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The effect of Sub-MIC of *Salvadora persica* on growth and virulence gene expressions in *Streptococcus mutans*

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ABSTRACT

Dental caries is a multifactorial infectious chronic disease caused by particular bacteria and their virulence products that causes demineralization and progressive deterioration of the dental enamel. Many studies have proven miswak to have a critical antibacterial impact, particularly on cariogenic bacteria and periodontal pathogens, in the oral cavity. This study aimed to investigate the effect of different concentrations of *Salvadora persica* plant extract on growth and virulence gene expressions at mRNA levels in *S. mutans*. A total of 191 clinical samples from tooth swabs were collected and sub-cultured on specific medium agar identified using biochemical and molecular approaches. MIC for the extract was determined and a bacterial growth curve was made to determine the growth phases and the optimum time for adding the extract at different concentrations. RT-qPCR technique was performed, and the REST-2009 software program was used for data analysis. Out of 191 swabs from the tooth 31 isolates were identified using several biochemical and molecular tests. Several *S. mutans* biofilm-related virulence genes and their Ct values were produced from RT-PCR under the effect of low and high doses of Meswak concentrations. Ct values and reaction efficiency were produced in RT-qPCR by Rotorgen3000, data then were analysed by REST-2009 software. Five isolates were selected to examine the effect of the extract on the mRNA levels using qPCR after growing them with both doses of the extract for about 30hrs. Levels of virulence gene mRNA were regulated differentially in cultures with added both extract doses. The isolates produced significantly lower virulence gene mRNA levels in cultures grown with both plant extract doses. The results produced in this study here provide new insights regarding several virulence gene expressions in *S. mutans* at the molecular levels when grown under different concentrations of *Salvadora persica* plant extract.

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Introduction

Throughout human history, natural products have been important sources of appealing and important therapeutic compounds for the invention and development of novel medications. Dental caries is a multifactorial infectious chronic disease caused by particular bacteria and their virulence products that causes demineralization and increasing deterioration of the dental enamel (1). The infection is produced by stationary plaque biofilms in which bacteria such as *Streptococcus mutans* (*S. mutans*) digest dietary carbohydrates and create acids (2). The most common cause of dental decay is *S. mutans*. Several lactobacilli have been linked to the progression of the lesion (3). *S. mutans*, a G+ve oral bacteria, has long been suspected of being the root cause of human dental caries, this stimulates the production of *S. mutans* biofilms, resulting in acidic microenvironments that demineralize tooth hard tissues, culminating in dental caries. Furthermore, virulence variables including acid and stress tolerance, cell persistence, genetic capacity, and bacteriocin production are implicated in dental caries progression (4). *S. mutans* is important in the development of virulent cariogenic biofilms in dental caries, a biofilm-induced oral disease, as a result, one of the most significant biological goals in preventing dental caries is to lower the bacterial burden in the oral cavity; dental varnishes are simple to apply and deliver an active component like fluoride or chlorhexidine to the teeth safely and efficiently (5). Adhesion is the first step in biofilm community formation, and it is the major causative agent of dental caries. Sucrose-dependent adhesion mechanisms mediated by extracellular enzymes [glucosyltransferase (GTF) and fucosyltransferase (FTF)] and glucan-binding proteins (GBPs) play important roles in the virulence of *S. mutans* (6). The adhesion mechanism needs the de novo creation of insoluble glucan from sucrose by *S. mutans* glucosyltransferase. As a result, medications that block the ability of *S. mutans* to attach may be useful in the fight against dental caries (7). GTFB and GTFC produce water-insoluble glucans that act as adhesion molecules that fasten the bacteria to the gums (6). Because of the rise in antibiotic resistance and antimicrobial side effects on one hand, and the safety, availability, and cheap cost of natural products on the other, a range of natural agents have been evaluated for caries prevention and included in dental products.

