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Phyto-assisted rapid biosynthesis of silver nanoparticles using leaf extract of *Croton caudatus* Geisel and assessment of their dye degradation and antibacterial activity.

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ABSTRACT

Over the past few decades, synthesis of silver nanoparticles using plant extract have gained importance due to its rapid, low cost and environmentally amiable nature. In the present study for the first time sunlight induced stable silver nanoparticles have been synthesized using aqueous leaf extract of *Croton caudatus* Geisel. 90 ml of 10^{-3} M of silver nitrate was mixed with 1ml of plant extract and exposed to bright sunlight. The colour of the reaction mixture changes from light green to dark brown which visually confirmed the formation of AgNPs. The UV-Vis spectra of AgNPs showed a peak at 423 nm. Further, the XRD analysis showed four intense peaks at 2θ = 38.17º, 44.37º, 64.54º and 77.65º respectively confirming fcc structure of AgNPs. The SEM analysis revealed the spherical shape of the synthesized AgNPs.The FTIR showed the possible biomolecules that may be involved in reducing and capping the stable silver nanoparticles. Furthermore the DLS analysis confirmed the particles size to be 37.25 nm and the zeta potential was recorded at -23.9 mV. Also the synthesized silver nanoparticles showed catalytic activity in the degradation of 4-nitrophenol dye by Sodium Borohydride. Moreover the synthesized silver nanoparticles were tested against different bacterial strains viz. *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and Micrococcus luteus* to evaluate its antibacterial activity. Thus, sunlight mediated biosynthesis of the silver nanoparticles from the leaf extract of *Croton caudatus* Geisel plant could be exploited in the field of medicine, agriculture and industries in near future.

KEYWORDS: Silver nanoparticles, catalytic activity, antibacterial activity, agriculture.

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1. INTRODUCTION

Nanotechnology is considered to be one of the emerging field in material science research. Nan particle are those particles that possess size less than 100 nm[1, 2]. Nan particles synthesis have attracted many researchers due to its stupendous physiochemical properties. Nan particles due to their nanoscale size and their surface to volume ratio enable it to be used in a wide range of applications such as in biomedicine, drug delivery[3, 4], electronics, agriculture [5], energy, medicine, environmental remediation [6, 7], catalysis, solar energy, water treatment, etc[8].

Various metals such as Au, Ag, Zn, Fe etc. have been used to produce metallic nanoparticles. Among all, the silver nanoparticles have gained considerable attention due to their remarkable physical, chemical and biological properties. It has been known from a long time that silver exhibits a strong antimicrobial activity[9, 10].Silver nanoparticles due to its wide range of antimicrobial activity as well as low toxicity in humans is used in various applications such as water purification system, textile industry, cosmetics, medical instruments, tropical ointments etc.[11, 12].Moreover, literatures suggest that silver nanoparticles arrest the growth of the bacterial cells by inducing DNA damage, inhibition of the metabolic enzymes, inducing apoptosis in the cell, damage to the mitochondria etc. [13, 14].

Literatures suggest that silver nanoparticles can be synthesised using chemical, physical and biological methods[15]. The chemical and physical method for synthesis of silver nanoparticles include chemical reduction, heat vaporization, electrochemical reduction [16], wet chemical method, pyrol method [17], Laser ablation, thermal decomposition, gamma radiation assisted etc. [18]. The chemical synthesis involves the use of toxic chemicals which are harmful toward environment as well as area not suitable to use in biomedical application [19]. The synthesis of silver nanoparticles using biological methods have gained interest among researchers due to its cost effective, simple and environmentally benign process [20, 21]. The biological methods include use of microorganisms, plant extract, enzymes [22], bacteria fungi etc. [23]. Using plant extract for the biosynthesis of silver nanoparticles is considered to be cost effective, rapid and ecofriendly. Advantage of using plant extract for the biosynthesis of silver nanoparticles over microorganisms are the latter require aseptic conditions as well as culture maintenance[24, 25]. Reports suggest that synthesis of silver nanoparticles using plant extract may be due to the presence of the biomolecules such as phenols, flavonoids etc. in the plant extract [26].

The word “Croton” was derived from Greek word “*Kroton*” which mean Ticks due to its seed resembles as ticks. *Croton caudatus* belongs to the family Euphorbiaceae. Literature suggests that the leaf of *Croton caudatus* is used as ethnomedicine for treating cancer in the Saikot region of Manipur [27]. According to the literature the leaves of the *Croton caudatus* are used to treat malaria, convulsion, ardent fever, arthritis, liver disorders, and sprains[28, 29]. In this study we report for the

first time the sunlight mediated biosynthesis of stable silver nanoparticles using leaf extract of *Croton caudatus* and assessment of its catalytic activity in the reduction of 4-nitrophenol. Furthermore the antibacterial activity of the synthesised silver nanoparticles was evaluated against bacterial strains such as*Escherichia coli, Bacillus subtilis, Bacillus cereus, Micrococcus luteus, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

2. MATERIALS AND METHODS

2.1Chemicals:

Nutrient agar was purchased from Himedia (Mumbai). Silver nitrate ($AgNO_3$) and Sodium Borohydride ($NaBH_4$)were purchased from Merck (Mumbai). P-Nitrophenol ($C_6H_5NO_3$) was purchased from Loba Chemie, Mumbai, India, respectively. The working stocks were prepared freshly using double distilled water.

