

Green Synthesis of Silver Nanoparticles from *Cleome Viscosa*: Synthesis and Antimicrobial Activity

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Abstract –The synthesis of metal and semiconductor nanoparticles is an expanding research area due to the potential applications for the development of novel technologies. In this work, we describe a cost effective and environment friendly technique for green synthesis of silver phyto nanoparticles and their antibiogram from 3mM silver nitrate solution through the extract of *Cleome viscosa* as reducing as well as capping agent. In the process of synthesising silver nanoparticles we observed a rapid reduction of silver ions leading to the formation of stable crystalline silver nanoparticles in the solution. The herbal leaves and their medicinal properties were already discussed in varieties of ayurvedic studies. The synthesis of silver phyto nanoparticles were prepared by adding silver nitrate solution [3mM] to the plant extract. Nanoparticles were characterised using UV-Visible absorption spectroscopy, FTIR, XRD, XRF, TEM AND SEM analysis. The biomass of plants produces their nanomaterials by a process called bio mineralisation. The silver phyto nanoparticles were isolated from these herbal leaves and tested for antimicrobial activity. The test cultures included in this study were *Staphylococcus aureus*, *E.Coli*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Salmonella*. The maximum inhibitory effect using 3mM silver nitrate against the microbes were obtained.

Keywords-Herbal extract, Silver nitrate, Silver nanoparticles, Antimicrobial activity.

I. INTRODUCTION

Nanotechnology concerns with the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled dispersity[1]. This provides an efficient control over many of the physical and chemical properties[2] and their potential application in optoelectronics.[3],[4], recording media[5],[6], sensing devices[7],[8], catalysis[9] and medicine[10-12]. To date, metallic nanoparticles are mostly prepared from noble metals (ie, Ag, Pt, Au and Pd)[13]. Among the noble metals, silver (Ag) is the metal of choice in the field of biological system, living organisms and medicine[14]. Green synthesis of nanoparticles is an emerging branch of nanotechnology[15]. The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the

synthesis of silver nanoparticles offers numerous benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols.[16]. Bio-inspired synthesis of nanoparticles provides advancement over chemical and physical methods as it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals[17].

Disease causing microbes that have become resistant to drug therapy are an increasing public health problem. Therefore there is an urgent need to develop new bactericides. Silver nanoparticles take advantages of the oligodynamic effect that silver has on microbes[18]. In the present study, reducing silver ions present in the aqueous solution of silver nitrate by the help of *Cleome viscosa* extract and their antibacterial assessment was performed to produce novel drugs to overcome drug resistance and adverse reaction.

II. MATERIALS AND METHODS

A. Materials

For the synthesis of silver nanoparticles, *Cleome viscosa* was collected from the Anna University Campus, Chennai, India. The extract was used for reducing and capping agent. Silver nitrate was purchased from Merck Limited, India. Lyophilised culture of microorganisms were procured from the department of Microbiology, King's Institute, Chennai. The nutrient media used here were supplied by Hi-Media Laboratories.

B. Methods

1) Preparation of the Extract

Extract have been prepared by using fresh leaves of *Cleome viscosa*, weighing 20grams. Washed thoroughly thrice in distilled water, cut into fine pieces, transferred into a 500ml Erlenmeyer flask with 100ml of distilled water and boiled for 10minutes. It was then filtered to obtain the plant extract.

2) Synthesis of Nanoparticles

3 mM solution of silver nitrate was prepared. 5ml of plant extract was mixed with 25ml of 3mM silver nitrate. The

formation of reddish brown colour was observed and λ_{max} at different time intervals were taken for 8 hours, using a UV-Visible spectroscopy. Then the solution is stored in room temperature for 24 hours for the complete settlement of nanoparticles. After 24 hours centrifuge the reaction mixture, discard the supernatant. Add 1ml of distilled water to the pellet and wash by using centrifugation. Collect the pellet by using acetone/ethyl acetate/Alcohol. Dry in the watch glass and store the nanoparticles.

