Research Article

Green Synthesis, Optimisation and Characterisation of Silver Nanoparticles Using Aqueous Root Extract from Exotic Tree Species *Casuarina equisetifolia* and Its Antibacterial Efficacy

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ABSTRACT

Casuarina equisetifolia is a widely cultivated tree species, for its quality fuel wood, that also serves as a rich source for potential phytoconstituent. The present study has demonstrated the bioinspiered synthesis of silver nanoparticle, using aqueous root extract of *Casuarina equisetifolia,* as a reducing agent. The biosynthesized Silver nanoparticles (AgNP's) were optimized for various parameters like temperature, pH, time and stability. Characterization of the optimized AgNP's, for its structural and morphological properties were carried out using UV-Vis spectroscopy, FTIR spectroscopic technique, TEM analysis and X-Ray diffraction (XRD). The UV wavelength of AgNP's was between 415–430 nm, optimized temperature lies between 60°C to 80°C, pH was neutral and time required for incubation was 30 mins, for the synthesis of an effective silver nanoparticles using aqueous root extract of *Casuarina equisetifolia* as a reducing agent and the synthesized nanoparticles was found to be stable without addition of any stabilizing agent. TEM analysis revealed that AgNP's synthesized were spherical in shape with an average particle size of 25nm. The FTIR study, supports the presence of certain biomolecules in the root extract which could be responsible for the stabilization of the synthesized silver nanoparticles. The average crystalline size was found to be 44 Å using XRD pattern. Further, these biosynthesized AgNP's were found to be highly effective antibacterial agent against, multi drug resistant pathogens *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus vulgaris*. Maximum antibacterial activity was obtained compared to the standard, Gentamycin. Thus, the study reveals that silver nanoparticles synthesized using aqueous root extract of *Casuarina equisetifolia* serves as an effective bioreductant for synthesis and also alternative for therapeutic approach in medical field.

Keywords: *Casuarina equisetifolia,* optimization, Temperature, FTIR, TEM, XRD, root extract.

INTRODUCTION

Nanotechnology is a technology that has the potential to revolutionize and transform multiple technologies at industrial level including agriculture, medicine, biotechnology energy services etc (National Nanotechnology initiative). Among the noble metals, silver is widely used in the synthesis of nanoparticles involving biological system, medicine and living organism.

The unique property, which is Surface Plasmon Resonance (SPR) occurs upon irradiating silver nanoparticles with light, that results in the oscillation of free electrons in the conduction band of nanoparticles, which is based on the size and shape of the particle synthesized.

Thus these properties allow silver nanoparticles to be employed in various fields for various application.

Researchers have developed potential silver nanoparticles, synthesized using aqueous leaf extract of *Aerva lanata*[1], leaf ethanol extract of *Pisonia grandis*[2], *Pimenta diocia*[3], *Ocimum* leaf extract[4], where in all these cases plant materials served as potential reducing agent.

Casuarina equisetifolia, an evergreen tree species, containing wider range of potential biomolecules, which still lacks scientific validation.

But the history of folk medicine reports that beefwood was used to treat various ailments like beri-beri, colic, cough, diarrhea, dysentery, headache, nerve problem, swelling and toothache.

Since this tree species is rich in several potential phytochemicals like alkaloids, flavonoids, triterpenoids, carbohydrates, tannins, phenols, gums[5], systematic investigations were done to evaluate antimicrobial, antioxidant and haemolytic activity of extracts of *Casuarina equisetifolia* in recent days.[6]

The current study explores the role of aqueous root extract of Casuarina *equisetifolia* in the novel *in vitro* synthesis of Silver nanoparticles.

The synthesized nanoparticles were optimized for various parameters like temperature, time, pH and stability.

On optimizing, the conditions required for the synthesis were standardized, the silver nanoparticles were analyzed for UV-Vis Spectroscopy, FTIR spectrum study, TEM micrograph and XRD to characterize the synthesized particles.

These biologically synthesized AgNP's were found to produce effective antibacterial activity against multi-drug resistant pathogens.

MATERIALS AND METHODS

Collection of Plant Material

Root samples of *Casuarina equisetifolia* were collected from, Pudhucherry Union territory, India. Collected root materials were made free from adhering soil matters and then surface sterilized using 1% Sodium hypochlorite, followed by shade drying. The dried materials were finely powdered and stored at 4°˙C for further investigations.

