

Inhibitory capacity of *Rhus coriaria* L. extract and its major component methyl gallate on *Streptococcus mutans* biofilm formation by optical profilometry: Potential applications for oral health

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Abstract. *Streptococcus mutans* (*S. mutans*) bacterium is the most well recognized pathogen involved in pathogenesis of dental caries. Its virulence arises from its ability to produce a biofilm and acidogenicity, causing tooth decay. Discovery of natural products capable to inhibit biofilm formation is of high importance for developing health care products. To the best of our knowledge, in all previous scientific reports, a colorimetric assay was applied to test the effect of sumac and methyl gallate (MG) on *S. mutans* adherence. Quantitative assessment of the developed biofilm should be further performed by applying an optical profilometry assay, and by testing the effect on both surface roughness and thickness parameters of the biofilm. To the best of our knowledge, this is the first study to report the effect of sumac extract and its constituent MG on biofilm formation using an optical profilometry assay. Testing antibacterial activity of the sumac extract and its fractions revealed that MG is the most bioactive component against *S. mutans* bacteria. It

reduced *S. mutans* biofilm biomass on the polystyrene surface by 68-93%, whereas 1 mg/ml MG was able to decrease the biofilm roughness and thickness on the glass surface by 99%. MG also prevented a decrease in pH level by 97%. These bioactivities of MG occurred in a dose-dependent manner and were significant vs. untreated bacteria. The findings are important for the development of novel pharmaceuticals and formulations of natural products and extracts that possess anti-biofilm activities with primary applications for oral health, and in a broader context, for the treatment of various bacterial infections.

Introduction

Streptococcus mutans (*S. mutans*) is the most well recognized causative agent implicated in the pathogenesis of dental caries (tooth decay), which is an infectious disease of human dentition still exhibiting high global prevalence (1-3). Virulence of this bacterium arises from its ability to form a biofilm on teeth and produce organic acids (acidogenicity) from dietary sucrose within the biofilm, causing tooth decay (4,5). The structural matrix of the biofilm consists of water-insoluble glucans synthesized from sucrose by several isoforms of the glucosyltransferase (Gtf) enzyme present in *S. mutans* bacteria (5). In this respect, *S. mutans* produces water-insoluble and partly water-soluble glucans using GtfB and GtfC enzymes encoded by *gtfB* and *gtfC* genes, respectively. The synthesized insoluble glucans possess a capacity to concentrate protons generated by the proton-extruding F-type ATPase (F-ATPase), thereby retaining acidogenicity of *S. mutans* biofilm (6). In addition, it has been reported that biofilm bacteria have up to a 1,000-fold more tolerance to antimicrobials than planktonic bacteria (7,8). Therefore, it is important to develop novel pharmaceuticals in order to inhibit *S. mutans* biofilm formation and its acidogenicity.

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Abbreviations: *S. mutans*, *Streptococcus mutans*; MSE, methanolic sumac extract; MG, methyl gallate; HPLC, high-performance liquid chromatography

Key words: *Rhus coriaria* L., sumac, methyl gallate, *Streptococcus mutans*, biofilm, acidogenicity, natural product

Natural products have been optimized to interact with biological systems through a long natural selection process (9), and because of this, nature is considered the best concocter of medicines and has been a source of medicines for millennia (10-13). During the last two decades, various plants have been tested for their antimicrobial activity and many of them exhibit significant antibacterial activity against *Streptococcus* species (14). In this context, *Rhus coriaria* L. (sumac) fruits contain many of the bioactive compounds that were characterized in detail by using high-performance liquid chromatography-diode array detector-hyphenated with tandem mass spectrometry (HPLC-DAD-ESI-MS/MS) in the investigation performed by Abu-Reidah *et al* (15). Among these bioactive components, methyl gallate (MG) has antibacterial and anti-biofilm effects for *S. mutans* bacteria, as reported by Kang *et al* (16). However, currently available studies did not provide sufficient data whether MG can suppress the development of *S. mutans* biofilm on polystyrene and glass surfaces as well as inhibit acidogenicity. In the scientific report of Kang *et al* (16), colorimetric assay was applied for testing the effect of MG on *S. mutans* adherence. Colorimetric assay is considered a well-established method for quantification of the biofilm biomass (17,18). It could be used as a pre-test for checking potential existence of the biofilm. Quantitative assessment of the developed biofilm should be further performed applying an optical profilometry assay, by testing the effect on both surface roughness and thickness parameters of the biofilm. To our best of knowledge, this is the first article reporting the effect of sumac methanolic extract and its constituent MG (bioactive phytochemical that was isolated from the crude extract and quantitated by HPLC) on biofilm formation by using an optical profilometry assay.

Materials and methods

Materials. *Rhus coriaria* L. was purchased from Al Alim Ltd. (Medicinal Herb Center, Zippori, Israel), and the fruits were ground to yield a reddish-purple powder. MG (analytical standard) was purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany).

Plant extraction. The air-dried, powdered fruit of sumac plant (25 g) was packed in an Erlenmeyer flask and extracted with 250 ml methanol (MeOH), sonicated for 2 h at 45°C, then left in a dark glass bottle at room temperature for 24 h for complete extraction. The methanolic extract was filtered and evaporated to dryness with a rotary vacuum evaporator.

