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Research Article

AN ECO-FRIENDLY APPROACH FOR SYNTHESIS OF SILVER NANOPARTICLES USING *IPOMOEA PES-CAPRAE* ROOT EXTRACT AND THEIR ANTIMICROBIAL PROPERTIES

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ABSTRACT

Objective: Silver nanoparticles (AgNPs) were obtained through green synthesis using *Ipomoea pes-caprae* root extract for the first time. The active biomolecules present in the roots of *I. pes-caprae*, are ergoline alkaloids, indolizidine alkaloids, benzenoids and phenolic compounds act as both the reduction and stabilization of AgNPs.

Methods: The synthesized nanoparticles were characterized using UV-Visible spectroscopy, Fourier transform infra-red spectroscopy, transmission electron microscope (TEM).

Results: A peak at 400 nm was obtained in UV-Visible spectroscopy confirmed the formation of AgNPs. TEM microgram confirms that AgNPs were in the nano range and spherical in nature, the size was observed to be 50 nm.

Conclusion: A higher zone of inhibition was observed in the AgNPs synthesized from the root extract of *I. pes-caprae* because of the active biomolecules capped on the AgNPs that has greater efficacy against bacteria.

Keywords: *Ipomoea pes-caprae*, Silver nanoparticles, Antimicrobial activity.

INTRODUCTION

Nanotechnology is the most momentous branch of technology that deals with the improvement in the new materials in all fields in our day to day activities, especially in the field of medicine. Nano predominantly deals with the materials that are in the range from 1 to 100 nm. Recently researcher's major concerns were synthesizing of metal nanoparticles that possess high novel physical, chemical, biological, electrical and magnetic properties. The most important property of the nanoparticle is the large surface to volume ratio where all the surface atoms present in the particles actively participating in the change in the property of the material [1]. The size, shape, and surface morphology of nanoparticle plays a key role in controlling the property nature of the materials so the materials can be used in the place where the possibility is highly questionable.

Two major approaches were used for the preparation of nanoparticles namely top-down and bottom-up. Various physical and chemical methods such as ball milling, chemical solution, co-precipitation, physical vapor deposition, chemical vapor deposition, sol-gel, micro-wave assisted, and electrochemical synthesis methods were available for the preparation of metal nanoparticles comes under the above mentioned approaches [2-8]. The chemicals used for the processes of nanoparticle synthesis were highly toxic, expensive and non-ecofriendly that limits their role, particularly in medical and food applications, compare with other applications (electronics, mechanical, etc.,) where it can be used as such. Hence, researchers were progressively aiming at biological methods of synthesizing of metal nanoparticles to use in the medical application. The major advantage of biological methods over other methods are simple, less expensive, openly applicable without any binders, no need for toxic chemicals, no toxic byproducts and most it is environment friendly [9,10]. In the biological approach, the synthesis of nanoparticles has been evolved from various sources that were available enormously in the earth such as microorganisms and terrestrial plants. The active ingredients found in the sources were responsible for the reduction of metallic ions to nanoparticles in green methods [11-13].

Silver is used extremely because of its significant property and recently contributing its role toward the medical application [14-16]. Silver nanoparticles (AgNPs) were reported to have an extraordinary anti-bacterial, anti-fungal and anti-inflammatory activity. Synthesizing AgNPs using biological methods helps the particles by direct usage for the medical treatment and other medicinal applications. Previously various plant source were used for the synthesis of AgNPs by green method like, *Acalyphaindica*, *Coriandrum sativum*, *Sorbusaucuparia* leaf, *Gliricidiasepium*, Rose, *Cinnamomumcamphora*, Aloe Vera, Neem, *Camellia sinensis, Magnolia kobus, Diopyros kaki* and Geranium etc. [17-20] All the parts of the source such as leaves, stem, flower, seeds, root and skin of the fruits were separately used earlier for the synthesis of AgNPs. Synthesized nanoparticles using green method were observed to contain the biomolecules of the plant extract on the surface that were found to be having high medical benefits that can be used as drugs, carrier for drugs, cosmetic, food and other pharmaceutical applications, etc. [21].