Derived from the roots or branches of the Arak tree (*Salvadora persica*) found in many countries, Miswak is one of many plants with antimicrobial potential (8). Tannic acid, alkaloids, eucalyptol, sulphur compounds and many

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other medically important components are all found in Miswak plant extract and have been associated to antibacterial, anti-tumor, anti-inflammatory, and wound healing benefits. The calcium concentration in its aqueous extract was also observed to be high, whereas fluoride levels were low (5). This plant's oil contains biological activity and is used to treat gall bladder disease, piles, polio, intestinal worms, gonorrhoea, and rheumatoid arthritis. The oil has also been utilized to manufacture candles, soaps, and as a substitute for coconut oil (9). Several clinical studies have proven the effects of diverse bacteria species in the oral cavity. Many studies have proven miswak to have a critical antibacterial impact, particularly on cariogenic bacteria and periodontal pathogens, in the oral cavity (10). This study aimed to investigate the effect of different concentrations of *S. persica* plant extract on growth and virulence gene expressions at mRNA levels in *S. mutans.*

Materials and Methods

Sample collection

A total of 191 clinical tooth swabs were isolated and sub-cultured on specific medium agar" mitis-salivairus base agar incubated at 37°C/ 72 hrs. The swabs were taken from the carious lesion with the tip of a sterile wooden toothpick. A toothpick was cut off and immediately dipped in 1 ml of sterile phosphate-buffered saline (HiMedia, India) and stored at 4 °C. The swab was vortexed to obtain a uniform suspension. Samples were then cultured using Mitis Salivarius Bacitracin (MSB) agar. MSB agar consisted of 15% sucrose, 1% agar, 0.0001% potassium tellurate solution and Mitis salivarius agar (HiMedia, India) supplemented with 0.2 units/ml bacitracin (HiMedia, India). Plates were left in the incubator anaerobically at 37° C/ 48-72 hours. The MSB agar is composed of mitis salivarius agar (HiMedia, India) and complemented with 15% of sucrose, 1% of agar, 0.0001% potassium tellurite solution and 0.2 units/ml of bacitracin (HiMedia, India). The plates were incubated anaerobically at 37°C for 48-72 hrs.

Gram stain and biochemical tests for the identification of samples

Gram staining is still the gold standard for bacterial identification and taxonomic classification. Most bacteria are divided into two groups based on cell wall composition using this differential staining procedure: Gram-positive bacteria (a thick layer of peptidoglycan accounts for 90% of the cell wall) - leaves a purple stain.

Oxidase test.

The oxidase test is one of the biochemical tests used to identify bacteria that produce cytochrome c oxidase, an enzyme in the electron transport chain. In the presence of cytochrome C oxidase, it oxidizes the reagent to produce purple or dark blue indophenol.

Mannitol test

A mannitol salt agar test isolates and identifies the presence of *S. mutans* in a clinical specimen, making it a selective, differential, and indicator medium.

Esculin hydrolysis tests

The Esculin Hydrolysis test is used to identify a wide range of microorganisms, including members of the Enterobacteriaceae family, the genera *S. mutans* and Listeria, non-fermentative gram-negative bacilli, and anaerobes. The test can be used to determine whether an organism can generate the esculinase enzyme.

Glucose fermentation test

The bacterial ability to generate organic molecules by metabolizing specific carbohydrates and related substances is a frequently used approach for identifying microorganisms. Different fermentation media are used to distinguish organisms depending on their capacity to ferment carbohydrates in the basal medium.

Starch hydrolysis test

The test microorganisms are cultivated on starch-containing agar plates in the starch hydrolysis test. If the bacteria can hydrolyze starch, it does so in the medium, particularly in the areas around their development, whereas the rest of the plate contains non-hydrolysed starch.

Colony PCR targeting *S. mutans*

Amplification of 16S rRNA from 22 isolates was performed to confirm bacterial identification. Five to ten colonies were selected from MSB agar plates and mixed with a mixture of GoTaq® colourless master mix (Promega, USA) and a set of primers (Table 1) Amplification was PCR was accomplished using Thermocycler (Thermo Fisher Scientific, USA) and the reaction conditions were heated for 5 min at 95°C, then 35 cycles of denaturation for 15 sec at 95°C, annealing for 30 sec at 55°C and extension for 1 min at 72°C, followed by 10 min at 72°C.

Preparation of Meswak plant extract.

