ABSTRACT

Objectives: Metal nanoparticle synthesis is fastest growing area of research in life science. Nanotechnology applied to reduce the material into nano size. Plant mediated synthesis of nanoparticle is ecofriendly compare to other methods of synthesis. Present investigation aims to produce silver nanoparticles by green synthesis using aqueous extract of *Bryum salakense*. Methods: The plant extract mixed with 1mM of AgNO₃ kept in light condition. The synthesized AgNps were characterized by UV-Vis Spectroscopy, FTIR, FESEM, EDAX, Zeta potential and XRD. Both plant extract and silver nanoparticles were tested against the human bacterial pathogens viz. *Staphylococcus lentus* and *Staphylococcus aureus* (gram positive) *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens* (gram negative).

Result: Colour changes during the incubation of extract with AgNO₃ from pale yellow to reddish brown indicating the silver nanoparticle formation. λ max at 443 nm in UV-Vis confirms the presence of AgNps. FTIR analysis revealed the various functional groups were acted as capping agent for the production of silver nanoparticles. FESEM and XRD analysis revealed the average size of the synthesized particles were between the 35nm and 40nm. Zeta potential for synthesized AgNps was found to be -33mv. It confirmed the synthesized AgNps have a high stability. Compare to plant extract, the silver nanoparticles showed a higher inhibition rate in all the tested bacteria. Conclusion: it is concluded that *Bryum salakense* could be a good source for synthesis the silver nanoparticles and also acted as good antimicrobial agent.
KEYWORDS: *Bryum salakense*, silver nanoparticles, UV-Vis, FTIR, FESEM, XRD and antibacterial activity.

INTRODUCTION

Nanotechnology is the most promising field of nanoscience and it is a key factor for the development of new technologies in the 21st century. The nanotechnology deals with the synthesis and application of the nanomaterials for the desired objects. A physicist Professor Richard Feynman was the pioneer of the nanotechnology and introduced by him in his historical talk “there is a plenty of room at the bottom” in the California institute of technology in December 29, 1959. Term of nanotechnology was first coined by Tokyo Science University professor Norio Taniguchi. Assimilation of nanotechnology by biotechnology commonly called as bio-nanotechnology. Due to the manifestation of bio-nanotechnology the nanomaterials were developed by biological methods. Apart from that the nanoparticles completely lacking of macro particles this is the reason behind the increasing demand of metal nanoparticles synthesis. Nanomaterials contribution predominantly in biological field such as drug delivery, cancer diagnosis, HIV inhibition, making of surgical devices. Different metals were used to synthesis the nanoparticles such as gold, silver, copper, zinc. Among these metals silver has been used widely because of their strong bactericidal activity. Silver was used to treat various diseases in ancient times. Now the nanoparticles are becoming more powerful with enhance and prolong effect. It is widely used as part of clothing, food containers, wound dressing, ointment. Several approaches were used to synthesis the metal nanoparticle such as chemical, physical, photochemical reaction, radiation assisted, microwave assisted biological method. Even though these much methods were existed for the nanoparticle synthesis, biological synthesis especially green synthesis was mostly preferred because of the easy way, ecofriendly and safe for human therapeutic use. Numerous works had been gone for the silver nanoparticle synthesis using lower and higher group of plants such as *Gracilaria birdiae*, *Ganoderma neo-japonicum*, *Fissidens minutus*, *Adiantum philippense*, *Catharanthus roseus*, *Ananas Comosus*, *Ficus carica*. In this view the present investigation attempts with a moss plant *Bryum salakense* to synthesis the silver nanoparticles and analyze its bactericidal activity.
MATERIALS AND METHODS

Description of plant: Plants in loose tufts. Stem erect, long, branched by about 2 subfloral innovation, long. Leaves smaller and sparse below, large and clustered in prominent comal tufts, erectopatent, curled. Margin recurved from base to more than two-thirds of length, entire below, serrulate at tip. Costa strong, reaching up to apex and slightly excurrent. Sporophyte not seen. The identification and confirmation of the plant is done with the help of the monograph of Gangulee (1974 - 1977).

Plant collection and Extract preparation
The moss plant *Bryum salakense* was collected from Kuzhivalavu of Kolli Hills, Eastern Ghats from Namakkal District, Tamilnadu, India. The plants were cleaned by running tap water to remove the debris and shade dried for a week. Dried plants were made into powder. About 10g of plant powder mixed with the 100ml of distilled water and boiled for 20 minutes. Then the extract filtered by whatman No. 1 filter paper. The extract were preserved in brown bottle and kept in dry and cool place.

