ORIGINAL ARTICLE

# Biosynthesis of silver nanoparticles using *Ailanthus excelsa* leaf extract and their antibacterial activity

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## Abstract

**Objective:** The objective of the study was to evaluate the antibacterial activity of silver nanoparticles (AgNP) synthesized from *Ailanthus excelsa* against human pathogens. **Materials and Methods:** Ultraviolet-visible (UV-Vis) spectrophotometry and transmission electron microscopy (TEM) were performed to confirm the formation and stability of AgNPs. Antibacterial activities of the synthesized AgNPs were determined using the agar well diffusion assay method. **Results:** UV-Vis spectrum of the aqueous medium containing AgNPs showed an absorption peak at around 425 nm for the yellow to brown colored AgNPs synthesized from 10 to 3 (M) silver nitrate and the fixed volume fraction ($f = 0.2$) aqueous leaf extract. TEM showed the formation of AgNPs with a size ranging from 15 to 25 nm. The X-ray diffraction patterns of AgNPs synthesized from leaf extract of *A. excelsa* clearly illustrates that the AgNPs were synthesized. The formed AgNPs showed good antibacterial activity against human pathogens. **Conclusions:** *A. excelsa* plant extract solution is potent for the green and eco-friendly synthesis of silver (Ag) nanoparticles, which provides efficient research applications.

**Key words:** Antibacterial, ecofriendly, green synthesis, silver nanoparticle

## INTRODUCTION

Nanotechnology is one of the most advanced technologies in the 21st century. Nanotechnology is widely used in various branches such as molecular nanotechnology, environmental nanotechnology, and bioscience nanotechnology.[1] In the field of nanotechnology, many tools and machines are used. Nanotechnology is a field that is developing day by day, making an impact in all spheres of human life and creating a growing sense of excitement in life sciences.[2] This current emerging field of nanobiotechnology is at the primary stage of development due to the lack of implementation of innovative techniques in a large industrial scale and yet has to be improved with modern technologies. Hence, there is a need to design an economic, commercially feasible as well environmentally sustainable route of synthesis of silver nanoparticles (AgNP) to meet its growing demand in diverse sectors. Various approaches available for the synthesis of AgNP include chemical and biological sciences. Consequently, with a wide range of application available, these AgNPs have the potential to make a significant impact to the society. The physicochemical properties of nanoparticles can be attributed to their high surface-to-volume-ratio and make an excellent candidate for biomedical application as types of the biological process occur at nanometer scales.[3] Biosynthesis of AgNPs using biological agents such as plant extracts has gained much attention in the area of nanotechnology in the last few decades.[4] In general, there are three main steps involved in the green synthesis method, that is, reaction medium selection, biological reducing agent selection, and selection of non-carcinogenic substances for the stability of nanoparticles.[5] Therefore, there has been a search for an inexpensive, reliable, safe, and "green" approach to the synthesis of stable metal nanoparticles with controlled size and

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shape. Yet, plant-mediated preparation of nanoparticles can be advantageous over another bio-based synthesis because the procedure of maintaining cell cultures can be omitted and it is also suitable for large scale production under non-aseptic environments.[6] Medicinal plants play an important role in human health care. About 90% of the world population, mainly in developed and under developing countries, rely on the use of traditional medicine which is predominantly based on medicinal plant material. Traditional medicine was lesser side effects. It is obvious that the traditional medicine medicinal plants play a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans as well as have given valuable components of medicines.[7] A biological approach using medicinal plant extracts for AgNPs synthesis has been suggested as a valuable alternative tool for biological synthesis. Biosynthesis of AgNPs has already been reported as clean, cost-effective, and non-toxic to environmental routes. Synthesis and characterization of nanoparticles are an important area of research as the selection of size and shape of nanoparticles provide efficient control over many of the biological properties.[8] Materials like plant leaf extracts were used for the green synthesis of AgNPs.[9] Green synthesis of nanoparticles has attracted considerable attention in recent years. In this regard, plant extracts and natural resources such as microorganisms and enzymes have been found to be good alternative reagents in nanoparticle synthesis. Parts such as leaf, bark, root, and stem have been used for the AgNP synthesis. Plant crude extract contains novel secondary metabolites such as phenolic acid, flavonoids, alkaloids, and terpenoids. These compounds are mainly responsible for the reduction of ionic into bulk metallic nanoparticle formation.[10] These primary and secondary metabolites are constantly involved in the redox reaction to synthesize eco-friendly nanosized particles.[11] Biosynthesis reactions can be modulated to transform the shape and size of nanoparticles using different metal concentrations and amounts of plant extract in the reaction medium.[12] AgNPs are having a good history in the field of antimicrobial properties. The AgNPs are vigorously involved in the antimicrobial activity against a lot of disease-causing foodborne and waterborne pathogenic bacteria and fungus.[13] In the present study, medicinal plants have been used for the synthesis of nanoparticles. The leaf of *Ailanthus excelsa* was collected and their aqueous extract was used to reduce the silver ions (Ag+) in aqueous solution. In this research, the synthesis of AgNPs from *A. excelsa* leaf extract and its antibacterial activity of these synthesized AgNPs against six bacterial pathogens are evaluated.

## MATERIALS AND METHODS

### Chemical and Reagents

The chemicals used in all experiments were obtained from Sigma (Bengaluru, India) and Merck (Mumbai, India). Minimum Essential Medium (HiMedia), fetal bovine serum (Cistron), trypsin, methylthiazol diphenyl-tetrazolium bromide, and dimethyl sulfoxide (DMSO) were obtained from sigma chemical company, St Louis, MO, USA. All of the other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

### Plant Materials

A total of six species of Indian medicinal plants *Coccinia grandis, Mimusops elengi, Euphorbia hirta, Momordica charantia, A. excelsa,* and *Boerhavia diffusa* were collected during the month of July 2013 from in and around Vellore District, Tamil Nadu, India. The taxonomic identification was made by Dr. Sutha, Department of Botany, Voorhees College, Vellore and confirmed by Dr. C. Hema, Department of Botany, Arignar Anna Government Arts College for Women, Walajapet, Vellore, India. The voucher specimen numbers were kept in our research laboratory for further reference.

