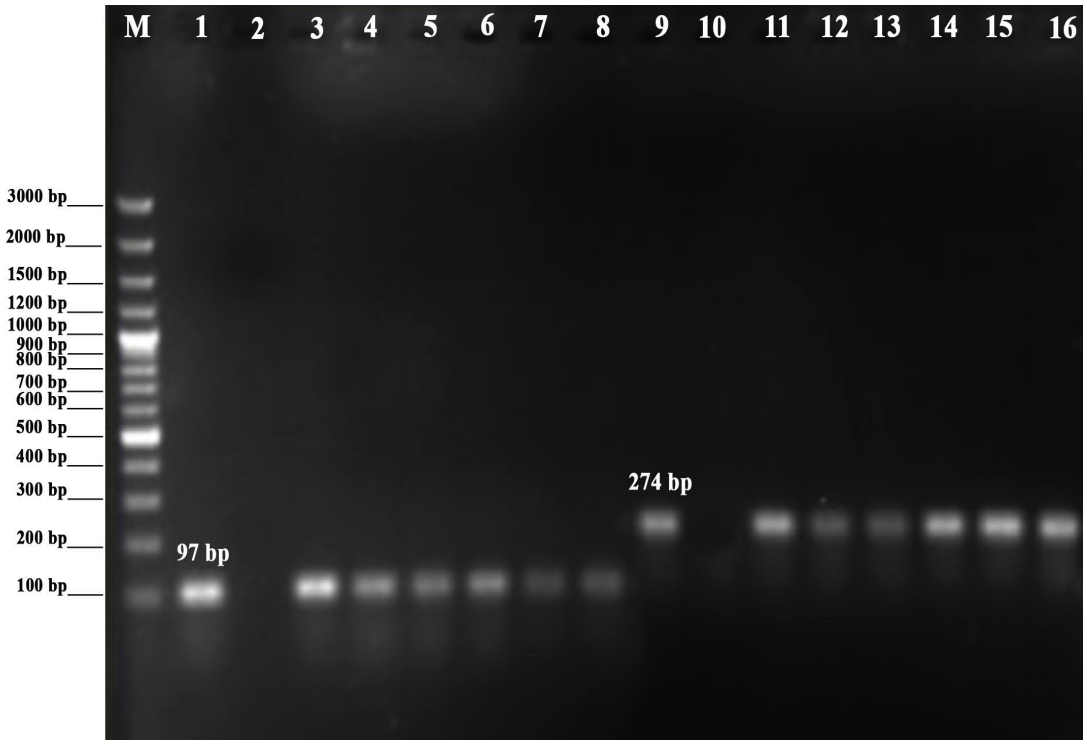


**Figure 1.**
Agarose gel electrophoresis of the PCR product of *16S rRNA* and *sdiA* genes for DNA extracted from analyzed *S.* Typhimurium isolate*S.* Lane M: GeneRuler™ 100 bp plus DNA ladder; lane 1 and 9: positive control for *16S rRNA* and *sdiA* genes (*S.* Typhimurium RITCC1730), respectively; lane 2 and 10: negative control for *16S rRNA* and *sdiA* genes, respectively; lanes 3-8 and 11-16: amplified fragments of *16S rRNA* and *sdiA* genes in the isolates.

