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PLANT MEDIATED GREEN AND FACILE SYNTHESIS OF SILVER NANOPARTICLES AND THEIR POSSIBLE APPLICATION AS ANTIMICROBIAL AGENTS

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ABSTRACT

In recent year green and eco-friendly synthesis of metal nanoparticles plays an important role in nanotechnology, medical science, industries, ceramics etc. The manuscript focuses on the green and facile synthesis of AgNPs from aqueous solution of leaf of *Cassia fistula*. UV-Visible spectra confirm the bio-formation of AgNPs at 412 nm with the witness of change in colour. Synthesized silver nanoparticle characterized further by FTIR, SEM, EDX and XRD spectroscopic technique. The shape of AgNPs is observed to be like oval, triangular and cylindrical respectively. EDX analysis confirmed the elemental percentage of silver 93.02%. FTIR shows shifting in bands from lower side to upside.The biosynthesized AgNPs shows antibacterial activity against *E. coli and B. subtilis* pathogens and it was found that AgNPs were more effective against *E. coli and B. subtilis* bacteria. The method used in this study for the biosynthesis of AgNPs from aqueous extracts of *Cassia Fistula* was green, reduce harmful chemical with no environmental hazards.

Keywords: Silver Nanoparticles, *Cassia Fistula*, Antimicrobial Study, Green Synthesis, UV-Vis, SEM, XRD

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INTRODUCTION

Nanotechnology is rapidly growing field in science in the recent years. Scientist among the world working on the green and eco-friendly synthesis of metal nanoparticles because of its wide application in many areas. Plant mediated biosynthesis of metal oxides` nanoparticles has been an ideal process compared to the chemical, physical etc methods. Now a days nanotechnology is the fastest growing filed because of its wide application in many areas like pharmaceutical industry, chemical industry, photocatalyst in various organic name reaction, photovoltaic, ceramic industry, dyes industry etc.[1-7] There are different processes for the synthesis different types of metal nanoparticles in the shape like trigonal, tetrahedral, pentagonal, hexagonal plates, nano-rods etc.[8] Metal nanoparticles can be synthesised by different processes like biological, chemical and physical. Considering the harmful impact on the environment by the processes like chemical and physical methods because they produce harmful b-products and sometimes these are non-biodegradable that why plant mediated biosynthesis of nanoparticles consider to be easy and eco-friendly method.[9-10] Plant-mediated biosynthesis of metal nanoparticles are considered as cost-effective, economic, eco-friendly and can easy scale up to pilot level.[11] The importance of biologically synthesis metal nanoparticles depends on the specific size, shape and structural morphology.[12] AgNPs has huge application in medicinal field, especially as antioxidant and antibacterial agent.[13] In recent year many microbial pathogen cause various diseases up to problem like COVID 19 etc. Thus, the scientist develops various alternative ways to protect the environment from the microbial pathogens, which involve application of AgNPs.[14]

Considering all the issue and importance, the current work was carried out in which AgNPs synthesised by biological process and characterised by various spectroscopical techniques. Synthesized AgNPs tested against gram-positive and gram-negative bacteria to know its antibacterial potential.[15-17]

Plant Description

Fabaceae is the family of *Cassia fistula* and it is well known as the golden shower tree. *Cassia fistula* tree is the national tree of Thailand by well-known golden shower tree and flower is the national flower. Bahawa is the common name of *Cassia fistula* in Maharashtra. In India there is wide application of medicinal plant in Ayurveda and in this *Cassia fistula* tree is known as disease killer. For the purification of blood, the fruits and bark of the *Cassia fistula* tree are used in Ayurveda. The Root of *Cassia fistula* tree has wide application in cardiac disorders, biliousness and rheumatic condition. As per some studied blood sugar can be reduced up to 30% by using the roots of the golden shower tree.[16]

EXPERIMENTAL

Plants and Chemicals

The fresh leaf of *Cassia fistula* was collected from the botanical garden of the college campus. Silver nitrate ($AgNO_3$) was purchased from Merk Chemicals. Throughout the experiment deionised water was used.