### Selection of Medicinal Plant

*A. excelsa* plants were obtained from a natural source. Five gram of plant leaf powder was milled using an ordinary grained plant; it was mixed with 50 ml of deionized distilled water in a 250 ml beaker and was kept overnight. The extract was filtered using Whatman No.1 filter paper three times and the extract was stored in 4°C. After filtration, clear leaf extract was obtained for further use. Silver nitrate ($AgNO_3$) Merck analytical grade was purchased from Sigma-Aldrich. All the aqueous solutions were prepared using deionized distilled water.

### Synthesis of AgNPs

In a typical reaction procedure, 5 ml of leaf extract was added to 20 ml of $10^{-3}$ (M) aqueous $AgNO_3$ solution which was heated up to 80°C; the resulting solution became brown in color after 15 min of heating. 5 ml, 10 ml, and 15 ml of the leaf extract was added to 25 ml of the aqueous solution of $AgNO_3$ ($10^{-3}$ M) and stirred vigorously for 5 min.[14] Reduction takes place slowly at 300 K and gets completed in 30 min by stable light brown color formation, depending on the intensity of color formation, respectively, to the volume of the extract added. Besides, at 373 K, AgNP was obtained by adding 25 mL of the extract to 100 mL $AgNO_3$ ($10^{-3}$ M). Furthermore, by adding 5 mL of the extract to 25 mL of the $AgNO_3$ solution, the AgNPs were synthesized by rapid reduction at 300 K at a pH of 8, which was found to be intense brown in color.[15]

### Characterization Studies

#### *Ultraviolet-visible (UV-VIS) spectra analysis*

The bioreduction of AgNPs was monitored by sampling the reaction mixture at regular intervals and the absorption maxima were scanned by UV-Vis spectra at the wavelength

of 200–700 nm in UV-2450 (Shimadzu) spectrophotometer operated at a resolution of 1 nm. The AgNPs exhibited unique and tunable optical properties on account of their surface plasmon resonance (SPR), dependent on the shape and size distribution of the nanoparticles.[16] The reduction of Ag+ ions was monitored by measuring the UV-visible spectra of the solutions after diluting 20 times of aliquot (0.2 mL) with Milli Q water. The solution mixture was subjected to centrifugation at 10,000 rpm for 45 min and the resulting pellet was dissolved in deionized water which was filtered through 0.22 µm Millipore filter. Following which pellet was dispersed in sterile distilled water to get rid of any uncoordinated biological molecules. The process of centrifugation and redispersion in sterile distilled water was repeated three times to ensure better separation of free entities from the metal nanoparticle.

### *X-ray diffraction (XRD) analysis*

The AgNP solution thus obtained was purified by repeated centrifugation at 5000 rpm for 20 min followed by redispersion of the pellet of AgNPs into 10 ml of deionized water. After the freeze-drying of the purified silver particles, the structure and composition were analyzed by XRD and scanning electron microscopic (SEM). The dried mixture of AgNPs was collected for the determination of the formation of AgNPs by an X'Pert Pro X-ray diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation in θ- 2 θ configurations. The crystallite domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer formula.

$$D= 0.94 \lambda / \beta \cos \theta \quad (1)$$

where D is the average crystallite domain size perpendicular to the reflecting planes, λ is the X-ray wavelength, β is the full width at half maximum (FWHM), and θ is the diffraction angle. To eliminate additional instrumental broadening, the FWHM was corrected using the FWHM from a large-grained Si sample.

$$\beta \text{ corrected} = (\text{FWHM2 sample- FWHM2si})1/2 \quad (2)$$

This modified formula is valid only when the crystallite size is smaller than 100 nm.[17]

### *Fourier-transform infrared (FTIR) spectroscopy analysis*

The synthesized AgNPs solution was centrifuged at 8000 rpm for 15 min and the obtained pellet was dried, lyophilized, and mixed with KBr pellets, and then it was subjected to a wide range of FTIR spectral analysis (Shimadzu, Japan).

### *TEM analysis*

In TEM analysis, the sample is first sonicated for 10 min and a drop of this AgNPs solution is loaded on a carbon-coated copper grid and the solution is allowed to evaporate for 10 min in room temperature and it is analyzed using HITACHI-H-7650 at an operating voltage of 80 kV. XRD is used to determine the crystalline structure. This study was made on the powder samples at room temperature 27°C on a Rigaku X-ray diffractometer (MiniFlex, UK).

### *SEM analysis of AgNPs*

SEM analysis was done using the Hitachi S-4500 SEM technique. Thin-film samples were prepared on a carbon-coated copper grid by just dropping a very small amount of the sample on the grid; extra solution was removed using a blotting paper; and then the film on the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min.

### *Energy dispersive X-ray (EDX) analysis*

EDX spectroscopy (EDS) analysis for the confirmation of elemental silver was carried out for the detection of elemental silver. The samples were dried at room temperature and then analyzed for the sample composition of the synthesized nanoparticles. The EDX analysis of the synthesized AgNPs using *A. excelsa* revealed the presence of a strong Slur signal which the presence of AgNPs. EDS takes advantage of the photon nature of light. In the X-ray range, the energy of a single photon is just sufficient to produce a measurable voltage pulse X-ray, the output of an ultra-low noise preamplifier connected to the low noise is a statistical measure of the corresponding quantum energy. By digitally recording and counting a great number of such pulses with in a so-called multichannel analyzer, a complete image of the X-ray spectrum is building up almost simultaneously. This digital quantum counting technique makes the energy dispersive spectrometry exceedingly reliable. A semiconductor material is used to detect the X-rays together with processing electronics to analyses the spectrum.