The extract was prepared according to (Wassel et al., 2017) with some modifications. One hundred grams of miswak powder were added to 100 mL of 95% ethyl alcohol and left for 6 days at room temperature. The solution was filtered through a 0.45 mm filter paper and left to evaporate and the remaining powder or gum at the bottom is the extract. The dried gum was dissolved in 50ml of carboxymethyl cellulose (CMC, 0.25% w/v). The extract was kept in tightly closed screw-capped containers at 4°C.

Determination of minimum inhibitory concentration (MIC)

The MIC of the extract was determined by mixing equal volumes of the plant extract and broth media in test tubes. Standardized inoculums of $1–2\times10^7$ CFU/ml of 0.1 ml were added to each tube and incubated anaerobically at 37°C/ 48-72 hrs. Plant extract MIC was determined as the lowest concentration allowing no visible growth (no turbidity) when compared with the control tubes.

Growth curve

The growth curve was generated to measure changes in absorbance over time. A few colonies of selected isolates were inoculated into 5 ml of BHI (Brain Heart Infusion) broth and incubated anaerobically at 37°C/ 72hrs. One ml of the culture was then taken and added to 100 ml of fresh liquid of the same medium and mixed. Growth was examined for approximately 72 hrs. To read the absorbance at 600 nm, 1 ml of bacterial suspension was aseptically taken and measured. Absorbance readings were taken every 60

minutes and the data was plotted to generate a growth curve.

Effect of the extract on gene expression at mRNA levels.

To determine the effect of the plant extract on the gene expressions cultures of 5 isolates were prepared under anaerobic conditions with and without low and high doses of the plant extract for 48hrs. Five isolates were selected on the bases of the presence of all 4 virulence target genes under study using PCR. Fifty millilitre of the culture was centrifuged for 10 min at 3,000 g at 4°C and the pellet was resuspended in TE buffer (Tris 10 mM, EDTA 1 mM, pH 8.0), followed by another centrifugation step after which the pellet was resuspended in TE buffer for the next step RNA extraction.

RNA extraction

RNeasy mini kit (Qiagen) was used for RNA extraction according to the instruction provided with the kit. For this, bacterial cells were grown as previously explained, and 50ml of the culture was centrifuged at 4000 g for 15 min. The pellet was mixed with 1.8 ml TE buffer (Tris 10 mM, EDTA 1 mM, pH 8.0) and incubated for 1-2 hrs at 37°C with occasional gentle mixing. Using 350 µL of lysed cells, RNA elution proceeded according to the manufacturer's instructions. DNase1 (Qiagen) was used by mixing 10 µL of the enzyme with 70 µL of the provided DNase I buffer and adding it to each RNA sample tube and was eluted in a volume of 50 µl. RNA samples were then checked for quality and quantity using a NanoDrop1000 spectrophotometer (Labtech) and stored at -20°C for short periods and -80°C for long-term storage.

cDNA synthesis

RNA samples were then subjected to reverse transcription to cDNA using the Superscript III transcriptase (Invitrogen) system according to the manufacturer's instructions. An amount of one µg of total RNA was converted in a total volume of 20µl containing random hexamer primers (150 ng/µl) (Invitrogen), made up to 11.0µl with RNase-free water. The suspension was then heated at 65ºC/ 5 minutes, put on ice for 5 minutes and centrifuged for one min. Then 1 x Reverse Transcriptase (RT) buffer (Invitrogen), 10nM DTT (Invitrogen), dNTP (0.5 mM) (Bioline), RNase inhibitor (Promega 2.0u/ µl), and Reverse Transcriptase enzyme (RT III) (10u/ µl, Invitrogen) as final concentrations, were added. The mixture of 20 µl was then incubated at 42ºC/ 90 min, followed by 72ºC/ 15 mins. Finally, PCR was performed for cDNA samples to confirm their synthesis was successful, samples were then stored at -20ºC.

