

# Anti-bacterial activities of silver nanoparticles synthesized from plant leaf extract of *Abutilon indicum* (L.) Sweet

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**Abstract** *Abutilon indicum* (L.) Sweet is an Asian phyto-medicinal plant and it has hypoglycemic and anti-bacterial activities. The nano silver was successfully synthesized from leaf extract using *A. indicum* (L.) Sweet. Nitrate reductase enzyme or other extra cellular proteins released from leaf extract involve in reduction of nitrate and formation of silver ions occurs. These proteins or enzymes act as template for the silver nucleation sites for the formation of silver nanocrystals and also act as capping agents (peptides or proteins) which control the size and shape of silver nanoparticles. FT-IR reveals that protein molecules can be bound to silver nanoparticles and XRD result confirmed crystal structure of nanoparticles. On the basis of our results, extract of *A. indicum* (L.) Sweet leaf protein molecules plays a vital role in reducing and capping of silver nanoparticles at 50 µl concentrations which act as anti-bacterial agent to control pathogenic microorganisms of *Klebsiella pneumonia*, *Salmonella typhi*, *Bacillus subtilis*, and *Proteus vulgaris*.

**Keywords** Nano silver · *Abutilon indicum* · XRD · FT-IR · Anti-bacterial activity

## Introduction

The plant-mediated synthesis is a rapid, flexible and suitable process for large-scale production of nanoparticles. Nowadays, plant parts like seed [1], leaf [2, 3], bark [4], stem [3, 5] and fruit [6] extracts have been effectively used for synthesis of nanoparticles. Among nanoparticles, silver nanoparticles

have been used enormously due to their potent anti-bacterial [7], anti-fungal activity [8]. *Cassia auriculata* is a common plant, profoundly used as anti-pyretic, hepatoprotective, anti-diabetic, anti-hyperglycemic, conjunctivitis, ulcers, leprosy, skin and liver diseases. Although there are several reports on synthesis of silver nanoparticles from plants and its anti-microbial activity [9]. Owing to the excellent anti-microbial properties, the silver nanoparticles have been widely used in food packaging [10], preservation [11], cosmetics [12], and medicine [13].

Silver nanoparticles have found tremendous applications in the field of high sensitivity bio-molecular detection, diagnostics, catalysis and micro-electronics. A number of approaches are available for the synthesis of silver nanoparticles for example, reduction in solutions, chemical and photochemical reactions in reverse micelles, thermal decomposition of silver compounds, radiation assisted, electro chemical, sono-chemical, microwave-assisted process, and recently via green chemistry route.

Silver nanoparticles were synthesized using dried areca nut (*Areca catechu*). The microwave exposed aqueous areca nut powder when treated with the aqueous silver salt solution yielded irregular shaped silver nanoparticles. The understanding of capping of biological moiety is derived from Fourier transform infrared spectroscopy and the thermo gravimetric analysis [14].

Green silver nanoparticles (AgNPs) have been synthesized using various natural products like *Azadirachta indica* [15], *Glycine max* [16], *Cinnamon zeylanicum* [17], *Camellia sinensi* [18] Peel extract of Pomegranate [19] and *Callicarpa maingayi* stem bark extract [20]. However, there is still a need for economic, commercially viable as well environmentally clean route to synthesize AgNPs. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment

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friendly, easily scaled up for large-scale synthesis and further there is no need to use high pressure, energy, temperature and toxic chemicals. The morphology and crystalline phase of the NPs were determined from UV–Vis spectroscopy, Fourier transform infrared (FT-IR) spectroscopy and X-ray diffraction (XRD) spectra.

The development of new resistant strains of bacteria to current antibiotics has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides. Silver has long been known to exhibit a strong toxicity to a wide range of microorganisms for these reason silver-based compounds have been used extensively in many bactericidal applications. Silver compounds have also been used in the medical field to treat burns and a variety of infections. Jiang et al. [21] reported that the Ag has long been recognized as having an inhibitory effect toward many bacterial strains and microorganisms commonly present in medical and industrial processes. AgNPs are reported to possess anti-fungal [22], anti-inflammatory [23], and anti-viral activity [24].

