

Original Research Article

<https://doi.org/10.20546/ijcmas.2020.902.221>

Synthesis and Characterization of AgNps Using Plant Extracts

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ABSTRACT

Keywords

Silver nanoparticles, green synthesis, *T. procumbans*, *E. hirta* and *A. indica*.

Article Info

Accepted:

15 January 2020

Available Online:

10 February 2020

An attempted was made for the green synthesis of silver nanoparticles using commonly available plants viz., *Tridax procumbans*, *Euphorbia hirta* and *Azardirect aindica*. The plants yielding silver nanoparticles successfully were characterized and compared. It is concluded that the plant source of *T. procumbans* is most preferable for the synthesis of nanoparticles having smallest particle size with spherical shape, crystalline nature, stable silver elemental composition etc.,

Introduction

Biological methods of nanoparticles synthesis using microorganisms like algae (Lengke *et al.*, 2006), bacteria (Husseiny *et al.*, 2007), fungi (Gajbhiye *et al.*, 2009), actinomycetes (Ahmad *et al.*, 2003) and plant or plant extracts (Sumitra, 2013) have been suggested as possible ecofriendly alternatives to chemical and physical methods. Using plants for nanoparticles synthesis can be advantageous over other biological processes by eliminating the elaborate process of

maintaining cell cultures (Shankar *et al.*, 2004). Further the green synthesis of AgNPs using plant sources which does not require the usage of high pressure, energy, temperature and toxic chemicals is considered as cost effective and ecofriendly method by Barrena *et al.*, (2009). Besides, Parikh *et al.*, (2008) and Bar *et al.*, (2009) opined that the green synthesis of nanoparticles can be suitably scaled up for large scale with well-defined size and shape. Moreover the ecofriendly nanoparticles were not reported to produce toxic wastes in the process of synthesizing of

silver nanoparticles. However, physical and chemical methods used to synthesize metal nanoparticles have certain disadvantages due to involvement of hazardous chemicals, high energy requirements and radiation.

The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction and to act as capping agent. The size of the nanoparticles synthesized using xerophytes, mesophytes and hydrophytes were in the acceptable range of 2- 50 nm (Jha *et al.*, 2009).

Hence, Morones *et al.*, (2005) concluded that the green synthesis of AgNPs has offered a consistent, nontoxic and ecofriendly approach for plant pathogen management due to their strong antimicrobial properties.

Materials and Methods

Preparation of plant extracts

The healthy fresh leaves of above *T. procumbens*, *E. hirta* and *A. indica* were collected from farm area of the Tamil Nadu Agricultural University, Coimbatore. The leaves were thoroughly washed with tap water to remove soil, dirt, etc. and finally with distilled water. Twenty five grams of the leaves were cut into small pieces and taken in 250 ml Erlenmeyer flask containing sterile distilled water and kept in a water bath at 90°C for 45 min. The extract was filtered using Whatman filter paper no.1 to obtain the aqueous plant extracts.

Preparation of silver nitrate solution

The silver nitrate (AgNO₃) was purchased from Hi-Media Laboratories Pvt. Ltd. was used in the present study. A concentration of 0.01M AgNO₃ solution was prepared by

dissolving 0.169g AgNO₃ in 100 ml double distilled water and used for the green synthesis of silver nanoparticles (AgNPs).

Synthesis of AgNPs

The above filtered aqueous leaf extracts of 25 ml was added individually to 100 ml of 0.01M of AgNO₃ in a 250 ml Erlenmeyer flask and boiled at 90°C for 10-15 min. Then subsequently content of the flask was stirred at 150 rpm at 30°C using magnetic stirrer.

The process was continued till the change of colour occurred from yellowish to dark brown indicating the completion process of AgNPs synthesis. Then the extract was centrifuged at 6000 rpm for 15 min and the resultant pellet was kept in hot air oven overnight at 60°C to make a fine powder of nanoparticles (Dhanalakshmi and Rajendran, 2012).