In the present investigation, *Ipomoea pes-caprae* plant was used for the synthesis of AgNPs for the first time using its root extract. To confirm the formation of nanoparticles, different characterization techniques were been used.

METHODS

Materials

AR grade of silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich, Mumbai, India. High Pure Double distilled water was used throughout the process. Both normal and whatmann No.1 filter paper were used for the filtration process. All glassware were washed and dried well before using.

I. pes-caprae

I. Pes-Caprae is also known as beach morning glory or goat's foot, and a principle sand binding plant belongs to the family of convolvulaceae (Fig.1). In ancient days *I. pes-caprea* used to treat a headache, jellyfish stink, dermatitis and it acts as an anti-inflammatory,

antispasmodic, analgesic, activity, antinociceptive action, and inhibition of platelet aggregation. It contains alkaloids, amino acids, monoterpenes, quinones, saponins, steroids, and triterpenes [22-25]. *I. pes-caprae* is a valuable medicinal plant found in the regions of tropic and subtropic lands. It grows on the upper part of the beach and sustains salt air. It is well known for its salt tolerant nature. It was used as a great remedy for the skin burns, ulcer, cancer, wound treatment and pain relief and broken bones in the ancient times because of high medicinal values that were being used by the tribal people. They are also used for the treatment of inflammation and gastrointestinal disorders. Various parts of this plant contain several chemical compounds such as alkaloids, benzenoids, etc. These plants were collected from the seashore of Chennai Beach area, Chennai, Tamil Nadu, India. The collected roots were washed well with running tap water and further with double distilled water before the extraction process.

Preparation of root extract

The well-cleaned roots were cut into smaller pieces using a sterilized knife thereafter allowed to dry in a hot air oven for 24 hrs at 40°C. The dried, cut roots were removed from the oven and cooled down to room temperature before grinding using a mechanical grinder. The powdered root was collected and filtered using mesh to collect very fine powder. The fine powders were then packed in airtight bags and stored for future use.

From the fine powder, 5 g was weighed in a beaker. To which 100 ml of double distilled water was poured into it. The mixture was stirred well using magnetic stirrer and heated around 60°C using a hot plate for 1 hr under reflux condition to avoid the liquid loss by evaporation. The extract obtained was then filtered out using Whatman filter paper, and the collected extract were kept in cold condition for the future synthesis process.

An eco-friendly method of synthesis of AgNPs

From the prepared plant extract, 20 ml of the aqueous plant extract of *I. pes-caprae* was added drop by drop to the 1 mM of $AgNO_3$ solution. The colorless silver nitrate solution turns to brown color that indicates the formation of AgNPs. Then the solution was stirred at 50°C for 3 h and then centrifuged at 15,000 rpm for 15 minutes. The supernatant was discarded and replaced by double distilled water during each time of centrifuge. The cycle was repeated for three times. The obtained suspension was stored as lyophilized power for future characterization and study the antimicrobial activity.

Characterization

Surface Plasmon resonance (SPR) properties of synthesized AgNPs were from the root extract of *I. pes-caprae* characterized by UV-Spectrometry analysis (UV-1800, Shimadzu) and the identification of functional groups which were involved in the reduction of silver ions into AgNPs would be characterized by Fourier transform infra-red (FTIR) spectrometry analysis (Jasco 520 instrument). The particles mean size distribution and surface charge of the nanoparticles were established by the particle size analyzer (Malvern zeta analyzer). Morphological analysis of AgNPs was characterized by transmission electron microscopy (TEM) (Hitachi2000) and crystalline size along with particle size was analyzed by X-ray diffraction (XRD) analyzer. Antibacterial studies were carried out against Gram-negative and positive bacteria.

RESULTS AND DISCUSSION

UV-Visible spectroscopy

Synthesized AgNPs and their stability in aqueous solution were determined by UV-Vis spectral analysis. Usually, UV-visible spectroscopy used to determine the size and shape controlled nanoparticles in aqueous solution [16]. Due to the excitation of SPR on AgNPs the synthesized solution turns into brown color [14,15]. As the leaf extracts of *I. pes-caprae* mixed with the $AgNO_3$ solution, colorless silver nitrate turned into amber brown that shows the reduction of the silver ion into AgNPs. UV-visible spectrum was recorded after the addition of plant extracts to the silver nitrate solution under continuous stirring at 60°C for 1 hr (Fig. 2). The UV-absorption spectra of AgNPs peak was observed at 430 nm, and the broadening UV peak indicates the polydispersity of AgNPs.

