Oral health and biochemical composition of saliva play important parts in pathogenesis of several diseases related either to oral cavity or internal body environment as a whole. Periodontitis is a highly prevalent adult gingival disease that leads to bone destruction and connective tissue attachment loss. The disease is not caused by a single bacterium, but by a group of bacteria. Quorum sensing is a phenomenon used by certain bacteria to coordinate virulence determinant production. In this study, we investigated the anti-quorum sensing ability of \textit{Salvadora persica} extracts on \textit{Enterococcus faecalis} from saliva samples of patients with different periodontitis problems. \textit{E. faecalis} is a potential pathogen, resistant to most of the antibiotics and one of many oral indigenous microbiota. In the practical section, anti-quorum sensing effect of the ethanolic extracts from fibrous branches of the plant was tested against \textit{Chromobacterium violaceum}. The growth of \textit{E. faecalis} extracted from saliva samples was detected using disk diffusion assays. The results showed considerable ability of extracts as quorum sensing quencher. Based on the findings of the research, it is suggested that the anti-quorum sensing ability of \textit{S. persica} extract on \textit{E. faecalis} makes it a suitable candidate for oral infections caused by \textit{E. faecalis}.

**Keywords:** Quorum quenching, \textit{Salvadora persica}, misvak, \textit{Enterococcus faecalis}, disk diffusion.

**INTRODUCTION**

The use of natural plant extracts as mouth wash and for oral hygiene as well as for their pharmaceutical benefits has been attracting increasing attention during the past two decades. \textit{Salvadora persica}, commonly referred to as Miswak, Arak, Meswak or toothbrush tree, is a popular chewing stick throughout the Muslim world. It is believed that chewing \textit{S. persica} was recommended by the Islamic prophet Muhammad. The use of fibrous branches of \textit{S. persica} was approved by the World Health Organization for oral hygiene due to their protective effect on
some oral pathogens. Despite the traditional use of *S. persica*, up to the present time few to no reports have been found about the antibacterial effects of the various parts of the plant.

Periodontitis is a highly prevalent adult gingival disease that leads to bone destruction and connective tissue attachment loss. Its severity ranges from gingivitis to various classes of periodontal disease. Periodontitis differs from many other types of infections since it is not caused by a single bacterium but by a group of bacteria. More than 500 different types of bacteria have been isolated from the oral cavity (1), most of which are innocuous. Periodontitis is associated with members of the indigenous oral microbiota, including *Aggregatibacter actinomycetemcomitans*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Enterococcus faecalis* and *Treponema denticola* (2). The relationship between periodontal microbiota and chronic periodontitis has recently been studied using real time polymerase chain reaction (3). It was revealed that mean count of *P. gingivalis* was significantly higher in patients with chronic periodontitis than in periodontally healthy individuals. It has also been found that there was a considerable association between *P. gingivalis* and chronic periodontitis (3). Enterococci are Gram-positive cocci that are able to survive very extreme conditions in nature. For example, *Enterococcus faecalis* was reported in the saliva of many chronic periodontitis patients (4). The bacterial plaques formed in the oral cavity – mainly on teeth surface, dental restorations, prostheses, and implants – could be composed of a broad range of bacterial species (5).

Bacteria communicate within themselves using some chemical signaling molecules, which are generally N-acyl homoserine lactones (AHLs, Fig. 1), in Gram-negative bacteria, and oligopeptides, in Gram-positive bacteria. In addition, both Gram-positive and Gram-negative bacteria produce a family of signaling molecules known as autoinducer-2 that they employ for their communication (6). Quorum sensing is a process that enables bacteria to communicate using some types of secreted signaling molecules known as autoinducers; thus, a population of bacteria becomes able to regulate gene expression collectively and therefore, to control behavior on a community-wide scale (7).

![Fig. 1 – General structure of N-acyl homoserine lactones (AHLs).](image)
Among many diverse mechanisms known for quorum sensing, two of the most common forms rely on acyl homoserine lactones and oligopeptides, in Gram-negative and Gram-positive bacteria, respectively (8). It is known that almost all QS bacteria produce and respond to their own unique AHLs specific to each species. These signaling molecules are sensed by cells allowing the whole population to initiate a concerted action once a critical concentration has been achieved. Some biochemicals were identified as bacterial cell-to-cell quorum sensing signal molecules in both Gram-negative and Gram-positive bacteria (9). Virulence could be regulated by quorum sensing in a number of different organisms, including the opportunistic pathogens Pseudomonas aeruginosa and Enterococcus faecalis. It has been suggested that quorum sensing can play an important role in bacterium-bacterium communication and therefore, in the formation of dental biofilms (10). The complexity of biofilms and the presence of putative pathogens may lead to production of quorum sensing signal molecules and development of periodontitis (11).