3) Analysis of Silver nanoparticles:

a) UV-Vis Spectra analysis:

The reduction of pure silver ions was observed by measuring the UV-Vis spectrum of the reaction at different time intervals taking 1ml of the sample, compared with 1 ml of distilled water used as blank. UV-Vis spectral analysis has been one by using An Elico spectrophotometer at a resolution of 1 nm from 200 to 1100 nm.

b) TEM analysis of silver nanoparticles:

Sample is dispersed in double distilled water. A drop of thin dispersion is placed on a "staining mat". Carbon coated copper grid is inserted into the drop with the coated side upwards. After about ten minutes, the grid is removed and air-dried. Then screened in JEOL JEM 100SX Transmission Electron Microscope at an accelerating voltage of 80kv.

c) FTIR Analysis:

Perkin-Elmer spectrometer FTIR Spectrum ONE in the range 4000–400 cm^{-1} at a resolution of 4 cm^{-1} was used. The sample was mixed with KCl procured from Sigma. Thin sample disc was prepared by pressing with the disc preparing machine and placed in Fourier Transform InfraRed [FTIR] for the analysis of the nanoparticles.

d) XRD Analysis:

X-ray diffraction (XRD) analysis of drop-coated films of silver nanoparticles in sample was prepared for the determination of the formation of silver nanoparticle by an X'Pert Pro X-ray diffractometer operated at a voltage of 40kv and a current of 30mA with $\text{Cu K}\alpha$ radiation.

e) SEM analysis:

After the preparation of the nanoparticles, the suspension of nanoparticles in water was used for SEM analysis by fabricating a drop of suspension onto a clean electric stubs and allowing water to completely evaporate. SEM observations were carried out on a ZEISS EVO 40 EP Electron microscope.

III. RESULTS

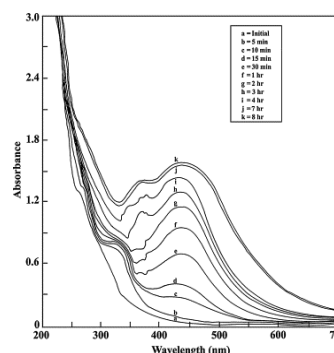


Figure 1. UV-Vis Absorption Spectrum of nanoparticles synthesized from *Cleome viscosa* extract at different time intervals

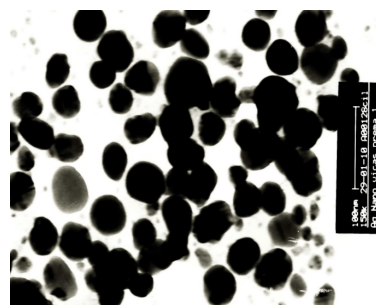


Figure 2. TEM Micrograph of nanoparticles synthesized from *Cleome viscosa* extract.



Figure 3. SEM images of nanoparticles synthesized from *Cleome viscosa* extract.

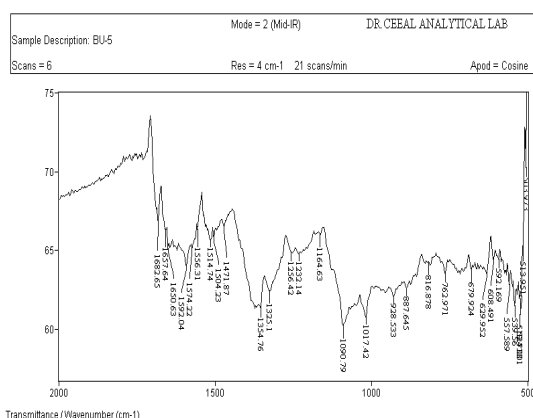


Figure 4. FTIR Spectra of nanoparticles synthesized from *Cleome viscosa* extract.

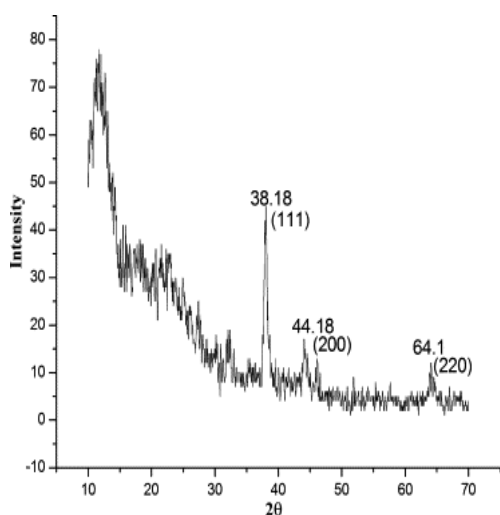


Figure 5. XRD patterns recorded for the nanoparticles synthesized from *Cleome viscosa* extract.

