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Effect of Silver Nanoparticles Green Synthesized by Using the *Quercus Infectoria* Extract on Some Dental Pathogens

Bahareh Nazemi Salman [1], Negin Aliyari [2], Alireza Yazdinezhad [3], Mahtab Mohammadi Qeydari [4], Seyede Solmaz Taheri [5]

[1] Pediatric Dentistry, Dental School, Zanjan University of Medical Sciences, Zanjan, Iran.
[2] Department of Oral Medicine, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran.
[3] Department of Pharmaceutical Biotechnology, School of Pharmacy, Zanjan University of Medical Sciences, Zanjan, Iran.
[4] Student Research Committee, Qazvin University of Medical Sciences, Qazvin, Iran.
[5] Department of Biostatistics and Epidemiology, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.

Corresponding author:
Seyede Solmaz Taheri, Department of Biostatistics, School of Allied Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

dr.taheri.solmaz@gmail.com
dr.solmaztaheri@zums.ac.ir

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Abstract

Background and Aim: Green synthesis through using plants such as *Quercus infectoria (Q. infectoria)* is a relatively novel technique for synthesis of nanoparticles. This study aimed to assess the effect of silver nanoparticles (SNPs) green synthesized by using the *Q. infectoria* extract on some dental pathogens.

Materials and Methods: In this in vitro study, SNPs were synthesized by using the *Q. infectoria* extract and silver nitrate. Formation of SNPs was confirmed by UV-visible spectrophotometry. Presence/absence and proliferation of *Streptococcus mutans* (*S. mutans*), *Streptococcus salivarius* (*S. salivarius*), *Streptococcus sobrinus* (*S. sobrinus*), *Lactobacillus acidophilus (L. acidophilus)*, and *Enterococcus faecalis* (*E. faecalis*) were evaluated by observing the tube turbidity following their culture in presence of SNPs. Also, different concentrations of *Q. infectoria* extract (1, ½, ¼, 1/8, and 1/16) were added to 5 bacterial plates, and the diameter of the growth inhibition zones was measured by a ruler. The results were reported descriptively.

Results: The minimum inhibitory concentration (MIC) of SNPs against *L. acidophilus* was lower than that for other pathogens. The highest antibacterial effect was observed in concentration of 1 against *L. acidophilus*, and ½ on *S. salivarius* and *L. acidophilus*. Also, *L. acidophilus* was the most sensitive and *E. faecalis* was the least sensitive microorganism to ¼, 1/8, and 1/16 concentrations. The 1/16 concentration caused no growth inhibition zone in *E. faecalis* plate.

Conclusion: Green synthesized SNPs had acceptable antibacterial activity against the tested microorganisms, and may be used as an antibacterial agent against these pathogens.

Keywords: Silver; Nanoparticles; Anti-Bacterial Agents; *Streptococcus mutans*; *Lactobacillus acidophilus*

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Introduction

Nanotechnology is currently a highly important topic of research [1]. Nanoparticles have shown unique characteristics in terms of size, morphology, and distribution [2,3]. The size

of nanoparticles is equal to or smaller than 100 nm. Nanoparticles have unique properties. Their small size results in a large surface/volume ratio, increasing their biochemical activity [4]. Nanoparticles are used in drug delivery systems, biology, gene transfer, chemical industry, and mechanics, among others [5]. Historically, silver metal (Ag) has antimicrobial effects and many applications in traditional medicine and even cooking. Silver has long been used in medicine due to its significant bactericidal and therapeutic properties [6]. Also, silver nanoparticles (SNPs) are more beneficial than free silver, due to providing a larger contact surface for exposure to microorganisms. SNPs have some important applications [7]. They are commonly used in medicine, and topical ointments for infection prevention in open wounds and burns [8]. There has been a growing interest in synthesis of SNPs due to their excellent antibacterial properties [9]. However, their exact mechanism of action remains unknown; although some studies have reported that the electrostatic force between the negatively charged bacterial cells and positively charged SNPs would result in their bactericidal activity [10,11]. Several methods are available for synthesis of SNPs, such as the physical and chemical methods and biological protocols [12]. These methods have subgroups such as recovery of silver salt compounds in liquid media, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, photolysis, and electrochemistry [13]. Synthesis of SNPs in plants is superior to physical and chemical methods because it is cost-effective and, like other biological methods, does not harm the environment [14]. Biosynthesis of SNPs by using plant extracts is a low-cost, biocompatible, and accessible technique [9]. Green synthesis does not harm the nature, and is easy to perform [14]. It does not require high temperature, energy, pressure, or chemicals [15]. Some chemicals used in production of nanoparticles may be toxic for the nature and/or the human body [16]. Recently, green synthesis by using herbal products such as Argemone mexicana leaf extract has been added to old methods for reduction of silver nitrate [17]. *Quercus infectoria* (*Q. infectoria*), gall oak, or Aleppo oak (*Q. infectoria* flowers used for treatment of postpartum wound infection in traditional Malay medicine) is among other plants used for this purpose [18,19]. Roy et al. [20,21] showed optimal efficacy of green synthesis for reduction of toxicity. Evidence shows that SNPs are not toxic for the human body and have the highest antimicrobial efficacy in low concentrations against the bacteria, viruses, and other eucaryotic microorganisms with no side effect [20,21].