We have previously examined the effect of Salvadora persica extract to prevent bacterial quorum sensing in vitro (12). In the present study, quorum quenching ability of extracts was screened on in vivo produced E. faecalis. The bacterium was extracted from oral fluid of chronic periodontitis patients; it was cultured and grown in the presence and absence of plant extracts. The anti-quorum sensing ability of extracts was examined by a number of molecular methods based on QS signal molecules (13).

**MATERIALS AND METHODS**

**Collection of saliva samples**

In order to obtain E. faecalis from in-vivo, saliva samples were collected from patients diagnosed with periodontitis. The study enrolled 35 patients, who received explanations regarding the purpose of the research and signed an informed consent. They were instructed to rinse their mouths for 60 seconds with 10 mL of sterile distilled water and pour their saliva samples into sterile tubes. The tubes were sealed completely, stored at 4°C and transferred to laboratory within 2 hours.

**E. faecalis selection and characterization**

Saliva samples were first centrifuged at 4°C for 15 min at 15,000 g and pellets were re-suspended in 1 mL of sterile nuclease-free water. Afterwards, the samples were plated onto bile esculinazide agar plates to select for enterococci, followed by aerobical incubation of the plates for about 48 hours at 37°C. Colonies susceptible to enterococci were purified by streak-plating onto a fresh esculinazide agar plate. Isolates were characterized as catalase-negative, non-motile, Gram-positive cocci if they were capable of growth in Todd Hewitt agar with 6.5% NaCl.
at 42°C. Analytical Profile Index 20 Strep identification kits (Bio Mérieux SA, Marcy-l’Etoile, France) and the 16-S rRNA technique were then employed for the specific identification of *E. faecalis*.

**Preparation of *S. persica* methanol extracts**

Two hundred grams of dried pulverized bark of the miswak plant were mixed with 500 mL of 20% methanol. The suspension was continually mixed using a magnetic stirrer for at least 12 hours at room temperature and then filtered twice on Whatman ashless paper (Cat. No. 1442). The mixture was centrifuged at 8000 rpm for one hour. The resulting supernatant was carefully separated and remained at ambient temperature for one hour, in order to let complete evaporation of the solvent. To make sure that residual methanol has been also removed, the extracts were air-pumped at room temperature for at least 10 hours. As methanol could also inhibit bacterial growth, aqueous solutions from the resulting powder were prepared using w/v ratios in deionized water. A stock solution (400 mg/mL) of the *S. persica* extract was prepared by mixing 200 grams of the resulting powder with 500 mL of 20% methanol (400 mg/mL). At the time of experiment, solutions of 300, 200, 100 mg/mL (needed for *E. faecalis* growth inhibition tests) and 60 mg/L (for late log assay) were prepared from this stock solution.

The reason for making various concentrations for the two purposes was to make sure that the lack of virulence factors expression, monitored by QPCR, occurs at an extract concentration at which the bacterial growth is not inhibited.

**Antibacterial effect of *S. persica* extracts**

Antimicrobial studies were carried out using *Enterococcus faecalis* isolated from saliva of patients suffering from chronic periodontitis. The disk diffusion method was used to *in vitro* compare antibacterial activity of *S. persica* solutions on *in vivo* produced *E. faecalis* using known antibiotics as positive controls. In practice, broth subcultures were prepared by inoculating, with one single colony from a plate, a test tube containing 5 mL of sterile nutrient Broth. The tubes were then incubated at 37°C for at least 24 hours.

The bacterial suspension was diluted to match of 10⁸ CFU/mL on the Mac Farland scale. Each suspension was spread on a Muller Hinton Agar medium by sterile swabs. Whatman filter paper disks (diameter 6 mm) dipped into *S. persica* solutions (concentrations 100-400 mg/mL) and two known antibiotic disks – vancomycin, 20 µg/mL and ampicillin, 10 µg/mL (Padtan Teb™ Iran) – were placed on the top of the agar surface. The diameter of the inhibition zone was measured after incubation of all plates at 37°C for 24 hours. A disk dipped in deionized water was used as negative control. Considering that the disk diameter was 10 mm, inhibition zones (including disk diameter) less than 10 mm were negative. Zone calculation was the average of three measurements. The t-test was
used with the GraphPad PRISM® software (GraphPad Software, Inc., San Diego, CA, USA) and p ≤ 0.05 differences were considered as significant.

