

A Simple Biosynthesis of Silver Nanoparticles from Aqueous Leaves Extract of *Acacia arabica* and their Antimicrobial Studies

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ABSTRACT

This paper describes a simple biosynthesis of silver nanoparticles (SNPs) from aqueous leaves extract of *Acacia arabica* (AA), a natural product. The formation of SNPs was confirmed and optimized by measuring surface plasmon resonance (SPR) peak around 420 nm using UV-visible spectroscopy (UV-Vis). The possible functional groups of AA leaves extract and their changes after treating with aqueous silver nitrate were evaluated by Fourier transform infrared spectroscopy (FTIR). The size of synthesized SNPs was measured by Zetasizer analyzer analysis and morphology was examined by scanning electron microscopy (SEM) in the nano range. The average size of SNPs is ~30 nm and monodispersed. Antibacterial activity of AA-SNPs was studied taking into account *Bacillus subtilis* (*bacillus*) and *Escherichia coli*. The characteristics of the facial AA-SNPs formed suggest biomedical application as chemical sensors in the future.

Key words: Biosynthesis, *Arabica aqueous*, Natural product, Silver nanoparticles, Antimicrobial activity.

1. INTRODUCTION

In recent years, an escalating percentage of nanomaterials have emerged, providing advancement in diverse fields [1-3]. As nature makes optimum use of materials and space, many inorganic materials are produced in biological systems [4]. Similar to such natural processes, plants [5-8], fungi [4], and bacteria [9] are found to be power sources for the production of nano-sized metal particles. Biomolecules are the sources of reductants that are found to have a potential gain over their protecting group counterparts [10]. The highest challenge in the nanoparticles study is to get the desired size and shape of the metal nanoparticles using benign starting materials. To achieve these results, scientists made several efforts to develop beneficial and green chemistry compound inspection protocols for the synthesis of nanomaterials with tailor-made structural properties [11,12].

At present, there has been a progressive need to derive an environmentally exonerate nanoparticle synthetic process that does not involve any toxic chemicals. However, polymeric metal nanoparticles are synthesized by reducing agents such as hydrazine, 1, 2-hexadecanediol, NaBH₄, and ascorbic acid [13,14] for the reduction of silver ions to SNPs (Ag⁺ to Ag⁰), which are extremely toxic in nature. Furthermore, natural plant extracts are used extensively as reducing agents as well as capping agents to inhibit the agglomeration and stability [15,16] of the synthesized nanoparticles. Interestingly the noble metallic nanomaterials are receiving more interest in recent research due to their interesting properties exhibiting wholly innovative and improved characteristic properties compared to the large particles of the bulk materials with specific characteristics, such as unique size distribution and required morphology.

The potentiality of a plant system in biologically assisted synthesis of metal nanoparticles called “green synthesis” and has now achieved a great role in the field of nanoscience and technology [17]. Green synthesis is chemistry which helps in the design, development, and

implementation of chemical products; also, it facilitates in a reduction or elimination the substances that are hazardous to human health and the environment [18]. Recently, there is a renewed interest in using a green chemistry approach to synthesize metal nanoparticles [19,20]. Sastry *et al.* [21,22] employed an in silico approach to investigate the synthesis of stable Ag, Au, and Ag-Au bimetallic nanoparticles using *Azadirachta indica* leaf broth and suggested that the flavanone and terpenoids are prime in responsibility for the stabilization of nanoparticles. Recent reports also suggested biosynthesis of Ag and Au nanoparticles from *Sansevieria roxburghiana* [23], *Tridax procumbens* leaf extract [24], *Plumeria obtusa* leaf extract [25], *Ficus racemosa* leaf extracts [26], and glucose and starch [27]. The biosynthesized nanoparticles such as Au, Ag, and Pt are widely used in medical and pharmaceutical applications [28] for catalysis [29], anti-bacterial, antiviral agents [30], and sensor applications [31].

In this paper, we report a simple biosynthesis of Ag metallic nanoparticles by the reduction of aqueous Ag⁺ ions through a green approach using the bark extract of AA at room temperature. Due to the nutritional, medicinal, and environmental properties and also the bioavailability of AA, it is chosen to produce nanoparticles with leaf extracts. Rapid biosynthesis of nanoparticles using AA leaf extract has been investigated. To the best of author’s knowledge, there have been no reports on the biosynthesis of nano-silver from the aqueous extracts of the AA leaf, also their spectrochemical and antibacterial studies.

