

ANTIBACTERIAL ACTIVITY OF GREEN SILVER NANOPARTICLES SYNTHESIZED FROM DRIED SENNA AURICULATA LEAF EXTRACT

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Abstract

Silver nanoparticles were synthesized using Silver Nitrate as a precursor and leaf extract of *Senna Auriculata* as stabilizing and reducing agents. Different concentration of Silver Nanoparticles were prepared from concentration (1mM, 2mM, and 3mM) with 20 ml of leaf extract. These prepared silver nanoparticles were characterized using UV-Visible, FT-IR, SEM, EDAX and XRD. The possible biochemical mechanism leading to the formation of silver nanoparticles was studied using FTIR. Antibacterial activity of silver nanoparticles were evaluated against *Serratia Marcens*, *Pseudomonas aeruginosa*, *Staph albus* and *Bacillus cereus* bacteria.

Keywords: *Senna Auriculata*, Ag nanoparticles, Nanomaterial, Green process, Antibacterial.

INTRODUCTION

Nanotechnology is one of the most interesting field which is used to describe the creation and utilization of materials with structural features between of atoms and bulk materials with at least one dimension in the nano range [1]. Silver nanoparticles have gained much popularity on account of their broad spectrum of antimicrobial activity and their surface plasmon resonance effects [2]. Silver nanoparticles have been extensively applied in surgical instruments, wound dressings, bond prostheses, electronics and bio sensing [3]. In addition, Silver nanoparticles are used in on the surface of various textiles, laundry additives, room sprays, and food storage containers.

Physical and chemical approaches [4] are being used extensively for production of metal and metal oxide nanoparticles. However, this production requires the use of very reactive and toxic reducing agents such as sodium borohydride and hydrazine hydrate, which cause undesired detrimental impacts on the environment, plant, animal life it supports. Various organism act as clean, eco-friendly [5] and sustainable precursors to produce the stable and well functionalised nanoparticles. These may include biomolecules such as plant extract [6], bacteria [7], and fungi, yeast viruses etc. The plant leaves *Senna Auriculata*, also known as Tanners Senna, also known as Avaram in Tamil is a shrub that belongs to the Caesalpiniaceae family and is distributed throughout hot deciduous forests of India [8].

The plant has been reported to possess antipyretic, hepatoprotective, antidiabetic, antiperoxidative and microbicidal activity. The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation. Bindhu *et.al* [9] synthesized that silver nanoparticles using *Hibiscus cannabinus* leaf extract, the prepared silver nanoparticles were monodispersed, spherical in shape with the average particle size of 12 nm and appears surface Plasmon peak at 446 nm. These nanoparticles shows good antimicrobial activity against *Escherichia coli*, *Proteus mirabilis* and *shigella flexneri*.

Plant extract has been used as reducing and capping agent for the synthesis of nanoparticles which could be advantageous over microbial synthesis because there is no need of the elaborated process of culturing and maintaining the cell. Plant leaf extract of *Ficus benghalensis* [10], Germanium leaf [11] and *Annona Squamosa* leaf [12] had been used for synthesis of silver nanoparticles, which lead to formation of pure metallic nanoparticles of silver and can be used directly. In this study, we have synthesized silver nanoparticles with the help of *Senna Auriculata* leaf extract by reduction of Ag^+ to Ag^0 from silver nitrate solution and investigated the antibacterial activity of nanoparticles against two gram positive and two gram negative bacteria, were evaluated by surface inoculation method.

MATERIALS AND METHODS

Preparation of leaf extract from *Senna Auriculata*

Silver nitrate, Double distilled water were purchased from SA Chemicals, Tirunelveli. *Senna Auriculata* leaves were collected from Nagercoil town. The *Senna Auriculata* leaves were collected and washed with distilled water. Leaves are allowed to shade dry at room temperature for 20 days to get fine powered. Take 20g of leaf powder in 250 ml beaker and it was kept at 60°C on magnetic stirrer. The extract was filtered and stored.