FTIR analysis of the plant extracts AgNPs

The root extract and the synthesized AgNPs were observed using FTIR as shown in Fig 3. FTIR was carried out to categorize the feasible biological macromolecules responsible for the reduction and

Fig. 1: *Ipomoea pes-caprae* plant

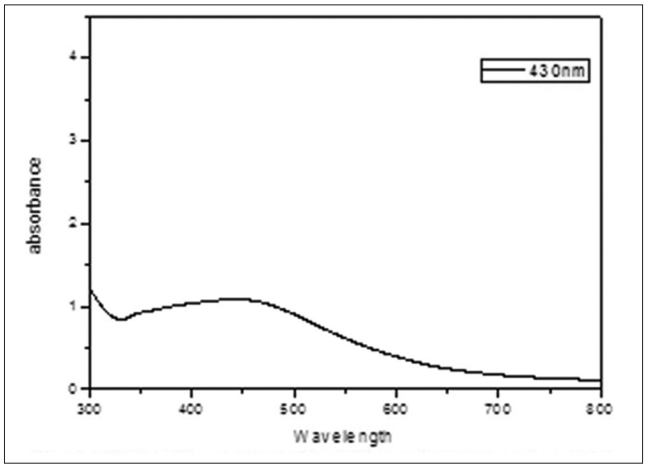

Fig 2: Ultra violet-Visible spectra of synthesized silver nanoparticles from the *Ipomoea pes-caprae* root extract

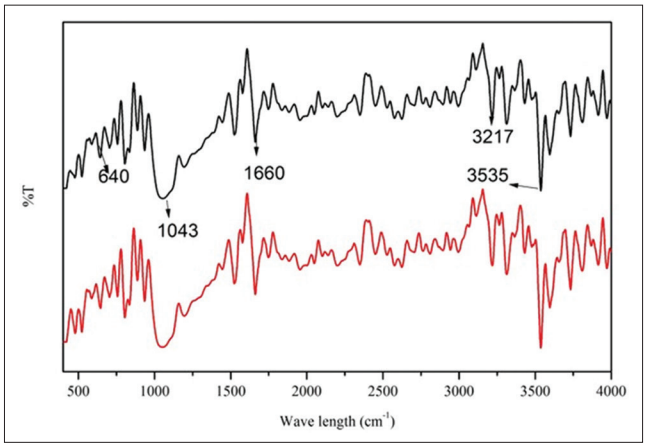

Fig. 3: Fourier transform infra-red spectra of (a) root extract and (b) synthesized silver nanoparticles

stabilization of AgNPs. The FTIR spectrum of AgNPs shows peaks at 3535, 3217, 1660, 1043 cm^{-1}. Among the obtained peaks 1660 cm^{-1} represent the C=C stretch vibration and its corresponding to the amide I bond of protein. The peak at 1043 cm^{-1} could be due to C-O stretch of (CH-OH) in cyclic alcohol. The peak at 635 cm^{-1} corresponds to the aromatic C-H vibrations that denote the involvement of free quinone (polyphenolic compounds) [25]. These results suggest that the polyphenolic compounds from the *I. pes-caprae* employed as a reducing and capping molecules. Satyavani reported the similar results for the FTIR spectrum [26]. The shallow peak at 2004 cm^{-1}match with the C=O amide stretching. The acquired result implies that the presence of several functional groups in the aqueous leaf extract as well isolated nanoparticles. Particularly, few groups on the AgNPs would suggest that the presence of plant polyphenols or polysaccharides may be responsible for the biotransformation of silver ions into AgNPs.