### Antibacterial Activity (Agar Well Diffusion Method)

The extracts obtained from plant material were studied for antibacterial activity. A loopful of standard strains such as *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3940), *Micrococcus luteus* (MTCC 106), *Enterobacter aerogenes* (MTCC 111), *Salmonella typhi* (MTCC-734), and *Pseudomonas aeruginosa* (MTCC 841) were inoculated in 30 ml of nutrient broth in a conical flask and incubated for 24 h to activate the strain. In agar well diffusion methods,[18] the media and the test bacteria cultures were poured into Petri dishes. The test strain 0.25 ml was inoculated into the media. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5 mm). The extract compound (50 µl) was introduced into the well and the plates were incubated at 37°C for 24 to 48 h. All samples were tested in triplicates. Microbial growth was determined by measuring the diameter of the zone of inhibition (ZOI). Ciprofloxacin (HiMedia, Mumbai, India) is a reference drug used as a control for test organisms.

### Determination of Minimum Inhibitory Concentration (MIC)

Antibacterial activities were measured using a dilution technique. The plant extract (100 mg) was solubilized in 1 ml of DMSO and serially two-fold diluted in Mueller–Hinton broth (HiMedia, India) to obtain a concentration range of 15.6–1000 mg/ml. The broth containing only DMSO diluted in the same way, which did not influence bacterial growth, was included as controls (ciprofloxacin). The bacterial strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an optical density of 0.05 at 530 nm (equivalent to 1 × 106 CFU/ml). This suspension was used as the inoculums for the test in the agar plates. Bacterial suspensions (100 µl) were inoculated using a micropipette. The MIC was defined as the minimal concentration of the plant extract which completely inhibited the visible growth (turbidity) of the bacteria in tubes.

## RESULTS

The screening on the leaf extract of medicinal plants to evaluate the optimal synthesis of AgNPs revealed that leaves extract of plants *A. excelsa* was found to be effective, reducing agents of Ag ions produce AgNPs of nanosized.

The formation and stability of AgNPs in aqueous leaf extract solution are conformed using UV/Vis spectral analysis. Extinction spectra of silver synthesized from different concentrations of $AgNO_3$ [Figure 1] and characteristic surface plasmon absorption bands are observed 425 nm for the yellow to brown colored AgNPs synthesized from $10^{-3}$ (M) $AgNO_3$ and the fixed volume fraction ($f$ = 0.2) aqueous leaf extract. The red with increasing concentration of $AgNO_3$ from $10^{-3}$ to $10^{-3}$ (M) and the corresponding color changes are observed from yellow to deep brown [Figure 2]. The SEM images of the samples obtained from the $AgNO_3$ solutions prepared at 800°C, confirm the existence of very small and similarly spherical silver nanoparticles [Figure 3a and b]. From the

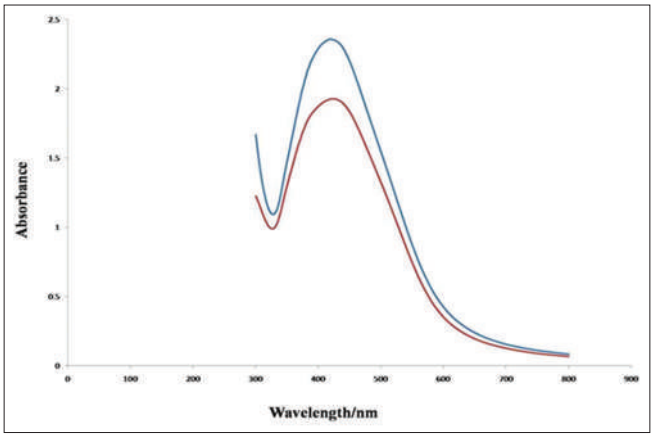


**Figure 1:** Ultraviolet-visible spectroscopy of synthesized silver nanoparticles using aqueous leaves extract of *Ailanthus excelsa*

SEM images, it can be observed that larger particles of $AgNO_3$ are formed due to the aggregation of silver nanoparticles which might be induced by the evaporation of solvent during sample preparations. This could have contributed to the variation in particle size. The elemental composition of powdered samples was determined. Transmission electron microscope (TEM) images of silver solution synthesized dried sample by treating $10^{-3}$ (M) $AgNO_3$ solution with 0.2 volume fraction of aqueous leaf extract showed that the particles were predominantly spherical in shape with a diameter ranging from 15 to 25 nm [Figure 4a and b]. The TEM images also show the selected area electron diffraction pattern and it suggests the presence of the synthesized AgNP. The XRD patterns of AgNP synthesized from the leaf extract of *A. excelsa* clearly illustrates that the AgNP were synthesized [Figure 5].

Fluorescence transmission infrared (FTIR)-spectroscopy measurements were carried out to identify the silver surface. The presence of the bonds 3329 present at O-H stretching frequency, 3455 present at N-H bond (Hydrogen bonding), 2762 present at aldehyde C-H bond, and the absorption peak at 2923 $cm^{-1}$ alkenes C-H bond were obtained. The strong absorption peak at 1674 $cm^{-1}$ was Alkene C = C group and therefore the stabilization of AgNP by the surface bound was possible in the green synthesis [Figure 6].

The fluorescent spectra of the colloidal nanoparticles synthesized from different $AgNO_3$ concentration with fixed volume fraction ($f$ = 0.2) of plant leaf extracts. A broad emission band having a prominent peak centered at 500 nm was observed for the plant leaf extracts as it excited at 420 nm [Figure 7]. It was anticipated that the emission was due to the presence of *A. excelsa* leaf extracts. Similar broad and shifted emission peak types were observed. However, the emission intensity gradually decreased with the increasing concentration of $AgNO_3$ and the increasing size of AgNP. This decreasing intensity suggests that due to the close proximity of emissive species with the emission of nanoparticles takes place through the transfer process. In other words, this emission behavior supports the involvement of *A. excelsa* leaf extracts in stabilizing the AgNP. In the present study is to evaluate the antibacterial activity of synthesized AgNP from *A. excelsa* leaf extract. Six bacterial pathogens *B. subtilis, S. aureus, M. luteus, E. aerogenes, S. typhi,* and *Pseudomonas aeruginosa* are used for experimental study [Figure 8; Plates 1-6]. The study concludes that the minimum inhibitory concentration