2.2 Preparation of aqueous leaf extract of Croton caudatus:

Freshleaves of *Croton caudatus*were collected from Guwahati, Assam region. The leaves were first washed thoroughlyunder tap water and then washed twice with double distilled to remove any impurities. The leaves were shade dried at room temperature for 7 days. The dried leaves were grinded to fine powder using an electric grinder.

15gram of fine powderof *Croton caudatus* leaves were mixed with 100 ml of double distilled water and boiled at 70ºC for 20 minutes. The extract was cooled and filtered using Whatman filter paper No. 1 (pore size 25µm). The extract was then stored at 4 ºC for further experiments.

2.3*Phytochemical analysis:*

The preliminary qualitative phytochemical analysis was performed on the aqueous leaf extract of *Croton caudatus*:

2.3.1 Carbohydrate Test:

Fehling's Solution:

1ml of solution A and 1ml of solution B was mixed in a test tube. 2ml of the mixture solution was added to 2ml of the aqueous leaf extract and heated at water bath for 2-3 minutes at 60ºC. A brick red precipitate was observed in the bottom of the test tube indicated the presence reducing sugar.

2.3.2: Cardiac glycosides:

2ml of Crude leaf extract was added to 2ml of glacial acetic acid. Few drops of 2% FeCl solution was added. Now the solution was poured into separate test tube containing conc. H_2SO_4. A brown ring appeared confirming the presence of cardiac glycosides.

2.3.3: Test for Protein

2ml of aqueous leaf extract of *Croton caudatus*was mixed with 2ml of 2% Ninhydrin solution and boiled at 60ºC for 15minutes. No colour change was observed [30].

2.3.2: Test for alkaloid:

20 mg of the dried powder of *Croton caudatus* leaf was mixed with 1% Hydrochloric acid and boiled in water bath for 5-7 minutes. The extract was boiled using filter paper. The filtrate was used for following tests:

Wagner's Test:

2g of Potassium and 1.27g of iodine was initially mixed in 5ml of double distilled water which was later diluted to 100ml. To 1ml of the filtrate few drops of Wagner's reagent was added. Formation of reddish brown precipitate indicated the presence of alkaloid.

Mayer's Test:

To few ml of filtrate 1ml of the Meyer's reagent (Potassium mercuric iodide solution) was added. Creamy coloured precipitate was formed which indicated the presence of alkaloids [31].

2.3.3: Test for Flavonoids:

Lead acetate Test:

To 1ml of aqueous leaf extract of *Croton caudatus* few drops of 10 % lead acetate was mixed. A yellow precipitate formation confirms the presence of flavonoids.

Alkaline reagent test:

2ml of 2% NaOH was added to the aqueous leaf extract of *Croton caudatus.* Formation of an intense yellow colour which become colourless upon addition of dilute H_2SO_4, thus confirmed the presence of flavonoids.

2.3.4: Saponin Test:

Foam Test:

5ml of the aqueous leaf extract of *Croton caudatus* was taken in a test tube and was shaken vigorously for 5 minutes. Formation of persistence foam indicated the presence of saponin[32].

2.3.5: Test for fixed oils:

Spot Test:

Few drops of leaf extract was put in filter paper and pressed with another filter paper. oil stains on the filter paper confirmed the presence of fixed oil in the aqueous leaf extract of *Croton caudatus*[33].

2.4 Biosynthesis of silver nanoparticles:

For the biosynthesis of the stable silver nanoparticles, silver nitrate ($AgNO_3$) was used as a precursor. To the 90 ml of 1mM silver nitrate solution, 10 ml of aqueous leaf extract of *Croton caudatus* was added and exposed to bright sunlight. The change in the colour of the solution was observed and recorded at certain interval of time. The synthesis of the stable silver nanoparticles was monitored periodically using UV-Vis Spectrometer. The AgNPs solution was centrifuged (Eppendorf AG Model No. 5430R) at 14,000 rpm for 20 minutes. The pellets obtained was washed 3 times with double distilled water to remove any unbounded biomolecules. The pellets were dissolved in double distilled water and stored at 4ºC for further characterization.

2.5 Reduction of 4 nitrophenol (Catalytic activity test of biosynthesis silver nanoparticles):

The biosynthesised silver nanoparticles was used as a catalyst to evaluate the reduction of 4-NP by $NaBH_4$. The chemical reaction was carried out in a 3.5 ml Quartz cuvette. For the reduction reaction , 1.5 ml of 0.002M 4-NP was mixed with 1.5 ml of double distilled water in the cuvette. To the solution mixture 1ml of 0.015M $NaBH_4$ was added along with 0.5ml of biosynthesised silver nanoparticles to act as a catalyst.The UV-Vis spectra of the reaction mixture was recorded after every 5 minutes in the range of 300 – 600 nmat 25º C using Analytikjena SPECORD 50 PLUS spectrophotometer[34, 35].

3. DETECTION AND CHARACTERIZATION OF SILVER NANOPARTICLES

3.1 UV-visible spectroscopy:

The synthesis of the stable silver nanoparticles using leaf extract of *Croton caudatus* was monitored by UV-Vis spectroscopy (Thermo fisher scientific Multiskan go Model no. 1510) in the spectrum range of 300 – 800 nm with water as reference.

