



RESEARCH ARTICLE

BIOGENIC SYNTHESIS OF SILVER NANOPARTICLES USING AZADIRACHTA INDICA AND ACORUS CALAMUSEXTRACT AND EVALUATION OF ANTI BACTERIAL ACTIVITY

B.NAGAMAHESHWARI¹ , M.SRINIVASA MURTHY² , SARITHA KODITHALA³ , VADDE SRIJASREE⁴ , THEEGALANANDHA KISHORE REDDY⁵ , UBBU SINDHU⁶ , YEDLAMADHUSREE⁷ 

¹Associate Professor, Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Near Ramoji film city, Yadadri Bhuvanagiri Dist., Telangana.

²Professor & Principal, Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Near Ramoji film city, Yadadri Bhuvanagiri Dist., Telangana.

³Associate Professor, HOD, Department of Pharmacognosy, Vignan Institute of Pharmaceutical Sciences, Near Ramoji film city, Yadadri Bhuvanagiri Dist., Telangana.

⁴⁻⁷Vignan Institute of Pharmaceutical Sciences, Near Ramoji film city, Yadadri Bhuvanagiri Dist., Telangana.

*CORRESPONDING AUTHOR

B.Naga Maheshwari

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ABSTRACT

In recent science Nanotechnology is a burning field for researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields. Nanoparticles are used immensely due to their small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, because this method is easier than the other methods, ecofriendly and less time-consuming. The Green synthesis was done by using the aqueous solution of *Azadirachta indica* leaf extract and AgNO₃. Silver was of particular interest for this process due to its evocative physical and chemical properties. A fixed ratio of plant extract to metal ion was prepared and the color change was observed which proved the formation of nanoparticles. The nanoparticles were characterized by UV-vis Spectrophotometer, FTIR, DLS, Zeta Analysis, XRD, and SEM. The nanoparticles were found to have the size ranges from 160-180 nm.

Keywords: Nanotechnology, Nanoparticles, Green Synthesis.

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INTRODUCTION

Nanotechnology is an emerging field of material science involving the design, synthesis, and manipulation of materials at the nanoscale, generally within the range of 1–100 nm. Nanoparticles exhibit unique physicochemical properties, including high surface area, catalytic activity, optical and electrical properties, magnetic behavior, and antimicrobial activity, which differ considerably from their bulk counterparts. Among various nanomaterials, silver nanoparticles (AgNPs) have received considerable attention because of their broad applications in medicine, pharmaceuticals, dentistry, textiles, electronics, food packaging,

environmental protection, diagnostics, and antimicrobial products. Silver nanoparticles are particularly valued for their strong antimicrobial activity, which is influenced by particle size, shape, surface charge, concentration, and morphology. They have been incorporated into wound dressings, medical devices, coatings, textiles, dental materials, sensors, and other functional materials [1-5]. Nanoparticles can be synthesized through physical, chemical, and biological approaches. Physical methods such as evaporation–condensation and laser ablation can produce relatively pure nanoparticles but may require high energy and specialized equipment. Chemical

synthesis commonly employs reducing and stabilizing agents such as sodium borohydride, sodium citrate, ascorbate, and other chemicals; however, these methods may generate toxic residues and environmentally undesirable by-products. Consequently, green synthesis has gained increasing importance as an eco-friendly alternative. Biological synthesis utilizes microorganisms or plant-derived materials containing natural reducing, capping, and stabilizing agents. Plant extracts offer several advantages because they are readily available, relatively safe, economical, and contain phytochemicals such as flavonoids, terpenoids, phenolics, aldehydes, ketones, quinones, and organic acids that can reduce Ag^+ ions to metallic Ag^0 and simultaneously stabilize the resulting nanoparticles. Compared with microbial synthesis, plant-mediated synthesis is generally simpler, faster, and does not require maintenance of microbial cultures, making it attractive for sustainable and large-scale nanoparticle production [6]. The antimicrobial activity of silver nanoparticles is attributed to several complementary mechanisms, although the exact mechanism is not completely established. AgNPs can interact with and damage microbial cell membranes, alter membrane permeability, interfere with cellular respiration, generate reactive oxygen species, release Ag^+ ions, and interact with sulfur- and phosphorus-containing cellular components, including proteins and DNA. These effects can ultimately result in disruption of cellular metabolism and microbial death. Despite their extensive applications, the potential toxicity of silver nanoparticles toward humans and the environment must also be considered. Studies have reported oxidative stress, mitochondrial dysfunction, cellular toxicity, and possible adverse effects following exposure to nanosilver, particularly at higher concentrations or under specific experimental conditions. Environmental exposure may also affect beneficial microorganisms and aquatic organisms. Therefore, while green-synthesized silver nanoparticles offer significant advantages as sustainable antimicrobial nanomaterials, their safety, dose-dependent effects, environmental fate, and long-term toxicity require careful evaluation before extensive biomedical and environmental applications [7-10].