Preparation of Aqueous Root Extract

Extract was prepared using the finely powdered root material. Powdered root material and distilled water was taken in the ratio 1:10 and boiled for 20 mins. The extract was then filtered using whatman No 1 filter paper and then centrifuged at 5000 rpm for 15 mins. Supernatant was stored at 4˙C for further analysis.

Synthesis of Silver Nanoparticle

For the present study, 1mM of $AgNO_3$ aqueous solution was prepared. For the synthesis of silver nanoparticles, root extract and 1mM $AgNO_3$ aqueous solution was mixed in the ratio 1:9, followed by incubation in dark condition for 24 hours. Formation of brown or wine red colour confirms the reduction of aqueous silver ions by aqueous root extract[7], to form silver nanoparticles.

Optimization of the Silver Nanoparticles

Temperature

The process of Bio reduction of metal ions, by the biomolecules in the extract is dependent on temperature at which the reaction takes place. To standardize an optimum temperature for the formation of silver nanoparticles 20˙C, 40˙C, 60˙C, 80˙C, 100˙C was fixed, as the temperature ranges, for the reaction trial. The absorbance was monitored using UV-Vis spectrophotometer from which the result was observed.

Time

Incubation time plays a crucial role in the formation of nanoparticles. The optimization of time was studied at different time interval viz. 10 mins, 20 mins, and 30 mins. Absorbance was measured spectrophotmetrically.

pH

The shape of the silver nanoparticles synthesized mainly depends on the pH value of the reaction solution.

Thus pH of the reaction was set as 4, 7 and 9 for the trial. pH was adjusted using NaOH solution (0.1N) and HCl solution (0.1N).

The results were read using UV-Vis spectrophotometer.

Stability

The stability of the synthesized nanoparticles at the optimized condition, was checked periodically at regular time interval for 30 days.

Characterization of Silver Nanoparticles

UV-Vis Spectrophotometer

Optical absorption spectra of silver nanoparticles present in the suspension were analyzed using Double beam UV-Vis spectroscopy at wavelength range 350 nm-500nm and the peaks were recorded.

FT-IR Spectroscopy

The synthesized silver nanoparticles were mixed with Potassium bromide in the ratio of 1:100 and the pellet was prepared.

This was taken for spectrum analysis and recorded on Shimazdu IR Affinity 1, Japan equipped with DLATGS detector with a mirror speed of 2.8mm/sec and with Diffuse Reflectance mode (DRS) as attachment operating at resolution of $4cm^{-1}$ was used.

The measurement range was 400-4000 cm^{-1}.

This study was done basically to know the fundamental vibration of the functional groups.

Transmission Electron Microscope

The dark wine red silver nanoparticles were centrifuged at 20,000 rpm for 15 mins and the pellet of Ag NP's was formed.

This was washed thoroughly to remove the attachment of residual Silver nitrate particles.

The pellet thus obtained was air dried and suspended in ethanol. This was observed in Transmission Electron Microscope Techai 10 Philips and photographed.

X-Ray Diffraction (XRD)

The powdered silver nanoparticles, were deposited on a microscopic slide and air dried overnight at room temperature using Cu-Kα1radiation source in powder diffractometer (XRD – Rich siefert 300 diffractometer).

X-ray diffraction (XRD) measurements were carried out with a Bruker D8 A vance diffract meter using Ni-filtered Cu Kα radiation (λ = 0.154 nm).

XRD patterns were recorded in the 2ϑ range 10–70° at a scan speed of 10° min^{-1} at room temperature.

The XRD analysis was done to determine and confirm the structure of silver nanoparticles.

Antibacterial Activity

In vitro antibacterial activity of the silver nanoparticles synthesized using the root extract of *Casuarina equisetifolia* were studied.

Three pathogens viz., *Pseudomonas aeruginosa, Staphylococcus aureus and Proteus vulgaris*, that are multiple drug resistant bacterial strains were used for antibacterial studies, obtained from the Department of Microbiology, Ethiraj college for women, Chennai. Bacterial test organisms were grown in nutrient broth for 24 hours.

These cultures were used to prepare bacterial lawn on the Muller Hinton Agar medium used for plating. Four wells of diameter 8mm were made on each bacterium inoculated plate.

