



Original Research Article

***Eichornia crassipes* mediated biofabrication of silver nanoparticles and spectroscopic evaluation of its catalytic efficacy in the degradation of azodyes**

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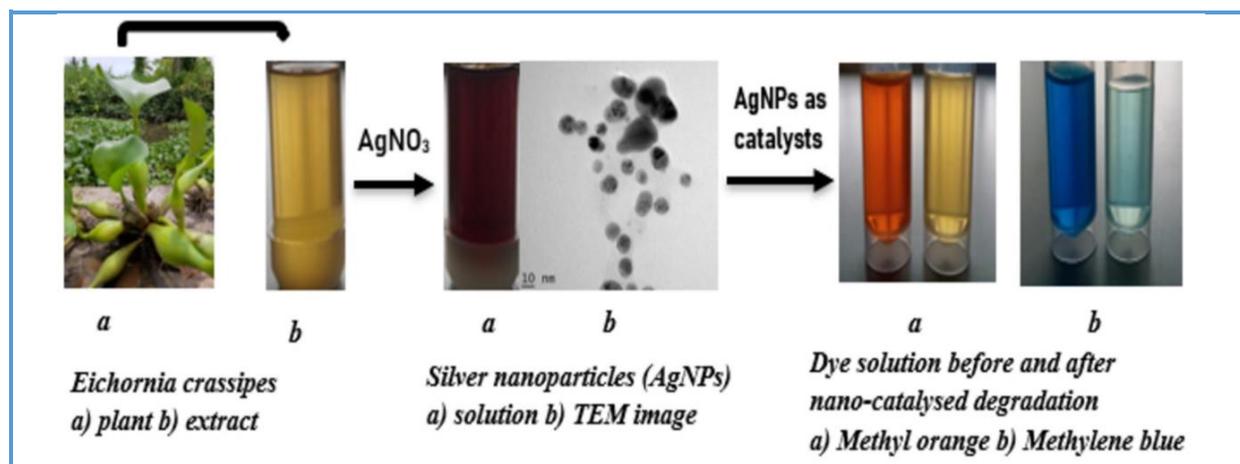
Silver nanoparticles
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Water hyacinth
Catalytic Degradation
Azodye

ABSTRACT

Aquatic resources explored for the biofabrication of nanoparticles are mainly the weed-plants owing to their wide distribution and abundance. *Eichornia crassipes* (Water hyacinth) is considered the most notorious aquatic weed due to its prolific spreading ability in water bodies. The present study investigated the ability of aquatic weed *Eichornia* in green synthesis of silver nanoparticles and the catalytic efficacy of the thus made nanoparticles in degradation of azodyes, a major class of industrial pollutants. The method adopted is entirely a green chemistry approach and is facile, economical, and efficient in mass production. The synthesis was confirmed by observation of SPR peak at 422 nm. FTIR revealed the presence of phenols, amino acids, and carbohydrates. TEM analysis confirmed the particle to be nearly spherical with a size distribution from 10-20 nm. The crystallinity of the particle was assessed using XRD technique and the crystalline size was calculated 14.64. The catalytic efficacy of the nanoparticle was then spectrophotometrically monitored. The degradation of pollutants was 92.46% and 91.9%, respectively for methyl orange and methylene blue. Thus, the biosynthesized silver nanoparticles using low-valued renewable resources like aquatic weeds proved a promising tool in aquatic bioremediation.

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Graphical Abstract



Introduction

Water, the ‘universal solvent,’ can dissolve more substances than any other liquid on earth. For this reason, our water bodies are under significant threat of frequently getting ‘impure’ by washing off all other impurities. Water contaminants, mainly carcinogenic and mutagenic pollutants and heavy metals, act as slow poisons with serious chronic effects [1]. Their sources can be either point or non-point in nature [2]. Most of the industrial pollutants reaching the water bodies are from point sources, through direct discharge of effluents from factories. This includes dyes from textiles and paper mills, toxins from fertilizer industries, and heavy metal ions. As per the 11th annual report on World’s Worst Pollution Problems published by Green Cross Switzerland and Pure Earth, dyes are the 10th worst pollutant affecting life on earth [3].

