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Antimicrobial Activity of Biomediated Silver Nanoparticles of Echinops echinatus and Its Characterization

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Abstract: *Silver nanoparticle plays very important role in biological activity, but it is very hazardous when synthesized by chemical method. In present work, we used aqueous plant extract of roots of Echinops echinatus for preparation of AgNPs. The bio reduction and stabilization of so formed silver nanoparticles was monitored by UV-visible spectroscopy. The synthesized AgNPs are also characterised by FTIR, SEM and XRD. The UV spectrum shows the Plasmon resonance at 424 nm. The particle grain size of AgNPs was calculated from the SEM. The nanoparticle imageshows antibacterial activity against both gram positive and gram negative organisms.*

Key words: *Nanoparticles, Echinops echinatus, FTIR, XRD and SEM.*

I. Introduction

Nanotechnology broadly refers to a field of applied science and technology of synthesizing nanoparticles whose special and unique properties could be attributed to their small sizes and large surface areas. Nano biotechnology is defined as a field that applies the nano scale principle and techniques to understand and transform bio systems (living and non living) and which uses biological principles and materials to create new devices and system integrated from the nanoscale.In recent years, the research is mainly focused on the metal nano particles due to their unique optical, electronic, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials. Physical, chemical and green synthesis methods are available for synthesis of silver nanoparticles, but the physical and chemical methods may be toxic and highly reactive and hence it is risk for environment and human. Therefore, to search an inexpensive, reliable, safe and "Green" method for synthesis of various metal nanoparticles with controlled size, shape and stability is highly warranted. Silver nanoparticles have proved to be most effective as it has good antibacterial and antifungal activities. The most important application of silver and silver nanoparticles is in medical industry such as tropical ointments to prevent infection against burn and open wounds. In the present study, we have synthesized silver nanoparticles using root extract of *Echinops echinatus*. Synthesized nanoparticles were characterized by UV- visible spectroscopy, FTIR, SEM and XRD. Further, the antimicrobial activity of synthesized silver nanoparticles against three ATCC cultures including gram positive bacteria such as Staphylococcus aureus and gram negative bacteria such as E.coli, Pseudomonas aeruginosa were explored.

II. Material and methods

2.1 Collection of plant material

The roots of *Echinops echinatus* plant were was collected from region of Sangamner tehsil, Dist. Ahmednagar, Maharashtra.

Figure 1- *Echinops echinatus plant*

2.2 Preparation of plant extract

Fresh roots of *Echinops echinatus* were collected and washed with distilled water. Roots were dried under sunlight and grinded to fine powder. 10 g of root powder of *Echinops echinatus*was transferred into 250 ml beaker containing 100 ml distilled water and boiled for 15 min, filtered with Whatmann Paper No. 41 and is collected.

2.3 Synthesis of silver nanoparticles

10 ml of 0.1 M of silver nitrate solution was prepared. 90 ml root extract was added separately to the silver nitrate solution. This mixture was placed under the sunlight for 2 hrs for accelerative bio reduction of $AgNO_3$. The brownish yellow colour indicated the formation of silver nanoparticles.

2.4 Purification of silver nanoparticles

The fully reduced silver nanoparticles solution was centrifuged at 10,000 rpm for 5 min. The supernatant was discarded. The residue was purified with distilled water.

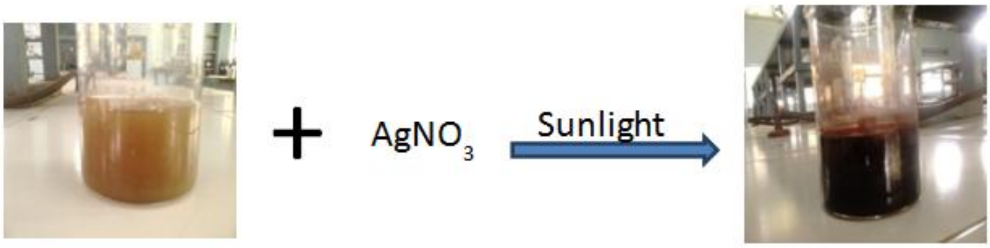


Figure 2-Formation of Silver Nanoparticles using *Echinops echinatus* root extract

III. Characterization of silver nanoparticles

3.1 UV-visible Analysis

Ultraviolet-visible analysis was done by using Chemito Spectrophotometer UV-2100 at the wavelength 300 – 700 nm. The colour transition from brownish yellow to dark brown confirmed the formation of Ag^0 from Ag^+

3.2 FTIR analysis of silver nanoparticles

The purified dried nanoparticles powder was analyzed by FT-IR-4100.

3.3 SEM and EDX analysis of silver nanoparticles

The dried powder was analyzed with SEM(JEOL JSM-6360) to investigate the morphology and size of the particles. EDAX analysis gave information of qualitative and quantitative status of elements.

3.4 X Ray Diffraction analysis

The crystalline nature of silver nanoparticles was confirmed by XRD (Brukar D8 Advanced diffractometer) analysis.