Characterization of the green synthesized AgNPs

Particle size analyzer with Zeta potential

The particle size analyzer (Make: HORIBA Nanoparticle analyzer SZ-100 Japan) was used to measure the size and distribution pattern of green synthesized AgNPs. A sample of 0.5 mg of AgNPs was dispersed in 20 ml distilled water and sonicated for 32 min (4 cycles) using ultrasonicator make of Citizon Digital Cleaner at 42,000 Hz frequency and 5 ml of the dispersed AgNPs taken in the cuvette was loaded in the particle size analyzer to determine the size of green synthesized AgNPs.

Stability of particles

The stability of the AgNPs was assessed in terms of Zeta potential using the above particle size analyzer allowing to run from - 200 mV to +200 mV and plotting the data in graph.

UV-Vis Spectroscopy

The UV-Vis spectroscopy was used to confirm the synthesis of AgNPs based on their optical properties. The wavelength of green synthesized AgNPs based on light absorbance in the range of 300 to 600 nm with resolution of 1nm was measured by loading the sample of 2 ml in quartz cuvette in UV-Vis spectroscopy (Make: Beckman-DU20).

Scanning electron microscope (SEM) with EDAX

The SEM was used to characterize the size and morphology of the nanoparticles by focusing a beam of high-energy electrons to generate a variety of signals at the surface of solid specimen of green synthesized AgNPs. A sample of 0.5 mg AgNPs was dusted on one side of the double sided adhesive carbon conducting tape. The tape was then mounted on 8 mm diaaluminium stub. The sample was observed at different magnification and the images were picturized(Make: FEI QUANTA 250, Netherlands).

Transmission electron microscope (TEM) with EDAX

The TEM was used to image the size and shape of green synthesized AgNPs. A sample of 0.5 mg of AgNPs was dispersed in 20 ml distilled water and sonicated for 32 min by using Citizon Digital Ultrasonic Cleaner at 42,000 Hz frequency. A drop of the resultant solution was placed on 300-mesh lacy carbon coated copper grid, dried and the images were captured at different magnifications (Make: FEI TECHNAI SPRIT, Netherlands).

X-Ray diffraction (XRD)

The result obtained from the XRD pattern was interpreted with standard reference of Joint

Committee on Powder Diffraction Standards (JCPDS card number 04-0783) for the characterization of AgNPs. For XRD analysis, one g of AgNPs was loaded in the sample holder of instrument and allowed to run at 40 kV voltage and 30 mA current with Cu K (a) radiation for the identification of nature of the particles (Shankar *et al.*, 2004). Then the diffractogram was recorded with 2θ value ranging between $10-80^\circ$ at a scanning speed of 0.080 for a step time of a second at room temperature (25°C).

Fourier transform infrared spectroscopy (FTIR)

The FTIR data measures the interaction between Ag and protein molecules. Therefore for FTIR analysis a sample of 0.5 g of AgNPs was used and the spectra was scanned in the range of $4000-400\text{ cm}^{-1}$ at a resolution of 4 cm^{-1} for the characterization of chemical functional groups present over the surface of green synthesized AgNPs.

Results and Discussion

Comparison of AgNPs based on their characterization

The characters *viz.*, particle size, shape, stability, structure, elemental composition etc. of green synthesised AgNPs using *T. procumbens*, *E. hirta* and *A. indica* were compared. Among them the most effective plant based AgNPs was selected based on desirable characters and advanced for their antimicrobial properties.

