Bio-inspired Synthesis of Silver Nanoparticles Using Andrographis paniculata Whole Plant Extract and their Anti-microbial Activity over Pathogenic Microbes

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Abstract

Andrographis paniculata is a medicinal plant enriched with bioactive compounds and displays multiple biological actions. Thus, the study planned to biosynthesize antimicrobial potent silver nanoparticles using whole plant aqueous extract of Andrographis paniculata (A. paniculata).

The synthesized silver nanoparticles were confirmed by color transformation and Ultraviolet-visible (UV-visible) spectrophotometry. The size and morphology of the silver nanoparticles were characterized by Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM). The stability of silver nanoparticles was detected by Fourier Transform Infra Red spectroscopy (FTIR). The effect of silver nanoparticles over bacterial strains such as B. subtilis, E. coli, P. aeruginosa, P. fluorescence, S. aureus, S. typhi and V. parahaemolyticus and pathogenic fungi such as A. flavus and A. niger were examined. The appearance of dark brown color and UV absorption range at 430 nm confirmed the synthesized silver nanoparticles. The silver nanoparticles showed spherical structure and their sizes were ranging from 14-80 nm under SEM and TEM observations. FTIR spectra of silver nanoparticles showed the peaks for functional groups, N-H, C=O, -C=C and mono-substituted ring which indicate the stability of synthesized silver nanoparticles. The obtained nanoparticles showed good inhibitory activity on all bacterial species, whereas it showed anti-fungal activity only on A. niger and had no effect on A. flavus.

The synthesis of silver nanoparticles using whole plant aqueous extract of A. paniculata would be helpful for the preparation of pharmaceutically useful drugs to destroy pathogenic microbes.

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1. INTRODUCTION

Synthesis of metal nanoparticles and their characterization has been an emerging field of nanotechnology since the past few decades because of their unique properties and potential application in the fields of physics, chemistry, biology and medicine [1]. Widely, nanoparticles are synthesized by different routes. However, the synthesis of nanoparticles by chemical methods is not environment friendly. Therefore, the synthesis of nanoparticles by biological route (using microorganisms, enzymes and plant extract) is the suggested alternative to the non eco-friendly methods [2]. The noble metals (Ag, Au, Pb, Pt and Hg) are widely used for the synthesis of nanoparticles [3]. Among the noble metals, silver is the metal of choice because it is used as a health additive in traditional medicine [4] and shows a strong toxicity over microorganisms. In addition, silver and its derivatives are widely used to treat many bacterial infections and burns [5, 6, 7, 8]. However, the silver ions or salts have limited application as antimicrobial agents due to its inadequate release. The study hypothesize that the above limitation can be overcome by using silver nanoparticles, as these are highly reactive species due to their large surface area [9].

The bioreduction of Ag+ to silver nanoparticles involves plant extracts and microorganisms [10, 11]. It has been shown that variety of plant extracts served as green reactants in silver nanoparticles synthesis [12, 13, 14, 15]. A recent study has demonstrated that the synthesized silver nanoparticles using leaf extract of A. paniculata displayed good anti-plasmodial activity [16]. However, no reports demonstrate whole plant aqueous extract of A. paniculata derived silver nanoparticles and its effect on human pathogens.

A. paniculata is an herbaceous plant, commonly known as “King of Bitters”. It belongs to the family Acanthaceae. In India, A. paniculata is predominantly used as a constituent in various ayurvedic formulations [17, 18]. A. paniculata contains major bioactive compounds such as diterpenoids, flavonoids and polyphenols [19, 20] and shows multiple biological actions [21, 22, 23]. Therefore, the study...
assumed that preparation of silver nanoparticles using *A. paniculata* would be useful to develop new antimicrobial drug(s) with increased efficiency against bacterial and fungal diseases. The study synthesized silver nanoparticles using aqueous extract of *A. paniculata* and was confirmed by color transformation and UV-visible spectrophotometry. The size and shape of nanoparticles were observed by SEM and TEM and the stability of nanoparticles was studied by FTIR. The antimicrobial activity of synthesized silver nanoparticles over bacterial and fungal pathogens was studied.

2. MATERIALS AND METHODS

2.1. Materials

Silver nitrate was purchased from Sigma Chemicals Company, MO, USA. All the other chemicals were purchased from Hi-Media Laboratories Pvt. Ltd (Mumbai, India). The medicinal plant, *A. paniculata* was collected from Siddha Medical College, Chennai, Tamil Nadu. The microbial (bacteria and fungi) cultures were obtained from Life Tech Research Institute, Chennai, Tamil Nadu, India.

2.2. Preparation of plant extract

The plant was washed with tap water, rinsed with distilled water and air dried for 2 h and cut into small pieces and allowed to dry at room temperature (37°C) for a week. The dried *A. paniculata* was ground to fine powder and stored at 37°C. About 5 g of *A. paniculata* powder was weighed and dissolved in 100 ml of distilled water and kept at 37°C for 24 h. The aqueous extract of *A. paniculata* was filtered and stored at 37°C for further studies.