Real-Time quantitative PCR (RT-qPCR)

RT-qPCR assays were performed in a total volume of 20 µl including 10.0 ng target cDNA, 1x buffer (Bioline), 1.5 mM MgCl2, 200 nM dNTP, 100 nM of each virulence gene primers and 0.08 µl Taq DNA polymerase (Bioline) and Syber green I fluorescent dye at 5 µM. This was added at the last minute as it is very light-sensitive. The reaction was then performed by Rotor-Gene 3000 (Corbett Research) under the following thermal cycling conditions: 95 °C for 10 min, then at 95 °C/ 20 s, 54–56 °C (depending on melting temperature of the primers) for 20 s and at 72°C for 20 s, with data collection in each cycle at 72°C. Relative expression using RT-PCR was used in this study, in which the expression of a target gene is standardised by a reference gene. RT-qPCR test was based on measuring the increase in a fluorescent signal that is relative to the amount of DNA formed throughout each PCR cycle. Each reaction was described by PCR cycles in which the fluorescent signal rises above the threshold background (threshold cycle Ct) value. The higher amount of targets in the starting material, the smaller the Ct value. This relationship between fluorescence and produced DNA abundance allows accurate quantification of target molecules.

Data analysis using (REST-2009)

Ct values and reaction efficiency were produced in RT-qPCR by Rotorgen3000, data then were analysed by REST-2009 software (Corbett Research). REST 2009 is a programming tool used to ascertain differences in target gene expression normalised by non-regulated reference genes (Corbett Research). The program tool was used to determine whether there were significant differences in gene expression profiles between bacterial cultures exposed to low doses and high doses of the plant extract under study.

Ethical statement

The research proposal was submitted to the ethics committee of the College of Health Sciences of Hawler Medical University, and official permission was taken from Khanzad Center for dental health in Erbil City for sample collection. The included subjects were given details about the aim of the study and following their agreements and permission they were enrolled; they were ensured that their identities would be kept anonymous.

Table 1. Specific primers used in RT-PCR (Li et al., 2020 and Moshe et al., 2007).

Gene	Primers	Description	Amplicon size
16S rRNA	F: AGCGTTGTCCGGATTTATTG R: CTACGCATTTCACCGCTACACA	Normalizing internal standard	156bp
ldh	F: GGCGACGCTCTTGATCTTAG R: GGTTAGCAGCAACGAGGAAG	Lactate dehydrogenase	236bp
gtfB,	F: AGCAATGCAGCCAATCTACAAAT R: ACGAACTTTGCCGTTATTGTCA	glucan production	96bp
gtfC	F: GGTTTAACGTCAAAATTAGCTGTATTAGC R: CTCAACCAACCGCCACTGTT	glucan production	122bp
brpA	F: GGAGGAGCTGCATCAGGATTC R: AACTCCAGCACATCCAGCAAG	Biofilm-regulation protein	148bp

Results

Out of 135 swabs from the tooth 31 isolates were identified using several biochemical tests as mentioned in the previous section. Identification of all isolates was confirmed by standard biochemical tests (Table 2) and staining methods. On agar with sucrose, such as Mitis Salivarius agar, colonies produced puddles of glucan, and a droplet of glucan appeared at the top of the colony, especially when plates were left to stand on the bench for 24 hrs (Figure 1A). The bacterium appeared purple-colored cells under the microscope when they gram stained as they are G+ve (Figure 1B).

Samples were further identified as *S. mutans* using several biochemical tests as shown in Table. 2.

Bacterial isolates were further identified by colony PCR (Figure 2) using specific primers (Table 1) and 16S rRNA gene sequencing. The PCR products of 5 isolates were submitted to Macrogen Company (238, Teheran-ro, Gangnam-gu, Seoul, Republic of Korea) for sequencing. Sequences were edited using Bioedite sequences software and compared with sequences reported in GeneBank (National Center for Biotechnology Information NCBI). The isolates 1, 4, 7, 15 and 25 showed 100% similarity to *S. mutans*.

Both doses of the extract were used at sub-MIC levels (low 64 µg/mL and high128 µg/mL), permitting bacterial cells to grow at the lower rate and it can be investigated if the extract has any impact on virulence gene expressions. The extracts were added growth exponential phase when the cells are functioning and at the same biological conditions. Growth curves following the addition of these concentrations of antibiotics were created for several isolates with similar patterns produced as shown in Figure 3 for isolate no. 1. Slowdown of the growth was noticed in samples subjected to the extract at both doses. Absorbance readings were taken at 5hrs intervals for 30 hrs post extract additions and plotted to create the growth curve and RNA extraction for mRNA levels and relative quantifications by qPCR. For data analyses, Ct values were produced from RT-PCR assays (Table 4) and used in the REST program.