During the dying and subsequent washing of textiles, many dye is lost in the effluent waters. These dyes are resistant to degradation by traditional wastewater treatment plants; therefore, they are released into the environment untreated, polluting surface and ground waters in locations where such

industries exist [4]. Reduction of the concentration of these pollutants in the discharge is the crucial point to cut the pollution. There arises a demand for the timely removal of these pollutants from getting dispersed in water and avoiding the so-induced biomagnification in higher trophic levels.

The advent of the technology proposed several sophisticated methods for wastewater treatment, one among them being nano-bioremediation. This relies on the basic principles of nanotechnology, a nascent branch of material science, and uses nanoparticles as effective pollutant sensors and catalysts in their removal.

Nanotechnology, one of the modern momentous multi-applicative technologies, deals with matter in nanoscale dimension. According to the U.S. Environmental Protection Agency (EPA), nanotechnology is defined as “the creation and use of structures, devices, and systems that have novel properties and functions because of their small size.” The National Nanotechnology Initiative (NNI, 2010) describes nanotechnology as “the understanding and control of matter at dimensions between approximately 1 and 100

nm, where unique phenomena enable novel applications.”

Nanoparticles, especially noble metal nanoparticles, are widely used in biomedical, pharmaceutical, environmental, electrical, and other similar technology fields [5]. The multi-applicative nature of nanotechnology necessitated in large-scale production of nanoparticles. Physical and chemical synthesis methods were the first used techniques that involved the use of several toxic chemicals and harmful radiations [6]. This makes these traditional processes a potential hazard. So there arose an urge to develop an eco-friendly method of nano-fabrication, and this resulted in the development of green synthesis, a complete green chemistry approach.

Green synthesis involves bio extracts instead of toxic chemicals and harmful radiations. The method is cost-effective, eco-friendly also have the practicality to be extended to a large scale [7]. Bio extracts can be taken from living cells like microbes, terrestrial plants, and aquatic plants. The extract's role is not only limited to the reduction of metal ions but extract's role is not only limited to the reduction of metal ions but also involved in stabilizing the particles by capping [8]. Plant-based sources are found better than microbes owing to the risk of maintaining microbial cultures [9]. Several weed plants have also been explored for the green synthesis of beneficial nanomaterials.

Aquatic weeds, especially water hyacinth, are considered harmful to the ecosystem. When left unchecked, they will completely blanket lakes and ponds, reducing water flow and blocking sunlight from reaching the water column, thus limits the primary productivity and starves and blocking sunlight from reaching the water column, thus limiting the primary productivity starving the entire ecosystem. An evaluation of phytochemicals of *Eichhornia crassipes* detected the presence of alkaloids,

flavonoids, steroids, tannins, phenolic contents, and anthraquinone in the extract [10]. The major secondary metabolites present in *Eichornia*, as reported by Sanaa *et al.*, (2010), are phenolic compounds (4.35%), terpenoids (1.53%), and alkaloids (0.98%) [11]. Since then, they have been widely studied for their antimicrobial and biomedical properties, but their environmental implications remain less discussed.

The present study involves a one-pot synthesis of silver nanoparticles through biogenic reduction and stabilization reactions mediated by the extract of aquatic weed *Eichornia crassipes* and the spectroscopic evaluation on the catalytic efficacy of the thus formed nanoparticles in the degradation of industrial pollutants like azodyes. Thus, the work aims to report the efficacy of a non-valued weed plant in aquatic pollution-remediation activities.

Experimental

Materials and methods

Silver nitrate (AgNO_3 , 99.9%), HCl, and NaOH pellets were purchased from Sigma-Aldrich and used as such without further purification. Methyl orange, Methylene blue, and NaBH_4 were brought from Merck India Ltd. All aqueous solutions were prepared using sterile Milli Q water. Before the experiments, all the glassware was acid washed and rinsed with Milli Q water.

