

Antibacterial Activity of Neem Extract and its Green Synthesized Silver Nanoparticles against *Pseudomonas aeruginosa*

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Azadirachta indica, generally called as neem, margosa or Indian lilac is an Indian subcontinent native species, it is well known for its various bioactivity. In this study, neem leaves were collected and extracted. The extract was subjected for TLC and antibacterial activity against *Pseudomonas aeruginosa*. The extract was subjected for green synthesis of silver nanoparticles and it was able to reduce silver to silver nanoparticle of size around 65nm. The silver nanoparticle was also exhibiting antibacterial activity against *Paeruginosa*.

Keywords: Neem extracts, silver nanoparticles, antibacterial activity.

Over two millennia, neem tree products have been utilized in India as traditional medicines due to their novel properties¹. Basically, neem is called *arishtha* in Sanskrit^{2,3} which means 'reliever of sickness'³ and in India, Neem is also called as the 'village pharmacy'⁴ for its properties to relieve from various infections, pains and fever. These most popularly known Neem tree is scientifically called as *Azadirachta indica* and is found in tropical regions indigenous to Indian subcontinents⁵. The other species closely related to *A. indica* is *Melia azedarac* also known as the Persian lilac³. The biological activities of neem include anti-inflammatory, anti-pyretic, hypoglycaemic, anti-fungal, anti-gastric ulcer, diuretic, antibacterial,

antitumour, antimalarial, anticancer, hypolipidemic etc^{1,3,6,7}. Thus in Ayurveda, Neem (Nimba) has been long used for the control of malarial fever, blood disorders, leprosy, wounds, eye disorders and ulcer⁸ and hence is referred to as 'Sarva-Rogha-Nirvarini' which means 'curing ailment' in Ayurveda^{3,8,9}. In a few experimental reports, the different approaches for the production of polymeric resins using neem is documented¹⁰. Neem is best known for its pesticide activity^{11,12}. One of the primary mode of action of neem against insects is the disruption of its metamorphosis and the bitter flavour aids in keeping them away from feeding the host plants making it an exemplary anti-feedent and repellent¹³. Recently neem has acquired a great deal of global attention towards it¹² for its versatile ability in medicine and agriculture. As a sign of global acceptance, US national academy of sciences has titled neem as 'The tree for solving global problems'⁹ and the ability of neem extracts to prevent from tooth decaying, inflammation of

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gums are proved by various tests in Germany and it is currently exploited as an active ingredient in toothpastes in Germany and India. In 1985, neem based insecticide called Margosan-O was accepted by the Environmental protection agency for non-food uses and ever since various other neem based products were also approved by various administrations and agency for food and feed crops¹⁴. Neem can also be used to produce nanoparticles for its ability to reduce the metallic ions to yield nanoparticles¹⁵. Silver salts is known widely for its anti-bacterial effects having high toxicity towards micro organisms and is used for the same in many applications like dental work, catheters and burn wounds^{16, 17}. The novel

properties of silver are also exploited in the field of nanotechnology. Nanoparticles are clusters of atoms that are very small ranging from the size 1-100nm and are known for their high surface area to volume ratio which aids its applications in many fields¹⁸. Silver nanoparticles (AgNPs) are used as anti-microbial agents in health, food, textile industries and their products are also approved by US FDA, US EPA, SIAA of Japan, FITI testing and research institute, etc¹⁹. Though there are various ways to synthesise nanoparticles, the most non-toxic and eco-friendly method is the bio-based method which utilises various biologically products such as microbes and plants^{20,21}. Hence this study focuses on the evaluation of the bioactivity of the neem extract and its ability to produce silver nanoparticles. The antibacterial effect of both the extract and the AgNPs is also discussed.

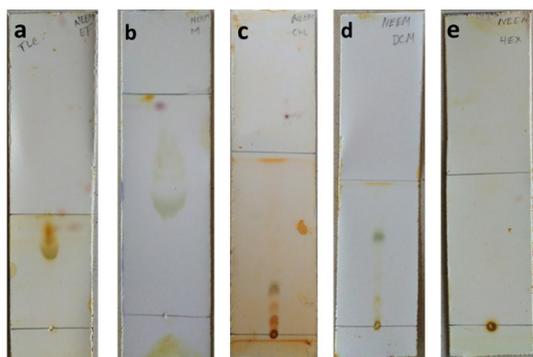
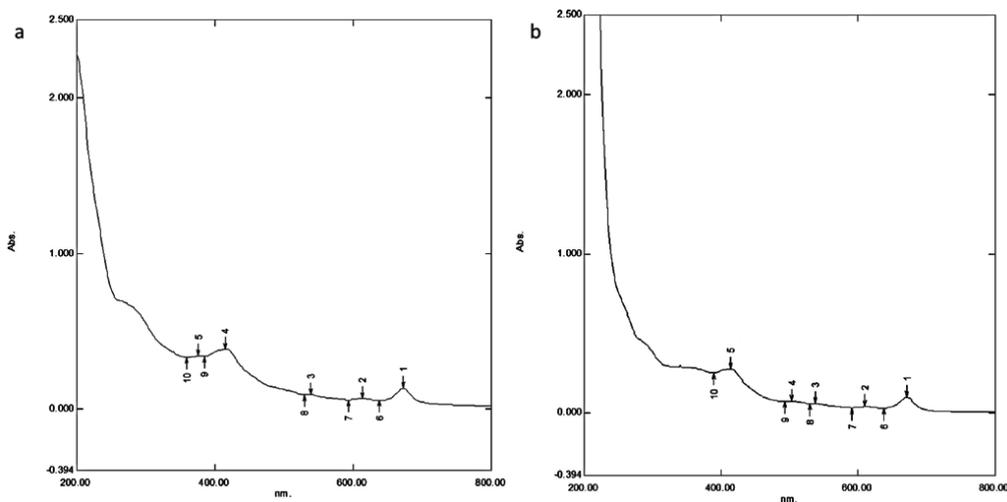


