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RESEARCH ARTICLE

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SYNTHESIS AND CHARACTERIZATION OF GREEN NANOPARTICLES FROM NEW SOURCE OF PLANT MATERIAL

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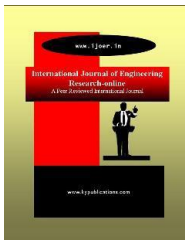

ABSTRACT
The green biosynthesis of nanoparticles has received increasing attention due to the growing need to develop safe, cost-effective and environmental-friendly technologies for nano-materials synthesis. In the process of synthesizing silver nano particles (AgNPs), it was observed that a rapid reduction of silver ions leading to the formation of stable crystalline AgNPs in the solution. Leaf extract (n-Hexane) from *Tiliacora acuminate* was used for the synthesis of AgNPs from silver nitrate solution. AgNPs were characterized by different techniques. Nanoparticles were characterized with the help of UV-Vis absorption spectroscopy analysis, Fourier Transform Infrared (FTIR) analysis, X-ray diffraction analysis (XRD), Scanning Electron Microscopy (SEM) & EDX analysis, and Transmission Electron Microscopy (TEM) analysis. The prepared AgNPs were monodispersed, spherical in shape with the particle size in a range of 30-45 nm
Keywords: AG NP Green synthesis, Characterization, FTIR, UV analysis SEM, EDX, TEM and XRD analysis

INTRODUCTION

The growing need of environmental friendly nanoparticles has attracted many researchers to use green synthesis methods of various metal nanoparticles [1] due to their interesting and remarkable properties with a variety of applications over their bulk material [2]. Considering the photochemical reduction, chemical reduction methods, electrochemical reduction, heat evaporation etc., the biological method is more advantageous [3]. In this biological method, the plant extract has been used as reducing agent and capping agent for the synthesis of nanoparticles [4] due to their reducing properties [5]. Some properties such as size, distribution, and morphology of the particles are clearly obtained from the nanoparticles [6].

Tiliacora acuminata (Lam.) Hook. f. & Thom. (Menispermaceae) (Figure 1), a wonder plant where all its parts are effectively utilized for various medicinal properties. The ethno medicinal uses of this plant include its use as an antidote for snake bite, anti-bacterial, antifungal effect, anti-inflammatory etc. purposes. Roots are rubbed between stones and mixed with water is given as a drink for the cure of venomous snake-bites. Taking into consideration, the medicinal importance of this plant, the methanol extract of leaf of *Tiliacora acuminata* were analyzed for the first time using HPLC, to identify the phytoconstituents present in it.

This work will help to identify the compounds of therapeutic value. A majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties [7]. The current investigation focuses on the n-hexane leaves extract of *Tiliacora acuminata* used to synthesize AgNPs using different experimental conditions and thereby enhancing the importance of plant sources and implementing green chemistry for the future research.

Figure 1: *Tiliacora acuminata* (Lam.) leafs and plant (inset)

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 roots of *Tiliacora acuminata* (Lam.) leaves in bulk collected in the month of August 2012 from Nallamala Forest area, Andhra Pradesh. Fresh leaves collected in bulk, cut in to small pieces washed and dried in sunlight for one month completely to eliminate surface moisture. Then leaf powder packed into envelops and kept in oven at 55^{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: A bout 150 g of leaf powder dipped into a beaker containing 200 ml acetone to remove chlorophyll and stirred at 2000x speed on magnetic stirrer. After that the leaf powder was filtered using Whatmann filter paper and filtrate was collected and dried in an air over for half an hour at 50^{o}C. The dry leaf powder material passed through sieve (100□). The coarse powdered drug (100 grams) was extracted in Soxhlet apparatus for 48 h with n-hexane as a solvent (2L), the extract obtained was concentrated under reduced pressure in rotatory evaporator below 60°C temperature to get semisolid sticky light green residue (15 gm). 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 *Tiliacora acuminata* (Lam.) leaves 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 thick 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 5000 rpm for 10 minutes with magnetic shaker. The supernatant was discarded and the pellet was air dried in the incubator.

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 Elmer Spectrum, 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. 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. Results and discussion

In this study, the silver nanoparticles were synthesized and studied using the extract of the leaves of *Tiliacora acuminata* (Lam.) .

3.1 UV-Visible spectral Studies

The reduction of silver ions to silver nanoparticles was confirmed by UV-Visible spectroscopy analysis and shown in figure 2. It is well known that the silver nanoparticles shows yellowish brown colour in water. These colours occur due to the observable fact of surface Plasmon excitations in the metal nanoparticles of the AgNPs, which is considered to be the primary signature of the formation of nanoparticles [8]. When the plant extract was added into the $AgNO_3$ solution the pale yellow colour solution was obtained. After 45 minutes, the colour changes from colourless to dark yellow. The absorption spectra of silver nanoparticles formed in the reaction mixture was obtained by the UV-Vis analysis at the range between 300-800 nm, the AgNPs has sharp absorbance with highest peak at 443 nm, which is comparable with the literature values and exhibits continuous rise in intensity without any change in the peak position as a function of time [9].