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2. MATERIALS AND METHODS

2.1. Materials

Silver nitrate (AgNO_3 99.9%) received from Sigma-Aldrich Chemie, Germany, *Acacia arabica* (AA) aqueous leaves were collected from the premises of Sri Venkateswara University, Tirupati, India. All other chemicals used are analytical grade and received from Sigma-Aldrich Chemicals Pvt. Ltd., Hyderabad, India. Throughout the experimentation, distilled water was used.

2.2. Extraction Procedure

Aqueous AA leaf extract was prepared by a green process technique, using the standard procedure described by Salem *et al.* [32]. Collected AA leaf was washed several times with double distilled water to remove the dust and foreign particles on the surface of leaves. Twenty grams of leaves samples were finely chopped and boiled in 100 mL double distilled water in 250 mL conical flask for 30 min. The AA extract was filtered with Whatman grade No.1 filter paper. The extract was centrifuged at 7500 rpm to remove the heavy molecular weight

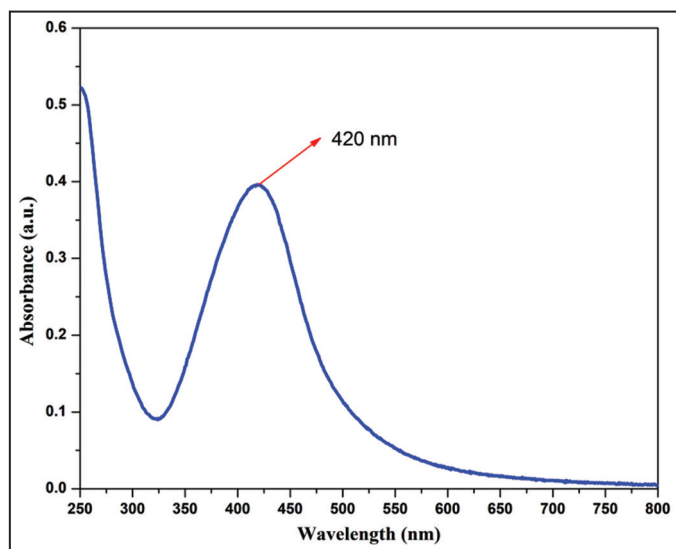


Figure 1: UV-Vis absorption spectra of AA leaf extract silver nanoparticles (AA-SNPs).

constituents. The pH of the extract was found to be 7.5. The collected extract was preserved at 4°C for further experimental analysis.

2.3. Biosynthesis of Silver Nanoparticles (SNPs)

Ten milliliters of aqueous AA leaf extract were added to 100 mL of 1×10^{-3} aqueous solution of AgNO_3 at room conditions. The color change from pale green to dark brown was observed within 40 min, indicating the formation of AA-SNPs. UV-Visible spectroscopy analysis showed the plasmon resonance (SPR) peak at around 415 nm.

2.4. Analytical Characterization

To confirm the formation of SNPs in the AA leaf extract, absorption studies of developed SNPs were carried out on an ultraviolet-visible spectroscopy (UV-Vis) (Shimadzu) for well-dispersed extract and AA-SNPs solution in the wavelength range 200–800 nm. Fourier transforms infrared spectroscopy (FTIR) is only one of the superlative analytical tools, which provides the possibility to identify the functional groups in the aqueous bark extract and produced AA-SNPs. The IR spectra of the AA-SNPs were measured using an FTIR spectrophotometer (ECO-ATR). To measure the transmission electron microscopy (TEM) (HR-TEM, JEOL JEM-2010, accelerating voltage of 200 kV) samples of the green-synthesized SNPs were prepared by placing a drop of the colloidal solution on carbon-coated copper grids, allowing the films on the TEM grids to stand for 2 min, removing the excess solution with blotting paper, and letting the grid dry before measurement.

