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RESEARCH ARTICLE

Green synthesis and Anti-bacterial activity of Silver Oxide nanoparticles prepared from Pinuslongifolia leaves extract

Zainub Khatun, Reena Shirley Lawrence, MOHAMMAD JALEES, Kapil Lawerence
Department of Chemistry, SHIATS, Allahabad (U.P.)-India

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Corresponding Author

Zainub Khatun

Abstract

There is an increasing commercial demand for nanoparticles due to their wide applicability in various areas such as electronics, catalysis, chemistry, energy, and medicine. Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. So the exploitation of various plant materials for the biosynthesis of nanoparticles is considered a green technology because it does not involve any harmful chemicals. The present study reported the green synthesis of silver nanoparticles using *Pinuslongifolia* leaves. Green synthesis of silver oxide nanoparticles (AgONps) was carried out using the aqueous extract of *Pinuslongifolia* leaves. The UV- Vis spectrum was recorded to monitor the formation of the nanoparticles, which exhibited a blue shifted absorption peak at 420 nm. The morphology structure and stability of the synthesized AgO nanoparticles were studied using scanning electron microscope (SEM). Further these biologically synthesized nanoparticles exhibited antibacterial activity against *E.coli* and *Staphylococcus aureus*.

INTRODUCTION

The term “nanoparticles” is used to describe a particle with size in the range of 1nm100nm,**(Simi *et al.*, 2007)** at least in one of the three possible dimensions. In this size range, the physical, chemical and biological properties of the nanoparticles changes in fundamental ways from the properties of both individual atoms/molecules and of the corresponding bulk materials. Nanotechnology is one of the modern techniques of the material science. The small sized nanoparticles mean they exhibit enhanced or different properties when compared with a bulk material. The extremely small size of nano particles having a large surface area relative to their volume, especially silver, have drawn the attention of scientist because of their extensive application in the development of new technologies in the area of electronics, material science and medicine at nanoscale level**.** New application of naoparticles and naomaterials are emerging rapidly **(Dhanalakshmi *et al*., 2012).** An array of physical, chemical and biological methods have been used for synthesis of noble metal naoparticles of particular shape and size for various application, but they remain expensive and involve the use of harzardous chemicals. An eco-friendly green mediated synthesis of inorganic naoparticle is fast growning research in the limb of nanotechnology **(Lalitha *et al*., 2013).**

The development of green methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology, because these methods are considered safe and ecologically sound the nanomaterial fabrication has been found to be an alternative to the conventional methods **(Yu, 2007)**. The green synthesis techniques are generally synthetic routes that utilize relatively nontoxic solvents such as water, biological extract, biological systems and microwave assisted synthesis. Silver nanoparticle have become the focus of intensive research owing to their wide range of application in the development of new techniques in the area of electronics, medicine, materials science due to good conductivity and chemical stability, selective coating of solar energy absorption, intercalation materials for electrical batteries, optical receptors, catalyst in chemical production.

The use of environmentally benign materials like plant leaf extract, bacteria, fungi and enzymes for the synthesis of silver nanoparticles offer numerous benefits of eco-friendliness and compatibility for pharmaceutical and other biomedical applications as they do not use toxic chemical absorbed on the surface that may have adverse effect in the medical application. Green synthesis provides advancement over chemical and physical method is no need to use high pressure, energy, temperature and toxic chemicals. Silver has long been recognized as having inhibitory effect on microbes present in medical and industrial process. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds **(Geethalakshmi and Sarada, 2010).**

A rapid and eco-friendly green method for the synthesis of silver nonoparticles from silver nitrate solution using Oleaeuropaea leaves extract. Effect of Oleaeuropaea leaves extract, silver nitrate concentration; reaction time and temperature on reaction rate were investigated **(Awwad *et al*., 2012).** The antibacterial activity of green synthesized AgNPs showed effective inhibitory activity against water borne pathogens *Listeria monocytogenes, Shigella and Staphylococcus aureus.* This reveals that silver nanoparticles could provide a safer alternative to conventional antimicrobial and antibacterial agents.

Materials & Methods

Extract preparation and nanoparticle generation

In the present study the leaves of *Pinuslongifolia leaves* were brought from the forest range at Lucknow and identified by NBRI, Lucknow.

