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Research Article

SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLE FROM *Erythrina indica*

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ABSTRACT

Silver nanoparticle (AgNPs) is synthesized using novel aqueous leaf extract of *Erythrina indica* as a reducing and stabilizing agent. This leaf contains bio molecules such as alkaloids, flavonoids, carbohydrates, amino acids and phenolic compounds. Formations of stable nanoparticles were observed after the addition of aqueous leaf extract into silver nitrate solution. The silver nanoparticles were characterized by UV-visible Spectroscopy (UV), Fourier Transform Infra-red Spectroscopy (FT-IR), Scanning Electron Microscope (SEM), Transmission electron microscope (TEM), Energy dispersive spectroscopy (EDX), Particle size analysis (DLS) and X-ray diffraction (XRD). A peak at 396 nm was obtained in UV-visible spectroscopy which is due to the surface Plasmon resonance. XRD proves the crystalline nature of silver nanoparticle. Highly mono dispersed silver nanoparticles with the range of 70 to 90nm are revealed from SEM analysis. TEM analysis also shows the particles are in nano size.

Keywords:*Erithrina indica*, AgNPs, bioreduction, antibacterial activity.

INTRODUCTION

Nanotechnology has been used in many engineering fields such as electronics, mechanical, biomedical engineering. Above all, it has led to the significant advancement in a biomedical field such as controlled drug/gene delivery, tissue engineering, bone replacement etc., [1, 2]. Nanoparticles usually speak of particles with a size up to 100 nm with increasing in surface to volume ratio, providing more active surface atoms to contribute the role in applications and improve the properties of the materials [3]. Nanoparticles have widely been applied for the treatments like cancer [4], diabetes [5], allergy [6], infection [7] and inflammation [8]. It exhibits completely new properties based on specific characteristics such as size, distribution of particles and morphology, as decreasing in size of the particle a very high surface to volume ratio is attained. There are two basic approaches for the synthesis of nano particles namely top down and bottom up, some of them like solid state reaction, chemical reaction, co- precipitation and sol gel method etc. Among all nanoparticles gold, copper, ion, palladium, zinc, quantum dots(CdS, ZnS), Silver nanoparticles plays a major role in the many fields particularly in medical applications. Silver metal had been given more importance next to gold in ancient days by our ancestors for medical application. In recent years, Silver nanoparticle had been used as silver coated bags for blood collection, drug delivery and other medical applications. It is used as an antimicrobial agent; it is applied in textiles, home water purification systems, medical devices, cosmetics, electronics, and household appliances [18]. Silver is a safe and effective bactericidal metal because it is non-toxic to animal cells and highly toxic to bacteria [19-20]. Chemical reduction method is most frequently applied for preparing silver nanoparticles. biological way of silver nanoparticle synthesis has proved to be much more better than that of chemical methods in being more environment friendly as well as cost effective [21].

In recent years nanoparticles have received considerable attention due to their wide range of applications in the fields of diagnostics, biomarker, cell labeling, antimicrobial agents, biological tagging, pharmaceutical applications, drug delivery systems, cancer therapy, biosensing and material chemistry[22]. Silver nanoparticle plays avital role in pharmaceutical industries and biotechnological application. It is one of the fastest growing materials due to their unique physical, chemical and biological properties; small size and high specific surface area. It also proved to have potential antibacterial and antifungal properties [23- 28].

The plant mediated green synthesis of nanoparticles is advantageous over chemical and physical method because it is environmentally Friendly and biocompatible, where it is not necessary to use high pressure, energy, temperature and toxic chemicals [29]. Various parts of plants such as leaf, stem, flower, seed, fruit and outer layer of the fruit were used to synthesis not only silver but also some other nanoparticles. The use of plants for the synthesis of nanoparticles is a rapid, low cost, eco-friendly, and single step method which can directly use for drug delivery and other similar application without any coating or core-shell techniques [30]. *Erythrina indica*contains some biomolecules such as alkaloids, flavonoids, carbohydrates, amino acids and phenolic compounds. A wide range of medicinal formulations have been developed from *E. indica* that exhibit analgesic, anti-arthritic, anti-hyper triglyceridemia, anti-inflammatory and muscle relaxing effects. It has been widely used in Indian traditional medicine for treating common ailments such as asthma, arthritis, diarrhea, fever, inflammation and leprosy. Its leaf were alsoused in liver ailment, rheumatism, relieve joint pain, and to kill tapeworm, roundworm and threadworm. In recent years, the green synthesis of metal nanoparticles has become a major focus of scientists. There are several reports on synthesis of AgNPs from plants.

MATERIALS AND METHODS

Materials

Materials with high grade AR silver nitratewaspurchased from Sigma- Aldrich, India, throughout the reaction Double distilled water were used. Whatman no.1 filter papers were used for filtration. All glass wares used were washed well and dried using hot air oven.

