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GREEN SYNTHESIS OF SILVER NANO PARTICLES USING AQUEOUS LEAF EXTRACT OF *SENNA ALEXENDRINA* AND STUDY OF ITS ANTIBACTERIAL ACTIVITY

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ABSTRACT

A simple, rapid, eco-friendly and cheap method for Green synthesis of silver nano particles was successfully developed using *Senna alexendrina* aqueous leaf extract,. Silver nitrate was reduced to silver ions by reduction activity of *Senna alexendrina* extract. The eco friendly AgNPs were highly stable and monitored through UV–Vis spectrophotometer, X-ray diffraction (XRD) and the selected area electron diffraction (SAED) patterns proved the crystalline nature of AgNPs with facecentered cubic (fcc) geometry. Morphological images confirmed the uniform distribution of spherical nanoparticles. Fourier transform infrared spectroscopy (FTIR) result expounds the functional groups of a leaf extract responsible for the bio-reduction of silver ions and their interaction between them. The biosynthesized AgNPs exhibited effective antibacterial activity against both Gram negative and Gram positive bacteria.

INTRODUCTION

Silver Nanoparticles have attracted significant attention due to their catalytic, optical, electrical, magnetic and antimicrobial properties. Silver nanoparticles (AgNPs) are reported to be an effective antimicrobial (Khan et al., 2014; Kumar et al., 2014),and antioxidant agents and are widely used in broad area of biological applications such as antitumor effect (Jeyaraj et al., 2013), detectors of metal pollutants (Balavigneswaran et al.,2014), dyes (Kumar et al., 2013), antibiotics (Singh et al.,2012) and nitro-aromatic compounds (Narayanan and Sakthivel, 2011) in industrial effluents. The conventional method of silver naoparticles involves the use of chemicals Which are environmental pollutants, the amount of nanoparticles produced is less and the process is expensive. In most of the cases, the silver nanoparticles formed are highly unstable and necessitate the addition of a separate capping agent which renders stability.

An alternative ecofriendly method for nanoparticles synthesis is assessed considering three aspects, the solvent, the capping agent and reducing agent. Green synthesis of silvernano particles has gained importance as it is easy,cheap and environmentally safe. The plant extracts mediated process for nanoparticles synthesis is good and advantageous compared to microorganisms because of exceptionally economic and eco-friendly aqueous reactions involved in this process which are carried out at almost low temperatures and involves short reaction times.The various biomolecules present in the plant extract such as enzymes, proteins, flavonoids, terpenoids and cofactors act as both reducing and capping agents (Tavakoli et al., 2015). The plant-mediated synthesis of nanoparticles is relatively fast as there is no need of maintaining specific media and culture conditions, unlike microbial synthesis. Green synthesis of silver nanoparticles has been reported with leaf extract from various plants such as *Azadirachtaindica* (Nazeruddin et al., 2014), *Delonixelata* (Sathiya and Akilandeswari, 2014), *Tephrosiapurpurea* (Ajitha et al., 2014), *Meliadubia* (Kathiravan et al., 2014), *Tribulusterrestris* (Ashokkumar et al., 2014), *Artemisia nilagirica* (Vijayakumar et al., 2013), *Boerhaaviadiffusa* (Kumar et al., 2014), *Ficusreligiosa* (Antony et al.,2013), *Piper pedicellatum* (Tamuly et al., 2013) .Hence, in the present study, the aqueous extract of *Senna alexendrina* was studied for the synthesis of silver nanoparticles. *Sennaalexandrina* belongs to family fabaceae its leaves are widely used as a laxative drugs. The leaves are known to have 8-O β-Dglucopyranoside of torachrysone, kaempferol-3-Ogentiobioside and also anthracenderivative named as neorhein, which is a new natural compound and has a structure of 1,7-dihydroxy-3-carboxyanthraquinone7 (Shmygareva AA et al., 2016). Literature reveals that there are no reports available for the synthesis of nanoparticles using the aqueous extract of *Senna alexendrina* leaves. Therefore, the objective of the present study was to synthesize and characterize the silver nanoparticles using the leaf extract of *Senna alexendrina*. In addition, the antibacterial activity was also investigated.