Preparation of extract

Eichornia (Water hyacinth) samples (Voucher no. DJ/2020-21/7/04-02-2021) were collected from Vembanad backwaters, Kerala. Vembanad lake is the longest lake in India and the largest in Kerala with 2033 square

kilometers. Sampling is done from the Cochin region of the lake (9.9105° N, 76.3173° E).

Collected samples were first washed thoroughly to remove all extraneous matter. The plant's leaves and petioles were taken and rewashed with distilled water, followed by Milli Q water. Samples were then chopped finely and shade dried for 7 days. From the dried sample, 10 g was added into 100 mL Milli Q water, stirred well, and kept in water-bath for 20 min at 60 °C. After cooling, the extracts were filtered using Whatman No. 1 filter paper, and the clear filtrate was further used for nanoparticle biosynthesis.



Figure 1. Photograph of *Eichornia crassipes*

Biosynthesis of nanoparticles

Optimal concentration of reactants and reaction conditions were established through an experimental series. For the optimized biosynthesis of silver nanoparticles, water hyacinth extract and 1 mM AgNO₃ are mixed in a ratio of 1:10. The pH of the reaction mixture was acidic and so corrected to alkaline (pH=11) using 1 M NaOH because no nanoparticles were observed at neutral and acidic pH. The reaction mixture is then kept on a magnetic stirrer at 1200 rpm with a temperature of 90 °C for 15 minutes.

Purification and Characterization of the biosynthesized nanoparticles

Before characterization, the nanoparticle is purified by repeated centrifugation and then freeze-dried. Centrifugation is done at 12000 rpm for 20 min at 4 °C followed by re-dispersion of the pellet in Milli Q water and freeze-drying. The lyophilized samples thus obtained were taken for further analysis.

The characterization of freeze-dried AgNPs was done using UV-Visible Spectroscopy, High-Resolution Transmission electron microscopy (HRTEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) techniques. UV-Visible spectral analysis was done using Thermo scientific Evolution 201 Spectrophotometer. TEM images were obtained using Tecnai G2 30 Transmission Electron Microscope. XRD measurement was made on a PANalytic X'PERT-PRO X-ray spectrometer. FTIR spectrum was recorded 100 at the wavelength range of 4000-550 cm⁻¹ on Perkin Elmer Spectrum 100.

Catalytic degradation of pollutants

The catalytic activity of biosynthesized nanoparticles was evaluated for the degradation reactions of azodyes (Methyl orange and Methylene blue) by NaBH₄. In a quartz cuvette of 1 cm path length, 1.4 mL of water, 0.3 mL of aqueous dye solution (2 mM), and 1 mL of newly prepared NaBH₄ solution (0.03 M) were taken. To this reaction mixture, 100 µL of nanocatalyst solution (1 mg/mL) was added, and progress of reduction was observed using a UV-Visible spectrophotometer at regular intervals. Control reactions were also performed in the absence of the nanocatalysts. To evaluate the degradation efficiency of nanocatalysts, the percentage of pollutant degradation was calculated using the following equation (1) where A₀ denotes initial UV

absorbance and A_t is the absorbance after time 't'.

$$\% \text{ degradation} = \left\{ \frac{A_0 - A_t}{A_0} \right\} \times 100 \dots \dots \dots (1)$$

Results and Discussion

The formation of AgNPs using Eichornia extract as reducing and stabilising agent (E-AgNPs) was primarily evidenced by the observation of color change of the reaction mixture from pale yellow to dark brown within 15 min when kept at a temperature of 90 °C (pH = 11), whereas control AgNO₃ solution kept without plant extract showed no change in color (Figure 2b). This characteristic change in color is attributed to the surface plasmon resonance of silver nanoparticles in the solution.