Preparation of green silver nanoparticles

Preparation of Silver nanoparticles was carried out using Silver Nitrate. 1mM of $AgNO_3$ was taken in a standard flask and makeup into 100ml. This setup was stirred at 70°C on magnetic stirrer for half an hour. Add 20ml of prepared *Senna Auriculata* leaf extract in burette and added drop wise in beaker. The color change from pale yellow to black precipitate indicates the presence of silver nanoparticles. The solution allowed to cool and centrifuged 7000 rpm, Nanoparticles are washed with deionized water and are allowed to dry oven at 90°C for 3 hours.

Characterization studies

Silver Nano particles were characterized by using UV-Visible, FT-IR, X-Ray diffraction (XRD) analysis and Scanning Electron Microscope (SEM). The functional group present in Silver nanoparticles were determined by using FT-IR spectroscopy. XRD is used to study phase composition of a sample and crystal structure. Scanning Electron Microscopy is used to determine particle size and distribution. Antibacterial activity against gram positive, gram negative bacterial strains, *Proteus* by surface inoculation method.

RESULT AND DISCUSSION

UV-Vis Spectroscopy

Absorption spectroscopy is a main tool to analyze the noble metal nanoparticles formation primary method to indicate the bio reduction of silver from aqueous silver nitrate solution to silver nanoparticles. 1 mM concentration of Silver nitrate was taken for the reduction of silver by *Senna Auriculata* leaf extract. Addition of silver nitrate solution reacts with leaf extract of *Senna Auriculata*, the intensity of color converted from pale yellow to black color was expected to the surface Plasmon resonance phenomenon. Silver nanoparticles were observed around in 400-430nm [13]. Silver nanoparticles prepared from leaf extract of *Senna Auriculata* were observed around in 416 nm. The reaction was maximum for 72 hours incubation.

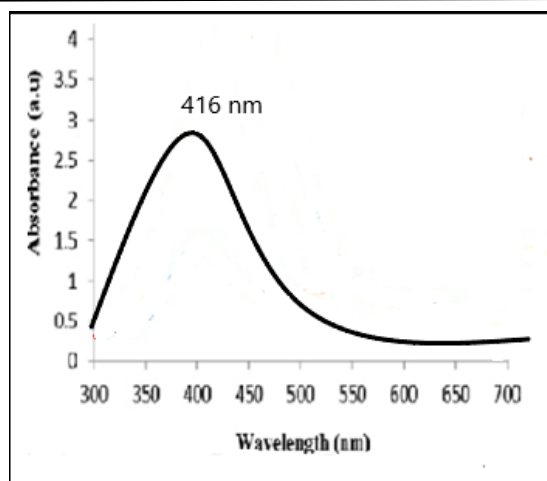


Figure 1: UV-Visible spectrum of Silver nanoparticle

FT-IR

FT-IR gives the information about functional groups present in the silver nanoparticles. A broad peak at about 3387 cm^{-1} due to the stretching vibration of hydroxyl group. The band at 2924 cm^{-1} attributed to the symmetric and asymmetric C-H Stretching vibration of aliphatic acids [14], The peak shift from 2367 to 2266 cm^{-1} becoming weak and the disappearance of 1720 cm^{-1} after the reaction with silver nitrate implicate that these groups may be involved in the process of reducing Ag^+ ions causing them to get oxidized to carboxylic acids. The absorption band at 1612 cm^{-1} can be attributed to C=C stretching vibrations of aromatic ring. The peak at 1381 cm^{-1} can due to O-H bending vibrations of polyols such as flavonoids present in the leaf.

