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Original Article

Antimicrobial Efficacy of Green Synthesis of Silver Nanoparticles against Cariogenic Pathogens – An *In vitro* Study

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ABSTRACT

Background: Nowadays, emergence of drug resistance might happen in the world. Hence, invention of new dental material had started by researchers for the same. The present study evaluated the antimicrobial property of green synthesized nanosilver particles against dental cariogenic microorganisms such as *Streptococcus mutans* and *Lactobacillus acidophilus*. **Materials and Methods:** An *in vitro* study had been designed to meet the objectives. Galla Chinensis ellagic acid powder synthesized nanosilver particles (GCAgNPs) synthesized nanosilver particles were used in this study. The cariogenic bacteria *S. mutans* (ATCC 25175) and *L. acidophilus* (ATCC 4356) were used in this study. The antimicrobial activity was detected at different concentrations (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, and 62.5 µg/ml) by means of qualitative and quantitative methods. **Results:** The results show a statistically significant difference between all the concentration (1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml) in Galla Chinensis synthesized silver nanoparticles (GCAgNPs) in *S. mutans* and *L. acdiophilus*. Intergroup comparison of GCAgNPs shows a statistically significant difference among all the concentrations against *S. mutans* and *L. acidophilus*. **Conclusion:** GCAgNPs show antimicrobial and antibiofilm activity against *S. mutans* and *L. acidophilus* microorganisms.

KEYWORDS: *Antimicrobial, green synthesis, nanosilver*

INTRODUCTION

Destruction of teeth is caused by pathogenic oral microorganisms. It affects most of the human beings in all generations in the world. From the 19th century onward, the usage of silver metal had gained more advantage as one of the components in silver amalgam restorative treatment in the field of dentistry as substituted as resin restorations.[1] From evolving of nanotechnology field, proved that nanosilver acts as an antimicrobial agent. It also proved the reduction of cariogenic biofilm in oral cavity. Hence, silver nanoparticle (AgNP) plays an important role in oral cavity because it used in most of the dental field.

Silver contains a (Kr)$4d^{10}$ $5s^{[1]}$ electron configuration, and 4d shell does not effectively protect the $5s^{[1]}$ electrons because of discovered reduction potential nature. Hence, silver is quite not reactive. In Group 11, silver has the lowest first ionization potential and most stable all nature of fluids. Because of filled 4 d orbital silver compounds has essential, and they do not appear from the organometallic compounds due to their covalent character. The coordination number is 2, if they form complexes.[2] The majority of silver ion forms bond with sulfur, nitrogen, and oxygen. As a result, Ag^+ can bind to enzymes with S-pending groups as well as nucleic acid N atoms.[3] In dentistry, silver base compounds have also been implicated. In the U. S. and japan countries were clearly stated that the linear complex Silver diamine fluoride considered as a caries arrestment compound. With having the development of nanotechnologies, AgNPs have gained attention in dentistry.[4,5]

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Nowadays, it is easy to produce silver nanoparticles with controlled size and morphology by adding capping agents. A green and biological synthesis can be used to monitor the nucleation and growth process. The structure, particle size distribution, morphology, and surface chemistry of nanoparticles can be changed to reach various locations in the oral cavity, allowing them to be conceived as multifunctional building blocks for dental materials and protocols.[6]

Silver and its compounds have antimicrobial properties due to the release of bioactive silver ions. In dentistry, antibacterial silver has been combined with fluorides to prevent and arrest dental caries. Silver ions' mechanism of action primarily targets cariogenic bacteria, while fluoride ions aid in the reconstruction of tooth structure. Silver is famous because of its low cost and effectiveness. The disadvantages of silver diamine fluoride (SDF) used in dentistry, because of deposition of silver chloride cause black staining of dental caries lesion has limited in clinical use. This can be reduced by using nano-sized silver particles and a capping agent to shield them.

According to Loo *et al*., ellagic acid reduces *Streptococcus mutans* glucan-mediated adhesion to saliva-coated hydroxyapatite.[7] Nanoparticles with diameters ranging from 1 to 100 nm may have unique properties. It contains spheres, rods, triangles, and wires, as well as capping agents such as citrate, polymer, and protein. These capping agents can shield nanoparticles while also stabilizing their chemical and biological properties. The use of AgNPs in the medical field has drawn attention to the dental field. AgNPs will stop cariogenic bacteria including *S. mutans* and *Lactobacillus acidophilus* from growing and adhering. To avoid dental caries, AgNPs have been introduced into most dental products. Researchers recently investigated the use of AgNPs in conjunction with fluoride or natural extract products to prevent caries, which involved the use of an antibacterial and remineralizing agent.[8]

Materials and Methods

Strains and chemicals used for the study

S. mutans (ATCC 25175) and *L. acidophilus* (ATCC 4356) were bought from Sudhagar biological chemicals, Chennai, and Galla Chinensis (ellagic acid powder) were bought from High Altitude Naturals for synthesis of nanosilver particles[9] and five samples were taken in this study.

