ANTI-BACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING PHYLLANTHUS AMARUS AQUEOUS & ANDROGRAPHIS PANICULATA ETHANOLIC EXTRACTS

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ABSTRACT

The use of metallic silver as an antimicrobial agent has been recognized for centuries. Silver nanoparticles (SNP) are now incorporated in apparel, wound dressings, appliances, cosmetics, paints and plastics for their antimicrobial properties. Generally, silver nanoparticles are prepared by a variety of chemical methods. In this study, we have used Phyllanthus amarus and Andrographis paniculata herbal plants leaves and stem extracts to synthesis SNP and were characterized by UV-Vis spectroscopy and Transmission Electron Microscopy (TEM). SNP capped with plant extracts gave absorption peak at 420 nm as expected for silver and broadening of peak were also noticed. TEM images suggested that they were of almost spherical shape and in the range of 7-60 nm in size. Antibacterial activity of plant extracts capped SNP were tested in Mueller-Hinton agar by well diffusion method against Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Escherichia coli and Salmonella Typhimurium. SNP of 1 mg/ml and 5 mg/ml of P.amarus exhibited almost similar antibacterial activity , whereas, 5mg/ml, not 1 mg/ml of SNP capped with A.paniculata exerted antibacterial activity. The highest zone of inhibition was noticed against B.subtilis followed by S.aureus and P.vulgaris and the least zone of inhibition was observed against E.coli and S.Typhimurium. Green synthesis of SNP is cost effective and environment friendly. Further studies are required to explore the possibility of use of P.amarus capped silver nanoparticles for the treatment of burn and wound.

Key words : Silver nanoparticles, antibacterial activity, P.amarus, A.paniculata, green synthesis

INTRODUCTION

The use of metallic silver as an antimicrobial agent has been recognized for centuries. Extensive research has been done to improve the efficacy of silver based antibacterial agents. Synthesis of nanosized drug particles with tailored physical and chemical properties is of great interest in the development of new pharmaceutical products. Silver nanoparticles (SNP) were incorporated in apparel, wound dressings, appliances, cosmetics, paints and plastics for their antimicrobial properties (Nithya et al.,2011;

Jain et al., 2009). Generally, nanoparticles are prepared by a variety of chemical methods such as chemical reduction, electrochemical techniques and photochemical reactions in reverse micelles. Use of plants in synthesis of nanoparticles is quite novel leading to truly green chemistry which provide advancement over chemical and physical method as it is cost effective, environment friendly, easy to scale up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals (Saxena et al., 2010). In this study, we have used P. amarus and A. paniculata extracts which are commonly used in Ayurvedha and Siddha medicines, to prepare silver nanoparticles and assessed their anti-microbial activity.

MATERIALS AND METHODS

Dried leaves and stems of A. paniculata and P. amarus were purchased from local Siddha and Ayurvedha medical shop. E. coli, S. aureus, P. vulgaris and B. subtilis were isolated from clinical specimen and characterized by cultural, morphological, staining and using various biochemical tests as per methods described in Quinn et al. (2004). S. Typhimurium (MTCC 3232) was procured from Microbial Type Culture Centre, Chandigarh. Chemicals such as silver nitrate, trisodium citrate and Mueller – Hinton agar were purchased from HiMedia Pvt. Ltd., India.

Preparation of crude extract: Dried leaves and stems were powdered using mortar and pestles and 5 g of powder was dissolved in 100 ml of distilled water or ethanol for P. amarus and A. paniculata respectively and kept at room temperature for 48 hours. Then, they were subjected to centrifugation at 6000rpm for 10 min and the supernatant was collected and allowed to evaporate to get dried crude extracts.

Preparation of Silver nanoparticles: Crude extracts of 5 mg, 10 mg, 25 mg and 50 mg were dissolved in distilled water or ethanol and were added drop by drop to 100 ml 1mM silver nitrate solution at 70-80°C under constant stirring. This process continues till the colour of the solution changes from green to yellowish brown. For chemical synthesis of SNP, 100 ml of 1mM silver nitrate solution was heated to boil and 10 ml of trisodium citrate solution was added drop by drop until it turns to yellow colour and allowed to cool. Later, they were centrifuged and the pellets were washed twice in distilled water and allowed to evaporate.

Characterization of SNP: Prepared SNP were dissolved in distilled water and were characterized by UV – Vis spectroscopy in 200-800nm wave length to demonstrate the surface plasmon resonance shift in the samples. The homogeneity and diameter of the nanoparticles were characterized by using TEM (Tecnai 10, FEI) operated at an accelerated voltage of 80kV. Samples were prepared by applying 30 µl of a diluted nanoparticle solution to a carbon coated copper grid (300 mesh). Excess solution was removed with filter paper, and the sample was allowed to dry.

