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BIOSYNTHESIS OF SILVER NANOPARTICLES FROM ACHYRANTHES ASPERA LEAF AQUEOUS EXTRACT

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ABSTRACT

The field of nanotechnology is one of the most active researches nowadays in modern material science and technology. Eco friendly methods of green mediated synthesis of nanoparticles are the present research in the limb of nanotechnology. In recent years, green synthesis of silver nanoparticles(AgNPs) has gained much interest from chemists and researchers. The present work leads to the synthesis of nanoparticles from $AgNO_3$ solution through aqueous leaf extract of Achyranthes aspera as reducing as well as capping agent. Synthesized nanoparticles are characterized through the XRD, SEM, UV-Vis Spectrophotometer and FT-IR. Green synthesized silver nanoparticle showed zone of inhibition against isolated Gram positive and Gram negative using bacteria such as Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Citrobacter and Proteus vulgaris. Based on the result obtained it can be said that the plant resources can efficiently used in the production of silver nanoparticle and it could be utilized in various fields such as biomedical, nanotechnology and so on.

Keywords: Green Synthesis, SEM, XRD, Silver Nanoparticles, Antimicrobial Property.

I INTRODUCTION

Indian greeneries are the chief and cheap source of medicinal plants and plant products. Recently, many such plants have been gaining importance due to their unique constituents and their versatile applicability in various developing fields of research and development. Nanobiotechnoloy is presently one of the most dynamic disciplines of research in contemporary material science whereby plants and different plant products are finding an imperative use in the synthesis of nanoparticles. In general, particles with a size less than 100nm are referred to as nanoparticles. NPs of noble metals like gold,silver and platinum are well recognized to have significant applications in electronics, magnetic, optoelectronics and information storage [1-4].Silver is one of the most commercialized nano-material with five hundred tons of silver nanoparticles production per year. [5].

They are also broadly applied in shampoos, soaps, detergents, cosmetics, toothpastes and medical and pharmaceutical products and are hence directly encountered by human systems [6,7]. The techniques for obtaining nanoparticles using naturally occurring reagents such as sugars, biodegradable polymer, plant extracts and microorganisms as reductants and capping agents could be considered attractive for nanaotechnology.[8]. Greener synthese of nanoparticles also provides advancement over other methods as they are simple, one step, cost-effective, environment friendly, easy to perform and sustainable [9].

The present work leads to the synthesis of nanoparticles from $AgNO_3$ solution through aqueous leaf extract of Achyranthes aspera as reducing as well as capping agent. Synthesized nanoparticles are characterized through the XRD, SEM, UV-Vis Spectrophotometer and FT-IR. Green synthesized silver nanoparticle showed zone of inhibition against isolated Gram positive and Gram negative using bacteria such as Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Citrobacter and Proteus vulgaris. Based on the result obtained it can be said that the plant resources can efficiently used in the production of silver nanoparticle and it could be utilized in various fields such as biomedical, nanotechnology and so on.

II EXPERIMENTAL

Typically, a plant extract- mediated bioreduction involves mixing the aqueous extract with an aqueous solution of the appropriate metal salt. The synthesis of nanoparticles occurs at room temperature and completes within a few minutes.

2.1 Preparation of Plant Extract

Fresh leaves of *Achyranthes aspera* were collected and washed several times with water to remove dust particles and then grinded into the marter. The extract was filtered and was stored in a room temperature.

Fig.1 ***Achyranthes Aspera Plant***

2.2 Green Synthesis of Silver Nanoparticles

Silver nanoparticles were prepared by simple precipitation method. Silver nitrate used as such (purchased from Merck, India). .2mM $AgNO3$ was dissolved in 100 ml distilled water in a glass beaker and stirred in a magnetic stirrer for several hours. After that 2ml of plant extract was added. The solution turns reddish brown in colour. Then the solution was filtered and the remaining substance was dried for 2 days.

