In Vitro Efficacy of Biosynthesized AgNPs against Streptococcus mutans causing Dental Plaque Formation

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Nanoparticles have been suggested as useful antibacterial and anti-plaque solutions for patients with dental caries. The purpose of this study was to compare the antimicrobial and antiplaque effects of silver nanoparticles synthesized by using Viola serpens plant. Furthermore, oral cavities are inhabited by both commensal and pathogenic bacterial species. In some conditions bacteria belonging to the indigenous or resident oral microorganisms can lead to infectious dental diseases. The total 40 samples were collected analyzed for the prevalence of Streptococcus mutans (S. mutans) in dental plaque. Total recovered isolates were 32 out of which 18 S. mutans were isolated, and the prevalence of recovered isolates was found to be 45%. In the present investigation, the evaluation of efficacy of biologically synthesized silver nanoparticles against recovered isolates was performed using agar well diffusion method and were found to be moderately effective against the three strains of S. mutans in comparison to reference drug.

Keywords: Dental Caries, Silver Nanoparticles, Antimicrobial Activity, Agar Well Diffusion

Introduction

The oral bacterial plaque is the major aetiological factor of the periodontal diseases and has also an important role in caries formation and inflammation of oral mucosa1. Oral cavities are inhabited by both commensal and pathogenic bacterial species. In some conditions bacteria belonging to the indigenous or resident oral microorganisms can lead to infectious dental diseases2. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by products of carbohydrate metabolism by Streptococcus mutans, a cariogenic bacterium3. The demineralization occurs within a bacteria-laden gelatinous material called dental plaque that adheres to the tooth surfaces and become colonized by bacteria4. Secondary infections are caused by Lactobacillus species, and yeasts such as Candida albicans4,5. Effective antimicrobial agents against these oral pathogens could play an important part in the prevention of dental caries. Antibiotics such as amoxicillin, penicillin, ciprofloxacin were highly effective in terms of maximum diameter of growth inhibition zones followed by chloramphenicol have been reported to effectively prevent dental caries in human drugs namely: ofloxacin, tetracycline, erythromycin, and gentamycin were found to be moderately effective against the three strains of S. mutans. The natural phytochemicals and silver nanoparticles synthesized biologically by using Viola serpens plant could offer an effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for dental caries and other oral infections.

Materials and methods

Sample collection

In the present investigation, the total 40 dental plaque sample were collected from Dental hospital Sharda University Greater Noida. The sample were collected aseptically in sterile 50 mL Oakridge tubes and inoculated in Brain Heart Infusion (BHI) broth Himedia/ M210-100G for 24 hrs at 37°C. Inoculated sample were streaked on Blood agar Himedia/M073-100 G.

Isolation and identification of bacterial strains

The isolates obtained were identified on the basis of colony morphology and biochemical reactions.

Biochemical tests

Biochemical characterization and identification of the S. aureus and P. aeruginosa were done using tests i.e. Indole Production Test, Methyl Red Test, Vouges – Proskauer Test (VP Test), Citrate utilization Test, Catalase Test, Coagulase test6.

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Extraction of plant material
Fresh and healthy leaves of *A. paniculata* and *C. asiatica* were collected, washed thoroughly with distilled water, incised into small pieces and air-dried. About 20 g of leaves of *M. nigra* were weighed and transferred into 500 ml beaker containing 100 ml distilled water, mixed well and boiled for 10 min. Filtered the extract through Whatman No.1 filter paper and collect the filtrate in a 250 ml Erlenmeyer flask.

Biological synthesis of Silver nanoparticles
Bio-reduction reaction was used for the synthesis of biological silver nanoparticles. The reaction utilized *Viola serpens* leaves extract as a reducing agent. 10 mL of plant extract was added dropwise into 200 mL of aqueous solution of 1mM AgNO₃ for reduction of Ag atoms into silver ions and was kept for 15–30 min at 60–65°C. Characterization of biologically synthesized silver nanoparticles was done by using UV-Vis Spectroscopy, scanning electron microscopy and X-ray diffraction.

Purification of biosynthesized silver nanoparticles
Fully reduced solution of AgNO₃ was purified by centrifugation (5000 rpm for 30 minutes) followed by 2 or 3 times thoroughly washing of solution using double distilled water to make the solution contamination free and dried in hot air oven at 55–60°C for 3 hrs. Prepared silver nanoparticles were stored and tested for antibacterial potentiality against MDR isolates.