Well A, B and C was loaded with 10µl, each with the concentration of 25µg, 50µg and 100 µg of silver nanoparticles synthesized using aqueous root extract of *Casuarina equisetifolia*, respectively.

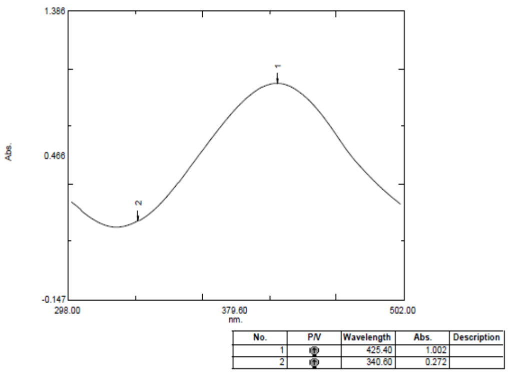
Well D was loaded with 10 µl of freshly prepared 1 mM silver nitrate solution. The plates were incubated for 24 hours at 37˚C. Gentamycin (100 µg) was used as a standard.

RESULTS AND DISCUSSION

Synthesis of Silver Nanoparticles

The synthesis of silver nanoparticles can be confirmed both visually by colour change and also spectrometrically with the help of UV –Vis Spectrophotometer. On addition of Root extract to Silver nitrate solution, reddish wine colour was formed, which is the characteristic feature of formation of silver nitrate particles (Fig 1). The formation of AgNP's was confirmed spectrometrically, by the SPR (Surface Plasma Resonance Band). In the current study absorbance peak was recorded at 425nm (Fig 2). This confirmed the formation of Silver nanoparticles, since, the characteristic SPR absorption band for the silver nanoparticles lies in the range of 400nm-475nm.[8]

Figure 1: Synthesized AgNP's using Aqueous Bark



No.	P/V	Wavelength	Abs.	Description
1		425.40	1.002	
2		340.60	0.272	

Figure 2: UV-Vis Spectra of Silver

Root Extract of *Casuarina equisetifolia* Nanoparticles

Optimization of Temperature

Increase in the temperature of the reaction mixture was followed by increase in the absorbance value. Fig 3 reveals a very small shift in the SPR value for temperature ranging at 60˚°C and 80 °C as 424nm and 425 nm respectively. Thus for our present study, temperature between 60˚°C and 80 °C (Fig 3) proves to be effective in the formation of silver nanoparticles, using aqueous root extract of *Casuarina equisetifolia*.

Increase in the temperature of the reaction, results in rapid reduction of Ag^+ ions and also Silver nanoparticles of smaller size are synthesized due to homogenous nucleation of silver nuclei.[9]

In the study conducted in optimizing the temperature for the synthesis of silver nanoparticles from aqueous leaf extract of *Garcinia mangostana*[10] and *Hippophae rhamnoides* Linn[11] maximum absorbance was observed at 75˚C, which is in close agreement with our study.

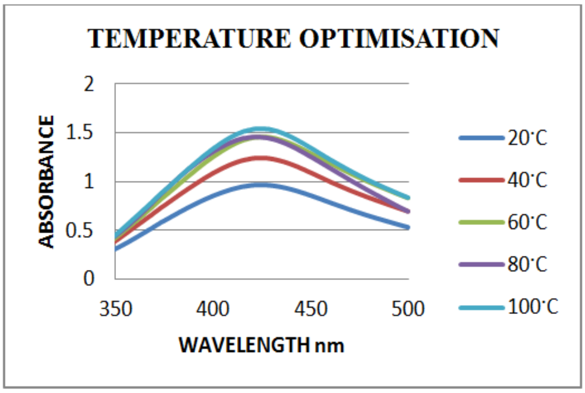


Figure 3: Uv-Visible Spectra of AgNP's showing effect of different reaction temperature

Optimisation of Time

Generally increase in incubation time is marked by the increase in absorbance value for the synthesis of silver nanoparticles. In the present study, the intensity of SPR and absorbance has increased with the increase in time period.[11] Thus, from the current investigation, the optimum time period for the incubation of synthesis of silver nanoparticles (at temperature range between 60˚°C and 80 °C, using aqueous root extract of *Casuarina equisetifolia* was standardized as 30 mins (Fig 4). Similar results were obtained during the formation of silver nanoparticles using aqueous leaf extract of *Acalypha indica*[12] and *Prunus armeniaca*[13] where the synthesis was achieved with the incubation period of 30 mins.