IV. Result and discussion

4.1 Uv-visible spectroscopy

It is known that silver NPs show the dark brown colour due to surface Plasmon resonance of silver NPs. The root extract were used to synthesize the silver NPs. This was confirmed by the dark brown colour and hence the Plasmon resonance obtained at 424 nm. The frequency and width of surface Plasmon band is dependent on the size and shape of silver NPs.

4.2 Fourier transform infrared spectroscopy

The FTIR measurements were carried out to identify the possible natural products for the reduction

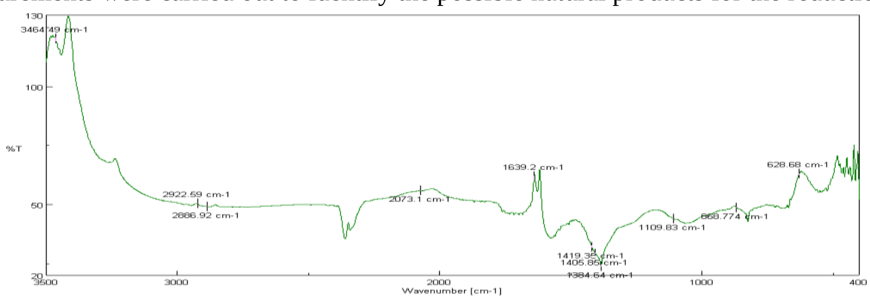


***Figure3**- FTIR Analysis of synthesized silver nanoparticles*

of silver ions to silver nanoparticles synthesized by *Echinops echinatus* roots extract. The FTIR absorption spectra of the sample is shown in Figure 3.The peaks are at 3464.49 cm^{-1}, 2922,59cm^{-1}, 2886.92cm^{-1}, 2073.1cm^{-1}, 1639.2 cm^{-1}, 1419.35 cm^{-1},1405.85cm^{-1}, 1384.64 cm^{-1}, 1109.83cm^{-1}, 868.779 cm^{-1}, 628.68 cm^{-1}, respectively. The band at 3464.49 cm^{-1} indicates O-H stretching vibration of phenolic group, the band at 2922.59 $cm^{-1}$is due to C-H stretching of alkane. The band at 1639.2cm^{-1} is due to N-H bend at of 1^0 amines. The band at 1405.85cm^{-1} is corresponding to C-C stretching vibration to aromatics. The band at 628.68 cm^{-1} is due to alkyl halides. The band at 1384.64 cm^{-1} is due to nitro N-O bonding.

4.3 Scanning Electron Microscopy (SEM)

SEM image of silver nanoparticles (figure 4) reveals that there is an aggregation of prepared silver nanoparticles with an average diameter of 100 nm.

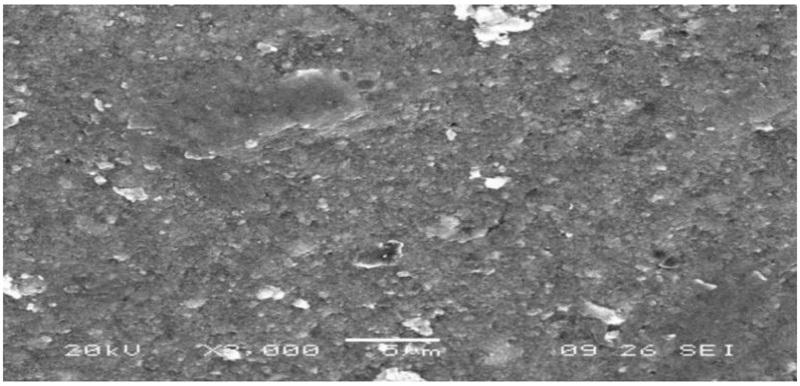


Figure 4 *- SEM Analysis of Silver Nanoparticles*

4.4Eenergy dispersive x-ray spectrophotometer (EDAX)

EDAX spectrum shows strong signal at 3KeV establishing the formation of silver nanoparticles (figure 5)and indicating 42.67% of Ag by weight (Table 1).

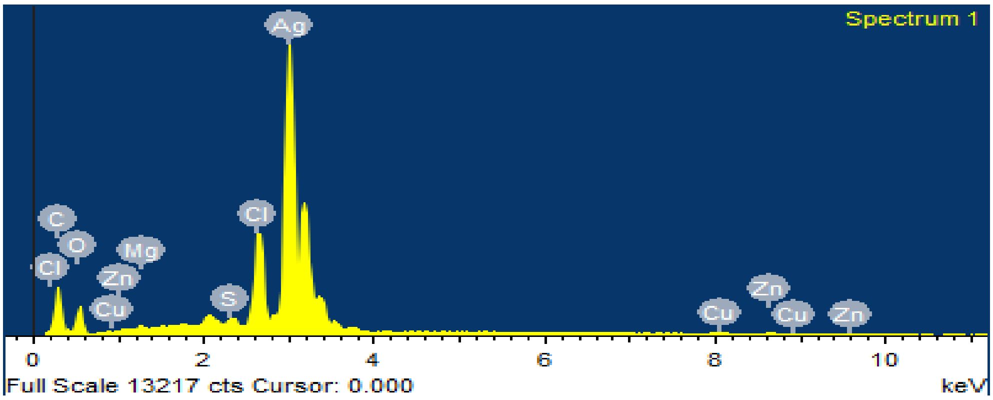


Figure5*- EDX spectrum of synthesized silver nanoparticles*

Table 1- The elemental composition of silver NPs synthesized from root of *Echinops echinatus* plant.