**Figure 2:** (a-c) Color change during phytoreduction from silver nitrate to silver nanoparticles over for *Ailanthus excelsa*

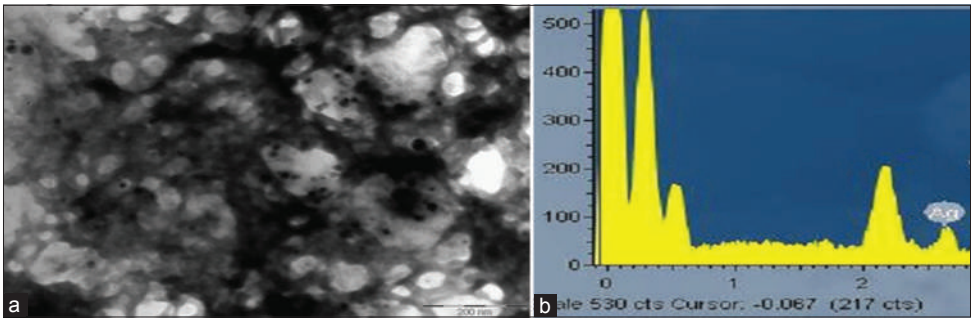


**Figure 3:** (a): Scanning electron microscopic micrographic of synthesized silver nanoparticles using aqueous leaf extract of *Ailanthus excels*. (b): Energy dispersive X-ray showing the chemical composition of synthesized silver nanoparticles the aqueous leaf extract of *Ailanthus excelsa*

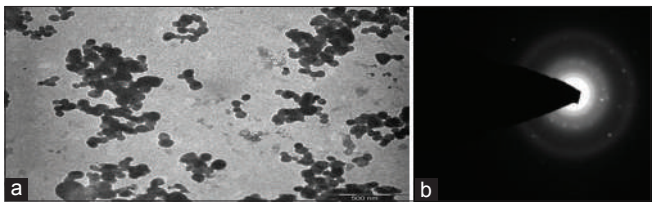


**Figure 4:** (a): Transmission electron microscopy (TEM) micrograph of the synthesized silver nanoparticles aqueous leaf extract of *Ailanthus excelsa* (b): TEM micrographic of selective area electron diffraction synthesized silver nanoparticles using aqueous leaf extract of *A. excelsa*

(MIC) results which revealed the details of mean MICs of AgNP from *A. excelsa* aqueous extract are 25 µg in six bacterial strains. Syntheses of AgNPs were considered for antibacterial activity against pathogenic microorganisms using standard ZOI. *Streptomycin, Gentamycin, Ampicillin,* and *Erythromycin* of 10 mg/mL concentration were used as an antibacterial agent. The synthesized AgNPs showed an inhibition zone against all the test organisms. Maximum ZOI was found due to the presence of *P. aeruginosa*, *Micrococcus luteus*, and *E. aerogenes*. A minimum of ZOI was found due to the presence of *B. subtilis, S. typhi,* and *S. aureus* in all the tested bacterial organisms [Figures 9; Plates 7-12].

## DISCUSSION

The aqueous leaf extracts were employed as reducing agents for the development of AgNP from $AgNO_3$ solution. The appearance of the color was due to the excitation of the surface plasmon vibrations, typical of AgNPs having λmax values which are reported in the visible range of 400–450 nm.[19] As the *A. excelsa* extract was mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to yellowish-brown due to the reduction of silver ion which indicated the formation of AgNP. The reduction of Ag ions to AgNPs could be followed by a color change and UV-Vis spectroscopy. The technique has proven to be very useful for the analysis of nanoparticles. Therefore, the progress in the conversion reaction of silver ions to

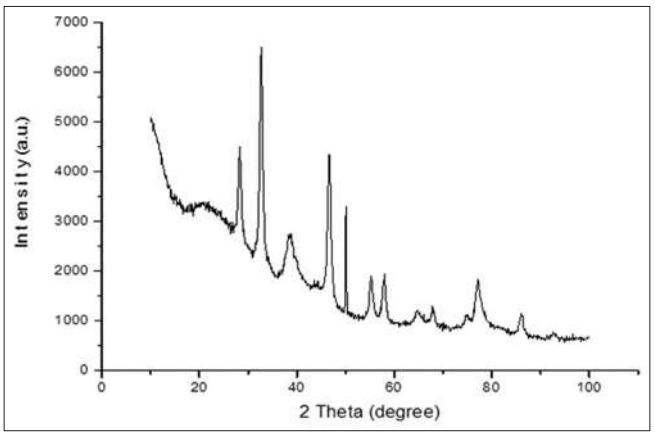


**Figure 5:** X-ray diffraction pattern of silver nanoparticle synthesized from aqueous leaf extract of *Ailanthus excelsa*

AgNP was followed by a color change and spectroscopic techniques. The photograph of sample solutions containing $AgNO_3$ and $AgNO_3$ in the presence of optimal amounts of aqueous leaf extract after completion of the reaction shows the appearance of a yellowish-brown color which confirms the existence of AgNP. Several approaches have been employed to obtain the better synthesis of AgNP plant crude extracts contain novel secondary metabolites such as phenolic acid, flavonoids, alkaloids, and terpenoids, which are mainly responsible for the reduction of ionic metal into bulk metallic nanoparticles.[10] Primary and secondary metabolites are constantly involved in redox reactions required to synthesize eco-friendly nanoparticles.[11] Biosynthesis reactions can be modulated to transform the shape and size of nanoparticles using AgNP and amounts of plant extract in the reaction medium.[20] The results revealed that aqueous leaf extract of $AgNO_3$ were recorded has 45°C and 1 mM $AgNO_3$, respectively. Pai *et al*.[21] reported an approximately similar result in their study absorption spectra (at 425 nm) of AgNP formed by reaction media. Kaushik and Joshi[22] reported that the synthesis of AgNP and characterization of UV-VIS spectrophotometer was given the absorbance peak at 425 nm which was showing the approximately similar result.