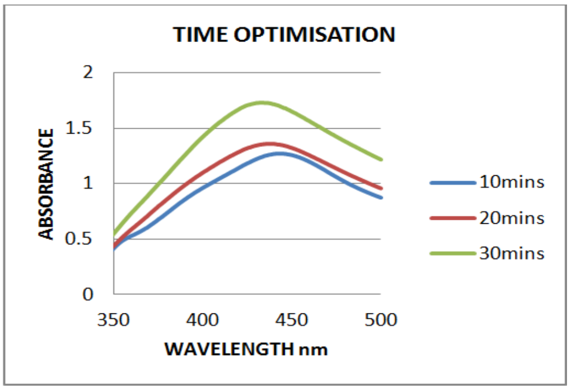


Figure 4: Uv-Visible Spectra of AgNP's showing effect of different reaction time

Optimization of pH

The effect of pH in the formation of silver nanoparticles, using aqueous root extract of *Casuarina equisetifolia* were investigated with the pH ranges of 4.0, 7.0 and 9.0. Maximum absorbance was recorded at neutral pH (433 nm) at the temperature range between 60·°C and 80 °C and an incubation period of 30 mins. The study in the biosynthesis of silver nanoparticles using Mangosteen leaf extracts, also supports neutral pH as the optimized condition for the efficient synthesis of AgNP's.[10] The particles size is expected to be larger in acidic medium compared to that of basic pH (Fig 5). Many studies have reported that size and shape of the bio synthesized nanoparticles can be altered by varying the pH of the reaction mixture.[9]

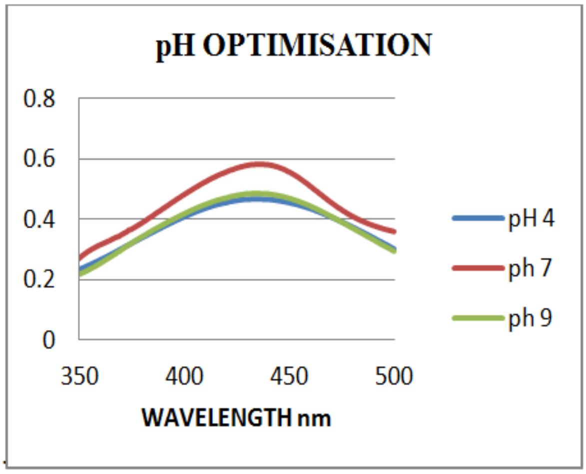


Figure 5: Uv-Visible Spectra of AgNP's showing effect of different pH

Stability

The AgNP's synthesized at temperature range between 60·°C and 80 °C, an incubation period of 30 mins and pH 7 was found to be stable upto the period of 30 days without the addition of any stabilizing agent, after which it leads to reduction in stability due to agglomeration of the nanoparticles.

Characterization of Silver Nanoparticles

UV-Vis Spectrophotometer

UV-Vis spectrophotometer analysis study was used to confirm the formation and stability of the silver nanoparticles synthesized using root extract of *Casuarina equisetifolia.* Excitation of surface plasma resonance, produced by the reaction mixture gives the characteristic colour to the silver nanoparticles synthesized.[7] In the current study, the SPR band lies between 420 nm-440nm, that confirms the dispersed state of the synthesized nano particles in the solution and also absence of clubbing of the particles.[7]

FTIR Analysis

FTIR spectrum analysis helps us to characterize the presence of various functional groups with respect to the vibrational bands, present in the aqueous root extract of *Casuarina equisetifolia,* that could be responsible for capping and reduction of the reduced silver nanoparticles (Fig 6). The broad peaks at 3452.73 cm-1 represent the presence of NH_2 group of primary amine or amide. Sharp peak at 1635.71 cm^{-1} corresponds to the medium intense band with stretching vibration of CO stretch. Absorbance band at 1345.51 cm^{-1} corresponds to the phenol or tertiary alcohol OH bend. The band observed at 1012.67 and 1056.07 cm^{-1} represents cyclohexane ring vibration. The absorption band at 1056.07 cm^{-1} corresponds closely to the C-O stretch. The vibrational bands that appear in the IR spectrum of the aqueous root extract could be observed in the IR spectra of the reduced AgNP's.