Further characterization studies are done using UV spectrophotometer, TEM, FTIR, and XRD. Figure 2a shows the UV Visible absorption spectrum of the solution shows a distinct peak at 422 nm, in the specific absorption range of silver nanoparticles, confirming the formation of AgNPs by reducing silver ions by the phytochemicals in the extract. The occurrence of a single SPR band indicates the monodispersed nature of the particles.

HR-TEM characterization technique is used to analyze the morphology of nanoparticles that have a significant influence on their multifaceted applications. Figure 3 shows TEM images of the silver nanoparticles, which are nearly spherical-shaped particles and almost homogenous in structure. Particles show a size variation in a range of 10-20 nm.

XRD analysis reveals the crystalline nature, phase purity, and preferential orientation of the green synthesized AgNPs through the principle of diffraction occurring in X-rays when passing through a material. The diffraction pattern obtained is cross-checked with the standard diffraction pattern of silver nanoparticles from JCPDS File no. 04-0783.

Figure 4 represents the XRD patterns of the AgNPs. The peaks obtained are at 28.870, 38.360, 44.380, 64.260, and 77.780. These are characteristics of face-centered cubic (fcc) structure of metallic silver and can be assigned to (1 1 1), (2 0 0), (2 2 0), and (3 1 1) crystallographic planes, respectively on comparing with a standard pattern (JCPDS File no. 04-0783). Among the peaks, the one that corresponds to (1 1 1) is prominent. Also, the average crystalline size of the biosynthesized AgNPs was determined using the Debye-Scherrer equation by considering the width of (1 1 1) peak and found to be 14.64 nm.

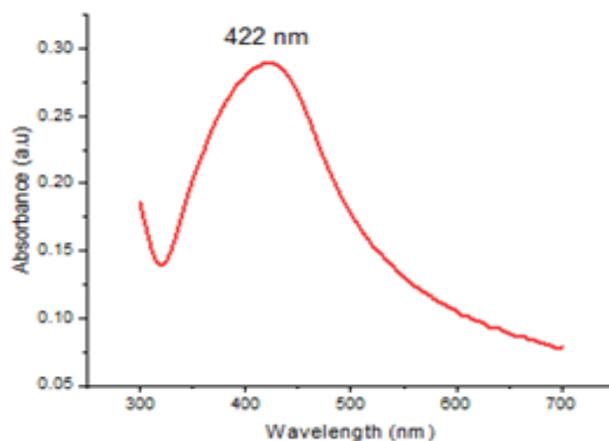


Figure 2. a) UV Visible absorption spectrum of E-AgNPs b) Digital images of aquatic weed extract, AgNO₃ solution, and nanoparticle solution

