Research Article

Phytosynthesis of Silver Nanoparticles by *Thunbergia fragrans* Roxb. and Their Characterization

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ABSTRACT

Phytosynthesis could be an alternative way to traditional chemical methods for the production of metallic nanomaterials in a clean, nontoxic and ecologically manner. In the present study, we aimed to develop a rapid method for the synthesis of silver nanoparticles using aqueous leaf extract of *Thunbergia fragrans* Roxb. 1mM solution of silver nitrate was treated with the aqueous extract of leaf leading to the formation of Ag-NP's was observed visually by change in color from colorless to dark brown color reaction mixture and confirmed by the surface Plasmon resonance peak at 416nm in UV-Vis spectroscopy. The FTIR data reveals the possible functional groups of biomolecules involved in bioreduction and capping for efficient stabilization of Silver nanoparticles. Atomic force microscopy and High resolution transmission electron microscopy studies showed that the spherical shape silver nanoparticles with size ranges from 5 nm to 50nm. Further characterization was done by XRD and EDS.

Keywords: *Thunbergia fragrans* Roxb., Phytosynthesis, FTIR, HR-TEM, AFM, XRD.

INTRODUCTION

Nanoparticles are viewed as the fundamental building blocks of nanotechnology.[1] The synthesis of nanoparticles with the desired quality and properties is one of the key issues in current nanotechnology[2]. Biosynthesis of nanoparticles is a kind of bottom up approach where the main reaction occurring is reduction/oxidation. The microbial enzymes or the plant phytochemicals with anti-oxidant or reducing properties are usually responsible for reduction of metal compounds into their respective nanoparticles. Biosynthesis of nanoparticles is advantageous over physical and chemical methods as it is a cost-effective and ecofriendly, non-toxic method.[3,4] In recent years, the biosynthesis of nanoparticles using plant extracts has gained more significance.

The major advantage of using plant extracts for silver nanoparticle synthesis is that they are easily available, safe, practical, scalable, nontoxic and avoidance of maintaining the microbial culture (Ayman A.). Silver nanoparticles have been synthesized using various plant extracts such as *Cinnamon camphora,*[6] Geranium,[7] Neem leaf broth,[8] *Aloe vera,*[9] Tamarind leaf,[2] *Phyllostachys* sp. Leaves,[10] *Acalypha indica*[11] and *Capsicum annum.*[12]

Among several noble metal nanoparticles, silver nanoparticles (Ag NPs) have attracted special attention because of its superior antimicrobial characteristics as compared to bulk silver.[13] Nanoparticles can act as antibacterial and antifungal agents, due to their ability to interact with microorganisms.[14]

The present study was designed to synthesize, characterize silver nanoparticles and to investigate the antimicrobial activity using leaves of medicinal plant *Thunbergia fragrans* Roxb. It is a perennial climbing twiner belongs to Acanthaceae family.

MATERIALS AND METHODS

All glass wares were washed with sterile water and dried in an oven before use. Analytical grade chemicals -Silver nitrate and sodium hydroxide were used. Experimental plant *Thunbergia fragrans* Roxb. collected from the Botanical Garden of Karnatak University Dharwad, Karnataka, India.

Preparation of Leaf Extract

Experimental plant leaves were washed 2-3 times with tap water followed by double distilled water to remove dust and impurities. Leaves were shade dried to remove the residual moisture and about 25gm. were cut into small pieces and boiled in glass beaker containing 250ml of sterile distilled water for 20 minutes.[15] The aqueous extract was separated by filtration with Whatman No. 1 filter paper and stored in refrigerator at 4^0C for further use.