Preparation of leaf extract

For extraction, fresh leaf of *Erithrina indica* was collected from Mannargudi, Tamilnadu India. Collected leaves were cleaned well with normal water and again cleaned with double distilled water. The leaf is dried under sun with closed pack to free from dust. The dried leaf is ground it to fine powders and 5g of powder is mixed with 100 ml of distilled water thenit is boiled to 60ºC for 15 min. After cooling down to normal room temperature, the extract was filtered through normal filter paper to get free from powder and again filtered using whatman filter paper to get clear leaf extract. The filtered extract is stored in refrigerator at 4 ºC and used for further synthesis process [31].

Synthesis of silver nanoparticle

100 ml of 0.001 M aqueous solution of silver nitrate was taken in a flask and 25ml of stock solution of leaf extract was added drop by

drop with 60ºC heating and continuous stirring for 20 min. The colorless solution changed into brown in colour which gives colloid silver nanoparticles. The colloidal solution is then centrifuged at 9000rpm, supernatant was collected and stored for further analysis [31].

Characterization

The prepared silver nanoparticle was confirmed using UV-visible spectrum with a range of300-700nm and was recorded on a UV-visible JascoV-550 spectrophotometer. FT-IR measurements were used to determine biomolecules present in AgNPs. The Ag nanoparticles were centrifuged at 9000rpm for 30min, Jusco 5300 model with the range of 400–4000cm^{-1}. SEM analysis was used to characterize surface morphology and EDX were used to find elemental composition in the reaction mixture. The synthesized AgNPs was evaporated onto a clean glass slide and covered left to dry completely at room temperature. SEM analysis was done using Supra Zeiss with resolution of 1nm at 30kV with 20mm Oxford EDS detector. Dynamic light scattering (DLS) was used for measurement of average hydrodynamic diameters and polydispersity indices (PDIs) of the particles, Malvern Zetasizer Nano-ZS, Malvern Instruments, UK. Sample is analyzed in triplicate at 20ºC at a scattering angle of 173º and double distilled water is used as a references dispersing medium. In TEM analysis the sample is first sonicated for 10 min and a drop of this AgNPs solution is loaded on carbon coated copper grid and the solution is allowed to evaporate for 10 min in room temperature and it is analyzed usingHITACHI-H-7650at an operating voltage of 80kV.X-ray diffraction is used to determine crystalline structure. This study was made on the powder samples at room temperature 27 ºC on a Rigaku X-ray diffractometer (Miniflex, UK).

Antimicrobial activity

The antimicrobial activity was done on various pathogenic bacteria. The test organisms used were *Escherichia coli, Staphylococcus aureus and pseudomonas aueruginosa.* Nutrient agar medium was used to cultivate Bacteria. Then the medium were sterilized by autoclave and transferred to petriplate. After solidification inoculated with a fresh growth of test stain, then AgNPs were loaded with different concentrations of 25 µl, 50 µl and 100 µl respectively. Then antibacterial assay plates were incubated at 37°C for 24h after the incubation time, zone of inhibition was calculated.

RESULTS AND DISCUSSION

UV- Visible spectroscopy

The nanoparticles synthesis reaction was started after the leaf extract of *Erithrina indica* was introduced into aqueous silver nitrate solution. After overnight incubation in dark room condition the colorless reaction mixture was turned into a dark brown color due to excitation of surface Plasmon resonance indicating the biotransformation of ionic silver to reduced silver [32]. It is observed that the maximum absorbance occurs at 396nm figure.1 .The reduction of silver ions occurred due to the water-soluble phytochemicals present in the leaf extract [33, 34].

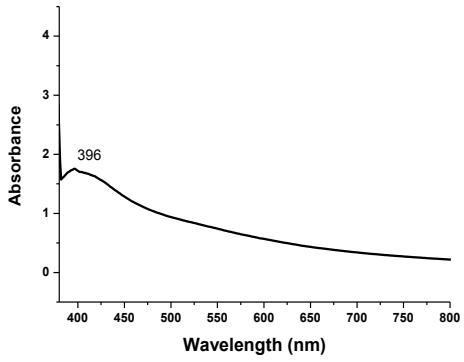

Fig.1: UV- Visible spectrum of AgNP

Fourier transforms infra- red spectroscopy

FTIR measurements were performed to identify the biomolecules responsible for capping, reducing and stabilizing the silver nanoparticles present in the leaf extract of *Erythrina indica.* Figure.(2a) shows the FTIR spectrum of the leaf extract, which clearly shows peaks at 3852, 3750 and 3469 cm^{-1} corresponds to the O-H stretching of hydroxyl groups [35]. The relatively strong absorption peak around 1645 cm^{-1} indicated the characteristics IR absorption of polysaccharides. Instead of showing peak in leaf extract, figure.(2b) in biosynthesized nanoparticles, peak at 3902, 3888, 3853 3766, 3647 and 3473cm^{-1}was assigned as –OH stretching in phenolic compounds, peak at 2927cm^{-1}it represent C-H and also peak at1646cm^{-1}represents C=O [36]. The vibrational bands corresponding to bonds O-H, C=C are derived from the compounds such as alkaloids-Erythrinan (reducing agent).(S.S. Hussain), flavonoids and phenolic compoundsin *Erythrina indica*. The obtained results suggest that the presence of various functional group in the leaf extract and as well as in synthesized AgNPs.