2.3 Characterization of AG NPS

2.3.1. X- Ray Diffraction (XRD) analysis

The phase variety and grain size of synthesized silver nanoparticles was determined by X-ray diffraction spectroscopy (Model pw1390) X-rays are collimated and directed into the sample. As sample and detector are rotator, the intensity of diffracted x-rays is recorded. A detector records and x-rays signal are converted into a count rate and it is displayed on a digital printer/monitor.

From XRD, the average particle size is determined by applying Debye-Scherrer's formula.

$$D = \frac{k\lambda}{\beta \cos\theta}$$

Where

$\lambda \rightarrow wavelength\ of\ x - ray$

$\beta \rightarrow full\ width\ haf\ maximum\ of\ the\ diffraction\ peak$

$\theta \rightarrow angle\ of\ diffraction$

$K \rightarrow$ *Scherrer's constant (0.89)*

2.3.2. Fourier Transform Infrared Spectroscopy (FTIR) analysis

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR Spectrometer. Then the sample mixture is poured into a petri dish and kept in a hot air oven until its getting dried off. After that the dried sample is scrubbed, powder form of sample is stored. Then it is used for region of 400-4000cm^{-1} of Ag-NPS from *Achyranthes Aspera* leaf extract.

2.3.3. Scanning Electron Microscope: (SEM)

The Morphological features of synthesized silver nanoparticles from *Achyranthes Aspera* plant extract were studied by SEM (EDS-INCA, Oxford instruments,UK).

SEM is a type of electron microscope that produces the images of a sample by scanning; it was a focused beam of electron. The electron's interacts with atom in the sample producing various signals that can be detected and that contain the information about the sample surface topography. SEM creates various images by focusing a high energy beam of electrons on to the surface of a sample and detecting signals from the interaction of the incident electrons with the samples surface.

2.3.4. UV Visible Spectrometer (UV)

The optical property of Ag-nanoparticles was determined by UV-Visible spectrometer (shimadzu) UV deals with the measurement of energy absorbed when electrons are promoted to higher energy levels. The ultraviolet spectrum is simply a plot between wavelength and absorbance (or) transmittance. The UV and visible regions have wavelength ranging from 190 to 700 nm. In this organic and inorganic molecules and functional groups are transparent in the portion of electromagnetic spectrum. Thus the UV spectroscopy involves the spectroscopy of photon in the UV – visible region. The UV spectrum results from transition between electronic energy level accompanied by changes both in vibration and rotational states.

2.4 Antimicrobial Activity

The antimicrobial activity of pathogens was established using agar well method. The bactericidal effect of silver nanoparticles has been attributed to their high surface to volume ratio and small size which allows them to interact very closely with microbial membranes.

The selected strains of bacteria-A panel of three pathogens (***Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Citrobacter, Proteus vulgaris***) were inoculated into 10 ml of sterile nutrient broth and incubated at 37°C for 16-18 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile nutrient agar plates. Agar wells were prepared with the help of sterilized cork borer with 10 mm diameter. Using a micropipette, different concentrations of the samples 50 µl, 75 µl, and 100 µl were added to different wells in the plate. The plates were incubated in an upright position at 37^{o}C for 24 hours. The diameter of inhibition zones was measured in mm and the results were recorded.

III RESULTS AND DISCUSSION

3.1 XRD Analysis

Fig 2 shows the XRD pattern of silver nanoparticles. The crystalline nature silver nanoparticles synthesized from Achyranthes aspera has been studied by XRD analysis. XRD spectrum of silver nanoparticles shows distinct diffraction peaks around 35.3^{o} which are indexed by the (100) of the cubic face centered silver. This sharp Bragg peaks might have resulted due to crystalline nature of silver nanoparticles.

Using Debye scherer formula the grain size of the synthesized silver nanoparticles was calculated and its value is 13nm.