Phytosynthesis of Silver Nanoparticles

For reduction of silver ions, 10ml of leaf extract was added to 90ml of 1mM aqueous $AgNO_3$ solution taken in Erleyenmeyer flask (250ml). Simultaneously, the reaction mixture was adjusted to pH 8 by using 1 N. NaOH. Then the flask containing reaction mixture was incubated at 40-60°C, resulting in the formation of pale yellow to dark brown solution indicating the synthesis of silver nanoparticles.[16]

Characterization of Nanoparticles

The reduction of metal ions was monitored by measuring the UV-Vis spectroscopy of the solution according to the method of Mie (1908), by the sampling of aliquots (3ml) of the aqueous component. The silver nanoparticles were measured in a wavelength ranging from 200-800nm. The UV-Vis spectroscopy measurement of silver nanoparticle was recorded on UV- Vis spectroscopy (Jasco V-670 UV-Vis NIR spectrophotometer) operated at resolution of 1nm. The solution containing reduced silver ions was centrifuged at 3000 rpm for 40 min to remove the unwanted biomass residue; the resulting suspension was then dispersed in 10ml of double distilled water and centrifuged again at the same condition. Redispersion and centrifugation process was repeated for 2-3 times to obtain silver nanoparticles free from any biomass residue. A sample taken from pellet was dispersed on a slide and dried slide was observed on contact mode of AFM. The pellet thus obtained was redispersed in double distilled water and oven dried at 60°C to obtain the powder. The powder was used for FTIR and HRTEM (TECNAI 20 G2-electron microscope), X-ray diffraction (XRD) analysis and SEM with EDX analysis (Fei Quanta 200 SEM EDAX Genius Xm 4).

RESULTS AND DISCUSSION

The present investigation was carried out to synthesize Ag-NPs using leaf extract of *Thunbergia fragrans* Roxb and to study their biological properties. Nanoparticles are generally characterized by their size, shape, surface area and dispersity. Homogeneity of these properties is important in many applications.[17]

Visual Observation of Silver Nanoparticles

The reduction of metal ions was primarily monitored by visual inspection of the reaction mixture.[18] Leaf extract was mixed with $AgNO_3$; pH was adjusted to 8 and incubated at 30-40°C. The color of the reaction mixture changes from pale yellow to dark brown within few seconds. The change in color has been attributed to excitation of surface Plasmon resonance of the metal nanoparticles.[19]

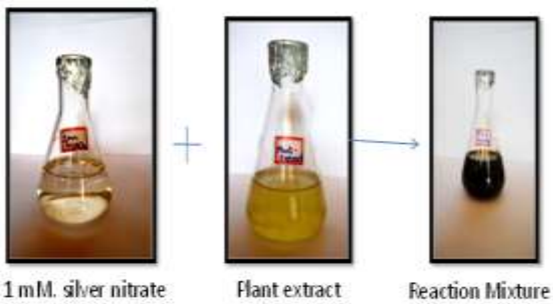

Figure 1: Visual observation of the formation of silver nanoparticle synthesis.

UV-Vis absorption spectroscopy is one of the main tools to analyze the formation of metal nanopartcles in aqueous solution.[20] The AgNPs formation was confirmed by UV-Vis spectrophotometric analysis. The analysis confirmed surface plasmon resonance peak at 416nm. (at pH 8) which is the characteristic of silver nanoparticles and clearly indicate the formation of nanoparticles in solution (Figure 2). Baishya reported the strong SPR band at 418 in case of *Bryophyllum pinnatum* (Lam).[21] Baishya, Kiruba reported the characteristic SPR of colloidal nanoparticles (ranges between 390 – 420) due to the Mie scattering in case of *Dodonaea viscose* leaf extract.[15,22] Silver nanoparticles were synthesized rapidly by using plant system.[23,24,25,26]

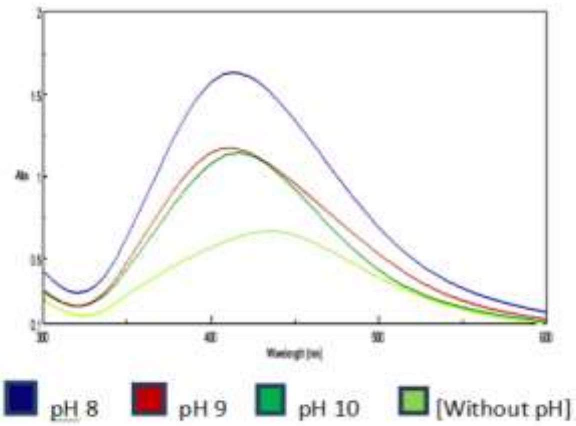

Figure 2: UV-vis absorption spectrum of Ag-NPs synthesized from *Thunbergia fragrans* Roxb. showing SPR peak at 416nm., 413nm., 411nm., and 435nm , pH8, pH9 and pH10, at without pH respectively.

