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Green Synthesis of AgNPs, Characterization and Antibacterial Activity from *Salvia Leucantha* Cav. Plant Aqueous Extract

R. Rajendran[1], A. Lakshmi Prabha[2]

Plant Tissue Culture, Photochemistry and Nanobiotechnology Lab, Department of Plant Science, Bharathidasan University, Tiruchirappalli, Tamilnadu, India

Abstract: ***The present study we proposed green synthesis of silver nanoparticles has been characterized and its antibacterial activity against human pathogen. The Lamiaceae family plant of Salvia leucantha Cav. for synthesis of silver nanoparticles and their efficacy in antibacterial activity against four human pathogens. The plant extract mediated synthesized silver nanoparticles were characterized by UV-Vis spectroscopy, FT-IR, XRD, SEM and EDS. The UV-Vis spectra depicts surface Plasmon resonance peak present at 431 nm. The three dimensional structure of silver nanoparticles was seen through SEM analysis. The observed FT-IR peaks showed functional groups and the stretch of bonds that responds to AgNPs synthesis. The XRD analysis showed that the silvernanoparticles are crystalline in nature and have face-centered cubic geometry. The synthesized nanoparticles showed remarkable antibacterial activity against Bacillus, Escherichia coli, Proteus vulgaris, and Streptococcus pneumonia. This present work provides a direct route of synthesis of environment benign silver nanoparticles via medicinally essential plant material.***

Keywords: *Salvia leucantha,* AgNPs, FT-IR, XRD, SEM, EDS

1. Introduction

The medicinal plants have been provided a broad range of natural products with various chemical structures and most of them having of biological activities. In reality, many of which have found significance 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 bulky scale manufacture as required in the pharmaceutical remedy industry (Rajendran and Lakshmi Prabha 2015). The *Salvia* genus belongs to the Lamiaceae family and its covers about 700 to 900 species are dispersed worldwide (Delamere *et al.,* 2007; Mossi *et al.,* 2011). Salvia species are generally utilized for their essential oils in the foods, drugs and perfumery industries (Goren *et al.,* 2006; Ozcan *et al.,* 2003; Ulubelen and Topcu, 1998).

The 121 pharmaceutical products were prepared based on the traditional knowledge obtained from different sources in last century. Plant derived drugs came into use in the current medicine through the uses of plant material as original cure in folklore or traditional systems of drug (Chopra *et al.,* 1956).

The synthesis of is sliver nanoparticles using *Agaricus bisporus* (white button mushroom) aqueous extract. The antibacterial activity of synthesized silver nanoparticles showed successful inhibitory activity against human pathogenic bacterial strains like *Escherichia coli*, *Staphylococcus sps*, *pseudomonas sps*, and *Bacillus sps*. (Narasimha, 2001).

The green syntheses of silver nanoparticles were synthesis from extract of *Sargassum tenerrimum.* The synthesized AgNPs were characterized by UV-Visible Spectroscopy, FT-IR, TEM and DLS. The results showed that the anti-bacterial activity of silver nanoparticles (Kumar et al., 2012). The silver nanoparticles effect against several types of pathogenic bacteria has been broadly studied (Panacek *et al.*, 2009).

In this, study was synthesized AgNPs using *S. leucantha* plant aqueous extract for reduction of Ag+ ions to AgNPs from $AgNO_3$ solution within 1 min of reaction time at 60°C temperature. Supplementary, green synthesized silver nanoparticles are found to be highly efficient against human pathogenic bacterial strains.

2. Materials and Methods

Material collection

In this study, *S. leucantha* plant was used and it was collected from the Udhagamandalam (Tamilnadu). The entire part of the plant has been used for silver nanoparticles synthesis.

Silvernanoparticles Synthesis (AgNPs)

Plant material and preparation of the extract

The *S. leucantha* fresh plant was dried for 25 to 30 days under shadow at room heat and ground into fine powder. 250 mg of plant powder was used to make aqueous extract using 100 ml of double distilled water. It was lightly heated and filtered through a Whatman filter paper.

Synthesis of Silvernanoparticles

1mM aqueous solution of Silver nitrate 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 light greenish yellow to golden brown.

