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RESEARCH ARTICLE

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SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *EMILIA SONCHIFOLIA (L.) DC* AERIAL PARTS

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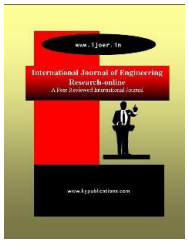

ABSTRACT
Plant mediated synthesis of metallic nanoparticles is an increasing commercial demand due to the wide applicability in various areas such as electronics, catalysis, chemistry, energy, cosmetics and medicine. The present study aimed to synthesis of SNPs (Silver Nanoparticles) or (Green-Silver) in aqueous medium using *Emilia sonchifolia* plant extract from aerial parts. The synthesized silver nanoparticles (SNPs) were characterized by the different techniques. Their morphology, elemental composition and crystalline phase were determined by scanning electron microscopy, TEM, SAED, XRD, energy dispersive X-ray spectroscopy and FT-IR analysis was used to confirm the presence of silver nanoparticles in the extract. UV–visible spectrum of the aqueous medium containing silver nanoparticles showed absorption peak at around 432nm. Fourier transform infrared spectra had shown that the biomolecule compounds were responsible for the reduction and capping material of silver nanoparticles and nearly 10-70 nm in diameter with spherical in shape by TEM, high crystalline with the Bragg peaks of (111), (200), (220), (311) and (222) plane as the predominant orientation by XRD. A SEM images showed that the silver nanoparticles formed were spherical in shape, with an average size of around 42nm.

Key Words: *Emilia sonchifolia* plant extract, Silver Nanoparticle, Characterization

INTRODUCTION

Silver nanoparticles (SNPs) are increasingly used in various fields, including medical, food, health care, consumer, and industrial purposes, due to their unique physical and chemical properties. These include optical, electrical, and thermal, high electrical conductivity, and biological properties [1]. Recently, AgNPs have been frequently used in many textiles, keyboards, wound dressings, and biomedical devices [2]. Nanosized metallic particles are unique and can considerably change physical, chemical, and biological properties due to their surface-to-volume ratio; therefore, these nanoparticles have been exploited for various purposes [3, 4]. In order to fulfill the requirement of AgNPs, various methods have been adopted for synthesis. Generally, conventional physical and chemical methods seem to be very expensive and hazardous [5]. Interestingly, biologically-prepared AgNPs show high yield, solubility, and high stability [1]. Among several synthetic

methods for AgNPs, biological methods seem to be simple, rapid, non-toxic, dependable, and green approaches that can produce well-defined size and morphology under optimized conditions for translational research. In the end, a green chemistry approach for the synthesis of AgNPs shows much promise.

After synthesis, precise particle characterization is necessary, because the physicochemical properties of a particle could have a significant impact on their biological properties. In order to address the safety issue to use the full potential of any nano material in the purpose of human welfare, in nanomedicines, or in the health care industry, etc., it is necessary to characterize the prepared nanoparticles before application [6,7]. The characteristic feature of nanomaterials, such as size, shape, size distribution, surface area, shape, solubility, aggregation, etc. need to be evaluated before assessing toxicity or biocompatibility [8]. To evaluate the synthesized nanomaterials, many analytical techniques have been used, including ultraviolet visible spectroscopy (UV-vis spectroscopy), X-ray diffractometry (XRD), Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), and so on [9,10].

Emih sonchifoha (L) DC (Compositae family) (figure 1), is a herbaceous plant found in India and other countries in Asia. In India it is used in folklore medicine, against inflammation, rheumatism, cough, cuts and wounds. The aqueous extract of this plant showed andmicrobial activity. The aerial part of the plant contains alkaloids, and flavaiioids. Plants belonging to Compositae showed antioxidant, antitumor and anticarcinogenic propemes. A detailed investgation has been made to ascertain the antioxidant, anti-inflammatory antitumor and anticarcinogenic property of this plant. The aqueous and methanolic extracts of the leaves of Emilia sonchifolia gradually exhibit antitumor activities. The n-hexane extract of E. sonchifolia has anticancer effect and is rich in terpenoids and terpenoids were evaluated for their potential antineoplastic activity in various human cancer cell lines such as gastric, pancreatic, and colon carcinomas [15].