Fig 1: *(a) Pinuslongifolia leaves (b) Pinuslongifolia leaves' powder*

For nanoparticle generation, we used plant leaves extract, prepared by mixing of 5g of plant powdered in 100ml of 95% ethanol, with 1-3 M aqueous solution of silver nitrate ($AgNO_3$) in 250 mL flask to give a pale yellow solution mixture at room temperature. The color of the solution mixture of silver nitrate and plant extract changes from pale yellow to deep color at 40℃ and 5 min of reaction time. Increasing the temperature of water bath to 60℃ and 10 min of reaction time, the color of mixture changed to deep brown color in 15 min of reaction time at 60℃ the deep brown color changed to grey-black due to excitation of surface Plasmon. This color indicates the reduction of Ag^+ ions to Ag nanoparticles. Unmodified Ag+ separated as supernatant by process of centrifugation.

Characterization of nanoparticles

Silver oxide nanoparticles were characterized by UV-Vis spectrophotometer spectrum. Spectrophotometric analysis of AgO nanoparticles was performed using SYSTRONICS, DOUBLE BEAM UV-VIS Spectrophotometer 2202 at Cytogene Research & Development, Lucknow.

Scanning Electron Microscope Analysis

The morphological features of synthesized silver nanoparticles from *Pinuslongifolia* plant extract were studied by Scanning Electron Microscope (Electron Probe micro Analyzer JEOL MODEL No JXA8100) at Allahabad University. The samples were characterized in the SEM at an accelerating voltage of 25.0KV.

Antibacterial activity and MIC determination: Antibacterial activity of the silver nanoparticles was evaluated against *E.coli, B.subtilis* and *S.aureus.*

Result & Discussion

Conformation of formation of nanoparticles

A 1mM aqueous solution of $AgNo_3$ when added to the extract of *Pinuslongifolia leaves* leads to color change of extract from colourless to brown. This color change is due to excitation of surface Plasmon vibration **(Irawwa *et al.*, 2012)**.

Fig 2: *Aqueous solution of 1mM AgNO3 with Pinuslongifolia leaves extract (A) before adding the extract and (B) after addition of extract*

The intensity of color increases with increase in time duration indicating the continuous reduction of silver ions. The formation of silver nanoparticle in the reaction mixture was confirmed by plasmon resonance of silver nanoparticles at 420nm by UV-Vis spectrophotometer. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The AgNPs were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of Ago nanoparticles. The UV-Vis spectrum of the synthesized Ag nanoparticles carried out using SYSTRONIC, Double Beam UV-VIS Spectrophotometer-2202. The absorption peak is shown in **Fig 3**. The formation of silver nanoparticle in the reaction mixture was confirmed by plasmon resonance of silver nanoparticles at 420nm by UV-Vis spectrophotometer. **Gavhane *et al.* (2012)** reported approximately similar result in their study absorption spectra (at 420nm) of silver nanoparticle formed by reaction media. **Sastry *et al.* (2003)** reported the synthesis of silver nanoparticles and characterization of UV-VIS spectrophotometer was given the absorbance peak at 430nm which was showing approximately similar result.

