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Phytomediated Synthesis of Silver Nanoparticles using *Dicrostachys cinerea* leaf extract and evaluation of its Antibacterial and Photo catalytic activity of Textile dye

Dongamanti Ashok*[1], Raju Sandupatla[1], Rama Koyyati[2]

*[1]Green and Medicinal Chemistry Laboratory, Department of Chemistry, Osmania University, Hyderabad-500007, Telangana, India.
[2]Forensic Science Unit, University College of Science, Osmania University, Hyderabad, Telangana, India-500007.

Abstract : The biosynthesis of nanoparticles have usual increasing attention due to the growing demand to produce secure, cost-effective and environmentally friendly technologies for nanomaterials synthesis. The acquainted study explains the green synthesis, characterization and their potential effect against harmful bacteria, photo catalytic degradation of dye used in the textile dyeing industry, of silver nanoparticles (AgNps) synthesized by using aqueous leaves extract of *Dicrostachys cinerea* plant. Formation of silver nanoparticles was supervised by UV-Visible Spectroscopy. The biomolecules responsible for the formation of AgNps was confirmed by using Fourier Transform Infrared Spectroscopy. The crystalline structure of synthesized silver nanoparticles from *Dicrostachys cinerea* was identified by using X-ray Diffraction studies. Scanning Electron Microscopy images dipicted Nano-sized Particles. The Elemental analysis of AgNps was carried out by Energy Dispersive Spectroscopy.The average size of the particles was calculated by using X-ray diffraction data, and Transmission Electron Microscopy images. The anti bacterial activity of silver nanoparticles was performed by using zone inhibition method and the photocatalytic activity of degradation of textile dye by using sunlight irradiation. The UV-Vis spectroscopy was exhibited exact peak at 406nm and the size of nano particles were identified as 24nm from the X-ray diffraction data and Transmission electron microscopic images. The shape of the nanosized silver nanoparticles was identified as spherical using scanning electron microscopy. The synthesized silver nanoparticles displayed efficient antibacterial activity against gram positive and gram negative bacteria, and it has exhibited photocatalytic activity for degradation of textile dye Green Pls. From the present study it is concluded that the formed silver nanoparticles are stable and showed significant antimicrobial activity, against four different harmful bacteria and also act as silver nanocatalyst for degradation of textile dye Green pls.

Keywords: Green biosynthesis, Silver Nanoparticles, *Dicrostachys cinerea,* X-ray diffraction, SEM-EDX, TEM, Antibacterial Activity, Zone inhibition method, Photocatalytic Activity, *Green Pls.*

Introduction

Synthesis of silver nanoparticles is a booming field trending in the current research owing to their varied properties. The various applications of silver nanoparticles includesensing devices, information storage[1],

recording media[2], biosensor design, catalysis[3], optoelectronics[4], wound healing[5],pollution control[6], genetic disorders detection[7] and determination of ct-DNA[8].Metal nanoparticles represents many improvise new properties based on their specific characteristics, such as size, shape, morphology, and distribution[9]. Traditional methods for the synthesis of silver nanoparticles include microwave-assisted process[10] chemical and photochemical reactions in reverse micelles[11], thermal decomposition[12], radiation assisted, electrochemical[13], sonochemical and reduction in solutions[14]. Synthesis of nanoparticles using physical and chemical methods has been found to possess several difficulties as they are toxic[15] and in maintaining the size and shape. In such a condition, biological synthesis[16, 17, 18] stand as an effective and convenient way for the synthesis of silver nanoparticles. Investigation of biosynthesis of AgNPs nanomaterials is presently getting enormous attention due to ever growing consequence of green chemistry practices; Green plants possess the broadest spectrum of synthetic activity and have been the source of many useful compounds. Many metals have been used for the synthesis of nanoparticles such as gold[19, 20] platinum, titanium[21, 22] copper, zinc, cobalt, magnesium, nickel and silver[23-26] etc. A survey on previous reports of nanoparticle synthesis reports that various parts of the plant[27] can be used for the synthesis of nanoparticles, those were leaves extract[28], flower extracts, fruit extracts, seed extracts[29,30,31] and micro organisms[32] including phototropic bacteria[33].