2.3. Bio synthesis of silver nanoparticles

About 5 ml of whole plant aqueous extract of *A. paniculata* was mixed with 25 ml of 1 mM silver nitrate solution and kept in shaker at 37°C for 24 h and the color change was observed. The bio reduction of silver ions in the solution was monitored by sampling the aqueous component after incubation period and the absorption maxima was scanned at different wavelengths (420-500 nm) using a UV-Visible spectrophotometer.

2.4. Characterization of silver nanoparticles

After the incubation period, the silver nitrate treated *A. paniculata* extract was centrifuged at 9,000 rpm for 15 min. The supernatant was taken for the analysis of size, shape and stability of the bioreduced silver nanoparticles using Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and Fourier Transform Infra Red Spectroscopy (FTIR).

2.5. SEM analysis of silver nanoparticles

Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid. The excess solution was removed using a blotting paper. The film on the SEM grid was dried under a mercury lamp for 5 min. The thin film on grid was examined using Scanning Electron Microscope.

2.6. TEM analysis of silver nanoparticles

Samples for TEM analysis were prepared by placing a drop of the silver colloidal solution on a TEM copper grid (200 meshes, carbon-coated, colloid on covered). The films on the TEM grids were dried and the excess solution was removed using blotting paper. TEM measurements were performed on TECNAI 10 Philips; the instrument was operated at an accelerating voltage of 80 KV. The size and shape of the bioreduced silver nanoparticles were obtained from TEM images.

2.7. FTIR analysis of silver nanoparticles

The AgNO₃ treated *A. paniculata* extract was centrifuged at 9,000 rpm for 25 min. The pellet was washed thrice with 20 ml of deionized water to get rid of free proteins/enzymes. The residue was dried and mixed with potassium bromide (KBr). The pellet was used for FTIR analysis in the range of 400-4000 cm⁻¹ at a resolution of 4 cm⁻¹.

2.8. Anti-microbial studies of silver nanoparticles

Antibacterial activity of *A. paniculata* reduced silver nanoparticles was analyzed by well diffusion method against bacterial species *B. subtilis, E. coli, P. fluorescens, P. aeruginosa, S. typhii, S. aureus* and *V. parahaemolyticus*. The antifungal activity was evaluated by disc diffusion method against *A. niger* and *A. flavus*.

2.9. Well diffusion method

The activity of aqueous *A. paniculata* extract and silver nanoparticles were compared using Agar-Well diffusion method [24]. The medium was sterilized at 120°C (15 lb/in²). About 30 ml of the medium (Nutrient Agar Medium) was transferred aseptically into each sterilized petri plates and kept at 37°C for solidification. The bacterial strains were spread on the petri plates using L-rod. On each plate, a single well of 3 mm diameter was made using a gel punch. The *A. paniculata* extract and its silver nanoparticles (5 µl) were added into the wells. The plates were incubated at 37°C for 24 h. The experiment was carried out in triplicates and the zone of inhibition was measured.

2.10. Disc diffusion method

The antifungal activity of *A. paniculata* extract and its silver nanoparticles was carried out by disc diffusion method [25]. About 5 µl of plain *A. paniculata* extract and its silver nanoparticles were loaded onto different filter paper discs. The discs were placed on the PDA medium containing fungal cultures and incubated for 48 h at 37°C. The zone of inhibition was recorded.

2.11. Statistical Analysis

Values are the means ± SD; (n=3). Data within the groups are analyzed by Student’s t-test. The levels of significance were: **P<0.05; ***P<0.01; ****P<0.001; ""P<0.0001; "" non significant.

3. RESULTS

3.1. Bio synthesis of silver nanoparticles

Figure 1 shows the color intensity of aqueous extract of *A. paniculata* incubated with silver nitrate solution in the beginning (a) and after 24 h (b) of reaction. Figure 1b revealed the bio reduction of Ag⁺ ions to silver nanoparticles by ingredients of *A. paniculata* extract. The synthesized silver nanoparticles’ maximum absorption range was measured using UV-visible spectrophotometry. The strong resonance (λmax) for *A. paniculata* derived silver nanoparticles was clearly observed at 430 nm (Fig. 2), suggesting the presence of silver nanoparticles.

3.2. Scanning electron microscope (SEM)

Figure 3a depicts the size and shape of silver nanoparticles. The average size of synthesized silver nanoparticles was...
found to be in the range of 40-80 nm. The silver nanoparticles showed spherical morphology under SEM observation.

Figure 1: Color transformation of *Andrographis paniculata* reduced silver nanoparticles. (a) A. paniculata extract and silver nitrate (initial), (b) dark brown color indicating the formation of silver nanoparticles (final).

