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Antimicrobial Activity of Silver Nanoparticles Synthesized by the Green Method Using *Rhus coriaria L.* Extract Against Oral Pathogenic Microorganisms

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Abstract

Background: Oral health is part of general health. Dental caries is the most common chronic disease worldwide. Considering the significance of plaque control, complications of chemical agents, and the optimal antimicrobial efficacy of nanoparticles, this study aimed to assess the antimicrobial activity of silver nanoparticles (AgNPs) synthesized by the green method using *Rhus coriaria L.* extract against oral pathogenic microorganisms.

Methods: In this in vitro experimental study, Rhus coriaria fruit was dried at room temperature. It was then ground, and its aqueous extract was obtained by the maceration technique. The effects of AgNPs synthesized by the green method in different concentrations were evaluated against *Enterococcus faecalis* (*E. faecalis*), *Streptococcus mutans* (*S. mutans*), *Streptococcus sobrinus* (*S. sobrinus*), *Lactobacillus acidophilus* (*L. acidophilus*), and *Streptococcus salivarius* (*S. salivarius*) using the well-plate technique. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were also calculated. Data were analyzed by the Wilcoxon test, Kruskal-Wallis test, and Mann-Whitney test.

Results: The MIC values were 1024 µg/mL for *S. mutans* and *E. faecalis*, and 512 µg/mL for *S. sobrinus*, *S. salivarius*, and *L. acidophilus*. The resistance of *S. mutans* and *E. faecalis* was higher than that of *S. sobrinus*, *S. salivarius* and *L. acidophilus*. According to the growth inhibition zones and MBC test results, S. salivarius had the highest resistance to AgNPs followed by *L. acidophilus*, *S. sobrinus*, *S. mutans*, and *E. faecalis*.

Conclusion: AgNPs synthesized by the green method using *Rhus coriaria* extract was effective against oral pathogenic microorganisms. Thus, they may be used in the formulation of mouthwash and toothpaste.

Keywords: Anti-infective agents, Nanoparticles, Bacteria, Plant extracts

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Introduction

Oral health has an impact on the quality of life since it can affect nutrition, speech, and facial esthetics. According to the World Health Organization, oral health is part of general health. Dental caries and periodontitis are among the most common oral diseases in Asia (1). Dental caries is the most common chronic disease worldwide, while

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↑What is "already known" in this topic:
Based on the literature review, a broad spectrum of animal studies has demonstrated the antimicrobial effect of sumac in one or a limited number of bacteria.Moreover, its antimicrobial mechanism is vague compared with the gold standard plaque-controlling agent.

→What this article adds:
For the first time, the effects of different concentrations of Rhus coriaria nanoparticles, which were synthesized by the green method, were evaluated on the most common cariogenic microorganisms.

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periodontal disease is the most common oral disease and the main cause of tooth loss (2). Many systemic conditions such as cardiac disease and miscarriage, may be related to periodontal disease (3, 4). Over 700 bacterial species colonize the oral cavity, approximately 50% of which are not cultivable (5). Cariogenic bacteria that play a role in the destruction of tooth structure include *Streptococcus mutans (S. mutans), Streptococcus sobrinus (S. sobrinus), Streptococcus salivarius (S. salivarius), Streptococcus Sanguinis (S. sanguinis)*, and *Enterococcus faecalis (E. faecalis)* (6). *S. mutans* is also involved in the development of angular cheilitis and parotiditis (3). Antimicrobial agents have been suggested as a simple, low-cost strategy for the prevention of oral diseases (2). Chlorhexidine (CHX) is the gold-standard chemical agent for plaque control. However, several complications (tooth discoloration, oral burning sensation, xerostomia, cytotoxicity, altered sense of taste, and microbial resistance) have been reported following its extensive use. Considering the global demand for new, easily available, cost-effective, and efficient antimicrobial agents with maximum efficacy and minimal complications, the focus was directed to herbal products (7). In this regard, nano-drugs were introduced and soon gained popularity due to higher substantivity, targeted tissue delivery, and fewer side effects (8).

Recently, silver nanoparticles (AgNPs) have been increasingly used as antibacterial agents.

The methods of synthesis of AgNPs include chemical and physical methods such as solgel, chemical reduction, physical vapor deposition, thermal decomposition, and microwave irradiation.

