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PLANT MEDIATED SYNTHESIS OF SILVER NANOPARTICLES FROM *LEONOTIS NEPETIFOLIA* L. AND ITS ANTIBACTERIALPROPERTY

Dr. Wadavkar D. S.*[1], Gadhave S. R.[2], Palave S. A.[3] and Chavan S. J.[4]

[1]Department of Botany, Dada Patil Mahavidyalaya, Karjat, Dist- Ahmednagar, 414402, Maharashtra, India.
[2,3,4]Post Graduate Research Center, Department of Botany, Tuljaram Chaturchand College of Arts, Science and Commerce, Baramati, Dist- Pune, 413102, Maharashtra, India.

***Corresponding Author: Dr. Wadavkar D. S.**
Department of Botany, Dada Patil Mahavidyalaya, Karjat, Dist- Ahmednagar, 414402, Maharashtra, India.

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ABSTRACT

Nanotechnology has now started to develop a new route for changing of our day to day life. The new eco-friendly "green" methods of synthesis are being discovered for increasing demands commercial nanoparticles. Plant mediated synthesis of nanoparticles offers single step, easy extracellular synthesis of nanoparticles. The synthesis of nanoparticles was confirmed by change in colour from pale green to reddish brown. Further, a peak between 400 nm to 440 nm was obtained on UV-Vis spectrometer which confirmed the biosynthesis of silver nanoparticles. XRD have been used to investigate the morphology of prepared AgNPs. The peaks in XRD pattern are associated with that of face-centered-cubic (FCC) form of metallic silver. FTIR was performed to identify the functional groups of carboxylic acid, carbonyl, hydrocarbon, alkane, hydroxyl, unhydride, ester, ketone, alkenes nitro, alcohol, phenol, ether and monosubstituted benzene molecule which form a layer covering AgNPs and stabilize the AgNPs in medium. The antibacterial activity against different bacteria likes *Escherichia coli*, *Bacillus subtilis*, *Salmonella abony*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and control were reported.

KEYWORDS: Antibacterial, *Leonotis nepetifolia*, Nanotechnology, Synthesis.

INTRODUCTION

Nanotechnology an emerging field of nanoscience deals with nano size particles having a size of 1-100 nm. These nonmaterials are considered as "smart" materials used for constructing nanocarriers, which plays a vital role in drug delivery systems and possess high biocompatibility nature (Shi *et.al.* 2014). Nanoparticles have received special attention due to greater surface area to volume ratio and highly reactive than macromolecules (Gnanajobitha *et.al.* 2012). Employing nanotechnology, green method for the synthesis of nanoparticles provides tremendous advantages as it is free of toxic chemicals and eco - friendly.

To synthesis stable metal nanoparticles with controlled size and shape, there has been search for inexpensive, safe, and reliable and "green" approach. The novel methods so called green/biosynthesis have been recently developed by a variety of plant extract such as *Coriandrum Sativum* (Sathyavathi *et.al.*2010), *Murraya koenigii* (Christensen *et.al.* 2011), *Ocimum Sanctum* (Singhal, *et.al.* 2011), *Petroselinum crispum* (Roy *et.al.* 2015) for the synthesis of metal nanoparticles.

Silver nanoparticles synthesized from plant extract results that silver capped with the functional groups present in the active phytoconstituents of the plant extract, acts as antioxidant agents and enhance the biological activity like anticancer effect (Hussen, and Siddiqi, 2014).

Among the various metal nanoparticles synthesized (such as silver, gold, iron, zinc and platinum), silver nanoparticles have gained more importance in the nanotechnology field. As silver in the nano size is safe inorganic and non-toxic agents and encompasses a wide range of applications such as antibacterial and antifungal effects (Ashok Kumar *et.al.* 2015).

MATERIAL AND METHODS

1. Collection of Plant Material

The fully matured leaves of *Leonotis nepetifolia* L. from family Lamiaceae were collected from Baramati district of Pune, Maharashtra, India. The leaves were thoroughly cleaned using water and dried in shade for 5 days.

2. Preparation of Plant Extract

The *Leonotis nepetifolia* L. leaves after shade dried for a period of 5days were blended and made into fine coarse powder. 1 gram of powder is mixed with 10 ml of 80% ethanol and boiled for 15 minutes. Then the extract is filtered using filter paper. Residue is removed and pure plant extract is obtained.