2.5. Antibacterial Activity

Antimicrobial activity of SNPs was performed by Bauer–Kirby's disk diffusion method. The Muller–Hinton Agar (M173 HiMedia, India) media was sterilized at 121°C and 15 lbs for 15 min in an autoclave and plates were prepared with a depth of about 4 mm. The cultures were transferred to the center of an agar plate independently and spread evenly over the surface with a sterile bent-glass rod (purveyor). Ten microliters of pure AA leaf extract along with biosynthesized AA-SNPs solution and 1×10^{-3} M AgNO_3 have impregnated onto filter paper disks of ~5 mm diameter (which were prepared using Whatman grade No.1 filter paper) under laboratory conditions, then placed onto cultured plates using sterile forceps. The plates were then incubated for 24 h at 37°C in an incubation chamber. The antimicrobial activity was evaluated in replicates by quantifying the zone of inhibition (ZOI) for

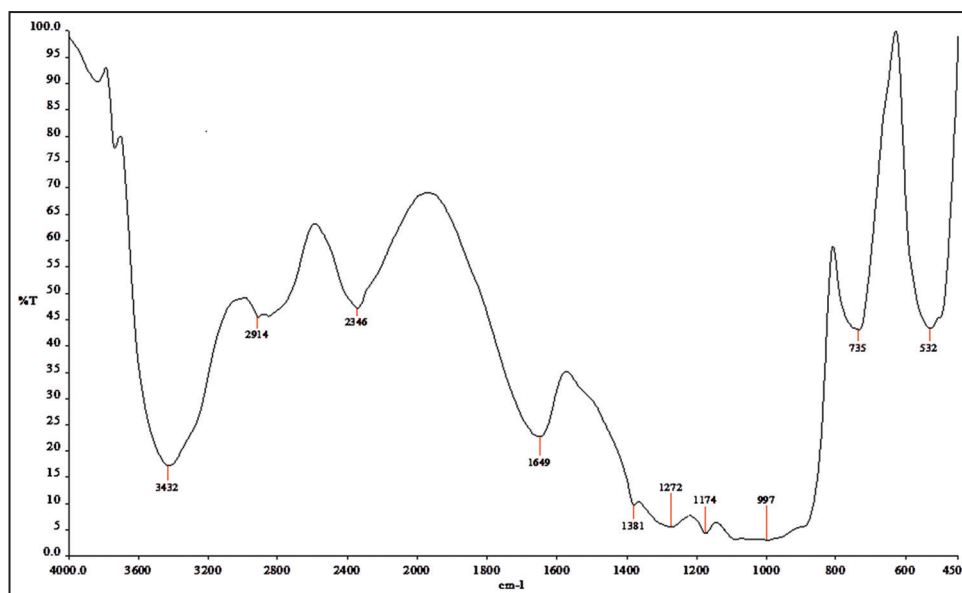


Figure 2: FTIR spectra of AA leaf extract bio-synthesized AA-SNPs.

the test organisms, namely, *Bacillus subtilis* (*Bacillus*) and *Escherichia coli* (*E. coli*), respectively. The inhibition zone diameter produced specifies the susceptibility or resistance of a bacterium to the extract.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Absorption Spectra

The bioactive chemical constituents, which were present in AA leaf extract and are responsible for formation of AA-SNPs, are identified in this study. Reduction of Ag nanoparticles during exposure to leaf extract could be detected by the color change. A color change from pale green to brown was observed within 5 min and for dark brown within 30 min. It may be due to the addition of aqueous AgNO₃ solution into AA leaf extract that the Ag⁺ ions were attracted by the -O⁻ group of biomolecules to form Ag complex. The formed complex was reduced to zero valence of silver (Ag⁰) by free electrons which were produced in the reduction process. The AA-SNPs are produced and stabilized by carboxyl (-COO⁻), hydroxyl (OH) groups present in the AA aqueous extract of leaf [33]. This phenomenon is the result of the surface plasmon vibrations of SNPs. It is due to the free electrons which are present at SNPs. In Figure 1, UV-visible spectra of band peaks produced surface

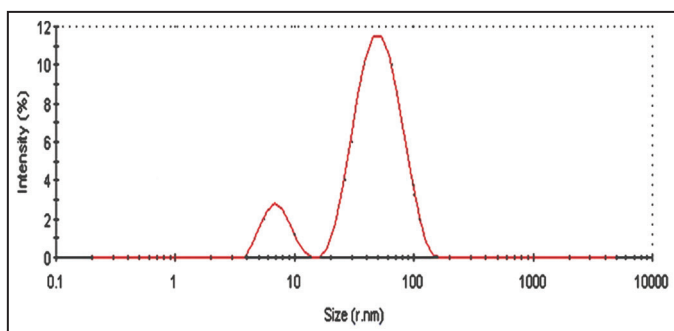


Figure 3: Size distribution of AA-SNPs by Zetasizer analyzer.

plasmon vibrations of AA-SNPs at 420 nm, which indicates the reduction of Ag⁺ into Ag⁰. It is a well-known fact that the optical properties of the metal nanoparticles are strongly dependent on their shape and size [34].