Fig. 1. TLC ran with different mobile phase and exposed to iodine a) Ethanol b) Methanol c) Chloroform d) Dichloromethane e) Hexane

Table 1. Antibacterial activity of neem extract against *Pseudomonas aeruginosa*

Concentration (µg/ml)	Zone of inhibition (cm)
+ve control	4.4
-ve control	-
5	-
10	-
15	0.4
20	0.6



a) Neem extract b) silver nanoparticle
Fig. 2. UV-Visible spectroscopy analysis

MATERIALS AND METHODS

Chemicals used

All the solvents and chemicals used, chloroform, ethanol, methane, dichloromethane, hexane, iodine, silver nitrate, DPPH (1, 1-diphenyl 2-picrylhydrazyl) were of analytical grade. Sterile distilled water was used throughout the procedure.

Sample collection and extraction

Leaves of *A. indica* was collected from Chennai, Tamil Nadu, India and cleaned, then it was shade dried. The powdered leaves were added with ethanol and left for 3 days. The solvent was then filtered using Whatmann no.1 filter and then dried.

Thin layer chromatography

Thin Layer Chromatography was

performed with TLC silica plate (Merk F245) for the sample and various solvents like chloroform, methane, ethanol, dichloromethane and hexane were used as mobile phase. The plates were exposed to iodine

Agar well diffusion assay

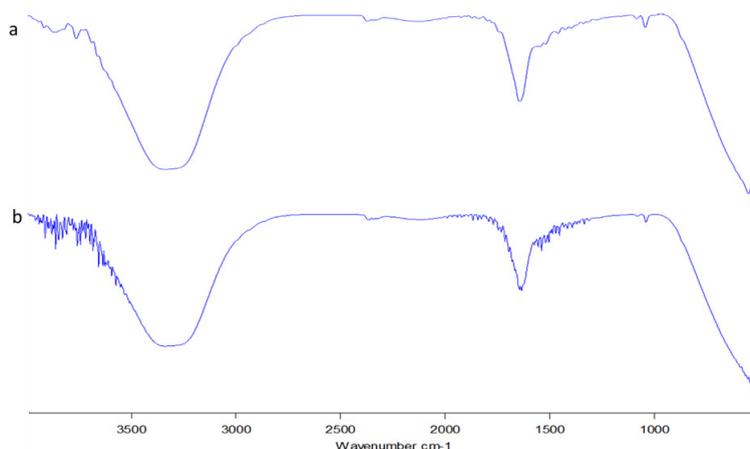
Agar well diffusion method was done to identify and evaluate the anti-bacterial activity of the extract obtained against *Pseudomonas aeruginosa*²².

Synthesis of AgNPs

The extract was mixed with 3mM Silver nitrate solution in water in the ratio of 1:5 and was kept in the dark for 24h in room temperature in order to produce and settle silver nanoparticles.