Instruments. A Waters Alliance e2695 separations module, 2998 photo diode array (PDA) and Empower version 3 software was used (Waters, Eschborn, Germany). The preparative HPLC system consisted of a 3535-quaternary gradient module and a 996 PDA detector.

¹H-NMR and ¹³C-NMR measurements of isolated MG from sumac was carried out on a Bruker Avance II 500 spectrometer, which was equipped with a 5 mm indirect detection probe with Z gradient.

The UHPLC system (Accela; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was equipped with an XBridge ODS-column (150x2.1 mm i.d.), 3.5-μm particle size

(Waters, Milford, MA, USA) and a mobile phase containing 0.1% formic acid (FA) as (eluent A) and MeOH containing 0.1% FA as (eluent B). Linear gradient elution was used starting with 95% A and continuously increased to 100% B in 20 min. The UHPLC system was coupled to LTQ Orbitrap XL system (Thermo Fisher Scientific, Inc.) equipped with an electrospray ionization (ESI) source. The positive ionization mode was used at a scan range of m/z 100-1000.

Chromatographic conditions. MG quantitation was run on a Waters HPLC ODS Column (XBridge, 4.6 ID x150 mm, 5 μm) with a guard column (Xbridge ODS, 20x4.6 mm ID, 5 μm). The mobile phase consisted of water (labeled A) and acetonitrile (labeled B) solvent mixture. The gradient was as follows: 95% A and 5% B at 0 min, held there for 2 min, then raised to 50% A and 50% B over 13 min, then to 10% A and 90% B over 1 min, held there for 3 min, and finally returned to 95% A, 5% B over 1 min. All of the samples were filtered with a 0.45 μm micro-porous filter. The PDA wavelengths ranged from 210 to 500 nm, and the monitoring wavelength of MG was 272 nm. The flow rate was 1 ml/min. The injection volume was 10 μl, and the column temperature was at room temperature.

The UHPLC was equipped with an XBridge ODS-column (150x2.1 mm i.d.), 3.5 μm particle size (Waters) and a mobile phase containing 0.1% formic acid (FA) as (eluent A) and MeOH containing 0.1% FA as (eluent B). Linear gradient elution was used starting with 95% A and continuously increased to 100% B in 20 min.

The prep-HPLC experiments were run on an ODS column (Agilent PrepHT C18, 22.2x250 mm, 10 μm). The linear gradient started at 98% A, where it stayed for 3 min; it was then raised to 5% A over 15 min, where it remained for another 4 min and was then returned to 98% A over 2 min. The flow rate used was 15 ml/min, the injection volume was 1,000 μl.

Methyl gallate standards preparation. Five different concentration levels of standards for MG were prepared: 10, 50, 100, 250 and 500 ppm in methanol diluents. These standards were used to construct a calibration curve to quantitate MG in sumac.

Sumac sample preparation for the preparative HPLC injections. Crude sumac was prepared by dissolving ~3,000 mg in 15 ml of methanol, sonicated for 3 min and then filtered via a 0.45 μm membrane filter before injection. The concentration of the final red solution was 200 mg/ml. This solution (1 ml) was injected into the preparative HPLC Chromatograph and six fractions were collected.

Bacterial strain and culture conditions. *Streptococcus mutans* UA159 (700610; American Type Culture Collection, Manassas, VA, USA) was selected for this study because it preferably colonizes humans. Stocks of this strain were maintained in 10% skimmed milk (Difco; BD BioSciences, Franklin Lakes, NJ, USA) at -70°C until use. Prior to experiments, *S. mutans* was cultured in Bacto™ Todd Hewitt broth (THB; BD BioSciences) under anaerobic conditions (95% N₂ and 5% CO₂) at 37°C for 18 h. Purity of the culture was checked on Mitis Salivarius agar (Difco; BD BioSciences)

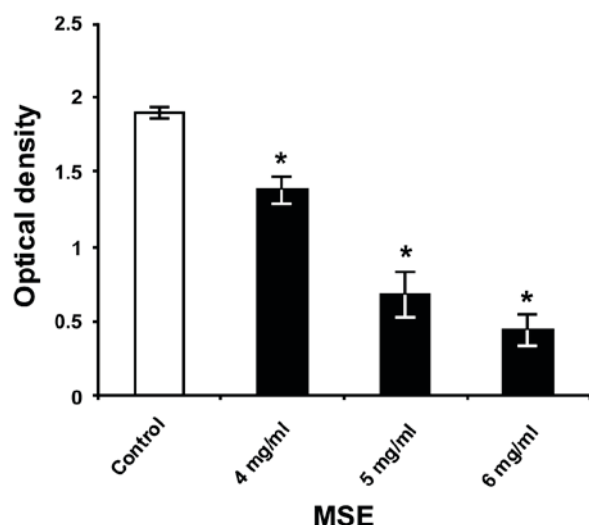


Figure 1. *Streptococcus mutans* biofilm biomass formed on the polystyrene surface after 24 h of incubation in THB containing 1% sucrose and different concentrations of MSE. Data are presented as the mean \pm standard error from three independent experiments (n=4-11). *P<0.05 vs. control group. MSE, methanolic sumac extract.