**Preliminary evaluation of S. persica AQS effect on C. violaceum**

All microbial culture procedures were performed in a Class 2 Biosafety Cabinet. *C. violaceum* ATCC 12472 was grown in Luria-Bertani (LB) agar and AQS of *S. persica* extracts were screened by standard disk diffusion assay. Plates were incubated at 37°C overnight (at least 18 hours) in a standard incubator. Extracts positive for AQS were further tested for bactericidal activity against *E. faecalis* using the same disk diffusion assay protocol but in Brain Heart Infusion (BHI) agar and blood agar plates, respectively. Incubation was done at 37°C overnight in a standard incubator.

**Late Log Phase Growth of E. faecalis in the presence of S. persica extracts**

The late log assay was performed as discussed earlier (12). In practice, oral *E. faecalis* was grown in Brain Heart Infusion broth and incubated at 37°C for about 8 hours to reach the late log phase. This was designed to act the inoculum source, from which 500 µL was then transferred into 4 mL BHI broth containing 500 µL of the prepared plant extract (concentration 60 mg/mL) with a total volume of 5 mL. 500 µL of 3-Bromofuran were used as positive control and 500 µL of deionized sterile distilled water as negative control. After about 10-11 hours (the late-log phase), 1 mL portions of the cells were separated for RNA and total DNA extraction as well as quantitative measurement of bacterial population. The remaining cells were centrifuged at 12,000 rpm (at least 10 min) and the resulting supernatant was separated to be used later.

**DNA extraction and polymerase chain reaction (PCR)**

Total DNA was extracted using InstageneTM Matrix (Bio-Rad Cat. 732-6030) under the earlier described conditions (12). In practice, the reaction mixture was composed of 1x KAPA Fast SYBR mix (Kapa Biosystems) and 0.3 µM each of primer (Sedgeley et al., 2005). The *E. faecalis*-specific polymerase chain reaction primer, which was designed previously for *in vitro* derived bacterium tests, was used in this study too (12). The PCR reaction was slightly modified from the previous work and performed for the *in vivo* (saliva) extracted *E. faecalis*. Briefly, in a 25-µL reaction mixture, a volume of 0.5 µL of the designed primer was added together with 2.5 µL of 10×PCR buffer. To the same mixture, appropriate volumes of Taq DNA polymerase from Gibco BRL, Gaithersburg (0.5 µL) and deoxyribonucleoside triphosphates (0.5 µL) were added. The denaturation step was performed at 95°C (15 min), then 35 cycles at 94°C (20 s), a primer annealing step at 50°C (45 s) followed by extension at 72°C (30 s) and finally 72°C (5 min). Amplicons were
analyzed using 1.5% agarose gel electrophoresis (Tris-borate EDTA buffer and 4 V cm⁻¹). The electrophoresis gel was then stained using 0.5 mg mL⁻¹ ethidium bromide followed by gel documentation under ultraviolet light.

**RNA extraction and qPCR conditions**

The total RNA from *E. faecalis* pellet was extracted using RNeasy Minikit (Qiagen, GmbH, Germany) after at least 10 minutes centrifugation. The procedure was based on the manufacturer’s recommended route. The relative purity of total RNA as well as its concentrations (µg/mL) was determined by spectrophotometry. The ratios of OD_{260nm}/OD_{280nm} were then used for calculations. For amplification of signaling molecules, cytolysin (*cylR1*) and gelatinase (*gelE*), two transcripts were designed (Table 1). The quantitative PCR mixture was composed of 1.0 µL of mRNA and a total volume of 20 µL, 1x iScript SYBR One-Step RT-PCR mix and 0.3 mM of each primer (12).

The relative quantity of bacterial population obtained through qPCR was compared to the data resulted from spectrophotometric assay for densities of cells at 620 nm.

**Table 1**

<table>
<thead>
<tr>
<th>Target</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>cylR1</em></td>
<td>TTTATTTTTTTATGGATATCATTTCTGTA</td>
<td>TCGCTCATCTTTTTTTGAATC</td>
</tr>
<tr>
<td><em>gelE</em></td>
<td>CGGAACATACTGCCGGTTAGA</td>
<td>TGGATTAGATGCACCCGAAAT</td>
</tr>
</tbody>
</table>

**Statistical analysis**

Each assay was repeated at least twice and the results were presented as mean ± SD values. The statistical difference (SD) between results was compared by un-paired t-test, and p values less than 0.05 were retained as significant; SDs between means were determined by Duncan’s multiple range tests; *P* values less than 0.05 were considered statistically significant.

