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AgNPs Synthesis, Characterization and Antibacterial Activity from *Salvia splendens* Sellow ex Roem. & Schult. Plant Extract

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Abstract: ***In recent years green synthesis of silver nanoparticles has been increased for diverse applications. In this study we proposed lamiaceae family plant of Salvia splendens Sellow ex Roem. & Schult. for synthesis of silver nanoparticles and their efficacy in antibacterial activity against four human pathogens. The direct method was chosen for silver nanoparticles synthesis using water extract of S. splendens. Green synthesized silver nanoparticles were characterized by UV-Vis spectroscopy, FT-IR, SEM, EDS and XRD. The UV-Vis spectra depicts surface Plasmon resonance peak at 437 nm. The three dimensional structure of silver nanoparticles was seen through SEM analysis. The observed peaks in FT-IR showed functional groups and the stretch of bonds that responds to nanoparticles synthesis. The XRD analysis showed that the AgNPs are crystalline in nature and have face-centered cubic geometry. The synthesized nanoparticles showed noteworthy antibacterial activity against Bacillus, Proteus vulgaris, Bacillus subtills and Staphylococcus aureus. This work provides a direct route of synthesis of environment benign silver nanoparticles via medicinal plant material.***

Keywords: AgNPs, *Salvia splendens*, FT-IR, SEM, XRD

1. Introduction

Plants have been provided a wide range of natural products with diverse chemical structures and most of them having of biological activities. Indeed, many of which have found applications in the health care processes. Plant based drugs have been afforded the challenge of developing syntheses of many bioactive compounds with structural complexity and the resulting multi-step syntheses rarely find application in large scale production as required in the pharmaceutical drug industry. Last century 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources. Plant derived drugs came into use in the modern medicine through the uses of plant material as indigenous cure in folklore or traditional systems of medicine (Chopra *et al.*, 1956).

The *Salvia* genus belongs to the Lamiaceae (mint) family and its covers about 900 species are worldwide dispersed (Delamere et al., 2007; Mossi *et al.*, 2011). Salvia species are mostly utilized for their essential oils in the foods, medicines and perfumery industries (Goren *et al*., 2006; Ozcan *et al*., 2003; Ulubelen and Topcu, 1998).

An eco-friendly route for the synthesis of is sliver nanoparticles using *Agaricus bisporus* (white button mushroom) extract. The antibacterial activity of synthesized silver nanoparticles showed effective inhibitory activity against pathogenic bacterial strains like *Escherichia coli*, *Staphylococcus sps*, *pseudomonas sps*, and *Bacillus sps*. (Narasimha, 2001).

Silver nanoparticles synthesis from extract of *Sargassum tenerrimum*. The synthesized silver nanoparticles were characterized by UV-Visible Spectroscopy, Fourier-Transform Infra-red Spectroscopy (FT-IR), Transmission Electron Microscopy analysis (TEM) and Dynamic Light Scattering (DLS). Altogether, extracts from seaweed were screened for phytochemicals followed by FT-IR prediction to reveal chemical functional groups present. The results showed that the anti-bacterial activity of silver nanoparticles (Kumar *et al.*, 2012). The silver nanoparticles effect against several types of bacteria has been extensively studied (Panacek *et al.*2009).

The present study was synthesized silver nanoparticles using *S. splendens* plant extract for reduction of Ag+ ions to AgNPs from silver nitrate solution within 1 min of reaction time at 60°C temperature. Further, biosynthesized silver nanoparticles are found to be highly effective against pathogenic bacterial strains.

2. Materials and Methods

Material collection

For the present study, *S. splendens* plant was used and it was collected from the Coonoor (Tamilnadu). The entire part of the plant has been used for AgNPs synthesis.

Silver Nanoparticle Synthesis

Plant material and preparation of the Extract

Fresh plant of *S. splendens* was dried for 20 to 30 days under shadow at room temperature and ground into fine powder. 200 mg of plant powder was used to make aqueous extract using 100 ml of distilled water. It was gently heated and filtered through a filter paper.

Synthesis of Silver Nanoparticles

1mM aqueous solution of Silver nitrate ($AgNO_3$) was prepared and used for the synthesis of silver nanoparticles. 100ml of plant extract was taken in a separate conical flask and 100**µl** of $AgNO_3$ was added then heated until the colour changed from yellow to brown.