Materials and methods

Preparation of leaf extract: Fresh leaves of *Senna alexandrina* were collected from Idupulayapaya region of Y.S.R. Kadapa district, Andhra Pradesh. Leaves were washed thoroughly with tap water and followed by a rinse with distilled water and later dried in shade. The dried leaves were powdered in an electric blender and sieved to remove any fibrous debri. This sieved powder (5 g) is added to 100ml of double distilled water and boiled at 60 °C for 5 min and then allowed to stand still for 24 hrs at room temperature. This aqueous leaf extract was filtered through Whatman's No. 1 filter paper and then concentrated by rotavapour (Heidolph) and stored at 4 °C till further use.

Biosynthesis of Silver Nanoparticles using *Senna alexandrina* aqueous leaf extract

Green synthesis of AgNPs was carried out by adding 50mg of dried leaf extract powder to 50 ml of 1mM $AgNO_3$ and vigorously stirred. This solvent mixture was heated at 80°C for 15 min and transferred to dark conditions for 24hrs.

Characterization of biosynthesized AgNPs

The synthesis of AgNPs was confirmed using UV–visible spectrophotometer with a resolution of 1 nm between 200 and 700 nm. After the biosynthesis, the AgNPs were separated by centrifugation of the solution at 15000 rpm for 15 min. The pellet of AgNPs were resuspended in water and purified by repeated centrifugation for five times to remove the traces of unused extract. The finally purified pellet was then dried in hot air oven. These silver nanoparticles were used for characterization using FTIR analysis to study the possible functional groups involved in synthesis and stabilization of AgNPs. The FTIR analysis was done in the range of 400–4000 cm^{-1} with the resolution of 2 cm^{-1}. The crystalline nature of synthesized AgNPs was confirmed by XRD analysis. The size and shape of the AgNPs were determined using transmission electron microscopy (TEM).

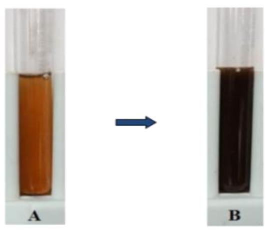
Antimicrobial activity: The antimicrobial activity of the biofabricated silver nano particles was evaluated using gram positive and gram negative pathogenic bacteria like *Salmonella. ebony*(MTCC 3384), *Bacillus subtilis*(MTCC10619), *klebsiella pneumonia* (MTCC 532) and *Peudomonas aeruginosa*(MTCC 1688) . The antimicrobial activity was carried out with 24 h active cultures by employing disc diffusion method (Ghassan et al. 2013). The bacterial inoculum(100ul) was spread on the surface of nutrient agar medium plates by swabbing. Sterile discs impregnated with 20 µl of synthesized AgNPs solution at a concentration of 100mg/ml were then placed on the surface of the bacterial cultyure seeded nutrient agar medium. Sterile disc without any treatment was used as negative control and standard antibiotic Ampicillin was taken as positive control. The agar plates were incubated at 37˚C for 24 h.

RESULTS AND DISCUSSION

UV–Vis analysis: The synthesis of AgNPs was initially observed by the change in color of aqueous leaf extract solution from light brown to dark brown color (Fig. 1). The color change is due to the excitation of Surface Plasmon Resonance (SPR) vibrations of AgNPs. The UV–Vis absorption spectroscopy (Fig. 2) showed the absorbance peak at 435nm which confirmed the synthesis of AgNPs owing to the surface plasmon vibrations of the excited, AgNPs. Presence of different biomolecules in which the leaf extract could be responsible for the capping and stabilization of AgNPs formed.

This characteristic SPR peak may also correspond to the spherical shape of AgNPs (Kotakadi et al. 2014)

Figure.1 Color change of *Senna alex andrina* aqueous leaf extract before (A) andafter (B) the synthesis of AgNPs



A. *S. alexandrina* aqueous leaf extract (Light Brown colour) B. *S. alexandrina* AgNps (Dark Browncolour)

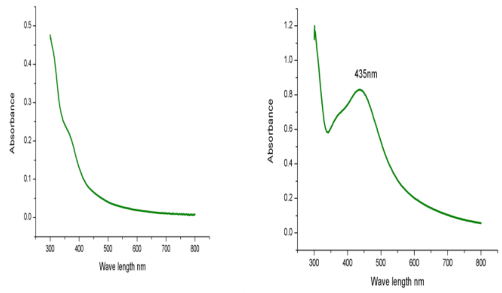
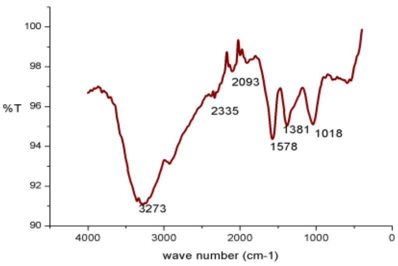


