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# Green synthesis of silver nanoparticles from *Azadirachta indica* leaves and its antibacterial activity

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**Abstract**
In the present study, *Azadirachta indica* leaves were taken for synthesizing silver nanoparticles and checked their antibacterial activity against *E.coli, Klebsiella, Pseudomonas, Staphylococcus* and *Acinetobacter*. The plant extract were analysed for the detection of the presence of protein, carbohydrate, flavonoids, terpenoids, glycosides, steroids, saponins, phenols and tannins. In this present study the antibacterial activity of green synthesized silver nanoparticles from guava leaf shows the zone of inhibition against all the five pathogens.

**Keywords:** Neem Leaves – extract – silver nanoparticles -antibacterial activity

## Introduction
According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Increasing incidence of multidrug resistant pathogenic microorganisms has lead to the search for new antimicrobial substances from various sources like the medicinal plants. Antimicrobial activities are due to the secondary metabolites synthesized by the plants such as phenolic compounds. Leaf is one of the highest accumulated plant part of such compounds
*Azadirachta indica* leaf extract is used in the synthesis of various nanoparticles like gold, zinc oxide, silver etc. The phytochemicals present in Neem are namely terpenoids and flavanones, which act as reducing as well as capping agent and helping in stabilizing the nanoparticles. When silver salt is treated with Neem leaf extract, the silver salt is reduced to AgNPs. The synthesized nanoparticles, which are capped with neem extract also exhibit enhanced antibacterial activity
Different parts of the plant *Azadirachta indica* is found to possess different level of antimicrobial activity against S*taphylococcus aureus, Enterococcus feacalis, Proteus mirabilis Pseudomonas aeuroginosa*, *Aspergillus fumigatus* and *Candida albicans* by Agar well diffusion method. The leaf extract exhibited strong antimicrobial activity against bacteria and fungi. Bark extract showed moderate and seed extract exhibited least antimicrobial activity (Raja Ratna Reddy *et al*., 2013) [4].
Phytochemical analysis of the plant extract is done to detect the presence of various biologically active plant metabolites such as phenols, flavanoids, alkaloids, coumarins, glycosides, tannins, saponins and steroids.
Our environment possesses varieties of plants and microorganisms. Plant leaf extract, bacteria fungi and enzymes for the synthesis of silver nanoparticles are majorly used and they offer numerous benefits such as eco friendliness and lots of compatibility for pharmaceutical and other biomedical applications as not been used any toxic chemicals for the synthesis of nanomaterials (Kanipandian *et al.,* 2014) [3]. Nanocrystalline silver particles are found too much of applications in the field of detection of biomolecules and diagnostics, antimicrobials and therapeutics catalysis and micro-electronics.
Green synthesis of Silver Nanoparticles using *Azadirachta indica* (Neem) extract at room temperature has been studied earlier and the results are good. (Namratha and Monica, 2013) [2].

## Material and Methods

Leaves of *Azadirachta indica* were collected and washed thoroughly in water to remove mud and dust particles and dried in the shade for about 10 days. The shade dried leaves were then powdered coarsely in mixer and stored in separate air tight containers at room temperature for further use. For acetone extraction, 20gms of coarsely powdered leaves were taken and 100 ml of the acetone was added. The solvent was then evaporated to dryness under reduced pressure and the extracted compound was used for the antibacterial assay. Phytochemical tests of plant extract was also done and for this 1gm of the extract of *Azadirachta indica* was mixed in 1ml of dimethyl sulfoxide solution.

The bacterial strains used for the test were *E. coli*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Acinetobacter*. Muller Hinton agar and Nutrient broth was used as the media for the culturing of bacterial strains. Loopful of bacterial cultures were inoculated in the nutrient broth and incubated at 370c for 24 hours. For Green synthesis of silver nanoparticles, about 40gms of the crude leaf powder was mixed with 200 ml sterile distilled water and kept in the shaker at 120 rpm for overnight. After overnight incubation, the flasks were allowed to stand still for about 30 minutes at room temp to settle. After settling the supernatant was to get the filtrate which is the aqueous plant extract.

To 9 ml of the aqueous plant extract of *Azadirachta indica* 45 ml of 1 mM silver nitrate solution was added. The flasks were covered with aluminum foil and incubated at room temperature in shaking condition in dark for 48 hours. After incubation the mixture was changed in colour from orange brown to dark brown.