Particle size analyser with zeta potential

The particle size analyzer used to estimate the average particle size of synthesized AgNPs revealed the particle size of 78.9, 87.6 and 40.4 nm with plant extracts of *T. procumbens*, *E. hirta* and *A. indica* respectively used for

the synthesis of AgNPs in the present study. The findings of the present study fall in line with the report of successful synthesis of AgNPs with the plants of *T. procumbens* (Ondari and Nalini, 2014) and *A. indica* (Gavhane *et al.*, 2012). The environmentally benign and chemically complex nature of plants which contains biomolecules such as proteins, enzymes, amino acids, alkaloids, terpenoids, phenols, flavanoids, tannins, quinines etc., were reported to be responsible for the successful synthesis of AgNPs (Kavitha *et al.*, 2013). The AgNPs size of 10 to 60 nm with spherical shape, stability, crystalline nature, higher content of silver elemental composition were considered as the desirable characters of green synthesized AgNPs (Shah *et al.*, 2009).

Zeta potential

The Zeta potential was used to measure the

physical stability and colloidal systems of synthesized AgNPs was registered as -41.7, -27.9 and -37.2 mV with *T. procumbens*, *E. hirta* and *A. indica* respectively.

The Zeta potential distributed with wide range of -41.7 mV indicated the highly stable in nature of NPs at highest level in the plant source of *T. procumbens* used for the green synthesis of AgNPs. It is reported that the stability of the green synthesized AgNPs is varying markedly (Kavitha *et al.*, 2013).

The finding is contradictory to the earlier report of low to moderate stability of the synthesized AgNPs using *T. procumbens* made by Ondari and Nalini (2014). However, the high stability of AgNPs synthesized using *T. procumbens* in the present study supported the preference of the same plant source for the synthesis of AgNPs.

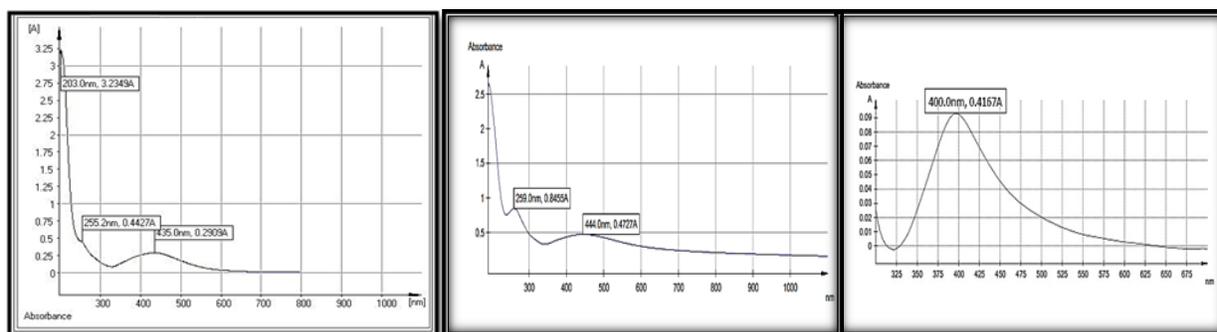


Figure.1 UV-Vis spectra analysis for AgNPs using *T.procumbens*, *E.hirta* & *A. indica*

UV-V is Spectroscopy

The green synthesized AgNPs initially showed yellowish color in aqueous solution due to excitation of surface plasma resonance of AgNPs. Then the color of the solution changed from yellow to brown on addition of plant extracts to silver nitrate. The color change indicated the formation of AgNPs. Further, it was confirmed with the wavelength of 435, 444 and 400 nm recorded

with the plant extracts of *T. procumbens*, *E. hirta* and *A. indica* respectively (Fig. 1). Since the registered wavelength falls within the prescribed range of 430-460 nm confirmed the formation of AgNPs.

Shankar *et al.*, (2004) reported the confirmation of formation of AgNPs could be made with absorbance peak ranging from 430 to 460nm. The AgNPs synthesized in the present study using *T. procumbens* had the

maxima absorbance of 435 nm which was within the range indicated. Bhati and Malik (2014) and Ondari and Nalini (2014) explained that there was reduction of pure Ag^+ to Ag^0 ions due to splitting of AgNO_3 into Ag^+ and NO_3^- during the synthesis of AgNPs. Hence change in colour in the reaction mixture was observed with progressive time. Apparently the metabolites in the leaf extract acted as e^- donor and reduced Ag^+ ions into Ag.