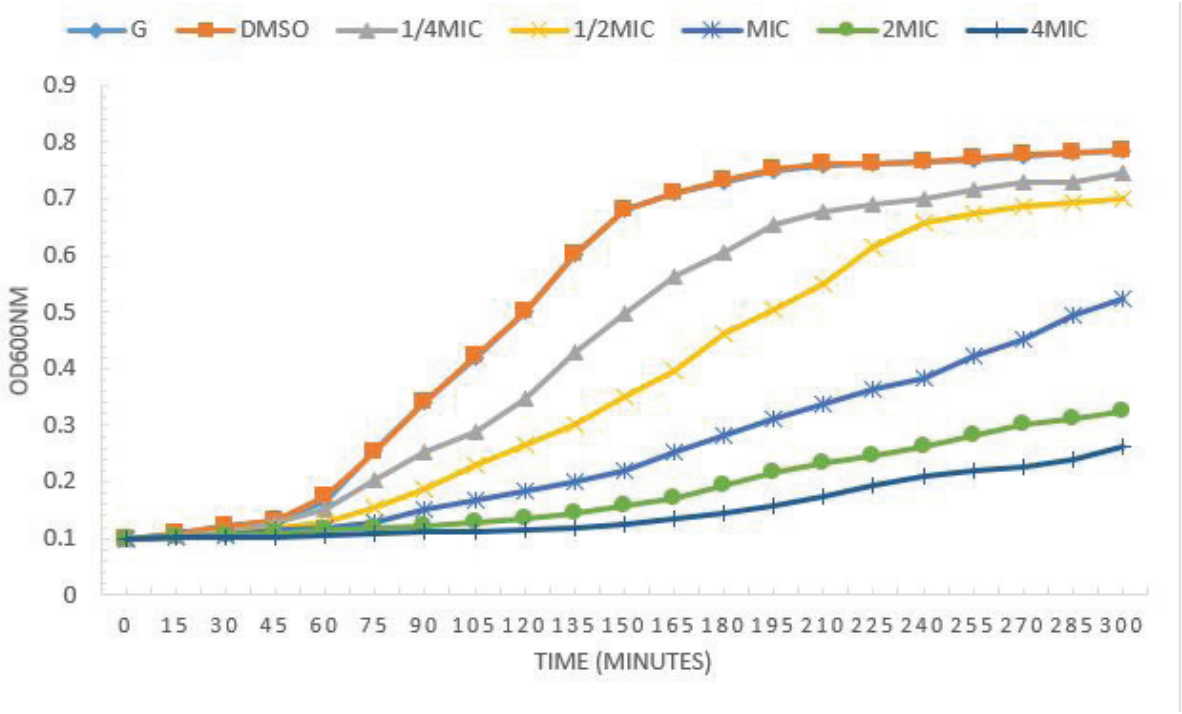


**Figure 2.**
Growth curve for *S.* Typhimurium RICC1730 in the presence or absence of SSHE. a) untreated *S.* Typhimurium; b) *S.* Typhimurium plus 10% DMSO; c) *S.* Typhimurium plus 0.58 mg $ml^{-1}$ SSHE; d) *S.* Typhimurium plus 1.17 mg $ml^{-1}$ SSHE; e) *S.* Typhimurium plus 2.34 mg $ml^{-1}$ SSHE; and f) *S.* Typhimurium plus 4.68 mg $ml^{-1}$ SSHE; g) *S.* Typhimurium plus 9.37 mg $ml^{-1}$ SSHE.

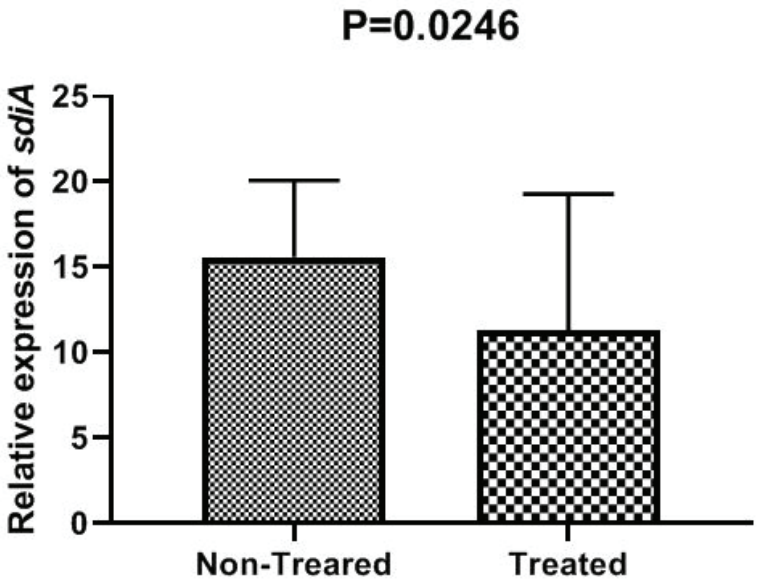


**Figure 3.**
Relative expression of *sdiA* in response to SSHE. Relative expression of *sdiA* mRNA was detected in 20 SSHE -treated and -nontreated *S.* Typhimurium using RT-qPCR, and it's expression in each sample was normalized to the level of *16S rRNA* mRNA expression in each sample.