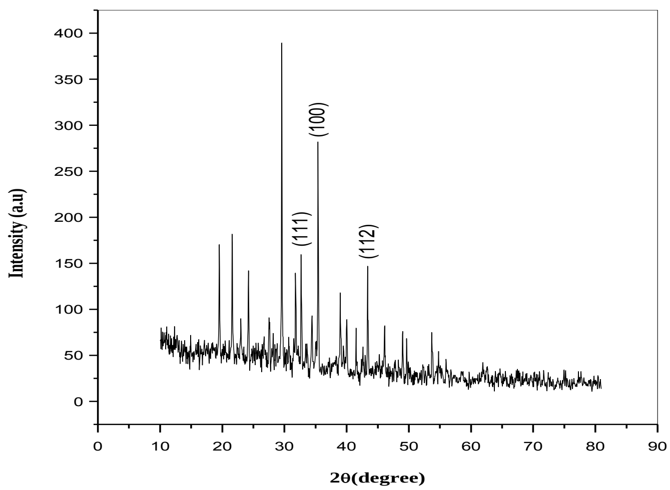

Fig 2: XRD spectra of silver nanoparticles

3.2 FTIR Analysis

The FTIR spectrum of silver nanoparticles was shown in fig 3. Figure shows that the absorption bands at above 3541.31cm^{-1} are may be responsible for the presence of O-H and N-H stretching vibrations. A band at 2904 .80 cm^{-1} is due to C-H stretching vibrations. The vibration band at 2395.59 cm^{-1} is due to O-H stretching vibrations. The band at 1762.94 cm^{-1} is characteristics stretching vibration of carbonyl functional group in acid and aldehyde. The vibration band at 1608.63 cm^{-1} shows the presence of carbonyl functional group in amide. Germinal methyl group shows the absorption band at 1377.17 cm^{-1}. The FTIR analysis showed that some of the functional group s present in the plant extract of Achyranthes aspera which may involved in the AGNPs synthesis.

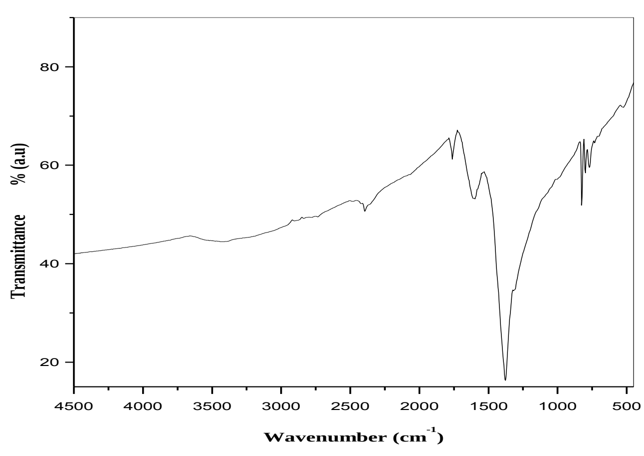

Fig3: FTIR spectra of silver nanoparticles

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3.3 SEM Analysis

Fig4 shows the FESEM image of silver nanoparticles. It exhibits that almost all the nanoparticles are of spherical shape. EDX spectrum of the silver nanoparticles is recorded using the electron beam of 10 KeV accelerating voltage as shown in **fig 4.a**. EDX Spectrum of silver nanoparticles consists of silver, oxygen, aluminum, chlorine, calcium with elemental composition of 73.1%, 22.6%, 0.76%, 2.80% and 0.5% respectively. The presence of oxygen, aluminum and chlorine in the EDX spectrum of silver nanoparticles is attributed from the precursor or the secondary planes of the samples.