**RESULTS AND DISCUSSION**

**AQS activity of *S. persica* extracts on *Chromobacterium violaceum***

The late log phase population of *E. faecalis* treated with *S. persica* extracts was comparably similar to, or higher than, the negative control. It is worth indicating that reduced population of *E. faecalis* is indicative of antibacterial activity exhibited by the extracts. This resulted in a decrease of virulence factor expression not secondary to AQS.
On the other hand, *C. violaceum* was inoculated on Luria-Bertani (LB) agar and purple isolated colonies (Fig. 2) were used for subsequent disk-diffusion assay for the AQS activity of *S. persica*. Care was taken to keep the mother plate culture free from any contamination. It is worth reminding that AQS screening for violacein pigment inhibition is a qualitative assay, which means that results are reported only as either present (violacein-free zone) or absent (no zone).

![Fig. 2 – Pure culture of *C. violaceum* on LB agar after overnight incubation at 37°C showing purple isolated colonies.](image)

In support of our previous study on *in vitro* produced bacterium (12), here too the disk-diffusion assay in the presence of *S. persica* extracts showed the occurrence of some zones free of purple pigmentation around the impregnated paper disks (results not shown). Based on this result, it could be suggested that the *S. persica* methanol extracts exhibited anti-quorum sensing and thus, prevented *C. violaceum* from producing violacein signal molecule.

The growth of *E. faecalis* at late log phase could provide enough time for population count. Experimental tubes containing *E. faecalis* grown in BHI broth was treated with 60 mg/mL methanol extracts. In order to determine quantitatively the negative effect of extracts on the late log population of *E. faecalis*, optical density (OD) at 620 nm was measured using a UV-visible spectrophotometer. It was found that cell densities recorded were higher than the negative control. Similar to our previous results for *in vitro* results (12), QPCR confirmed that the bacterial population was not negatively affected by the extracts. Quantified 16S rDNA region specific only for *E. faecalis* revealed copy numbers greater than the negative control (figure not shown). Having found that the methanol extracts of *S. persica* did not affect the late log phase growth of *E. faecalis*, AQS assays were suggested as a goal for subsequent research.

The result of the LB agar disk-diffusion assay was consistent with our previous finding about *in-vitro* derived *E. faecalis* (12). It was indicative of the fact that the *S. persica* extract exhibited AQS activity. The production of violoxanthine by *C. violaceum* was significantly scavenged in the presence of disks dipped in *S. persica* extract (figure not shown).
Antibacterial effect of *S. persica* extracts on *E. faecalis*

Stock culture of *E. faecalis* was subcultured and initially tested for purity and identity by Gram staining, catalase test and 16S rDNA PCR prior to disk-diffusion assay. Considering that the bacterium is derived from oral fluid, it is very important that *E. faecalis* is free from contamination by other Gram positive and Gram negative bacteria to get reliable results.

*E. faecalis* isolation and characterization

Figure 3A shows the positive response of cocci to Gram staining. In Fig. 3B, the negative catalase is confirmed by the absence of O₂ bubbles from 3% H₂O₂. It is worth indicating that the formation of any small bubbles is the result of a positive catalase test, and neither positive or negative controls were needed.

On the other hand, PCR amplification of the 16S rDNA 138-bp sequence region, specific only for *E. faecalis*, confirmed the identity of amplicons using primers specific only for *E. faecalis* (Fig. 4).
As mentioned above, quantitative polymerase chain reaction (QCR) procedure was used to quantitatively obtain the actual expression of two important virulence factors, i.e., cytolysin (cylR1) and gelatinase (gelE). The results obtained in this part of the research revealed no detectable signals, even through setting a very low threshold by suitable software (Fig. 4).

Although both vancomycin and ampicillin inhibited the growth of *E. faecalis*, methanol extracts of *S. persica* did not significantly exhibit antibacterial activity against *E. faecalis*. Table 2 shows the diameter of inhibition zone (mm) ±SD in the presence of antibiotic and extracts disks. It can be seen that the values increase with decreasing concentration of extract. Instead, the methanolic extracts interfered with the bacterial quorum sensing through inhibition of violacein production in *C. violaceum*, as stated earlier. As it can be noticed from Table 2, the diameter of the inhibition zone for *S. persica* 100 mg/mL treatment has very similar values with those for Amp treatment. Therefore, the extract solution with a concentration of 100 mg/mL could inhibit the growth of *E. faecalis* with the same power as Amp, which means that, at only very low concentrations, extracts of *S. persica* react as antibacterial agents. Based on this finding, we chose the highest concentrations of extract with almost no antibacterial effect for our quorum sensing studies.