3. Synthesis of Silver Nanoparticle

5mM aqueous solution of silver nitrate (AgNO3) was prepared and used for the synthesis of silver nanoparticles.10 ml of *Leonotis nepetifolia* extract was mixed with 1 ml of aqueous solution of 5mM silver nitrate for the reduction into Ag+ ions and incubated overnight at room temperature in dark.

Plate 1: ***Leonotis nepetifolia*** **L.**

Plate 2: A. Plant extract B. Silver Nitrate C. Plant extract + silver nitrate colour change (Reddish brown).

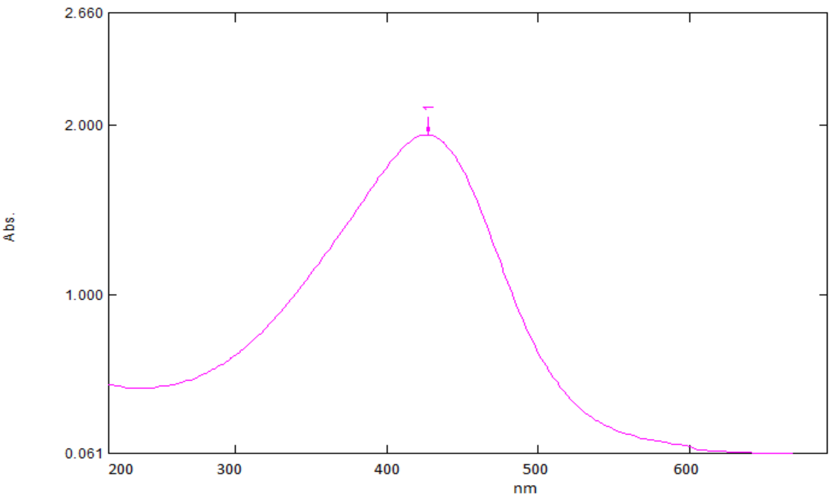

Fig. 1: UV-Visible Spectrum of Silver Nanoparticles

4. UV-Visible spectroscopy analysis

The bioreduction of pure Silver nanoparticles are monitored using UV-Vis spectroscopy at regular intervals. During the reduction *Leonotis nepetifolia* samples was taken and centrifuged at 12,000 rpm. The supernatant was scanned by UV 300-400 nm.

5. Fourier transform infrared spectroscopy (FTIR)

A known weight of sample (mg) was taken in mortar and pestle and ground with 2.5 mg of dry potassium bromide. The powder obtained was filled in 2 mm interval diameter micro cup and loaded on FTIR set at 26 ^{0}C ± 1 ^{0}C. The samples were scanned using infrared in range of 4000-500 cm^{-1} using FTIR. The spectrum obtained was compared with reference chart to identify functional groups present in sample.

6. X-RAY Diffraction Analysis (XRD)

XRD is an analytical technique used to determine phase crystallinity of the material. The XRD pattern was obtained by placing the prepared samples on a glass slide and dried under hot air oven at 50 °C. The samples were dried and analyzed under the XRD instrument (PAN analytical, XPERT- PRO diffractometer) with a Cu source at 1.5406 A^{o} wavelength as X - ray source in thin film mode.

7. Antibacterial Activity

The antibacterial activity of *Leonotis nepetifolia* L. plant extract was evaluated by disc diffusion method. Muller Hinton agar medium was prepared and poured into the petriplates and allowed to solidify. Then it was inoculated with a swab of culture and spread throughout the medium uniformly with a sterile cotton swab. Two wells are bored in a plate and in which one well are induced with100 µl of plant extract (sample) and in another well 100µl of distilled water (control) were loaded and antibiotic (ampicillin) was also placed. The test bacterial *Escherichia coli*, *Bacillus subtilis*, *Salmonella abony*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were included in this study to assess the susceptibility pattern of the compounds. The plate was incubated 37 ºC for 24 hrs for observing inhibition zone.

RESULT AND DISCUSSION

The chemical reduction of aqueous solution of silver nitrate is the most widely used method for the synthesis of silver nanoparticles. In the present study the formation of silver nanoparticles by using *Leonotis nepetifolia* L. was determined. The appearance of the dark brownish colour suggested the formation of silver nanoparticles.