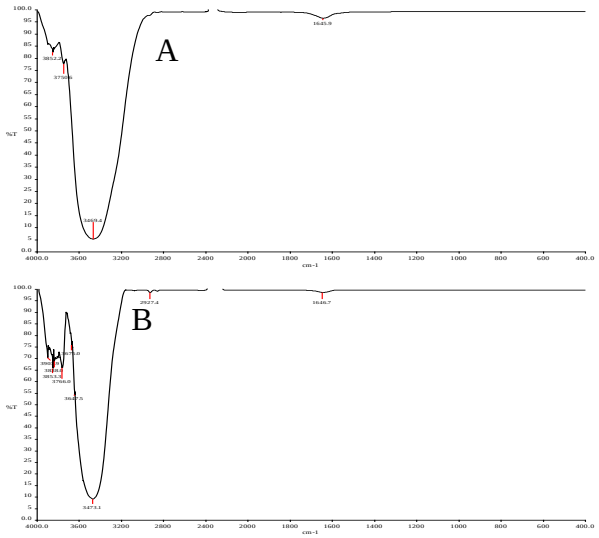

Fig.2:FT-IR spectrum of leaf extract of (a), Silver nano particle (b)

Table: 1.FT-IR

S.No N	Peak (cm^{-1})	Stretching bonds	Compounds
1.	3902,3888,3853,3766,3647 and 3473	NH/OH	Phenolic compounds
2.	2927	C-H	Flavonoids(indicanine)
3.	1646	C=O	Alkaloids(erythrinan)

Particle size distribution

The average size of the particles, size distribution, and polydispersity index (PDI) of the synthesized AgNPs were determined by particle size analyzer, and the results are shown in figure 3. It shows the average particle diameter is 156nm and Polydispersity index is 0.263. The average particle size and PDI revealed that the produced AgNPs were monodispersed [30].

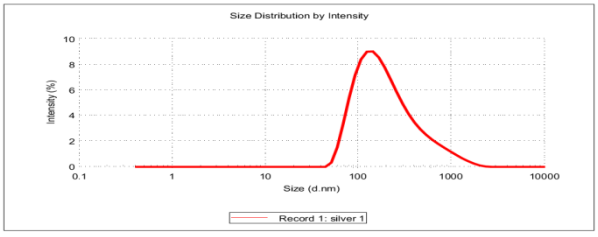

Fig.3: Particle size distribution

Table: 2.Particle size analysis

S.No	Parameters	Values
1.	Average particle diameter	156nm
2.	Poly dispersity index	0.263

Scanning electron microscope

The morphology and size of particles were determined by SEM. Figure 4(a) shows that the particles are in spherical shape with range from 70 to 90nm.The synthesis of silver nanoparticles by *erithrina indica* is further characterized by EDX analysis, which gives the additional evidence for the reduction of silver nanoparticles to elemental silver[32]. The optical absorption peak is seen approximately at 3 keV, which is typical for the absorption of metallic silver nanocrystals due to surface Plasmon resonance, which confirms the presence ofnanocrystalline elemental silver. The spectrum shows strong silver signal along with weak oxygen and chlorine peak, which may be originating from the biomolecules that are bound to the surface of nano silver particles can be observed in Figure. 4b. [37]

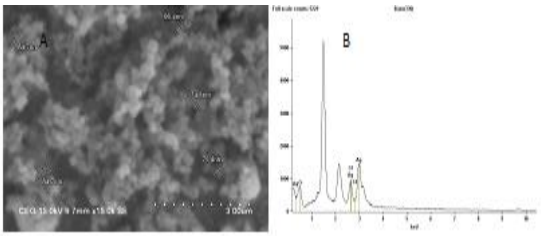

Fig.4: (a) SEM image of synthesized AgNPs. (b) EDX spectroscopy AgNPs.