Characterization of silvernanoparticles

UV-spectrophotometer analysis (UV)
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 aqueous extract were changes from light greenish yellow to golden brown within 5 minute at microwave heat and then to dark brown.

Fourier Transform Infrared Spectroscopy Analysis (FTIR)
Further characterization of AgNPs involved FT-IR 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 12,000 rpm for 10 min and the resulting suspension was dispersed in sterile double distilled water. The centrifuging and redispersing process was repeated 3 times. Finally the silver nanoparticles were analyzed by using FT-IR.

Scanned Electron Microscope Analysis (SEM)
In SEM analysis 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.

X-Ray Diffraction Analysis (XRD)
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, Escherichia coli, Proteus vulgaris, and Streptococcus pneumoniae* 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: Syed Moideen and Lakshmi Prabha, 2014). 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. leucantha* 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.

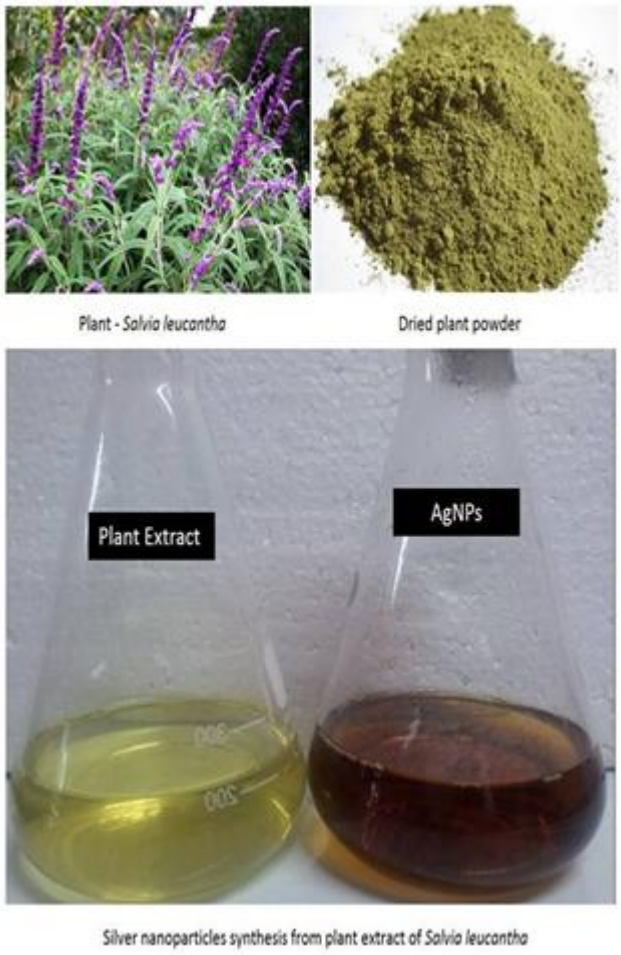

Figure 1: Reduction Silver Nanoparticles indicating by colour change

UV-VIS spectral analysis of silver nanoparticles

Silver nanoparticles were synthesized using plant extract of *S. leucantha* Reaction between biomolecules and silver nitrate takes place and silver nitrate 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 431nm (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 (Syed Moideen and Lakshmi Prabha, 2014).

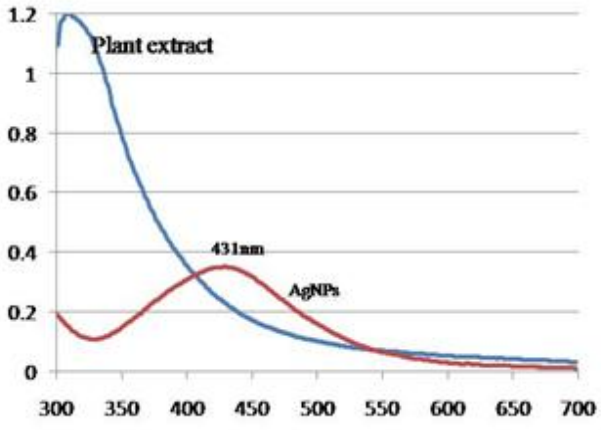

Figure 2: UV spectrum shows peaks corresponding to production of silvernanoparticles by *S. leucantha* Plant extract

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. leucantha*. The spectral bands were interpreted for identification of functional moieties of organic compounds adhering to the silver nanoparticles (Syed Moideen and Lakshmi Prabha, 2014: Helen Mary Piramila *et al.,* 2014). The FTIR spectrum of *S. leucantha* (Figure 3) peaks was showed in the following table 2.

