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Original Article

ANTIBACTERIAL ACTIVITY OF BIOLOGICALLY SYNTHESIS SILVER NANOPARTICLES FROM LEAF EXTRACT OF *ALANGIUM SALVIFOLIUM* (L.F) WANG

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ABSTRACT

Biologically Synthesis process of silver nanoparticles using plant extract is simple, cost-effective, and ecofriendly. In the present study, silver nanoparticles (AgNPs) were rapidly synthesized using *Alangium salvifolium* leaf extract. The nanoparticles obtained have been characterized with various techniques like UV-Visible spectrum, Scanning Electron Microscopy (SEM), Energy Dispersive Spectroscopy (EDAX), X-ray Diffraction (XRD) and Atomic Force Microscopy (AFM) and also tested antibacterial. These techniques showed the formations of AgNPs with an average size of 24.2 to 52.5 nm and also find the spherical shaped nanoparticles. The synthesized AgNPs had the potential to mitigate the bacterial proliferation against *Staphylococcus aureus, Pseudomonas auroginosa, Bacillus thuringiensis, Escherichia coli, Klebsiella pneumonia* and *Salmonella typhi*. The results were compared with the Ciprofloxacin as positive controls. It is concluded that the *Alangium salvifolium* is one of the best sources for synthesis of AgNPs and also showed good anti bacterial activity.

Keywords: *Alangium salvifolium*, AgNPs, Leaf extract, Antibacterial activity.

INTRODUCTION

Silver has been special place in the history of the elements because it is one of the first five metals discovered and used by humans. Archaeological evidence suggests that people have been using silver for at least 5000 years. Silver is the best conductor of heat and electricity of all known metals, so it is sometimes used in making solder, electrical contacts and printed circuit boards. Silver has also been used to create coins, although today other metals are typically used in its place. Sterling silver, alloy containing 92.5% silver, is used to make silverware, jewelry and other decorative items. Silver has been recognized as having an inhibitory effect towards many bacterial and fungal strains. The most widely used and known application of silver are in the medical industry. In addition, silver containing consumer products are now used in sporting equipment (1). Nanoparticles usually referred as particles with a size up to 100 nm and it exhibit completely new or improved properties based on specific characteristics such as size, distribution and morphology. Nanoparticles play an indispensable role in drug delivery, diagnostics, imaging, sensing, gene delivery, artificial implants and tissue engineering (2). Recently, biosynthetic methods have been investigated as a new way for the production of AgNPs. Biological methods are currently gaining importance because they are eco-friendly, cost effective and do not involve the use of any toxic chemicals for the synthesis of nanoparticles (3). Silver nanoparticles are known for drug delivery, Food industries (4) as on anti-inflammatory (5), antiviral activity (6), antimicrobial (7), anti-cancer (8), antiarthritic activity (9) and larvicides (10). The green synthesis of AgNPs has been reported using the extract of plants such as *Svensonia hyderobadensis* (11), *Murraya koenigii* (12), *Punica granatum* (13) *Abrus precatorius* (14), and *Salicornia brachiata* (15).

Alangium salvifolium (L.f) Wang belongs to family Alangiaceae, it is a medium seized tree and the tree is called as 'Ankol' by the local people this is thorny tree distributed in dry regions in plains and lower hills of Central India. It grows to a height of about 3 to 10 meters (16). The plant is used in traditional medicinal practices. It is used in Ayurveda for the treatment of rheumatism and hemorrhoids. It is a popular folk medicine and has been studied for its anti-inflammatory, antimicrobial, antifertility and cardiotonic activities (17). Its dried seeds, has traditionally been used to treat various ailments in Asia (18). Traditionally, *Alangium salvifolium* seeds have been reported to exhibit a variety of biological activities, including antidiabetic, anticancer, diuretic, anti-inflammatory, antimicrobial, laxative, and antiepileptic activity (19-20). Root is used in diarrhea, paralysis, piles and vomiting (21). They are acrid, astringent, emollient, anthelmintic, thermogenic diuretic and purgative. Root is useful for external application in acute case of rheumatism, leprosy and inflammation and internal application in cases of bites of rabbit and dogs (22). Antibacterial compound was isolated from the flower of *Alangium salvifolium* (23).

The present study, we have explored the *Alangium salvifolium* for green synthesis of AgNPs by using its leaves and characterized these nanoparticles with SEM, EDAX, XRD, AFM and UV-Vis Spectroscopy. Furthermore, the synthesized AgNPs were evaluated for antibacterial activity.