The disadvantages of these methods include high cost, use of high levels of energy, and production of hazardous toxic chemicals. New methods based on green synthesis were recently proposed to overcome such limitations. The green synthesis method uses plant extracts and microorganisms that are not toxic to humans or the environment to synthesize nanoparticles. The synthesis of nanoparticles by using plant extracts has received more attention compared with the use of microorganisms due to the lack of limitations related to the culture of microorganisms and easy availability (9, 10). In addition, using plants for the synthesis of nanoparticles has other advantages, such as the use of safer solvents, milder response conditions, higher feasibility, and various surgical and pharmaceutical applications (11-16).

Sumac, with the scientific name *Rhus coriaria L.* (*R. coriaria*) is endemic to Iran and is cultivated in Tabriz, Zanjan, Tehran, Qazvin, Qom, and Hamadan cities (17). It is used as a spice and also an appetizer. It can decrease stomach acid due to its high tannin, flavonoid, organic acid, protein, fiber, sal volatile, nitrate, nitrite, and antioxidant content (18). The *R. coriaria* fruit is used as a disinfectant for the treatment of constipation and for the reduction of serum triglyceride levels, blood pressure, and uric acid (19). AgNPs are used for the treatment of infectious diseases due to optimal antibacterial effects (12, 13). Evidence shows that AgNPs have stronger antimicrobial effects than gold against *S. mutans*, and they are believed to have lower cytotoxicity (20).

Some studies showed that an aqueous extract of *R. coriaria* inhibited *S. mutans* biofilm formation on stainless steel orthodontic wire (21, 22). Another study indicated higher antibacterial activity of the mixture of aqueous extracts of *R. coriaria, Punica granatum,* and *Rosa damascena*on than 2% CHX against *S. mutans* (23). Furthermore, another study evaluated the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of sumac extract against some microorganisms and reported higher sensitivity of Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*) (24) AgNPs containing these herbal compounds had stronger antimicrobial effects on *S. mutans* as compared to gold and zinc nanoparticles (25). Thus, the synthesis of nano-drugs has attracted the attention of researchers due to benefits such as longer durability, targeted tissue delivery, and fewer side effects.

Considering the significance of plaque control in oral health, complications of chemical products, optimal efficacy of nanoparticles, and disinfecting effects of *R. coriaria*, this study aimed to assess the effects of AgNPs synthesized by the green method against oral pathogenic microorganisms. Given that their optimal efficacy is confirmed, *R. coriaria* and nano-technology may be used for the production of oral antibacterial products in order to improve the general health of the population.

Methods

This experimental study was approved by the university ethics committee (code:IR.ZUMS.REC.1397.156). This in vitro study evaluated the antimicrobial effects of *R. coriaria* on common cariogenic microorganisms, including 1683 *S. mutans* (ATCC 35668), 1601 *S. sobrinus* (ATTC 27607), *S. salivarius* (ATCC 19258), 1237 *E. faecalis* (NCTC 8213) and 1643 *L. acidophilus* (DSM 20079) approved by the National Center for Genetic and Biological Resources of Iran in lyophilized form.

R. coriaria fruit was collected from Zanjan city and dried at room temperature away from sunlight. Dried fruits were then kept refrigerated until maceration. Dried fruits were ground and their aqueous extract was obtained by the maceration technique. For this purpose, 5 g of the fruits were ground and transferred to the maceration apparatus to obtain their aqueous extract under a vacuum without solvent evaporation (4003 Laborota; Heidolph, Germany). The solvent (125 mm of deionized water) was then added and stirred at 25ºC for 30 minutes to obtain the aqueous extract (21, 22). Maceration was performed at 50ºC and 80ºC. After 30 minutes, the herbal extract was centrifuged (K330, Sigma, Germany) at 6000 rpm for 30 minutes. The contents of the Falcon tube were then filtered using a #1 Whatman filter paper. The obtained extract was then frozen at -20ºC.

Synthesis of AgNPs

According to a previous study, AgNPs were synthesized using different concentrations of *R. coriaria* extract by reduction of silver nitrate (26).

A sampler was used to add 50, 100, 200, 400, and 800 µL of the extract to tubes #1 to 5. Next, 8 mL of 1 mM

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silver nitrate salt was added to each tube to obtain 1:10, 1:20, 1:40, 1:80 and 1:160 dilutions. According to previous tests, the best dilution for AgNPs synthesized by the green method was 1:40. After manually stirring the mixtures, the samples were placed in the dark. The extract was then filtered and exposed to silver salt.