Scanning electron microscope (SEM) with EDAX

The morphology of the synthesized AgNPs using *T. procumbens*, *E. hirta* and *A. indica* respectively were observed through SEM. The observations revealed that the AgNPs were spherical in their shape and agglomerated. The EDAX profile showed the highest silver elemental composition of synthesized AgNPs and it was highest (97.91%) with *T. procumbens*.

It was followed by *E. hirta* (86.3 %) and *A. indica* (78.12 %) respectively. The observations made through SEM revealed spherical shape of the AgNPs using all the above three plants with agglomeration. The observations made through SEM also confirmed the spherical shape of AgNPs. Dhanalakshmi and Rajendran (2012), Bhati and Malik (2014) and Ondari and Nalini (2014) also observed similar spherical shape of green synthesized AgNPs using *T. procumbens*.

The same authors reported that the elongated large spherical nanoparticles as seen with the presently green synthesized AgNPs was due to agglomeration and it was caused by the accumulation of two or more reducing moieties bound on the surface of the preformed nuclei.

Transmission electron microscope (TEM) with EDAX

The observations made through the TEM revealed the spherical shape of AgNPs with size ranging from 15.5 to 31.8 nm, 35 to 49 nm and 26.7 to 43.2 nm in respect of *T. procumbens*, *E. hirta* and *A. indica* respectively (Fig 2). Therefore, it is inferred that the AgNPs size was lowest with *T. procumbens* among the three plant extracts used for the green synthesis of AgNPs. The TEM EDAX observations showed the specified peaks indicating the possession of silver in AgNPs using the above three plant sources (Ondari and Nalini, 2014 and Gavhaneet al., 2012).

Further, the TEM EDAX analysis undertaken to determine the elemental composition also showed that there was highest silver elemental composition (97.91%) in *T. procumbens* mediated synthesized AgNPs. The elemental composition of silver was lesser in AgNPs synthesized using certain plants (Khan et al., 2013). Therefore, the higher elemental composition of silver as observed with *T. procumbens* mediated synthesized AgNPs in the present study made the plant as desirable source for the synthesis of AgNPs.

X-Ray diffraction (XRD)

The X-Ray Diffraction (XRD) pattern profile of all the three plants mediated synthesized AgNPs samples quite consistently matched well with the standard Ag nanopatterns.

The XRD patterns recorded in the 2θ range of $10-80^\circ$ with the synthesized AgNPs indicated the crystalline nature of AgNPs by showing strong diffraction peaks at 28.60° , 32.50° , 38.38° , 44.56° , 46.42° , 55.57° , 64.78° and 77.02° for *T. procumbens*, 27.65° , 31.98° , 37.62° , 45.13° , 53.22° , 55.32° , 61.30°

and 71.66° for *E. hirta* and 27.74° , 32.05° , 26.39° , 45.17° , 52.96° , 55.24° , 61.32° and 71.70° for *A. indica*. The *T. procumbans* mediated synthesis of AgNPs showed the maxima at $2\theta=32.50^\circ$ which corresponding to the d spacing of 2.75 \AA , *E. hirta* showed the maxima at $2\theta=31.98^\circ$ with corresponding d

spacing of 2.76 \AA and *A. indica* also showed the maxima at $2\theta=32.05^\circ$ with corresponding d spacing of 2.75 \AA .

All the above plant extracts exhibited the diffraction pattern which is characteristic to indicate crystalline nature of AgNPs.