Antibacterial Analysis:

The inhibition rate of 25 μ l-150 μ l of *Cleome viscosa* extract nanoparticles against *S.aureus* increased from 6mm to 23mm, against *P.vulgaris* it increased from 7mm to 25mm, against *V.cholerae* it increased from 7mm to 28mm and against *P.aeruginosa*, it increased from 8mm to 32mm respectively.

IV. DISCUSSION:

Reduction of silver ions present in the aqueous solution of silver complex during the reaction with the ingredients present in the *Cleome viscosa* plant leaf extract have been seen by the UV-Vis spectroscopy and found that UV-Vis spectrograph of the colloidal solution of silver nanoparticles has been recorded as a function of time by using a quartz cuvette with water as reference. Maximum absorbance was seen at 455nm, indicating that the formation of spherical silver nanoparticles in majority or anisotropic particles whose appearance and ratio increases with time but the

UV-Vis spectra for the leaf extract alone showed no absorption in the spectral window between 400-700nm, similar to the work of [19], where the UV-vis spectra show no evidence of absorption in the spectral window 400-800 nm for the as-harvested actinomycete cells (curve 1), whereas the actinomycete cells exposed to AuCl₄⁻ ions show distinct absorption at around 540 nm. Transmission Electron Microscopy was utilized to characterize the particles and their sizes and distribution by taking micrograph from drop-coated films of the silver nanoparticles shows that most of them are spherical with the average size range from 50 nm to 7 nm which could be correlated with the morphology of the nanoparticles which is highly variable, with spherical and occasionally triangular nanoparticles observed in the micrograph [20]. Moreover the nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent. This corroborates with the previous observation by [21] in their study on *F. oxysporum*. SEM analysis shows uniformly distributed silver nanoparticles on the surface of the cells are observed. However, it does not indicate that all the nanoparticles are bound to the surface of the cells, because those dispersing in the solution may also deposit onto the surface of the cells. An XRD pattern obtained for the silver nanoparticles shown in Fig.5 shows a number of Bragg reflections corresponding to (111), (200) and (220) sets of lattice planes are observed, as the presence of intense peaks corresponding to the (111), (200), (220) and (311) Bragg reflections of gold (identified in the diffraction pattern) agree with those reported for gold nanocrystals [22], which may be indexed based on the structure of silver. The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. The FTIR spectra indicate various functional groups present at different positions. The appearance of peaks in the amide I and amide II regions characteristic of proteins/enzymes that have been found to be responsible for the reduction of metal ions when using the plant extract for the synthesis of silver nanoparticles similar to the use of microorganisms such as fungi for the synthesis of metal nanoparticles [23] indicates the binding of the nanoparticles with proteins. The IR peaks for amide I and amide II arise owing to carbonyl stretch and -N-H stretch vibrations in the amide linkage of the proteins. IR spectroscopy study has confirmed that the carbonyl group of amino acid residues and peptides of proteins has a stronger ability to bind metal, so that the proteins could most possibly form a coat covering the metal nanoparticles (i.e. capping of AuNP) to prevent the agglomeration of the particles, and thus, the nanoparticles are stabilized in the medium.

The mechanism of the antimicrobial action of silver ions is closely related to their interaction with thiol (sulfhydryl) groups (24-27), although other target sites remain a possibility (28,29).

V. CONCLUSION

It is concluded that the extract of *Cleome viscosa* is capable of producing silver nanoparticles extracellularly and are quite stable in solution. Achievements of such a rapid time scale for the synthesis of nanoparticles by various

methods of analysis increases the efficiency of synthetic procedures using environmentally benign natural resources as an alternative to chemical synthesis protocols at low cost. Also it was confirmed that the composite release of silver at a core is capable of rendering antimicrobial efficacy and proved to be active against the microbes.

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