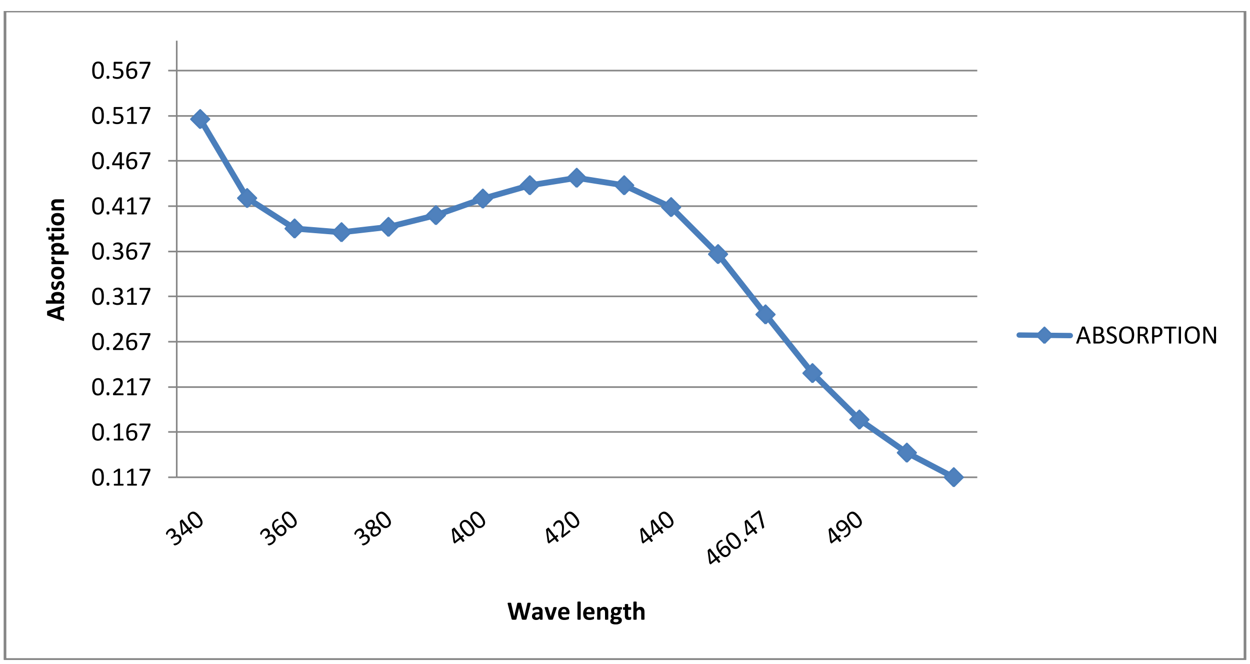


Fig 3: *UV-Vis spectrum of synthesized AgNp*

Scanning Electron microscopy (SEM) analysis of AgNPs

The SEM images of the AgNPs are shown in Figure 4. It is seen that AgNPs of different shapes were obtained in case of leaf extracts being used as reducing and capping agents. This reducing and capping agents of the extracts formed approximately spherical, triangular NPs, respectively which may be due to availability of different quantity and nature of capping agents present in the leaf extracts. An insight into the morphology and size details of the silver nanoparticles was done using Scanning Electron microscopy. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes of several µm in case of 10000rpm at 25mints and 30000rpm at 25 minutes the size is of several nm. The size of the prepared nanoparticles was more than the size of nanoparticle which should be; i.e.; between 1-100 nm. The bound proteins in the surface of the nanoparticles lead to increased size which was more than the desired size. The result showed that the particles were of spherical shape, the shape varies due to the concentration increased.

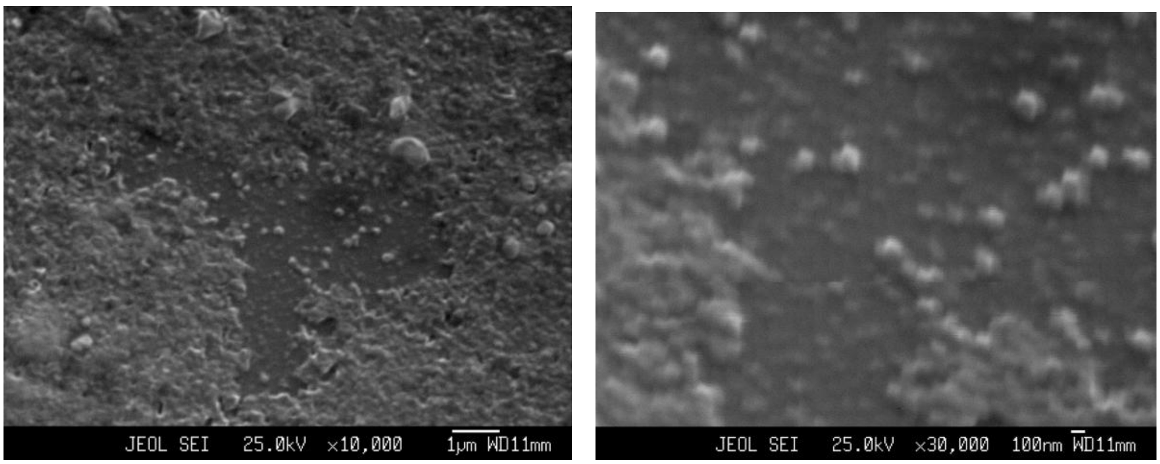
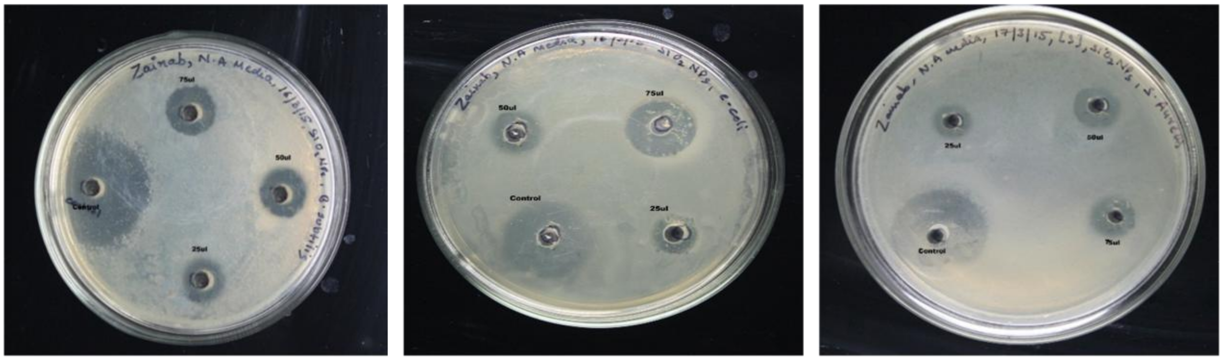