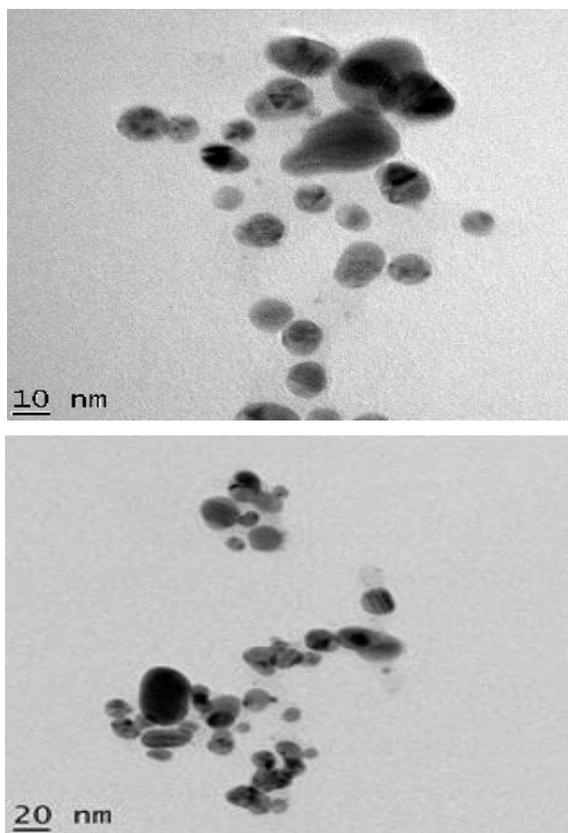


Figure 3. TEM images of E-AgNPs

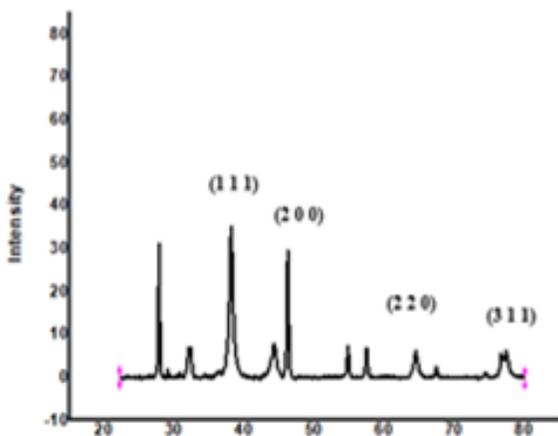


Figure 4. XRD spectra of E-AgNPs

Figure 5a demonstrates the FTIR spectra of *Eichornia* extract and (b) mediated AgNPs. Prominent peaks found in the extract spectra are found to replicate in that of the nanoparticle, also with slight variation. A peak at 3464

represents O-H stretching vibrations of the phenolic or carboxylic group. The peak at 1726, owing to the vibration of C=O functional groups, indicates the presence of aldehydes, ketones, and carboxylic acids. A peak at 1057 indicates ether linkages in the phytochemicals, and that at 662 corresponds to glucopyranose ring formation in carbohydrates. Thus, the obtained FTIR spectrum suggests that the extract's reduction and stabilization functions are backed up by flavonoids, phenols, and carbohydrate groups [12].

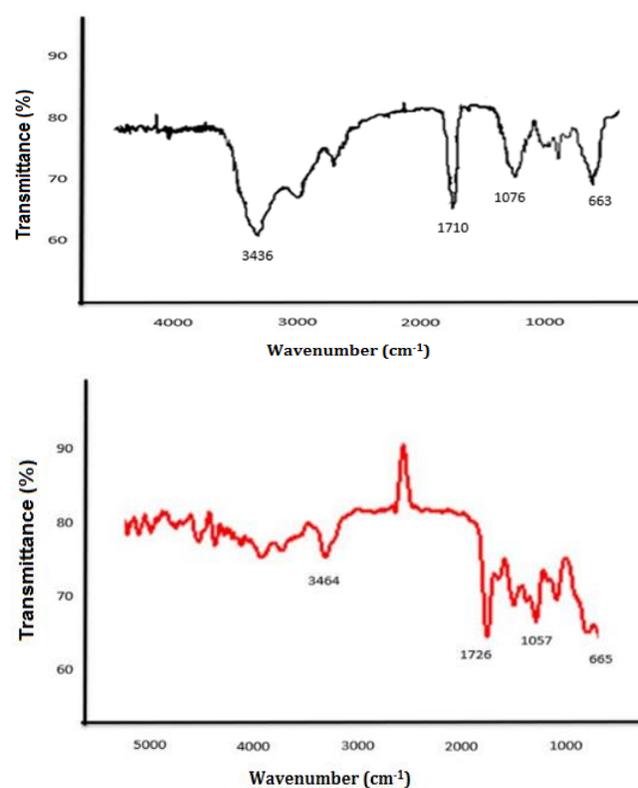


Figure 5. a) FTIR spectra of *Eichornia* extract b) FTIR spectra of E-AgNPs

Degradation of azodyes

a) Methyl orange (MO)

Methyl orange (Sodium 4-[(4-dimethyl amino) phenyl diaziny] benzene sulphonate), a member of the largest class of dyes called azo

dyes, is a widespread acid-base indicator extensively used in dyeing and chemical laboratories. An aqueous solution of the dye is reddish-orange with an absorption maximum at 464 nm. The complex chemical structure of the dye makes its removal from the aquatic system quite challenging.