*hendica* essential oil in Gram negative bacteria was in the range of 3.5-15 mg/ml$^{-1}$ (21). Serrano et al. showed that the essential oil and ethanol extracts of *Satureja* contain several compounds with antimicrobial properties (26). The composition of the essential oil isolated from aerial parts of *S. sahendica* has previously been reported. Thirty-three component were identified in *S. sahendica* essential oil. In fact, the major components in *S. sahendica* essential oil were thymol, P-cymene, γ-terpinene and α-terpinene, respectively (27). The high antimicrobial activity of *S. sahendica* essential oil could be explained by the higher percentage of thymol that is well known to has antibacterial activity (28). Most studies on the mechanisms of thymol indicate that it disturbs the structure of cellular membranes, altering their function, and reacts with the active sites of enzymes in Gram positive and Gram negative bacteria (29, 30). The ethanol and methanol extracts of other *Satureja* species were assessed against some Gram-positive and Gram-negative bacteria. The MIC values of *S. bachtiarica* ethanol extract against Gram negative bacteria have been reported in the range of 100-200 mg/ml$^{-1}$ (31) and the corresponding value of methanol extract of *S. khuzestanica* were in the range 4-10 mg/ml$^{-1}$ (32). In the another study, the MIC values of methanol extract from the aerial parts of *S.* hortensis plants against Gram negative bacteria were ranging from 0.03 to 0.25 mg/ml$^{-1}$ (33). Although it has been reported that essential oils of plants contain more antimicrobial substances than their extracts including methanol, ethanol and water extracts, but the results of our study indicate that hydroalcoholic extract of *S. sahendica* can be stronger than essential oil and other type of extract*S.* These differences can be due to ecological factors or species variation*S.* In total, these data indicate the possibility that SSHE may find an application as an antibacterial agent against salmonellosi*S.* However, conducting further studies with more focus on antimicrobial mechanisms of this extract is suggested. For this reason, in this study the attention has been given to it's QS inhibition activity.

A number of studies have shown that extracts from traditional medicinal plants could interfere with bacterial cell-cell communication and can be used for the development of novel anti-infective measures (34-36). Overall, plant extracts or compounds usually target the bacterial QS system via three different ways: stopping the signaling molecules from being synthesized by the luxI encoded AHL synthase, degrading or modifying the signaling molecules, and/or targeting the luxR signal receptor (17, 37). In this study, we have quantitatively assessed the expression level of *sdiA* gene (one of LuxR family member) in SSHE -treated, and non SSHE treated *S.* Typhimurium isolate*S.* According to the results, *sdiA* gene was found to be significantly down-regulated in SSHE treated isolates as compared to the matched non-treated one*S.*

Many Gram-negative bacteria, including *Salmonella*, employ the QS system to control the expression of several virulence genes (38). One of the most significant regulators of *S.* Typhimurium QS system was SdiA which responds to AHLs produced by other bacterial species (39). Thus, downregulation in the expression of this gene was associated with a decrease in expression of the genes involved in QS system. Genes that are controlled by SdiA include: the *pefI*/*srgC* operon, the *srgE* gene, and the *sirA* gene (7). Hence, reducing the expression of the *sdiA* gene can affect the expression of these genes, and subsequently on their virulence factors, including flagella formation (motility), fimbria formation, bacterial invasion, biofilm production, type 3 secretion system, and the phenotypes derived from genes located on the pathogenic islands 1 and 4 (40-43).

Overall, the results of this study showed that SSHE has antimicrobial and anti-QS effects by decreasing the expression of *sdiA* gene. Although there is no documented scientific report on the Quorum Quenching (QQ) potential of SSHE, we found that SSHE also possessed significant anti-QS activity against *sdiA* gene. These results can be in agreement with the results of other studie*S.* The results of Sharifi et al., 2018 revealed that *S. hortensis* essential oil can act as an anti-QS agent against *Staphylococcus aureus* (44). Likewise, in the study of Bacha et al. two of the eighteen plant extracts were found interfering with bacterial QS (18). In another study, Adonizio et al. reported that medicinal plants and their extracts exhibited anti QS activities (45).