3.2 X-ray diffraction analysis (XRD):

The X-ray diffraction (XRD) spectra of the biosynthesised silver nanoparticles was recorded by Bruker AXS, Germany, D8 Advance, operated at a voltage of 40kV and a current of 40mA with Cu Kα radiation.

3.3 Fourier transforms infrared spectroscopy (FTIR):

The crude leaf extract of *Croton caudatus* and the dried pellets of the synthesised silver nanoparticles were subjected to FTIR analysis by Thermo Nicolet 6700 FTIR Spectrophotometer in the range of 650-4000 cm^{-1} so as to compare the data and reveal the possible functional groups that were responsible for the biosynthesis of AgNPs.

3.4Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDAX) analysis:

The biosynthesized silver nanoparticles were subjected to SEM analysisby Zeiss Sigma 300to determine the shape of the particles. The EDAX spectra of the biosynthesised silver nanoparticles were recorded using Element EDAX instrument.

3.5Zeta Potential and Dynamic Light Scattering (DLS):

The average size distribution and the zeta potential of the synthesised silver nanoparticles were measured by means of Dynamic Light Scattering (DLS) in the range between 0.1 – 1000 μmat 25 ºC using Zetasizer Nano Series-ZS90.

3.6 Antibacterial activity of AgNPs:

The antibacterial activity of the biosynthesized silver nanoparticles using the leaf extract of *Croton caudatus* was performed using disc diffusion method as described by Bauer *et al*. with few modifications [36].The bacterial strains were cultured in nutrient broth for 24 hours at 37ºC having (1×10^5)CFU/ml. The bacterial culture was then spread overnutrient agar plates. Sterile disc of 6 mm in diameter containing AgNPs and aqueous leaf extract of *Croton caudatus*were placed on to the agar plates. $AgNO_3$and gentamic in were used as negative and positive was control respectively. The plates were then cultured for 24 hours at 37 ºC. The zone of inhibition was measured.

4. RESULTS AND DISCUSSION

4.1 UV-Vis spectroscopy:

In the present study, synthesis of silver nanoparticles was carried out using leaf extract of *Croton caudatus.* 10 ml of the aqueous leaf extract of silver nanoparticles was mixed with 90ml

of1mM silver nanoparticles and kept under the bright sunlight (Figure 1). The change of the solution mixture from light yellow to dark brown indicated the formation of silver nanoparticles (Figure2b). The UV-Vis spectra of the biosynthesised silver nanoparticles showed an intense peak at 425 nm (Figure 2) due to the Surface plasmon resonance exhibited by the biosynthesised silver nanoparticles[37].The UV-Vis spectra of biosynthesised silver nanoparticles at different interval of time (as shown in Figure3) depicted that the absorption spectra of the AgNPs increases with increase in time. There was no significant increase in the absorption spectra after 27 minutes which may be due to complete conversion of Ag^{+} ions into AgNPs[38].

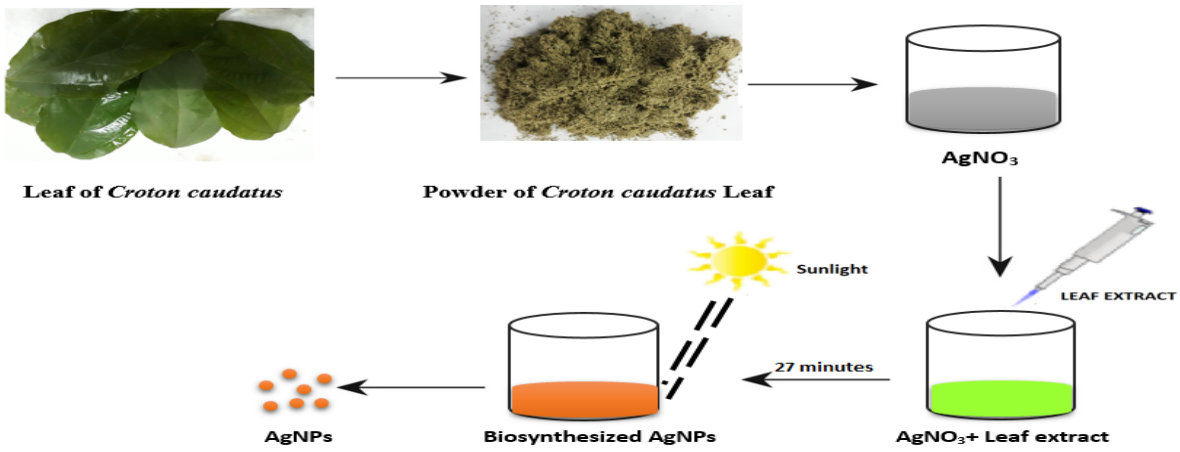

Figure1: Schematic diagram for the biosynthesis of AgNPs from leaf extract of Croton caudatus.