For the reduction reaction of MO by NaBH_4 , the biofabricated nanoparticle was used as catalysts. The catalytic efficacy of the biosynthesized E-AgNPs in the reaction was monitored spectrophotometrically by observing the intensity of the peak at 464 nm. The reaction was done both in the presence and absence of nanocatalysts. In the absence of catalyst, there is no characteristic change in the color, or any significant decrease in the intensity of the characteristic peak was observed (Figure 6b).

After adding nanocatalyst, the reaction spontaneously starts and gets completed in minutes, The disappearance of the reddish-orange color and decreased absorbance value at 464 nm on adding nanocatalysts, were shreds of evidence of the catalytic degradation of the dye (Figure 7a).

The degradation process of methyl orange using E-AgNPs has completed within 5 minutes with a %-degradation of 92.46%. The color of the reaction mixture lightens to a feebly colored solution (Figure 7b). On completion of the process, a weak absorption peak was observed at 402 nm E-AgNPs which is supposed to be the SPR band of the catalyst AgNPs. During the degradation process, this band was not highlighted because of the strong absorbance band of methyl orange; however, when the concentration of dye reduces, and the peak weakens, the SPR band of catalyst gets recognizable. However, after the hydrogenation reaction, the SPR band of E-AgNPs underwent a blue shift from 422 nm to 402 nm. According to Princy, 2019 this can be concluded as due to the

injection of electrons onto catalyst's surface by the sodium borohydride during the catalytic hydrogenation, leading to its modification [13].

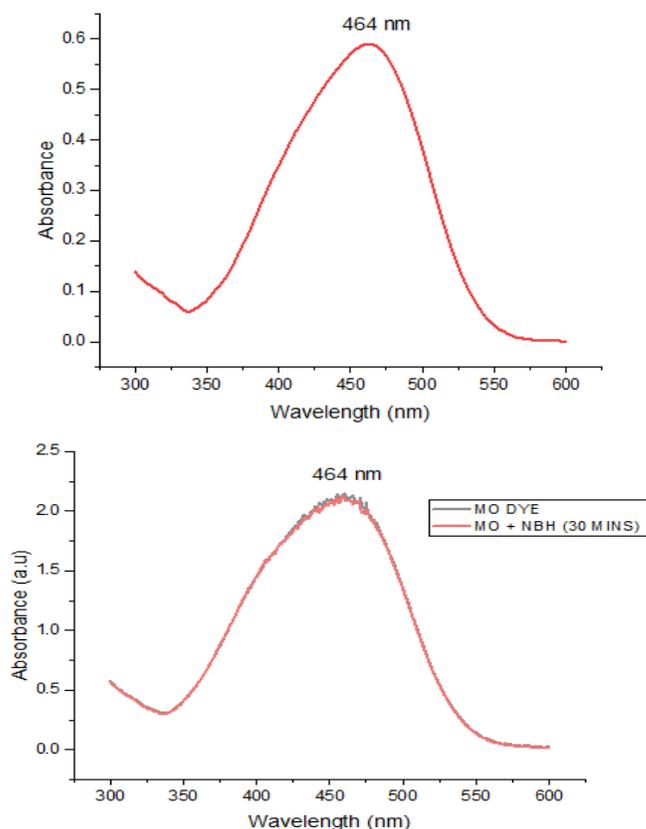
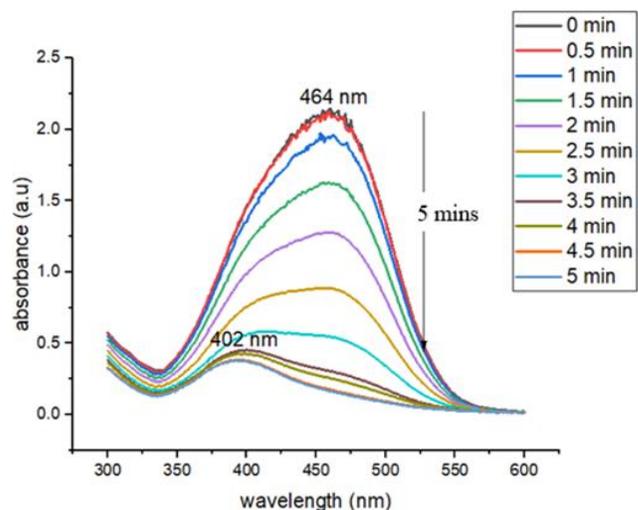