Fig.2 (a) *S. alexandrina* Aqueous Leaf extract (b) *S. alexandrina* AgNPs

Figure 2. The Characteristic SPR peak of synthesized AgNPs as seen by UV–vis absorption spectroscopy

FTIR analysis: The FTIR spectroscopy was carried out to investigate the surface chemistry composition of AgNPs capped by the biomolecules in *S.alexandrina* leaf extract. The FTIR spectrum (Fig. 3) showed peaks at 3273, 2335, 2093, 1578, 1381, 1018 cm^{-1}. The peak at 3273 cm^{-1} could be assigned to N–H stretching vibrations of the secondary amide of the protein and the peak 2361 cm^{-1} corresponding to N–H stretching/C–O stretching vibrations (Kumar and Mamidyala 2011) Mahitha 2011). The peak at 1578 cm^{-1} corresponds to asymmetric C=O stretching vibration and/or aromatic C=C stretching vibration (Valentina and Boris 2013). The peak at 1381 and 1025 cm^{-1} could be assigned to C–O stretching and O–H deformation of phenolic OH groups (Valentina and Boris 2013; Monali 2009). Based on the FTIR studies, it is reported that phenolic compounds present in the aqueous extract could be responsible for the reduction of silver ions (Ag) into AgNPs . Proteins could be responsible for both synthesis and stabilization of AgNPs. But the exhaustive mechanism of the synthesis of nanoparticles in this bio-based reduction by leaf extract is to be further elucidated.

Figure 3. The FTIR spectra of AgNPs produced by aqueous extract of



S.alexandrina

XRD analysis: The XRD analysis of the synthesized AgNPs in (Fig.4) shows four distinct diffraction peaks at 38.26, 44.33, 64.53and 77.62 corresponding to (111), (2 2 0) and (3 1 1) lattice planes of the face centered cubic (fcc) lattice of silver. This also revealed the crystalline nature of AgNPs. The mean average size of the particle was calculated as 22nm using the Deseherrer formula(Avg D = 0.9ʎ/βcosθ) (Veerasamy, R et al,2015)

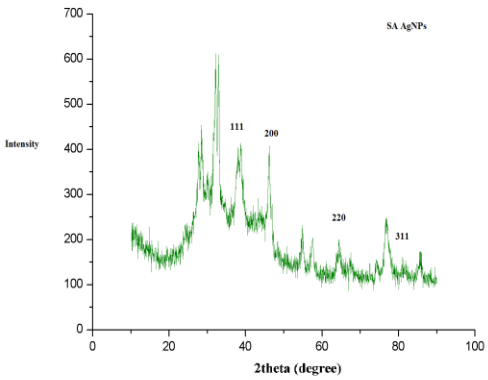


Fig. 4 XRD pattern obtained for AgNPs produced by *S. alexandrina* leaf aqueous extract

TEM analysis: From the TEM micrograph (Fig. 5) it is clear that the synthesized AgNPs were well dispersed and their shape is roughly spherical with the size ranging 50–100 nm. A small percentage of AgNPs in solution was partially aggregated but uniform in their size and shape. The TEM results are consistent with many earlier reports (Tran TTT et al. 2013).

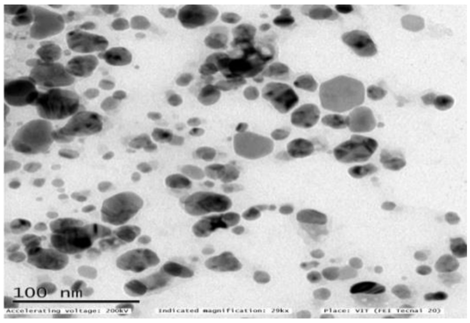


Fig.5. TEM micrograph of *Senna alexandrina* Silver nanoparticles size ranging 50 to100nm

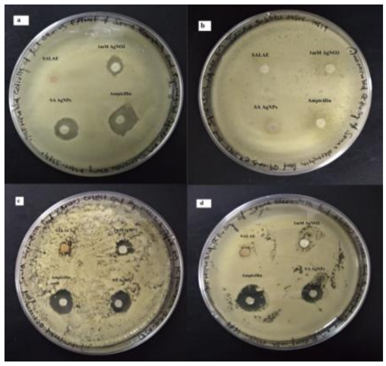


Figure 6. Antibacterial activity of *S.alexandrina* leaf aqueous extract and its synthesized AgNPs. (a).*Salmonella .ebony*(b).*Bacillus.subtilis* (c).*Klebsiella .pneumoniae* (d)*Pseudomonas aureginosa*

Table1: The Zone of Inhibitions of *S. alexandrina* aqueous leaf extract and its synthesized AgNPs.