MATERIALS AND METHODS

Preparation of plant extract

Alangium salvifolium leaves were collected from S.V.U. Botanical Garden, Tirupati, Andhra Pradesh, India. The leaves were washed thoroughly thrice with distilled water and shade dried for 10 days. The fine powder was obtained from dried leaves by using kitchen blender. The leaf powder was sterilized at 121^{0}C for 5 min. 5 g of powder was taken into a 250-ml conical flask and 100 ml of sterile distilled water and boiled for 15 min at 100^0 C. Then the leaf extract was collected in a separate conical flask by a standard filtration method.

UV-Vis Spectra analysis

The reduction of pure silver ions was monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot analysis was done by using UV-Vis spectrophotometer UV-2450 (Shimadzu).

X-ray diffraction (XRD) analysis

The practice size and nature of the silver nanoparticle were determined using XRD. This was carried out using Shimadzu XRD-6000/ 6100 model with 30 kV, 30 mA with Cuk $\propto$ radians at 2θ angle. X-ray powder diffraction is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analyzed material is finely ground – and average bulk composition is determined. The particle or grain size of the particles on the silver nanoparticles was determined using Debye Sherrer's equation.

$D = k\lambda / \beta (Cos\theta)$

SEM analysis of silver nanoparticles

Scanning Electron Microscope (SEM) analysis was carried out by using Hitachi S-4500 SEM Machine. Thin films of the sample were prepared on a carbon coated copper grid by just dropping a very small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry.

EDAX measurements

In order to carryout EDAX analysis, the drop of bark extract with reduced silver nanoparticles was dried on coated with carbon film and performed on Hitachi S-3400 N SEM instrument equipped with thermo EDAX attachments.

AFM measurements

The silver nanoparticles extracted through above protocol were visualized with an Atomic Force Microscope.

A thin film of the sample was prepared on a glass on the slide was allowed to dry for 5 min, the slides were then scanned with the AFM (Nano Surf® AG, Switzerland, and Product: BTO 2089, BRO).

Antibacterial activity

The clinical pathogenic strains of *Staphylococcus aureus*, *Pseudomonas aeuroginosa*, *Escherichia coli*, *Bacillus thuringiensis*, *Klebsiella pneumonia* and *Salmonella typhi* disc diffusion method was carried out by using Standard protocol (24).

Overnight bacterial cultures (100 µL) was spared over Muller Hinton Agar (Hi Media Laboratories Private Limited, Mumbai, India) plates with a sterile glass L-rod. 100 µL each extracts were applied to each filter paper disc Whatman No. (5 mm dia) and allowed to dry before being placed on the agar. Each extract was tested to triplicate and the plates were inoculated at 37^{0}C for 24 hours after incubation. The diameter of inhibition zones were measured and tabulated.

Fig. 1: Synthesis of SNPs: a. Plant extract, b. Plant extract with silver nitrate (SNPs) the color change of leaf extract of *Alangium salvifolium*

Table 1: Antibacterial activity of *Alangium salvifolium*

S. No.	Organisms	SNPs	Plant extract	$AgNO_3$	Control
1.	*Salmonella typhi*	12.3 ± 0.13	5.1 ± 0.11	12.0 ± 0.12	35.5 ±0.15
2.	*Bacillus thuringiensis*	15.4 ± 0.11	6.3 ± 0.10	15.3 ± 0.10	40.4 ± 0.10
3.	*Escherichia coli*	15.5 ± 0.12	6.5 ± 0.12	12.4 ± 0.14	34.5 ± 0.15
4.	*Pseudomonas auroginosa*	16.8 ± 0.16	5.4 ± 0.11	14.5 ± 0.12	57.4 ± 0.18
5.	*Klebsiella pneumonia*	19.5 ± 0.14	5.3 ± 0.14	12.0 ± 0.10	38.4 ± 0.14
6.	*Staphylococcus aureus*	10.6 ± 0.10	6.5 ± 0.11	18.6 ± 0.17	50.5 ± 0.10