Characterization of Silver Nanoparticles

UV-spectrophotometer analysis

Bio reduction of aqueous Ag^{+} ions can easily be followed by UV–Vis spectrophotometer, and one of the most important features in optical absorbance spectra of metal nanoparticles is surface plasmon band, which is due to collective electron oscillation around the surface mode of the excitations of their surface plasmon response (SPR), when dissolved in water. The plant extract were changes from light green to brownish yellow within 5 minute at microwave temperature and then to dark brown.

FTIR Analysis

Further characterization of silver nanoparticles involved **F**ourier **T**ransform **I**nfrared **S**pectroscopy by the range 450-4000 cm^{-1} of $4cm^{-1}$. To remove any biomass residue or compound that was not the capping legend of the nanoparticles, the residual solution of 100ml after reaction was centrifuged at 10,000 rpm for 10 min and the resulting suspension was dispersed in sterile distilled water. The centrifuging and redispersing process was repeated three times. Finally the nanoparticles were analyzed by using FTIR.

SEM Analysis

SEM analysis was done using software controlled **S**canned **E**lectron **M**icroscope. The energy of electron beam current was continuously adjusted from 1 pA to 1µA to suit the type of examination in progress. Thin film of the sample was prepared on a carbon coated copper grid by just dropping very small amount of the sample on the grid. The film of the SEM grid was allowed to dry by putting it under a mercury lamp for 5 min. Then each sample was analyzed by the SEM.

Energy-Dispersive X-ray Spectroscopy (EDS)

In order to carry out EDS analysis, the plant extract reduced silver nanoparticles were dried and drop coated onto copper plate and performed on TESCAN-SEM instrument equipped with a Thermo EDS attachments.

XRD Analysis

The air dried nanoparticles were coated onto XRD grid and analyzed for the formation of silver nanoparticle by Philips X-Ray Diffractometer with Philips PW 1830 X-Ray Generator operated at a voltage of 40kV and a current of 30mA with Copper Potassium alpha radiation. The diffracted intensities were recorded from 10' to 80' of 20 angles.

Antibacterial Activity

The antibacterial assays were done on human pathogenic bacteria like *Bacillus, Proteus vulgaris, Bacilus subtills and Staphylococcus aureus* by standard disc diffusion method. Luria Bertani (LB) broth/agar media was used to cultivate bacteria. Fresh overnight cultures of inoculum (100 µl) of each culture were spread on to LB agar plates. Sterile paper discs of 5mm diameter containing 20µl, 30µl, 40µl and 50µl 1mM AgNPs and Positive Control *Ampicillin*, along with five discs were placed in each plate.

3. Results

Silver Reduction

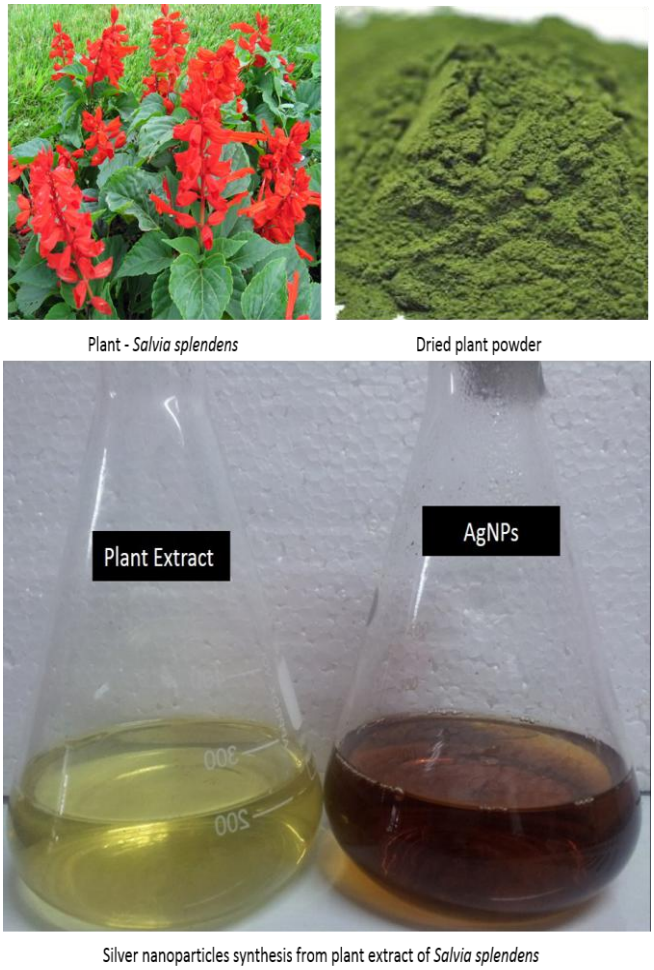
Silver nanoparticles are synthesized using plat extract showed yellowish-brown color in aqueous solution due to excitation of Surface Plasmon vibrations in silver nanoparticles (Jae and Beom, 2009). Reduction of silver ions to silver nanoparticles could be followed by a periodical color change and it was given below (Table 1& Figure 1).