Preparation of the Extract

For the reduction of Ag^+ ions to Ag^0 Fresh leaf *Cassia fistula* extract were used. To remove dust and suspended particles leaf was washed twice with deionised and later on dried under sunlight for the preparation aqueous leaf extract. The fine powder of leaf was prepared by grinding the dried leaf in grinder. 5 g fine powder of leaf added to 100 ml double distilled water in Erlenmeyer flask and boil for 10 min before decanting. The obtained extract stored at 4 ^{0}C for further studies after filtration using Whatman no.1 filter paper.

Synthesis of Silver Nanoparticles

0.01 mM aqueous solution of silver nitrate and different concentration of leaf extract were prepared separately. To the 10 ml of 0.01 mM $AgNO_3$ aqueous extract of leaf were added. After 20 min light yellow colour solution changes to dark brown which indicates the precipitation of silver nanoparticles. The resultant microscopical dispersed insoluble particles of silver was characterized by the UV-Vis spectrophotometer.

Characterization of Silver Nanoparticles

UV-Visible Spectral Analysis

The formation of AgNPs was analysed by UV-Visible absorption spectrophotometer model 117 in the range of of 200-800 nm.

Fourier Transform Infra-red Analysis

The FTIR spectroscopy was used for the characterization of biosynthesized AgNPs. 2 mg of silver nanoparticles mixed with 200 mg of KBr and pressed into a pellet. FT-IR spectra at a resolution of 4500-350 cm^{-1} was recorded after subjecting the sample in to sample container.

Scanning Electron Microscopy

Scanning electron microscopy considered the gold standard for the analysis of metal nanoparticles. To study the shape, size and morphology of biosynthesized AgNPs we used this technique. The analysis was performed by keeping a small quantity of sample on the grid. The remaining unnecessary solution was removed using a blotting paper and then the film on the SEM grid were allowed to dry for analysis.

Anti-bacterial Activity

Anti-bacterial activity of biosynthesized AgNPs was determined by disc diffusion method for *E. coli and B. subtilis.* Culture spread plate method was used for inculation at 37°C for 24 hours.

RESULTS AND DISCUSSION

UV-Visible Absorption Studies

The analysis was done in the range of 200-800 nm for Ag Nanoparticles. Plasmon absorbance of silver nanoparticles at 412 nm peak using UV–visible spectrum indicates and confirm the bio-reduction of silver-to-silver nanoparticles.

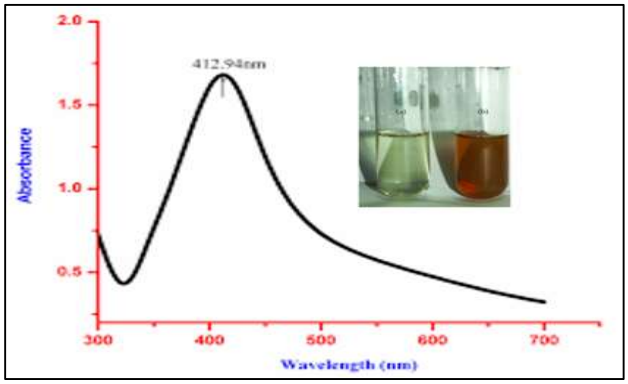

Fig.-1: UV–visible Spectrum of the Biosynthesis of AgNPs

FTIR Studies

The aqueous extract FTIR spectrum and synthesized silver nanoparticles depicted in Fig.-2. In FTIR the peaks are observed at 443, 615, 1072, 1285, 1419, 1598, 3151 and 3781 cm^{-1} respectively. Aqueous extract after reacting with silver nitrate peaks gets transformed from lower to the higher range, such as 461, 621, 1080, 1390, 1610, 3163 and 3884 cm^{-1}. Shifting in 3785 to 3881 cm^{-1} indicated the O–H stretching in carboxylic acids.