Spectrophotometry

One milliliter was collected from a 1:40 test tube by a sampler and transferred to a microtube. It was then centrifuged at 6000 rpm for 15 minutes. The obtained sediment was rinsed with deionized water twice, and eventually, 1 mL of deionized water was added to the sediment. The obtained sample was vortexed for 5 minutes. Next, 1 mL of the solution was added to 3 mL of deionized water. To read the optical density, the spectrophotometer was first calibrated (blanked) with deionized water. Next, the sample was poured into a quartz cuvette and its absorbance was read at 300-700 nm wavelength.

The zeta potential (electric potential in colloidal systems that indicates the stability of the synthesized particles and also the amount of repulsion between two adjacent particles) affects the distribution and absorbance of particles. The optimal zeta potential is approximately ±30. The stability of the particles was determined by controlling the zeta potential of the surface of the particles. For this purpose, a 3600 ZEN device (Zetasizer Nano, UK) was used with the non-invasive backscatter technology to precisely, reliably, and reproducibly measure the size of AgNPs in 0.6 nm to 6 µm scale. This device uses the phase analysis light scattering to measure the zeta potential with adequate sensitivity and accuracy.

The extract was standardized by determining its optical density. After measuring the absorbance of AgNPs at 300-700 nm wavelength, the size of nanoparticles was measured by the zeta sizer. The refraction coefficient of the synthesized solution was measured by a refractometer (T1, Atago, Japan), which showed a refraction coefficient close to zero. Next, a spectrophotometer (T80 UV-Vis, Jenway, UK) was used to measure the absorbance of the solution at 633 nm wavelength. After assessing the production of AgNPs by spectrophotometry, the size of synthesized AgNPs was measured by the zeta sizer. The size of particles in the 1:40 solution was measured due to the higher concentration of AgNPs and the high efficiency of this particular dilution. The size and potential of synthesized AgNPs were measured for up to 72 hours by the zeta sizer.

For phase identification, the crystalline structure and size of synthesized AgNPs were analyzed by X-ray diffraction (XRD; Advanced Model, BrukeRaxs) with Cu-K radiation source at 1.54A° and 40 KV. For morphological assessment of surfaces and measuring the size of AgNPs, atomic force microscopy (JPK Co., Germany) was also performed. Eventually, the Debye-Scherrer equation was used to calculate the mean diameter of crystals based on the width half of different spectral peaks.

Positive and negative controls (*R. coriaria* extract and chemically reduced AgNPs, respectively) were also used to determine the validity and reliability of the experiment.

The following microorganisms were evaluated in this study: *S. mutans* (ATCC 35668), *S. salivarius* 1448 (CIP53.158), *S. sobrinus* 1601 (ATCC 27607), *L. acidophilus* 1643 (DSM20079), and *E. faecalis* 1237 (NCTC8213). The reason behind the selection of these particular microorganisms was their prominent role in oral and dental conditions.

The culture medium for *streptococci, E. faecalis*, and *L. acidophilus* was trypticase soy agar, Mueller Hinton agar, and Man Rogosa and Sharpe agar, respectively. The microbial culture was performed by the pour plate method or the triplicate method. In other words, for assessment of bacterial proliferation, they were dissolved in an aqueous medium and were then poured into a petri dish. Different concentrations of AgNPs synthesized by the green method, *R. coriaria* extract alone, and chemically synthesized AgNPs in 100 µg/mL concentration were separately added to the abovementioned bacteria in vitro (21-23).

The MIC and MBC values and the diameter of growth inhibition zones caused by AgNPs synthesized by the green method, *R. coriaria* extract alone, and chemically synthesized AgNPs were measured to assess their antimicrobial efficacy.

MIC

The microbial samples were first cultured in a liquid culture medium at room temperature for one day. After the turbidity of the aqueous medium, the specimens were isolated on the culture medium to ensure their purity. Next, the extracts were diluted in 12 tubes containing a liquid culture medium by the ½ dilution technique. In other words, half of the primary concentration was collected and added to the liquid culture medium to obtain a concentration of half of the primary concentration. This process was continued and the amount removed from the final concentration was discarded. The number of bacteria was the same in all tubes equal to 1.5×10^5 colony-forming units/mL (23). After the addition of nanoparticles and incubation for 24 hours at room temperature, the turbidity of the tubes was evaluated to assess bacterial proliferation. Since MIC is defined as the minimum concentration inhibiting the activity of microorganisms, the concentration of the first tube with no turbidity was recorded as the MIC for the respective bacterial strain and reported as µg⁄mL by the macro-titer method.