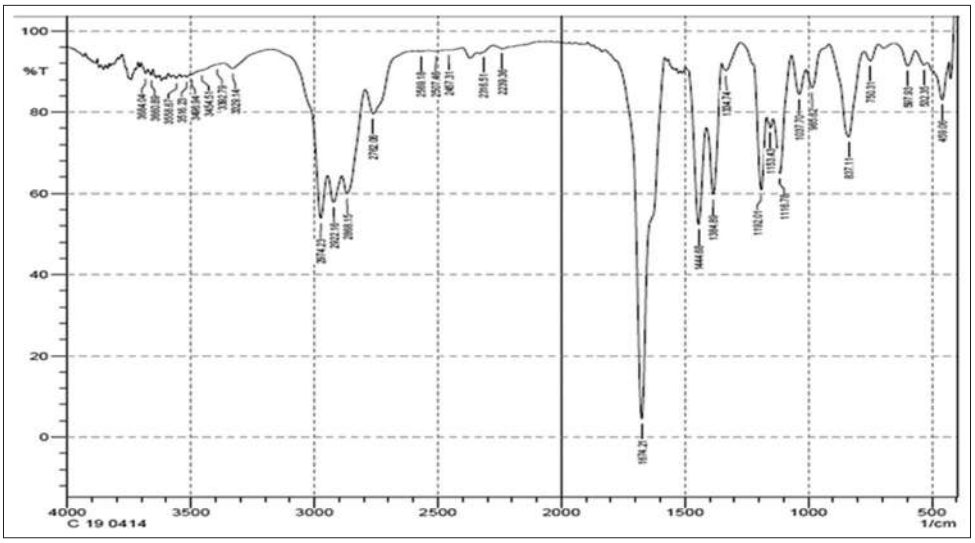


**Figure 6:** Fourier transforms infrared spectroscopy for leaf extract of *Ailanthus excelsa*

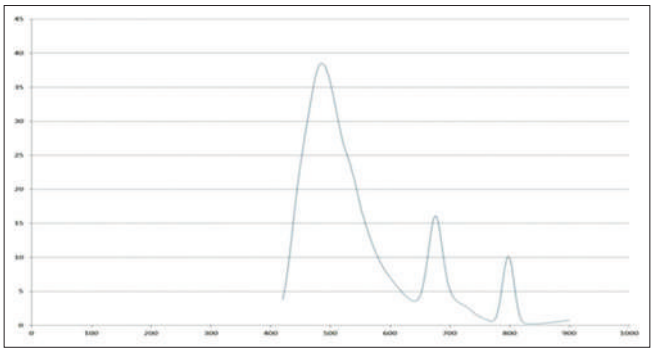

**Figure 7:** Fluorescence spectroscopy of synthesized silver nanoparticles the aqueous leaf extract of *Ailanthus excelsa*

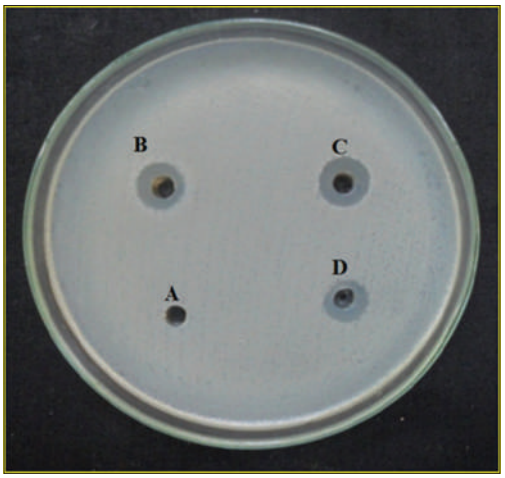


**Plate 1:** Antibacterial activity of 1 mM silver nanoparticles synthesized from *Ailanthus excelsa* against *Bacillus subtilis* by agar well diffusion method. A-Crude silver nanoparticles, B-Silver nitrate, C-Reference drug, D-Optimized silver nanoparticles

XRD is mostly used for determining the chemical composition of the crystalline nature of materials. The frequency and width of the surface plasmon absorption are depends on the metal nanoparticles detecting the presence of AgNP in plant extracts. This can be achieved using XRD to examine the diffraction peaks of the plant.[23] In the present study, the X-ray pattern of synthesized AgNP matches the FCC structure of the bulk silver and there were no obvious other phases found in the XRD patterns. The XRD results clearly show that the AgNP formed by the reduction of Ag+ ions by the *A. excelsa* is spherical in nature. In the present study, the TEM was noticeable that the edges of the nanoparticles were lighter than the centers, telling that molecules such as proteins in *A. excelsa* capped the AgNP most of the AgNP in TEM images were not in physical contact, but they were separated by a fairly uniform interparticle distance.[24] In the present study, SEM micrographs of nanoparticles showed that the nanoparticles synthesized were spherical shaped, highly distributed with aggregation. The aliquot of AgNP solution was placed into a drop coated copper grid and the sample was allowed to dry. The SEM images were recorded at different magnification to find the individual particles. The synthesized AgNP were observed in a spherical shape. The spherical shaped of nanoparticles was obtained with approximate size ranging 41–72 nm. Similar studies were carried out elsewhere wherein increased the plant extracts concentration modulated the size of the AgNP synthesized.[25] The EDX profile revealed a strong silver signal along with weak oxygen and carbon peak, which may have originated from the biomolecules that are bound to the surface of AgNP.[26] This analysis revealed that the nanostructures were formed solely of silver. The FTIR spectrum of the band intensities in different regions of the spectrum of the synthesized AgNP using the aqueous leaf extract of *A. excelsa* revealed that the bands due to 3329 present at O-H stretching frequency, 3455 present at N-H bond (hydrogen bonding), 2569 present at S-H group stretch, 2762 present at aldehyde C-H bond, and the absorption peak at 2923 $cm^{-1}$ alkenes C-H bond were obtained. The strong absorption