Dichrostachys cinerea belongs to Mimosaceae, is a semi-deciduous to deciduous tree up to 7 m tall with an open crown leaves bipinnate. The plant is thorny shrub found almost throughout India. It is commonly distributed in all arid zone of Indian dry regions. The bark is used to alleviate headache, toothache, dysentery, elephantiasis Brushed young shoots used in the treatment of ophthalmia, astringent, rheumatism and urinary calculi. The leaves are particularly useful and can be taken to treat epilepsy and can also be taken as a diuretic and laxative, and its powder can be used in the massage of bone fractures and used as fodder[34], the tannins isolated from *Dichrostachys cinerea* were used as bactericides against various bacteria[35].

In the current investigation, for the first time silver nanoparticles were successfully synthesized using *Dichrostachys cinerea* leaf extract, characterization, and applications like antibacterial and photocatalytic activity of silver nanoparticles has been reported. Synthesized silver nanoparticles characterized by various instrumental techniques such as UV-Vis, FTIR, XRD, SEM-EDX, and TEM. The phytochemicals presented in the plant responsible for the synthesis of silver nanoparticles by reducing the metal ions to stable metals, as well as they coated on the surface of the particles as a thin layer to act as a capping agent. Therefore, the objectives of the present study are, (i) to Response surface methodology mediated optimization the AgNPs synthesis using *Dichrostachys cinerea*(ii) to characterize the synthesized AgNPs, (iii) to evaluate the anti-bactericidal activity, and to study the photocatalytic activity of synthesized AgNPs. Because of its applications this silver nanoparticles can be used as antibacterial agents in medical industry and also for bioremediation of chemical textile dyes, as it is useful important in the polluted water treatment.

Material and Methods

Dicrostachys Cenerea is easily available in Osmania University campus, Hyderabad, India. The leaves were collected locally. The bacterial test strains were procured from IMTECH, Chandigarh. The media for the growth of bacterial strains was purchased from Himedia laboratories, Mumbai, India. Silver Nitrate (AgNO3) was purchased from SD fine chemicals.

Preparation of Plant Extract

The leaves were washed thoroughly with distilled water in order to remove the adhering foreign particles. Then the leaves are dried in shade for one week which was then grounded to a fine powder, 5gm of the plant material is added to 500 ml of double distilled water and incubated overnight facilitating the phytochemical extraction. Then the extract is filtered by WhatmannNo.1 filter paper, collected the filtrate and stored at 4 ^{0}C until further use. **Fig. 1 a)** showing the image of the plant selected for synthesis of AgNps and **Fig. 1 b)** showing the pale yellow colour of prepared aqueous leaves extract solution of *Dichrostachys cinerea.*

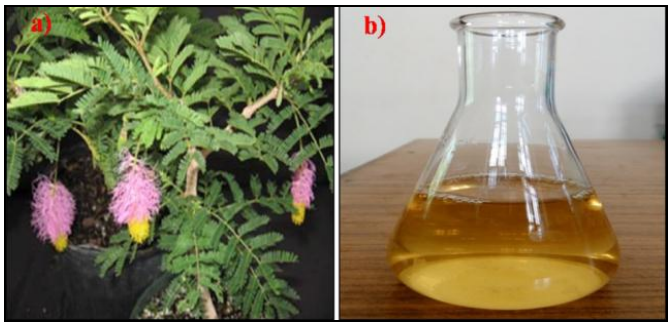

Figure 1: a) *Dicrostachys cenerea* plant b) Prepared leaves extract of *Dicrostachys cinerea* showing pale yellow colour of aqueous solution

Preparation of silver nitrate solution

A fresh stock solution of 1 mM $AgNO_3$ (molecular weight: 169.87 g/mol, assay: 99%) was prepared by dissolving requisite solid $AgNO_3$ in double-distilled water and stored in an amber colour bottle which prevents auto-oxidation of silver.

Biosynthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, the ratio of better volumes was tested by adding leaves extract of *Dichrostachys cinerea* and AgNO3 at different concentrations as 1:0.5, 1:1, 1:2, and 1:3 respectively and incubated for 4 hours at room temperature. After reaction all samples were recorded by UV-Vis spectroscopy, according to UV the sharp peak was noticed at 406 for the ratio of 1:2, which indicates the small sized, more number of particles. So for the preparation of silver nanoparticles, 500 ml of plant extract was directly added to 1000 ml of $AgNO_3$ solution and kept reaction at room temperature. After reaction, the solution was centrifuged at 10000 rpm for 15 min, pellet was collected and centrifugation was repeated twice followed by washing with distil-water to get rid of extra biological material.

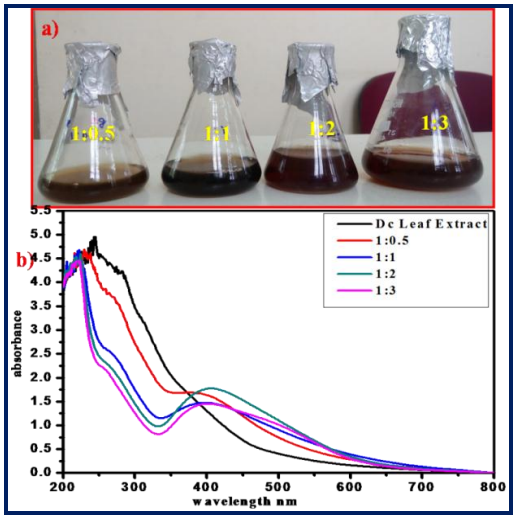

Figure 2: a) Displaying different ratios of volumes of Leaves Extract and $AgNo_3$ as 1:0.5, 1:1, 1:2, and 1:3 continuously. b) UV–VIS spectroscopy of synthesized silver nanoparticles with different ratios of volumes of Dc leaves Extract and $AgNo_3$

Characterization Studies

Visual Examination

Formation of silver nanoparticles was confirmed by converting the pale yellow colour of the solution to dark brown colour, due to surface Plasmon resonance.

UV-VISIBLE Spectrum

The UV spectra of the biosynthesized nanoparticles were recorded by using an UV 2600 UV-VIS Spectrophotometer, Shimadzu by continuous scanning from 200 to 800 nm and the distilled water was used as the reference for the baseline corrections.

FTIR Analysis

FTIR Analysis carried out to identify the phytochemical biomolecules in *Dicrostachys cinerea* plant leaves which were responsible for reduction of silver ions to stable silver nanoparticles as well as they acting as capping agents. 10 µL of the formed silver nanoparticles from the leaf extract was subjected to FTIR analysis using IR Affinity-1 Shimadzu model instrument, with 4500-500 cm^{-1} wavelength range, spectrophotometer in the diffuse reflectance mode at a resolution of $4cm^{-1}$ in KBr pellets.

XRD Analysis

The crystalline structure of synthesized silver nanoparticles was confirmed by x-ray diffraction studies. X-Ray Diffraction analysis of silver nanoparticles was measured by using Philips Xpert PRO, Instrument with Cu Kα X-Ray source with voltage 40 kv, with the scanning rate 2^0 min^{-1} in $1\theta = 2\theta$ configurations.

Scanning Electron Microscopy-Energy Dispersive Spectroscopy

The biosynthesized silver nanoparticles were subjected to Zeiss 700 Scanning electron microscope to determine their morphology. A Thin film of the sample was prepared by dropping a small amount of the sample on the carbon coated carbon grid, then the film on the SEM grid was allowed to dry by keeping under a mercury lamp for 5 min and the images were taken. The EDS microanalysis system which automatically identifies the elements which were corresponds to the peaks in the energy distribution.

TEM Analysis

A drop of the silver nanoparticle sample is placed on a piece of par film of the carbon-coated copper grid and was allowed to settle for 5-10 minutes, and drain the excess with the help of filter paper. Then the grid is washed with distilled water and stained with 2% uranyl acetate and allowed for absorption for few seconds. The grid is then observed under Transmission electron microscopy Hitachi, H-7500 model and the images were recorded.