Synthesis and Characterization of silver nanoparticles
To synthesize the silver nanoparticles 10ml of aqueous plant extract were added with 90ml of 1mM AgNO₃ solution taken in conical flask and kept in a sunlight condition for an hour. The reduced particles were analyzed by UV-Visible spectrophotometer (Perkin-Elemer Lamda 35) to obtain the absorbance spectrum at the range of 200 – 800nm. The particles were subjected into FTIR (Perkin Elmer) spectral region between 4000 cm⁻¹ and 400 cm⁻¹ to find the presence of functional groups. The size of the synthesized AgNps was determined by FESEM and elemental analysis carried out by EDAX (Carl Zeiss, SUPRA 55 model). The stability was assessed by the Zeta potential analysis by zeta sizer (Malvern instrument Ltd). The crystallinity of the AgNps was determined by the XRD analysis with the advance diffractometer (Bruker Ax D8). XRD values had been calculated using Debye-Scherrer’s formula D = k λ / β. Cosθ.

Antibacterial activity
Antibacterial activities of the moss extract and reduced AgNPs were studied by disc diffusion method. Gram positive bacteria such as *Staphylococcus aureus*, *Staphylococcus lentus* and gram negative such as *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens* were selected for the study. The bacterial cultures were maintained in the Nutrient broth. Sterile discs were prepared by whatman no.1 paper. Sterile discs were incubated in silver
nanoparticles solution and also in plant extract for two to three days. Nutrient agar media plates were prepared and inoculated with bacterial strain. Then incubated discs were placed on the medium. The plates were kept for incubation at 37°C for 24 h and the zone of inhibition were measured. Chloromphenical was used as a positive control.

RESULTS AND DISCUSSION

Visual observation of nano synthesis: B. Salakense extract mixed with the 1mM AgNO₃ solution. The colour change of pale yellow to reddish brown colour was observed in the mixture of plant extract and AgNO₃ solution after one hour of incubation in the direct sunlight (Figure-1). Colour change indicates the reduction of silver ions and confirmed the silver nanoparticle formation. This is the preliminary confirmation of nanoparticle formation.

![Figure 1: A) plant extract (B) Silver Nitrate C) silver nanoparticles.](image)

UV-Visible Spectrophotometer: The synthesized BsAgNps (Bryum salakense silver nanoparticles) were subjected to UV-Vis spectroscopy to identify the maximum absorbance of silver nanoparticles. Due to the surface plasmon resonance the colloidal silver exhibits the maximum absorbance in the range of 443nm (Figure-2). Amit Kumar Mittal et al., (2012) reported that Rhododendron dauricum showed the maximum absorbance in the range of 400–450 nm.¹²⁶
FTIR Spectroscopy: Fourier transform infrared analysis was performed to determine the possible functional group responsible for the reduction of silver ions. The FTIR spectrum of plant powder (Figure 3a) showed many peaks likewise 3781 and 3403 cm$^{-1}$ corresponds to the O-H stretch of alcoholic group. The peaks in the regions of 2927 and 2856 cm$^{-1}$were assigned to C-H stretching is the vibration of alkane compounds. The peaks observed at 2338 and 2132 represent the C=O and C=C stretching of alkyne and alkane respectively. 1737 cm$^{-1}$peak correspond to the C=O stretching of ester group. The peaks at 1632 and 1384 cm$^{-1}$ were assigned to C=O and C-H stretching and bending vibration of amide and alkane respectively. 1338, 1203, 1245, 1159 cm$^{-1}$ peaks correspond to C-N stretching is the vibration of amine group. The spectrum (Figure 3b) showed the BsAgNps peaks at 3780 cm$^{-1}$ were assigned to OH stretching of alcohol. Peak at 3402 cm$^{-1}$ were assigned to N-H stretching of amine. The peaks at 2927 and 2856 correspond to C-H stretch of alkane compounds. Peak at 2427 was assigned to OH stretching of carboxylic acid. 1384 peak shows the C-H aldehyde stretch. Thus the FTIR analysis indicated the carbonyl compounds of amine, alcohol, aldehyde and carboxylic acids were adsorbed strongly to the metal. So it might be acted as the capping agent for the production of silver nanoparticles. The result were matched with the Raman SuKirtha et al., (2012) *Melia Azedarach* having the carbonyl compounds acted as the capping agent for the production of silver nanoparticles.\[27\]
Figure 3a: FTIR spectrum of *Bryum salakense*.

Figure 3b: FTIR spectrum of *Bryum salakense* silver nanoparticles.

**FESEM analysis:** FESEM (Field Emission Scanning Electron Microscopy) image indicated the size of the BsAgNps mediated synthesized silver nanoparticles. The particle size ranges between the 35nm and 40nm (Figure 4). The result were accordance with the Thilagam et al., (2013) they got the particle size for the *Bixa orellana* silver nanoparticles between 35 and 65nm.\[28\]
EDAX analysis: EDAX (Energy Dispersive Analysis of X Ray) is an elemental analysis. EDAX spectrum reveals the quantity of silver element in BsAgNps (figure 5) and showed the strong signal in the silver region and confirms the silver nanoparticle formation. The silver nanoparticle showed the sharp peaks in 3kev due to the surface plasmon resonance and 4.59% quantity of silver were detected in synthesized particles. Javad Baharara et al., (2014) reported the similar result in Achillea biebersteinii flower extract mediated synthesized silver nanoparticles where the silver element was observed in the 3Kev [29].