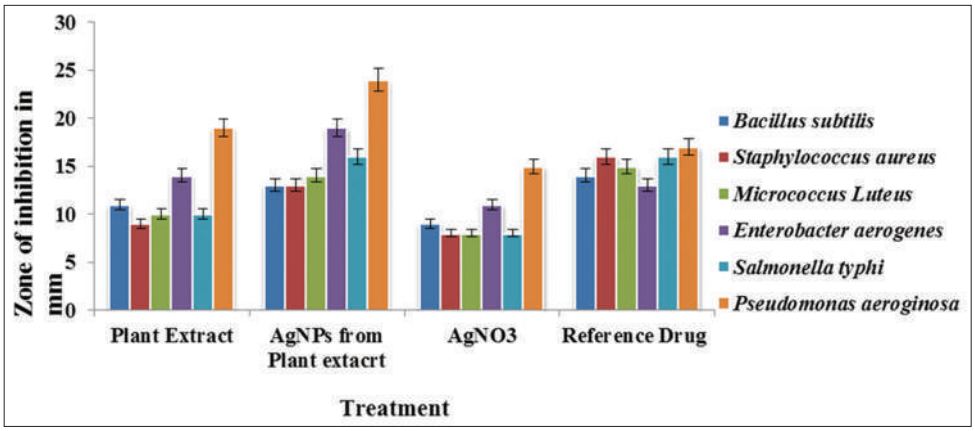


**Figure 8:** Antibacterial activity of silver nanoparticles by reacting 1 mM silver nitrate aqueous solution of *Ailanthus excelsa*

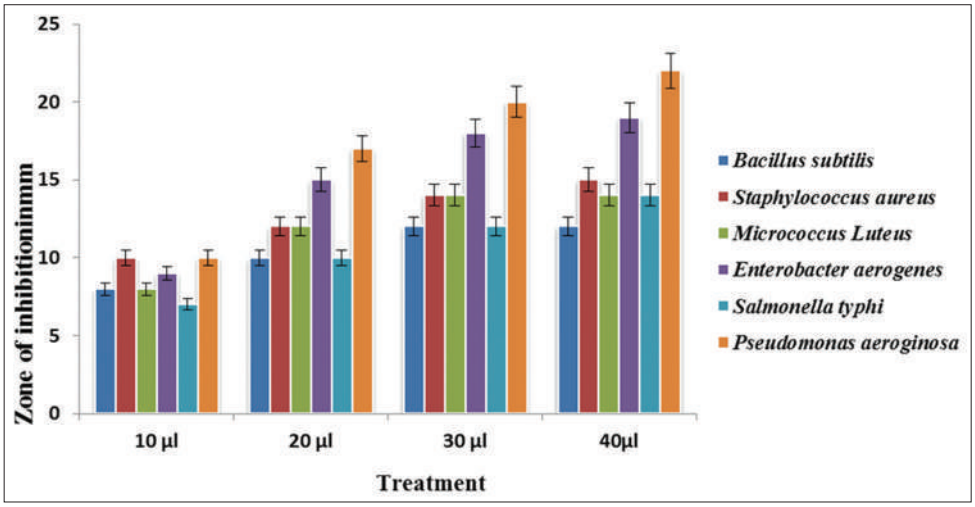


**Figure 9:** Determination of minimum inhibitory concentration (MIC) for 1 mM silver nanoparticles synthesized from *Ailanthus excelsa*

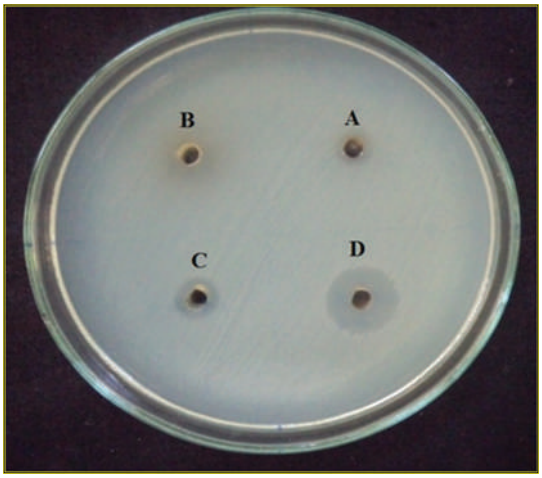


**Plate 2:** Antibacterial activity of 1 mM silver nanoparticles synthesized from *Ailanthus excelsa* against *Staphylococcus aureus* by agar well diffusion method. A-Crude silver nanoparticles, B-Silver nitrate, C-Reference drug, D-Optimized silver nanoparticles

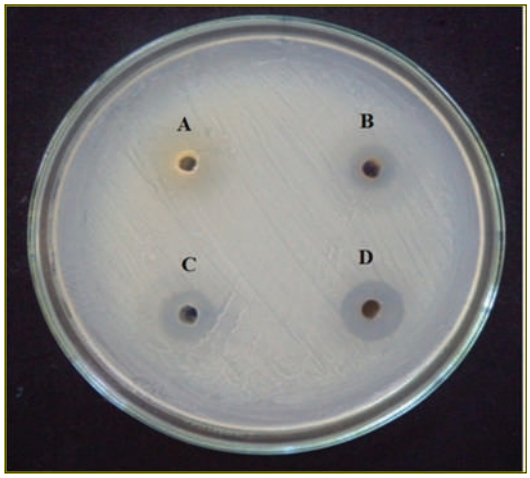


**Plate 3:** Antibacterial activity of 1 mM silver nanoparticles synthesized from *Ailanthus excelsa* against *Micrococcus luteus* by agar well diffusion method. A-Crude silver nanoparticles, B-Silver nitrate, C-Reference drug, D-Optimized silver nanoparticles

peak at 1674 cm-1 was alkene C = C group and therefore the stabilization of AgNP by the surface bound was possible in the green synthesis. An FTIR spectrum technique was used for detecting the functional group in medicinal plants.