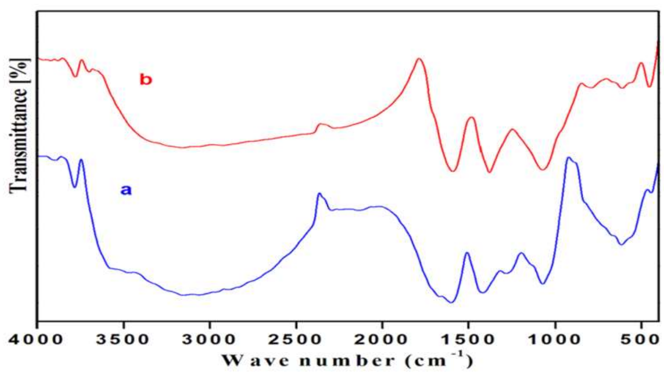

Fig.-2: FTIR Spectrum of Cassia Fistula (a) Pre-reaction and (b) Post- reaction with Silver Nitrate

Scanning Electron Microscopy

The silver nanoparticle SEM images are shown in Fig.-3. From the SEM image it was observed that the shape of AgNPs is like oval, triangular and cylindrical respectively. This is because of different types of capping agents present in the leaf extract. This is also supported by the data of FTIR were shifting in peaks observed.

Energy Dispersive X-ray Spectroscopy

The EDX spectrum depicted in Fig.-4 confirm the presence of elemental silver. The peak observed at 3 keV indicates the absorption of AgNPs. The EDX spectrum shows the signal of silver while weaker signals from Cl and Al atoms. It was nearly clear the data obtained from EDX spectrum; the elemental percentage of silver was 93.02%.

Anti-bacterial Activity

The biosynthesized AgNPs from the aqueous leaf extract of *Cassia fistula* shows high inhibition activity against both *E. coli and B. subtilis* shown in Table-1. The significant difference was observed in bacterial inhibition from 5 µl/ml to 15 µl/ml in biosynthesized AgNPs case.

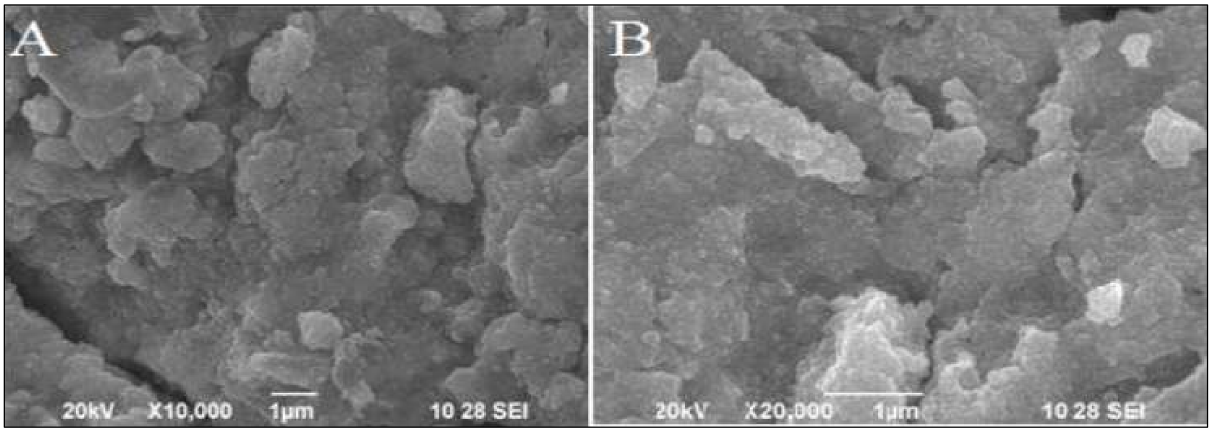

Fig.-3: SEM Monograph of AgNPs: at X10,000 magnification and at X20,000 magnification