MBC

The MBC test is somehow equal to the MIC test but in a solid culture medium such that the same MIC concentrations were applied to the discs that were placed on the solid culture medium. Twelve wells were prepared and the concentration of the first well with no microbial growth was recorded as the MBC value. In this study, MBC and the diameter of growth inhibition zones were determined by the dilution and disc diffusion techniques, respectively. The diameter of growth inhibition zones was reported in millimeters (mm). The sensitivity of standard bacteria and antibiotic-resistant microorganisms was determined by the well-plate technique (Fig. 1).

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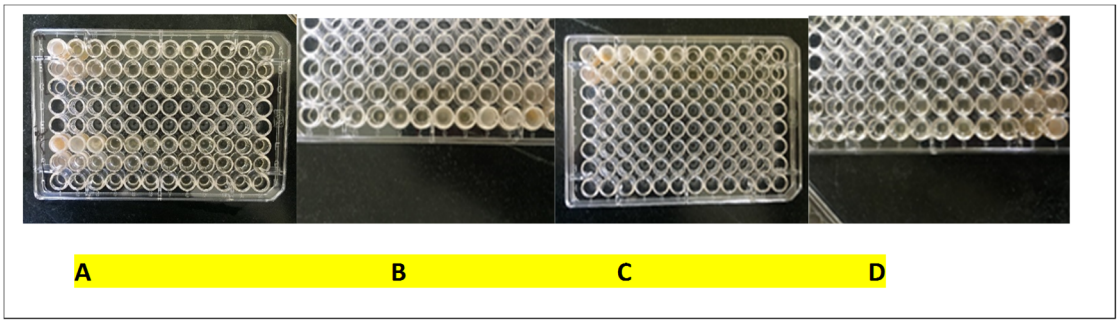

Fig. 1. **MBC test: A;** *S. mutans* (upper row), *S. sobrinus* (lower row). **B;** *S. salivarius*. **C;** *E. faecalis*. **D;** *L. acidophilus*

Measuring the diameter of growth inhibition zones

The plates containing different microorganisms were prepared, different concentrations of *R. coriaria* extract and synthesized AgNPs were added, and the plates were placed at room temperature for 24 hours; the diameter of the growth inhibition zones was then measured by a ruler.

To more precisely assess the antimicrobial activity of AgNPs synthesized by the green method, the extract and chemically synthesized AgNPs were separately tested as controls to assess the efficacy of green synthesis for optimization and increasing the antimicrobial activity.

AgNPs synthesized by the green method, extract alone, and chemically synthesized AgNPs all underwent the MIC, MBC, and growth inhibition tests. The results obtained in assessing the growth inhibition zones were analyzed using SPSS version 24. Data were analyzed by the Wilcoxon test, Kruskal-Wallis test, and Mann-Whitney test.

Results

The XRD results indicated that the AgNPs synthesized by the green method using *R. coriaria* extract were spherical in shape, and separate from each other, and had optimal dispersion. Their size ranged from 30 to 100 nm. The maximum absorbance of AgNPs was recorded at 415-450 nm, and particularly at 420 nm wavelength.

The MIC of *R. coriaria* extract was found to be 512 µg/mL against *S. mutans, E. faecalis* 1024, *S. salivarius, S. sobrinus,* and *L. acidophilus*. The results showed that *L. acidophilus, S. salivarius*, and *S. sobrinus* had the lowest resistance to AgNPs synthesized by the green method using *R. coriaria* extract. A comparison of the diameter of growth inhibition zones and the inhibitory effects of different concentrations revealed that the diameter of growth inhibition zones for *S. mutans, S. salivarius, S. sobrinus, E. faecalis*, and *L. acidophilus* decreased by a reduction in concentration from 1 to 1:16 (Table 1).

The results regarding the effects of AgNPs synthesized by the green method, *R. coriaria* extract alone, and chemically synthesized AgNPs with 100 µg/mL concentration on different microorganisms and the diameter of growth inhibition zones are presented in Table 2 and 3.