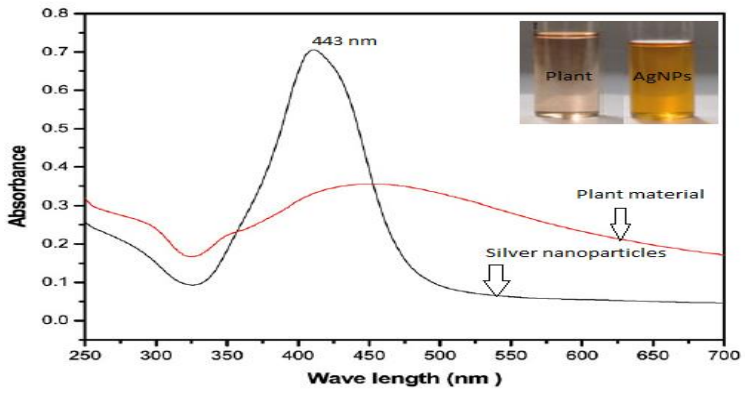

Fig. 2: Color change (inset) and UV-Visible spectra of prepared AgNPs

3.2. XRD Analysis

Analysis through X-ray diffraction was carried out to confirm the crystalline nature of the particles, and the XRD pattern showed numbers of Braggs reflections that may be indexed on the basis of the face cantered cubic structure of silver. A comparison of our XRD spectrum with the standard confirmed that the silver particles formed in our experiments were in the form of nanocrystals, as evidenced by the peaks at 2θ values of 37.21, 42.81, 63.94, and 79.42 corresponding to (111), (200), (220) and (311), respectively Bragg reflections of silver. The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag^+ ions by the *T.acuminata* (Lam.) leaves extract are crystalline in nature. As mentioned in the method section, the silver nanoparticles once formed were repeatedly centrifuged and redispersed in sterile distilled water prior to XRD analysis, thus ruling out the presence of any free biological material that might independently crystallize and giving rise to Bragg reflections. It was found that the average size from XRD data and using Debye-Scherer equation was 32 ± 2.78 nm. The presence of structural peaks in XRD patterns and average crystalline size around 34 nm clearly illustrates that AgNPs synthesized by our green method were nanocrystalline in nature. The XRD pattern of the dried AgNPs obtained by *T.acuminata* (Lam.) leaves extract is shown in Fig. 3. A number of Bragg reflections with 2θ values of 37.21°, 42.81°, 63.94°, and 79.42° correspond to the (111), (200), (220), and (311) sets of planes are observed which may be indexed as the band for face center cubic (FCC) structures of silver. The XRD patterns thus clearly illustrates that the AgNPs synthesize by the present green method are crystalline in nature. The average particle size of silver nanoparticles synthesized by the present green method can be calculated using Debye-Scherrer equation [10].

$$D = \frac{K\lambda}{\beta \cos \theta}$$

Where D = the crystallite size of AgNPs particles

λ = the wavelength of x-ray source (0.1541 nm) used in XRD

β = the full width at half maximum of the diffraction peak.

K = the Scherrer constant with value from 0.9 to 1.

θ = the Bragg angle.

The presence of structural peaks in XRD patterns and average crystalline size around 32 nm clearly illustrates that AgNPs synthesized by our green method were nanocrystalline in nature. The XRD patterns displayed in this work are in good agreement with the earlier research reported for green synthesis of silver nanoparticles [11]