In the present study, we report the synthesis of silver nanoparticles reducing the silver ions present in the solution of silver nitrate by the cell-free aqueous leaf extract of *Abutilon indicum* (L.) Sweet and their role as high antimicrobial activity.

## Materials and methods

### Biosynthesis of silver nanoparticles

Biosynthesis of silver nanoparticles was done according to the method of Song and Kim [25]. *A. indicum* (L.) Sweet plant leaves were collected from botanical garden of our Department, Tiruchirappalli-24, Tamilnadu, India and air dried for 10 days and then was kept in the hot air oven at 60 °C for 24–48 h. The leaves were ground to a fine powder. 1 mM silver nitrate solution was added to plant extract to make up a final volume of 200 ml and centrifuged at 18,000 rpm for 25 min. The collected pellets were stored at 4 °C. The supernatant was heated at 50–95 °C. A change in the color of solution was observed during the heating process.

### Anti-bacterial activity

A loop full of strain was inoculated in 25 ml of nutrient broth in a conical flask and incubated at room temperature on a rotary shaker for 24 h for the growth of bacteria. Nutrient agar medium (Himedia) was used for the anti-bacterial susceptibility study. The bacterial assay was performed by agar well diffusion method [26]. The media were poured into Petri dishes. After the media get solidified, the 60 µl of test strains was spreaded using L-rod and the well was made in the plates

with the help of a cup-borer (8.5 mm). The well was filled with 50 µl of the synthesized AgNPs from plant extract and then plates were incubated over night at 37 °C. The bacterial growth was determined by measuring the diameter of the zone of inhibition. The experiment was done three times and the mean values were presented. The sterile disc was maintained as control for each bacterial strain.

### Characterization of nanoparticles

#### UV spectroscopy analysis

About 5 mg of the deposit of silver NPs was sonicated in 10 ml water and the suspended nanoparticles were immediately measured in UV–visible Spectrophotometer (50 ANALYTIKJENA).

#### FT-IR spectroscopy analysis

The silver nanoparticles were analyzed by FT-IR spectroscopy. The FT-IR spectrum was taken in the mid IR region of 400–4,000  $\text{cm}^{-1}$ . The spectrum was recorded using Attenuated Total Reflectance (ATR) technique. The sample was directly placed in the zinc selenide crystal and the spectrum was recorded in the transmittance mode.

#### XRD analysis

The silver nanoparticles were analyzed by X-ray diffractometer (X'pert PRO PAN analyzer) with Syn Master 793 software to identify the nanoparticles product. The XRD pattern was recorded using computer controlled XRD-system, JEOL, and Model: JPX-8030 with CuK radiation (Ni filtered = 13418 Å) at the range of 40 kV, 20 Å. The 'peak search' and 'search match' program built in software (syn master 7935) was used to identify the peak table and ultimately for the identification of XRD peak. XRD analysis was carried out in Central Electrochemical Research Institute (CERI), Karaikudi.

#### Particle size analysis

10 mg of synthesized nanoparticles were taken and suspended with distilled water. Then this medium was characterized by particle size analyzer.