Effect of Physicochemical Parameters on the Nanoparticles Synthesis

Various parameters such as concentration of the leaf extract and $AgNO_3$, pH, temperature and incubation time were optimized for the reduction of Ag+ ions to AgNPs using *Thunbergia fragrans Roxb.*extract. Reaction mixture containing 90 ml. silver nitrate (1 mM.) solution with 10 ml. leaf extract incubated (pH 8) at 40-60°C temperature for 20 min, resulted in yielding absorption pattern at 416 nm. (at PH. 8) in UV–Vis spectrum. The maximum yield of AgNPs is with 1 mM, this concentration was selected for further studies. Among the various parameters, pH is one of the fundamental factors in nanoparticle synthesis. Among 8, 9, 10 pH, the reaction started rapidly at pH 8 of the reaction mixture (as observed by the change in color). At acidic pH, the yield of nanoparticle synthesis was very low and the sizes of AgNPs were relatively larger[25]. However, at pH higher than 7, a large number of nanoparticles with comparatively smaller diameters formed due to the presence of a considerable number of reactive functional groups to bind with silver ions. At pH 11 agglomerations of nanoparticles was observed. In summary, it may be concluded that starting from pH 8, the yield of nanoparticles increased whereas the size remained smaller till pH 10 and upon further enhancement of pH, formation of agglomerates was noticed. The optimal pH for nanoparticle synthesis was preferred to be pH 8; our results are in conformity with Kiruba who reported the formation of AgNPs with adjustment of pH 8-10. Acidic condition suppresses the formation of AgNPs (pH 2 and 4); whereas the slight basic condition enhances the formation of the nanoparticles (pH 6-8). Large sized nanoparticles were formed at lower pH which is indicated by the color change and aggregation in the solution, but small and highly dispersed nanoparticles were formed at pH 8-10, which is in agreement with the early reports.[15,27]

Summary of various optimized parameters for the synthesis of silver nanoparticles.

- Concentration of leaf extract: 10 ml. (for 90 ml. $AgNO_3$)
- Concentration of silver nitrate: 1 mM.
- pH of the medium: 8
- Temperature of the medium: 40-65°C (for 20 min.)
- Incubation time 24 hrs.

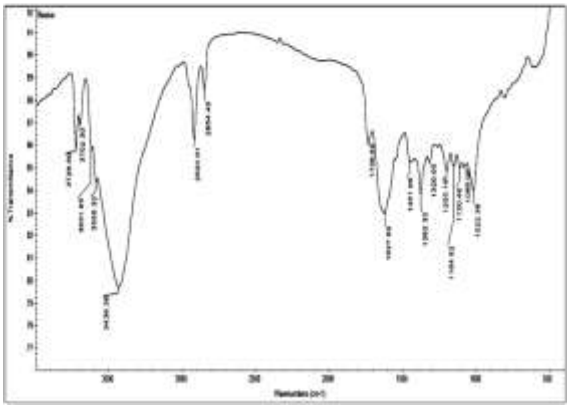

Figure 3: FT- IR spectrum of silver nanoparticles.