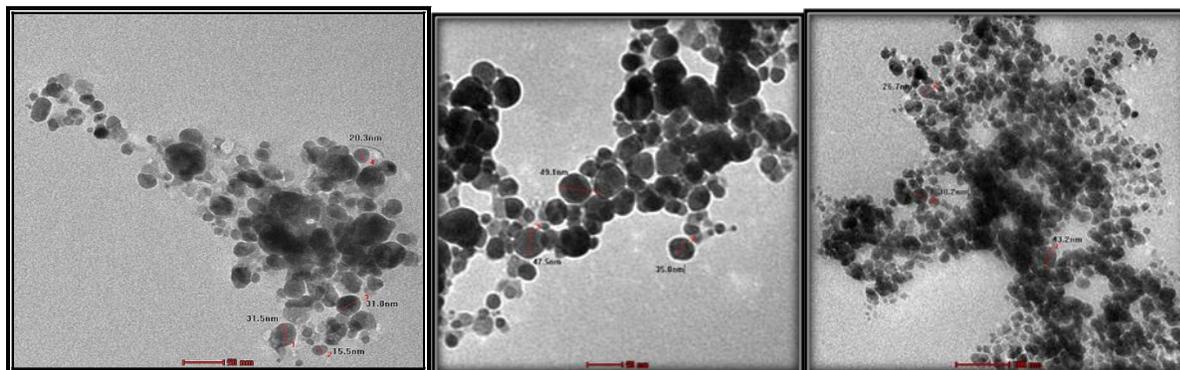


Figure.2 TEM image of green synthesized AgNPs with *T. procumbans*, *E. hirta* & *A. indica*

The XRD data confirmed that the AgNPs was crystalline in nature as the values matched well with the standard reference of Joint Committee on Powder Diffraction Standards (JCPDS Card No.04-0783) of silver nanoparticles. The sharp and clearly visible peaks of XRD profile confirmed that the particles were crystalline. The sharp peak indicated by the X-ray diffraction pattern confirmed the crystalline nature of green synthesized AgNPs using all the above three plants in the present study and the findings confirmed the earlier report of Shankar *et al.*, (2004); Sileikaite *et al.*, (2009); Geoprincy *et al.*, (2011) and Bhati and Malik (2014).

Fourier transform infrared spectroscopy (FTIR)

The FTIR spectral analysis showed the presence of possible functional groups of phytochemicals in plant extracts used for the synthesis of AgNPs as per standard reference of Infrared (IR) charts. The synthesized AgNPs using leaf extracts of *T. procumbans* and *E. hirta* exhibited intense absorption

peaks at 867.92 , 1639.41 and 3357.90 cm^{-1} corresponding to strong stretching of C–H aromatics. The medium stretching band indicated the presence of N–H primary and secondary amides and the broad band denoted as –OH stretching indicated the presence of alcohols and phenols as functional groups of biomolecules of the plant extracts. Similarly *A. indica* showed the peak at 1641.34 , 2360.75 and 3359.83 cm^{-1} with corresponding medium stretch of –C=C–alkenes.

The medium and broad stretching of H–C=O: C–H aldehydes and –OH alcohols and phenols indicated that biomolecules present in the extracts acted as reducing, capping and stabilizing agents for the development of AgNPs. The FTIR analysis confirmed the presence of different functional group of phytochemicals in plants used for the synthesis of AgNPs. The phytochemicals were believed to act as reducing agent for the conversion of Ag^+ to Ag^0 and to act as capping and stabilizing agent of AgNPs in the present study.

Table.1 Comparison of characters of green synthesized AgNPs using different plants

S.No	Characters	Instruments used	Plants		
			<i>T. procumbens</i>	<i>E. hirta</i>	<i>A.indica</i>
1	Particle size	Particle Size Analyzer	78.9 nm	87.6 nm	40.4 nm
2	Particle stability	Zeta potential	-41.7 mV	-27.9mV	-37.2mV
3	Confirmation of AgNPs synthesis	Uv-Vis Spectroscopy	Confirmed with absorbance peak at 435 nm	Confirmed with absorbance at 444 nm	Confirmed with absorbance at 400 nm
4	Shape	SEM	Spherical with agglomeration		
		TEM	Spherical with size of 15.5 - 31.8 nm	Spherical with size of 35- 49 nm	Spherical with size of 26.7 - 43.2 nm
5	Silver elemental composition (%)	SEM (EDAX)	97.91	86.83	78.12
6	Nature of particles	XRD	Crystalline		