Anti-bacterial studies

To evaluate applications, the antimicrobial activity of *Dicrostachys Cenerea* mediated silver nanoparticles has been studied. The procedure followed for evaluation of antibacterial action was similar to that of Manisha *et al*[36]. Well diffusion method, all sterilized labware was used to perform this study. Fresh cultures of four different bacteria such as *staphylococcus aureus, bacillus subtillis, Pseudomonas putida, klebsiella pneumonia* was prepared. Four media plates were prepared by inoculation of 20 ml of sterilized nutrient agar media and spread with 50 µL of specific bacteria to each plate, then wells were punched and respective samples were added where ampicillin drug used as a control sample, then plates were incubated at 37 ^{0}C for 12 hrs.

Photocatalytic activity

Photocatalytic activity of biosynthesized silver nanoparticles was performed by using degradation of the textile dye Green Pls, under solar irradiation. The degradation of the textile dye was monitored by UV-VIS Spectroscopy. 100 ml dye solutions was prepared by dissolving 1 mg of the dye in 100 ml beaker with distilled water, Then 50 ml of the dye transferred in another 100ml baker and marked as Control. 10 mg of the bio synthesized silver nanoparticles weighed and added to the dye solution and marked as Sample, and kept for magnetic stirring at 500 rpm under sunlight irradiation. Control dye solution was kept under same conditions for comparing any colour change. For every 30 min of time intervals, 4 ml of colloidal dye mixture was taken and centrifuged at 5000 rpm for 10 min to collect supernatant dye solution, and subjected to UV-VIS spectroscopy for evaluating the degradation of the dye in the presence of silver nanoparticles.

Results & Discussion

Silver nanoparticles were formed by the reduction of Ag+ into Ag^0 by the addition of plant extract to the solution of 1 mM of AgNO3. After incubation, the change in the colour from pale yellow to brown was exhibiting in **Fig. 2a)** indicated the formation of the colloidal AgNPs. The change in the colour was due to surface Plasmon resonance[37] which is an optical property exhibited by silver nanoparticles. The formation of silver nanoparticles was confirmed by UV-Vis absorption spectra at 200-800 nm where a band was observed at 406 nm showing in **Fig. 2 b).**The absence of extra peaks suggesting that nanoparticles are not agglomerated which can be confirmed by SEM and TEM Images which was stated in Venkatesham *et al*[38]. The FTIR Analysis carried out to identify the phytochemical biomolecules in *Dicrostachys cinerea* plant leaves which were responsible for the reduction of silver ions to stable silver nanoparticles as well as they acting as capping agents to prevent agglomeration of formed particles. The main peaks of FTIR data in **Fig. 3** observed at 3331, 1638, 725 which may be due to O-H Stretching or N-H Stretching (alcohols, phenols); -C=C- stretch or N-H Bend (alkenes.1 ^{0} amines), N-H wag or C-H oop (1 ^{0}, 2 ^{0}amines or aromatics) respectively, same results were observed in articles[39-43]. The crystalline structure of synthesized silver nanoparticles was confirmed by X-ray diffraction studies. The diffraction peaks which were showing in **Fig. 4** of silver nanoparticles at 2θvalues, 37.62, 45.78, 63.97, 76.38 these values are assigned to lattice planes of (111) (200) (220) (311) which were represented to face centric cubic (FCC) crystalline structured silver nanoparticles. The XRD data was well matched with the Joint Committee on Powder Diffraction Standards (JCPDS file No. 04-0783). The extra peaks observed, which were weaker than silver were assigned to crystallization of other biological materials found on the surface of AgNps[44], the same kind of results were reviewed in the articles[45, 46].The results from this procedure indicating the formed silver nanoparticles were nanocrystal of nano-size[47, 48]. The particle size of synthesized silver nanoparticles was calculated by using Debye-Scherer formula. The crystalline size of silver nanoparticles ranges between 5-80 nm. Average sizes of DCAgNPS were found to be 20 nm. The same is approximately matched to the size of particles calculated with TEM images.