Characterization of AgNPs

The neem extract and the nanoparticles



a) Neem extract b) Silver nanoparticle
Fig. 3. FTIR analysis

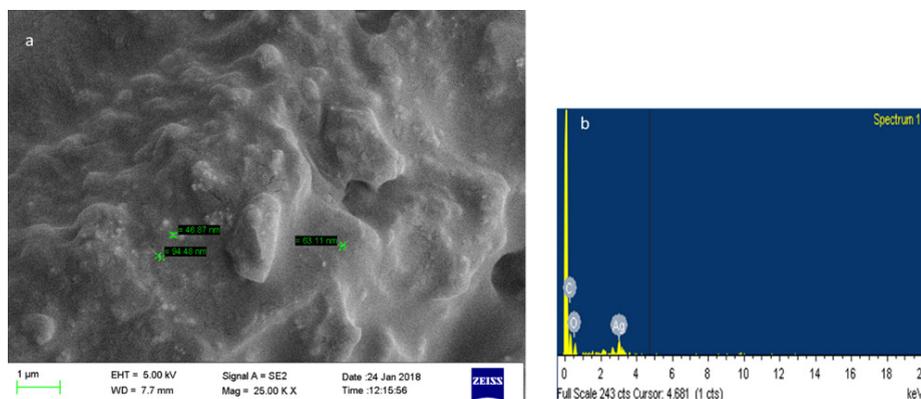


Fig. 4. SEM-EDX analysis of silver nanoparticle (a) SEM (b) EDX

Table 2. Antibacterial activity of silver nanoparticles

Concentration ($\mu\text{g/ml}$)	Zone of inhibition (c m)
+ve control	2.7
-ve control	-
2	0.4
4	0.5
8	0.6
16	0.6

so produced were subjected to UV-Visible spectroscopy (Shimadzu 1800, Japan) and Fourier Transform Infra-red Spectroscopy (Shimadzu, Japan). Silver nanoparticles were subjected to EDX spectroscopy and Scanning electron microscopy (SEI and BSI).

Agar well diffusion assay of AgNPs

The nanoparticles were subjected to agar well diffusion assay to evaluate the antibacterial activity of it against *Pseudomonas aeruginosa*²² with a concentration range from 2 to 16 $\mu\text{g/ml}$. The positive control used was Ciproflaxacin.

RESULTS AND DISCUSSIONS

Thin layer chromatography

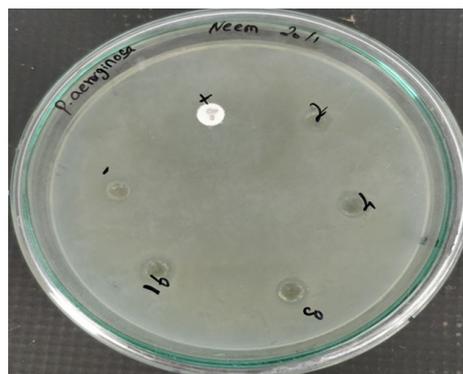
The ethanol extracted samples were run using TLC with various mobile phases and the plates were exposed to iodine. Four bands were seen when chloroform was used as the mobile phase (Figure 1c).

Agar well diffusion assay

When the extract was subjected to antibacterial assay, it was found that the concentrations above 15 $\mu\text{g/ml}$ were acting against the bacterial cultures from the zone of inhibition (Table. 1).

Characterisation of AgNPs

The absorbance spectra for the neem extract and the nanoparticles were obtained and it was found that the peaks near 360, 375, 380 and 440nm in the spectrum obtained for neem extract (Figure 2a) had a shift to 385, 440, 495 and 500nm in the spectrum obtained for the AgNPs (Figure 2b).

**Fig. 5.** Antibacterial activity of silver nanoparticle against *Pseudomonas aeruginosa*

In another study²⁴ on the biologically synthesised nanoparticles, absorption maxima was seen at the range of 440-500nm which is in par with the current study.

The IR spectrum of both the neem extract and the neem based silver nanoparticles were evaluated (Figure 3a, b). They both had a few bands in common with amide groups (at around 1640 cm^{-1})²⁵, hydroxyl group (around 3330 and 1045 cm^{-1})²⁶. The extract was found to have band at 1085 which corresponds to the -C-N bonds of alcohols, esters, ethers and carboxylic acid while the AgNP had bands at 1339 and 1396 which corresponds to the C-N stretching of aromatic amine groups²⁷. From the IR spectrum it can be confirmed that the amino acid residue and the protein groups present might have the strongest ability to bind with the metal nanoparticle successfully aiding in both formation and stabilisation of the particles²⁸.

The spectrum obtained confirms the presence of silver in the analysed sample with the presence of the typical range for the absorption of metallic silver nanocrystals at 3KeV (Figure 4b). The weak signals showing the presence of C, O suggests the capping of AgNPs with the organic compound from neem extract^{29,30}.

The Scanning electron micrographs of the silver nanoparticle shows the size of the ranging from 46 to 94nm and the presence of highly aggregated nanoparticles. (Figure 4a). In a study, less than 20nm spherical nanoparticles were produced using neem gum³⁰ and 12nm sized nanoparticles were prepared using neem plant extracts³¹.

Agar well diffusion assay of AgNPs

From Figure 5, it is evident that the antibacterial activity of the AgNPs increased with the increase in concentration when checked against *Pseudomonas aeruginosa*. This shows that the nanoparticles are bactericidal.

CONCLUSION

From the above study it is clear that the neem extract and its silver nanoparticles have antibacterial activity against *P.aeruginosa*. The nanoparticles were found to be capped by the organic compounds present in the neem extract which could be a reason for the production of nanoparticles and also for its stability.

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