XRD analysis of AgNPs

Fig. 4 shows the XRD pattern of AgNPs. The low intense peaks at 32.11°, 37.97°, 46.07°, 64.25°and 77.45° (2θ) which are responsible for (311), (111), (103), (220) and (311) Bragg reflection, respectively and these values were found to be matched with JCPDS file NO. 04-0783. Broadening of XRD peaks was responsible for the decrease in silver metal ion concentration and an increase in the formation of AgNPs. The peak at 12° represents the presence of organic molecules in the AgNPs [27]. Based on this result, green synthesized AgNPs could be a face centered and cubic structured [28]. It clearly indicates that the *I. pes-caprae* reduced $AgNO_3$ into AgNPs with crystalline nature. The broadening of XRD peaks represents the particle size with nano range [19]. Mahdieh *et al*. also obtained the same result in their investigation of AgNPs synthesis from *Spirulinaplatensis* [20].

TEM

The morphology of synthesized AgNPs was analyzed using TEM, it is shown in Fig. 5. From this Fig. 5, the AgNPs size and shape was found to be spherical in shape, and the particles size was observed in the range from 10 to 50 nm. The synthesis of AgNPs depends upon the nature of the plant extract. When the plant extract concentration increases the size of the particles, be decreased [21]. TEM micrograph clearly shows that silver was reduced to nano size and all particles are free from agglomeration. Indicates that chemical compounds present in the plant extract act not only reducing agent but also it stabilizes and controls the size and shape of the AgNPs.

Antibacterial activity

To demonstrate the behavior of the AgNPs in antimicrobial activity, the synthesized AgNPs were investigated on various bacteria. In the study, Kirby- Bauer disc diffusion method was performed against pathogenic bacteria. *Escherichia coli, Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) were used to study the performance of the AgNPs against the bacteria. The zone of inhibition is shown in Table 1, which clearly shows that the green synthesized AgNPs has the maximum zone of Inhibition. It was found to be 25 mm for *S. aureus,* 23 mm for *P. aeruginosa* and 22 mm for *E. coli.* In this study, the zone of inhibition for the standard commercial antibiotic (Gentamicin) was found to be less when compared to the AgNPs synthesized from the extract against *S. aureus,* found to be the same to the AgNPs against *P. aeruginosa* and found to be more to the AgNPs against *E. coli.* From the result, it was observed that the bactericidal action of the zero valent nanoparticles was found to be more when compared to gentamicin antibiotics against *S. aureus* and *P. aeruginosa.* From the Table 1, the antibacterial effects of AgNPs were found to be increased with the increase in the dose (concentrations). The highest zone of inhibition was observed as 25 mm at 75 µL of AgNPs concentrations on the gram negative Staphylococcus aureus. The antimicrobial work established previously by Satyavani *et al.* in AgNPs synthesized from the *I. pes-caprae* leaf extract clearly state that the zone of inhibition was found to be less when compare with the present investigation in all three bacteria (Table 1) [26]. Higher the zone of inhibition was observed in the AgNPs synthesized from the root extract of *I. pes-caprae* because of the active biomolecules capped on the AgNPs that has greater efficacy against bacteria.

CONCLUSION

In this investigation for the first time roots extract of *I. pes-caprae* were used to synthesize the AgNPs successfully using the green method. Here in this established work, we observed that even the extract