Table 1: Synthesized silver nanoparticles using aqueous extract of *S. splendens* and its Periodical colour change from pale yellow to dark brown with 1mM Silver nitrate

Time	Plant sample
0-second	-
10- second	+
20- second	++
30- second	+++
40- second	+++
50- second	++++
1- minute	++++

Note: Heated at 60ºC.

- No colour change
+ Colour change
++ Pale yellow
+++ Tinge brown
++++ Brown colour



Silver nanoparticles synthesis from plant extract of *Salvia splendens*

Figure 1

UV-VIS spectral analysis of silver nanoparticles

Silver nanoparticles were synthesized using plant extract of *S. splendens* Reaction between biomolecules and silver nitrate takes place and $AgNO_3$ reduced to form silver nanoparticles. The synthesized silver nanoparticles were characterized by UV Spectrometer at the range from 300 to 700 nm. UV-Visible spectrum for synthesized nanoparticles from plant extract heated at 60°C temperature for 1 minutes and the plant sample absorption spectrums were showed at 437nm (Figure 2). In the entire control sample, peak formation was not observed. The peak formations in the samples were mainly because of the reduction of silver nitrate and the bioreduction of plant molecules in the solution. These observations clearly indicate that, the plant molecules play an important role in the reduction of silver nitrate to silver nanoparticles (Kumar *et al.*, 2012).

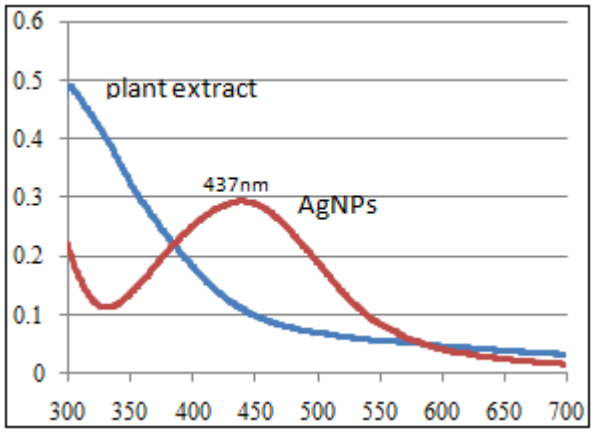


Figure 2: UV spectrum shows peaks corresponding to production of silvernanoparticles by *S. splendens* Plant extract

FTIR analysis of silver nanoparticles

FTIR analysis was carried out to identify the possible biomolecule responsible for the reduction of the silver ion and capping agent of bioreduced silver nanoparticles synthesized by the *S. splendens*. The spectral bands were interpreted for identification of functional moieties of organic compounds adhering to the silver nanoparticles (Kumar *et al.*, 2012). The FTIR spectrum of *S. splendens* (Figure 3) peaks were showed in the following table 2.

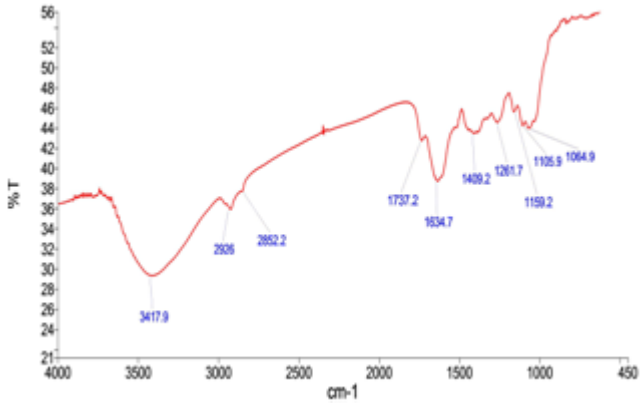


Figure 3: FTIR spectrum of synthesized silver nanoparticles using aqueous extract of *S. splendens*