Sterile paper discs were separately impregnated with Galla Chinensis synthesized AgNPs (GCAgNPs) each at concentration of 1000, 750, 500, 125, and 62.5 µg/ml and air-dried aseptically. Overnight, broth cultures of *S. mutans* (ATCC 25175) and *L. acidophilus* (ATCC 4356) were calibrated to 0.5 McFarland standards and used as an inoculum on brain–heart infusion broth (HiMedia). On the dry surface of Mueller–Hinton agar plate, a lawn culture of the inoculum was formed (HiMedia). Galla Chinensis synthesized nanosilver particle impregnated discs of different concentrations were mounted at a distance of 2 cm on inoculated media. After an overnight incubation period in a capnophilic setting, the plates were examined for a zone of inhibition around the medicated paper discs.

Determination of minimum inhibitory concentration of Galla Chinensis synthesized silver nanoparticles against cariogenic microorganisms through serial dilution method

GCAgNP antibacterial function was determined using a serial dilution procedure in brain–heart infusion broth. We put 1 ml of AgNP suspension in test tubes from each stock solution and diluted it by twofold serial dilution. Any of the test tubes carrying antimicrobial agents received 5 µl of bacterial aliquot. The test tubes were properly shaken before being incubated at 37° C for 24 h. Visual inspection was used to assess the Minimum Inhibitory Concentration of both antibacterial agents, which was then confirmed using spectrophotometry (OD – 570). To reduce defects, the experiment was repeated five times.[10]

Testing the antibiofilm activity

The ability of green synthesis of nanosilver particles to inhibit the biofilm of cariogenic microorganisms was assayed on microtiter plate.

Testing the antibiofilm activity of Galla Chinensis synthesized silver nanoparticle (A_{570})

Biofilms of *S. mutans* and *L. acidophilus* formed on microtiter plate were observed for the inhibitory activity of GCAgNP at various concentration. The wells were stained and destained after incubation of biofilms with the synthesized nanosilver particle, and the optical density of the destaining solution was determined at A_{570} using a microtiter plate reader. The test was carried out twice. As a monitor, uninoculated medium was used. The control wells' mean A_{570} value was subtracted from the test wells' mean A_{570} value.

Statistical analysis

Data obtained were entered in Microsoft Excel and analyzed using Statistical analysis was performed with IBM Statistical Package for Social Science (SPSS) Statistics for Windows, Version 16.0 (IBM Corp, Chicago, IL). Independent *t*-test was used to analyze the mean difference between both organisms in each concentration, and one-way ANOVA and *post hoc* tests were used to find the mean difference within concentrations. $P < 0.05$ was considered to be statistically significant.

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Results

Table 1 shows there is no significant difference found between *S. mutans* and *L. acidophilus* in the experimented concentration of GCAgNP.

Table 2 Minimum Inhibitory Concentration (MIC) shows. At a concentration of 1000 µg/ml, 500 µg/ml had no significant difference found with a mean 0.08±0.11, 0.13±0.03 (S.mutans) than 0.07±0.11, 0.12±0.16 (L. acidophilus) whereas 250 µg/ml , 125 µg/ml ,62.5 µg/ml had significant difference found with a mean 0.33±0.24, 0.42±0.16 , 0.53±0.12 (L. acidophilus) than 0.19±0.15, 0.25±0.01, 0.41±0.008 respectively (S.mutans).

Table 3 Antibiofilm activity shows At a concentration of 500 µg/ml had no significant difference found with a mean 0.07±0.012 (*S. mutans*) than 0.09±0.14 (*L. acidophilus*) whereas 1000 µg/ml,250 µg/ml, 125 µg/ml,62.5 µg/ml had significant difference found with a mean 0.05±0.007, 0.12±0.02,0.15±0.011, 0.23±0.01 (*L. acidophilus*) than0.03±0.01, 0.09±0.01,0.09±0.012, 0.28±0.02 respectively *S. mutans*).

Table 4 shows that a statistically significant difference has been observed between all the concentrations in GCAgNPs in all parameters (zone of inhibition, minimal inhibitory concentration, and antibiofilm activity).

Further, intergroup comparison of GCAgNPs shows statistically significant by using *post hoc* Tukey tests among all the concentrations against *S. mutans* and *L. acidophilus.*

Discussion

S. mutans was the first microorganism which causes the initiation of dental caries and *L. acidophilus* which

Table 1: Zone of inhibition activity of Galla Chinensis synthesized nanosilver against *Lactobacillus acidophilus* and *Streptococcus mutans* by using agar well diffusion method in different concentrations

Concentration (µg/ml)	Mean±SD		*P*
	Streptococcus mutans (GCAgNPs* in mm)	*Lactobacillus acidophilus* (GCAgNPs in mm)	
1000	18±1.58	19±1.58	0.347 (NS)
500	16±1.58	17±1	0.266 (NS)
250	15±1	15±1	1 (NS)
125	13±1	13±1	1 (NS)
62.5	12±1	12±1	1 (NS)

SD: Standard deviation, GCAgNPs: Galla Chinensis synthesized silver nanoparticles, NS - Non significant with p value greater than 0.05