Thus the FTIR analysis serves as an evidence for the presence of potential biomolecules that was responsible for the reduction of Ag ions and also for capping and stabilization of synthesized silver nanoparticles using aqueous root extract of *Casuarina equisetifolia.*

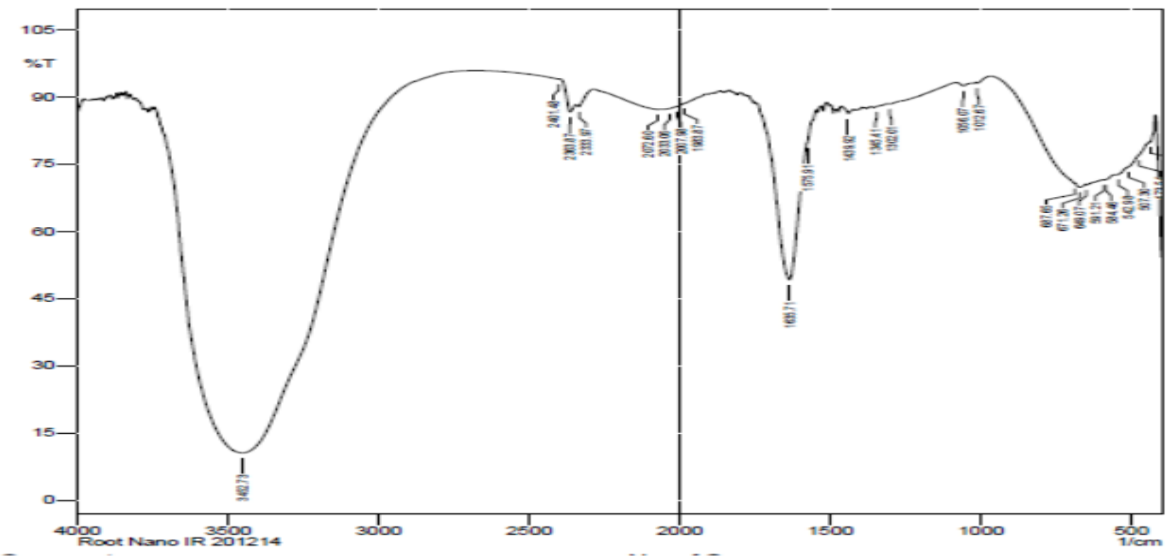


Figure 6: FTIR spectrum of the Silver Nanoparticles synthesized using aqueous root extract of *Casuarina equisetifolia*

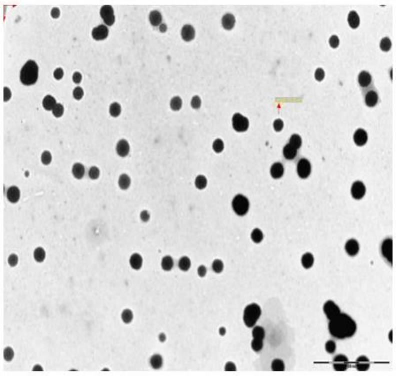


Figure 7: TEM Micrograph

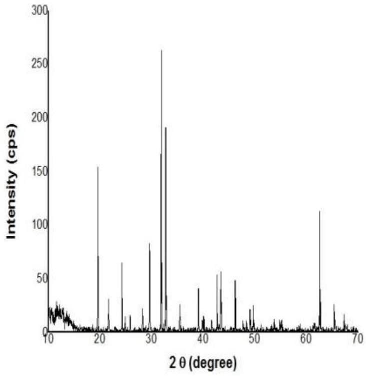
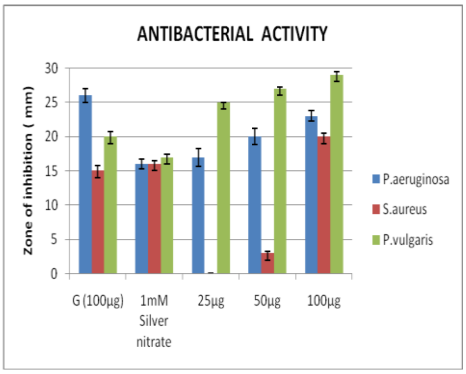


Figure 8: XRD Analysis



Values are mean inhibition zone (mm) ± SD of three replicates.