1. UV-VIS Spectroscopy analysis

The synthesized Silver nanoparticles using *Leonotis nepetifolia* plants extracts (Fig. 1) were detected by UV Vis Spectrophotometer. The UV-Vis spectrum of colloidal solution of Silver nanoparticles from *Leonotis nepetifolia* has maximum absorbance peak at 420 nm, which is proved the synthesis of silver nanoparticles in the colloidal solution. The position and shape of the Plasmon absorption depends on the particles size, shape and the dielectric constant of the surrounding medium. The particle showed gradual decrease between 300 – 600 nm.

2. Identification of functional groups using FTIR

FTIR analysis was used for the characterization of the extract and the resulting nanoparticle (Fig.2). FTIR absorption spectra of soluble extract reduction of Ag ions. Absorbance bands in the region of 500 - 4000 cm^{-1} are 3287, 2902, 1776, 1732, 1701, 1624, 1525,1361,1338,1209,1189 and 497 cm-1 .These absorbance bands are known to be associated with the stretching vibrations for O-H carboxylic acid, C-H alkane stretching, (RC(O))2O unhydride stretch, C=C alkenes stretch, C=O unhydride and ester stretch, CH_3—C(=O)—CH_3 ketone stretch, No_2 nitro stretch , O-H stretching for alcohols and phenols, R–O–R ether stretch and monosubstituted benzene stretching. The total disappearance of this band the bioreduction due to the fact that mainly responsible for the reduction of Ag ions, whereby they themselves to leading abroad peak at 3287 cm^{-1}.

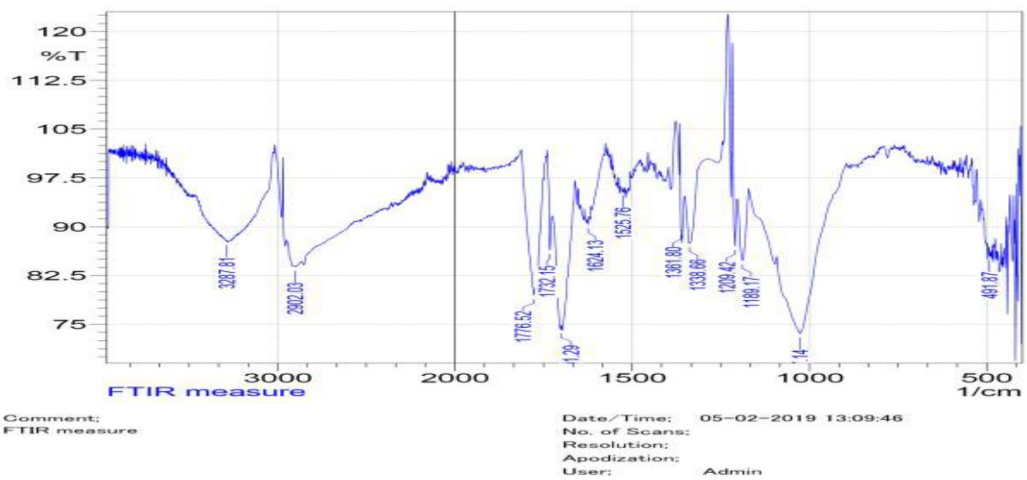

Figure 2: FTIR spectrum of *Leonotis nepetifolia* leaf extract.

3. X-RAY Diffraction Analysis (XRD)

The XRD pattern of synthesized AgNPs using *Leonotis nepetifolia* leaf extract was shown in Fig. 3. The XRD was done to determine the crystalline nature of AgNPs and the resulted peaks representing (111), (200), (220) and (311) face centered cubic structure of silver.

The XRD technique is used to identify the crystalline nature by comparing the obtained pattern with the reference library and assigning its nanocrystalline nature. In the present research work, the XRD patterns of the silver reaffirmed the presence of silver. It also proved the crystalline nature of the material.