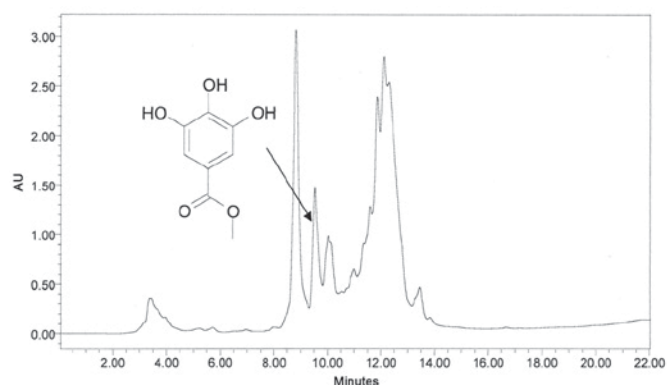


Figure 2. A typical preparative high performance liquid chromatography chromatogram of a crude methanol mixture of sumac-1,000 μ l was injected at a flow rate of 15 ml/min. The monitoring wavelength was 272 nm. The intervals of the fractions were as follows: I (1-8 min), II (8-9.1 min), III (9.1-9.9 min), IV (9.9-12.6 min), V (12.6-16 min), and VI (16-22 min). Fraction III is methyl gallate.

and Columbia agar with 7% sheep blood (E&O Laboratories, Bonnybridge, Scotland).

Biofilm formation and treatments. To evaluate the effect of treatments, *S. mutans* biofilm formation was performed on polystyrene and glass surfaces in separate experiments. Prior to each experiment, the optical density (OD) of the bacterial culture was adjusted to 0.2 at 630 nm, using a microplate-reader spectrophotometer. For biofilm formation on the polystyrene surface, 24-well, flat-bottomed, polystyrene cell culture plates (Sarstedt, Nümbrecht, Germany) were filled with THB containing 1% sucrose, and then solutions of methanolic sumac extract (MSE), prepared in sterile distilled water (Milli-Q water), were added to appropriate wells in the plates at final concentrations of 4, 5 and 6 mg/ml. Solutions of MG, also prepared in Milli-Q water, were added to appropriate wells in the plates at final concentrations of 0.55, 0.7,

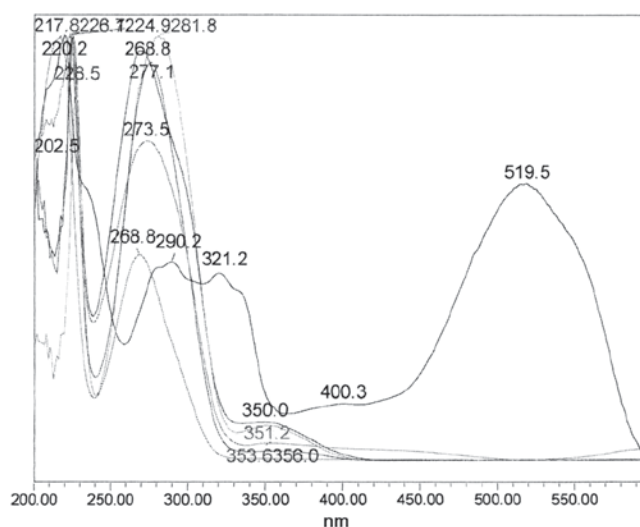


Figure 3. Overlay of ultraviolet-visible spectra of the eluted peaks of the crude methanolic extract of sumac in the range of 210-500 nm.

0.85 and 1 mg/ml. Afterwards, *S. mutans* bacteria was added to the wells at a final dilution of 1:100, and all of the plates were incubated anaerobically at 37°C for 24 h. Quantification of the formed biofilm (biomass) was performed using a colorimetric assay. The same experimental procedures were used for biofilm formation on the glass surface, except that sterile glass slides of 1-mm thickness, cut from standard microscope slides (76x26 mm; Thermo Fisher Scientific, Inc.), were inserted vertically into wells containing only MG at the above indicated concentrations prior to inoculation of bacteria. Quantitative assessment of the developed biofilm was further performed applying an optical profilometry assay. In these experiments, plate wells without bacterial cells were used as blank controls, and the untreated bacteria served as experimental controls.

Colorimetric assay. After 24 h of incubation, THB was discarded from plates, wells were rinsed with distilled water to remove loosely bound bacterial cells, and then adherent bacteria were fixed with 95% ethanol. For quantification of biofilm biomass, the fixed and air-dried *S. mutans* biofilm in plate wells was stained with 1 ml/well of 0.01% crystal violet solution (Merck KGaA) for 15 min, and then the bound dye was extracted using 1 ml/well of 33% acetic acid solution (Merck KGaA) for 30 min. Afterwards, 200 μ l extracted dye solution from each well was transferred to appropriate wells in an optically clear, flat-bottomed 96-well microplate. The OD of the samples was measured at a wavelength of 595 nm with a microplate-reader spectrophotometer. Background staining was corrected for by subtracting amount of the staining in blank wells.