Figure 9: Antibacterial activity of the synthesized nanoparticles

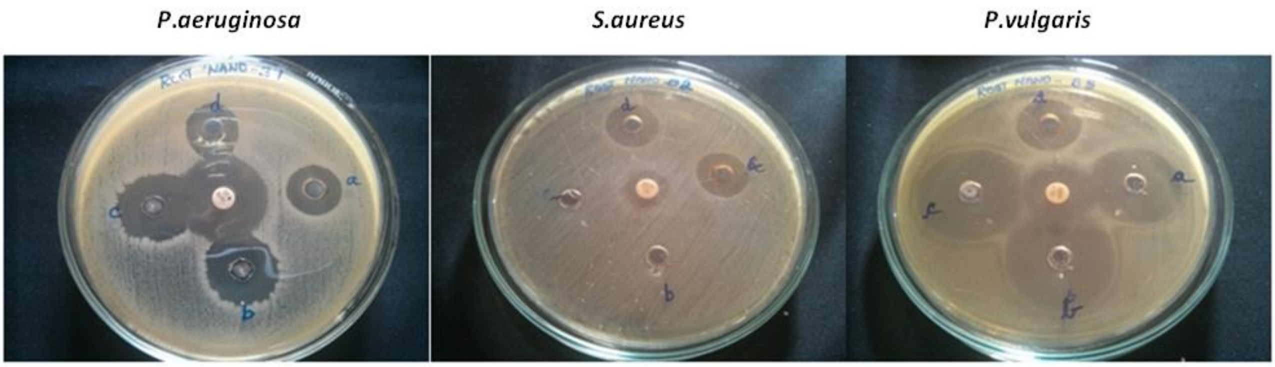


Figure 10: Antibacterial activity of the synthesized nanoparticles

TEM Analysis

Figure 7 shows the TEM micrographs of the silver nanoparticles synthesized. The micrograph shows variation in the particle size. The average size of the particles was 25nm (Fig 6). Few agglomerated silver nanoparticles are seen that may be due to the process of sedimentation.[10]

XRD Analysis

To confirm and identify the crystalline nature of SNPs using X-ray diffraction pattern were recorded from the 2θ upto 10 to 80 degrees.

The figure shows, that the characteristic 2θ values are 19.61º, 31.89º and 32.77º[7]. The average crystalline size was found to be 44 Å using Scherrer's formula, $d = 0.9 \lambda / B \cos\theta$.

Antibacterial Activity

Antibacterial activity of synthesized silver nanoparticles against *Pseudomonas aeruginosa, S. aureus* and *Proteus vulgaris*, were studied and compared with the standard. *Pseudomonas aeruginosa*, *Proteus vulgaris* was more susceptible to synthesized Ag nano particles, whereas *S. aureus* showed very minimal level of susceptibility even at higher concentration. *Proteus vulgaris* showed maximum zone of inhibition of 28mm ± 0.7 at 100 µg concentration (Fig 9 & 10).

Strong evidences are there since ages unknown, for its dual activity that silver ions and silver based compounds have effective antibacterial property, which intensified to make use of Ag nanoparticles as an efficient antibacterial agent, with the help of plant extract.[15]

The silver nanoparticles synthesized using plant extracts, attaches to the surface of the bacterial cell membrane, results in the disruption of the cell membrane to penetrate the cell, which ultimately results in the death of the bacterial cell[15] of multi drug resistant bacterial strains.

CONCLUSION

It is clear from the above results, that aqueous root extract of *Casuarina equisetifolia* can be employed in the potential silver nanoparticles, using 1mM Silver nitrate solution. Optimization of the processing condition is very important to synthesize perfect sized and shaped nanoparticles.[3] Thus the optimized processing conditions are: Temperature: 60°C - 80°C; pH: neutral; Time:30 mins and was found to be stable for a period of 30 days. Silver nanoparticles synthesized using optimized conditions have been characterized by UV-Vis Spectroscopy, FTIR spectrum study and TEM micrograph. FTIR study revealed the presence of certain functional groups that are in association with biomolecules like phenols, flavonoids, tannin, alkaloids which would have been the key factor for capping and stabilization for the synthesized silver nanoparticles. The average crystalline size was found to be 44 Å using XRD. The average size was 25 nm, which was interpreted using TEM micrograph. Our current investigation showed a simple, economical and rapid route to synthesis of silver nanoparticles, which could be employed as a new range of antibacterial agent in biomedical and pharmaceutical field, as well as bio protectant in disease management of pests and pathogens.