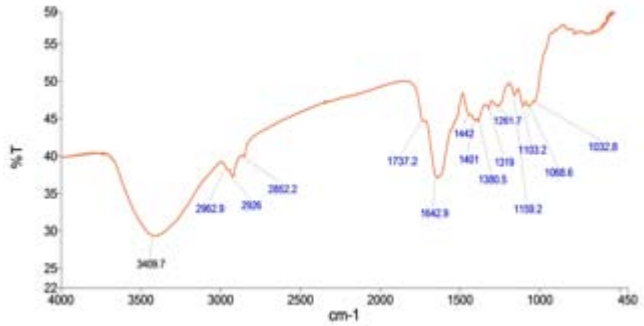

Figure 3: FTIR spectrum of synthesized silver nanoparticles using aqueous extract of *S. leucantha*

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

S. No	*Wave Number*	*Molecular Motion*	*Functional Group*
1.	3409.7	N-H stretch	Heterocyclic amine
2.	2962.9	C-H (asymmetric/ symmetric)	Methylene
3.	2952	C-H	Alkanes
4.	1737.2	C=O	Aldehydes, ketones
5.	1642.9	N-H-bend	Secondary amine
6.	1442	C=C=C stretch	Aromatic ring
7.	1401	C-H	Trimethyl
8.	1380.5	C-H(asymmetric/ symmetric bend)	Dimethyl or Isomethyl
9.	1159.2	C-N	Amines
10.	1261.7	C-N stretch	Aromatic primary amine
11.	1103.2	C-O stretch	Cyclic ether
12.	1068.6	C-H in plane bend	Aromatic
13.	1032.8	O-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 (Syed Moideen and Lakshmi Prabha, 2014: Helen Mary Piramila *et al.,* 2014).

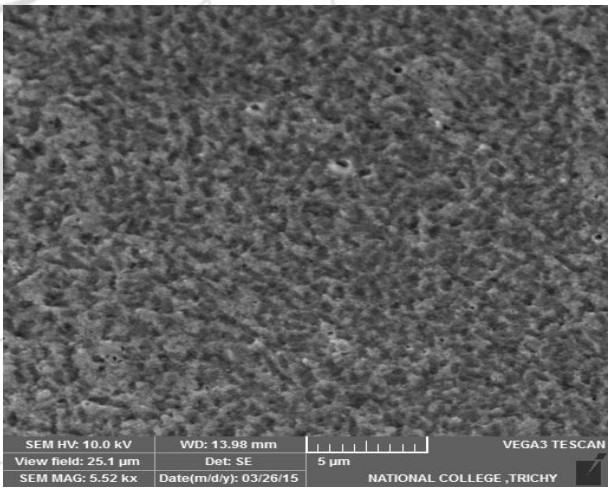

Figure 4: SEM image of synthesized silver nanoparticles using aqueous extract of *S. leucantha*

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 (Helen Mary Piramila *et al.,* 2014).

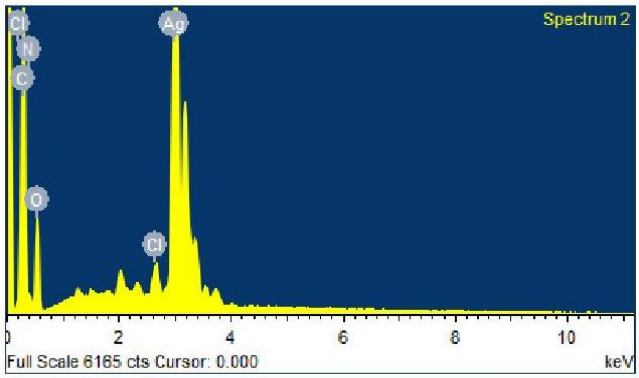

Figure 5: EDS spectrum of synthesized silver nanoparticles using aqueous extract of *S. leucantha*

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[12]. 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).

