# Green synthesis of Silver Nanoparticles using *Premna tomentosa* leaf extract and its effective antibacterial activity

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***Abstract***

*Green synthesis of silver nanoparticles was done at room temperature using Premna tomentosa leaf extract. Silver nanoparticle formation was preliminarily confirmed through UV Spectroscopy and characterized by FTIR, XRD, TEM and SEM. The $\lambda_{max}$ was observed at 424nm in UV spectroscopy results. The XRD and EDAX spectrum confirmed the presence of silver ions and the crystalline nature of synthesized silver nanoparticles. The synthesized silver nanoparticles were spherical, hexagonal and irregular in shape. FTIR results have revealed the functional groups present in the synthesized nanoparticles such as C-N stretch, N-O symmetric stretch, C-H stretch, N-H bend, C-H group, O-H stretch and H bond.*

*TEM and SEM results showed a clear image of nanoparticles in the size range 100nm and 200nm. In the application part, we focused on the antibacterial activity of synthesized nanoparticles on six pathogens namely Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio parahaemolyticus and Proteus mirabilis. The maximum zone of inhibition was found in Vibrio parahaemolyticus followed by Staphylococcus aureus and Pseudomonas aeruginosa.*

**Keywords**: *Premna tomentosa*, Plant extract, Synthesis, Silver Nanoparticles, Antibacterial.

## Introduction

Nanoparticle applications are found to be the best alternative for various applications due to their surface area specificity, smaller size and carrier ability to release the drug on targets[17]. When we focus on their fields of applications, they are used as combinations in medicine, biology, physics and chemistry for the betterment and easy delivery of drugs on targets, antibacterial activity and stability of materials. Nanoparticles are diverse and they mainly depend upon their chemical nature, shape, dispersion state, surface modifications and dispersion medium[1].

Among other metals, silver is most preferably used for nanoparticle synthesis. Silver has various salient features like less toxicity used as medicine in open wounds and ulcers[3], as a disinfectant and against cancer[15], effective against many infectious diseases[22]. Green synthesis of nanoparticles was found to be cost-effective[5], easy to produce in adequate quantity and a very promising way for synthesis. In recent decades, there are many reports on metal nanoparticles being prepared from plant extracts like Neem, Geranium, Aloe vera, Cinnamomum, Mushroom, Pear fruit, *Magnifera indica* and *Magnolia kobus*. Silver metal is found to be easy for the synthesis of nanoparticles and is thus commonly used. Silver nanoparticles were found to be useful in the areas of molecular biology and molecular imaging[8].

Premna belongs to a family named *Verbenaceae*. It is locally called Kolakkatti thekku and Malai thekku. They have many traditional and medicinal uses. The leaves are being used for the treatment of dropsy; their bark and oil from the bark are specially used for treating stomach problems. With Premna and some other herbs, it is possible to make 'Pidangunaari kudineer,' a Siddha herbal drink. Premna genus consists mostly of trees and shrubs[14]. *Premna tomentosa* has been used for many disorders as a diuretic, for dropsy, diarrhea, stomach aches etc. using leaves, stems, bark and roots. Plants which have medicinal value, have high security of usage as drugs[21].

In ancient days silver has been used against microbes to kill and prevent infection from them. Even though much research was done, the clear mechanism of silver nanoparticles with the microbes is not well understood. Silver nanoparticles get attached to the surface of the bacterial cell wall and cause damage through cell disruption. Nanoparticles are more stable compared to the microbes[19] and act as stabilizers when using Cacumen leaf extract[27].

In a particular study, it is found that silver nanoparticles can penetrate through the bacterial cell wall, disrupt the components inside them and stop their function overall. Another method is by releasing the silver cations from silver nanoparticles which acts as bactericidal[24]. Through nanoparticles, novel compounds are easily absorbed[6]. Silver nanoparticle mechanisms include the reduction of toxicity due to DNA damage and stress[15] and have biomedical applications as well[7]. Aqueous silver nitrate acts as a precursor when nanoparticles are synthesized from apple extract[11]. Rubidium oxide nanoparticles exhibited good antibacterial activity against both gram-positive and gram-negative bacteria[13].