Transmission electron microscope

TEM analysis is used to determine size and shape of the particle. Figure.5. Studies confirmed that AgNPs are not agglomerated, well-dispersed and almost spherical in shape with particles size in nano range. The results indicate that the average particle size of the synthesized silver nanoparticles is highly influenced by the concentration of leaf extract. If the concentration of leaf extract is increased in the reaction mixture, the particle size is decreased. It shows that the particles were about nano size with smooth surface. [36]

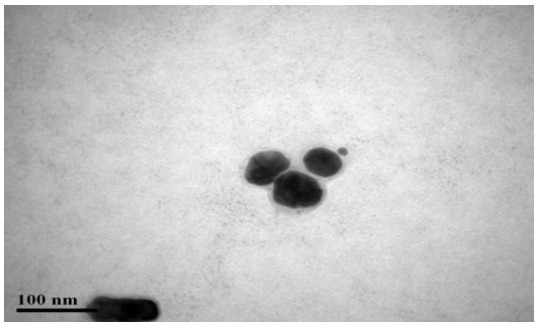

Fig.5: TEM image of synthesized AgNPs

X-ray diffraction

Figure.6shows the X-ray diffraction (XRD) patterns of dried silver nanoparticles synthesized using *Erythrina indica* leaf extract at room temperature. The XRD patterns of AgNPs indicated that the structure of silver nanoparticles is face cubic center (fcc) [37]. In addition, the XRD peaks could be attributed to the crystallographic planes [38]. All the prominent peaks at 2 theta values of about 28º, 32º and 46ºrepresenting the (2 2 0), (3 1 1) and (4 2 0) Bragg's reflections of 'fcc' structure of silver. Hence, from the XRD result, it is clear that AgNPs formed using *Erythrina indica* leaf were essentially crystalline. The average Nano crystalline size has been estimated by using well known Debye–Scherrer formula,

$D = K\lambda / \beta \cos\theta$

Where, D is particle diameter size, k is a constant (k=1), λ is wavelength of X-ray source (1.5405 nm), and βis the full width at half maximum (FWHM). The average crystalline size according to Debye–Scherrer equation calculated is found to be 8nm.

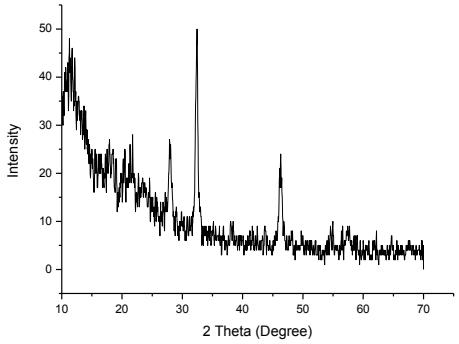

Fig.6: X-ray diffractogram of AgNPs.

Antimicrobial activity of AgNPs

Antimicrobial activity of biosynthesized silver nanoparticles were studied against pathogenic bacteria (clinical isolates) using agar well diffusion method and zone of inhibition were depicted in Figure.7 and Table 3. Wells were loaded with different concentrations of 25µl, 40 µl, and 100 µl of AgNPs respectively. Maximum zone of inhibition (9mm) was observed with *P. aeruginosa* at 25 µl of AgNPs. Next was staphylococcus aureus 7mm at 50µl of AgNPs concentration and E.coli showed least zone of inhibition of 2mm at 100 µl. At minimum concentration of 25 µl amongst pathogenic bacteria, P. aeruginosa showed maximum inhibition zone of 9 mm. [39, 40]

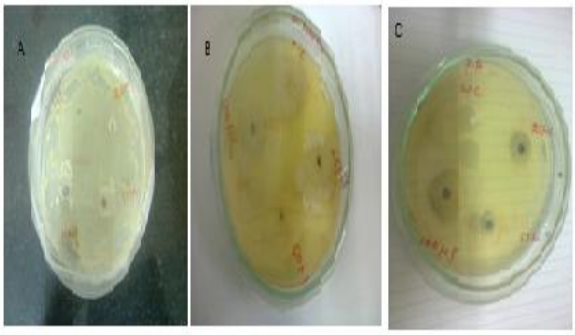

Fig.7. Antimicrobial activity of AgNPs (a) *E.coli*, (b) *Staphylococcus aureus* and (c) *Pseudomonas aeruginosa*

Table.3: Inhibition zone of AgNPs

S.No	Pathogenic bacteria	Zone of inhibition 25µl 50 µl 100 µl
1.	*E.coli*	3mm 6mm 2mm
2.	*P. aeruginosa*	9mm 3mm 7mm
3.	*S. aureus*	7mm 3mm 6mm

CONCLUSION

In this study, a green approach for the synthesis of AgNPs using *Erythrina indica* leaf extract was developedthe simplest and efficient method to obtain AgNPs without engaging any harmful chemicals as reducing agent. The biomolecules like alkaloids, flavonoids, carbohydrates and amino acids present in the leaf extract which is responsible for reduction of silver bulk to silver nanoparticle which is revealed from FT-IR studies. The presence of bio-organic components acts as a stabilizer for AgNPs probably. Crystalline nature of the particle was confirmed using XRD. The biosynthesized silver nanoparticles were found to have a pronounced antimicrobial

activity against *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* .

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