Optical profilometry assay. Following 24 h of incubation, glass slides with adherent *S. mutans* biofilm were removed from plate wells, air-dried and further analyzed using a non-contact optical imaging profilometer Sensofar PL μ 2300 system (Terrassa, Spain), using a x50 confocal objective with a view field of 253x190 μ m. Primarily, six regions of the glass slide were scanned seeking to evaluate surface roughness per slide

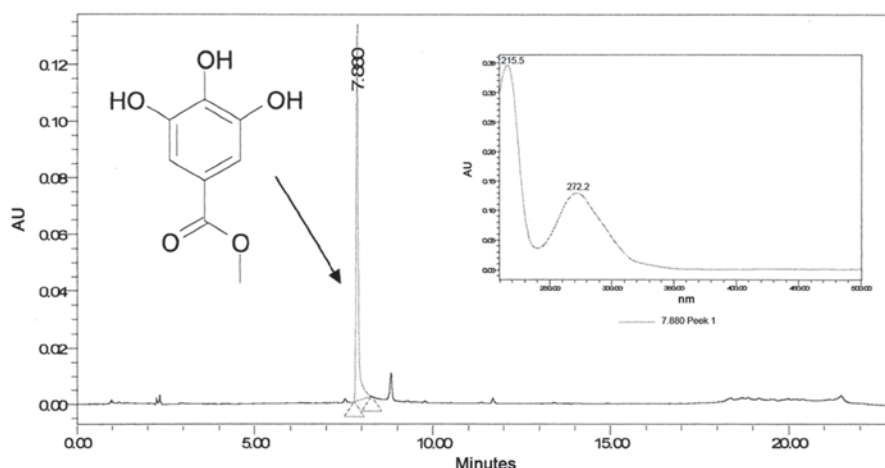


Figure 4. Chromatogram of the reinjected fraction III on analytical high performance liquid chromatography, which contained relatively pure methyl gallate at 7.88 min, with its corresponding ultraviolet-visible spectrum (210-500 nm), presented in the right corner of the figure.

halfway from bottom to top of visible biofilm. Afterwards, a vertical scratch was artificially made down the glass surface by a scalpel in the middle of every slide covered with biofilm, and then five regions of the glass slide were scanned to assess the biofilm thickness per slide halfway from the bottom to the top of the visible biofilm. The bottom of the scratch served as a reference point for accurate measurement of the biofilm thickness. All images were captured in a vertical scanning mode, and data of the collected images were further processed using Gwyddion software (version 2.40; <http://gwyddion.net>) to calculate the surface roughness and biofilm thickness parameters. A median filter (10 pixels or 3 μm) was selected to remove the errors of form and waviness. Root mean square roughness (R_q), as the most critical parameter, was calculated to evaluate quantitatively the slide surface roughness, which indicated adherence of bacteria. The R_q parameter is an average of the measured height deviations taken within the evaluation length and measured from the mean line. Indeed, R_q represents standard deviation of the surface profile heights, and it is calculated according to ISO 4287/1-1997 standard by the following formula:

$$R_q = \sqrt{\frac{1}{N} \sum_{j=1}^N r_j^2}$$

Where N is the number of points within a sampling length, and r_j is the height value at point j . To measure the biofilm thickness, which indicated the maturity of biofilm, the height of the artificially produced vertical scratch on each slide with adherent bacteria was used. Calculation of the biofilm thickness involved generation of the height distribution graph curve from the entire area of the scanned region containing the scratch, followed by Gaussian function fitting as defined here:

$$f(x) = y_0 + a \exp[-(x-x_0)^2/b^2]$$

Where y_0 is the peak height, a is the amplitude (height) distribution, x_0 is the peak position, and b is the standard deviation. Background for the parameters of surface roughness and biofilm thickness was corrected for by subtracting the R_q and thickness values of a blank glass slide.

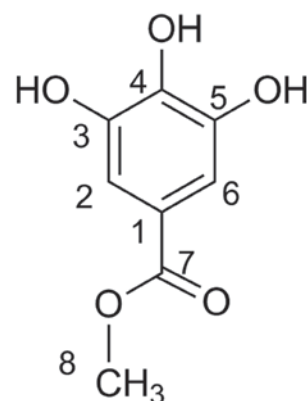


Figure 5. Numbered carbon atoms in methyl gallate for nuclear magnetic resonance spectroscopy analysis.

Biofilm acidogenicity. *S. mutans* biofilm formation and treatments were performed using the same procedures as described above. After 24 h of incubation, the biofilm growth medium (THB) was collected from the wells of all plates and transferred to 1.5 ml microcentrifuge tubes. The pH of *S. mutans* biofilm growth medium collected in 1.5 ml microcentrifuge tubes was measured with a microelectrode connected to a benchtop pH meter (Knick 766 Calimatic) at room temperature. The microelectrode was calibrated using standard pH buffers (pH 4.01 and 7.00) prior to and following each measurement.