Dental caries is a common chronic disease worldwide [22]. Microbial plaque is the main cause of development of dental caries. Thus, green synthesis of SNPs by using the *Q. infectoria* plant may be able to prevent dental caries and periodontal disease given that their antibacterial activity against oral pathogens such as *Streptococcus mutans* (*S. mutans*), *Streptococcus salivarius* (*S. salivarius*), and *Lactobacillus acidophilus* (*L. acidophilus*) is confirmed [23]. In the study conducted by Almatroudi et al. [6], SNPs inhibited the growth and proliferation of *S. mutans* in planktonic form and eliminated *S. mutans* biofilm. Dos Santos Junior et al. [24] demonstrated the bactericidal activity of SNPs against *S. mutans*. Thus, it appears that SNPs can effectively prevent dental caries. Considering the significance of green synthesized SNPs and applications of nanotechnology in dentistry [25], this study aimed to assess the antimicrobial effects of green synthesized SNPs using the *Q. infectoria* extract on some dental pathogens.

Materials and Methods

This study was approved by the ethics committee of Zanjan University of Medical

Sciences (A-12-623-18). This in vitro study was conducted in three steps. In the first step, *Q. infectoria* plant was collected from Zagros mountains and dried aways from sunlight at room temperature. Dried plant was refrigerated until extraction. Its extract was obtained by the maceration technique using a solvent at three different temperatures. For this purpose, 5 g of the plant was ground in a mortar and transferred to an Erlenmeyer flask; 125 mL of deionized water was added to it and stirred for 30 minutes at 25°C in a rotary shaker incubator on average speed. The same process was repeated for extraction at 50°C and 80°C as well. After completion of incubation, the extract was centrifuged at 600 rpm, and the contents of the Falcon tubes were filtered using #1 Whatman filter paper. The extracts were frozen and stored at -20°C.

In the second step, different concentrations of the *Q. infectoria* extract were tested to optimize SNPs and obtain their maximum concentration. To synthesize SNPs, the aqueous extract at different temperatures and silver nitrate salt were used. Accordingly, 5 tubes were coded and a sampler was used to transfer 50, 100, 200, 400 and 800 mL of the extract to the test tubes #1 to #5, respectively. Next, 8 mL of 1 mM silver nitrate salt was added to each test tube to create 1:10, 1:20, 1:40, 1:80, and 1:160 dilutions. The samples were then manually mixed and placed in a dark chamber. For UV light spectrophotometric analysis, 1 mL of each test tube content was collected by a sampler and transferred into a microtube. The microtubes were centrifuged at 6000 rpm for 15 minutes, and the obtained sediment was rinsed with deionized water twice. Finally, 1 mL of deionized water was added to the sediment, and the obtained mixture was vortexed for 5 minutes. Next, 1 mL of the solution was added to each cell along with 3 mL of deionized water. To read the absorbance, the device was blanked using deionized water. The samples were added to the quartz cell, and their absorbance was read at 300-700 nm wavelength.