Elemental analysis and chemical characterization of green synthesized silver nanoparticles from *Azadirachta indica* was analysed by Energy-dispersive X-ray spectroscopy. Silver nanoparticle synthesized was sent to SITRA Coimbatore for the analysis of the synthesized silver nanoparticles by Energy-dispersive x-ray spectroscopy. EDX can be used to confirm the composition and distribution of the nanoparticles through spectrum and elemental mapping by using an EDX spectrometer

Anti-bacterial activities of AgNPs were tested separately using disc diffusion method (Bauer *et al*., 1966) [1]. The suspensions of the bacterial strains were prepared corresponding to 0.5McFarland scale and swabbed on to the surface of sterile Mueller–Hinton agar plates. The different concentrations (5, 10, 15, 20µl) of green synthesized silver nanoparticles were transferred to the wells. Then the plates were incubated at 37 °C for overnight in upright position. The assessment of antibacterial activity was done based on the measurement of the diameter of inhibition zone formed around the disc.

## Result and Discussion

Plant leaves is used as medicines against gastroenteritis, diarrhoea and toothache for replace antibiotics The present study screened the antibacterial effects of silver nanoparticles of Guava. The major active compounds present in these extracts. The results obtained in various tests are tabulated in Table: 1

**Table 1:** Phytochemical analysis of acetone extract of *Azadirachta indica*

| S. No | Test | Result |
|---|---|---|
| 1. | Test for protein: | |
| | Ninhydrin test | +ve |
| 2. | Test for carbohydrates: | |
| | Fehlings test | +ve |
| | Benedicts test | -ve |
| | Molishs test | -ve |
| | Iodin test | +ve |
| 3. | Test for phenols & tannins | -ve |
| 4. | Test for flavanoids: | |
| | Alkaline reagent test | -ve |
| 5. | Test for saponins | +ve |
| 6. | Test for glycosides: | |
| | Libermanns test | -ve |
| | Salkowski test | +ve |
| | Keller-killani test | +ve |
| 7. | Test for dteroids | +ve |
| 8. | Test for terpanoids | -ve |

The color change seen in the mixtures due to the reaction between the silver nitrate and the leaves indicating the synthesis of silver nanoparticles was recorded by visual observation. With regard to *Azadirachta indica*, the leaves extract and silver nitrate mixture before and after incubation showed a color change from orange brown to dark brown (figure:1)indicating synthesis of silver nanoparticles. The synthesized silver nanoparticles (AgNPs) exhibited different colors in aqueous medium as a result of surface plasmon vibrations. In this present study the antibacterial activity of green synthesized silver nanoparticles from guava leaf shows the zone of inhibition against all the five pathogens under study (Figure2).


**Fig 2:** Synthesis of Silver nanoparticles

Energy dispersive X-ray (EDX) spectrometer established the existence of elemental sign of the silver and homogenous allocation of silver nanoparticle. Examination of AgNPs by Energy dispersive X-ray (EDX) spectrometer established the existence of elemental indication of the Ag and homogenous distribution of AgNPs. The pointed sign peak of Ag powerfully established the reduction of $AgNO_3$ to AgNPs. The upright axis expresses the number of X-ray counts while the parallel axis shows energy in KeV. Detection lines for the main release energy for Ag were clarified and these communicate with peaks in the spectrum, thus giving

affirmation that Ag has been properly recognized and present in the solution (Table 2 and Figure 3)

**Table 2:** % of silver in EDR analysis

| Element | Weight% | Atomic% | Compd% | Formula |
|---|---|---|---|---|
| C K | 25.57 | 32.52 | 93.71 | CO2 |
| Si K | 0.75 | 0.41 | 1.61 | SiO2 |
| K K | 0.86 | 0.34 | 1.04 | K2O |
| Ag L | 3.40 | 0.48 | 3.65 | Ag2O |
| O | 69.42 | 66.26 | | |
| Totals | 100.00 | | | |