Silver nanoparticles from *Cassia tora* leaf extract showed good antibacterial activity on gram-negative bacteria compared to gram-positive bacteria. Due to their thin layer

of peptidoglycan, the activity is high. It is seen in *Puesdomonas aeruginosa* and *Staphylococcus aureus*[20]. From the leaf extract of *A. calcarata*, nanoparticles were synthesized and they were found to have good optical properties from Melia plant extract.[18] *Onasma* sp root extracts[28] can treat wounds and burns. The concentration variation studies on extract were studied and the result revealed that increasing the concentration of extract increases the particle size too[5].

The nanoparticles are highly pure, crystalline in nature,[24] maintain aseptic surroundings throughout the process[23] and prevent bacterial infections[4]. Gold nanoparticles are used for cancer therapy from Stevia leaf extract[10]. Zinc oxide nanoparticles were synthesized through the green synthesis of *A. linearis* extract[12]; Ni nanoparticles from alfalfa extract[25] are used as a reducing, oxidizing agent; Pongamia leaf extract[12] performs photo degradative activity[26] and reduces organic pollutants[16]. Overall metal nanoparticles have found favor in the field of agriculture and the food sector[9].

## Material and Methods

Silver nitrate, ethanol, nutrient agar and Mueller Hinton agar of analytical grade were brought from Hi Media, India. All the glassware used in the experiment were brought from Borosil, pre-autoclaved and used.

**Plant Source:** The leaves of *Premna tomentosa* were collected from ATRI, Thirthamalai, Tamilnadu, India. Authentication of the plant was done at the Institute of Herbal Science, Plant Anatomy Research Centre. Fresh leaves were collected and shade dried for two weeks. Then the dried leaves were ground with an electric blender to a fine powder and stored in an air-tight container for further experimentation.

**Silver Nanoparticle Synthesis:** 10g of powdered sample was mixed with 100ml of Milli-Q water. It is kept in the water bath for 10min at $80^0$C. After that filtration was done using Whatmann no.1 filter paper and the extract was kept for Ag-NPs synthesis[24]. To synthesize silver nanoparticles from *P. tomentosa* leaf extract, 10ml of leaf extract was added to 90ml of 1mM silver nitrate solution. It was then incubated at 37˚C in a shaker for 24h. Colour change was observed from yellow to dark brown. Then preliminary conformation was observed through UV spectroscopy. Purification was done by repeated centrifugation @ 8,000rpm for 10min in Oak ridge tubes and then the pellet was washed twice with Milli-Q water. Finally, the pellet was powdered in a Muffle furnace. Obtained Ag-NP powder was stored for further studies.

**Characterization: S**everal techniques and methods are available for nanoparticles characterization, however we have used UV spectroscopy, FTIR (Fourier Transform Infrared Spectroscopy), XRD (X-Ray Diffraction), TEM (Transmission Electron Microscope) and SEM (Scanning Electron Microscope). UV spectroscopy is the preliminary conformation of whether the silver nanoparticles are synthesized or not.

The principle of this technique is based on the absorption of ultraviolet rays by the nanoparticles as outlined by Beer Lambert's law and taking it's OD (Optical Density). The synthesized liquid nanoparticles sample was diluted and the readings were taken by UV Spectroscopy (Jasco V-670), between 200-800 nm. The liquid sample was dried in the Muffle furnace at 350˚C for further characterization.

To study the functional groups and chemical composition, the dried sample was given to FTIR. FTIR (Fourier Transform Infrared Spectroscopy) is a validated technique; the principle is based on the absorption of infrared rays by the particles. The frequency range is mostly taken between 4000-400 $cm^{-1}$. This technique is highly useful for identifying the organic molecules present in the sample. XRD (X-Ray Diffraction) is mostly used to find a particular particle's atomic and molecular structure. The principle is based on the diffraction of an x-ray beam by crystalline atoms and diffracts into many directions.