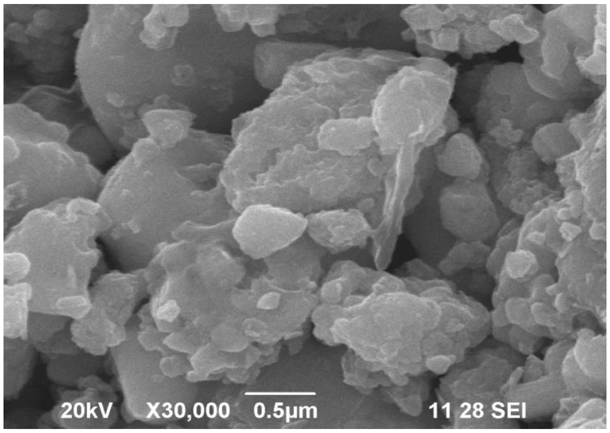

Fig 4: SEM image of silver nanoparticles synthesize by Achyranthes Aspera

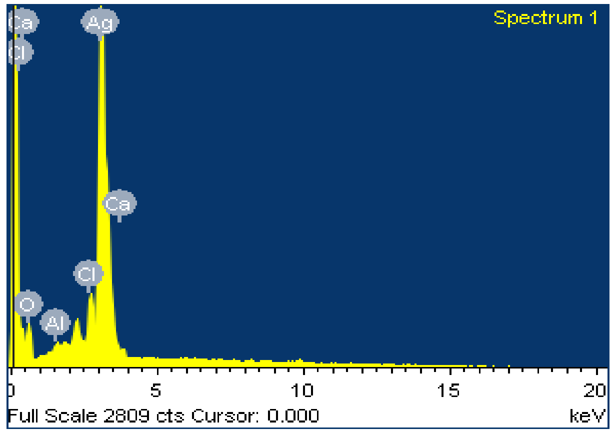

Fig 5.A EDX Spectrum of Silver Nano Particles

3.4 UV-Visible Spectrometer

Fig 5 shows the reduction of silver ions to silver nanoparticles during exposure to plant extracts was observed as a result of the colour change. The colour change is due to the surface Plasmon resonance phenomenon. The metal nanoparticles have free electrons which give the SPR absorption band due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The sharp bands of silver nanoparticles were observed around 488 nm in case of *Achyranthes aspera* .

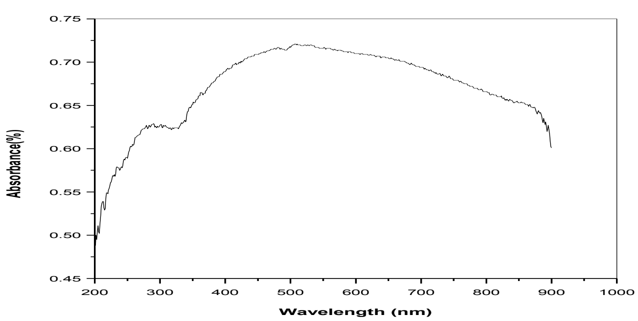

Fig 5 : UV-spectra of silver nanoparticles

3.5 Antimicrobial study on silver nanoparticles

The antimicrobial study of green synthesized AgNps were established against both gram negative and gram positive pathogenic bacteria such as such as staphylococcus aureus (gram positive), ***E-coli*** (gram negative), klebsiella pneumonia (gram negative), citrobactor (gram negative), proteus vulgaris (gram negative) using agar well method as show in **table l**. **Fig 6** shows the zone of inhibition (ZOI) for different pathogen of AgNps. This result was effective when the concentration of silver nanoparticles was observed to be increased with increase in the zone of inhibition. However the zone of inhibition was observed to be more in gram negative bacteria when compared to gram positive bacteria. The maximum ZOI values were observed as 11mm in E-coli bacteria for 100μl concentration of AgNps shown in **table 1**.The Zone of inhibition values observed by klebsiell pneumonia and citrobactor were found to be 12 mm as shown in **table 1**. The Zone of inhibition values observed by staphylococcus aureus and proteus vulgaris were 18 and 14 respectively in shown in **table 1.**