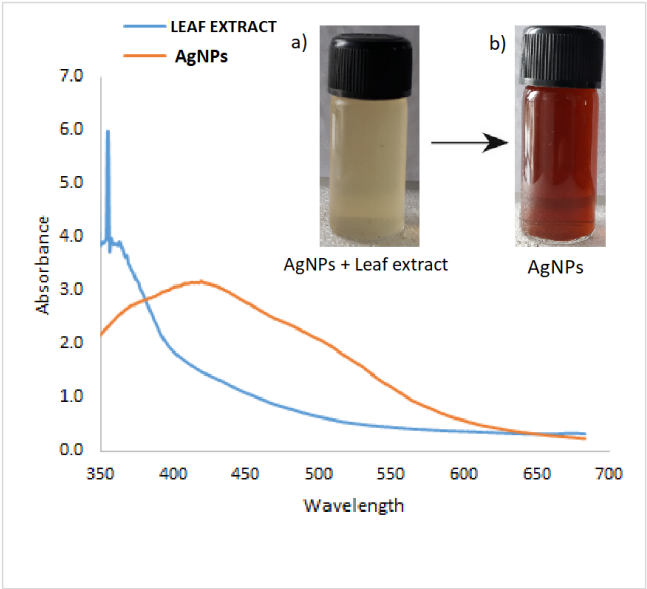

Figure 2: UV-Vis spectra of biosynthesised AgNPs a) Mixture of $AgNO_3$ and Leaf extract of Croton caudatus b) Biosynthesised AgNPs.

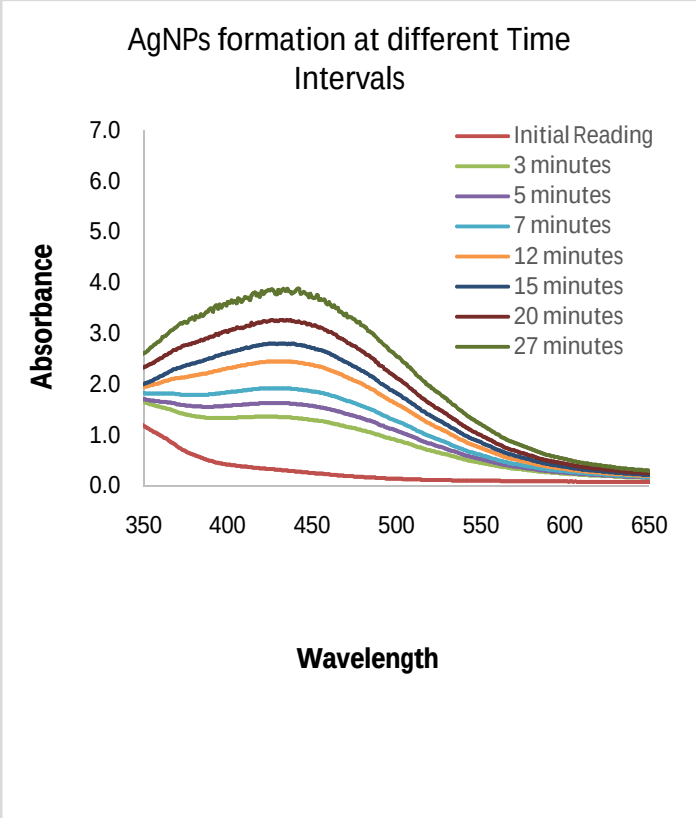

Figure 3: Time-dependent UV-Vis spectra of biosynthesized AgNPs.

4.2 Phytochemical Analysis:

The Phytochemical screening of the extract revealed theBio-organic composition of the *Croton caudatus*leaf. The phytochemicals that are present in the aqueous leaf extract of *Croton caudatus* are depicted in Table 1. *Croton caudatus*leaf consists of Alkaloid, Cardiac Glycosides, Flavonoids, Saponin, Carbohydrates etc.

Table 1: Result of Phytochemical Analysis:

Phytochemical	Types of test	Leaf extract
Alkaloid Test	Mayer's Test	+
	Wagner's Test	+
Cardiac Glycosides	Keler-Killiani Test	+
Flavonoid Test	Alkaline Reagent Test	+
	Lead Acetate Test	+
Saponin Test	Foam Test	+
Carbohydrate	Fehling's Test	+
Fixed Oil Test	Spot Test	+
Protein and Amino acids Test	Ninhydrin Test	-

(+)Present, (-) Absent

4.3 X-ray Diffraction (XRD) analysis:

The biosynthesized silver nanoparticles from the leaf extract *Croton caudatus*were subject to XRD analysis as shown in the Figure 4. The XRD analysis showed four intense and sharp peak at 2θ

= 38.17º, 44.37º, 64.54º and 77.65º which corresponds to (111), (200), (220) and (311) planes. Thus from the XRD data it has been found that the synthesized AgNPs are crystalline structure with fcc structure.

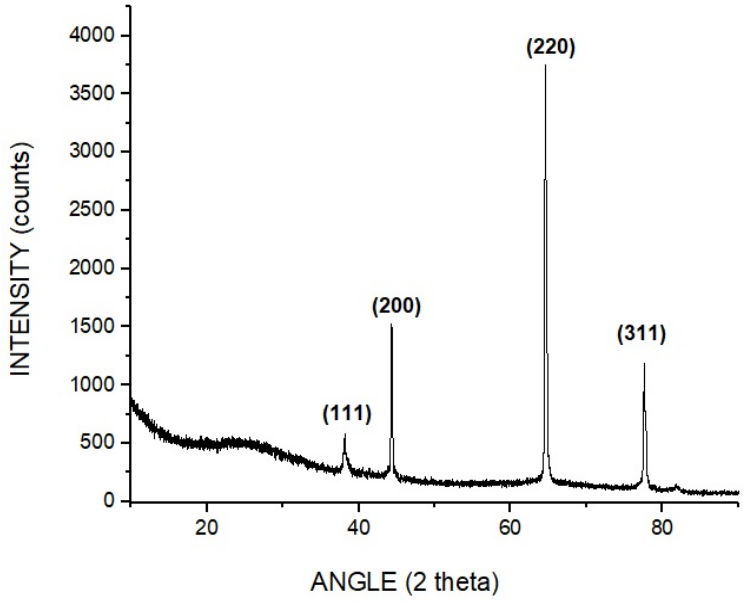

Figure 4: XRD pattern of biosynthesised AgNPs.