<table>
<thead>
<tr>
<th>Type of growth inhibitor</th>
<th><em>S. persica</em> methanolic extract</th>
<th>Amp</th>
<th>Van</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (mg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>12.0±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>14.0±0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>14.5±0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>15.0±0.07</td>
<td>15±0.08</td>
<td>17±0.04</td>
</tr>
<tr>
<td>Negative control</td>
<td>10 µg/ml</td>
<td>20 µg/ml</td>
<td></td>
</tr>
<tr>
<td>Diameter of inhibition zone (mm) ±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PS. Broth subcultures were prepared by inoculating, with one single colony from a plate, a test tube containing 5 mL of sterile nutrient Broth. The tubes were incubated at 37°C for at least 24 hours.

These results were consistent for both BHI and BHI-blood agars, respectively. Absence of anti-bactericidal activity is very important for the succeeding assays to prove that inhibition of virulence factor expression is secondary to AQS and not due to death of *E. faecalis*. Spectral images of *E. faecalis* growth lawns on both BHI and BHI-blood agars were documented to clearly show that colonies did grow at the boundaries of the filter paper disks (Fig. 5) visually confirming growth. According to this figure, *S. persica* extracts contained in paper disks did not inhibit the growth of *E. faecalis* in either BHI (A) or BHI-blood (B). These results, also summarized in Table 2, confirmed that the extracts are not antibacterial, but instead, they are possibly anti-quorum sensing, as confirmed later in the study.
Fig. 5 – Spectral imaging (100 x) of *E. faecalis* colonies (black arrows) close or next to the filter paper disks dipped in *S. persica* extracts (white arrows) from BHI (A) and BHI-blood (B) agars.

**Effect of *S. persica* extracts on the late log phase growth of *Enterococcus faecalis* extracted from saliva of periodontitis sufferers**

Growth of *E. faecalis* at late log phase was necessary to allow time for proper population count required for the occurrence of quorum sensing. As discussed in the material and method section, representative tubes of *E. faecalis* grown in BHI broth were treated with 60 mg/mL extracts. To quantitatively determine that the extracts did not drastically affect the late log population of *E. faecalis*, optical density at 620 nm was measured by spectrophotometry. Table 3 shows the cell densities recorded for all 35 samples compared to the negative control, deionized distilled water. It can be seen that in all cases, OD value is higher than the negative control, indicating the presence of *E. faecalis* late log population.

**Table 3**

Spectrophotometric readings of oral *E. faecalis* optical densities measured at 620 nm compared to negative control (H₂O). All tests were performed in triplicate.

<table>
<thead>
<tr>
<th>Number of samples with equal ODs</th>
<th>Optical density (OD) at 620 nm ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.502 ± 0.02</td>
</tr>
<tr>
<td>8</td>
<td>0.474 ± 0.05</td>
</tr>
<tr>
<td>7</td>
<td>0.488 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>0.480 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>0.485 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>0.521 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>0.496 ± 0.04</td>
</tr>
<tr>
<td>(H₂O)</td>
<td>0.345 ± 0.02</td>
</tr>
</tbody>
</table>

The same results were observed when total DNA was extracted from the respective tubes. The optical densities (ODs) were then measured at 260 nm and 280 nm. It was found that the extracted total DNAs were relatively pure, as confirmed by their respective absorption at 260 nm and 280 nm, *i.e.*, the 260/280
ratios (Table 4). In this research, the ratio of 1.7 to 2.0 ± 0.2 was considered as acceptable.

Table 4

Total DNA quantitation and purity check at 260 nm (nucleic acids) and 280 nm (protein contamination), respectively. All tests were performed in triplicate.