PREPARATION OF PLANT EXTRACT From *Azadirachta indica* (Neem) leaves:

Fresh leaves of *Azadirachta indica*, (Fig 4) were collected from N.I.T. RKL campus, and washed several times with water to remove the dust particles and then sun dried to remove the residual moisture and grinded to form powder. Then plant extract was prepared by mixing 1% of plant extract with deionized water in a 250ml of (Borosil, India) conical flask. Then the solution was incubated for 30 min. and then subjected to centrifuge for 30 min. at room temperature with 5000 rpm. The supernatant was separated and filtered with (mm filter paper pore size) filter paper with the help of vacume filter. Then the solution was used for

the reduction of silver ions (Ag^+) to silver nanoparticles (Ago) [11].



Fig. 4: Picture of *Azadirachta indica* leaves



Fig. 5: Picture of *Acorus calamus* rhizomes

From *Acorus calamus* (Sweet flag) rhizome

Dried rhizomes of *Acorus calamus* (Fig 5) were brought from Pondicherry and grinded to form powder. Then plant extract was prepared by mixing 1% of plant extract with deionized water in a 250ml of (Borosil, India) conical flask. Then the solution was incubated for 30 min. and then subjected to centrifuge for 0 min. at room temperature with 5000 rpm. The supernatant was separated and filtered with (mm filter paper pore size) filter paper with the help of vacume filter. Then the solution was used for the reduction of silver ions (Ag^+) to silver nanoparticles (Ago) [12-13].

Synthesis of Silver Nanoparticles

Four concentration ratios of plant and metal ions were prepared (30:1, 60:1, 120:1 & 240:1) by increasing the concentration of plant extract concentration in the solution. 0.17% of 1mM AgNO_3 metal ion was added in the prepared plant extract. Then the bio reduced aqueous component was used to measure UV-Visible spectra of the solution [14-18].

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Vis Analysis

The optical property of Ag-NPs was determined by UV-Visible spectrophotometer (Perkinelmer, Lambda35, Germany). After the addition of AgNO_3 to the plant extract, the spectra were taken in different time intervals up to 24Hrs. between 350 nm to 500 nm. Then the spectra were taken after 24Hrs. of AgNO_3 addition.

FTIR Analysis

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (Perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 750C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method.

XRD Analysis

The phase variety and grain size of synthesized silver nanoparticles was determined by X-ray diffraction spectroscopy (Philips PAN analytical). The synthesized silver nanoparticles were studied with $\text{CuK}\alpha$ radiation at voltage of 30 kV and current of 20 mA with scan rate of 0.030/s. Different phases present in the synthesized samples were determined by X'pert high score software with search and match facility. The particle size of the prepared samples was determined by using Scherrer's equation as follows

$$D \approx 0.9\lambda / \beta \cos\theta$$

Where D is the crystal size, λ is the wavelength of X-ray, θ is the Braggs angle in radians and B is the full width at half maximum of the peak in radians [19-25].

SEM Analysis

The morphological features of synthesized silver nanoparticles from neem plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs. of the addition of AgNO_3 the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV

DLS & Zeta-Potential Analysis

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min. at 25°C with 5000 rpm and the supernatant was collected. The supernatant was diluted 4 to 5 times and then the particle distribution in liquid was studied in a computer-controlled particle size analyzer (ZETA sizer Nano series, Malvern instrument Nano Zs) [26-28].

RESULTS & DISCUSSION

UV-Vis SPECTROPHOTOMETER ANALYSIS

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed because of the color change. The color change is due to the Surface Plasmon Resonance phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 421 nm in case of *Azadirachta indica* (Figure: 07, Figure: 08, Figure: 09, & Figure:10) whereas the bands for *Acorus calamus* were observed around 384 nm (Figure:6). From different literatures it was found that the silver nanoparticles show SPR peak at around 420 nm. From our studies we found the SPR peak for *Azadirachta indica* at 421 nm whereas for *Acorus calamus* it was at 384 nm. So, we confirmed that *Azadirachta indica* leaf extract has more potential to reduce Ag^+ ions into Ag nanoparticles than *Acorus calamus* extract, which leads us to further research on synthesis of silver nanoparticles from *Azadirachta indica*

leaf extracts. The intensity of absorption peak increases with increasing time. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles. The insects to Figure: 07, Figure: 08, Figure: 09 & Figure:10 represent the plots of absorbance at λ_{max} (i.e., at 420 nm) versus time of reaction. The reduction of the metal ions occurs rapidly; more than 90% of reduction of Ag^+ ions is complete within 4 Hrs. after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solutions even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticle solutions with time.