$D = K\lambda/\beta \cos\theta$

The SEM image at different magnifications in **Fig. 5** showed relatively spherical shape nanoparticles formed. In **Fig. 6** of Energy Dispersive Spectroscopy analysis the peak indicates the presence of elemental silver signal was confirmed, the presence of C, O, Cl peaks indicates the other organic compounds that cover the silver aggregates. The sample contained a high concentration of AgNPS was 71.09% and the atomic % was 30.62 % which was showed in **Table 1**: The TEM images of the nanoparticles formed in **Fig. 7** mostly spherical and near Spherical shapes can be observed within 5-80 nm size range. The **Fig. 8** showing the size distribution of synthesized silver nanoparticles, which can be indicated the mostly of particles distributed in 20-30 nm range.

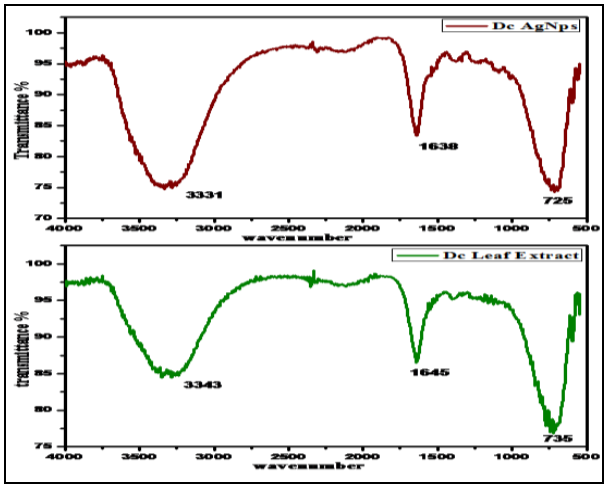

Figure 3: FTIR spectra of Dc Leaves Extract and silver nanoparticles synthesized from *Dicrostachys cinerea (DcAgNps)*

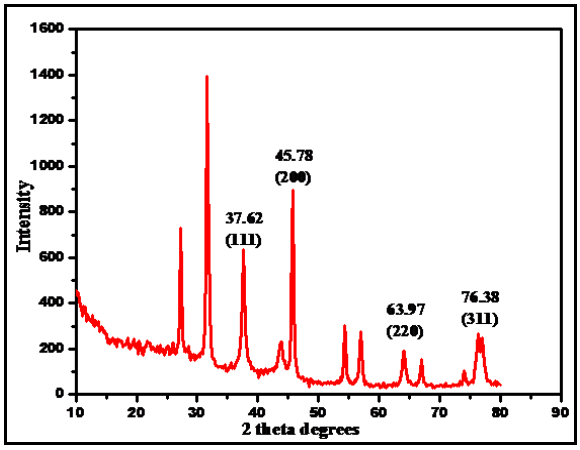

Figure 4: XRD pattern of DcAgNps synthesized from *Dicrostachys cinerea* leaf extract

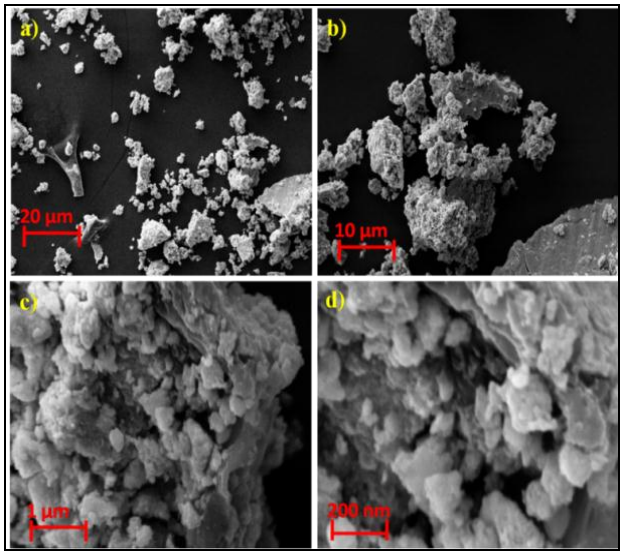

Figure 5: SEM images of synthesized silver nanoparticles from *Dicrostachys cinerea (DcAgNPs)* at various magnifications.