Gene expression of biofilm-associated genes

Virulence genes used in this study were known to be mostly involved in the biofilm formation of *S. mutans* and compared their expression under the two conditions with and without adding plant extracts. Gene expression at mRNA levels of all the tested genes (Table 1) for RT-qPCR was standardized using the reference gene 16S rRNA as previously used (6). No significant changes were noted in the expression of the 16S rRNA gene under both conditions nor in the different isolates tested. Each test was carried out with two separate RNA samples in duplicates. To

Table 2. Identification of *S. mutns* by biochemical tests.

No	Test	Results
1	Gram- stain	Positive
2	Oxidase	Positive
3	Esculyn lysis	Positive
4	Mannitol	Positive
5	Starch	Negative
6	Glucose	Positive

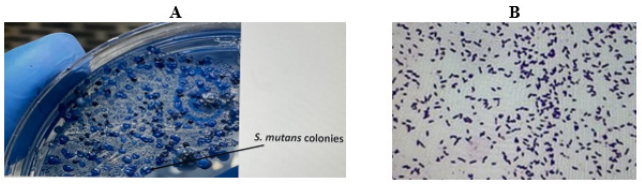

Figure 1. A: *S. mutans* colonies on MSB agar and **B:** Gram-positive, purple stained.

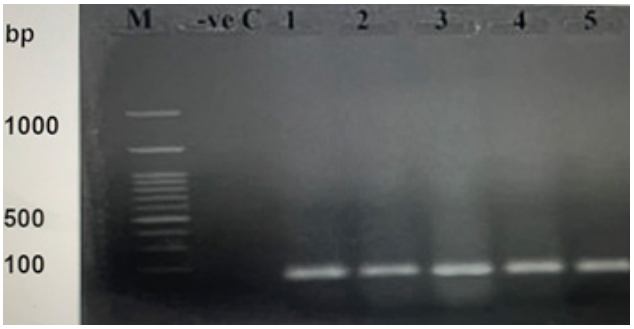

Figure 2. Amplification of a 156-bp 16s rRNA gene of *S. mutans* isolates on 1.5 % agarose gel electrophoreses, M (Marker), -ve Control (no template), isolates 1-5 referred by lanes 1-5.

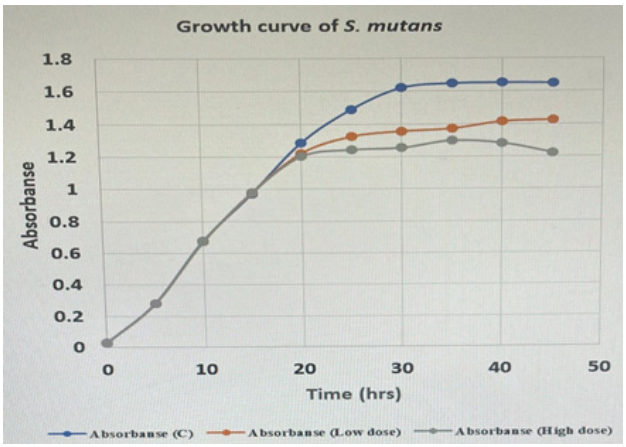

Figure 3. *S. mutans* growth curves of isolate no. 1, with adding plant extract (Low 64 µg/mL and high dose 128 µg/mL) and control (C: no added extract) groups.

Table 3. Determination of MIC.

Conc. (µg/mL)	Isolate number				
	1	4	7	15	25
1024	0	0	0	0	0
512	0	0	0.009	0.015	0.003
256	0.008	0.015	0.053	0.053	0.059
128	0.765	0.201	0.124	0.293	0.233
64	0.87	0.244	0.279	0.336	0.363

Table 4. Several *S. mutans* virulence genes and their Ct values were produced from RT-PCR under the effect of low and high doses of Meswak concentrations (the presented values are only for 3 isolates).