Figure 2: UV visible spectra of bio-synthesized silver nanoparticles. The UV-absorption spectra obtained for *A. paniculata* derived silver nanoparticles. A characteristic peak at 430 nm wavelength is clearly observed, confirming the formation of Ag nanoparticles.

3.3. Transmission electron microscope (TEM)
Transmission electron microscopy experiment proved the formation of silver nanoparticles, shown in Figure 3b. Most of the silver nanoparticles were spherical in nature and often agglomerated into small aggregates, comprising of 4-5 particles each (Fig. 3b). The obtained nanoparticles were quite uniform in size and ranging between 14-26 nm.

Figure 3a: Scanning Electron Microscopic images of bio-reduced silver nanoparticles. SEM images of silver nanoparticles synthesized by A. paniculata extract. The particle size ranges from 40-80 nm under SEM observation.

Figure 3b: Transmission Electron Microscopic images of bio-reduced silver nanoparticles. TEM images of silver nanoparticles synthesized by *A. paniculata* extract. The particle size ranges from 14-26 nm under TEM observations.

3.4. Fourier Transform Infrared Spectroscopy (FTIR) analysis
The FTIR spectra of extracts (aqueous *A. paniculata* extract and silver nanoparticles) were recorded before (Fig. 4a) and after (Fig. 4b) adding silver nitrate. The FTIR spectrum (Fig. 4b) of silver nanoparticles showed peaks at 3368, 2066, 1637 and 689 cm⁻¹. This represents the different functional groups of adsorbed biomolecules on the surface of nanoparticles. It indicates the influence of organic moieties on the formation of silver nanoparticles and its stabilization.

Figure 4a: FTIR spectra of *Andrographis paniculata* extract. FTIR spectrum of plain whole plant extract.

Figure 4b: FTIR spectra of bio-reduced silver nanoparticles. FTIR spectrum of silver nanoparticles. The peaks in the graph represent the different functional groups responsible for the stabilization of silver nanoparticles.

3.5. Antimicrobial activity
The zone of inhibition for *A. paniculata* extract and its silver nanoparticles on bacterial and fungal species are
shown in Table 1. A. paniculata derived silver nanoparticles displayed significant (P<0.0001) inhibition on the growth of E. coli, P. aeruginosa, S. typhii, V. parahaemolyticus and P. fluorescence when compared with A. paniculata extract treated cultures. The zone of inhibition for B. subtilis (P<0.001) and S. aureus (P<0.01) was also statistically significant when compared with inhibitory potential of A. paniculata extract. The growth of pathogenic fungus A. niger was significantly (P<0.01) retarded on incubation with A. paniculata reduced silver nanoparticles whereas, the nanoparticles did not show any effect on the growth of A. flavus.

### Table 1. Effect of A. paniculata and silver nanoparticles on the growth of bacterial and fungal species

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Zone of Inhibition (cm)</th>
<th>A. paniculata extract</th>
<th>Silver nanoparticles (5 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>0.4±0.03</td>
<td>1.6±0.15***</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>0.3±0.02</td>
<td>0.9±0.06***</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>0.5±0.2</td>
<td>1.1±0.08***</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0.6±0.05</td>
<td>1.0±0.03***</td>
<td></td>
</tr>
<tr>
<td>Salmonella typhii</td>
<td>0.2±0.01</td>
<td>0.7±0.08***</td>
<td></td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>0.3±0.03</td>
<td>0.8±0.06***</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas fluorescence</td>
<td>0.4±0.04</td>
<td>1.0±0.06***</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.5±0.03</td>
<td>1.2±0.21**</td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>0.1±0.02</td>
<td>Nil ns</td>
<td></td>
</tr>
</tbody>
</table>

Values are the means ± SD; (n=3). The levels of significance were: **P<0.05; ***P<0.01; ****P<0.001; *****P<0.0001; ns non significant. Data within the groups are analyzed by student’s t test.

### 4. DISCUSSION

The present research work used A. paniculata for the biosynthesis of silver nanoparticles and studied their effect on microbial growth. The whole plant material was dried and ground to fine powder before subjecting to crude phytochemical extraction. The active phytochemical components are expected to be more concentrated in dry preparation than in fresh plant material [26]. Most of the plant materials contain various water soluble anionic components such as thiocyanate, nitrate, chlorides, sulphates, starches, tannins, saponins, terpenoids, polypeptide and lectins [27]. Therefore, the study extracted active components of A. paniculata using water for the preparation of nanoparticles.