3.2. FTIR Analysis

Figure 2 shows that the FTIR analysis of bark aqueous AA leaf extract mediated AA-SNPs and carried out to identify possible functional groups which may be attributed to the process of bio-reduction of (Ag⁺) silver ions to silver nanoparticles (Ag⁰). The analysis of AA-SNPs displayed several absorption peaks at 2931, 1519, and 1043 cm⁻¹ in Figure 2. The peak at 3412 cm⁻¹ showed that the -OH stretching groups of a phenolic compound may be involved in bioreduction and to form the AA-SNPs. The absorption band at 2941 cm⁻¹ indicated that -CH₂- and -CH₃ symmetrical vibration of aliphatic hydrocarbons and the bands at 1727 cm⁻¹ could be assigned to the -C=O stretching of aliphatic esters. An intense band at 1043 cm⁻¹ indicates the -C-N- stretching vibrations of aliphatic amines. Moreover, the peak at around 1490 cm⁻¹ which is N-O symmetrical stretching of -C=C-NO₂. The fingerprint information evidenced that the carbonyl groups were bound to silver ions and could act as capping agents to prevent the agglomeration and provides stability to the nanoparticles.

3.3. Particle Size Distribution Analysis of Biosynthesized AA-SNPs

A particle size experiment of AA-SNPs was carried out by means of dynamic light scattering (DLS). Particle size ascertainment of the produced nanoparticles was shown under heading like size distribution by intensity. Figure 3 demonstrates that the size of AA-SNPs dispersed was drifted widely from 10 nm to 60 nm, the average particle size is expected to be in the range of ~30 nm. Allocation of particle size by magnitude gives a spherical-shaped group. In an earlier study, SNPs size distributions were obtained in the range of 2 nm to 14 nm using *Salacia mulbarica* leaf extract [16].

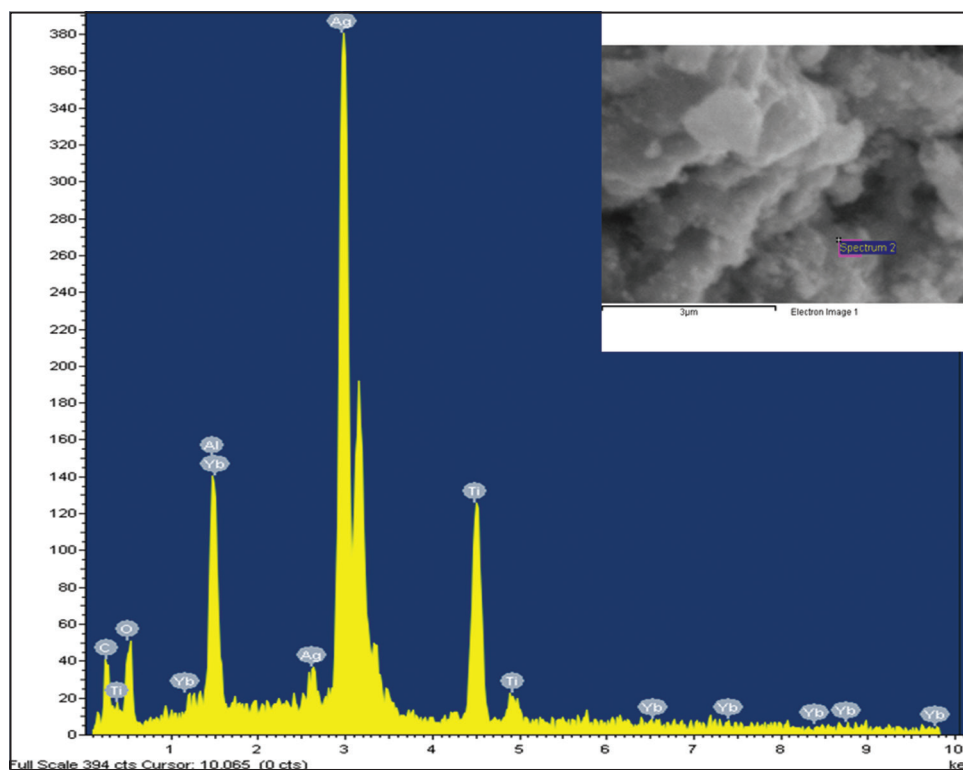


Figure 4: EDX spectrum and scanning electron microscopy image of the AA-SNPs (Insert).