Table 1: FT - IR absorption peaks value and their functional groups

S. No.	Absorption Peak (cm^{-1})	Functional Groups
1	3631.65	O-H stretch
2	3438.36	O-H Stretching
3	2924.01	C-H Alkanes (stretch).
4	2854.40	C-H Alkanes (stretch).
5	1738.69	C=O Aldehydes.
6	1627.99	C=C group or aromatic ring
7	1461.96	$-CH_2-$ (bend).
8	1382.32	-C-O Stretching
9	1320.65	C-N Amines.
10	1022.38	C-C Ring stretching

The FTIR analysis was carried out to identify the possible interfacial groups between the capping agents and silver nanoparticles. The study showed sharp absorption peaks located at 3631.65, 3438.36, 2924.01, 2854.40, 1738.69, 1627.99, 1461.96, 1382.32, 1320.65, 1205.18, 1164.62, 1120.46, 1088.08 and 1022.38cm^{-1}. FTIR spectrum also shows band at 3631.65 and 3596.32cm^{-1} indicates that O-H stretch and Free O-H stretch respectively. The band at 3438.36 cm^{-1} identified as N-H stretch, primary & secondary amines and amide. The band at 2924.01 and 2854.40 cm^{-1} identified as C-H Alkanes (stretch). The band at 1738.69 cm^{-1} identified as C=O Aldehydes. The bands at 1627.99, 1461.96, 1382.32 and 1320.65 cm^{-1} identified as C=C Alkenes, $-CH_2-$ (bend), C-X Fluorides Stretch and C-N Amines respectively. The bands at 1205.18, 1164.62, 1120.46, 1088.08cm^{-1} identified as C-O primary, secondary, or tertiary structure to an alcohol. The band at 1022.38 cm^{-1} identified as C-N Stretching vibration of amines. This result suggests that the biological molecules could probably perform a function involving the formation and stabilization of Ag NPs through free amine groups in the proteins.[28]

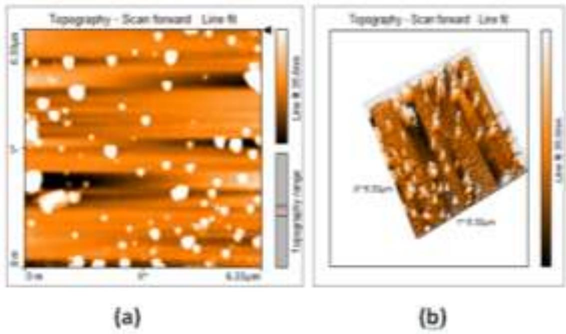

Figure 4: AFM images of silver nanoparticles synthesized from *Thunbergia fragrans Roxb.*(a) Topography and (b) 3D image

The biogenic silver nanoparticles were characterized by AFM. The topographic image of silver nanoparticles was shown in Figure 4(a) where the formation of spherical silver nanoparticles and their agglomeration was clearly observed. Figure 4(b) represents three dimensional views of synthesized silver nanoparticles. The size of the silver nanoparticles ranges from 5 -50 nm. AFM images were taken with silicon cantilevers with force constant and the particle size was measured using line profile. This could be attributed to the fact that the compounds present in the leaf extract were responsible for the particle morphology and were kinetically controlled.[29]

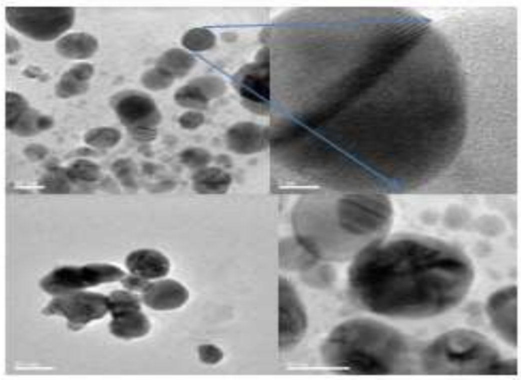

Figure 5: HR-TEM image of silver nanoparticle synthesized from *Thunbergia fragrans Roxb.*

The silver nanoparticles were further characterized by HR-TEM micrograph, these Silver nanoparticles showed spherical shape with the size range from 5 to 50 nm. (Figure 5). Further, it also shows that the biomolecules of leaf extract bound the nanoparticles as capping agents to hinder further oxidation of nanoparticles. HRTEM results confirmed that all the particles (AgNPs) exist in the nanoscale range and possess spherical shape.