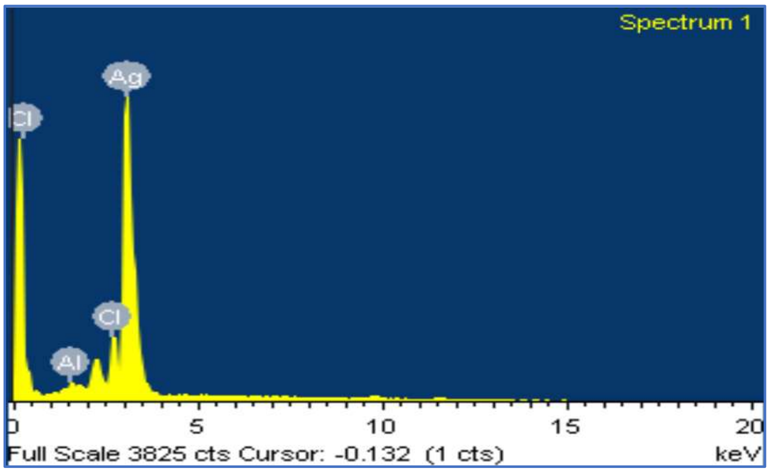

Fig.-4: EDAX Spectrum of Biosynthesised AgNPs

Table-1: Antibacterial Activity of Biosynthesised AgNPs

Name of Bacterium	ZOI of AgNPs (mm)			
	Control 2 mg/ml (ofloxacin)	5 µl/ml	10 µl/ml	15 µl/ml
E. coli	17.3	9.2	9.5	10.2
B. subtilis	17.2	9.7	9.7	10

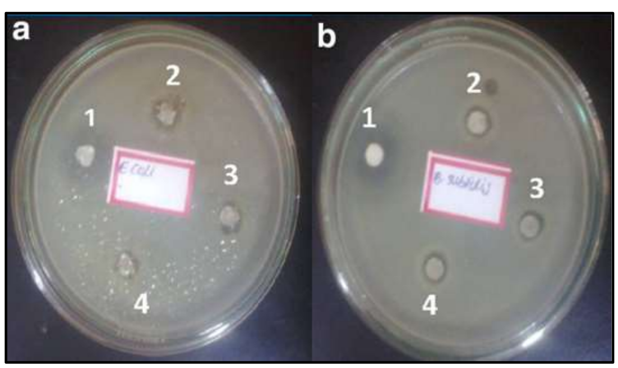

Fig.-5: Antibacterial Activity of AgNPs against *E. coli and B. subtilis* Strains [control (1), 5 µl/ml (2), 10 µl/ml (3), 15 µl/ml

CONCLUSION

In the present study leaf of *Cassia Fistula* was a source for the bio-reduction of silver-to-silver nanoparticles. Such biosynthesis process is green because it does not require any harmful chemical, cost-effective, require less man power so it can be easily scale up to pilot level. The biosynthesized silver nanoparticles confirm by the UV–Vis, FTIR, SEM and EDAX spectroscopical analysis. UV-Vis spectra confirm the bio-formation of AgNPs at 412 nm with the witness of change in colour. The biomolecules present in aqueous extract are liable for reduction and capping of silver nanoparticles are authenticated and verify by the FTIR spectra. EDX analysis confirmed the elemental percentage of silver 93.02%. The biosynthesized AgNPs shows antibacterial activity against *E. coli and B. subtilis* pathogens and it was found that AgNPs were more effective against *E. coli and B. subtilis* bacteria. Finally, is could be concluded that biosynthesis of AgNPs process from leaf aqueous extracts of *Cassia Fistula* was green, reduce harmful chemical, no environmental hazards and could be a potential source for the preparation of drugs in the pharmaceutical industry.

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