Fig 4: *SEM analysis shows that size and shape of the silver nanoparticles synthesized by Pinuslongifolia at 1µm and 100nm.*

Antibacterial assay

Biosynthesized silver nanoparticles were analyzed for their antimicrobial activity against *E. coli, B.subtilis and S.aureus* by agar well diffusion method. It was observed that microbial growth of 75µg/ml concentration of AgNPs showed maximum radius of zone of inhibition of 14mm against *B. subtilis* followed by 13mm against *S. aureus* and 16mm against *E.coli.* Thus showing that the increased concentration lead to higher Zone of inhibition. The antibacterial activity of silver nanoparticles was tested by the agar well diffusion methods. The presence of an inhibition zone clearly indicated the antibacterial effect of silver nanoparticles. As it was shown in the study of **Sharma *et al.* (2004)*;* Malabadi *et al.* (2012),** and **Lakshmi *et al.* (2014)** that by increasing the concentration of silver nanoparticles in wells, the growth inhibition has also been increased, similar case was observed in this study. The size of inhibition zone varied against different bacteria depending upon the size and the concentrations of silver nanoparticles as shown in the **Fig 6.**



E.Coli S.aureus B.subtilis

Fig 5: *Antibacterial activity of AgNPs by agar well diffusion method*

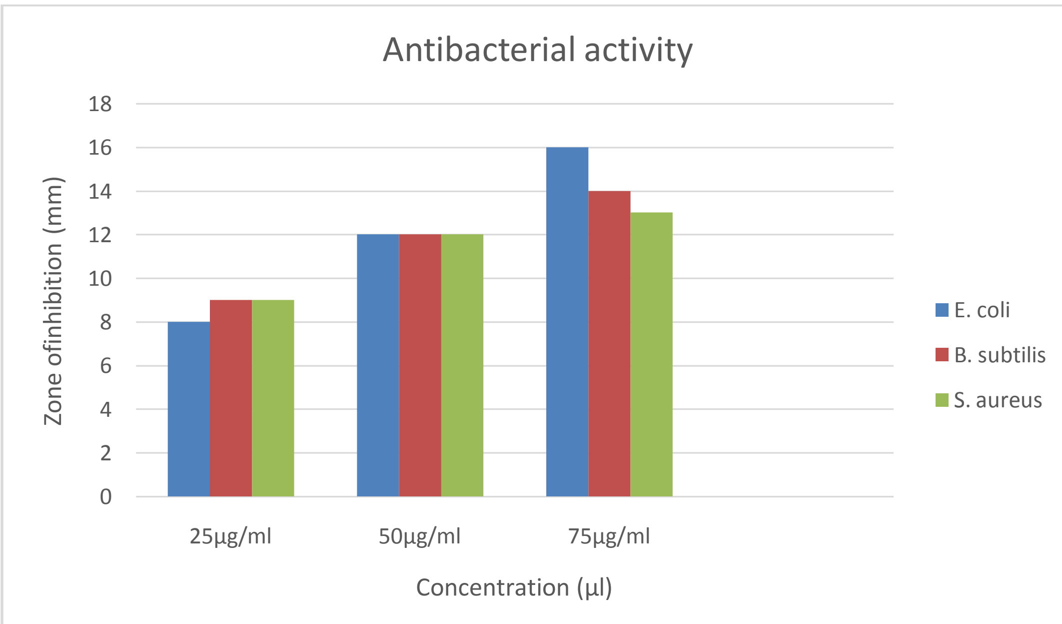


Fig 6: *Comparative graph of E.coli, B.subtilis, S.aureus.*

Minimum inhibitory concentration

The lowest concentration of plant extract that retained its inhibitory effect resulting in no growth (absence of turbidity) of a microorganism was recorded as the MIC values of the extract. Green synthesized silver nanoparticles were analyzed for their antibacterial activity against ***Escherichia coli, Bacillus subtilis, Staphylococcus aureus at*** **different concentration by agar well diffusion method.** It was observed that microbial growth of different microbes was independent on AgNP concentration. It was found that the MIC for silver nanoparticles that inhibited the visible growth of *Bacillus subtilis* was 25µg/ml.