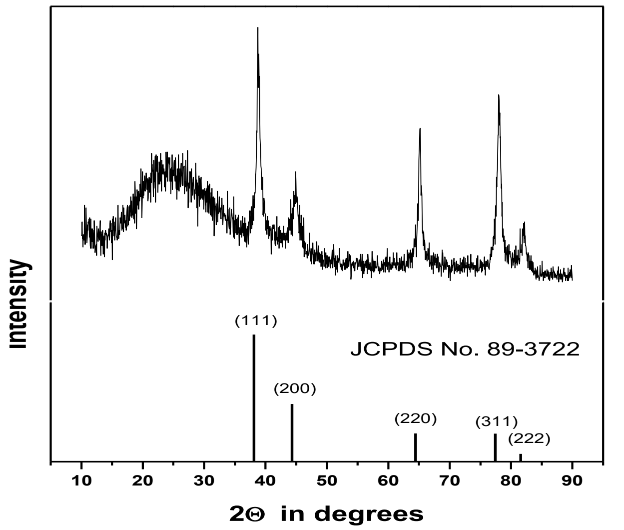

Figure 6: X-ray Diffraction Spectrum of Silver Nanoparticles from *Thunbergia fragrans* Roxb.

A representative XRD profile of the silver nanoparticle displaying the structural information and crystalline nature of the silver Nanoparticle synthesized from aqueous extract of *Thunbergia fragrans* Roxb. XRD analysis the crystalline nature of AgNPs was confirmed by the analysis of XRD pattern as shown in plate 9 and 10. The XRD spectrum showed four distinct diffraction peaks at 38.28°, 44.33°, 64.33°, 77.53° and 81.58° corresponding to lattice plane value indexed at (111), (200), (220) (311) and (222) planes of face centered cubic (FCC) silver with a lattice parameter of a = 4.08 Å which were in good agreement with reference of FCC structure from joint committee of powder diffraction standard (JCPDS) Card No 89-3722.

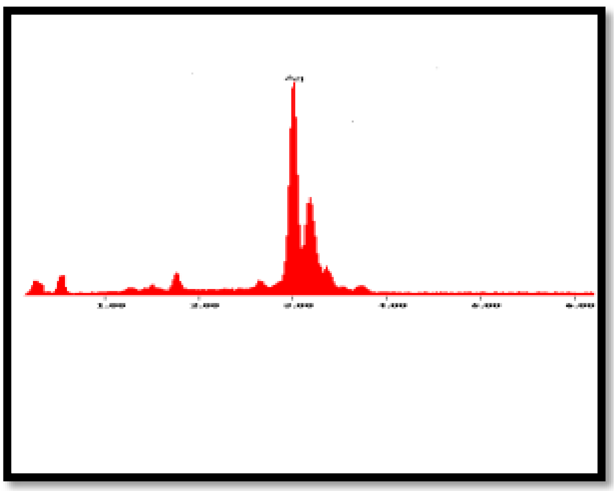

Figure 7: EDS spectra of silver nanoparticles synthesized by leaf extract of *Thunbergia fragrans* Roxb.

EDX analysis was conducted to confirm the elemental composition of the sample.

The EDS images (Figure 7) confirmed the presence of significant amounts of elemental silver in the nanoparticles. The optical absorption band of the EDX peak in the range of 3–4 keV is typical for the absorption of metallic silver nanoparticles.[30]

CONCLUSION

In this study, a simple, ecofriendly and economic biological procedure has been developed to synthesize Silver nanoparticles.

These were synthesized by bio-reduction of Silver ions using the leaf extracts of *Thunbergia fragrans* Roxb. The nanoparticles were fully characterized by various techniques including UV–Vis spectroscopy, AFM, HR-TEM, XRD, EDS and FTIR.

Role of phytochemicals significant in reduction and stabilization of the silver nanoparticles.

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