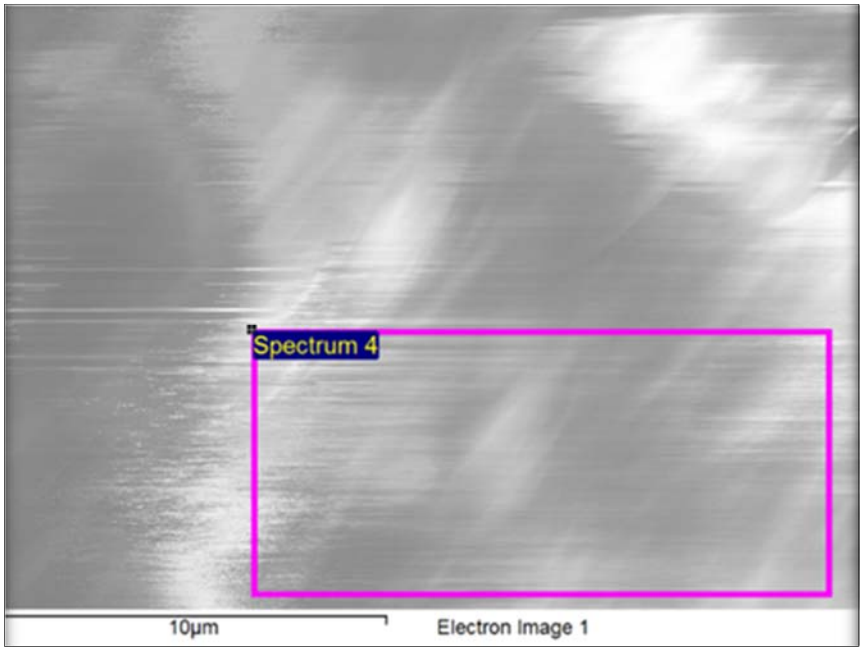



**Fig 3:** Distibution of Ag in silver nanoparticles in EDR study pattern

A good antibacterial activity was seen against all the pathogens to the silver nanoparticles synthesized from *Azadirachta indica* leaves extract. From this Maximum zone was against I (14mm) and minimum zone of inhibition was towards *Pseudomonas* (10.5 mm) (Figure:4) Standard antibiotic disc Tetracycline 30 µg/ml was also used in the study to check the comparative effect of silver nanoparticle.

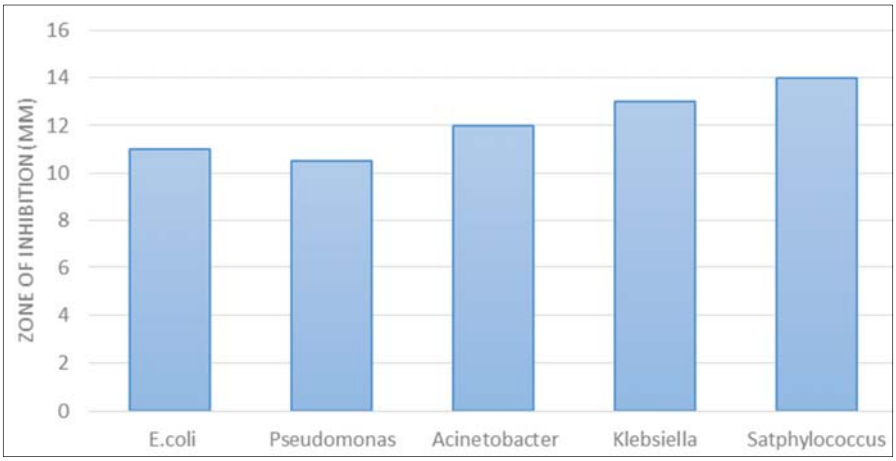
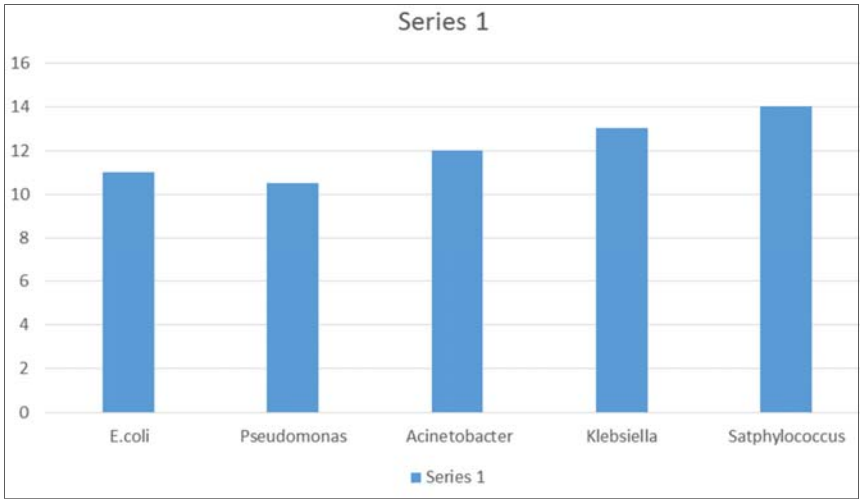



**Fig 4:** Antibacterial Activity




| ***E. coli*** | **11** |
|---|---|
| *Pseudomonas* | 10.5 |
| *Acinetobacter* | 12 |
| *Klebsiella* | 13 |
| *Satphylococcus* | 14 |

**Conclusion**

Green synthesis of silver nanoparticles was done by using *Azadirachta indica* leaf extract and 1mM $AgNO_3$ solution.The synthesized silver nanoparticles was analysed and confirmed by Energy-dispersive x-ray spectroscopy. The antibacterial activity of synthesized silver nanoparticles on *E.coli, Klebsiella, Psuedomonas, Staphylococcus,* and *Acinetobacter* in different concentration was assessed by well diffusion method. From the results, it was concluded that leaves extract of *Azadirachta indica* Linn. plant was shown effective and efficient result against bacterial pathogen used. *Azadirachta indica* leaves could serve as good source of antibacterial agents.

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