Therefore, the powdered sample was given to XRD (BRUKER D8 Advanced, Germany) for crystal structure analysis and confirmation of synthesized silver nanoparticles. TEM (Transmission Electron Microscope) is an advanced microscope in which the particles can be viewed at very high resolution. The electrons are transmitted through the sample; the images are formed due to the interaction between the electrons and the sample which can be viewed through the camera.

For structure identification and to view the sample in high resolution, the powdered sample was studied with TEM (FEI TECNAI G2, Netherlands). SEM (Scanning Electron Microscope) is a microscopic technique to gain knowledge on surface topology and composition of the sample by scanning with a focused beam of electrons. Hence, to know the morphological structure, the powdered sample was studied with SEM.

**Antibacterial Activity:** Antibacterial activity was studied with synthesized nanoparticles by the well diffusion method. The pathogenic bacteria used in the study are *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Vibrio parahaemolyticus* and *Proteus mirabilis.* 50µl of the sample with varying concentrations is added and Milli-Q water was maintained as blank. The plates were then kept in the refrigerator for half an hour for diffusion. Then they were stored in an incubator at 37˚C for 24hrs for zone formation. After incubation, the zones were observed and measured[2].

## Results and Discussion

Synthesis of silver nanoparticles from *Premna tomentosa* and its characterization was done.

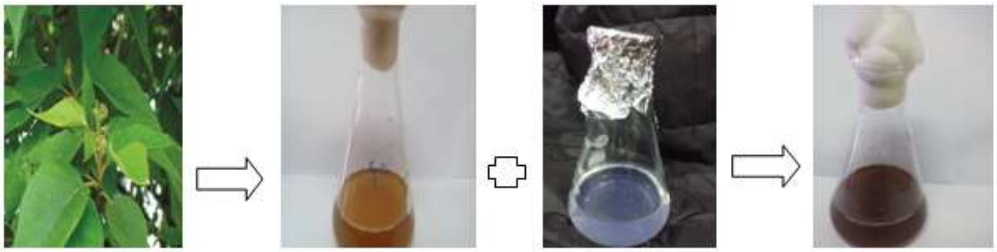

Figure1a Figure1b Figure1c Figure1d

**Figure 1: Synthesis of Silver Nanoparticles**

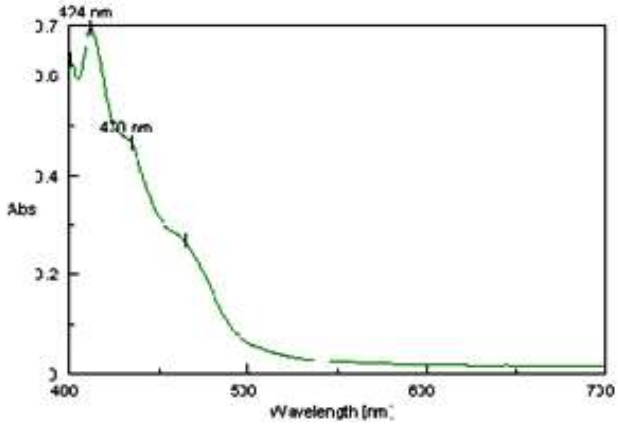


**Figure 2: UV Spectroscopy image**

Figure 1a is the *Premna tomentosa* plant, figure 1b shows the leaf extract from *Premna tomentosa*, figure 1c shows 1mM silver nitrate and figure 1d shows the synthesized silver nanoparticles. The overall picture depicts the synthesis of silver nanoparticles from the plant *Premna tomentosa*.