Statistical analysis. Data were analyzed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA). Differences between the control (untreated) and treatment groups were evaluated applying one-way analysis of variance followed by a post hoc least significant difference test for multiple comparisons. Data are presented as the mean \pm standard error. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Effect of the MSE on *S. mutans* biofilm formation on polystyrene surface. The primary assessment of crude MSE efficiency for the inhibition of *S. mutans* biofilm formation showed its

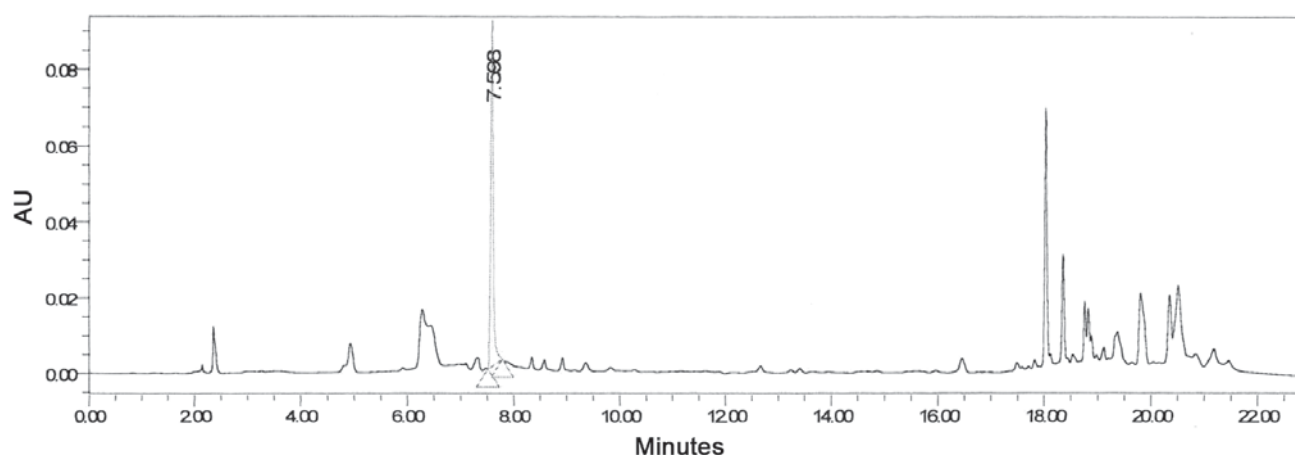


Figure 6. Chromatogram showing the methyl gallate peak in the methanol extract, with its corresponding ultraviolet-visible spectrum (210-500 nm).

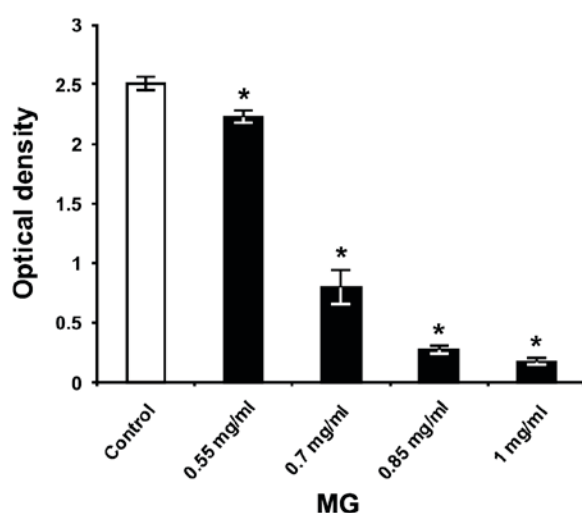


Figure 7. *Streptococcus mutans* biofilm biomass formed on the polystyrene surface after 24 h of incubation in THB containing 1% sucrose and different concentrations of MG. Data are presented as the mean \pm standard error from three independent experiments (n=10-11). *P<0.05 vs. control group. MG, methyl gallate.

capacity to reduce significantly the biofilm biomass on the polystyrene surface in a dose-dependent manner (Fig. 1). The greatest inhibitory effect of crude extract was achieved using a concentration of 6 mg/ml, which decreased *S. mutans* biofilm formation by 77%, compared to the formation of untreated bacteria (P<0.05) cultured in THB with 1% sucrose.

Analysis of methanol extract of sumac and isolation of the bioactive phytochemical. Sumac is very rich in phenolic and anthocyanin compounds. These classes of compounds are known to exhibit a range of biological activities. In the attempt to isolate the active ingredients from sumac, several analytical HPLC runs were performed using different mobile phases. Upon scaling up using preparative HPLC, separations were carried out by collecting six fractions, which were subjected to antibacterial assays. A typical preparative HPLC chromatogram of the crude methanol mixture and its corresponding overlaid UV-Vis spectra, in the range of 210-500 nm, are presented in Figs. 2 and 3, respectively.

The ultraviolet-visible spectra demonstrated many phenolics that possess typical absorption maximums between 268.8 and 277.1 nm. There was also a very strong absorption maximum at 519.5 nm, which is a typical anthocyanin compound that is responsible for the red pigment in sumac (Fig. 3).