Isolate	Samples	Virulence genes			
		ldh	*gtfC*	*gtfB*	*brpA*
		20.42	25.35	23.77	19.34
	Control	22.46	21.19	23.65	18.13
		20.76	25.27	24.25	19.34
		23.07	26.52	25.01	23.05
1	**Low dose 64 µg/mL**	24.03	25.57	24.95	22.94
		23.08	26.59	25.10	23.07
		23.42	28.35	26.77	22.34
	High dose 128 µg/mL	24.46	27.19	26.65	21.13
		23.76	28.27	27.25	22.34
		19.79	19.62	21.16	18.65
	Control	21.14	22.09	22.10	19.46
		19.73	20.67	21.09	18.65
		23.19	25.11	24.18	21.09
4	**Low dose 64 µg/mL**	23.08	25.07	25.25	21.04
		24.03	26.09	24.17	20.87
		26.84	26.69	28.22	25.71
	High dose 128 µg/mL	26.72	29.51	26.13	23.72
		26.78	29.72	28.16	25.01
		20.63	22.69	21.56	19.88
	Control	20.52	22.67	21.34	19.65
		20.47	22.56	21.32	19.46
		22.09	24.18	24.24	21.41
7	**Low dose 64 µg/mL**	22.24	24.16	24.20	21.56
		22.15	24.22	24.19	21.44
		27.63	28.62	28.74	25.78
	High dose 128 µg/mL	25.52	27.98	26.61	25.69
		27.47	28.52	28.78	23.69

investigate if the virulence gene expressions were similar or if different bacterial cells were grown with and without 2 doses of the plant extract, comparative real-time RT-PCR analysis was then used. For this RNA samples with equal amounts from each culture for quantification of the mRNA levels of these virulence genes.

Relative expression of *S. mutans* virulence genes

Data were analysed to compare target genes (virulence genes) with reference gene. The data for isolate no. 1 is showed below, as an example to explain the change of virulence gene mRNA levels with both doses of the plant extract used in this study. The isolate was positive for all 4 target genes under study. Cells of *S. mutans* isolate 1 were grown with and without both concentrations of the extract starting from 15hrs to 48 hrs. REST 2009 software program was used to analyse results and to measure statistically significant changes for the virulence genes tested. High-dose treatment had different effects on virulence gene mRNA levels. The extract clearly caused a significant decrease in mRNA levels of all 4 virulence genes (Figure 4). Comparable results were found in samples exposed to low doses of the extract.

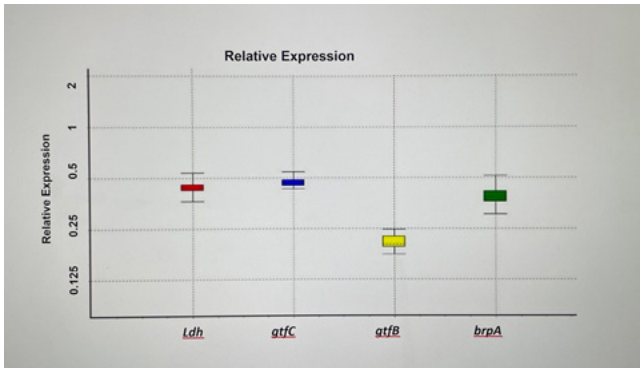

Figure 4. Relative Expression Ratio of four target genes in isolates No.1 exposed to the high dose of Miswak plant extract.

Virulence gene expression in cultures exposed to low and high doses of the plant extract.

Five isolates were selected to examine the effect of the extract on the mRNA levels using qPCR after growing them with both doses of the extract for about 30hrs. These isolates were selected on the bases that they have the studied virulence genes as it confirmed by PCR. mRNA concentrations of virulence genes were regulated differentially in cultures with added both extract doses. The isolates produced significantly lower virulence gene mRNA levels in cultures grown with both plant extract doses (Table 5).

Table 5. Fold changes of virulence gene mRNA levels analysed by REST-2009.

Isolate	Fold changes under the effect of low dose 64 µg/mL				Fold changes under the effect of high dose 128 µg/mL			
	ldh	***gtfC***	***gtfB***	***brpA***	***ldh***	***gtfC***	***gtfB***	***brpA***
1	0.35	0.34	0.69	0.10*	0.2*	0.09*	0.16*	0.16*
4	0.15*	0.06*	0.16	0.31*	0.01*	0.09*	0.02	0.02*
7	0.43*	0.47*	0.20	0.39*	0.02*	0.03*	0.01*	0.04
15	0.02*	0.09*	0.08*	0.03*	0.03*	0.07*	0.06*	0.06*
25	0.09*	0.05*	0.06*	0.01*	0.06*	0.07*	0.02*	0.03*

Star symbols indicate significant reduction ($p<0.05$).