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REFERENCES

1. Balashanmugam P. Synthesis of Plant-Mediated Silver Nanoparticles Using *Aerva lanata* leaf aqueous extract and evaluation of Its Anti-bacterial activities. Indo American Journal of Pharm Research. 2014, 4(01).
2. Jannathul Firdhouse M, Lalitha P and Shubashini K. Sripathi. "Novel Synthesis of Silver Nanoparticles using leaf ethanol extract of *Pisonia grandis* (R. Br)" Der Pharma Chemica, 4(6), 2012, 2320-2326.
3. Akshay Rajeev Geetha., Elizabeth George., Akshay Srinivasan, and Jameel Shaik. "Optimization of green synthesis of silver nanoparticles from leaf extracts of *Pimenta dioica* (Allspice)." The Scientific world Journal, Article Id 362890, Volume 2013, 5 Pages.
4. Mallikarjunaa K, Narasimhab G, Dillipa G.R, Praveen, Shreedhar C, Sree Lakshmic C, Reddy B. V. S, Deva Prasad Raju B. "Green synthesis of silver nanoparticles using *Ocimum* leaf extract and their characterization" Digest Journal Of Nanomaterials and Biostructures, Vol. 6, No 1, January-March 2011, P. 181-186.
5. Bukowska B., Kowalska S. Phenol and catechol induce pre-hemolytic and hemolytic changes in human erythrocytes. *Toxicol. Let.* 152, 2004, 73-84.
6. Ramanathan T, Gurudeeban S, Satyavani K and Kathiresan K. PP24: Pharmacological studies: antimicrobial, antioxidant and antiaggregant activities of coastal she oak (*Casuarina equisetifolia*) Proceedings of International Conference on Environmental Security for Food and Health @ Kanyakumari, 16-18 February, 2012.
7. Kirthika P., *Dheeba B., Sivakumar R., Sheik Abdulla S. "Plant mediated synthesis and characterization of silver nanoparticles" International Journal Of Pharmacy And Pharmaceutical Sciences. Vol 6, Issue 8, 2014.
8. Brajesh Kumar, Kumari Smita, Luis Cumbal, Alexis Debut. "Synthesis of silver nanoparticles using Sacha inchi (*Plukenetia volubilis* L.) leaf extracts", Saudi Journal of Biological Sciences (2014), http://dx.doi.org/10.1016/j.sjbs.2014.07.004
9. Mostafa M.H. Khalil A, Eman H. Ismail A, B, Khaled Z. El-Baghdady C, Doaa Mohamed. "Green synthesis of silver nanoparticles using Olive leaf extract and its antibacterial activity" Arabian Journal Of Chemistry, 7, 2014, 1131–1139.
10. Ravichandran V., Tiah Z.X, Subashini G, Terence F.W.X., Eddy F.C.Y., Nelson J and Sokkalingam A.D. "Biosynthesis of silver nanoparticles using Mangosteen leaf extract and evaluation of their antimicrobial activities". Journal of Saudi chemical Society, 15, 113-120, 2011.
11. Bashir Ahmed, Javid Ali, Shumalia Bashir. "Optimisation and effects of different reaction conditions for the bioinspired synthesis of silver nano particles using *Hippophae rhamnoides* Linn. Leaves aqueous extract". World Applied Sciences Journal, 22(6), 836–843, 2013.
12. Krishnaraj C., Ramachandran R, Mohan K and Kalaichelvan P.T. "Optimization for rapid and synthesis of silver nanoparticles and its effect on phytopathogenic fungi". Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 93, 2012, 95-99.
13. Preeti D. and Mukhopadhyay M. "*In-vitro* free. radical scavenging activity of biosynthesized gold and silver nanoparticles using *Prunus armeniaca* (apricot) fruit extract". Journal of Nanoparticles Research, 15, 2013, 1366-1376.
14. Vijay Kumara P.P.M, Pammib S.V.N., Pratap Kolluc, Satyanarayanad K.V.V, Shameema U. "Green synthesis and characterization of silver nanoparticles using *Boerhaavia diffusa* plant extract and their anti bacterial activity" Industrial Crops and Products, 52, 2014, 562–566.
15. Dipankar C, Murugan S. "The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts" Colloids and Surfaces B: Biointerfaces, 98, 2012, 112–119.

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