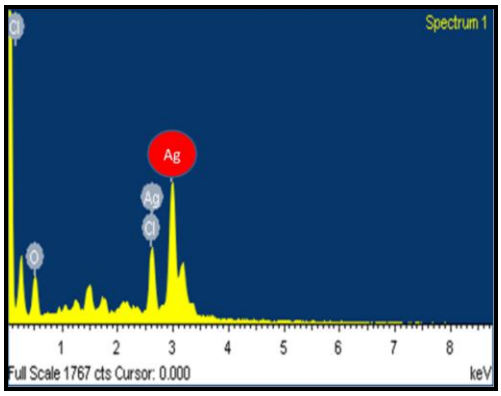

Figure 6: Energy dispersive X-ray spectrum (EDS) of DcAgNps synthesized using the leaf extract of *Dicrostachys cinerea*

Table 1: The table showing high concentration of element silver percentage

Element	Weight%	Atomic%
O	17.78	53.32
Cl	11.12	15.06
Ag	71.09	31.62
Total	100	100

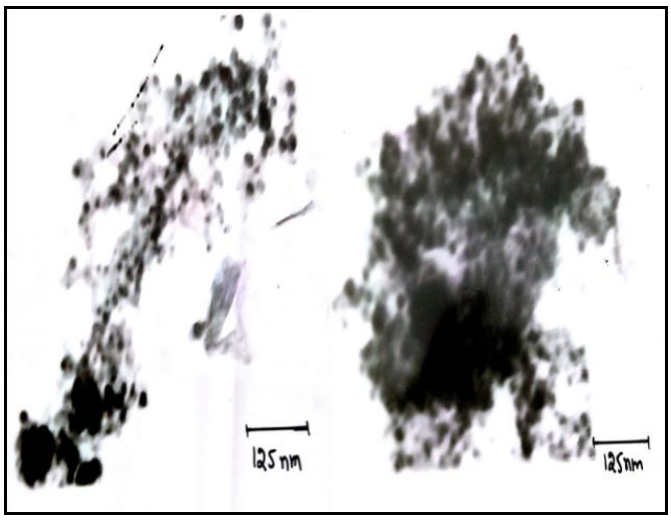

Figure 7: TEM images of synthesized silver nanoparticles from Dicrostachys *cinerea(DcAgNps).*

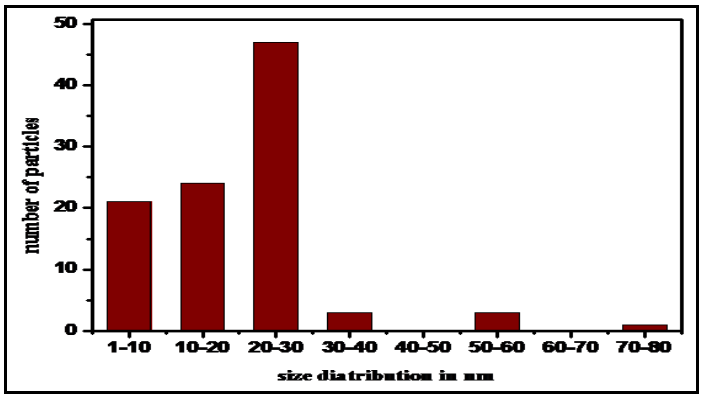

Figure 8: Histogram showing the size distribution of silver nanoparticles synthesized from *Dicrostachys cinerea*

Antibacterial activity

The well-known inhibitory effect of silver has been recognized for several years and used for various medical applications[49]. The **Fig. 9** ofSilver nanoparticles exhibited antibacterial activity against all the four bacterial strains used in the study. Clear zones of inhibition were seen although smaller zone of inhibition was found when compared to the standard antibiotic ampicillin, the results are tabulated as in **Table 2** which was indicating the diameter of inhibition zone in mm for four different bacteria with respected samples. The antibacterial activity images of synthesized silver nanoparticles from *Dicrostachys cinerea* leaves extract were clearly depicting inhibition zone in mm of diameter[50]. Zone of inhibition for synthesized silver nanoparticles for four bacteria *staphylococcus aureus, bacillus subtillis, Pseudomonas putida, klebsiella pneumonia* detected as 23mm, 20mm, 17mm, and 18mm respectively, The 3D diagram of **Fig. 10** revealing the same with respect of samples used in this evaluation.