Microrganisms/ Samples 1mg conc	Salmonella ebony (MTCC 3384),	Bacillus subtilis (MTCC 10619),	Klebsiella pneumonia (MTCC 532),	Peudomonas aeruginosa (MTCC 1688)
SALAE	1.5±0.05	0	2.41±0.8	1.2±0.8
SA AgNPs	4.5±0.05	0	4.2±0.5	3.3±0.5
AgNO3	2.56± 0.14	0	3.6±0.3	2.3±0.5
Ampicillin	6.73±0.08	0	6.3±0.6	5.6±0.3

The data represented above as Mean±SE

Antimicrobial Activity: The renowned inhibitory effect of silver has been known for many years and used for various medical applications (Geethalakshmi and Sarada, 2012). The biofabricated and synthesized silver nanoparticles obtained from *Sennaalexandrina*leaf aqueous extract were screened for antibacterial activity against human pathogens

Salmonella ebony (MTCC 3384), *Bacillus subtilis*(MTCC 10619), *Klebsiella pneumonia* (MTCC 532) and *Peudomonas aeruginosa*(MTCC 1688). The anti bacterial activity of crude plant extacts were found to be less compared to the silver nanoparticles as seen by zones of inhibition (ZOI) in Fig.6 and Table1. The zone of inhibition of aqueous leaf extract on *Salmonella ebony* showed (1.5±0.05), *Klebsellapneumonia* (2.41±0.8), and on *Pseudomonas aeruginosa*(1.2±0.8).Where as synthesized silver nanoparticles treated on *Salmonella ebony* showed (4.5±0.05mm), *Klebsiella pneumonia (*4.2±0.5mm), and on *Pseudomonas aeruginosa*(3.3±0.5mm). The potent antibacterial properties of AgNPs may be attributed to the released silver ions, which could have an interaction with microorganisms by means of their attaching to the surface of the cell membranes of bacteria, penetrating into the bacterial cells, and affecting the membrane permeability and respiration. In the bacterial cells, AgNPs could even interact with sulfur- and phosphorus-containing compounds like DNA to give rise to the deadly impairment of microorganisms.Patil et al., 2012claimed that the cell death arising out of exposure to SNPs might be due to the cytoplasmic membrane disorganization and the consequent leakage of various biomolecules such as amino acids, protein and carbohydrates. Moreover, they indicated that the cell death could be because of inhibition of various essential enzyme. The change in membrane permeability caused by the action of silver nanoparticles as a function of conductivity was studied (Krishnaraj et al. 2010). Their study concluded that the high conductivity of cells treated with SNPs was due to the release of cellular components present inside the cells.

CONCLUSION: Aqueous leaf extract of *Senna alexendrina* mediated, green method of synthesizing silver nanoparticles was successfully developed. It was found out that the various biomolecules present in the leaf extract were responsible for the formation and stability of the SNPs. The size, morphology, crystalline structure and the stability were characterized by UV–Vis spectroscopy, scanning electron microscopy coupled with energy dispersive spectroscopy, Xray diffraction and dynamic light scattering respectively. The functional groups present in the SNPs were analyzed by Fourier Transform Infrared Spectroscopy. The SNPs synthesized in the present study displayed antibacterial activity against human pathogens .The biogenic AgNPs exhibited good antibacterial activities against *E. coli* and *S. aureus*. In the present study, we have synthesized AgNPs of 50–100 nm in size with spherical shape using aqueous leaf extract of *Senna alexendrina* for first time. AgNPs synthesized by aqueous leaf extract were very distinct with very small size, well-defined shape, and well dispersed crystalline nature and clearly proved their biomedical importance by exhibiting strong antimicrobial activity on bacteria. Thus AgNPs possess important applications in biomedical or pharmaceutical industry.

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