**UV-Spectroscopy:** Preliminary conformation of silver nanoparticle formation was confirmed through UV spectroscopy. Figure 2 shows the UV spectroscopy reading of synthesized silver nanoparticles from *Premna tomentosa*. The peak is found maximum at 424nm and it is due to the surface plasma resonance of synthesized silver nanoparticles which confirmed the presence of silver.

**FTIR, XRD, TEM, SEM and EDAX:** Figure 3 shows the FTIR image of synthesized silver nanoparticles from *Premna tomentosa*. FTIR library search revealed the peaks. The peak 555.50cm$^{-1}$ is found to be silver nanoparticles, the peak 777.31cm$^{-1}$ for the C-H group(aromatics), the peaks 1043.49, 1111.00 for C-N stretch (aliphatic amines), the peak 1325.10 for N-O symmetric stretch (nitro compounds), the peak 1382.96, 2924.09 for C-H stretch (alkanes), the peak 1564.27 for deprotonated carboxylic groups, the 1633.71 for N-H bend (amines), the peak 3348.42 for O-H stretch, H bonded (alcohols and phenols).

Figure 4 depicts the XRD image of the synthesized silver nanoparticles and confirms the crystalline nature of silver nanoparticles. The unique peaks at 38, 44, 64 and 77 correspond to the lattice value 111,200,220 and 311 denoting the face-centered cubic nature of the nanoparticles.

Figure 5 (left side) shows the TEM image of a single silver nanoparticle at 20nm. The size is around 12- 15nm and adjacent to it shows a clump of silver nanoparticles at 100nm. From the figure, we can see the spherical shape of silver nanoparticles. Figure 5 (right side) shows the diffraction pattern linear towards the electric beam perpendicular to the individual nanospheres.

Figures 6a and 6b show the Scanning electron microscopic images of silver nanoparticles at 200nm and 100nm ranges. We can visualize a single nanoparticle in the first one and a cluster of nanoparticles in the second image.

Figure 6c shows the EDAX image of silver nanoparticles which shows the ratio of elements present in our nanoparticles. The result reveals our prepared nanoparticles have 6% of carbon, 14% of oxygen and the remaining 80% silver.

**Antibacterial Activity:**
Figure 7 depicts the zone of inhibition results against selected bacterial pathogens using silver nanoparticles. From figure 8, we can conclude that all six pathogens show good antibacterial activity starting from *Vibrio parahaemolyticus* to *Escherichia coli*.