Fraction III (Fig. 2) contains pure compound, which, upon reinjection using analytical HPLC-PDA, reached a peak at 7.88 min with a maximum wavelength of 272 nm (Fig. 4).

Accurate high-resolution mass spectroscopy (HR-MS) and ^1H -NMR and ^{13}C -NMR disclosed the identity of the peak at 7.88 min to be MG (Fig. 5). The HR-QTOF-MS using the ESI in the positive mode gave a protonated molecular ion of $[\text{M}+\text{H}]^+$ at m/z 185.0452 Da (calculated for $\text{C}_8\text{H}_9\text{O}_5^+$, m/z 185.0450 Da). ^1H -NMR (CD_3OD) gave peaks at δ : 3.8 (3H s, $-\text{OCH}_3$), 7.05 (2H s, H-2/ H-6). ^{13}C -NMR (CD_3OD) gave peaks at δ : 52 (C-8), 109 (C-2/C-6), 120.5 (C-1), 139 (C-4), 145.3 (C-3/C-5), 168 (C-7).

Analytical HPLC was used to construct a calibration curve of MG at 5 levels (10, 25, 50, 100, 250 and 500 ppm) with a coefficient of determination (R^2) of >0.999. The concentration of MG in the methanol extract was identified to be 4.85 ppm. Many other peaks that have a more lipophilic character were eluted between 18 and 22 mins (Fig. 6).

Effect of MG on *S. mutans* biofilm formation on polystyrene and glass surfaces. In an effort to evaluate anti-biofilm activity, the isolated MG (fraction III) was further tested on *S. mutans* strain UA159 because of its well-defined features to form biofilms on various solid surfaces (19,20). As presented in Fig. 7, all of the concentrations of MG significantly reduced *S. mutans* biofilm biomass on the polystyrene surface, compared with untreated bacteria (P<0.05) in THB containing 1% sucrose. The anti-biofilm activity of MG occurred in a dose-dependent manner, exhibiting its highest effect at concentrations of 0.7, 0.85 and 1 mg/ml, with biofilm inhibition at 68, 89 and 93%, respectively. These results confirmed the findings reported by Kang *et al* (16), where the researchers demonstrated using beaker-wire tests that 1 mg/ml MG significantly reduced the wet weight of *S. mutans* (strain Ingbritt) biofilm biomass in comparison with untreated bacteria, after 24 h of incubation in medium containing 5% sucrose.