4.4. Fourier transform infrared spectroscopy:

The FTIR analysis of both the leaf extract of *Croton caudatus* and the synthesised silver nanoparticles was carried out to identify the possible biomolecules involved in reduction and capping of the AgNPs as shown in Figure 5.

The FTIR spectra of the leaf extract of *Croton caudatus* showed intense peak at 3399cm^1(N-H Stretching), 2937.01 cm^1can be attributed to C-H stretching, 1618cm^1corresponds to C=O groups, 1510cm^1 may be due to– C=C- from aromatic group, 1395 cm^1 is due to NH_2 stretching, 1245 cm^1may be due to –C-O- bending, 1075 cm^1 corresponds to –C-N bending, 923 cm^1 can be attributed to –C-H- group. The peak 773 cm^1 and 620 cm^1 appears may be due to –C-Cl- stretching of alkyl halides. The FTIR absorption spectra of biosynthesised silver nanoparticles showed peaks at 3360 cm^1, 2921 cm^1, 1627 cm^1 and 1364 cm^1which is due to N-H stretching, C-H stretching and C-C stretch aromatic. The peaks 1038 cm^1 can be attributed to C-N stretching vibration of the amine. Therefore, the overall peak from the FTIR analysis indicated that various biomolecules that are present in the crude leaf extract of*Croton caudatus* are responsible for both reducing and capping of the synthesised AgNPs.

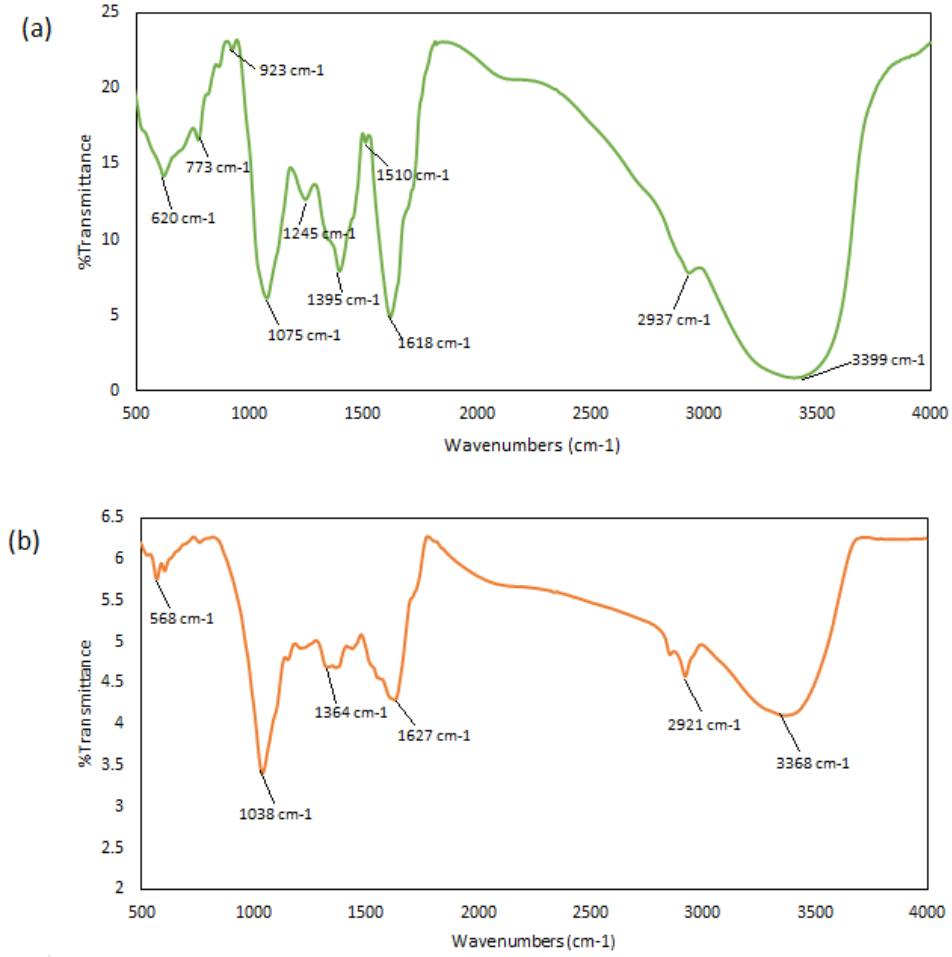

Figure 5: FTIR spectra of (a) aqueous leaf extract of Croton caudatus (b) Biosynthesised AgNPs.