Elements	**Weight %**	**Atomic %**
C K	27.96	53.87
O K	22.78	32.95
Mg K	0.21	0.20
S K	O.41	0.29
Cl K	4.75	3.10
Cu K	0.69	0.25
Zn K	0.52	0.19
Ag L	42.67	9.15
TOTAL	100.0	

4.5 X-ray diffraction analysis (XRD)

The XRD pattern for given sample is shown in Fig.6. X ray diffraction studies revealed that the given sample is crystalline in nature. In the XRD pattern of silver nanoparticles, the diffraction peaks at 38.10^0,44.52^0,64.32^0 and 77.43^0 can be assigned to Face Centered Cubic(FCC).The major planes correspond to (111), (200), (220) and (311) planes of silver is observed and compared with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards(JCPDS), Silver file. No. 04-0783. Along with silver nanoparticle diffraction peaks, pattern also exhibits several other diffraction peaks which may be assigned to the Ag_2O phase. Further investigation in this regard is required.

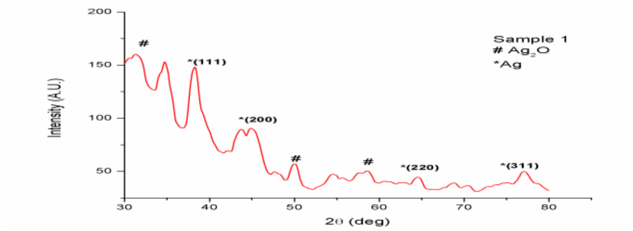
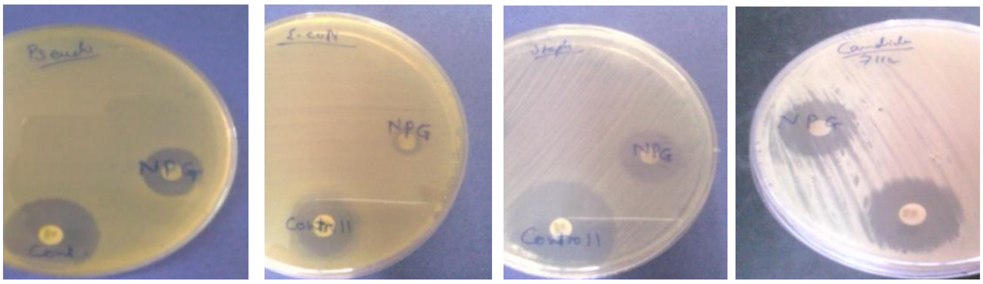


Figure 6 - XRD Analysis of *Echinops echinatus* silver nanoparticles

V. Anti microbial activity

In this study antimicrobial activity of silver nanoparticles were investigated against three ATCC cultures including both gram negative and gram positive bacteria such as E. coli, Pseudomonas aeruginosa and Staphylococcus aureus and Candida sp. (Figure 7).



(A)P. aeruginosa **(B)E.Coli** **(C) S. aureus** **(D) Candida sp.**

Figure 7 - (A-D) Antibacterial activity of synthesized silver nanoparticles

The maximum activity that is 18 mm zone ofinhibition was obtained against P. aeruginosa, 17mm zone of inhibition against Staphylococcus and 10 mm zone of inhibition against E. coli. using Gentamicin and Nystatin as Control 1 and Control 2 respectively (Table 2).

Table 2 – Evaluation of Antibacterial activity of silver nanoparticles synthesized by *Echinops echinatus*.

Sample	**E. coli ATCC 25922**	**P. aeruginosa ATCC 27853**	**S. aureus ATCC 25923**	**Candida Sp.**
NP	10mm	18mm	17mm	21mm
Control 1	17mm	24mm	28mm	-
Control 2	-	-	-	21mm

VI. Conclusion

Present work shows that the aqueous extract of root of *Echinops echinatus* can be efficiently used for silver nanoparticles. Formation of biomediated silver nanoparticles is confirmed by the colour change and Plasmon resonance at 424 nm. The SEM shows aggregation of prepared silver nanoparticles with an average size 100 nm. EDAX shows the elemental composition of synthesized silver nanoparticles. As revealed from antimicrobial study, biuomediated silver nanoparticles can be used for effective growth inhibition of above said microorganisms and hence applicable to diverse medical devices.

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