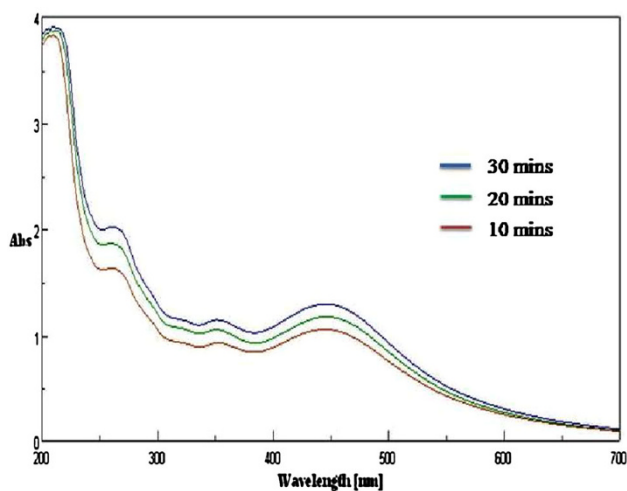
## Results and discussion

### Biosynthesis of silver nanoparticles

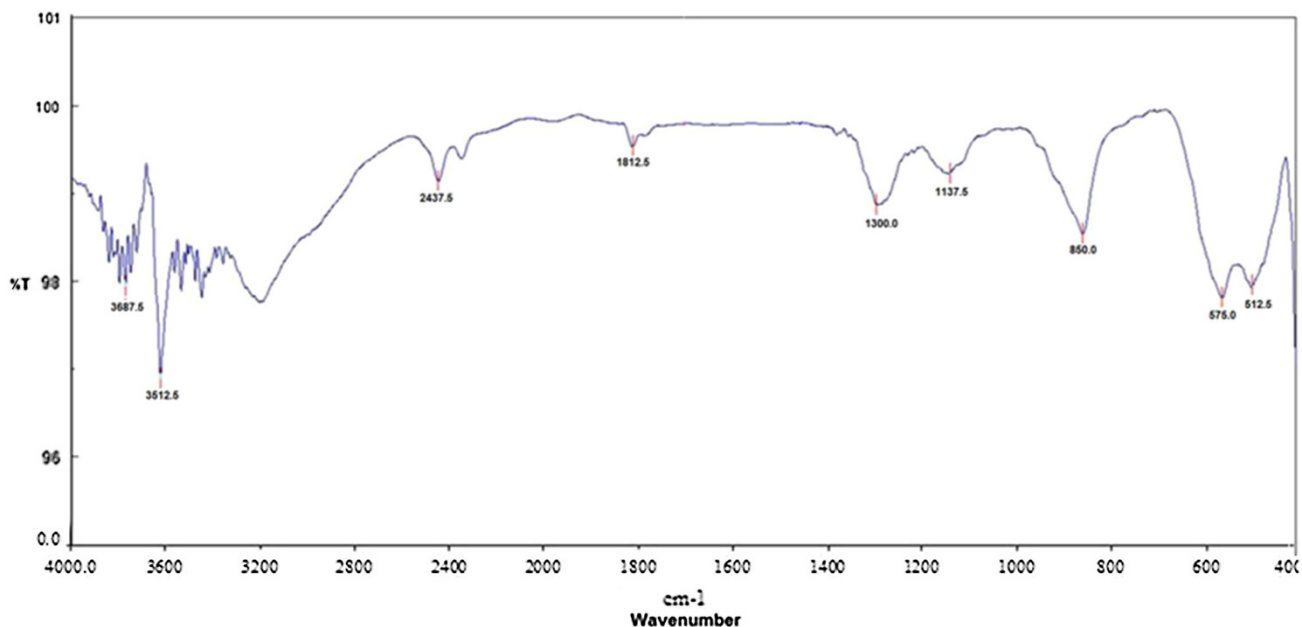
When the leaf extract incubated with silver nitrate, it was turned pale brown in color (Fig. 1) because of reduction



**Fig. 1** Biosynthesis of silver nanoparticles using plant leaf extract of *A. indicum* (L.) Sweet



**Fig. 2** UV-visible spectrum of synthesized silver nanoparticles



**Fig. 3** FT-IR analysis

reaction appeared in the biological synthesis process. The confirmations of these nanoparticles are as followed by different characterizations.

#### UV spectroscopy analysis

UV-visible spectra of silver nanoparticles were taken in water medium. The strong absorption peaks at 435 nm are associated with the silver nanoparticles (Fig. 2). It can be assumed that leaf protein acts as a template for the synthesis of silver nanoparticles and as well as stabilizing of nanoparticles. The formation of AgNPs was followed by measuring the surface-plasmon resonance (SPR) of the *A. indicum* (L.) Sweet and Ag/*A. indicum* (L.) Sweet emulsions over the wavelength range of 200–700 nm (Fig. 2). The SPR bands are influenced by the size, shape, morphology, composition and dielectric environment of the prepared nanoparticles [27]. Previous studies have shown that the spherical AgNPs contribute to the absorption bands at around 400–420 nm in the UV-visible spectra [27, 28]. These absorption bands were assumed to correspond to the AgNP's extra-fine nature, with relatively small size.