Discussion

There has been a recent reappearance of interest in the use of plant extracts and their effect on oral health. Their perspective on antibacterial impact could be of use in the avoidance and cure of oral diseases. Oral hygiene is one of the most efficient approaches to prevent dental carries, such as the use of mouthwash (11), which is the ideal vehicle in which to incorporate antimicrobial molecules to prevent bad breath and decrease plaque biofilm (12). Apart from antimicrobial properties, mouthwash can also include chlorhexidine, cetylpyridinium chloride, and important oils. These molecules can be regarded as curative because they include active components intended to decrease such conditions as gingivitis, dental carries and periodontitis (13). Limiting oral pathogens such as *S. mutans*, their growth and virulence properties are crucial to safeguarding oral health. Frequent cleaning with toothpaste has been reported to reduce periodontal disease and tooth decay (12). Due to the emergence of antibiotic resistance among oral pathogens, it is important to study their control of these pathogens in particular *S. mutans* by plant extracts. The pathogen utilizes a number of mechanisms such as tolerance to pH, consumption of several carbon sources, biofilm formation and adhesin production (14).

The main components of Miswak are sulphur, alkaloids, and butanediamide which have antibacterial activity, chlorides and fluorides help in enamel remineralization and others (15). Examining gene expression in bacteria and its effects on the host has already been reported a lot (15-18). The analysis of the previous reports supports the anti-cariogenic properties of polyphenols on cariogenic Streptococci, probably due to a direct effect of polyphenols against *S. mutans*; an interaction of polyphenols with microbial membrane proteins inhibiting the adherence of bacterial cells to the tooth surface; and the inhibition of glucosyl transferase and amylase in bacteria. Surprisingly, for all differentially expressed virulence genes, the abundance was lower in the extract-exposed cells than in the control. In cells exposed to the extract, a significant reduction in the abundance of all 4 virulence genes at mRNA levels was found. The most interesting effect of the extract on *S. mutans* was the reduction in the mRNA expressions of virulence genes under study. In this study, we demonstrate that *S. persica* plant extract could decrease the growth of *S. mutans* and degrade its mature biofilms, similar results reported that the extract has antibacterial activity against oral pathogens by reducing their growth (5). Our findings also show a significant decrease in certain virulent genes (*ldh, gtfC, gtfB* and *brpA*) expressed by *S. mutans*. The reduction of virulence gene expressions was higher with the effect of a higher dose of the extract. Overall data produced from this study suggest the possible use of *S. persica* extract to reduce the pathogenesis of *S. mutans.*

Hence, the main components of *S. persica* such as benzyl isothiocyanate can be used as a good antibacterial compound for oral hygiene. The extract can also be used as an alternative to other mouth washes such as chlorohexidine, which has many disadvantages including its unpleasant taste and tooth yellowing (14). Regarding the acute toxicity of the plant extract used in this study such as benzyl isothiocyanate need also to be tested at different concentrations in human subjects before its marketing. Though, since it has been used for a long time without known harmfulness, its use is highly recommended for oral hygiene.

In the current study, we have concluded that these plant extracts require ongoing investigations, particularly through antimicrobial efficacy and user satisfaction studies. Because mouthwash solutions are part of the cosmetic industry, non-prescription and chosen by spontaneous consumer demand. Taste is therefore still an important factor as it impacts consumer compliance, especially when continuous use is mandated. Therefore, suppressing the ability of *S. mutans* to form biofilm may be an appealing approach to prevent dental caries. Isolation and purification of phytoconstituents from these plants may yield significant novel antimicrobials, as plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose without any adverse effects that are often associated with synthetic compounds.

Interest conflict

The authors declare that they do not have any conflict of interest in the research work and publish the article.

Author's contribution

Salah Tofik Jalal Balaky designed the plan idea and was responsible for the interpretation of the results, and supervised the whole project and revised the final manuscript. Ali Idris Jamal performed the experimental work and wrote the manuscript. Both authors read the manuscript and were approved to be responsible for all parts of the work and agreed on the final manuscript.

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