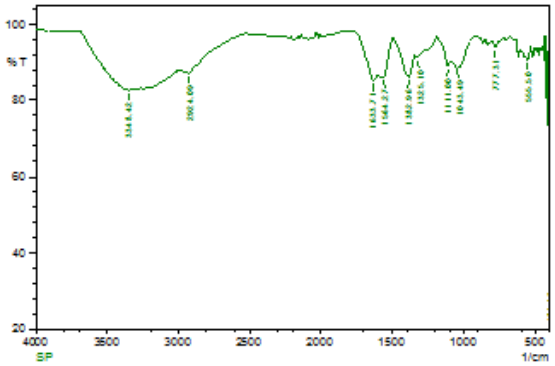


**Figure 3: FTIR image**

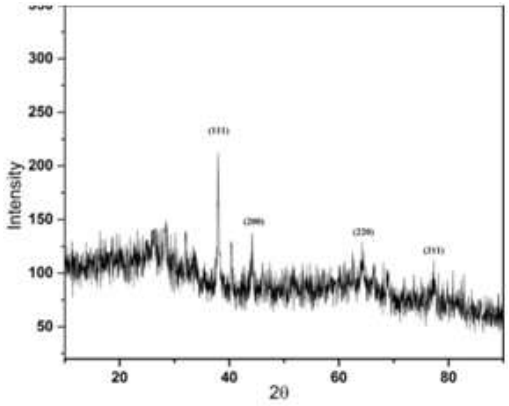


**Figure 4: XRD image**

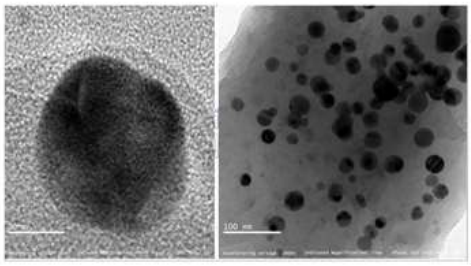

**Figure 5: TEM image and TEM diffraction pattern**

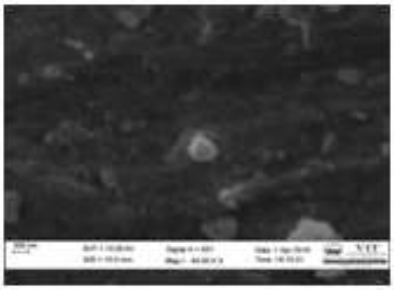

**Figure 6a: SEM image at 200nm**

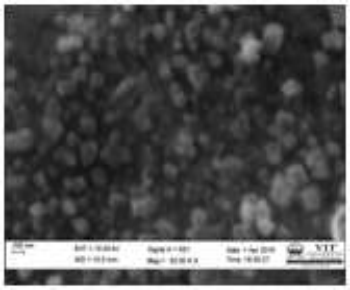

**Figure 6b: SEM at 100nm**

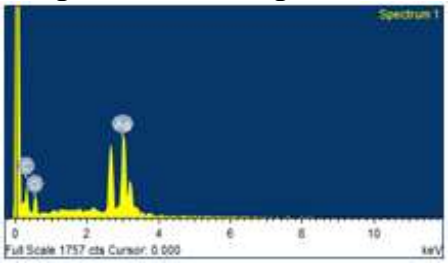


**Figure 6c: EDAX image**

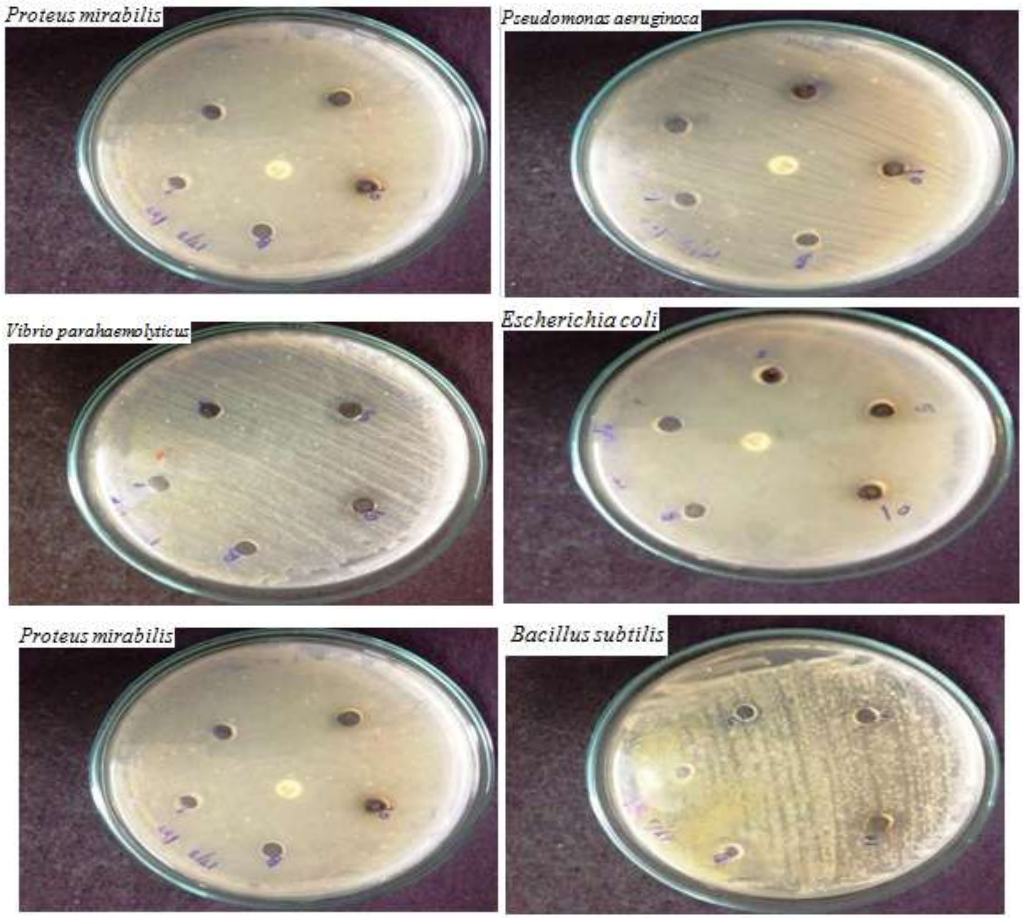


**Figure 7: Antibacterial Activity of silver nanoparticles**

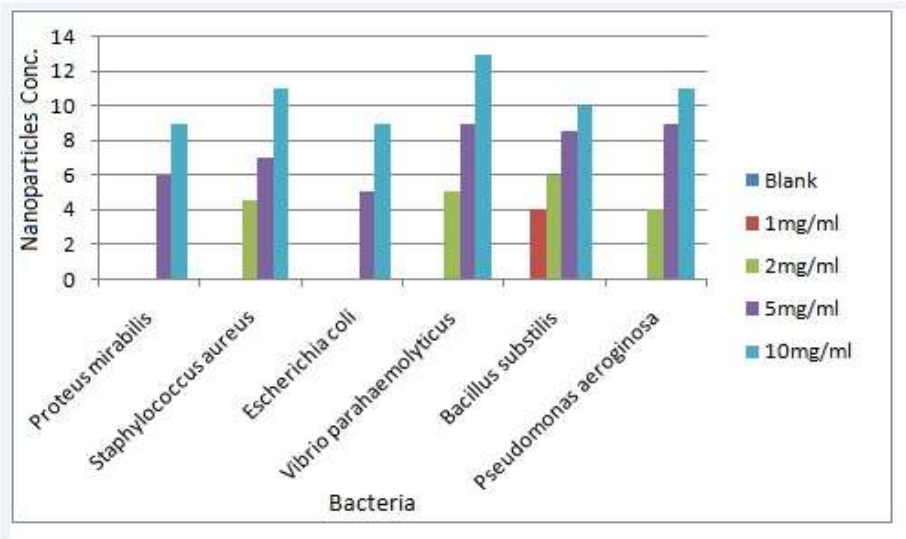


**Figure 8: The minimum inhibitory concentration of silver nanoparticles against tested bacteria species**

## Conclusion

In the present study, the *Premna tomentosa* plant which is an important medical tree, has been identified and silver nanoparticles have been synthesized from the leaf extract. The formation was confirmed through various characterization methods. Antibacterial activity was performed as an important application using six commonly used pathogens. All six pathogens were susceptible to the phytochemicals present in the leaf extract and showed a good zone of inhibition.

Among all the six pathogens, the synthesized silver nanoparticles showed maximum antibacterial activity against *Vibrio parahaemolyticus*, this was followed by *Staphylococcus aureus* while exhibiting comparatively lesser antibacterial effect on *Proteus mirabilis* and *Escherichia coli*. Altogether, the silver nanoparticles

synthesized by our plant extract showed good antibacterial activity against all six bacterial pathogens, due to the phytochemical constituent's present in the sample. This signifies that *Premna tomentosa* plant extract can be used as drug for various medicinal purposes. Nano-drug delivery is very effective in opening the door for further research of *Premna tomentosa* phytochemicals to be taken up as lead compounds for the drug industry.

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