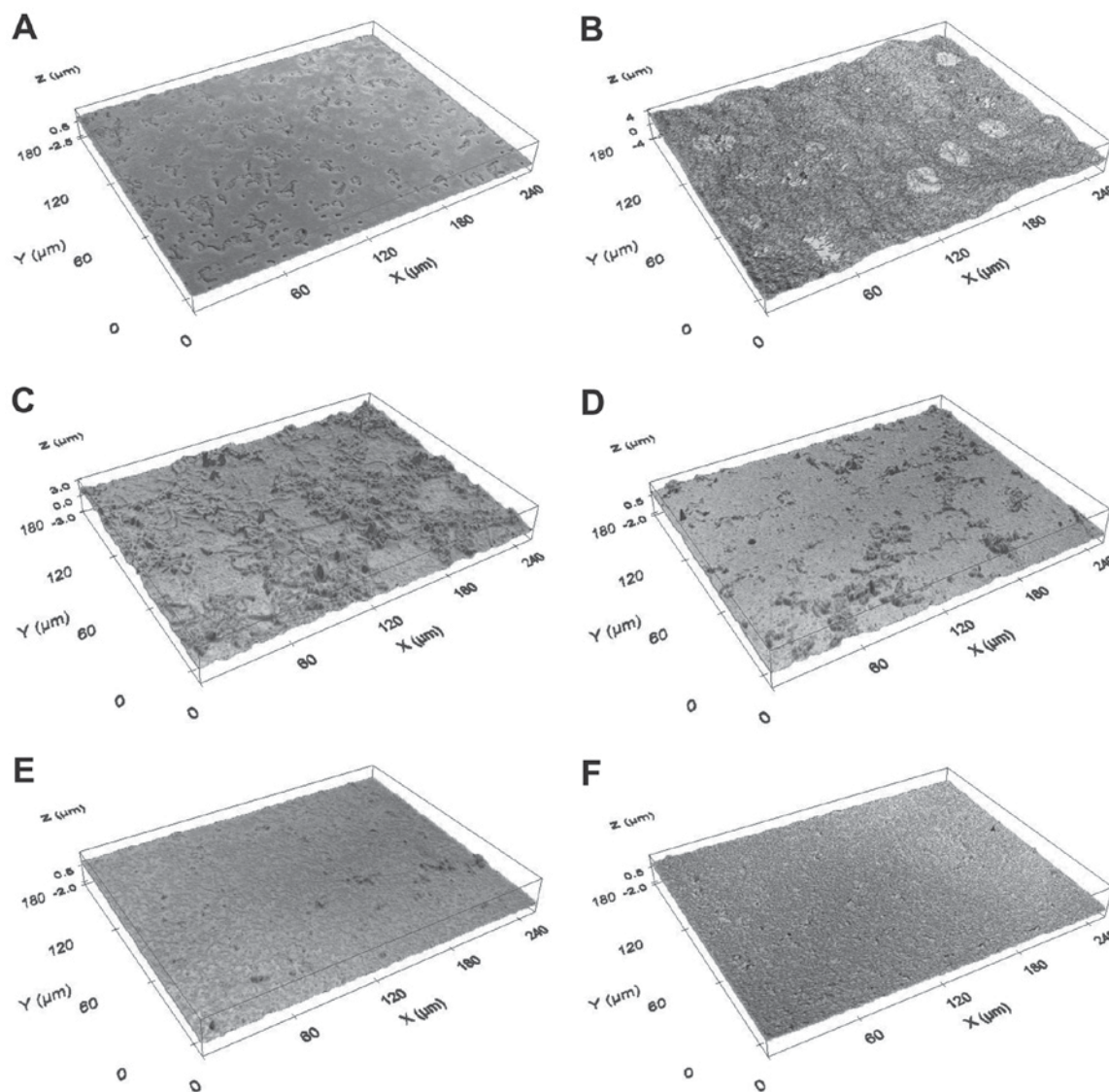


Figure 8. Optical profile of the glass slides with *Streptococcus mutans* culture biofilm after 24 h of incubation in the presence of different concentrations of MG. Glass slide surface of bacteria incubated (A) without MG, in the absence of sucrose, (B) without MG, in the presence of 1% sucrose, and treated with (C) 0.55, (D) 0.70, (E) 0.85 and (F) 1.00 mg/ml MG. Magnification, x50. MG, methyl gallate.

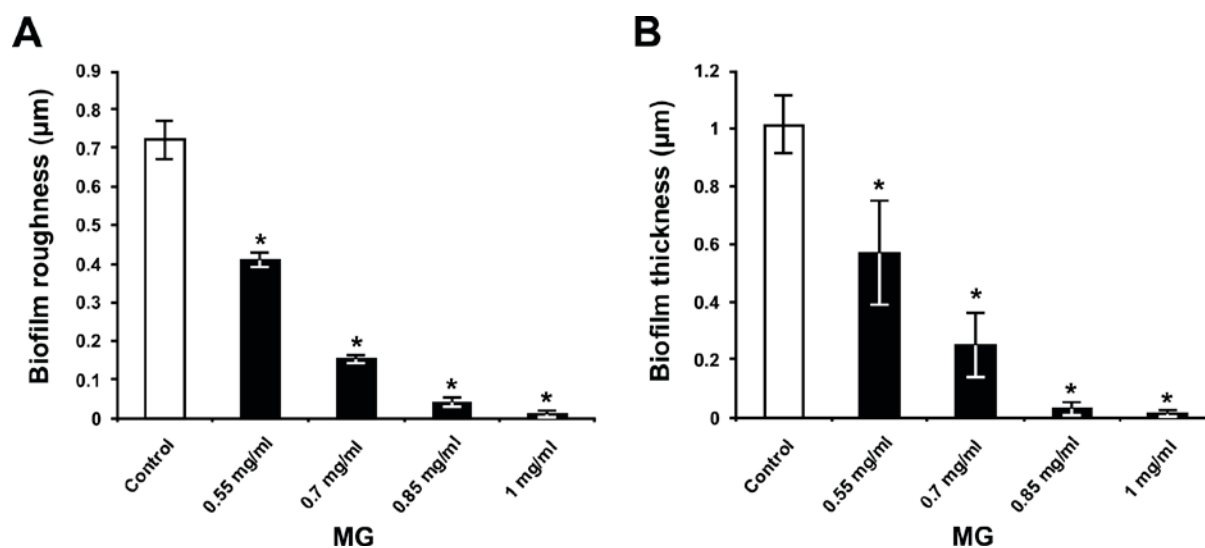


Figure 9. Quantities of *Streptococcus mutans* biofilm formed on the glass slide surface after 24 h of incubation in THB containing 1% sucrose and different concentrations of MG. (A) the surface roughness parameter R_q of the biofilm on glass slides and (B) the biofilm thickness. Data are presented as the mean \pm standard error from three independent experiments (n=18, biofilm roughness; n=15, biofilm thickness). *P<0.05 vs. control group.

Table I. pH levels of the *Streptococcus mutans* biofilm growth medium after 24 h of incubation in the presence of 1% sucrose and different concentrations of methanolic sumac extract.

Experimental group	pH
Blank	7.20±0.04 ^a
Control	4.07±0.03
MSE (4 mg/ml)	4.77±0.09 ^a
MSE (5 mg/ml)	5.21±0.11 ^a
MSE (6 mg/ml)	5.30±0.07 ^a

Data are presented as the mean ± standard error from three independent experiments (n=4-11). ^aP<0.05 vs. control group.

Table II. pH levels of the *Streptococcus mutans* biofilm growth medium after 24 h of incubation in the presence of 1% sucrose and different concentrations of methyl gallate.