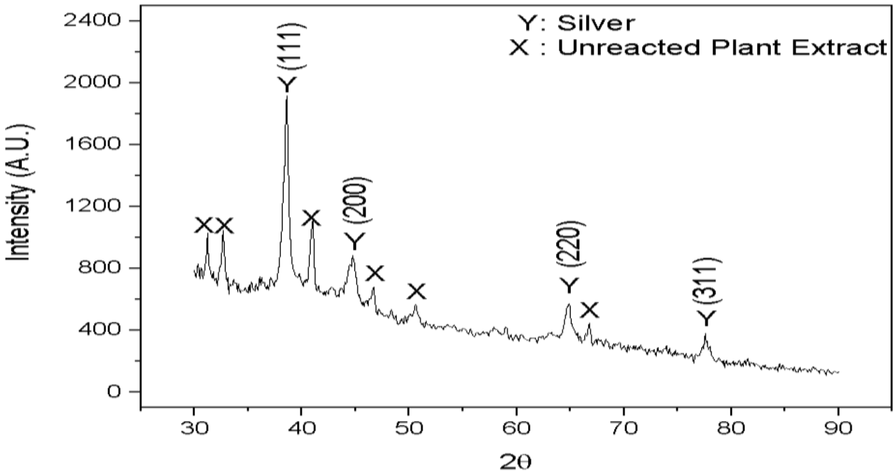

Fig. 3: XRD pattern of AgNPs.

4. Antibacterial Activity

Silver nanoparticles exhibit antibacterial properties against *Escherichia coli, Bacillus subtilis, Salmonella abony, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibacterial activity of AgNO3 nanoparticles produced *Leonotis nepetifolia* L. was studied against various Gram positive and Gram negative strains. Green synthesis of silver nanoparticles of *Leonotis nepetifolia* showed very strong inhibitory actions against *E. coli* than other bacterial species but less in *B. subtilis.*

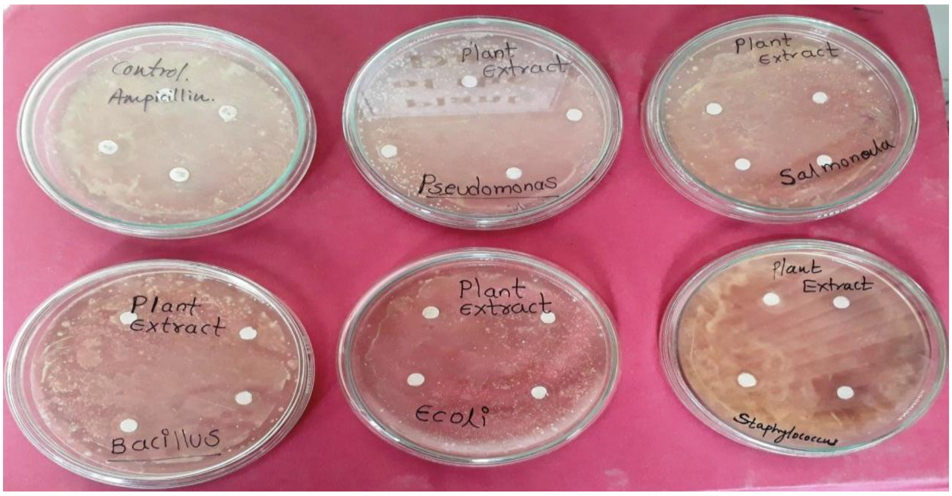

Plate 3: Antibacterial activity of silver nanoparticles against *Escherichia coli, Bacillus subtilis, Salmonella abony, Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Table 1: Antibacterial activity of the silver nanoparticles.

Sr. No.	Microorganism	Zone of inhibition (mm)
1	Ampicillin (control)	7.5
2	*Staphylococcus aureus*	8.0
3	*Salmonella abony*	7.0
4	*Bacillus subtilis*	6.5
5	*Pseudomonas aeruginosa*	7.0
6	*Escherichia coli*	9.5

CONCLUSION

The synthesized Silver nanoparticles using *Leonotis nepetifolia* plants extracts were detected by UV Vis Spectrophotometer. The UV-Vis spectrum of colloidal solution of Silver nanoparticles from *Leonotis nepetifolia* has maximum absorbance peak at 420 nm, which is proved the synthesis of silver nanoparticles in the colloidal solution. The XRD peaks ascribed with FCC structure of silver. The FT-IR spectrum ascribed the biological molecules which perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium. So, it can be summarized that, green synthesis is an effective and eco-friendly method of producing metal nanoparticles.

The activities were tested against various bacterial microorganisms including *Escherichia coli*, *Bacillus subtilis*, *Salmonella abony*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. These results evidently show that the inclusion of *Leonotis nepetifolia* extracts improves the solubility of AgNPs, which led to a significant enhancement in the toxicity of the NPs against the assessed microorganisms.

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