Antibiogram: Mueller-Hinton agar plates were prepared and allowed to solidify. 3mm well was made using gel punch. 1 x 10^6/ml of bacterial suspension were uniformly swabbed over the surface. Plant crude extracts and chemically synthesized SNP were also included in this study. 1 mg and 5 mg SNP was dissolved in sterile distilled water and 10 µl of different SNP preparation were added into the well. The plates were incubated at 37°C for 24 hours, after which the average diameter of the inhibition zone was measured with a ruler.
RESULTS AND DISCUSSION

The green synthesis of SNP through plant extracts were carried out. Aqueous extract of *P. amarus* and alcoholic extract of *A. paniculata* were used to reduce the silver ions to silver nanoparticles. As the extracts were added to the aqueous solution of silver ion complex, it started to change the colour from green to yellowish brown due to the reduction of silver ion which may be the indication of formation of silver nanoparticles. The aqueous silver ions when exposed to herbal extracts were reduced in solution, there by leading to the formation of silver hydrosol (Thirumurugan *et al.* 2010).

The reduction of pure silver ions were confirmed by measuring the UV-Vis spectrum of the reduction media. The UV-Vis spectrum of colloidal solution of SNP synthesized from *P. amarus* and *A. paniculata* have absorbance peak at 420 nm and the broadening of absorbance peak indicated that the particles are poly dispersed. The weak absorption peak at shorter wave lengths may be due to the presence of several organic compounds which are known to interact with silver ions. In contrast, the chemical reduction of silver ions using citrate gave a sharp peak at 420nm and there was no weak absorption peak. The secondary metabolites present in plant extracts may be responsible for the reduction of silver ions and synthesis of nanoparticles. The second biogenic route is the energy (or) electron released during glycolysis (photosynthesis) for conversion of NAD to NADH led to transformation of AgNO₃ to form nanoparticles and another mechanism were releasing of an electron when formation of ascorbate radicals from ascorbate reduces the silver ions (Ahmad *et al.* 2011).

TEM micrographs of the prepared SNP were shown in Fig 1A, B & C. The SNP prepared using plant extracts were of almost spherical shape having average diameter of 30 nm and ranged between 7 nm to 60 nm. These TEM images suggest that there was no clustering of nanoparticles as they were well separated from each other. Five mg crude extracts reduced silver nitrate into silver nanoparticles more efficiently with uniform particle size than of 10, 25 and 50mg crude extracts as characterized in TEM. Hence, for further studies, 5 mg crude extracts reduced SNP were used. Chemically synthesized SNP showed a mixture of spherical and rod shape (Fig 1D).

Antibacterial activity of silver salts has been noticed since ancient times. But with the advent of nanoparticle, the use of silver in nanoparticle form has opened new treatment avenues. Here, SNP prepared using plant extracts have been investigated against *S. aureus*, *B. subtilis*, *E. coli*, *S. Typhimurium* and *P. vulgaris* by the well diffusion method. The diameter of inhibition zone against various bacteria is represented in Table 1. Aqueous extract of *P. amarus*, alcoholic extract of *A. paniculata* and chemically synthesized SNP as such did not show any anti-bacterial activity against all the used bacteria. The highest antimicrobial activity was observed against *B. subtilis* followed by *S. aureus* and *P. vulgaris* (Fig 2a,b & c). The least sensitivity was noticed against *E. coli* and *S. Typhimurium* (Fig 2d & e) for SNP prepared using both *P. amarus* and *A. paniculata*. *A. paniculata* at 1 mg/ml did not exhibit any anti-bacterial activity whereas, 5 mg/ml exhibited anti-bacterial activity as that of 5 mg/ml of *P. amarus*. But, *P. amarus* at 1 mg/ml exerted anti-bacterial activity against all the bacteria used in this study. Thus it is proven from this study that the SNP synthesized
using *Phyllanthus amarus* extracts seems to be promising and effective antibacterial agent against various bacteria.