To our knowledge, this is the first report which introduces SSHE as an effective antibacterial medicinal plant agent against *S.* Typhimurium isolates and shows noticeable anti-QS effect. Nevertheless, further studies about the safety and toxicity of this extract are needed to evaluate possible clinical applications in the therapy of infectious diseases.

In conclusion, the results of this study revealed that MICs of SSHE can be used to control the expression of virulence genes involved in QS system of *S.* Typhimurium. Also, the present study introduced SSHE as an anti-QS agent with natural origin against *S.* Typhimurium. Nevertheless, more efforts are required to conduct clinical trials of these compounds in the future.

### *Plant material and extraction procedure*

*S. sahendica* plants were collected from the wild growing plants at the full flowering stage, from East Azerbaijan province of

Iran. The plant was authenticated by Herbal Museum of the Faculty of Pharmacy, Tabriz University of Medical Science, Tabriz, Iran. A voucher specimen (No. 4035) has been deposited at the Herbarium of Medicinal Plants, Faculty of Pharmacy, Tabriz University of Medical Science, Tabriz, Iran.

The collected plant materials were air-dried under shade and the plant leaves were separated from the stem. Then dried leaves were powdered and stored in an airtight container.

## Preparation of the ethanol extract

SSHE were prepared with aqueous ethanol (70%; v/v) by using Soxhlet extractor for 72 h at a temperature not exceeding the boiling point of the solvent. The ethanol extracts were filtered through Whatman filter paper (No.1) and then concentrated vacuo at 40 °C by means of rotary evaporator. The residues obtained were stored in a freezer at -80 °C until future test*S.*

## Bacterial strains

*S.* Typhimurium RITCC1730 was obtained from Razi Institute Culture Collection Center. A total of 20 local clinical isolates of *S.* Typhimurium, which were isolated from poultry flocks, were provided from Faculty of Veterinary Medicine, Tehran, Iran. These isolates had previously been identified by biochemical and molecular tests to be representative of *S.* Typhimurium (46).

## Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of SSHE against the *S.* Typhimurium isolates described above were determined according to the CLSI (Clinical and Laboratory Standards Institute) procedures (47). The stock solution extract of *S. sahendica* (300 mg/ml) was dissolved in 10% dimethyl sulfoxide (DMSO; Sigma-Aldrich). The MIC was defined as the lowest concentration of extract that prevented resazurin colour change from blue to pink. The minimum bactericidal concentration (MBC) values were determined by removing 100 µL of bacterial suspension from subculture, demonstrating blue color in wells and inoculating on Muller Hinton agar (MHA) plate*S.* MBC was recorded as the lowest concentration of SSHE, which killed 99.9% of bacterial inoculate after a 24-hour incubation at 37 °C (19). The assays were repeated in triplicate.

## Confirmation of presence of 16S rRNA and *sdiA* genes by PCR

PCR amplification was carried out in a 25 µL reaction mixture containing 2 µL of the DNA as the template, 12.5 µL of 2X PCR master mix (3 mM MgCl2, 0.04U/ µL Taq polymerase, reaction buffer, 0.4 mM of each dNTPs), 1 µL (0.4 Mm) of the forward and reverse primers (Sinaclon, Iran) (Table 1). PCR amplification was conducted in a Touchgene Gradient (Model FTGRAD2D, UK). The cycling program consisted of: denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 1 min. A final extension was performed at 72 °C for 10 min. Both positive and negative control reactions were included in each PCR amplification experiment. For negative controls template DNA was replaced with sterile water. *S.* Typhimurium RITCC1730 was used as positive control. PCR products were resolved by electrophoresis in 2% (w/v) agarose gel stained with SYBR Safe DNA gel stain. Visualizations were made using a UV transilluminator (BTS-20, Japan), and the 100 bp plus DNA ladders were used as molecular size markers.