The purpose of this research work is to provide information on the recently discovered green synthesis process of SNPs using extracts of *Emih sonchifoha* L. and reduction of Ag^{+} ions to Ag^{o} nanoparticles from $AgNO_3$ solution within 30 minutes of reaction time at room temperature. The size of SNPs can be managed from the range of 10 nm to 70 nm by changing the concentration of both plant extracts and $AgNO_3$ and characterized by UV-Vis spectra, XRD, SEM-EDX, TEM, and FTIR analysis.

Scientific classification
Kingdom: Plantae
Order: Asterales
Family: Asteraceae
Genus: Emilia
Species: E. sonchifolia
Binomial name: Emilia sonchifolia (L.) DC. ex Wight

Figure 1: Emilia sonchifolia (L.) DC specimen

2. Materials and Method

All chemicals and reagents had analytical grade. Silver nitrate, n-hexane with high purity purchased from Sd Fine/Merck India Chemicals, India.

2.1 Apparatus and Instruments: The conventional Soxhlet extraction apparatus was used, which consists of a condenser, a Soxhlet chamber, and an extraction flask. The extractor thimble was permeable one with 44

mm internal diameter and 200 mm external length. The rotary evaporator was used for evaporation of solvent of extracted material.

2.2 Sampling and extraction

***Plant Material*:** Fresh aerial parts (Leaf, Stems, Flowers except roots) of *Emih sonchifoha* (L) DC in bulk collected in the month of July 2011 from agricultural fields of local area of Nidubrolu town, Andhra Pradesh. 30x10 cm roots were collected cut in to small pieces, washed and dried in sunlight for one month completely to eliminate surface moisture. Then plant material packed into envelops and kept in oven at 60^{o}C temperature for further dryness. Dried material was grinded separately in a mortar obtained fine powder and sieved; which was then kept in plastic bags for further use.

Preparation of plant extract: The dry plant powder material of *Emih sonchifoha* passed through sieve (100). The coarse powdered drug (100grams) was extracted in Soxhlet apparatus for 48 h with ethyl acetate and n-Hexane (60:40) combination, the extract obtained was concentrated under reduced pressure in rotatory evaporator below 75°C temperature to get Then the filtered extract was stored in refrigerator at 4°C for further use in synthesis of silver nanoparticles.

2.3 Synthesis of AgNPs (SNPs): The synthesis of silver nanoparticles was done by mixing *A. hispidum* root extract and 1 mM of aqueous silver nitrate solution ($AgNO_3$) in the ratio 1:20 added to plant extract ethanolic solution and heated at 80 ± 2°C until the colour of the solution was changed from colour less to light brown (Figure 2). Resulted solutions were settled for 24 hours in dark to avoid any further photochemical reactions, after that the solution was centrifuged at 3000 rpm for 30 minutes with magnetic shaker. The supernatant was discarded and the pellet was air dried in the incubator.

2.4 Separation of silver nanoparticles: The synthesized silver nanoparticles were separated by centrifugation using a REMI centrifuge at 10,000rpm for 15min. The supernatant liquid was re-suspended in the sterile double distilled water. The process was carried out thrice to get rid of any unco-ordinated bio molecules. After, the desired reaction period, the supernatant liquid was discarded and the pellets were collected and stored at 4◦C for further use

The bioreduction of Ag^{+} ions was monitored by periodic sampling by the UV spectrophotometer. The AgNPs in the freeze-drying bottle were suspended in ultrahigh purity water for all characterization methods and antibacterial assays. During biosynthesis of silver nanoparticles when stem extract was added to 100 ml of 1 mM $AgNO_3$ salt, the ionization took place as follows:

$AgNO_3(aq) \leftrightarrow Ag^{+}(aq) + NO_3^{-}(aq)$

$e^{-} + Ag^{+} \rightarrow Ag^{o}$

It is assumed that the silver ions enter inside the plant cell via the H^{+}ATPase protein embedded in the thylakoid membrane by an electro genic pump. Synthesis of silver nanoparticles is a photochemical reduction reaction.