Note: ± indicates standard deviation

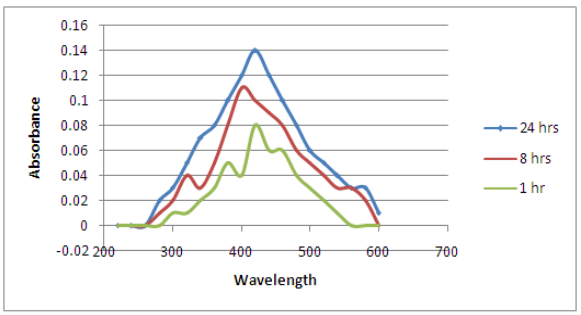


Fig. 2: UV-Visible spectroscopy of synthesized silver nanoparticles of *Alangium salvifolium* leaf extract

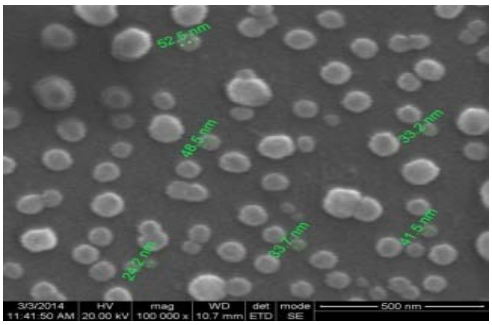


Fig. 4: SEM images of synthesized silver nanoparticles from *Alangium salvifolium* leaf extract

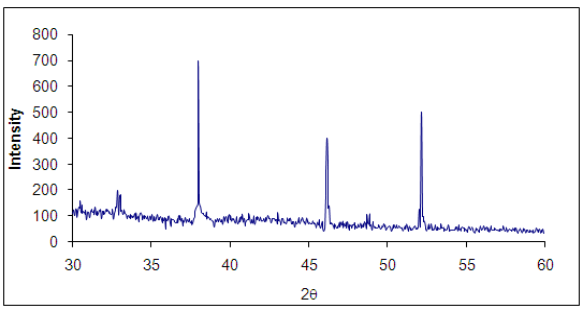


Fig. 3: XRD image of Synthesis of SNPs from leaf of *Alangium salvifolium*

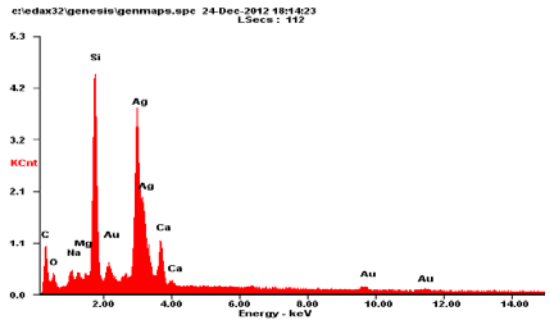


Fig. 5: EDAX information of synthesized silver nanoparticles from *Alangium salvifolium* leaf extract

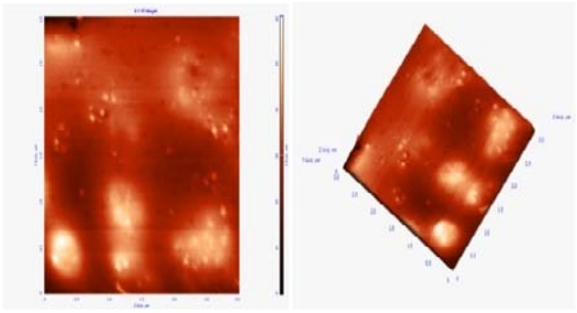
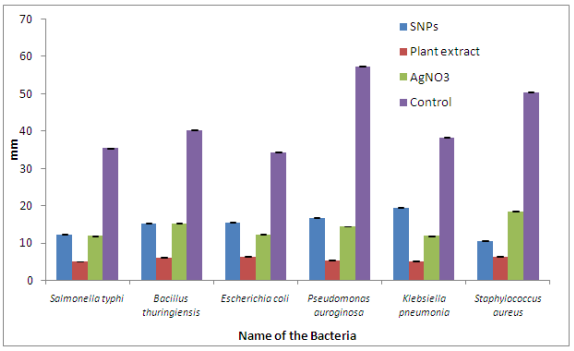