<table>
<thead>
<tr>
<th>Number of samples with equal ODs</th>
<th>OD at 260 nm</th>
<th>OD at 280 nm</th>
<th>260/280 ratios</th>
<th>DNA µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>0.122</td>
<td>0.076</td>
<td>1.605</td>
<td>610</td>
</tr>
<tr>
<td>8</td>
<td>0.065</td>
<td>0.031</td>
<td>2.097</td>
<td>325</td>
</tr>
<tr>
<td>6</td>
<td>0.075</td>
<td>0.037</td>
<td>2.027</td>
<td>352</td>
</tr>
<tr>
<td>5</td>
<td>0.082</td>
<td>0.041</td>
<td>2.00</td>
<td>410</td>
</tr>
<tr>
<td>4</td>
<td>0.110</td>
<td>0.068</td>
<td>1.604</td>
<td>398</td>
</tr>
<tr>
<td>2</td>
<td>0.093</td>
<td>0.046</td>
<td>2.002</td>
<td>372</td>
</tr>
<tr>
<td>H2O</td>
<td>0.038</td>
<td>0.023</td>
<td>1.652</td>
<td>190</td>
</tr>
</tbody>
</table>

DISCUSSION

The rapid increase of antibiotic resistance, as well as various side effects they cause opened a wide range of research area investigating the mechanism of resistance and replacing natural derived medications against pathogenic microorganisms. Among many known synthetic antibiotics, vancomycin is known as one of the most effective compounds against *E. faecalis*. However, the occurrence of vancomycin-resistant *E. faecalis* observed and reported during the last few years has needed sustained search for natural products (14). It is known that *E. faecalis* is the primary etiologic agent of chronic periodontitis. Therefore, the use of natural medications which are effective on this bacterium could be impressive for a periodontist. In recent years, scientists are trying to develop natural products with anti-quorum sensing ability (15). This type of compounds can exhibit their effect by targeting several pathways of bacterial metabolism with significant reduction in development of resistance.

*Salvadora persica* is a native plant in Iran and the Middle East countries, traditionally used for tooth cleaning, and the present research was aimed to examine its anti-quorum activity against *E. faecalis*. Literature survey has shown that only few studies attempted to characterize antimicrobial compounds in *S. persica*; on the other hand, there is currently no published research on experiments investigating its potential as an anti-quorum sensing medication. It is known that periodontal pathogens, including *E. faecalis*, produce quorum sensing signal molecules (10). In support of our work, we found anti-quorum sensing activity exhibited by pharmacological agents reported in literature (11). However, in the case of natural plants extracts, only antibacterial properties have been discussed (16). The activity of *S. persica* extracts on various bacteria has shown that the concentration of natural extracts exhibits a direct effect on bacterial growth.
inhibition (17). On the other hand, in the case of natural extracts, less attention was paid to the anti-quorum sensing ability.

The term “quorum sensing” refers to a special process demonstrated by some bacteria to control gene expression. In general aspects, the process depends on the density of bacterial cell population. In this regard, bacteria synchronize their behavior by synthesizing auto-inducers using synthases as their biocatalyst. Several bacterial cellular functions – for example, virulence gene expression, formation of thin biofilms, resistance to antibiotics as well as a special type of behavior, i.e., bioluminescence – are regulated by quorum sensing.

It is worth emphasizing that our findings highlight the new original experimental results on in vivo derived bacteria, as compared with the experimental original results already found in 2011 by our research team (12).

This study clearly confirmed an anti-quorum sensing activity of S. persica methanol extracts against oral derived E. faecalis. This activity was initially screened by inhibition of violacein production in C. violaceum.

CONCLUSIONS

Based on a broad literature survey, the present research, together with a previous one (12), are novel reports showing the presence of anti-quorum sensing molecules in S. persica against both oral extracted and lyophilized E. faecalis. However, the molecules and all anti-quorum sensing molecules in general have a broad range of activities targeting virtually all bacterial species depending on the Gram nature of bacterium which may possess different quorum sensing pathways. The two important virulence factors, i.e., cytolysin (cylR1) and gelatinase (gelE), act as signaling molecules for representing quorum sensing occurrence.

In conclusion, the anti quorum sensing activity of S. persica extracts could be effective in preventing the development and growth of many other pathogens found in the salivary fluid, such as Streptococcus mutans and numerous other pathogens. This type of behavior increases its activity to prevent chronic periodontitis by inhibiting formation of bacterial plaques and biofilms. A remarkable benefit of the plant extract is that its natural source makes it almost free from toxic chemicals.

As a final remark, it is suggested that further studies are needed to target the effect of S. persica anti-quorum sensing on the global expression of virulence transcriptome using DNA microarray. The next step is to specifically identify the effective molecules acting as specific quorum sensing signals, purify and study their activities individually or in synergy against oral pathogens, including their cytotoxicity to mammalian cells. These are certainly necessary and important steps prior to clinical trials of extracts on human subjects.

Acknowledgments. We sincerely thank the scientific and practical advice provided by all the professors in the Department of Periodontology, Tehran University of Medical Science, Iran.
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