4.5. Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDAX) analysis:

The SEM analysis as shown in the figure 6revealed the spherical shape of the biosynthesised silver nanoparticles and most of the particles were aggregated. Moreover the EDAX analysis (Figure 7)showed a strong signal at 3 ke V, due to the surface plasmon, of the biosynthesised silver nanoparticles. Apart from Ag other elements such as Cl, O, C are also present which may be the surface biomolecules responsible for reducing and capping of the AgNPs generating from the leaf extract of *Croton caudatus.*

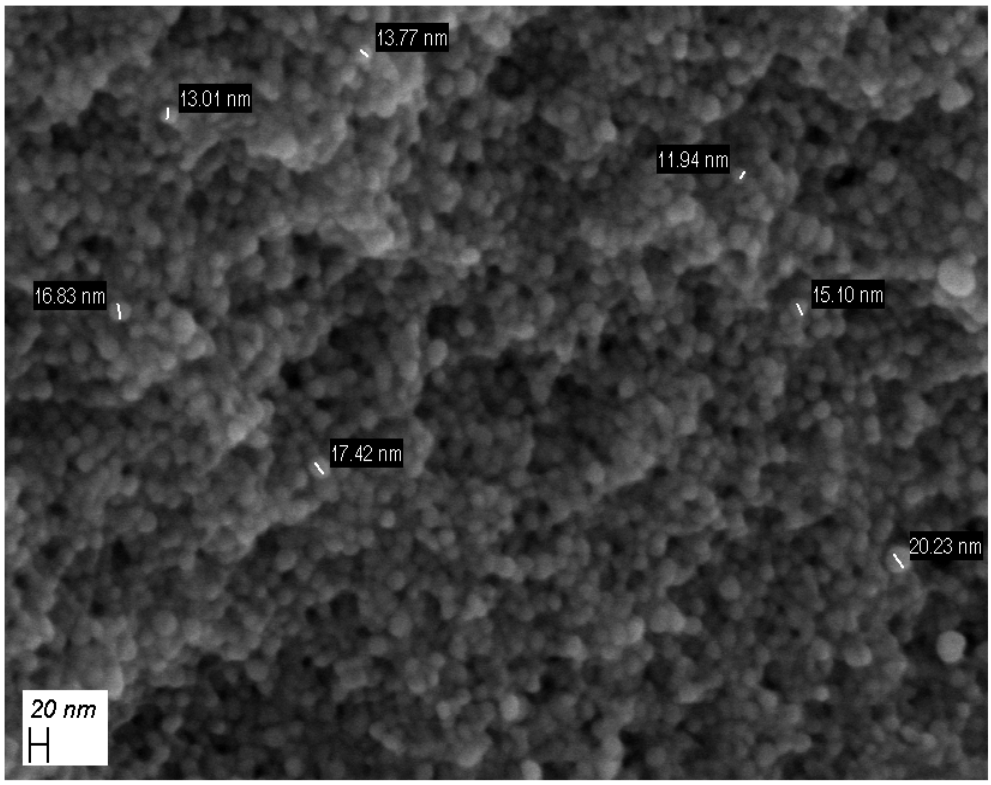

Figure6: SEM micrograph of biosynthesised AgNPs.

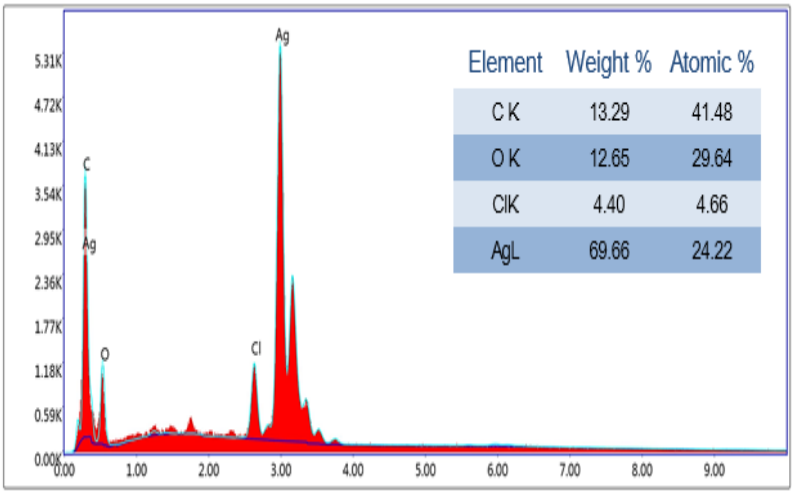

Figure 7: EDAX profile of biosynthesised AgNPs.

4.6. Zeta Potential and Dynamic Light Scattering (DLS):

The average size distribution and the Zeta Potential of the biosynthesised silver nanoparticles form the leaf extract of *Croton caudatus* is shown in the figure8. The average size distribution of the synthesisedsilver nanoparticles was 37.25 nm. The zeta potential of the biosynthesised silver nanoparticles was recorded with a sharp peak at -23.9 mV. The negative value of the zeta potential thus confirms that the particles repulse each other and thus proves to be stable[39].