Fig. 6: AFM image of leaf AgNPs of *Alangium salvifolium*



Graph 1: Antibacterial activity of *Alangium salvifolium*

RESULTS AND DISCUSSION

The colour of the freshly prepared aqueous extract obtained from the leaf of *Alangium salvifolium* changed, when added the silver nitrate solution. The appearance of a brown colour indicated the reduction of silver ion to metallic silver nanoparticles (25). The signatory brown colour was obtained, which resulted due to the excitation of surface plasmon resonance vibrations of the silver nanoparticles formed (26). Surface Plasmon are create on the boundary of metal, these represent quantized oscillations of surface charge produced by an external electric field (27). Due to SPR, the AgNPs showed yellowish to brown colour (fig-1). The formation and stability of the reduced silver nanoparticles in the colloidal solution was monitored by a UV–Vis spectrophotometer. The intensity of the biosynthesized AgNPs showed increased absorbance in various time intervals of 1h, 8h, and 24 h. A strong, broad peak was observed between 415, 429 and 440 nm. The increase in the intensity may have been due to the excitation of SPR and the reduction of $AgNO_3$, Where the increasing time interval of 1h, 8h and 24 h is well documented by various metal nanoparticles. The particle increase in size, the absorption peak usually shifts towards red wavelengths (28). Increase of absorption indicates that amount of silver nanoparticles increase, the stable position of absorbance peak indicates that new particles do not aggregated. The AgNPs of *Alangium salvifolium* the stable absorption peak at 440 nm on 24 h in travels (Fig-2).

XRD is commonly used for determining the chemical composition, crystal structure and purity of a material. Fig-3 shows the XRD pattern of the AgNPs synthesized using the *Alangium salvifolium* leaf extract. The observed peaks confirm that the obtained silver nanoparticles exist in a crystalline phase. A number peaks occur at 38^0, 46^0, 52^0 2θ values due to reflections from the 111, 200, 220 plans respectively. This can be indexed to face centered cube silver. Therefore, detecting the presence of silver nanoparticles in plants extracts can be achieved by using XRD to examine the diffraction peaks of the plant (29). The X-ray diffraction results clearly show that the silver nanoparticles formed by the reduction of Ag^+ ions by the *Alangium salvifolium* are crystalline in nature. Scanning Electron Microscopy (SEM) provided further insight into the morphology and size details of the silver nanoparticles. The results showed that the diameter of prepared nanoparticles was about 24.2 to 52.5 nm and shape was spherical as shown in fig-4. Energy dispersive X-ray spectrometer (EDAX) was employed to determine the silver concentration of the nanoparticles. AgNPs generally show a typical absorption peak at approximately 3 keV due to the surface plasma resonance phenomenon. From the EDS analysis (Fig- 5), the distinct peak detected at 3 keV confirmed the presence of elemental silver in the nanoparticles. AFM analysis the AgNPs was clearly distinguishable owing to their size difference (Fig-6). AFM images have given average sizes of AgNPs of *Alangium salvifolium* is 38 nm with three dimensional structures.

Antibacterial activity

The application of silver nanoparticles as an antibacterial agent was investigated and exhibited better antibacterial activity against all human pathogens. In the present study the antibacterial activity of AgNPs was carried out against various pathogenic microbes such as gram negative and gram positive bacteria of *E. coli, K. Pneumoniae, P. vularis, P. aeruginosa, B. subtiles* and *S. typhi* by using disc diffusion method. The extraction without silver nanopartices served as control. The leaf aqueous extract of *A. salvifolium* showed broad spectrum of antibacterial activity. The diameter of inhibition zone around each disc with AgNPs is represented and each disc contains of 10 µl at AgNPs solution. The AgNPs of *Alangium salvifolium* shows highest antibacterial activity was observed against *Klebsiella* followed by *Pseudomonas, E. coli, Bacillus* and *Salmonella* and lowest inhibition show against *Staphylococcus* (Table-1 and Graph-1)

The mechanism of inhibitory action of silver nanoparticles on microorganisms is not very well known. However, several mechanisms have been proposed to explain the inhibitory effect of silver nanoparticles on bacteria. It is assumed that the high affinity of silver towards sulfur and phosphorus is the key element of the antimicrobial effect. Due to the abundance of sulfur containing proteins on the bacterial cell membrane, silver nanoparticles can react with sulfur-containing amino acids inside or outside the cell membrane, which in turn affects bacterial cell viability. It was also suggested that silver ions (particularly Ag+) released from silver nanoparticles can interact with phosphorus moieties in DNA, resulting in inactivation of DNA replication, or reacting with sulfur containing proteins, leading to the inhibition of enzyme functions which results in loss of cell viability and eventually resulting in cell death (30-31).

CONCLUSION

Alangium salvifolium leaves are good source for synthesis of silver nanoparticles and also showed the good antibacterial activity against several human pathogens.

CONFLICT OF INTERESTS

Declared None

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