#### FT-IR spectroscopy analysis

FT-IR spectrum recorded from the film of silver nanoparticles formed after 24 h of incubation with the protein molecules. The bands seen at 3,280 and 2,924  $\text{cm}^{-1}$  were assigned to the stretching vibrations of primary and

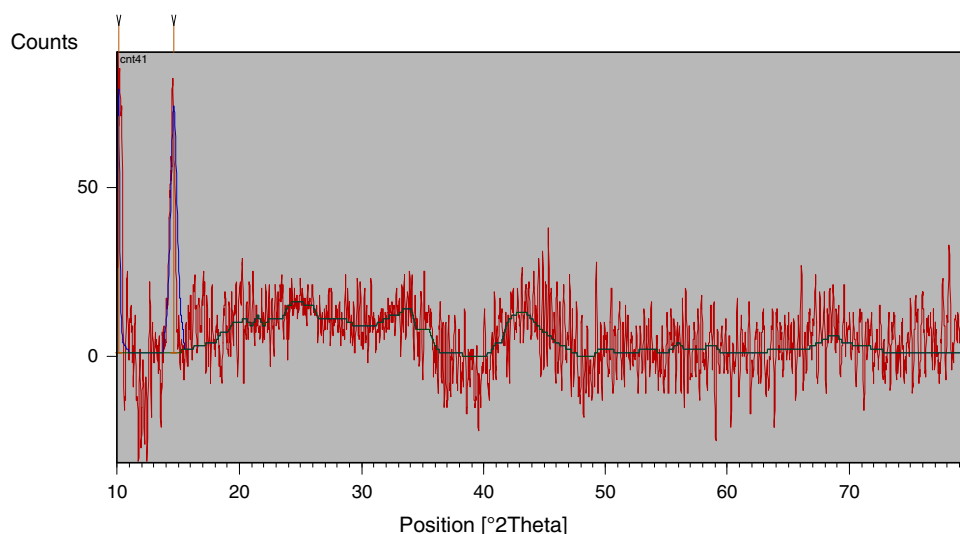
secondary amines, respectively. The corresponding bending vibrations were seen at 1,651 and 1,548  $\text{cm}^{-1}$ , respectively. The two bands observed at 1,379 and 1,033  $\text{cm}^{-1}$  can be assigned to the C–N stretching vibrations of aromatic and aliphatic amines, respectively (Fig. 3). The presence of protein as a stabilizing agent which surrounds the silver nanoparticles. It is reported earlier that proteins can bind to nanoparticles either through free amine groups or cysteine residues in the proteins and via the electrostatic attraction of negatively charged carboxylate groups in enzymes present in the cell wall of mycelia. The overall peaks from FT-IR observation confirm the presence of protein in the samples of silver nanoparticles. It can be assumed that nitrate reducing

plant leaf provides nitrate reductase enzyme (protein). The silver ions were reduced in the presence of nitrate reductase, leading to the formation of silver nanoparticles and stabilized by capping peptide.

#### XRD analysis

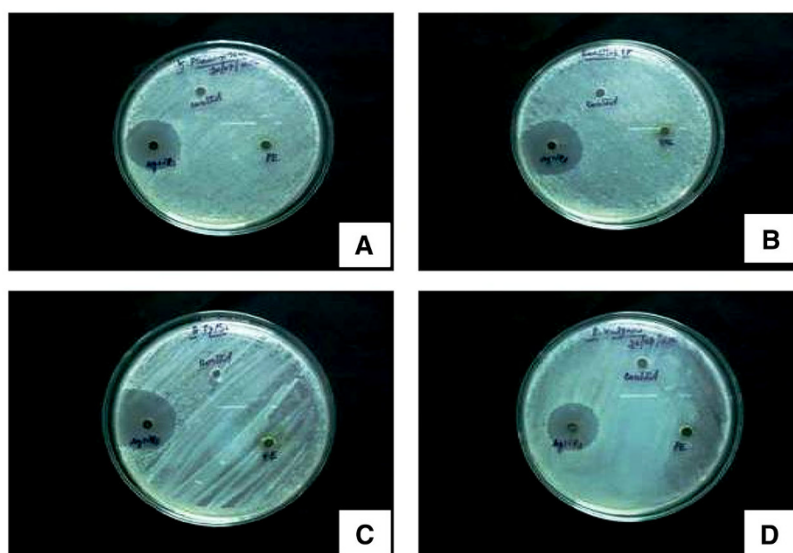
The XRD pattern of synthesized silver nanoparticles using *A. indicum* (L.) Sweet leaf extract was recorded and typical XRD pattern is shown (Fig. 4). The peaks are indexed as  $2\theta$  positions, 10.0884 and 14.6254 plans of silver by comparing with JCPDS data. Apart from these peaks responsible for silver nanoparticles, the recorded XRD pattern shows