In the third step, the samples were sent to a microbiology laboratory for evaluation of antimicrobial activity of SNPs where the antibacterial activity of 1, ½, ¼, 1/8, and 1/16 concentrations of SNPs against the standard-strain *S. mutans* 1683 (ATCC 35668), *Streptococcus salivarius* (*S. salivarius*) 1448 (CIP53.158), *Streptococcus sobrinus* 1601 (*S. sobrinus*) (ATTC27607), *L. acidophilus* 1643 (DSM20079), and *Enterococcus faecalis* (*E. faecalis*) 1237 (NCTC8213) was evaluated. The bacteria were cultured in a liquid medium overnight at room temperature. After observing turbidity, streptococci were cultured on tryptic soy broth agar, *E. faecalis* was cultured on Mueller Hinton agar, and *L. acidophilus* was cultured on MRS agar and isolated to ensure their purity. Next, the extracts were diluted in 11 tubes containing liquid culture medium. For this purpose, ½ of the pure concentration was collected and added to the liquid culture medium to obtain 1:2 dilution. This process was continued until the last concentration, half of which was discarded. The bacterial count in all tubes remained constant equal to 1.5×10^5. Next, the extracts were added and stored at room temperature for 24 hours. Subsequently, the turbidity of the tubes was evaluated to determine proliferation or no proliferation of the bacteria. Turbidity of a culture was defined as the 24-hour bacterial count in bouillon absorbing light [27]. The minimum inhibitory concentration (MIC) is the lowest concentration of SNPs showing no bacterial growth. The minimum bactericidal concentration (MBC) is the minimum concentration of SNPs causing elimination of 99.9% of the bacteria [28] (Figure 1). Also, the growth inhibition zones were measured to determine the MBC.

The results were reported descriptively.

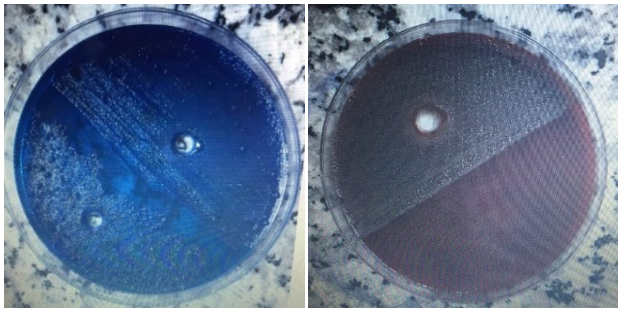

Figure 1. Bacterial culture to determine the inhibition zones (minimum bactericidal concentration)

Results

In the first test, the MIC of *L. acidophilus* was lower than that of other bacteria (Tables 1 and 2). In the second test, pure concentration created the largest growth inhibition zone and showed the highest effect on *L. acidophilus*. The ½ concentration had the greatest effect on *S. salivarius* and *L. acidophilus*, and the smallest effect on *E. faecalis*. The ¼ concentration had the highest effect on *L. acidophilus*. The same was observed for lower concentrations. Also, *L. acidophilus* showed a growth inhibition zone diameter of 14, 12, 11, 8, and 7 mm around 1, ½, ¼, 1/8, and 1/16 concentrations, respectively; while, these concentrations caused equal or smaller growth inhibition zones in other microbial cultures. Nonetheless, only *E. faecalis* did not show a growth inhibition zone around 1:16 concentration (Table 3).

Table 2. MIC of SNPs for *S. mutans, S. salivarius, S. sobrinus, E. faecalis, and L. acidophilus*

Bacteria	MIC (µg/mL)
S. mutans	1024
S. salivarius	1024
S. sobrinus	1024
E. faecalis	1024
L. acidophilus	512

Table 3. Inhibition zones of *S. mutans* caused by different dilutions of SNPs

Dilution	*S. mutans inhibition zone (mm)*
1	12
1/2	10
1/4	7
1/8	5
1/16	5
Control	45

The control group inhibition zone was measured without SNP

Also, *L. acidophilus* was more sensitive than other microorganisms in both tests. A lower MIC would indicate the need for a lower concentration of SNPs for inhibition of microorganism. The smallest growth inhibition zone belonged to *E. faecalis* such that the growth inhibition zone diameter around 1, ½, ¼, 1/8, and 1/16 concentrations was 10, 8, 6, 5, and 0, respectively. In fact, *E. faecalis* was resistant to 1:16 concentration (62.5 µg/mL) of SNPs. Other microorganisms were at the midpoint of this spectrum.