Table 2: FTIR Interpretation of synthesized silver nanoparticles using aqueous extract of *S. splendens:*

S. No	*Wave Number*	*Molecular Motion*	*Functional Group*
1.	3417.9	N-H stretch	Heterocyclic amine
2.	2926	C-H (asymmetric/symmetric stretch)	Methylene
3.	1634.7	N-H medium bend	Secondary amine
4.	1409.2	C-H	Trimethyl
5.	1261.7	C-N stretch	Aromatic primary amine
6.	1159.2	C-N	Amines
7.	1105.9	C-O stretch	Cyclic ether
8.	1064.9	C-H in plane bend	Aromatic

SEM Analysis

Scanning electron microscopic analysis of the silver nitrate solution (Control) and reduced form of silver nitrate solution were clearly distinguishable owing to their size difference. It was clear from the SEM pictures that control of silver nitrate particles were more than 1000nm size, where as silver particles in the bioreduced colloidal suspensions measured 15-20nm in size. Figure 4 is the SEM of bioreduced silver nitrate.

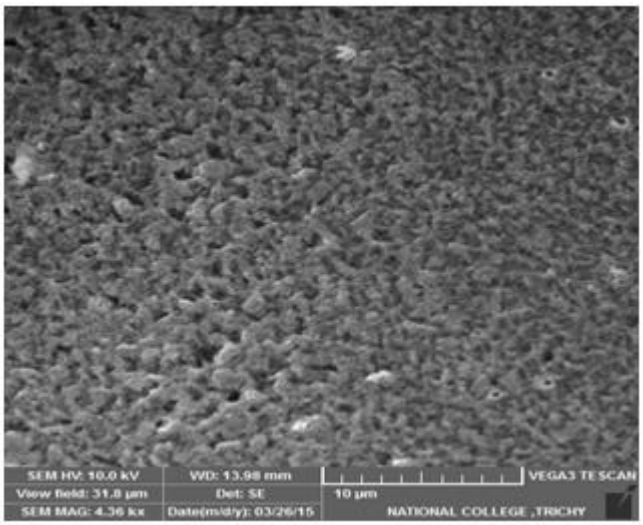


Figure 4: SEM image of synthesized silver nanoparticles using aqueous extract of *S. splendens*

Energy-Dispersive X-ray Spectroscopy Analysis

The EDS spectrum (Figure 5) showed high silver signals. The vertical axis shows the counts of the X- ray and the horizontal axis shows energy in keV. The strong signals of silver correspond to the peaks in the graph confirming presence of silver nanoparticles.

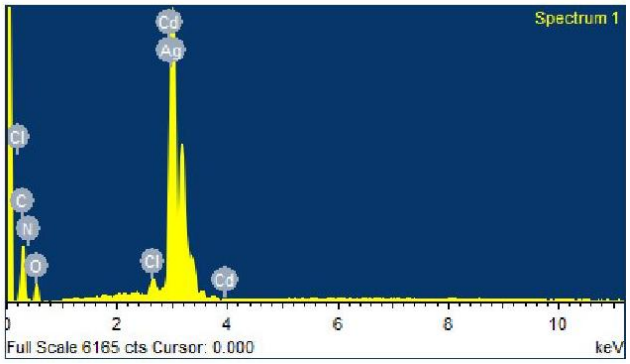


Figure 5: EDS spectrum of synthesized silver nanoparticles using aqueous extract of *S. splendens*

XRD Analysis

XRD analysis showed distinct diffraction peaks which can be indexed the angle vales of (111), (200), (220), (311) crystalline planes of nano silver. This analysis revealed the orthorhombic crystals of silver nanoparticles. The high peaks in the analysis indicated the active silver composition with the indexing (Figure 6).