**Fig. 4** XRD pattern of silver nanoparticles



**Fig. 5** Screening of anti-bacterial activity

### Anti-Bacterial Activity against Pathogenic Microorganisms by using synthesis of Silver nanoparticles

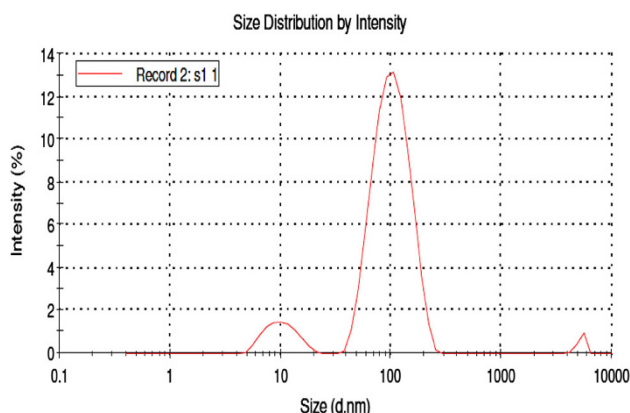


**A.** *Klebsiella pneumoniae* **B.** *Bacillus subtilis*  
**C.** *Salmonella typhi* **D.** *Proteus vulgaris*

**Table 1** Anti-bacterial activity against different pathogenic bacterial strains

Bacterial strains	Zone of inhibition (mm)
<i>Klebsiella pneumonia</i>	23.00 ± 0.57
<i>Bacillus subtilis</i>	26.67 ± 0.88
<i>Salmonella typhi</i>	<b>32.00 ± 1.20</b>
<i>Proteus vulgaris</i>	22.67 ± 0.60

Bold values indicate the highest zone of inhibition ± standard error

**Fig. 6** Particle size analysis

additional unassigned peaks. This may be due to the formation of the crystalline bio-organic compounds/metallo proteins that are present in the *A. indicum* (L.) Sweet leaf broth. Similar observations were reported for the silver nanoparticles synthesized using *P. graveolens* leaf extract [29]. The detailed investigation on this crystalline phase existing with the silver nanocrystals is in progress.

#### Anti-bacterial activity

The anti-bacterial activity of silver nanoparticles is reported to a large extent. The silver nanoparticles are synthesized and also show anti-bacterial activity against four strains of laboratory pathogens viz. *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi*, and *Bacillus subtilis* (Fig. 5). The zone of inhibition measured is summarized in Table 1. From the table, it is proved that the nanoparticles synthesized are good candidates their usage as and/or in anti-bacterial drugs. Each experiment was performed for three times. The data shown represent the mean ± SE. The data were analyzed statistically using SPSS (version-16) software.

#### Particle size analysis

The particles size analysis of silver nanoparticles of *A. indicum* (L.) Sweet (Fig. 6). The sizes of particle in diameter 106 nm were noticed.

## Conclusion

We have prepared silver nanoparticles using leaf extracts of *Abutilon indicum* L. Sweet, a plant abundantly found in Asia, as reducing agent. *A. indicum* (L.) Sweet has more medicinal properties, especially in anti-microbial activity. The above plant source involved nitrate reduction with help of nitrate reductase. This plant material released extracellular protein molecules. It acts as template for the silver nucleation sites for the formation of nanocrystals. FT-IR reveals that protein molecules can be bound to nanoparticles and XRD result confirmed silver crystal structure. The plant source may act as capping agents (peptides or proteins) which control the size and shape of silver nanoparticles. On the basis of our results, leaf protein molecules play a vital role in reducing and capping of silver nanoparticles.

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