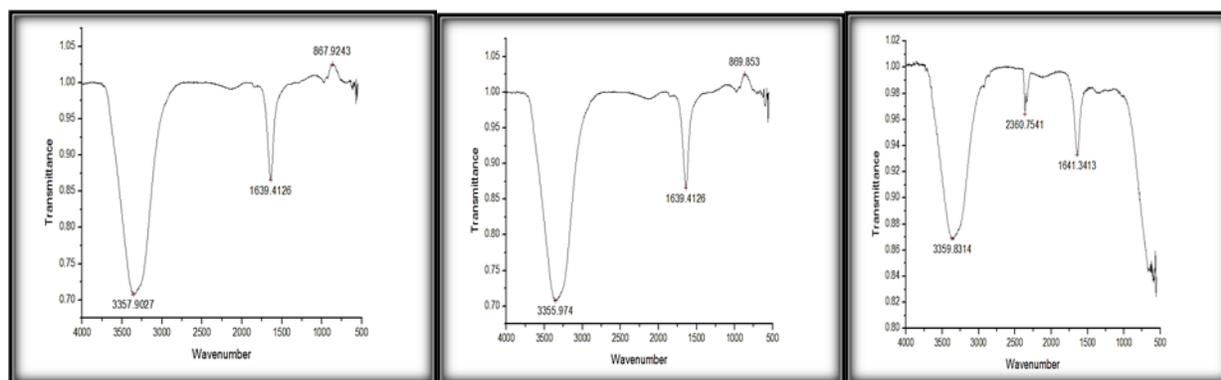


Figure.3 FTIR spectral analysis of synthesized AgNPs in *T. procumbans*, *E. hirta* & *A. indica*

The findings support the earlier reports of Tripathy *et al.*, (2010); AbdurZahir *et al.*, (2012); Ondari and Nalini (2014) and Bhati and Malik (2014). In this regard the above authors speculated that the phytochemicals *viz.*, phenols, aldehydes, aromatic groups of the amino acid residues and proteins had strong ability to bind with metal for acting as reducing, capping and stabilizing agent of green synthesized AgNPs. Further, it is reported to be responsible for the prevention of agglomeration of green synthesized AgNPs. Therefore, it is concluded that the phytochemicals/biomolecules present in the

above plant sources played a dual role in the formation and stabilization of the green synthesized AgNPs. Among the three plants chosen for the synthesis of AgNPs the *T. procumbens* was considered as most preferable plant source owing to the following reasons. As per particle size analyzer the average particle size of AgNPs was within the limit of 100 nm (Ravikumar and Sathish Kumar, 2014) *viz.*, *T. procumbens* (78.9 nm), *E. hirta* (87.6 nm) and *A. indica* (40.4 nm) used for the green synthesis of AgNPs.

Whereas the particle size of AgNPs using *T.*

procumbens was found to be the least (15.5 to 31.8 nm) as per the TEM observations in the present study and it is coinciding with the report of Nagati *et al.*, (2012) who stated that the size of synthesized AgNPs within 50 nm could be optimum size for silver nanoparticles.

The smallest size of nanoparticles having the highest surface volume ratio which enhance the surface energy is lethal to biological organisms as reported by Shrivastava *et al.*, (2007). Therefore, the *T. procumbens* is highly preferred than other plant sources tried for the green synthesis of AgNPs.

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How to cite this article:

Surega. R., B. Anita, S. Ramakrishnan, K. Gunasekaran and Nakkeran. S. 2020. Synthesis and Characterization of AgNps Using Plant Extracts. *Int.J.Curr.Microbiol.App.Sci*. 9(02): 1939-1947 doi: <https://doi.org/10.20546/ijcmas.2020.902.221>