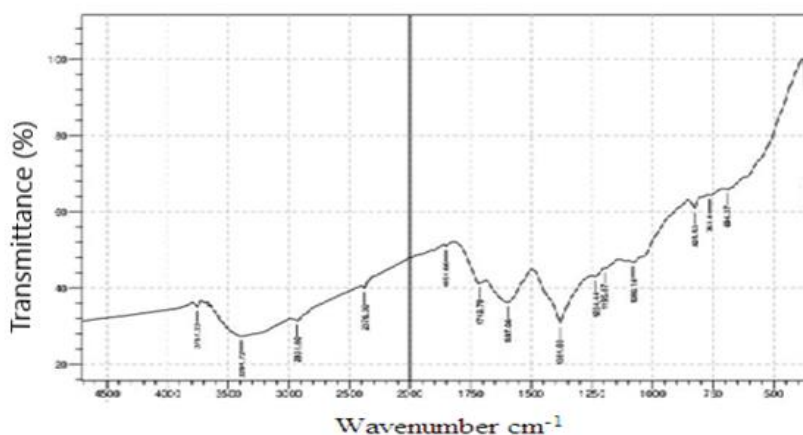


Figure 2: FT-IR spectrum of Silver nanoparticles

The band at 1234 cm^{-1} which is due to the C-O-H bending vibrations. The bands at $800\text{-}600\text{ cm}^{-1}$ region correspond to C-H out of plane bend which are characteristic of aromatic phenols. The peak located at 1051 cm^{-1} may be due to C-OH of carboxylic acids which shifts to 1032 cm^{-1} representing the involvement of these functional groups in the nanoparticle synthesis. The FT-IR spectra indicate that the functional groups aldehyde, ketone, carboxylic acid and phenol may be responsible for the reduction and stabilizing of silver nanoparticles.

X-Ray Diffraction

A XRD Profile of biosynthesized silver nanoparticles is shown in figure 3. The diffraction peaks

observed at 38.25° , 44.39° , 64.72° , 77.55° corresponds to the crystal lattice plane of (111), (200), (220) and (311), peaks which were due to the presence of biomolecules in the nanoparticles. The prominent peaks are observed at 38.25° , 44.39° , 64.72° , 77.55° matches the plane value of (111), (200), (220) and (311), which was in crystalline nature and further on the basis that can be revealed as FCC structure of silver [14]. The average crystallite size of synthesized silver nanoparticles is calculated by Scherrer equation and found be 34.60 nm.

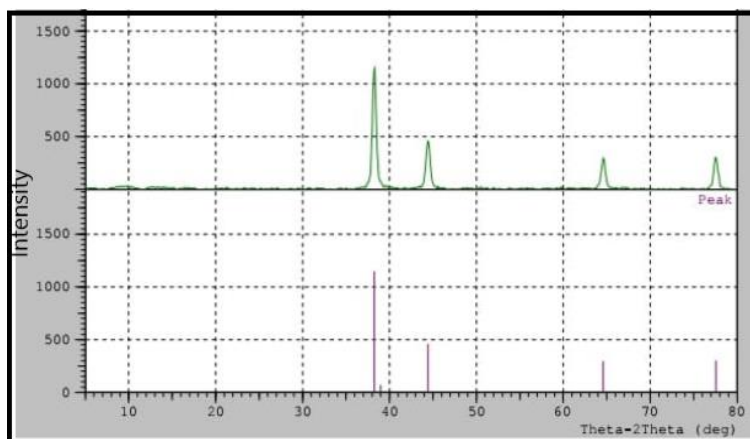


Figure 3: XRD spectrum of Silver nanoparticle

SEM

SEM analysis were employed to indicate the size and shape of the nanoparticles. SEM micrographs of silver nanoparticles are given in figure.4. It was observed that the silver nanoparticles are agglomerated spherical in shape with a uniform size about 25-35 nm. The particle size attained from SEM images is well correlated with the particle size resolute from XRD using confer to the Scherrer formula and the average of the synthesized nanoparticles was in the range of 25-35 nm.

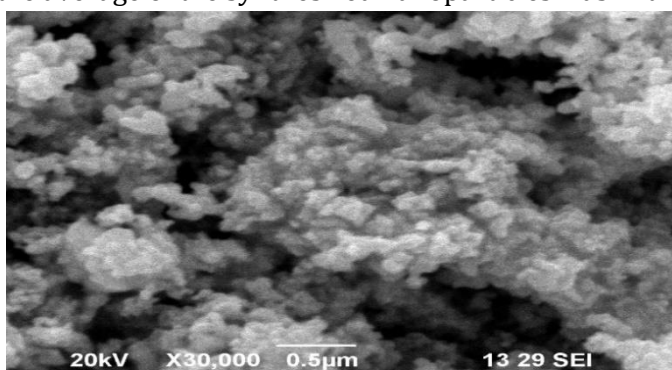


Figure 4: SEM images of Silver nanoparticle

EDAX

The result of Energy dispersive X-ray analysis gives a clear idea about the elements present in the biosynthesized nanoparticles. The EDS spectrum of silver nanoparticles prepared from dried leaf extract of Senna Auriculata as shown in figure 5. The presence of Ag nanoparticles formed using dried Senna Auriculata leaf extract showed strong signal energy peaks for silver atoms in the range 2-4 Kev [15] and 94% of Ag atom present by using these leaf extract which indicates the reduction of silver ions to elemental silver. In general, metallic silver nanoparticles show common optical absorption peak at 3 Kev.