Silver nanoparticles prepared using various plant extracts such as *Allium cepa* (Saxena et al. 2010), *Argimone mexicana* (Khandelwal et al. 2010), *Artocarpus heterophyllus* (Thirumurugan et al. 2010), *Sempervivum tectorum*, *Sempervivum hybridens*, *Boswellia ovalifoliolata*, *Shorea tumbugaia* (Savithramma et al. 2011) were evaluated for their anti-microbial activities and found that various extracts exhibited different spectrum of activities against Gram positive, Gram negative bacterial and fungal species. Ahmad et al. (loc cit) prepared silver nanoparticles using *Desmodium trifolium* extract and found that they were more active against Gram negative bacteria. They explained that the inhibition of growth of micro-organisms may be due to the presence of peptidoglycan, which is a complex structure and contains teichoic acids or lipoteichoic acids which have a strong negative charge. This charge may contribute to the sequestration of free silver ions. Thus gram positive bacteria may allow less silver to reach the cytoplasmic membrane than the gram negative bacteria. However, SNP capped *P. amarus* and *A. paniculata* extracts exhibited the highest zone of inhibition against *Bacillus* and *Staphylococcus* which are Gram positive organisms.

The mechanism of the bactericidal effect of SNP is not well known. Silver ions work against bacteria in a number of ways. Silver has been described as ‘oligodynamic’ due to its ability to exert bactericidal effect at very low concentrations. Silver ions are known to be highly reactive and could readily bind with a variety of negatively charged molecules including inorganic anions, proteins, RNA and DNA. Therefore, the antibacterial property of silver ions has been attributed to its interaction with thiol as well as other groups such as carboxylates, phosphates, hydroxyls, imidazoles, indoles and amines either singly or in combination so that multiple events could happen that simultaneously interfere with microbial processes such as respiration and DNA synthesis (Lee et al. 2007; Warinoicharoen et al. 2001). Silver ions are bound to the bacterial inner and outer cell wall, altering the function of the bacterial cell membrane. Sereemaspun et al., (2008) suggested that the inhibition of oxidation based biological process by penetration of metallic nano sized particles across the microsomal membrane. The high specific surface-to-volume ratio of SNPs increases their contact with microorganisms, promoting the dissolution of silver ions, thereby improving biocidal effectiveness. The ability of SNPs to release silver ions is key to their antibacterial activity (Stobie et al. 2008).

Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles. Biosynthesis of nanoparticles using plant extracts is the favourite method of green, eco-friendly production of nanoparticles and exploited to a vast extent because the plants are widely distributed, easily available, safe to handle and with a range of metabolites. The results show that SNP prepared by *P. amarus* can be used as effective growth inhibitors to various microorganisms. However, further studies on cytotoxicity, anti-viral properties of SNP are required to explore the possibility of using medicinal plants mediated synthesis of SNP for the successful treatment of jaundice, burns and wound and prevent bacterial colonization of prosthesis, catheters and dental materials.
Fig 1: Transmission Electron Microscopy images of silver nanoparticles

A - SNP of *P. amarus* at 1 mg/ml
B - SNP of *P. amarus* at 5 mg/ml
C - SNP of *A. paniculata* 5 mg/ml
D - SNP of chemical synthesis

Fig 2: Anti-bacterial activity of silver nanoparticles synthesized using plant extracts and chemical synthesis

2a. *B. subtilis*

2b. *S. aureus*
Anti bacterial activity of silver nanoparticles synthesized using *Phyllanthus amarus*

2c. *P.vulgaris*

2d. *E.coli*

2e. *S.Typhimurium*

A - *A.paniculata* crude extract
AS1 - SNP of *A.paniculata* at 1mg/ml
PS1 - SNP of *P.amarus* at 1mg/ml
Ag - SNP of chemical synthesis

P - *P.amarus* crude extract
AS5 - SNP of *A.paniculata* at 5 mg/ml
PS5 - SNP of *P.amarus* at 5 mg/ml
C - control
### Table 1: Zone of inhibition (mm) of plant extracts and silver nanoparticles synthesized using plant extracts tested against various bacterial species

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Substance</th>
<th>B.subtilis</th>
<th>S.aureus</th>
<th>P.vulgaris</th>
<th>E.coli</th>
<th>S.Typhimurium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Chemical SNP</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Aqueous extract of P.amarus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>PASNP 1 mg/ml</td>
<td>13</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>4.</td>
<td>PASNP 5 mg/ml</td>
<td>21</td>
<td>16</td>
<td>15</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>5.</td>
<td>Alcoholic extract of A.paniculata</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>APSNP 1 mg/ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>APSNP 5 mg/ml</td>
<td>20</td>
<td>17</td>
<td>13</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

PASNP: *P.amarus* silver nanoparticles  
APSNP: *A.paniculata* silver nanoparticles

### REFERENCES


Anti bacterial activity of silver nanoparticles synthesized using Phyllanthus amarus