Antibacterial assay of AgNPs
Standardization of inoculums
Twenty four (24) hours old culture of bacteria was suspended in sterile test tube with sterile normal saline using sterile wire loop to form turbidity that match with OD 0.5 scale of McFarland’s standard (1.5 × 10⁸ cells/ml). The cell culture was inoculated by seeding the plates with bacterial suspension on prepared Mueller-Hinton agar. The zones of inhibition were measured. The assay was done in triplicates to minimize errors. Results were interpreted using guideline by (NCCLS 2000).

Antibacterial activity of biosynthesized silver nanoparticles
Agar well diffusion method was used to study the antibacterial activity of biosynthesized silver nanoparticles derived from banafsha against *Streptococcus mutans* causing dental plaque formation. The antibacterial activity was done by agar well diffusion assay. Cork-borer was used to prepare 6 mm diameter wells. The wells were filled with varied dilutions of AgNPs extract from its 20mg/mL stock solution. Silver nitrate (10µg/mL) was used as positive control and DMSO was used as a negative control. Petri-plates were incubated for 24 hrs at 37°C. The zone of inhibition of microbes was measured. Each experiment was done in triplicate.

**Table 1 — Zone of inhibition (mm) obtained in agar well diffusion method for *S. mutans* isolates**

<table>
<thead>
<tr>
<th>Components</th>
<th>Zone of Inhibition (mm)</th>
<th>S-01</th>
<th>S-02</th>
<th>S-03</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>NZ</td>
<td>NZ</td>
<td>NZ</td>
<td>NZ</td>
</tr>
<tr>
<td>Plant extract</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. paniculata</em></td>
<td>18</td>
<td>17</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td><em>C. asiatica</em></td>
<td>10</td>
<td>12</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Silver nanoparticle</td>
<td>19</td>
<td>18</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime (CTX)</td>
<td>30µg</td>
<td>NZ</td>
<td>NZ</td>
<td>11</td>
</tr>
</tbody>
</table>

Note: mm=millimeter, NZ=No Zone, Control=DMSO
Comparative evaluation of antibacterial potency of silver nanoparticles and aqueous plant extract with a standard antibiotic cefotaxime (CTX) 30 µg was done. In our earlier reported study, Silver nanoparticles were synthesized by using Viola serpens and characterization was done by using UV-Vis Spectroscopy, SEM and XRD techniques. In our current study, we have evaluated the antibacterial activity of AgNPs microbiologically by using agar well diffusion method against 3 pathogenic MDR strains of S. mutans. In our study, biosynthesized AgNPs showed remarkable antibacterial efficacy against isolated pathogenic strains of S. mutans (Figure 1).

Determination of minimum inhibitory concentration (MIC)

MIC is defined as the lowest concentration of drug that completely inhibits the growth of the microorganism in 24 hrs. In this experiment, the MIC of biosynthesized silver nanoparticles was determined. i.e., (64mg/ml) as shown in Figures 2 & 3. In an earlier study biofouling was discussed i.e. a complex of community of microorganism that can damage the structure and function of polymers. In a similar study, positive effect of silver nanoparticles against different bacteria during orthodontic treatment was described. An algal based green synthesis of silver nanoparticles was done earlier and found much efficient approach. One earlier study revealed that factors such as pH and Temperature affects synthesis of silver nanoparticles. The emergence of antibiotic resistance was depicted in an earlier study. Nanoparticles have potential anti-biofilm ability that helps to reduce biofilm formation. Because of the emergence of the problem of antibiotic resistance in oral infection and the side effects of antibiotics in human organs, infections in oral cavity need the new strategies for treatments. The present study demonstrated the potential of nanoparticles to control the formation of biofilms within the oral cavity (Table 2).

**Conclusion**

Since the tested extract of Andrographis paniculata and biosynthesized silver nanoparticles were highly effective against the recovered isolates, purification and toxicological studies of the plant and in vivo trials should be carried out so that it can be used as a potential source for the development of a phytomedicine to act against dental plaque causing bacteria. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of modern drugs should be emphasized for the control of dental plaque.

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**References**