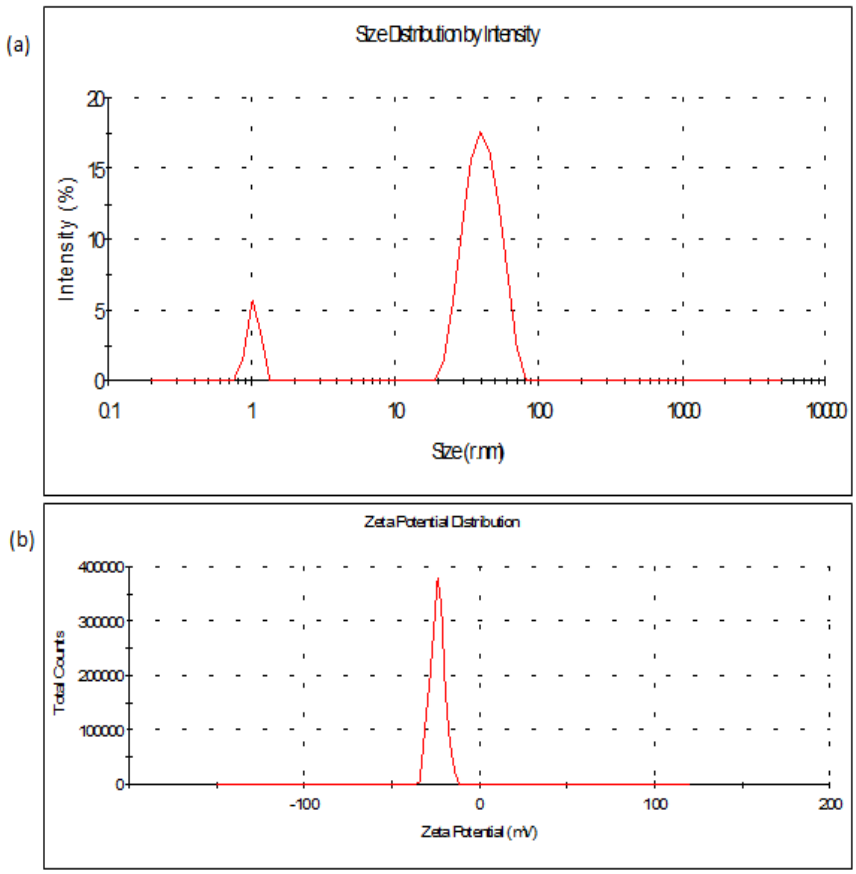

Figure 8: a) DLS and b) Zeta potential result of the synthesised AgNPs.

4.7. Reduction of 4 nitrophenol (Catalytic activity test of biosynthesis silver nanoparticles):

The catalytic activity of the biosynthesised silver nanoparticles in reduction of 4-Nitrophenol to 4-Aminophenol is a very much important due to the harmful impact of 4-Nitrophenol which is being used for the preparation of various organic compounds in various industries which cause serious health issues.

The reduction of 4-nitrophenol by $NaBH_4$ is a slow reaction. Initially the $NaBH_4$ breaks down the water molecule to release hydrogen as mentioned in the reaction. Further the reaction is carried out by the hydrogen that produce hydrogen gas which gives rise to bubbles in the reaction mixture. The slivernanoparticles facilitates the reaction as a transporter of hydrogen molecules between $NaBH_4$ and 4 – NP. Also there is an increase in the peak at 290 nm which is due to the formation of 4 Aminophenol.

$$\mathbf{NaBH_4 + H_2O \rightarrow NaBO_2 + 4H_2}$$

In the present study the biosynthesized silver nanoparticles acts as a catalyst by transporting between $NaBH_4$ and 4–NP and reduced 4-NP dye from 0 to40 minutes of the reaction which was recorded by UV-Vis as shown in Figure 9 [40, 41].

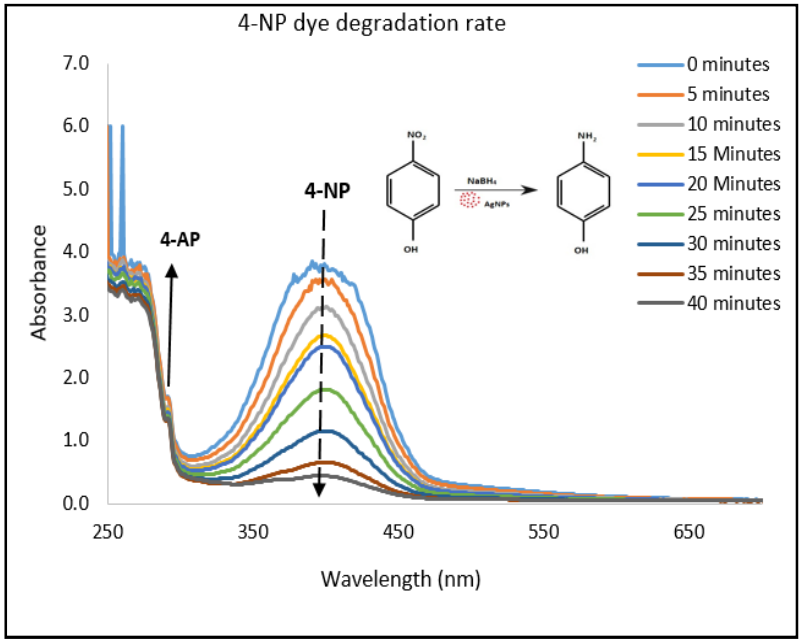

Figure 9: UV-Vis spectral image for the degradation of 4-NP dye by $NaBH_4$ with biosynthesised AgNPs as catalyst.