Comparison of the diameter of growth inhibition zones caused by the AgNPs synthesized by the green method, R. coriaria extract alone, and chemically synthesized AgNPs by the Kruskal-Wallis test (Kruskal-Wallis H=14.473, df=2) showed that irrespective of the type of bacteria and dilution, the three materials had different effects. However, since different dilutions were effective against different

Table 1. Diameter of growth inhibition zones and the inhibitory effect of different concentrations on different microorganisms

Concentrations/ microorganisms	*S. mutans*	*S. salivarius*	*S. sobrinus*	*E. faecalis*	*L. acidophilus*
1	13	15	14	12	14
1/2	11	13	11	10	12
1/4	9	11	10	8	11
1/8	7	9	9	5	8
1/16	6	8	8	5	7

Table 2. Diameter of growth inhibition zones and concentrations on different microorganisms

Concentrations	*R. coriaria* extract	Silver nanoparticles (AgNPs)	*R. coriaria* nanoparticles
100	19	17	12
50	18	16	11
25	17	16	9
12.5	14	12	7
6.25	12	10	6

Table 3. Descriptive statistics regarding the diameter of growth inhibition zones caused by AgNPs synthesized by the green method, *R. coriaria* extract alone, and chemically synthesized AgNPs based on the concentration, type of microorganisms, and diameter of growth inhibition zone

Concentrations	Low level	High level	Mean±SD	count	Microorganisms	Low level	High level	mean±SD
100	8	21	17.73±3.84	15	*S. mutans*	6	19	13.06±4.04
50	6	19	13.13±3.97	15	*S. salivarius*	0	20	10.86±5.91
25	6	18	12±4.12	15	*S. sobrinus*	8	19	12±3.67
12.5	0	16	9±4.7	15	*E. faecalis*	0	14	7.93±4.30
6.25	0	16	8.13±4.34	15	*L. acidophilus*	7	21	12±4.45

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microorganisms, dilution, and type of bacteria were also considered in data analysis as shown in Tables 4 and 5.

Considering the P values < 0.05, the materials had significant effects on the five types of microorganisms tested in this study (Table 5).

The mean diameter of growth inhibition zones caused by the AgNPs synthesized by the green method was larger than the diameter of growth inhibition zones caused by *R. coriaria* extract alone, indicating higher antimicrobial efficacy of AgNPs synthesized by the green method compared with *R. coriaria* extract alone.

Comparison of the diameter of growth inhibition zones caused by *R. coriaria* extract alone and AgNPs synthesized by the green method (grouping variable test; Mann Whitney U=135.500, Wilcoxon W: 460.500, Z=-3.444, and asymp. Sign. 2-tailed=0.001) showed that irrespective of the type of bacteria and concentration, the difference among the groups was significant (Table 6).

The difference in growth inhibition zones caused by *R. coriaria* extract alone, and AgNPs synthesized by the

Table 4. Comparison of the diameter of growth inhibition zones of different bacteria caused by *R. coriaria* extract in different concentrations

Statistics Test [a,b]	Concentrations					Microorganisms				
	100	50	25	12.5	6.25	*S. mutans*	*S. salivarius*	*S. sobrinus*	*E. faecalis*	*L. acidophilus*
Kruskal-Wallis H	3.418	3.544	3.544	3.956	4.114	8.193	9.958	6.359	8.268	9.800
Asymp. Sig.	0.181	0.170	0.170	0.138	0.128	0.017	0.007	0.042	0.016	0.007

[a]. Kruskal Wallis Test, [b]. Grouping Variable

Table 5. Diameter of growth inhibition zones (mm) of different bacteria in different concentrations

Concentrations (µgr⁄mL)	*S. salivarius*			*S. sobrinus*			*E. faecalis*			*L. acidophilus*			S. mutans		
	R. coriaria extract	AgNPs	*R. coriaria* nanoparticles	*R. coriaria* extract	AgNPs	*R. coriaria* nanoparticles	*R. coriaria* extract	AgNPs	*R. coriaria* nanoparticles	*R. coriaria* extract	AgNPs	*R. coriaria* nanoparticles	*R. coriaria* extract	AgNPs	*R. coriaria* nanoparticles
100	20	10	15	19	15	14	8	14	12	21	11	14	19	17	12
50	18	8	13	19	14	11	6	13	10	18	10	12	18	16	11
25	18	7	11	17	10	10	6	12	8	18	10	11	17	16	9
12.5	14	0	9	14	9	9	0	10	5	16	8	8	14	12	7
6.25	12	0	8	12	9	8	0	10	5	16	7	7	12	10	6