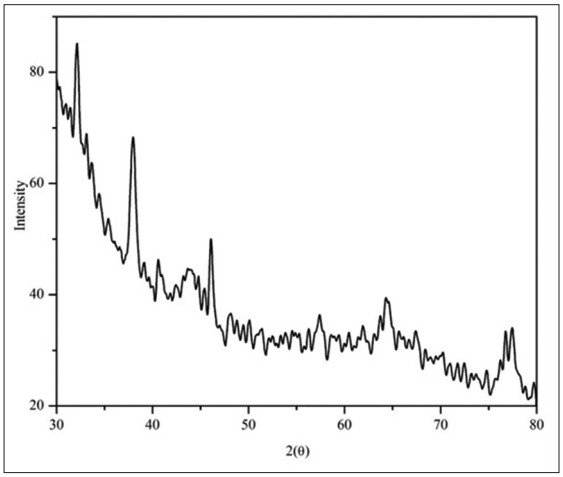

Fig. 4: X-ray diffraction of synthesized silver nanoparticles

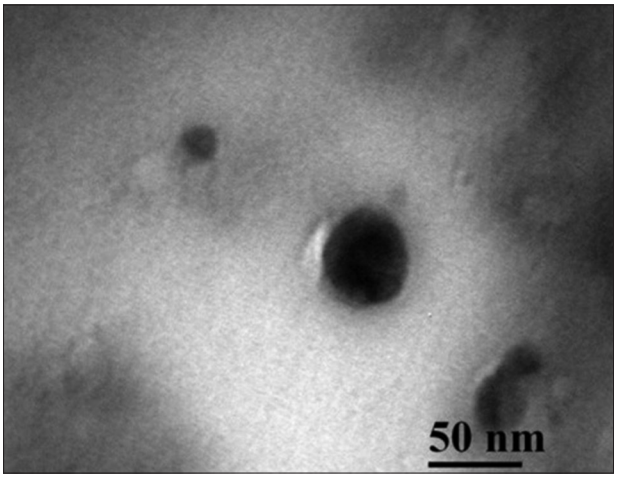

Fig. 5: Transmission electron microscopy micrograph of synthesized silver nanoparticles

Table 1: Zone of inhibition for biosynthesized AgNPs from *I. pes-caprae* against different bacterial species (mm)

Name of the bacteria's	Control	Gentamicin	Different concentration of AgNPs			
			(25 µl)	(50 µl)	(75 µl)	(100 µl)
Staphylococcus aureus	1	20±1.0	17±0.5	20±1.0	25±1.3	21±1.0
Pseudomonas aureoginosa	1	24±1.3	18±0.5	19±1.0	23±1.3	24±1.3
Escherichia coli	1	27±1.3	15±0.5	19±1.0	19±1.0	22±1.3

I. pes-caprae: Ipomoea pes-caprae, AgNPs: Silver nanoparticles, *S. aureus: Staphylococcus aureus, P. aureoginosa: Pseudomonas aureoginosa, E. coli: Escherichia coli*

from the root of *I. pes-caprae* can be used to the synthesis the AgNPs. The preparation time of AgNPs was reduced to 3 hrs from that of the previously established works. The obtained AgNPs was confirmed that they were in spherical in shape and size in the range of <30 nm from the TEM analysis. From the antimicrobial studies it was clear that the AgNPs synthesized from the roots extract of *I. pes-caprae* have larger zone of inhibition that proves that the active biomolecules present on the surface of AgNPs were also contributing against the microbes. Thus, the prepared AgNPs the roots extract of *I. pes-caprae* can be directly used as a carrier for drugs.