The reduction of silver ions to silver nanoparticles by A. paniculata extract was measured using UV-visible spectrophotometry. It was observed that the initial color of silver nitrate treated A. paniculata extract turned from light to dark brown after 24 h of the reaction, which indicates the formation of silver nanoparticles. The color transformation of A. paniculata extract treated silver nitrate might be due to vibrations in surface plasmon of silver [28]. The strong broad peak located at 430 nm indicates the reduction of Ag+ ions which further confirmed the formation of silver nanoparticles. It is corroborated to the findings of Vilchis-Nestor et al. [13], who have reported that the noble metal silver displays characteristic absorbance at around 430 nm. It has been suggested that polyol components are mainly responsible for the reduction of silver ions [29]. Thus, the study suggest that water-soluble active constituents like polyphenols, flavonoids and terpenoid contents of A. paniculata extract might reduce Ag+ into Ag0.

The synthesized silver nanoparticles using A. paniculata were well distributed as aggregates in solution. TEM images reveal that the A. paniculata silver nanoparticles were spherical in morphology which correlated with the results of Santhoshkumar et al. [30]. The green synthesis of noble nanoparticles using plant or fruit extracts and bio organisms lead to the formation of crystalline nanoparticles with variety of shapes and sizes ranging from 1 to 100 nm. Interestingly, the size of A. paniculata reduced silver nanoparticles were found to range from 14-80 nm under SEM and TEM observation. It has been suggested that the size and shape of nanoparticles are mainly determined by various factors such as the nature of plant extract and its concentration, metal salt, pH, temperature, extent of reaction time and the mixing ratio of plant extract and metal salt [31].

The functional groups of compounds adsorbed on the silver nanoparticles were identified using FTIR studies. The plain extract of A. paniculata shows a strong peak at ~3306 cm⁻¹, 684 cm⁻¹ indicating the presence of hydroxyl group and C=S group respectively. The peaks at 2364 cm⁻¹ and 2345 cm⁻¹ indicating the presence of aliphatic cyanide/ Nitrile group (C≡N) in aliphatic /aromatic compounds. The peaks for O-H and C=S groups disappeared in silver nitrate treated A. paniculata extract. It indicates water soluble polyols and C≡N group present in the A. paniculata extract might be adsorbed on the surface of Ag0 and thereby induced the catalytic reduction of Ag0 to Ag0 nanoparticles. The peak at 3368 cm⁻¹ is assigned to N-H group from peptide linkage and the peak at 690 cm⁻¹ is assigned to monosubstituted ring which indicate that the A. paniculata reduced silver nanoparticles might be surrounded by proteins and aromatic ring of polyphenol/flavonoids. The band at 1637 cm⁻¹ persist in both A. paniculata extract and A. paniculata derived silver nanoparticles and this is due to the stretch vibration of carbonyl groups (C=O) with a benzene ring in A. paniculata. It has been shown that peaks at 1620-1636 cm⁻¹ represent carbonyl groups (C=O) present in polyphenols such as catechin gallate, epicatechin gallocate and theaflavin [32]. Another report has assigned that the peak at 1636 cm⁻¹ is due to -C=C- aromatic stretching [33] This suggests that silver nanoparticles might be capped by water soluble secondary plant metabolites like flavonoids.

The silver nanoparticles exhibited excellent antibacterial and antifungal activity when compared with aqueous
extract of *A. paniculata*. This indicates the antimicrobial potential of *A. paniculata* derived silver nanoparticles. The antibacterial property of silver nanoparticles has been investigated against *S. aureus, P. aeruginosa* and *E. coli*. [34, 35]. It has been proven that the antibacterial property of silver nanoparticles is size dependent. The silver nanoparticles <10 nm in size could attach to cell membrane and thereby disturb membrane functions and its interaction with biomolecules like proteins and DNA leading to more damage. [36, 37]. The study suggests that the membrane interacting ability and its function modulating nature of silver nanoparticles could be the reason(s) for bactericidal activity of *A. paniculata* reduced metal nanoparticles [38]. It has been shown that bioreduced silver nanoparticles also exhibit good fungicidal effect over *A. flavus, A. niger* and *A. fumigates* [39, 40]. *A. paniculata* derived silver nanoparticles displayed antifungal action over *A. niger* and not over *A. flavus*. The fungicidal effect of silver nanoparticles is due to the inhibitory action of plant metabolite reduced silver and the suggested mechanism involves cytoplasm granulation, cytoplasmic membrane rupture and inactivation and/or inhibition of intracellular and extracellular enzymes [41]. The antibacterial and antifungal activity of biosynthesized silver nanoparticles could be due to the water soluble active components of *A. paniculata*; which might possibly interact with silver and thereby generate potent antimicrobial silver nanoparticles. The biosynthesized silver nanoparticles displayed good antimicrobial activity over tested human pathogens. From a technological point of view, the obtained silver nanoparticles have potential applications in the medical field and this simple product has several advantages such as cost effectiveness, compatibility for medical and pharmaceutical applications, as well as, large-scale commercial production.

**Conflict of interest statement**

We declare that we have no conflict of interest.

5. REFERENCES

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