2.4 Characterization techniques

- UV–visible spectroscopy: The formation of dark brown color during the synthesis was confirmed as the formation of AgNPs. The reduction of the pure AgNPs was recorded under UV–visible spectroscopy using ELico model UV–visible spectrophotometer between 300 nm and 700 nm. The UV–visible spectra of the plant leaf extract and silver nitrate solution were also recorded.
- FTIR analysis was done using Perkin Elemer Spectrum-1, and was used to identify the chemical constituents in the region of 400-4000 cm^{-1} of the Ag-NPs
- XRD measurement: XRD measurements of Ag-NPs were cast into glass slides were done by Phillips PW 1830 instrument. The operating voltage of 40 kV and current of 30 mA with Cu kα radiation of 0.1541 nm wavelength, in the 2θ range 10- 80º, step size 0.02/θ.
- The morphology of the Ag-NPs was analyzed using an SEM. The powdered Ag-NPs were uniformly spread and sputter coated with platinum in an ion coater for 120 seconds, then observed by SEM JEOL-JSM 6360 MODEL, JAPAN). The size distribution of the nanoparticle was obtained by counting

150 particles from an enlarged SEM image.32 Elemental analysis of the powdered Ag-NPs was conducted using an EDX detector (EDS, EDAX Inc., Mahwah, NJ, USA) attached to the SEM machine.

- TEM analysis of Ag-NPs: Sample for TEM analysis was prepared, as mentioned in IR sample preparations. The sample was first sonicated (Vibronics VS 80) for 5 minutes. Ag-NPs were loaded on carbon coated copper grids, and solvent was allowed to evaporate under Infra light for 30 minutes. TEM measurements were performed on Phillips model CM 20 instrument, operated at an accelerating voltage at 200 kV.

3.0 Results and Discussion

3.1 UV Visible spectroscopic studies

In the present study, reduction of silver ions present in the aqueous solution of silver nitrate during the reaction with the ingredients of *Emih sonchifoha plant* extract has been seen by the UV-Vis spectroscopy ranging from 300 to 600 nm. The maximum absorption was obtained at 432 nm (Figure 2). The formation of the AgNPs during the reduction process is indicated by change in the color of the reaction solution from colorless to dark brown which can be visually observed (Fig. 2). The bioreduction of $AgNO_3$ ions in solution was monitored by periodic sampling of aliquots (0.1 mL) of aqueous component and measuring UV-Vis spectra of the solution. UV-Vis spectra show no evidence of absorption in the range of 400–800 nm for the plant extract and the plant extract solution exposed to $AgNO_3$ ions shows a distinct absorption at around 432 nm which corresponds to surface plasmon resonance (SPR) of silver nanoparticles established at 420 nm in previous studies [11]. It is observed that the silver SPR band occurs initially at 430 nm; after completion of the reaction, the wavelength of the SPR band stabilizes at 434 nm. Green synthesized AgNPs were stable for six months without shifting the surface plasmon absorbance band [12, 13]. This suggests that the phytochemical present in *Emih sonchifoha* plant extract acts as a reducing agent.