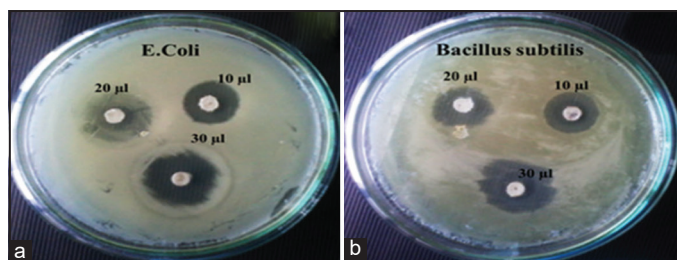


Figure 5: Photographs showing bacterial colonies in Petri plates (a) zone of inhibition refers to the *Escherichia coli* and (b) zone of inhibition refers to the *Bacillus subtilis* of the biosynthesized AA-SNPs.

3.4. Scanning Electron Microscopic (SEM) and EDX Analysis

Elemental analysis and SEM images were captured with a dry sample of the bio-fabricated AA-SNPs and the results were placed in Figure 4. A sharp, intense peak labeling with Ag indicates the synthesized composition having a high amount of silver nanoparticles present in it. The SEM image of the AA-SNPs had a smooth surface area with an average size of around 30 nm which associates with DLS. The size distribution, morphology of nano-sized particles, and surface morphology in solution are important factors in evaluating the toxicity of the biosynthesized SNPs [35]. SEM image reveals that the particles are in smaller size distribution, monodispersity, and with an average size of ~30 nm.

3.5. Antimicrobial Activity of AA-SNPs

In the present study, AA leaf extract mediated SNPs were tested against *Escherichia coli*, *B. subtilis* in different volumes. The zone of inhibition (ZoI) in diameter was discerned by the disk diffusion method and the results showed visible and clear zones (Figure 5) for disks loaded with aqueous solutions AA-SNPs colloids. For this analysis, 1 mg of AA-SNPs was dispersed in 5 mL of double distilled water and the suspension was allowed to stir on magnetic stir for 30 min for the uniform distribution of the AA-SNPs throughout the water. The zones of inhibition were developed against both Gram-negative (*Escherichia coli*) and Gram-positive (*B. subtilis*) and Gram-negative (*Escherichia coli*). The ZoI was increasing with an increase in the volume of the aqueous colloidal suspension of the AA-SNPs in both Gram-negative and Gram-positive species. Figure 5 showed that AA-SNPs exhibit effective ZoI against *E. coli* (Figure 5a) and moderate inhibition against *B. subtilis* (Figure 5b). Hence, in the present study, *Escherichia coli* showed better activity than *B. subtilis* toward green synthesized AA-SNPs.

4. CONCLUSION

The present study demonstrates bio-reductive synthesis of nano-sized silver particles using AA leaf extract. The AA leaf aqueous extracts appear to be environmentally friendly so that this procedure could be used for the rapid production of silver nanoparticles. SNPs were synthesized by the green approach reported in this study using AA leaf extracts. AA extract could act not only reducing the Ag ions but also controlling the size, that is, ~30 nm. Further, the SNPs formation conformed by UV-Visible, FTIR, Zetasizer, EDX, and SEM studies. In the future, the selection of such plants may create a new platform for realizing the potential of herbal medicines in nanoscience for drug delivery and biomedical applications. The antibacterial activity of AA-SNPs suspension showed enhancement of the activity toward *Escherichia coli* and *B. subtilis*. Hence, the developed procedure has several advantages, such as controlling the size of metal nanoparticles, being eco-friendly and cost-effective.

5. CONFLICTS OF INTEREST

Nil.

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