Table 6. Comparison of the diameter of growth inhibition zones of bacteria caused by *R. coriaria* extract and *R. coriaria* nanoparticles in different concentrations

Statistics Test [a]	Microorganisms					Concentrations				
	S. mutans	*S. salivarius*	*S. sobrinus*	*E. faecalis*	*L. acidophilus*	100	50	25	12.5	6.25
Mann-Whitney U	0.500	3.000	1.500	6.500	0.000	5.000	5.000	5.000	5.000	5.000
Wilcoxon W	15.500	18.000	16.500	21.500	15.000	20.000	20.000	20.000	20.000	20.000
Z	- 2.514	- 1.991	- 2.312	- 1.269	2.627	- 1.581	-1.591	- 1.581	-1.591	-1.591
Asymp. Sig. (2-tailed)	0.012	0.047	0.021	0.205	0.009	0.114	0.112	0.114	0.112	0.112
Exact Sig. 2*(1-tailed Sig.)	0.008[b]	0.056[b]	0.016[b]	0.022[b]	0.008[b]	0.151[b]	0.151[b]	0.151[b]	0.151[b]	0.151[b]

[a]. Grouping Variable: bacteria, [b]. Not corrected forties

Table 7. MIC and MBC of the materials against the tested bacteria

Bacteria	*R. coriaria* extract	Pipe number	*R. coriaria* nanoparticles	Pipe number	Chemical AgNPs	Pipe number
S. mutans	1024	2	1024	2	8	9
S. salivarius	512	3	512	3	64	6
S. sobrinus	512	3	512	3	8	9
E. faecalis	1024	2	1024	2	16	8
L. acidophilus	512	3	512	3	32	7

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green method was significant for all bacteria except for *E. faecalis*. However, a more precise analysis of the data based on the concentration and irrespective of bacterial type revealed no significant difference.

The diameter of growth inhibition zones caused by chemically synthesized AgNPs and AgNPs synthesized by the green method (grouping variable test; Mann Whitney U=269.000, Wilcoxon W: 594.000, Z=0.849, and asymp. Sign. 2-tailed=0.396) irrespective of bacterial type and concentration showed no significant difference (P>0.05). Thus, pairwise comparisons of bacteria and dilutions were not performed (Table 7).

Discussion

This study showed that *L. acidophilus, S. salivarius,* and *S. sobrinus* had the lowest resistance to AgNPs synthesized by the green method. In line with this finding, Naz et al. showed antibacterial properties of copper nanoparticles synthesized by the green method using Rhus punjabensis extract (21). Similarly, Dastjerdi et al. and Abbasi et al. showed the antibacterial effects of the aqueous extract of *R. coriaria* on *S. mutans* (22, 23). The diameter of the growth inhibition zones and MIC and MBC of different concentrations of materials against different bacteria were evaluated. Abbasi et al. reported MIC and MBC values of 2.5 and 5 mg/mL, respectively for the extracts. Similar to the study by Abbasi et al. (22) Babpour et al. evaluated the effect of an aqueous extract of *R. coriaria* on *S. mutans*, and *S. sanguinis*, and reported a MIC of 250 µg/mL (23, 24). The MIC values in the study by Abbasi et al. were significantly higher than those reported by Dastjerdi et al.; however, the MBC values in the study by Dastjerdi et al. were higher than those reported by Abbasi et al. This finding may be due to the fact that Dastjerdi et al., similar to the present study, performed MIC and MBC tests on other oral streptococci (*S. sobrinus, S. sanguinis*, and *S. salivarius*). Assessment of the antimicrobial effect of different concentrations of materials was a strength of the present study compared with that of Dastjerdi et al. On the other hand, in line with the present findings, Dastjerdi et al. reported minimum MIC (0.390 mg/mL) and MBC (1.5 mg/mL) against *S. sobrinus*. Variations in the reported values may be due to different extraction methods, types of solvent, methods of determination of MIC and MBC, different microorganisms tested, and different geographical locations of plant collection. The *S. sobrinus* strain (ATCC 27607) in the present study was similar to that used by Dastjerdi et al., while other microorganisms tested in the study by Dastjerdi et al., [*E. faecalis* (CIP 55142), *S. sanguinis* (ATCC 10556), *S. salivarius* (ATCC 9222), and *S. mutans* (ATCC 35608)] were different from those used in the present study. The sumac fruit was collected from a riverside in Chalous, Mazandaran, Iran, and the samples were kept at 35°C for 16-24 hours in their study, which was different from the current study. Another study showed that the extract of sumac fruit peel had stronger antimicrobial activity than the extract derived from the fruit seeds (22). Thus, fruit peel extract was used in the present study. Another study showed that the aqueous extract of *R. coriaria* significantly inhibited the proliferation of *Staphylococcus aureus, Listeria monocytogenes,* and *Escherichia coli* at 4 and 25°C (24). However, in the present study, assessments were performed only at 25°C. Variations in the results of studies can be due to different mechanisms of actions of extracts against the microorganisms (27). For instance, they may prevent the growth and proliferation of bacteria and acid production by *S. mutans* (23). Radmehr et al. showed that tannin and phenolic compounds are the most important components responsible for the antimicrobial activity of *R. coriaria* (28). Abbasi et al. attributed the antibacterial activity of the aqueous extract of *R. coriaria* to its tannin and polyphenol contents (23).