Zeta Potential analysis: The zeta potential analysis was used to measure the surface stability of nanoparticle and electrophoretic mobility of the synthesized silver nanoparticles. The zeta potential value for the BsAgNps is -33mv. The high zeta potential value shows the high electric charge on the surface of the nanoparticles. Negative values prevented the aggregation.
of the particles and it lead to stabilization of the particles. So the result revealed the synthesized particles was stable and also having an high electrostatic repulsion among the particles (figure 6). Anderson Passos de Aragao et al., (2016) got the near zeta value in *Gracilaria birdiae* silver nanoparticles.[19]

Anderson Passos de Aragao et al., (2016) got the near zeta value in *Gracilaria birdiae* silver nanoparticles. [19]

![Zeta Potential Distribution](image)

**Figure 6: Zeta potential image of biosynthesized silver nanoparticle.**

**Table 1**: Determination of crystalline size of AgNP’s by using Debye-Scherrer’s equation.

<table>
<thead>
<tr>
<th>Pos. [°2Th.]</th>
<th>FWHM Left [°2Th.]</th>
<th>d-spacing [Å]</th>
<th>Particle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.114</td>
<td>0.717</td>
<td>2.05122</td>
<td>12.4</td>
</tr>
<tr>
<td>37.956</td>
<td>0.593</td>
<td>2.36812</td>
<td>14.8</td>
</tr>
<tr>
<td>32.078</td>
<td>0.449</td>
<td>2.78754</td>
<td>19.2</td>
</tr>
<tr>
<td>27.670</td>
<td>0.460</td>
<td>3.22130</td>
<td>18.5</td>
</tr>
</tbody>
</table>

**XRD (X Ray diffraction) analysis**: XRD analysis used to determine the size and crystallinity of the particles (figure 7). Debye – Scherrer’s formula is commonly used to determine the crystallinity of the nanoparticles. $D = \frac{k \lambda}{\beta \cos \theta}$. Where, $D$ – Average crystalline size (nm), $k$ – Dimensionless shape factor (0.9), $\lambda$ – X ray wavelength (0.1541 nm), $\beta$ – Angular / line broadening at FWHM of the XRD peak at the diffraction angle, $\theta$ – Diffraction angle (Table 1). The peaks were obtained in $2\theta = 44.11^\circ$, 37.95°, 32.07° and in 27.67°. So the XRD analysis shows the average size of the particle is 65 nm and the crystallinity is face centered cubic structure. Thilagam et al., (2013) reported alike results in the XRD analysis of *Bixa orellana* silver nanoparticles.[28]
Antibacterial activity: Antibacterial activity was examined in both plant extract and synthesized nanoparticles. They were tested against five different human pathogens like gram positive bacteria like *Staphylococcus lentus* and *Staphylococcus aureus*. Gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae* and *Serratia marcescens*. Compare to the plant extract the silver nanoparticles exhibit the greater inhibitory effect on bacteria (Figure 8). All the tested bacteria responded and showed the zones of inhibition. Specifically *Escherichia coli* showed a high rate of inhibition (Table 2). Venkata Subbaiah Kotakadi et al., (2013) tested the synthesized AgNps from *Catharanthus roseus* against the bacteria and obtained the same result.[23]

Table 2: Zone of inhibition of synthesized silver nanoparticles.

<table>
<thead>
<tr>
<th>samples</th>
<th>Zone of inhibition (mm) mean *</th>
<th><em>Escherichia coli</em></th>
<th><em>Klebsiella pneumoniae</em></th>
<th><em>Serratia marcescens</em></th>
<th><em>Staphylococcus lentus</em></th>
<th><em>Staphylococcus aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant extract</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>AgNPs</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Control #</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

1)*Mean of the triplicate

2) # Standard antibiotic (Chloromphenicol)
CONCLUSION

Present investigation demonstrated the green synthesis of silver nanoparticles from a bryophyte *Bryum salakense* (moss plant). Colour change confirmed the nanoparticle formation. Maximum absorbance obtained in 443nm and the average particle size ranges from 35nm to 40nm. BsAgNps were found to be crystalline in nature. Zeta potential analysis supports the higher stability of the synthesized BsAgNps. BsAgNps showed a higher range of inhibition zones in all the tested bacteria. So this plant *Bryum salakense* proved its ability in
fabrication of silver nanoparticles and also it could act as an efficient antimicrobial agent. Hence this plant may be used in the pharmaceutical field to develop a new drug.

ACKNOWLEDGEMENT

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REFERENCES