Experimental group	pH
Blank	7.47±0.02 ^a
Control	4.28±0.02
MG (0.55 mg/ml)	6.14±0.17 ^a
MG (0.7 mg/ml)	7.17±0.03 ^a
MG (0.85 mg/ml)	7.27±0.01 ^a
MG (1 mg/ml)	7.28±0.02 ^a

Data are presented as the mean ± standard error from four independent experiments (n=10-11). ^aP<0.05 vs. control group.

The optical profilometry technique, applied in analysis of the surfaces of glass slides with *S. mutans* biofilm, was used to confirm the results of the colorimetric assay. Firstly, compared with bacteria incubated without MG and sucrose (Fig. 8A), where the R_q and thickness parameters for the untreated bacteria grown without sucrose were 0.04±0.01 and 0.02±0.01 μm , respectively, presence of 1% sucrose in THB induced adherence of the bacteria to glass slides and subsequent maturation of the biofilm (Fig. 8B). Secondly, in the THB with 1% sucrose, exposure of *S. mutans* to 0.55, 0.7, 0.85 and 1 mg/ml MG (Figs. 8C-F, respectively) decreased formation of the biofilm on glass surface in a dose-dependent manner. Quantification revealed that surface roughness parameter R_q of the biofilm (Fig. 9A) and biofilm thickness (Fig. 9B) were increased in control bacteria (Fig. 9); however, MG treatment inhibited this effect in a dose-dependent manner ($P<0.05$; Fig. 9). In this respect, MG concentrations of 0.85 and 1 mg/ml exhibited the greatest effects for reducing the surface roughness parameter R_q of biofilm, by 94 and 99%, respectively (Fig. 9A). Furthermore, these concentrations of MG decreased *S. mutans* biofilm thickness by 97 and 99%, respectively (Fig. 9B).

Effect of MG on *S. mutans* biofilm acidogenicity. The pH measurements of the collected *S. mutans* biofilm growth medium demonstrated that bacteria grown in THB with 1% sucrose produced organic acids from fermentation of

this carbohydrate, leading to an ~1.8-fold decrease in pH, compared with the blank group (Tables I and II). This substantial decrease of pH indicated increased acidogenicity in the *S. mutans* biofilm. However, exposure of the bacteria to crude MSE led to augmentation of pH in a dose-dependent manner, though not up to the pH levels of the blank group (Table I). In contrast, treatment of *S. mutans* bacteria with the isolated MG significantly prevented a decrease in the pH level, compared with untreated bacteria grown in THB with 1% sucrose ($P<0.05$), by increasing the pH almost up to the pH levels of the blank group (Table II). This preventive effect of MG occurred in a dose-dependent manner, and MG concentrations from 0.7 to 1 mg/ml increased pH by 96-97%. Therefore, similarly to reduction of the biofilm biomass, crude MSE was unable to fully suppress the pH decrease in the *S. mutans* biofilm due to reduced concentration of the biologically active compound within it. However, the isolated MG was able to almost completely inhibit the acidogenicity of the *S. mutans* biofilm.

Discussion

The present study demonstrated that the dominant and most antibacterial active compound of *Rhus coriaria* L. is MG. Using an *in vitro* system with *S. mutans* bacteria, it was demonstrated that MG inhibits growth of bacteria, suppresses the biofilm development on different solid surfaces (polystyrene, glass) and prevents the pH decrease in biofilm. Notably, these actions of MG occurred in a dose-dependent manner. It should be noted that Vahid-Dastjerdi *et al* (21) demonstrated the significant effect of *Rhus coriaria* L. water extract in reducing the expression of *S. mutans* *gtfB*, *gtfC* and *gtfD* genes. Another study performed by Thimothe *et al* (22) demonstrated that red wine grape phenolic extracts, containing high amounts of gallic acid, may inhibit the activity of GtfB and C enzymes at 70-85% (22). Furthermore, in the same study, these phenolic extracts also suppressed *S. mutans* F-ATPase activity by 30-65%. In addition, the findings reported by Choi *et al* (23) indicated that MG isolated from *Galla Rhois* may attenuate the action of proton-driven ATPases in *Salmonella* spp. bacteria. Finally, it is noteworthy that, in the presence of sucrose, *S. mutans* adhesion to glass surface and subsequent formation of the biofilm is mainly dependent on the activity of Gtfs, especially those synthesizing water-insoluble glucans (24). Taking into consideration the results of the present study and the studies outlined herein, it may be reasonable to hypothesize that the anti-biofilm effect of MG occurred because of the downregulation of glucosyltransferases, and the decrease of acidogenicity was due to inhibition of F-ATPases in *S. mutans* bacteria. However, the exact mechanisms for these biological effects of MG require elucidation through further studies.

In conclusion, the present study demonstrated that *Rhus coriaria* L. (sumac) contains the biologically active antibacterial natural compound MG. Additionally, MG was revealed to inhibit *S. mutans* biofilm formation on polystyrene and glass surfaces, and suppress the acidogenicity of the *S. mutans* biofilm. Taken together, these findings are important for the development of novel pharmaceuticals and formulations of natural products and extracts that possess anti-biofilm

activities with primary applications for oral health, and in a broader context, for the treatment of various bacterial infections.

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