Table 1. Dilutions of SNPs for evaluation of their MIC

Tubes	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Material control**	**Bacterial control**
M (µg/mL)	2048	1024	512	256	128	64	32	16	8	4	2	1	-	-
CM (µL)	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Number of Bacteria X 10^{-5}	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

The actual number of bacteria is 150000 or 1.5x $\mathbf{10^5}$

CM: Culture Medium

M: Material

Discussion

Nature is an important source of products recently used in medical fields [29]. In the past two decades, due to the resistance of microorganisms against the existing synthetic medications, attempts have been made to use organic products [30]. SNPs are extensively used in medicine and different industries due to their high electrical and thermal conductivity, chemical stability, and antibacterial effects. The chemical methods of production of SNPs cause chemical toxicity, which may compromise their medical applications [31].

Several studies have evaluated green synthesis of nanoparticles by using plant extracts to determine their antimicrobial activity [5,32-34]. SNPs were synthesized in the present study by extraction of *Q. infectoria* extract and reaction with silver nitrate salt. Use of herbal extracts such as the Croton sparsiflorus morong leaves, ziziphus Jojo leave extract, and Argemone Mexicana leave extract has been suggested for the synthesis of silver, copper, and zinc nanoparticles [23,32-35]. UV-visible spectrophotometry was used in the present study to confirm the synthesis of SNPs although some others used X-ray diffraction or scanning electron microscopy for this purpose [36]. Next, the antibacterial activity of the synthesized extract was evaluated against five pathogenic microorganisms mainly responsible for dental caries, formation of dental biofilm, and periodontal disease. Some other studies also evaluated the activity of SNPs against *S. mutans* [5] and *E. faecalis* [5,36] while other oral microorganisms have been less commonly evaluated in the literature. Also, MIC and MBC tests were performed for evaluation of antimicrobial activity of the synthesized SNPs. In the present study, MBC was reported in millimeters while another study reported it in percentage [37].

In the present study, no comparison was made between the efficacy of synthesized SNPs and commonly prescribed antibiotics, and only the antibacterial activity of their different concentrations against different pathogens was compared; while, another study compared their antibacterial activity with some antibiotics [38]. Urnukhsaikhan et al. [5] used penicillin G and chloramphenicol as the control group. Soltani et al. [36] demonstrated that Gram-negative bacteria such as Escherichia coli had optimal sensitivity to green synthesized nanoparticles. Similarly, Rabbi et al. [37] showed optimal antibacterial activity of the synthesized SNPs against Gram-positive and Gram-negative bacteria; however, their antibacterial activity was significantly greater against Gram-negative bacteria such as Escherichia coli and Klebsiella pneumoniae compared with Gram-positive microorganisms such as Staphylococcus aureus. This difference can be due to differences in cell wall structure of Gram-positive and Gram-negative bacteria [37]. Busi et al. [39] evaluated the antibacterial activity of green synthesized SNPs against Pseudomonas aerosols and demonstrated that green synthesized SNPs had antimicrobial activity against both Gram-positive and Gram-negative bacteria but this effect was more prominent on Gram-negative bacteria.

Enterococci showed resistance against the toxicity of SNPs in 1/16 (62.5 µg/mL) concentration. Other microorganisms had moderate sensitivity to SNPs. The same was reported by Busi et al. [39], who demonstrated that the MIC of SNPs was the highest for Staphylococcus aureus with a MBC of 62.5 µg/mL. Nonetheless, in vitro results are often different from the in vivo findings. In general, it has been confirmed that higher concentrations have a greater effect on microorganisms, and it appears that use of SNPs can decrease oral diseases.

The main limitation of this study was that it only evaluated some planktonic bacteria and made no comparison with the commonly prescribed antibiotics; whereas, bacteria in the form of biofilm cause dental caries or oral infections. Thus, future studies should assess the antimicrobial activity of SNPs against dental biofilm in comparison with other antimicrobial agents.

Conclusion

Green synthesized SNPs had acceptable antibacterial activity against the tested microorganisms, and may be used as an antibacterial agent against these pathogens.

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