## Growth curves

*S.* Typhimurium RITCC1730 was grown to an optical density of 0.1 at 600 nm in Muller Hinton Broth (MHB), and was distributed as 100ml volumes into six 500ml Erlenmeyer flask*S.* SSHE (dissolved in 10% DMSO) was added to six of the cultures to obtain final concentrations of 0.25 MIC, 0.5 MIC, 1 MIC, 2 MIC, and 4 MIC respectively. The control cultures included the addition of 10% DMSO alone. The cultures were incubated further, and cell growth was monitored spectrophotometrically in the optical density at 600 nm. Three ml samples of each culture were collected immediately at 15 min intervals after the addition of SSHE. In addition, the total number of viable bacteria was estimated by plating dilutions of the culture on MHA without antibiotic and counting the numbers of CFU after 24 h at 37 °C.

## Treatment with SSHE

To obtain RNA for investigating the effects of SSHE on *sdiA* gene expression, *S.* Typhimurium RITCC1730 and each of the clinical isolates of *S.* Typhimurium were grown overnight at 37 °C in 10 ml of MHB. Two 250 ml Erlenmeyer flasks, each of which contained 100 ml of MHB, were inoculated with an overnight culture to an initial OD600 of 0.1. Subsequently, the tock solution (SSHE), prepared in 10% DMSO was added to one of the cultures (experimental culture), giving a final concentration of 0.5 MIC. The other culture containing 10% (v/v) DMSO lacking SSHE was used as the control. All bacterial suspensions (both experimental and control suspensions) were further incubated for 60 min at 37 °C. RNA isolation was then performed at this time. Three independent bacterial cultures for SSHE treatment or control condition were prepared as biological replicates for RNA isolation on different days.

## RNA Extraction and cDNA Synthesis

Four hundred µL of the bacterial suspension were removed and combined with 800 µL of RNA protect Bacteria Reagent (Qiagen, Valencia, Calif., U.S.A.) to minimize RNA degradation immediately before harvesting for RNA isolation, then cells were collected by centrifugation and kept at -80 °C.

Extraction of the total RNA was carried out from both treated and non-treated bacteria using the Qiagen RNeasy mini kit (Qiagen) according to the manufacturer's instructions, and then each RNA sample was treated with RNase-free DNase I (TaKaRa Bio Inc., Shiga, Japan) to remove contaminating DNA. The RNA quality and quantity was monitored by agarose gel electrophoresis and by measuring the absorbance at 260 and 280 nm using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

Extracted RNAs were stored at -70°C until required for the experiment*S.* The cDNA synthesis of each sample was measured using a cDNA synthesis kit (Yekta Tajhiz Azma, Iran), following the manufacturer's instruction*S.* All the samples were stored at -20 °C until used in the analysis.

## Relative Quantitative Real-Time PCR

YTA qPCR Probe Master Mix (Yekta Tajhiz Azma, Iran) was used for amplification and the real-time PCR analysis, according to the manufacturer's instruction*S.* The primer pairs listed in table 1.

The amplification was done with the Applied Biosystems Step One Plus (Thermo Fisher Scientific, ABI, U.S.). Each reaction mixture (20 µL) was prepared as follows: 10 µL of 2X One-Step SYBR RT-PCR Buffer III (Takara), 0.4 µL of TaKaRa Ex Taq HS (5 U

**Table 1.**
Primers used in the PCR reactions

| Primer | Sequence | Size | References |
|---|---|---|---|
| *16s rRNA* for | AGGCCTTCGGGTTGTAAAGT | 97 bp | (Lee et al., 2009) |
| *16s rRNA* rev | GTTAGCCGGTGCTTCTTCTG | | |
| *sdiA* for | AATATCGCTTCGTACCAC | 274 bp | (Halatsi et al., 2006) |
| *sdiA* rev | GTAGGTAAACGAGGAGCAG | | |

µL-1), 0.4 µL of PrimeScript RT enzyme Mix II, 0.4 µL of each primer (4 mM), 0.4 µL of ROX reference dye I, 2 µL template RNA and 6 µL of RNase-free dH2O. Amplification and detection were performed with StepOne Real-Time PCR System (Applied Biosystems, Waltham, USA). Cycling condition included 42˚C for 5 min, 95˚C for 10 sec and then 40 cycles of 95˚C for 5 sec and 60˚C for 34 sec. To verify the identity of the amplified product, post-amplification melting curve analysis was conducted as follows: 95˚C for 15 sec followed by stepwise elevation of the temperature from 60˚C to 95˚C at a rate of 0.3˚C per sec with continuous fluorescence collection.