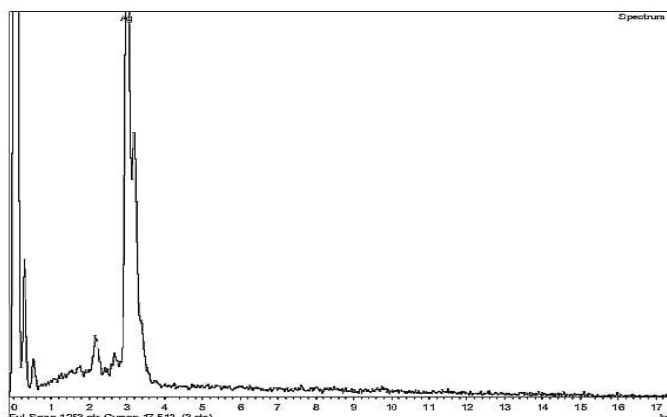


Figure 5: SEM images of Silver nanoparticle

PHYTOCHEMICAL ANALYSIS OF SENNA AURICULATA LEAF EXTRACT

Biochemical screening results showed that steroids, Tannins, Sugar, phenol, flavonoids were present in the dried leaf extract of Senna Auriculata. This can be attributed to the absorption of phenolic compound such as tannic acid product on the silver nanoparticles surface which may be responsible for the capping and particles compensation. The present study suggests the role of phenols in the reduction of Ag ions to silver nanoparticles.

ANTIBACTERIAL ACTIVITY

Silver nanoparticles was one of the most important antibacterial substances [16]. Antibacterial activity was investigated against *Serratia Marcens* (G-) *Pseudomonas aeruginosa* (G-) *Staph albus* (G+) *Bacillus cereus*(G+) for silver nanoparticles prepared from Senna Auriculata leaf extract in Ethanol medium and Silver nanoparticles in Acetone medium were evaluated by well diffusion method. Zone of inhibition of Senna Auriculata leaf extract also examined for all four bacterial strains. The maximum zone of inhibition were took place in the *Pseudomonas aeruginosa* (G-) as 18 mm. The diameter of inhibition zones around each well with silver nanoparticles and plant extract for both strains represented in Table. 1.

Silver in ethanol medium selected gram negative bacteria showed higher shown of inhibition compared to selected gram positive bacteria. Senna Auriculata leaf extract itself had the ability to resist bacterial growth, which is shown in Table 1.leaf extract are found to have highest antimicrobial activity when compared to silver nanoparticle. Amikacin were used in control against both strains.

Table 1. Antibacterial activity of the Silver nanoparticle

Samples	Ag Ethanol	Ag Acetone	S.A Leaf
<i>Serratia Marcens</i> (G-)	16	14	10
<i>Pseudomonas aeruginosa</i> (G-)	18	12	11
<i>Staph albus</i> (G+)	15	14	9
<i>Bacillus cereus</i> (G+)	12	13	8

These results can be interpreted on the basis of all bacterial action of silver nanoparticles on their size, quantity and arrangement. Thus, decreases in the silver nanoparticles size can lead to an

increase in ability to enter cell membrane and thus improving the antibacterial activity. Silver nanoparticles showed efficient antimicrobial activity when compared to the other salts to extremely large surface area, which provides better contact with pathogens.

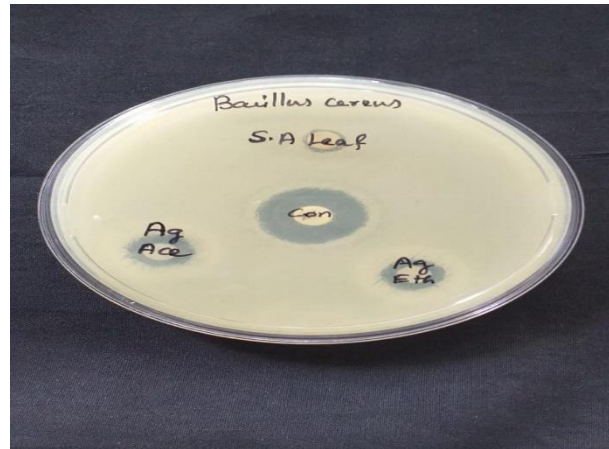


Figure 6: Sensitivity of AgNPs against *Bacillus cereus* (G+)