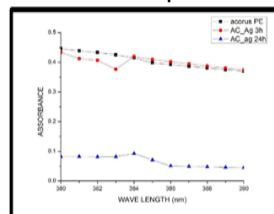


Figure 06: UV-vis spectra of Acorus Calamus at different time interval

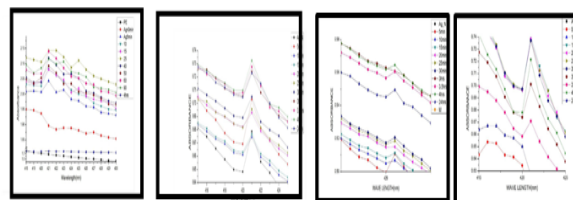


Figure 07: UV-vis spectra of Azadirachta indica 30:1, 60:1, 120:1, 240:1 ratio at different time intervals

On the behalf of UV-vis data it was cleared that *A. indica* reduces metal ions better than *A. calamus*. So, the further characterizations were carried out with *A. indica*.

SEM ANALYSIS

SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes of several μm in case of 30:1, 120:1 & 240:1 ratio whereas in 60:1 ratio the size is of several nm. (Figure: 11, Figure: 12, Figure: 13 & Figure: 14). The size of the prepared nanoparticles was more than the size of nanoparticles which should be; i.e.; between 1-100 nm. The size was more than the desired size because of the proteins which were bound in the surface of the nanoparticles. The result showed that the particles were of spherical shape in case of 30:1, 60:1, and 120:1 ratio but sheet shape in case of 240:1 ratio. The shape varies due to the concentration increased in 240:1 ratio.

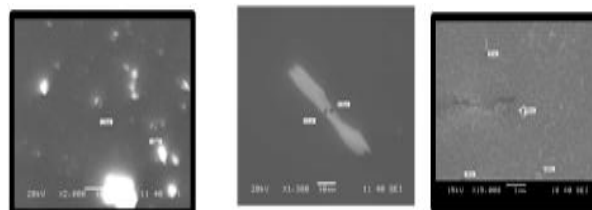


Figure 08: SEM image for 30:1, 60:1, 120:1 Ratio silver nanoparticle respectively.

DLS ANALYSIS

The particle size distribution (PSD) of synthesized silver nanoparticles of different ratios like 30:1, 60:1, 120:1, and 240:1 is shown in the figures. (Figure: 15, Figure: 16, Figure: 17 & Figure: 18). According to the figure: 15 the colloidal solution of silver nanoparticles of ratio 30:1 contains particles of different sizes, some with average sizes ranging from 5 nm to 180 nm. But in case of 60:1, the solution contains particles of uniform sizes ranging from 68 nm to 396 nm. The average size of nanoparticles is 160 nm. The particle size in case of 120:1 ratio from 78 nm to 255 nm with mean particle size of 169 nm. Similarly, the sizes of nanoparticles in case of 240:1 ratio range from 91 nm to 220 nm with average size of 164 nm. If we compare the above four results, we can conclude that the ratios like 60:1, 120:1, 240:1 give uniform distribution of particles but 30:1 ratio does not obey this principle. Among them, 60:1 ratio is very appropriate since it gives lowest average size of nanoparticles.

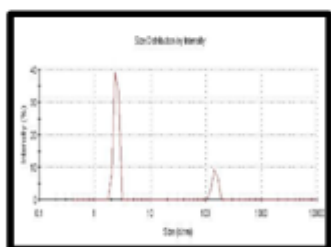


Figure 09: DLS result for 30:1 Ratio silver nanoparticle.

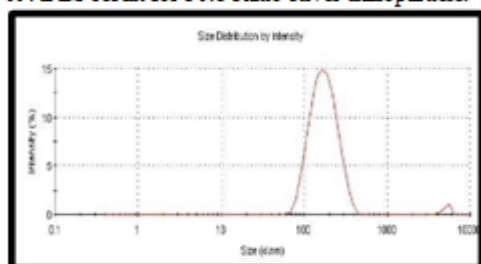


Figure 10: DLS result for 60:1 Ratio silver nanoparticle.