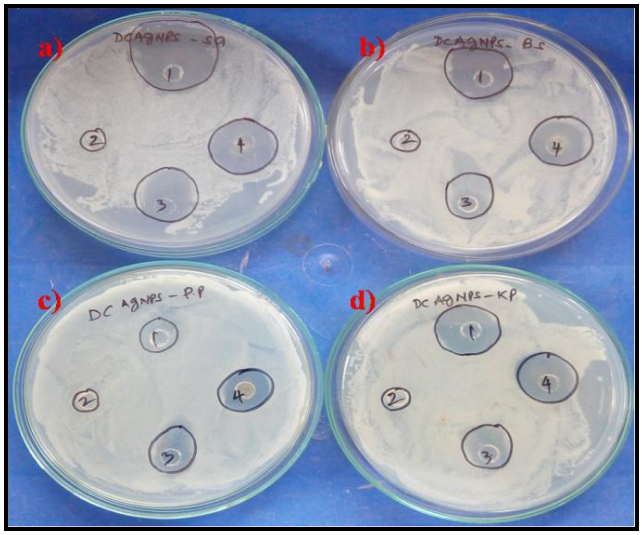

Figure 9: illustrates the Zone of Inhibition for different bacteria, a) *Staphylococcus aureus b)Bacillus subtillis* c) *Pseudomonas putida* d) *Klebesiella pneumonia* for the samples indicating, 1-Plant extract, 2-$AgNO_3$, and 3- DCAgNPS, 4-Ampicillin in each of the plate in 20 µL.

Table 2: showing zone of inhibition in mm of Ampicillin, DC plant extract, $AgNo_3$ DCAgNps for different bacteria

Bacteria	1.DC plant extract 20 µ*L*	2.$AgNo_3$ 20µ*L*	3.Dcagnps 20 µ*L*	4.Ampicillin 20µL
Staphylococcus aureus	8	20	23	28
Bacillus Subtillis	9	15	20	22
Pseudomonas putida	7	14	17	12
Klebsiella pneumonia	8	15	18	20

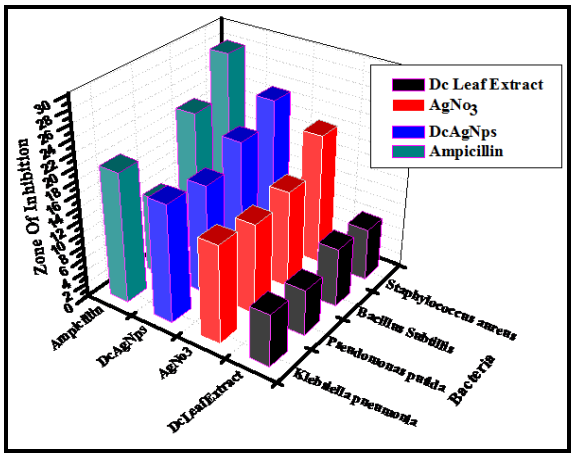

Figure 10: 3D diagrams displaying the zone of inhibition in mm for various bacteria.

Photocatalytic activity

Photocatalytic activity of green synthesized silver nanoparticles from the *Dichrostachys cinerea* leaves extract was evaluated by degradation of the textile dye Green pls under sunlight irradiation. The green Pls dye initially gives deep green colour in dissolving distilled water. The degradation of dye solution was usually observed by gradual decreases in the colour of the dye from deep green to colourless, which was shown in **Fig. 11a)** and it is monitored by UV-VIS spectroscopy. The characteristic absorption peaks for Green pls was noticed at λ_{max} 608 nm. The degradation of the dye in the presence of biogenic Ag nanoparticles was verified by

the decrease of the peak intensity during some minutes of exposure in sunlight confirmed by UV-Vis spectra as shown in **Fig. 11b)**. The gradual decreasing in peak intensity (hypochromic shift) was indicating the gradual degradation of the dye solution. The complete degradation of the dye was observed at 180 minutes of reaction, the same was confirmed by the UV-VIS Spectral data. The dye solution marked as control showing no colour change during the reaction. The % of dye degradation was calculated by using below formulae for each–time interval. The result of dye degradation % of textile dye for each time period was tabulated in **Table 3** and The **Fig. 12** of graph displaying the gradual degradation of textile dye with effect of DcAgNps as a time function.