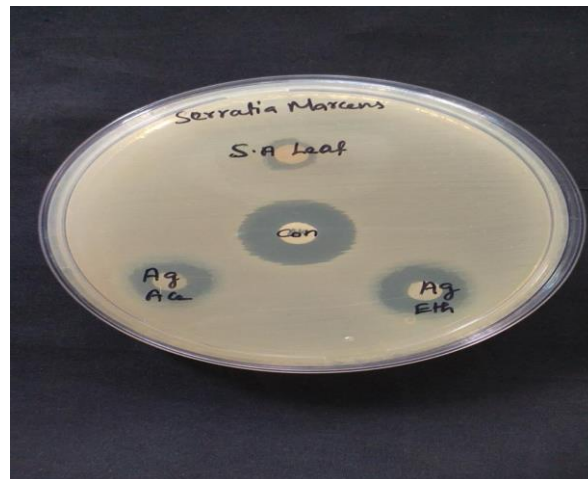


Figure 7: Sensitivity of AgNPs against *Serratia Marcens* (G-)

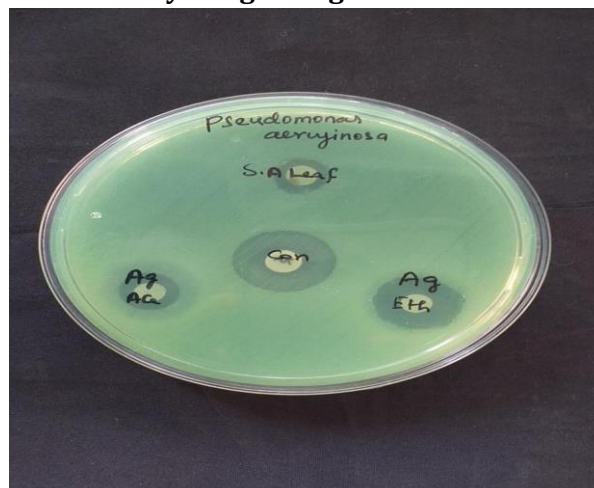


Figure 8: Sensitivity of AgNPs against *Pseudomonas aeruginosa* (G-)

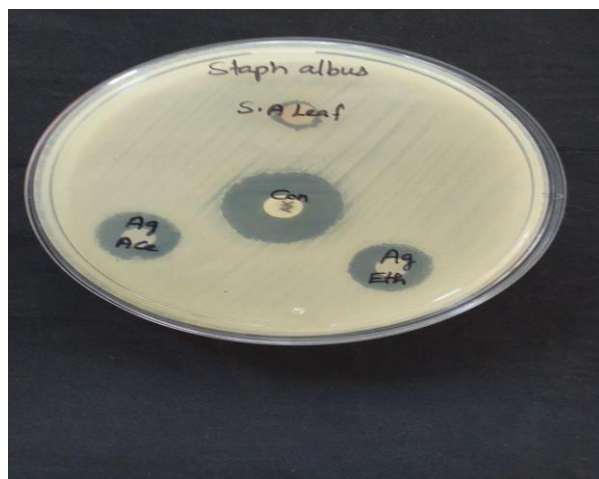


Figure 9: Sensitivity of AgNPs against *Staph albus* (G+)

CONCLUSION

The green synthesis of Silver nanoparticles has been achieved using the bio reducing agent *Senna Auriculata* leaf extract. Antibacterial for silver nanoparticles in acetone medium and Silver nanoparticles in ethanol medium by well diffusion method. The maximum zone of inhibition were took place in the *Pseudomonas aeruginosa* (G-) as 18 mm. Silver in ethanol medium, selected gram negative bacteria showed higher shown of inhibition compared to selected gram positive bacteria. *Senna Auriculata* leaf extract itself had the ability to resist bacterial growth as 14 mm in *Serratia Marcens* (G-) and *Staph albus* (G+).

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