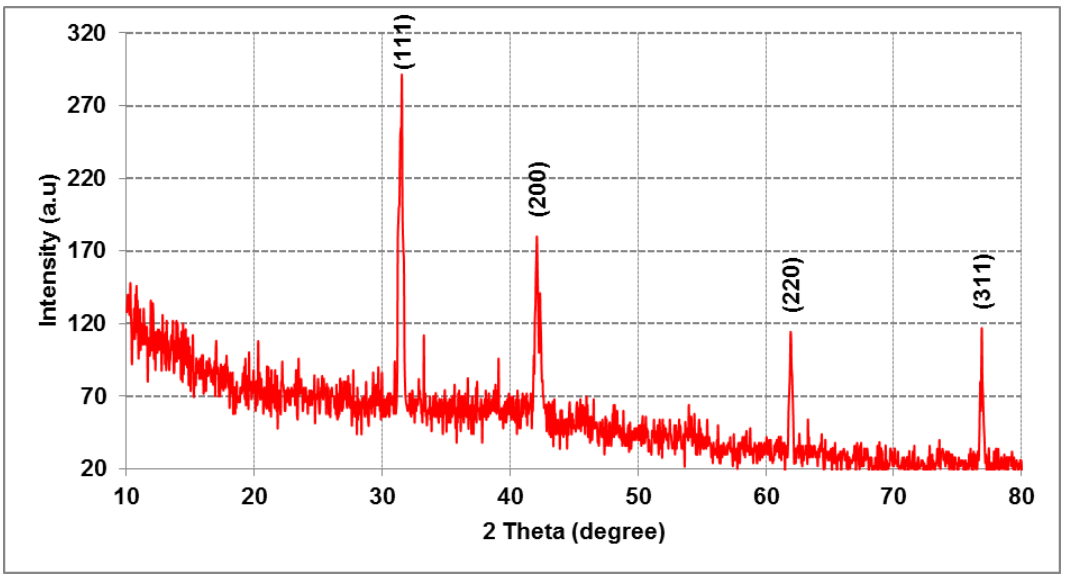

Figure 3. XRD pattern of AgNPS synthesized by *Tiliacora acuminata* (Lam.) leaves extract

3.3 FT-IR analysis

FT-IR analysis measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and the capping of AgNPs synthesized by the leaf extract of *T.acuminata* (L). The solution after complete reduction was centrifuged at 2000 rpm for 30 minutes to isolate the AgNPs free from the compounds present in the solution. FTIR spectrum of biosynthesized AgNPs and n-hexane extract showed absorption peaks at observed at different wavenumbers shown in figure 4.

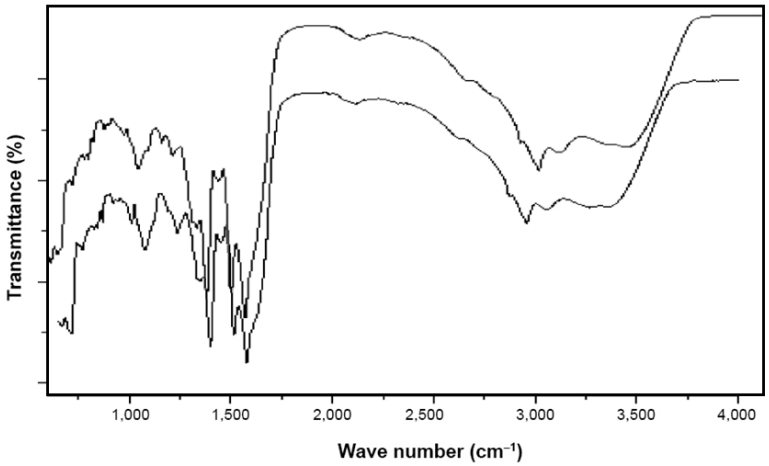

Figure 4. FTIR spectra of *T.acuminata* (L) leaves extract ((below line) and capped AgNPs (above line)

FTIR spectroscopy is useful in probing the chemical composition of the surface of the silver nanoparticles and the local molecular environment of the capping agents on the nanoparticles. In leaf extract, the peaks are observed at 445, 617, 1075, 1287, 1421, 1602, 3157 and 3785 cm^{-1}, respectively. After reaction with $AgNO_3$, the peaks are shifted to a higher wave number side, such as 456, 614, 1074, 1382, 1592, 3158 and 3881 cm^{-1}. The peak at 445 cm^{-1} of the extract is shifted toward a higher wave number side at 456 cm^{-1} due to the O–Si–O network and ring opening vibration. The band observed at 617 cm^{-1} is shifted to the lower side at 614 cm^{-1}, which corresponds to C–Cl stretching in the alkyl group. The strong intense peaks at 1382 cm^{-1} correspond to C–N stretch vibrations, as well as to the amide I bands of proteins in the leaf extract. The flavonoids present in the leaf extract are powerful reducing agents which may be suggestive of the formation of AgNPs by reduction of silver nitrate. The flavonoid compounds in the water extract of *T.acuminata (L)* might be actively involved and responsible for the reduction of Ag^+ to Ag^0. he involvement of water-soluble flavonoid in the reduction of metal ions using plant extracts is also evidenced from another study [12]

3.4 SEM-EDX analysis

SEM analysis showed an image of high density Ag-NPs synthesized by *T.acuminata* (L) leaves extract and is shown in Figure 5. The white individual spots present in the SEM photograph are silver nanoparticles while the larger spots are the aggregate of silver nanoparticles. The spherical and uniform Ag-NPs have been observed with diameter ranging from 15 nm to 60 nm, most of silver nanoparticles present having diameter 34nm. The capping agent indicates the stabilization of the nanoparticles because they were not in direct contact even in the aggregated condition. During SEM measurements the larger silver nanoparticles may be due to the aggregation of the smaller ones. EDX spectrophotometer analysis established the existence of element Ag signal of AgNPs. EDX analysis revealed strong signal of Ag region and is in Figure 5. Metal silver nanocrystals generally show typical optical absorption peak approximately at 3.7 kev. There were other peaks for C, O and Na suggesting that they are mixed precipitates present in the plant extract.