Dye degradation % = $[C_0\text{-}C_t/C_0] \times 100$ Where

C_0= initial concentration of green pls dye

C_t= after t hours of time, the concentration of the dye under sunlight.

Dye degradation % was calculated as the concentration of the dye solution is directly proportional to the observation value. The degradation mechanism involves the excitation of conduction electrons of metallic Ag in dyes as the earlier reports[51, 52], through exposure in sunlight when the photons from sunlight irradiation hit the biosynthesized silver nanoparticles which were added to sample dye solution, existing in the colloidal mixture, and the electrons at the particle surface are excited[53]. These excited electrons were accepted by oxygen molecules in green Pls dye solution, and they converted as oxygen anion radicals. The oxygen anion radicals break the dye into simpler dye molecules which lead degradation of the textile dye[54, 55].

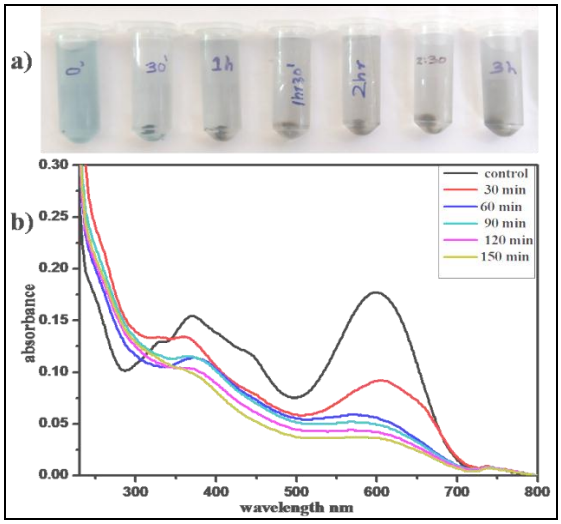

Figure 11: a) Image shows changes in from green to colourless of textile dye after every 30 min of interval. b) UV-VIS Spectroscopy of degradation of textile dye Green Pls

Table 3 showing % of degradation of textile dye

Time interval (min)	Initial OD	Final OD	% of degradation
30	0.1791	0.0842	52.98
60	0.1791	0.0550	69.29
90	0.1791	0.0470	73.75
120	0.1791	0.0421	76.49
150	0.1791	0.0332	82.02
180	0.1791	0.00	100.0

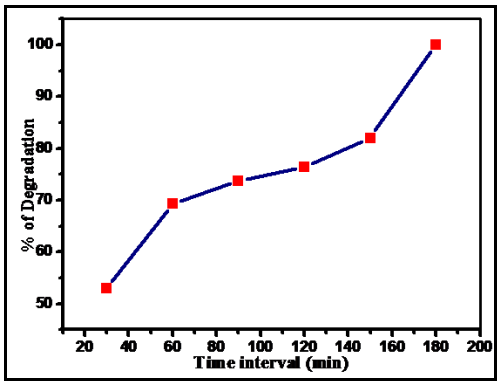

Figure 12: Graph displaying the % of degradation of textile dye at different exposure of time Intervals.

Conclusion

In conclusion, bio green synthesis of silver nanoparticles has materialized as a simpler and better preference than physical and chemical procedures as it is fast, clean and eco-friendly alternative that does not involve any chemicals. The present work shows a green synthetic method using *Dichrostachys Cinerea* leaves extract used as an effective reducing and capping agent for the formation of silver nanoparticles. Synthesized silver nanoparticles from silver nitrate solution using *Dichrostachys Cinerea* leaf extract and the biosynthesized Ag nanoparticles were found to be spherical in shape with an average diameter of 24 nm. The formed silver nanoparticles are stable and showed significant antimicrobial activity, against four different harmful bacteria and also act as silver nanocatalyst for degradation of textile dye Green pls.

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Conflict of Interest The authors declare that they have no conflict of interest.

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