All the samples were analyzed in triplicate and in order to confirm that there was no background contamination, a cDNA template-free negative control was included in each run, and the *16S rRNA* gene was used as internal control. To minimize data variation in separate runs, paired treated and non-treated samples from the same isolate were examined on the same run*S*. The expression of the target gene *sdiA* was determined as relative to the expression of the endogenous control gene *16S rRNA* by using the comparative CT (ΔΔCT) method of RT-qPCR.

### *Statistical analysis*

All the experiments were performed in triplicate and repeated for three times and the data are expressed as the mean ± SD. The statistical calculations were performed using GraphPad Prism software (version 8). A paired *t*-test was used to analyze the data. A *p*-value of $< 0.05$ was considered to be statistically significant.

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## Author Contributions

Conceived and designed the experiments: SH, JSH. Performed the experiments: SH, RSH. Analyzed the data: SH. Research space and equipment: SH, JSH, RSH. Contributed reagents/materials/analysis tools: SH, JSH, RSH. Wrote the paper: SH, JSH.

## Conflict of Interest

The authors declare that there is no conflict of interest in relation to the publication of this paper.

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Abstracts (in Persian)

# اثر ضد کوئوروم سنسنیگی و ضد باکتریایی عصاره هیدروالکلی مرزه سهندیکا بر روی جدایه های طیوری سالمونلا تایفی موریوم

**راضیه شارچی، جلال شایق*، سمیه حسین زاده**

۱گروه دامپزشکی، واحد شبستر، دانشگاه آزاد اسلامی، شبستر، ایران.
* نویسنده مسئول

## چکیده

کوئوروم سنسینگ مکانیسم وابسته به تراکم سلولی است که توسط بسیاری از باکتریهای بیماری زا جهت تنظیم بیان ژن های حدت، مورد استفاده قرار می گیرد. مهار یا متوقف کردن این سیستم توسط داروهای گیاهی به عنوان یک استراتژی جدید برای مبارزه با باکتری-های مقاوم به آنتیبیوتیک محسوب می گردد. هدف از این مطالعه، بررسی فعالیت ضد باکتریایی عصاره هیدروالکلی مرزه سهندیکا بر روی نمونه های سالمونلا تایفی موریوم جدا شده از طیور و همچنین ارزیابی اثر این عصاره بر روی بیان ژن مرتبط با کوئوروم سنسینگ (sdiA) در این جدایه ها بود. عصاره هیدروالکلی مرزه سهندیکا با استفاده از روش سوکسله تهیه شد و حداقل غلظت مهارکنندگی (MIC) و حداقل غلظت کشندگی (MBC) آن با استفاده از روش ریز رقیق سازی در محیط مایع مشخص گردید. برای آنالیز بیان ژن sdiA در جدایه های سالمونلا تایفی موریوم تیمار شده با غلظت تحت مهاری عصاره هیدروالکلی مرزه سهندیکا در مدت زمان ۶۰ دقیقه، از روش real-time PCR کمی استفاده شد. مقدار MIC عصاره هیدروالکلی مرزه سهندیکا در برابر جدایه ها در محدوده ۶۸/۴ ۰-۲۹/۰ میلی گرم بر میلی لیتر و میزان MBC در برابر جدایه ها ۷۵ تا ۱۵۰ میلی گرم بر میلی لیتر بود. همچنین نتایج نشان داد که بیان ژن sdiA در جدایه های سالمونلا تایفی موریوم تیمار شده با عصاره هیدروالکلی مرزه سهندیکا نسبت به جدایه های تیمار نشده به طور معنی داری کاهش یافته است. بطور کلی نتایج نشان داد که عصاره هیدروالکلی مرزه سهندیکا دارای فعالیت ضدباکتریایی و ضدکوئوروم سنسینگی است و میتواند برای کنترل بیان ژن های حدت در باکتری های بیماری زا از جمله سالمونلا تایفی موریوم بکار رود.

## واژگان کلیدی

مرزه سهندیکا، عصاره هیدروالکلی، ژن sdiA، بیان ژن، سالمونلا تایفی موریوم