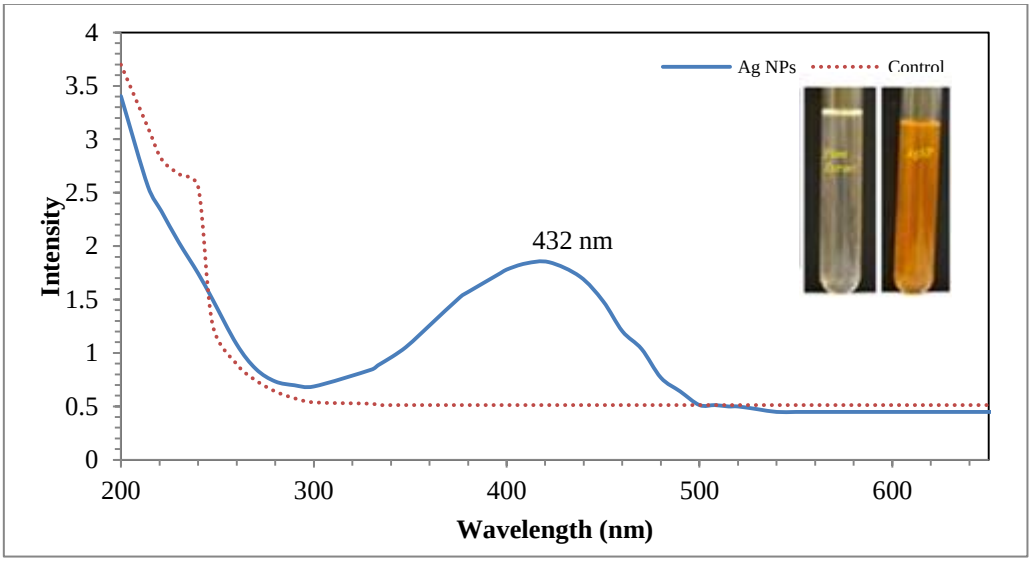

Figure 2: UV-Vis absorption spectrum of (a) *Emih sonchifoha* plant extract and biosynthesized SNPs (visual observation during reduction process (inset)

3.2 FTIR Studies

FTIR has become an important tool in understanding the involvement of functional groups in relation between metal particles and biomolecules which is used to search the chemical composition of the surface of the silver nanoparticles and identify the biomolecules for capping and efficient stabilization of the metal nanoparticles. FTIR measurements were carried out to identify the possible biomolecules in the *Emilia sonchifolia* plant extract. These biomolecules are responsible for the reduction and stabilization of silver nanoparticles.