4.8. Antibacterial activity of AgNPs:

The synthesised silver nanoparticles were tested for their antibacterial activity against different bacterial strains such as *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and Micrococcus luteus* using disc diffusion method as shown in the figure 10. The antibiotic gentamicin was used as a positive control. The result of the antibacterial activity of the synthesised AgNPs is shown in Table 2. The synthesised AgNPs showed high inhibitory effect compare to crude leaf extract of *Croton caudatus*against all the bacterial strains. Reports suggest that there are various mechanism which inhibits the growth of bacteria in the presence of AgNPs such as inducing efflux of intracellular phosphate, Silver ions cause DNA mutation in the bacterial DNA, destruction of the outer membrane leading to release of the intracellular contents etc.[42]

Table 2: Antibacterial activity of biosynthesised AgNPs:

Sl. No.	Bacterial Strains	Gentamicin	Leaf Extract (cm)	AgNPs (cm)
1.	*Bacillus subtilis*	2.8 cm	1 cm	1.4 cm
2.	*Escherichia coli*	2.8 cm	0.9 cm	1.5 cm
3.	*Staphylococcus aureus*	2.9 cm	1 cm	1.1 cm
4.	*Pseudomonas aeruginosa*	3 cm	0.5 cm	1.8 cm
5.	*Bacillus cereus*	2.9 cm	1 cm	1.4 cm
6.	*Micrococcus luteus*	3 cm	1 cm	1.8 cm

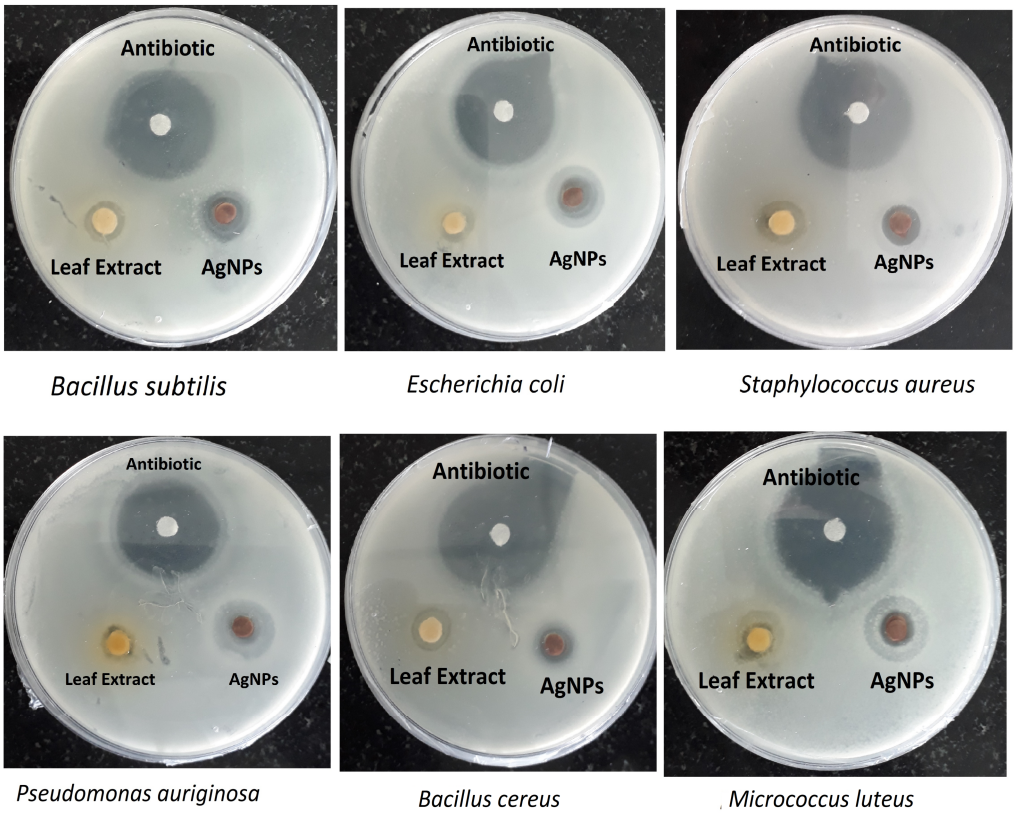

Figure 10: Antibacterial activity of the synthesised AgNPs using leaf extract of Croton caudatus.

5. CONCLUSION:

From the present study it can be concluded that stable silver nanoparticles can be synthesised using aqueous leaf extract of *Croton caudatus* under the influence of sunlight which is a rapid, simple and eco-friendly process.Moreover, the UV-Vis analysis of the synthesized AgNPs showed an intense peak at 425 nm, which confirmed the formation of silver nanoparticles. The stable AgNPs were further characterized using various techniques such as XRD, FTIR, SEM, EDX and DLS. The average size of the AgNPs was 37.25nm with –23.9 mV zeta potential and were spherical in shape. In addition the synthesisedAgNPs showed a high inhibitory effectagainst pathogenic bacterial strains viz. *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus and Micrococcus luteus*as compare to crude leaf extract of *Croton caudatus*. Thispreliminary study provides a rapid, simple and cost effective approach for the synthesis of silver nanoparticles from leaf extract of *Croton caudatus* that could be used as an antibacterial as well as a dye degrading agent in near future. Moreover the synthesised AgNPs can further be exploited in pharmaceuticals, agricultural etc. applications.

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