Table 2: Minimum inhibitory concentration activity of Galla Chinensis synthesized nanosilver against *Streptococcus mutans and Lactobacillus acidophilus* in different concentrations

Concentration (µg/ml)	Mean±SD		*P*
	Streptococcus mutans (GCAgNP MIC*)	*Lactobacillus acidophilus* (GCAgNP MIC)	
1000	0.08±0.11	0.07±0.11	0.203 (NS)
500	0.13±0.03	0.12±0.16	0.355 (NS)
250	0.19±0.15	0.33±0.24	0.001 (S)
125	0.25±0.01	0.42±0.16	0.001 (S)
62.5	0.41±0.008	0.53±0.12	0.001 (S)

SD: Standard deviation, GCAgNP: Galla Chinensis synthesized silver nanoparticle, MIC: Minimum inhibitory concentration, NS- Non significant p value with greater than 0.05, S- significant with p value less than 0.05

Table 3: Detection of antimicrobial biofilm activity of Galla Chinensis synthesized nanosilver against *Streptococcus mutans and Lactobacillus acidophilus* using optical density readings in different concentrations

Concentration (µg/ml)	Mean±SD		*P*
	Streptococcus mutans (GCAgNP* biofilm OD at 570 nm)	*Lactobacillus acidophilus* (GCAgNP biofilm* OD at 570 nm)	
1000	0.05±0.007	0.03±0.01	0.006 (S)
500	0.07±0.012	0.09±0.014	0.07 (NS)
250	0.09±0.01	0.12±0.02	0.001 (S)
125	0.09±0.012	0.15±0.011	0.001 (S)
62.5	0.28±0.02	0.23±0.01	0.001 (S)

SD: Standard deviation, GCAgNP: Galla Chinensis synthesized silver nanoparticle,NS- Non significant with p value greater than 0.05, S- significant with p value less than 0.05

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Table 4: Statistical analysis of Galla Chinensis synthesized nanosilver particles against *Streptococcus mutans and Lactobacillus acidophilus* (one-way ANOVA)

	Mean square	Significant
Streptococcus mutans		
GCAgNPs (mm)	28.500	<0.001
GCAgNP MIC	0.086	<0.001
GCAgNP biofilm OD at 570nm	0.043	<0.001
Lactobacillus acidophilus		
GCAgNPs (mm)	41.000	<0.001
GCAgNP MIC	0.194	<0.001
GCAgNP biofilm OD at 570 nm	0.028	<0.001

GCAgNP: Galla Chinensis synthesized silver nanoparticle, MIC: Minimal inhibitory concentration, significant with p value less than 0.05

causes the progression of dental caries in the tooth structure. Accumulation of microorganisms thereby increases pH, which causes the removal of calcium and mineral content in the tooth structure, which further causes tooth cavity, which leads to food lodgment, sensitivity, and pain.

SDF is most common and effectively prevents the dental caries, but it can cause discoloration of tooth. According to Garcia-Contreras *et al*., 2011,[11] silver has antimicrobial activity against a wide range of bacteria, fungi, protozoa, and viruses, including antibiotic-resistant strains. Nanoparticles, according to Hwan *et al*., 2011,[12] target more than one site in bacteria, such as the respiratory chain or cell division system, decreasing the likelihood of bacteria developing resistance. As it comes into contact with a cell's DNA and RNA, it interacts with the soft bases of nucleic acids, disrupting the related nucleic acids by interactions with sulfur and phosphorus groups.

Garria (2011, 2013) stated the importance of green synthesis of AgNPs in dental practice. From these results, we evaluated the tested silver nanoparticle more effectively act as antimicrobial and antibiofilm activity against *S. mutans* and *L. acidophilus*. Shalhav *et al*.[13] more than one assay or procedure should be used in the field of testing antibacterial properties of dental products, according to the proposal. As a result, a broth dilution procedure on microtiter plates was used to assess antimicrobial activity and determine the minimum inhibitory concentration of the nanosilver against *S. mutans* and *L. acidophilus*.

The agar diffusion test yielded a qualitative bacterial sensitivity value, while the broth dilution test yielded quantitative data. The serial dilution procedure was used to assess the MIC of antibacterial agents in this analysis. This approach is more precise and easier to understand than the disc diffusion test.[14] The majority of previous researches focused on the antibacterial properties of AgNPs against a smaller number of oral pathogenic bacteria, and evidence on the bactericidal properties of green synthesized nanoparticles is also restricted. In another study,[15] zone of inhibition of *S. mutans* had the weakest response to N-single nucleotide polymorphisms with the diameter of 14.7 ± 0.03. However, in GCAGNPs, the results show a higher antibacterial activity. We have not found any research that compares green synthesized nano-AgNPs and ellagic acid as a reducing agent with antibacterial properties against a variety of oral pathogenic bacteria, and ours may be the first of research.

Conclusion

Plant extracts from Galla Chinensis were used to make AgNPs, which is a cost-effective, safe, and environmentally friendly process. Based on this result, GCAgNPs have better antimicrobial and anti-biofilm activity against *S. mutans* and *L. acidophilus*.

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Conflicts of interest

There are no conflicts of interest.

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