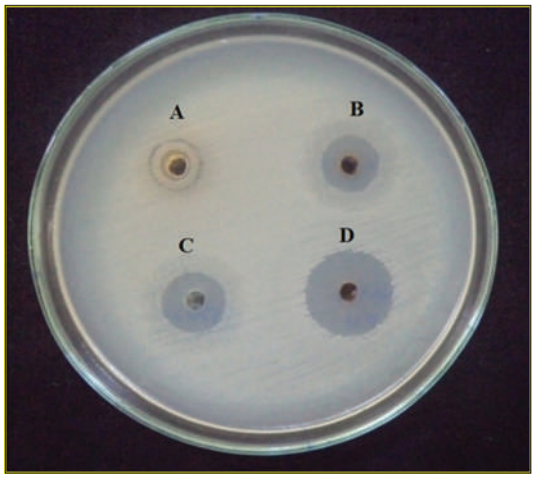


**Plate 4:** Antibacterial activity of 1 mM silver nanoparticles synthesized from *Ailanthus excelsa* against *Enterobacter aerogenes* by agar well diffusion method. A-Crude silver nanoparticles, B-Silver nitrate, C-Reference drug, D-Optimized silver nanoparticles

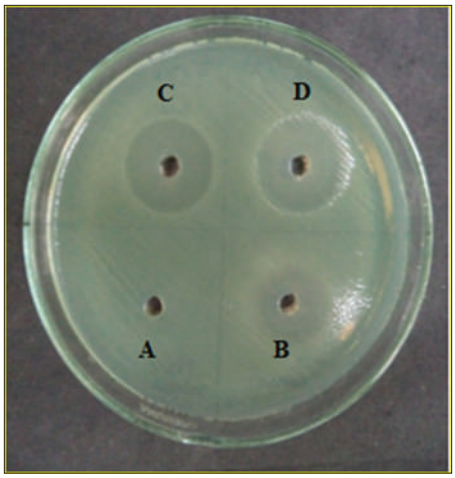


**Plate 6:** *Antibacterial* activity of 1 mM silver nanoparticles synthesized from Ailanthus excelsa against *Pseudomonas aeruginosa* by agar well diffusion method. A-Crude silver nanoparticles, B-Silver nitrate, C-Reference drug, D-Optimized silver nanoparticles

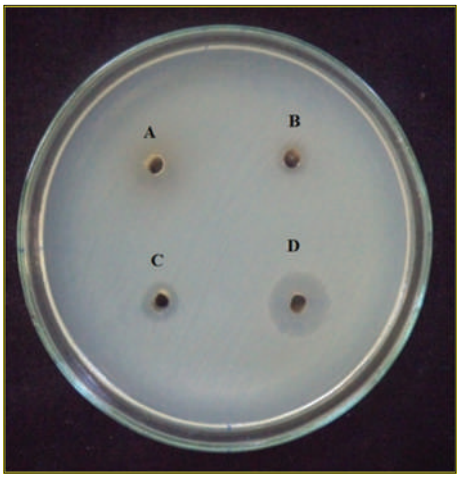


**Plate 5:** Antibacterial activity of 1 mM silver nanoparticles synthesized from *Ailanthus excelsa* against *Salmonella typhi* by agar well diffusion method. A-Crude silver nanoparticles, B-Silver nitrate, C-Reference drug, D-Optimized silver nanoparticles

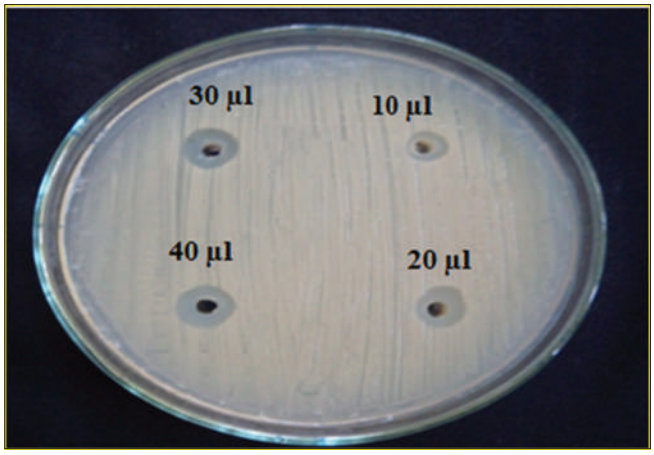


**Plate 7:** Minimum inhibitory concentration test for green synthesized 1 mM silver nanoparticles from *Ailanthus excelsa* against *Bacillus subtilis*

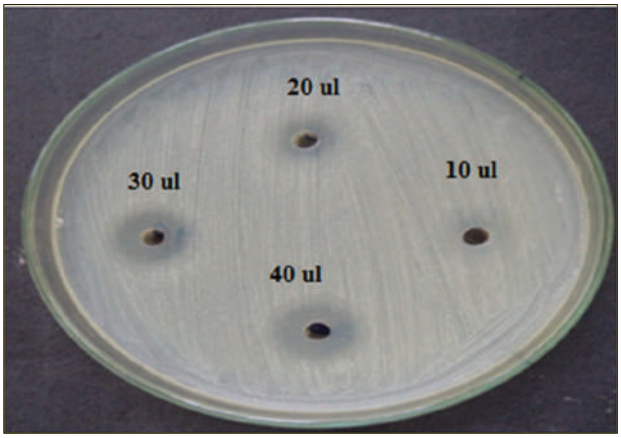


**Plate 8:** Minimum inhibitory concentration test for green synthesized 1 mM silver nanoparticles from *Ailanthus excelsa* against *Staphylococcus aureus*

AgNP which is readily soluble in water has been exploited as an antibiotic agent for many decades.[27] The exact mechanism of the antibacterial effect of silver ions is partially understood. Literature survey reveals that the positive charge on the Ag ion is crucial for its antibacterial activity. The antibacterial activity is probably derived through the electrostatic attraction between negative charged cell membrane of microorganisms and positive charged of Ag ions.[28] The antibacterial activity of green synthesized nanoparticle in the present study evaluated using standard ZOI microbiology assay. The nanoparticle showed an inhibition zone against all the six bacterial species. To understand the antibacterial mechanisms of the AgNP on the microbes organism studied by the hypothesis proposed recently by Kim *et al*.,[29] it is well known that smaller AgNP can penetrate into the cell membranes and make the microorganism inactivate easily.[30] In the present study revealed that AgNP synthesized using aqueous leaf extract *A. excelsa* showed augmented antibacterial activities.