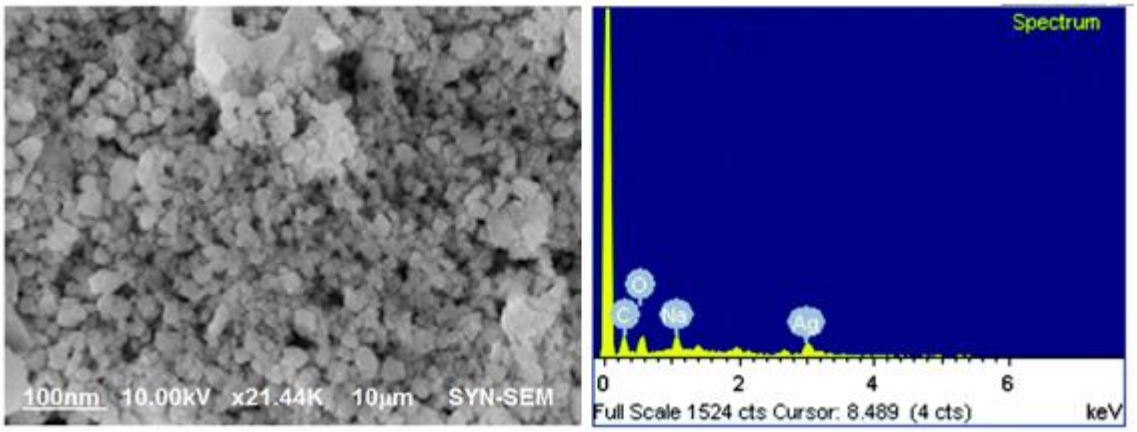

Figure 5: SEM photograph of silver nanoparticles (LEFT) & EDX spectrum of silver nanoparticles (RIGHT)

3.5 TEM-Particle distribution studies

Figure 6 exhibits the TEM images of the silver nanoparticles produced *T.acuminata* (L) leaves extract and 1mM $AgNO_3$. It was detected that the nanoparticles are sometimes spherical and rarely cubical also determined abnormal distribution of particles. The size of the particles extended from 15 to 60 nm, and the mean particle size was around 35 nm (Fig. 6).

The Selected-Area Electron Diffraction (SAED) patterns given reveal bright dots (figure 6), indicating that the nanoparticles are crystalline in nature. The data obtained corroborates other studies on green synthesis of metal nanoparticles

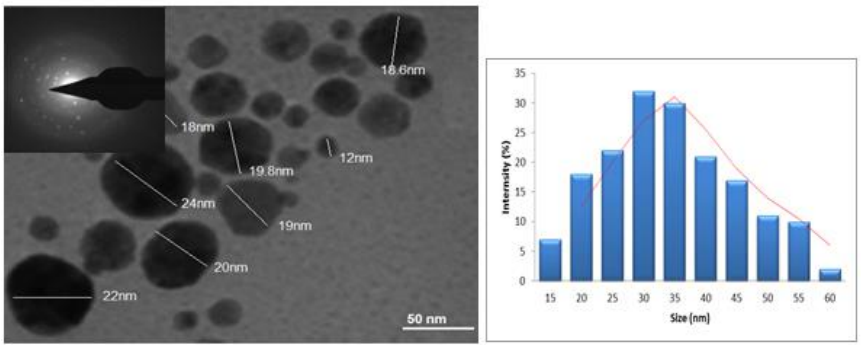

Figure 6: TEM & SAED studies (inset picture is SAED pattern) & The AgNPs size distribution histogram

Conclusion

Silver nanoparticles (AgNPs) were successfully obtained from bioreduction of silver nitrate solutions using *T.acuminata* (Lam.) leaf extract. Owing to varying properties of AgNPs obtained from this plant leaf also varied in size. AgNPs have been appropriately characterized using UV-Vis spectroscopy, XRD, SEM, TEM and EDX analysis. Results denoted *T.acuminata* (Lam.) leaf extract to be a better reducing agent in comparison. FTIR analysis revealed the efficient capping and stabilization properties of these AgNPs. Besides, they also aided in plant germination and growth by sequestering nutrients for them and could hence be implemented for agricultural purposes.

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