Figure 6. a) UV spectrum of methyl orange (MO) dye. b) UV-Visible spectra showing the reduction of MO by NaBH_4 without catalyst



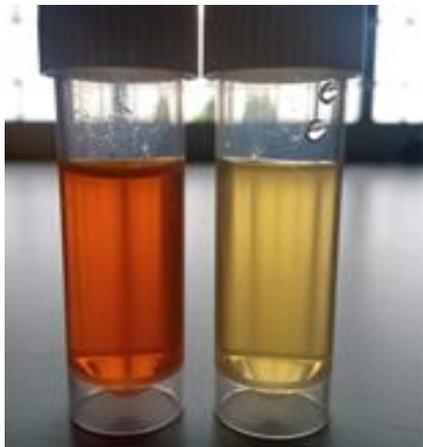


Figure 7. a) UV-Visible spectra indicating the NaBH_4 reduction of MO using E- AgNPs catalyst b) Digital image of MO dye before and after degradation using E- AgNPs catalyst

b) Methylene blue (MB)

Methylene blue, a thiazine dye also known as Swiss blue, was first prepared in 1876 as a textile dye. It became an important stain in laboratories on realizing its potential in biological staining. The cationic MB dye is a water pollutant that consumes the dissolved oxygen in the water and endangers the aquatic system [14]. The dye has a characteristic absorption peak at 664 nm and a shorter band at 612 nm (Figure 8a). An aqueous solution of the dye is deep blue. Removing the dye from polluting the aquatic system can be done by various methods. Various methods can remove the dye from polluting the aquatic system. Treating effluents before discharge to the environment through reduction reaction using NaBH_4 is one of the promising methods. Still, the reaction is ultra-slow in the absence of a suitable catalyst. Figure 8b represents the reduction of MB using NaBH_4 without the addition of nanocatalysts.

But, after the addition of nanocatalysts, it is observed that degradation reaction favors and the color of the reaction mixture turns pale blue to nearly colorless within minutes, indicating almost complete removal of the dye (Figure 9b).

The hydrogenation reaction occurring here converts methylene blue to leuco- methylene blue, a less toxic reaction product that is nearly colorless [15]. Figure 9 represents the degradation reaction after adding E-AgNPs, which is completed in a period of 14 min by 91.9%.