Table 1: *Initial MIC of AgNPs against bacteria*

Conc. (µl/ml)	**AgNPs (µg/ ml) against bacteria**		
	S a	**B s**	**E c**
100	-	-	-
50	-	*	-
25	*	+	*
12.5	+	+	+

6.25	+	+	+
MIC	50	25	50

(+) Growth (*) MIC and (-) growth

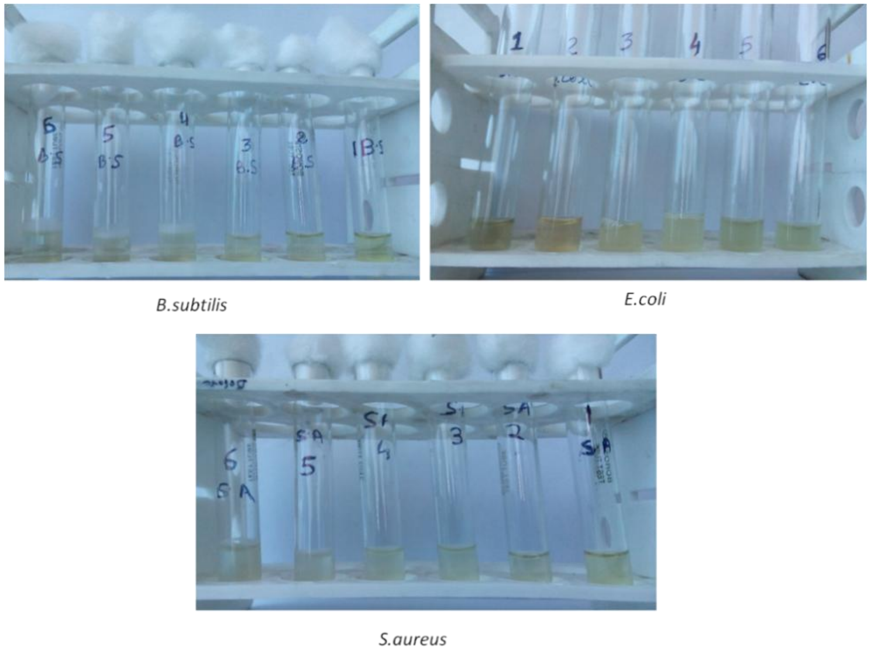


Fig 7: *MIC of AgNPs against bacteria*

Conclusion

The exploitation of various plant materials for the biosynthesis of nanoparticles is considered a green technology because it does not involve any harmful chemicals. The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Eco friendly methods of green mediated synthesis of nanoparticles are the present research in the limb of nanotechnology. In recent science Nanotechnology is a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics, and all other known fields. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But the biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, ecofriendly and less time consuming.

The present study reported the green synthesis of silver nanoparticles using *Pinuslongifolia* leaves. Green synthesis of silver oxide nanoparticles (AgNps) was carried out using the aqueous extract of *Pinuslongifolia* leaves. The UV-Vis spectrum was recorded to monitor the formation of the nanoparticles, which exhibited a blue shifted absorption peak at 420 nm. The morphology structure and stability of the synthesized Ag nanoparticles were studied using scanning electron microscope (SEM). Further these biologically synthesized nanoparticles exhibited antibacterial activity against *E.coli* and *Staphylococcus aureus*.

The rapid biological synthesis of silver oxide nanoparticles using *Pinuslongifolia* leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 1-100 nm. The size were as the nanoparticles were surrounded by a thin layer of proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., which were found from the characterization using UV-VIS spectrophotometer, SEM, techniques. All these techniques it was proved that the concentration of plant extract to metal ion ratio plays an important role in the shape determination of the nanoparticles. The higher concentrated nanoparticles had sheet shaped appearance whereas the lower concentrations showed spherical shaped. The sizes of the nanoparticles in different concentration were also different which depend on the reduction of metal ions. From the technological point of view these obtained by silver oxide nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production These nanoparticles showed a broad spectrum antimicrobial activity against both Gram positive and Gram negative bacteria. Investigation on the antibacterial activity of synthesized silver nanoparticles using *Pinus* extract against *S.aureus*, *B.subtilis and E. coli*. has high potential as antimicrobial agent in pharmaceutical, food, and cosmetic industries.

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