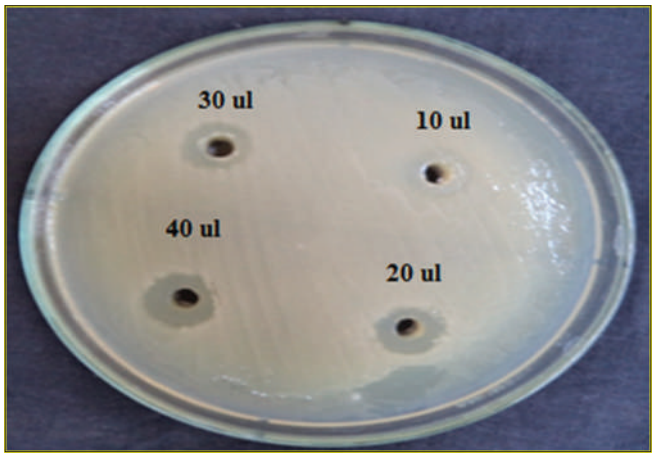


**Plate 9:** Minimum inhibitory concentration test for green synthesized 1 mM silver nanoparticles from *Ailanthus excelsa* against *Micrococcus luteus*

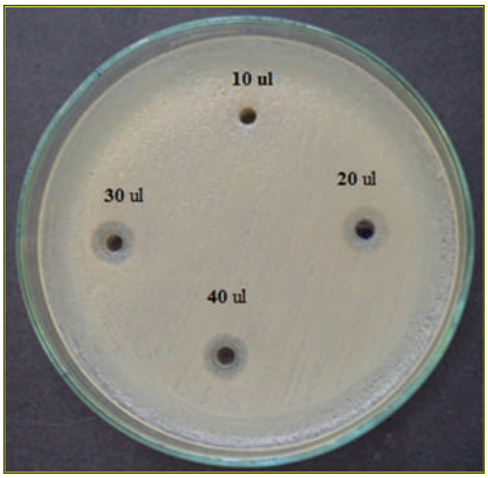


**Plate 11:** Minimum inhibitory concentration test for green synthesized 1 mM silver nanoparticles from *Ailanthus excelsa* against *Salmonella typhi*

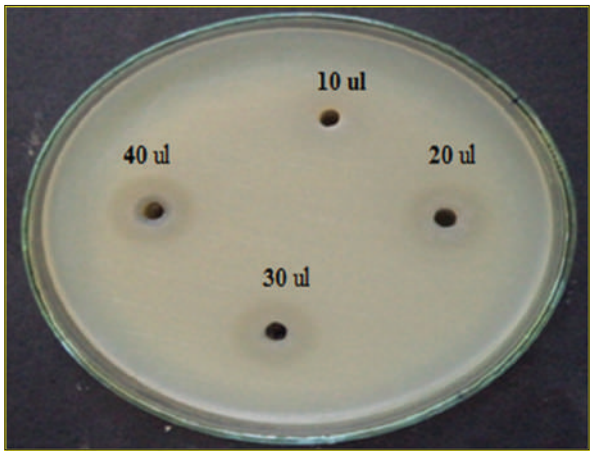


**Plate 10:** Minimum inhibitory concentration test for green synthesized 1 mM silver nanoparticles from *Ailanthus excelsa* against *Enterobacter aerogenes*

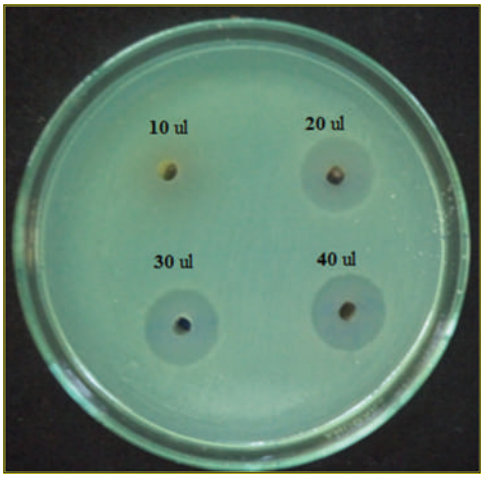


**Plate 12:** Minimum inhibitory concentration test for green synthesized 1 mM silver nanoparticles from *Ailanthus excelsa* against *Pseudomonas aeruginosa*

The antibacterial effect of the AgNP synthesized is directly proportional to the aqueous leaf extract of *A. excelsa* concentration for the highest ZOI. This might be attributed to the results obtained in the present study wherein increased leaf concentration in the reduced smaller size AgNP.

## CONCLUSION

The study of green synthesis of AgNPs has been used by various research institutes and medicinal researches. The study on the green synthesis of AgNP using the leaf extracts of medicinal plants and the characterization of the synthesized AgNP showed that leaf extracts of *A. excelsa* of 1 mM $AgNO_3$ yield stable AgNP in solution *A. excelsa* plant extract solution are potent for the green and eco-friendly synthesis of silver (Ag) nanoparticles which provide efficient research applications. The syntheses of synthesized AgNP were analyzed through techniques such as UV, SEM, EDAX, TEM, XRD, FTIR, and fluorescence spectroscopy. It showed that the AgNP were spherical in shape with an average size of 50 nm. The green synthesized AgNP using the leaf extract of *A. excelsa* exhibited antibacterial and MIC. Green synthesis of silver nanoparticles was revealed to be the most significant in bacterial effect and thus it could be used as a (potential) bacterial pathogen for various medical applications.

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