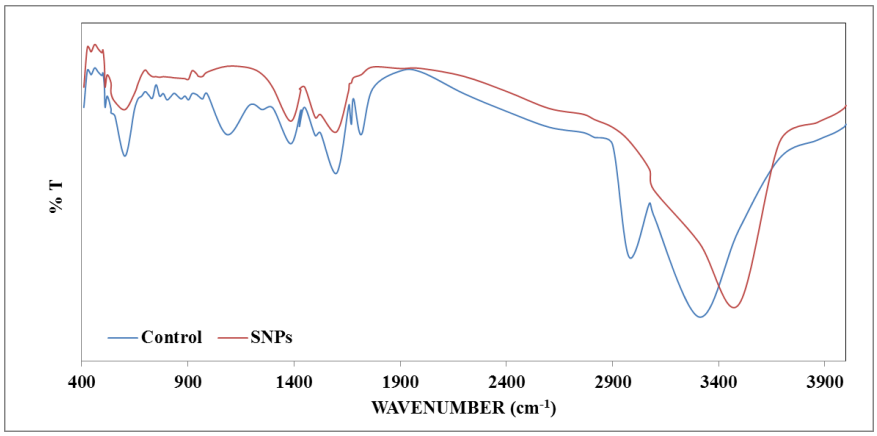

Figure 3: FTIR spectra of plant extract and synthesized silver nanoparticles

The band intensities in different regions of the spectrum for plant extract and silver nanoparticles were analyzed and are shown in Figure 5. FTIR spectrum shows different major peak positions at 3464, 2922, 2886, 2073, 1639, 1419, 1405, 1384, 1109, 868, 861 and 628 cm-1. The similarities between the spectra with some marginal shifts in peak position, clearly indicate the presence of the residual plant extract in the sample as a capping agent to the silver nanoparticles. The peak located at 1639 cm-1 could be assigned to C=O stretching or amide bending. The broad and intense peak at 3464 cm-1 corresponds to OH stretching vibrations of phenol/carboxylic group present in extract. A peak observed at 2922 and 2886 cm-1 is due to C-H stretching of alkanes. The peak at 1384 cm-1 assigned to nitro N-O bending and a peak at 1109 cm^{-1} to C-O-C stretching aromatic ring. It showed peak in the range of 628 cm-1 relating to the alkyl halides band especially the C-Cl bond. Therefore, it may be inferred that these biomolecules are responsible for capping and efficient stabilization of synthesized nanoparticles.

3.3 XRD Studies

The crystalline nature of SNPs was confirmed by the X-ray diffraction analysis. The number of Bragg reflections was showed due to the face-centered cubic structure of silver by the analysis of X-ray diffraction. Figure 3 shows the typical X-ray diffraction pattern for the specimen. The diffraction peaks are broad, suggesting that the sample consists of very small particles. The major peaks (Figure 4) of the prepared SNPs are observed. Five broad peaks with values of, and corresponding to the (111), (200), (220), (311), and (222) planes of the bulk Ag, respectively, which can be assigned to Ag^{o} face-centered cubic structure (FCC) structure. The XRD pattern shows that the samples are single phase, and no other distinct diffraction peak, except the characteristic peaks of FCC phase Ag^{o}, was found.

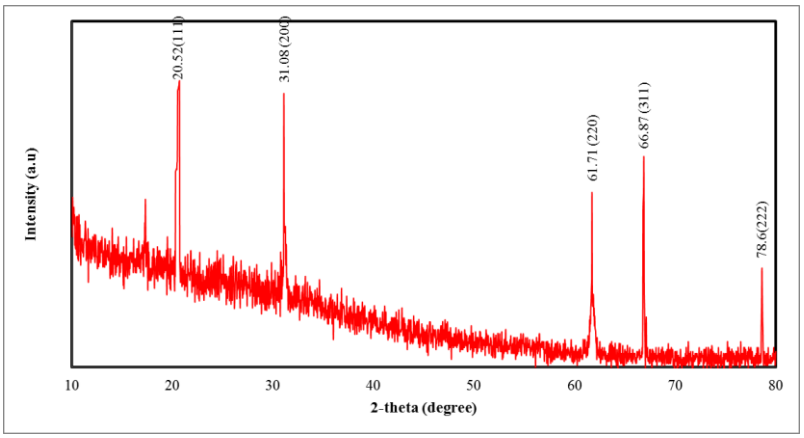

Figure 4: XRD patterns of Ag nanoparticles.

From the full width at half maximum, the grain size for the sample can be calculated from half widths of the major diffraction peak (111) according to Scherrer formula, where is the grain size, is the Scherrer constant

related to the shape and index (hkl) of the crystals, is the wavelength of the X-ray (Cu K,), is the diffraction angle, and is the corrected full width at half maximum (in radian). The average crystallite size was found to be about 42nm, which is well consistent with the average particle diameter obtained from TEM images of Figure 6.

3.4 SEM – EDX Analysis

A SEM employed to analyse the morphology and size details of the silver nanoparticles that were formed. From (Fig. 5) it was showed that the silver nanoparticles formed were spherical in shape, with an average size of around 40nm and uniformly distributed silver nanoparticles on the surface of the cells was observed.