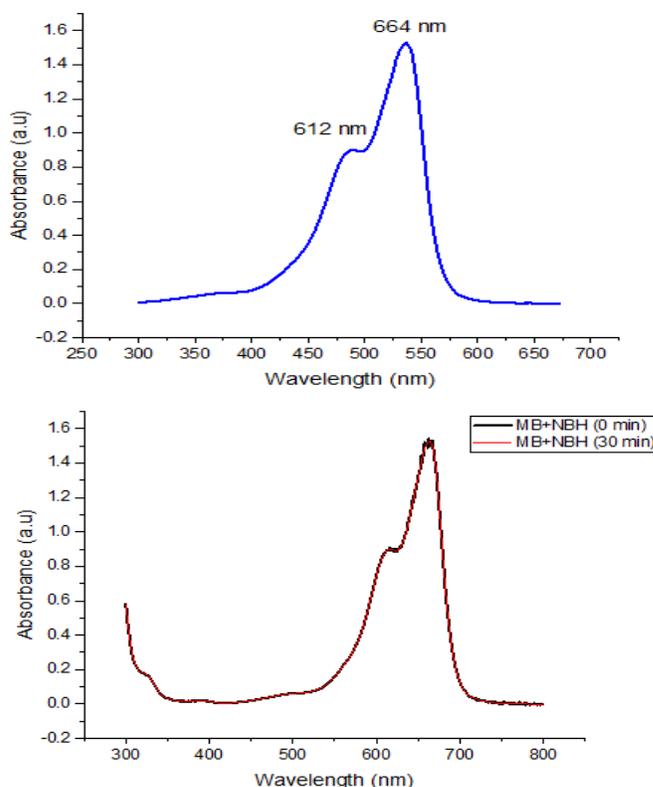
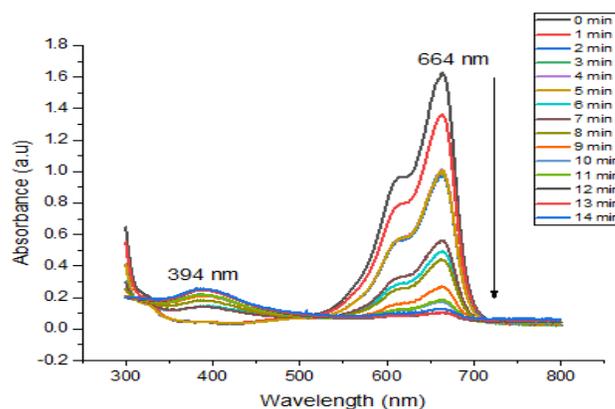


Figure 8. a) UV spectrum of Methylene Blue (MB) dye b) UV-Visible spectra showing the reduction of MB by NaBH_4 without catalyst



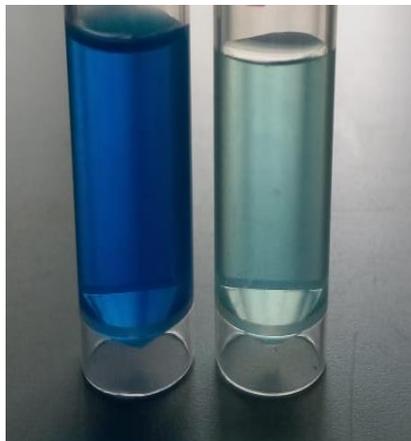


Figure 9. a) UV-Visible spectra indicating the NaBH_4 reduction of MB using E- AgNPs catalyst b) digital image of MB dye before and after degradation using E- AgNPs catalyst

Conclusions

The present study reported an environmentally benign, facile, and economical way of synthesizing silver nanoparticles through a green route. Aqueous extract of weed plant *Eichornia* is proved efficient in reducing precursor metal ions and further stabilizing the formed nanoparticles. The synthesis is completed in 15 min, and characterization using various techniques revealed the particles to be monodispersed, homogenous, crystalline, and stabilized by phytochemicals in the *Eichornia* extract. The synthesis was confirmed by observing a characteristic SPR peak at 422 nm. TEM analysis confirmed the particle to be nearly spherical with a size distribution from 10-20 nm. The crystallinity of the particle is assessed through the XRD technique, and the crystalline size is calculated as 14.64. FTIR analyzed the functional groups attached to the particle's surface and revealed the presence of phenols, amino acids, and carbohydrates. Silver nanoparticles thus formed were then employed as a catalyst in the degradation of azodyes using NaBH_4 . The catalytic efficacy of nanoparticles was spectrophotometrically monitored. The %

degradation of pollutants was 92.46 and 91.9 respectively for methyl orange and methylene blue. The reactions were quick, simple, and effective in removing pollutants. The method followed here is an entirely green chemistry approach with enormous potential for large-scale application. Thus, the biosynthesized silver nanoparticles using *Eichornia crassipes*, a low-valued renewable resource, proved to be a promising tool in aquatic environment protection.

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Disclosure Statement

No potential conflict of interest was reported by the authors.

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