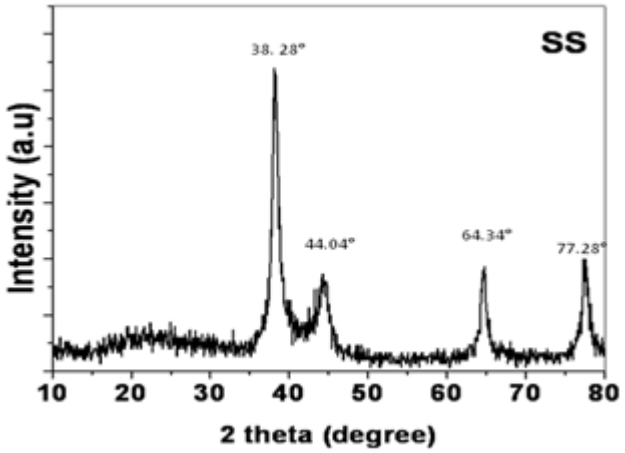


Figure 6: XRD spectrum of synthesized silver nanoparticles using aqueous extract of *S. splendens*

Antibacterial Activity

In this study, silver nanoparticles of plant extracts and positive control (Ampicillin) were tested on bacterial strains *Bacillus, Proteus vulgaris, Bacillus subtilis* and *Staphylococcus aureus*are shown in Figure 7. The formation of clear zone (restricted bacterial growth) around the cavity is an indication of antibacterial activity (Kumar *et al.*, 2012). The diameter of zone of inhibition was determined at concentrations, respectively (Table 3). Among various concentrations (1mg/1ml) 20µl, 30µl, 40µl and 50µlwere tested, all concentration showed clear zone of inhibition.

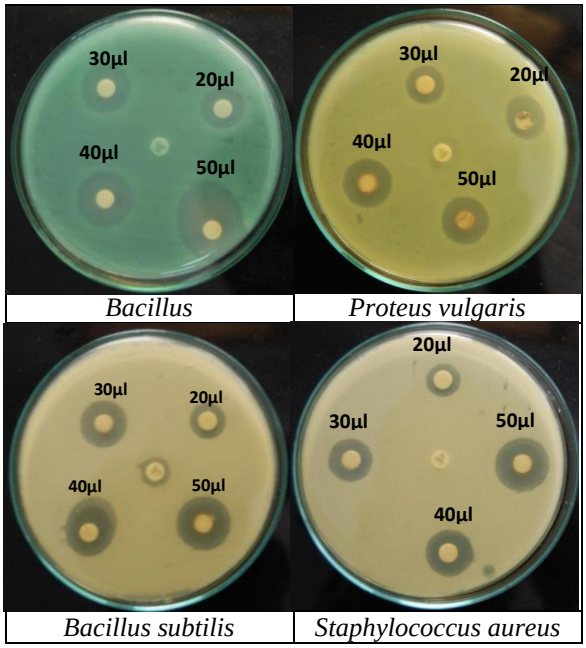


Figure 7: Antibacterial activity of silver nanoparticles produced from aqueous extract of *S. splendens* show inhibition zones with Bacterial Pathogens

Table 3: Antibacterial activity of silver nanoparticles from aqueous extract of *S. splendens:*

S. No	Name of the bacterial species	Zone Of Inhibition (mm)				
		Control Ampicillin	AgNPs			
			20µl	30µl	40µl	50µl
1.	*Bacillus*	_	13	18	20	23
2.	*Proteus vulgaris*	-	12	14	17	18
3.	*Bacillus subtilis*	10	12	16	18	21
4.	*Staphylococcus aureus*	-	12	16	18	21

4. Conclusion

The present study deals with silver nanoparticle synthesized using *Salvia splendens* plant extracts reveals the synthesis was noted by the colour change from pale yellow into brown colour. UV spectral analysis indicates the peaks at 437nmin the studied samples. Functional group analysis shows the presence of Heterocyclic aimne, Methylene, Secondary amine, Trimethyl, Aromatic primary amine, Amines, Cyclic ether and Aromaticsuggest that, these biomolecules plays an important role in the reduction of silver nitrate to silver nanoparticles. Silver nanoparticles samples were characterized by using SEM, EDS and XRD. The synthesized silver nanoparticles at less concentration effectively inhibited the growth and multiplication of pathogenic microbes like *Bacillus, Proteus vulgaris, Bacillus subtilis* and *Staphylococcus aureus.*

Therefore in the present work it is concluded that synthesizing silver nanoparticle by using *Salvia splendens* plant extract is a rapid, low-cost method and it is an eco-friendly.

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