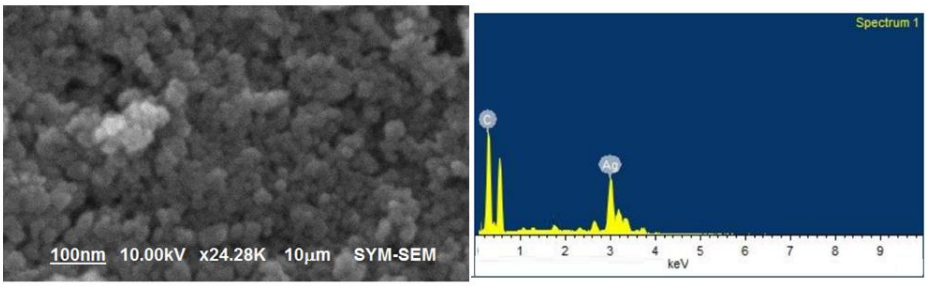

Figure 5: SEM image of silver nanoparticles synthesised using *Emih sonchifoha* plant extract and EDX spectrum of synthesised SNPs (right)

Figure 5 shows the energy dispersive spectrum of the synthesized nanoparticles, which suggests the presence of silver as the ingredient element. Metallic silver nanoparticles generally show a typically strong signal peak at 3 keV, due to surface plasmon resonance [14]. Figure 5 (right) shows the quantitative information of biosynthesized SNPs. This is one of the advantages of nanoparticles synthesized using plant extracts over those synthesised using chemical methods.

3.5 TEM-SAED studies

The shape, size and morphology of the synthesized silver nanoparticles were elucidated with the help of transmission electron microscopy (TEM) further confirming the formation of silver nanoparticles. The size was in the range of 30-55 nm; and the shape of the nanoparticles was spherical and irregular in shape with moderate variation in size, confirming the results obtained by DLS. The average particle size was determined by DLS method, and it was found to be 40 nm as revealed in the size distribution graph.

The selected area electron diffraction (SAED) pattern obtained from the nanoparticles represented silver having face centred cubic (fcc) crystallographic structure. The different diffracting planes were indexed as shown in figure 2(b). Figure 6 shows a cluster of silver nanoparticles at higher magnification. The particles were found to be spherical or polyhedral in shape, having a mean size about 35-50 nm. This polyhedral shape could be attributed to the controlled reaction rate, resulting in initial nucleus formation and subsequent growth. Figure 6 represents the size distribution plot, suggesting that most of the particles conformed to the size range of 35-50 nm

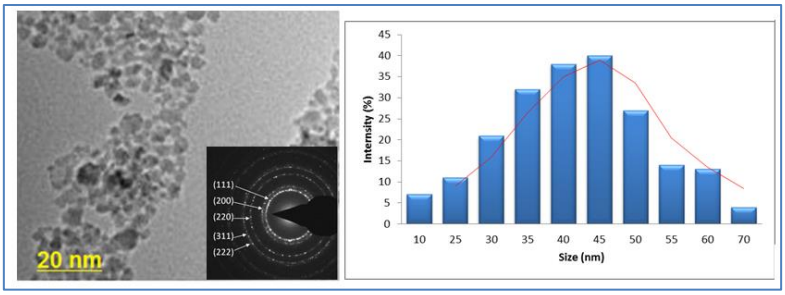

Figure 6: TEM micrographs showing spherical silver nanoparticles, and the SAED pattern and particle size distribution showing most of the particles in the size range of 30-50 nm (Right side image)

4 Conclusion

In conclusion, there has been an exponentially increasing interest in biological synthesis of AgNPs. In this study, AgNPs were synthesized by an ecofriendly and rapid method using *Acanthospermum hispidum* root extract. *A. hispidum* root extract has been used as a reducing agent for the synthesis of silver nitrate into silver nanoparticles. Green synthesized silver nanoparticles are confirmed by color change which was characterized by UV-Vis spectroscopy at 439nm. Further characterization with SEM and TEM analysis shows the spherical AgNPs of particle size ranging from 29 to 33 nm. FTIR showed the structure, the respective bands of the synthesized nanoparticles, and the stretch of bonds. EDX showed the elemental composition of synthesized silver nanoparticles.

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