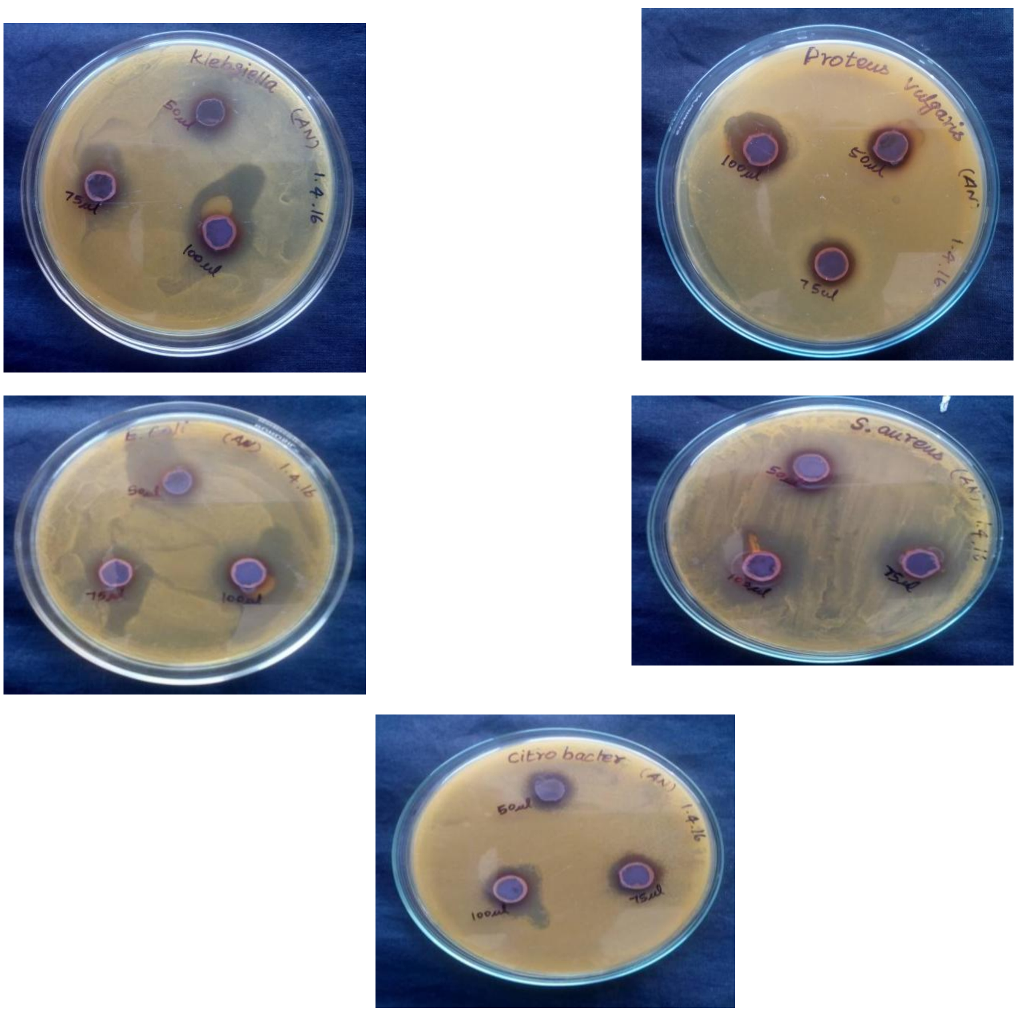

Fig6 Antimicrobial activity of AgNPs

Table 1: Zone of inhibition of AgNPs (mm)

Microbes	Zone of Inhibition (mm)		
	50 µl	**75 µl**	**100 µl**
Escherichia coli	8	9	11
Klebsiella pneumoniae	12	14	12
Staphylococcus aureus	5	12	18
Citrobacter	8	08	12
Proteus vulgaris	5	07	14

IV CONCLUSION

The silver nanoparticles were successfully synthesized by using *Achyranthes aspera* plant to use antimicrobial study which provides cost effective, easy and proficient way for synthesis of silver nanoparticles. The synthesized silver nanoparticles were analyzed using FT-IR, XRD, UV and SEM. Silver nanoparticles were effective utilized for the antimicrobial activity study. The maximum ZOI was found to be more in gram positive bacteria when compared to gram negative bacteria. The *Achyranthes aspera* plant may be effectively utilized for the production of silver nanoparticles with economically for many pharmaceutical applications.

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