REFERENCES

1. Leela A, Vivekanandan M. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. Afr J Biotechnol 2008;7(17):3162-5.
2. Horwat D, Zakharov DI, Endrino JL, Soldera F, Anders A, Migot S, *et al*. Chemistry, phase formation, and catalytic activity of thin palladium-containing oxide films synthesized by plasma-assisted physical vapor deposition. Surf Coat Technol 2011;205(2):S171-7.
3. Dillon AC, Mahan AH, Deshpande R, Alleman JL, Blackburn JL, Parillia PA, *et al*. Hot-wire chemical vapor synthesis for a variety of nano-materials with novel applications. Thin Solid Films 2006;501(1-2):216-20.
4. Sobhani M, Rezaie HR, Naghizadeh R. Sol-gel synthesis of aluminum titanate (Al2TiO5) nano-particles. J Mater Process Technol 2008;206:282-5.
5. Nadagouda MN, Speth TF, Varma RS. Microwave-assisted green synthesis of silver nanostructures. Acc Chem Res 2011;44(7):469-78.
6. Wani IA, Ganguly A, Ahmed J, Ahmad T. Silver nanoparticles: Ultrasonic wave assisted synthesis, optical characterization and surface area studies. Mater Lett 2011;65(3):520-2.
7. Starowicz M, Stypula B, Banas J. Electrochemical synthesis of silver nanoparticles. Electrochem Commun 2006;8(2):227-30.
8. Kima KD, Hanb DN, Kim HT. Optimization of experimental conditions based on the Taguchi robust design for the formation of nano-sized silver particles by chemical reduction method. Chem Eng J 2004;104(1-3):55-61.
9. Balaji DS, Basavaraja S, Deshpande R, Mahesh DB, Prabhakar BK, Venkataraman A. Extracellular biosynthesis of functionalized silver nanoparticles by strains of *Cladosporium cladosporioides* fungus. Colloids Surf B Biointerfaces 2009;68(1):88-92.
10. Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi AA. Rapid synthesis of silver nanoparticles using culture supernatants of enterobacteria: A novel biological approach. Process Biochem 2007;42(2):919-23.
11. Kumar KP, Paul W, Sharma CP. Green synthesis of gold nanoparticles with *Zingiber officinale* extract: Characterization and blood compatibility. Process Biochem 2011;46(10):2007-13.
12. Dubey SP, Lahtinen M, Sillanpaa M. Tansy fruit mediated greener synthesis of silver and gold nanoparticles. Process Biochem 2010;45(7):1065-71.
13. Sukirtha R, Priyanka KM, Antony JJ, Kamalakkannan S, Thangam R, Gunasekaran P, *et al*. Cytotoxic effect of Green synthesized silver nanoparticles using *Melia azedarach* against in vitro HeLa cell lines and lymphoma mice model. Process Biochem 2012;47(2):273-9.
14. Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces 2010;76(1):50-6.
15. Noginov MA, Zhu G, Bahoura M, Adegoke J, Small C, Ritzo BA, *et al*. The effect of gain and absorption on surface plasmon in metal nanoparticles. Appl Phys B 2006;86(3):455-60.
16. Shrivastava S, Dash D. Applying nanotechnology to human health. J Nanotechnol 2009;12:240-3.
17. Sathyavathi R, Krishna MB, Rao SV, Saritha R, Rao DN. Biosynthesis of silver nanoparticles using *Coriandrum sativum* leaf extract and their application in nonlinear optics. Adv Sci Lett 2010;3(2):1-6.
18. Shameli K, Ahmad MB, Yunus WM, Ibrahim NA. Synthesis and characterization of silver/talc nanocomposites using the wet chemical reduction method. Int J Nanomedicine 2010;5:743-51.
19. Khanna PK, Sing N, Charon S, Subbrarao VV, Gokhole R, Mulik UP. Synthesis and characterization of Ag/PVA nanocomposite by chemical reduction method. Mater Chem Phys 2005;93(1):113-7.
20. Mahdieh M, Zolanvari A, Azimee AS, Mahdieh M. Green biosynthesis of silver nanoparticles by *Spirulina platensis*. Sci Iran 2012;19(3):926-9.
21. Khalil MH, Ismail EH, El-Magdoub F. Biosynthesis of au nanoparticles using olive leaf extract. Arab J Chem 2012;5(4):431-7.
22. Achary PM Subudhi S, Das CC. Laboratory evaluation of Ipomoea leaf extract in the control of *Culex quinquefasciatus* population. Environ Ecol 1993;11(3):519-22.
23. Carrick J, Chan KC, Cheung HT. A new phyto-chemical survey of Malaya chemical screening. Chem Pharm Bull 1968;16:2436-41.
24. De Souza MM, Madeira A, Berti C, Krogh R, Yunes RA. Antinociceptive receptive properties of the methanolic extract obtained from Ipomoea pes-caprae (L.) R. Brit. J Ethanopharmcol (Ireland) 2000;69(1):85-90.
25. Krishanan R, Maru GB. Isolation and analyses of polymeric polyphenols fractions from black tea. Food Chem 2006;94(3):331-40.
26. Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T. *Impomoea pes-caprae* mediated silver nanoparticles and their antibacterial effect. Sci Int 2013;1(5):155-9.
27. Kalainila P, Subha V, Ernest Ravindran RS, Enganathan S. Synthesis and characterisation of silver nanoparticles from *Erythrina indica*. Asian J Pharm Clin Res 2014b;7 Suppl 2:39-43.
28. Saranyaadevi K, Subha V, Ernest Ravindran RS, Renganathan S. Synthesis and characterisation of copper nanoparticle using *Capparis zeylanica* leaf extract. Int J Chem Tech Res 2014b;6(10):4533-41.