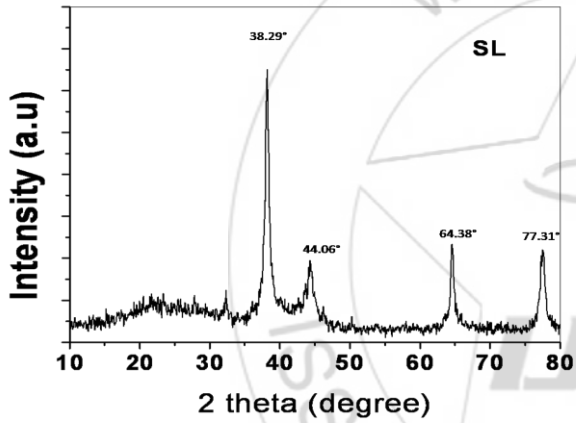

Figure 6: XRD spectrum of synthesized silver nanoparticles using aqueous extract of *S. leucantha*

Antibacterial activity

In this study, silver nanoparticles of plant extracts and positive control (Ampicillin) were tested on bacterial strains *Bacillus, Escherichia coli, Proteus vulgaris,* and *Streptococcus pneumoniae* 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 (Syed Moideen and Lakshmi Prabha, 2014: Helen Mary Piramila *et al.,* 2014).

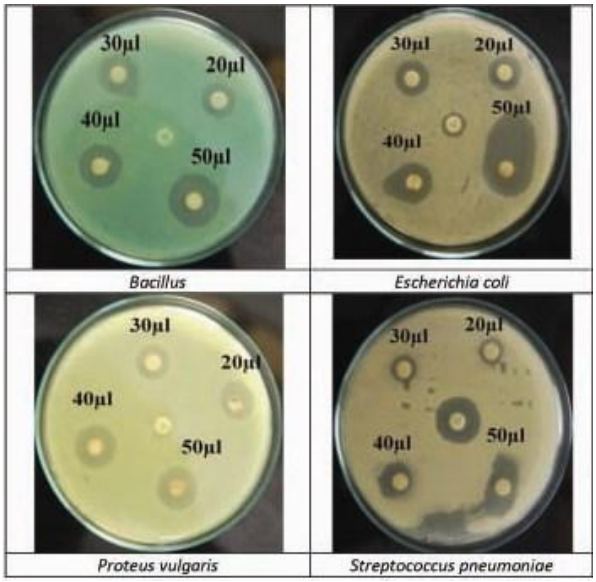

Figure 7: Antibacterial activity of silver nanoparticles produced from aqueous extract of *S. leucantha* show inhibition zones with Bacterial Pathogens

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

S. No	Name of The Bacterial Species	Zone of Inhibition(nm)				
		Control Ampicillin	AgNPs			
			(20µl)	(30µl)	(40µl)	(50µl)
1.	*Bacillus*	_	**11**	**15**	**16**	**20**
2.	*Escherichia coli*	**9**	**15**	**16**	**18**	**21**
3.	*Proteus vulgaris*	-	**13**	**15**	**17**	**18**
4.	*Streptococcus pneumoniae*	**19**	**11**	**12**	**15**	**18**

4. Conclusion

In this study deals with silver nanoparticle synthesized using *Salvia leucantha* plant extracts reveals the synthesis was noted by the colour change from pale yellow into brown colour. UV spectral analysis indicates the peaks at 431nmin the studied samples. Functional group analysis shows the presence of Heterocyclic amine, Methylene, Alkanes, Aldehydes, ketones, Secondary amine, Aromatic ring, Trimethyl, Dimethyl or Isomethyl, Amines, Aromatic primary amine, Cyclic ether and Aromatic 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, Escherichia coli, Proteus vulgaris,* and *Streptococcus pneumoniae.*

Therefore in the current effort it is concluded that synthesizing silver nanoparticle by using *Salvia leucantha* plant extract is a hasty, low-cost method and it is an eco-friendly.

5. Acknowledgement

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Author Profile

Dr. A. Lakshmi Prabha, Associate Professor, Department of Plant Science, School of Life Sciences, Bharathidasan University, Tiruchirappalli – 620 024. Tamilnadu, India.