In line with the present findings regarding the green synthesis of AgNPs using different plants, Khalili et al. confirmed the antibacterial activity of AgNPs synthesized by the green method using olive tree leaves (29). Jancy Mary et al. showed that Morinda pubescens is a suitable source for green synthesis of AgNPs compared with their chemical production (30). Moreover, Manish Dubey et al. reported that green synthesis of AgNPs by using papaya tree leaves had lower cost and toxicity than chemical synthesis (31). Yuet Ying Loo et al. showed optimal antimicrobial activity of AgNPs synthesized by the green method against Gram-positive and Gram-negative bacteria (32). Another study also confirmed the antibacterial effects of AgNPs (40-60 nm) against Gram-positive and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*) by the diffusion, dilution, and MIC testing (33). Savithramma et al. indicated that AgNPs synthesized by using medicinal plants had optimal antimicrobial activity against bacteria and fungi (32). In line with the present study, Zargar et al. concluded that the green synthesis of AgNPs by using Vitex Negundo L. yielded superior results compared with their chemical synthesis (30). Moreover, AgNPs synthesized by the green method showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*. Savithramma et al. confirmed the optimal antibacterial activity of AgNPs synthesized by using plant leaves against drug-resistant bacterial species and indicated that the formation of AgNPs significantly increased with temperature rise (34). However, in the present study, the effect of AgNPs synthesized by the green method was evaluated against common oral pathogens at room temperature but in different dilutions. Not assessing different temperatures was a limitation of this study (35).

Different approaches are available regarding the use of alcohol in the production of mouthwashes. Ustrell-Borràs et al. discussed that use of alcohol-based mouthwashes is not an independent risk factor for the development of head and neck cancer (36). However, it may increase the risk of the development of such cancers in association with some other risk factors. But Satpathy et al. reported that the use of a mouthwash with a high percentage of alcohol was associated with pain and earlier, more severe, and longer burning sensation (37) The study results of Vandana Gupta et al. revealed no statistical as well as biological significant adverse responses of both alcohol-based and alcohol-free mouth rinses at clinicopathological lev-

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els. (38). Furthermore, the benefits of alcohol-containing mouthwashes have not been scientifically confirmed (26).

Assessment of the inhibitory effects of different concentrations of the aqueous extract of sumac fruit against different oral pathogens was a strength of this study. It appears that mouthwashes containing sumac extract produced by the method explained in this study would probably not have the adverse effects of mouthwashes containing alcohol on the oral cavity (23).

To eliminate the limitations (limited availability of plants, insolubility of nanoparticles, and difficult anaerobic culture of bacteria), only the extract of sumac fruit collected from a specific location at a specific time was evaluated in this study, and standard microbial strains were used. Since clinical isolates were not used in this study, further clinical studies in the oral environment are required. Also, different temperatures should be assessed in future studies, and the most efficient protocol should be determined.

Conclusion

The results showed that AgNPs synthesized by the green method were effective against oral pathogens and inhibited their growth and proliferation. Considering the confirmed antimicrobial effects of sumac extract against cariogenic microorganisms, it may be used in the formulation of oral healthcare products such as mouthwashes, toothpaste, or dental floss to benefit from its antimicrobial properties.

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Ethics Committee Approval

The required permissions for conducting this study were obtained from Zanjan University of Medical Sciences (code: IR.ZUMS.REC.1397.156).

Conflict of Interests

The authors declare that they have no competing interests.

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