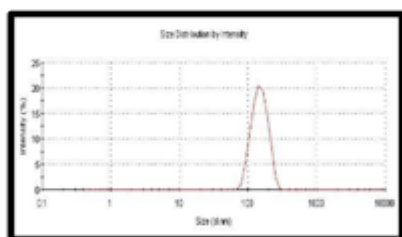


Figure 11: DLS result for 120:1 Ratio silver nanoparticle.

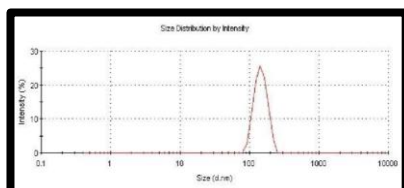


Figure 12: DLS result for 30:1 Ratio silver nanoparticle.

ZETA POTENTIAL ANALYSIS

The Zeta potential measurements of silver nanoparticles synthesized with different ratios like 30:1, 60:1, 120:1, and 240:1 is 15.5mV, 1.92mV, 6.12mV, and 2.45mV respectively. From the analysis the order of stability of nanoparticles synthesized from different ratios is 30:1 > 120:1 > 240:1 > 60:1. Nanoparticles are very small in size for which they are energetically very unstable. Therefore, the particles undergo agglomeration/aggregation to stabilize themselves. So, there were some potential charges on the surface of the nanoparticles which made them stable. These charge potential we got from this analysis. Zeta potential (Surface potential) has direct relation with the stability of a form/structure as mentioned below (Table: 03)

Table: 03: A table showing the stability of the NPs according to the potential charge

Zeta potential [mV]	Stability behavior of the colloid
From 0 to ± 5	Rapid coagulation or flocculation
From ± 10 to ± 30	Incipient instability
From ± 30 to ± 40	Moderate stability
From ± 40 to ± 60	Good stability
More than ± 61	Excellent stability

FTIR ANALYSIS

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles (in case both 60:1 and 120:1 ratio showed the band between 3490-3500 cm⁻¹ corresponds to O-H stretching H-bonded alcohols and phenols. The peak found around 1500-1550 cm⁻¹ showed a stretch for C-H bond, peak around 1450-1500 cm⁻¹ showed the bond stretch for N-H. Whereas the stretch for Ag-NPs was found around 500-550 cm⁻¹. Therefore, the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins have the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. Carbonyl groups proved that flavanones or terpenoids are absorbed on the surface of metal nanoparticles. Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or π -electrons in the absence of other strong ligating agents

in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in reducing metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids. These issues can be addressed once the various fractions of the neem leaf extract are separated, identified and individually assayed for reduction of the metal ions. This rather elaborate study is currently underway.

XRD ANALYSIS

XRD spectrum showed distinct diffraction peaks around 380, which are indexed by the (100) of the cubic face-centered silver. These sharp Bragg peaks might have resulted due to capping agent stabilizing the nanoparticle. Intense Bragg reflections suggest that strong X-ray scattering centres in the crystalline phase and could be due to capping agents. Independent crystallization of the capping agents was ruled out due to the process of centrifugation and redispersion of the pellet in millipore water after nanoparticles formation as a part of purification process. Therefore, XRD results also suggested that the crystallization of the bio-organic phase occurs on the surface of the silver nanoparticles or vice versa. Generally, the broadening of peaks in the XRD patterns of solids is attributed to particle size effects. Broader peaks signify smaller particle size and reflect the effects due to experimental conditions on the nucleation and growth of the crystal nuclei.

CONCLUSION

The rapid biological synthesis of silver nanoparticles using *Azadirachta indica* leaves extract provides environmentally friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were spherical and sheet shaped and the estimated sizes were 160-180 nm. The size was bigger as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-vis spectrophotometer, SEM, DLS, Zeta Analyzer, XRD, and FTIR techniques. All these techniques were proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance whereas the lower concentrations showed spherical shape. The sizes of the nanoparticles in different concentrations were also different which depend on the reduction of metal ions. From the data of DLS it was found that the 30:1 ratio solution had sharp nanoparticles of around 5 nm, and some have around 180 nm and the had the potential of around 15.5 mV. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has

several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

FINANCIAL SUPPORT

None

DECLARATION COMPETING INTEREST

Not Declared

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None

INFORM CONSENT AND ETHICAL CONSIDERATION

Not Applicable

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