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Research Article

Green Synthesis of Silver Nanoparticles Using Equisetum Plant Extract and Its Antimicrobial Activities and Dye Degradation Behaviour

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Abstract: Silver nanoparticles were prepared using the green synthesis method using *Equisetum* plant extracts. The plant extracts reduce silver ions into silver nanoparticles at ambient conditions. The process is straightforward, rapid, cost-effective and environment friendly. The silver nanoparticles were characterized by UV-visible spectroscopy, IR spectroscopy, SEM and XRD study. The average particle sizes are in the range of 10–20 nm. The UV–visible spectra of the silver nanoparticles exhibit an absorption band around 350 nm which suggests that the size of the silver nanoparticles is small. IR spectra show the characteristic peaks of flavonoids, terpenoids, and fatty acids of the plant extract. The antimicrobial activity of green synthesized silver nanoparticles was carried out using the disc diffusion method against human pathogens. Small-size silver nanoparticles show high antimicrobial activity in this work. The synthesized silver nanoparticles have also been chosen for the catalytic degradation of different dyes.

***Keywords*:** Ag nanoparticles, green synthesis, XRD, SEM, antimicrobial activity, dye degradation

1. Introduction

In recent times, nanotechnology has become one of the most important research fields with versatile applications in science, engineering, biomedical and pharmaceutical sectors. Nanoparticles are unique in characteristics as their properties depend upon their size and morphology [1–3]. Nanoparticles play a crucial role in electronics, biology and medicine due to their versatile properties such as optical, antibacterial and catalytic properties. In recent times, noble metal nanoparticles have attracted much attention from researchers because of their interesting physical, chemical and biological properties [4,5]. Silver nanoparticles have various applications in the area such as drug delivery, imaging, anti-viral agents in the health industry, textile coatings, catalysts in chemical reactions and several environmental applications. Silver nanoparticles have received considerable attention in the antibacterial research field compared to other inorganic metal nanoparticles [6–8].

Synthesis of nanoparticles involves various techniques like physical, chemical and biological processes. In the case of the chemical process, the synthesis of nanoparticles requires high pressure, temperature and toxic chemicals. Chemicals are

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used as reducing agents and capping agents that may adversely affect various processes. Synthesis of silver nanoparticles by conventional chemical and physical processes may increase environmental and biological risks due to the involvement of poisonous reducing agents in the synthesis procedure. The preparation of nanoparticles using the green synthesis process has several advantages because of their cost-effectiveness and harmless nature [9–13]. Synthesis of silver nanoparticles using plant extracts is an interesting current research field. Here, plant extracts provide reducing agents and capping agents [14–16]. At the same time plant extracts are free from toxic chemicals [17–20]. Synthesis of silver nanoparticles using plant extracts is considered to be an eco-friendly and rapid strategy.

Equisetum is commonly known as Horse tail and it belongs to the Equisetaceae family. It is a vascular plant. *Equisetum* is used as an Ethnomedical plant and it is found in the eastern Himalayan hill region. *Equisetum* is an herbal remedy and it was used traditionally to prevent bleeding and heal ulcers and wounds. It is also helpful for treating tuberculosis and kidney problems. Secondary metabolites present in various parts of this plant provide reducing and capping agents as they are natural reservoirs of terpenoids, alkaloids, flavonoids and tannins [21].

In recent times, water has been contaminated due to the different types of industrial activities. Various types of dyes are used by industries to impart color [22,23]. Such types of dyes released by industries contain highly toxic chemicals that are responsible for decreasing oxygen levels, circulation of carcinogenic agents and causing many water-borne diseases [24,25]. It also decreases the sunlight penetration into the water bodies affecting aquatic living organisms [26].

The degradation of dye molecules to the nontoxic components is a crucial factor before its discharge to nearby water bodies. Therefore, suitable eco-friendly treatment of wastewater containing various types of dyes is needed. In recent times, metallic nanoparticles have been utilized to degrade toxic dyes. Green synthesized silver nanoparticles have been used for the catalytic degradation of different types of dyes. Various researchers reported silver nanoparticles which were synthesized via the green route exhibiting efficient dye degradation potential [27–29].

In this paper, we have reported an environmentally friendly and cost-effective pathway for the green synthesis of silver nanoparticles using *Equisetum* plant extracts. The bio-synthesized silver nanoparticles are characterized by UV-visible, IR spectroscopy, SEM and X-ray powder diffraction analysis. The reported silver nanoparticles show antimicrobial activity against a series of human pathogenic bacteria *Chromobacterium violaceum,* gram-positive *Enterococcus faecalis, E. coli, Enterobacter cloacae, Pseudomonas aeruginosa, Escherichia fergusonii,* and *Salmonella enterica* and the results exhibit that silver nanoparticles synthesized via green route using *Equisetum* plant extracts have a potential to inhibit the growth of bacteria. The silver nanoparticles also show antifungal activity against *Aspergillus niger* fungus. Synthesized silver nanoparticles have also been used for the degradation of different types of dyes.

2. Experimental section

2.1 *Materials*

Silver nitrate was purchased from E Merck. All other reagents were commercially available and were of reagent grade and used to receive without further purification.

2.2 *Synthesis*

2.2.1 *Preparation of equisetum leaf extract*

Equisetum plants were collected from Himalayan ranges and were identified taxonomically. The plants were washed several times with double distilled water. 5 gm of the sliced pieces were taken in a beaker and heated with 100 mL double distilled water for around 30 min. The solution was cooled and filtered. The extracts were collected for the preparation of nanoparticles.

2.2.2 *Synthesis of silver nanoparticles and their purification*

Silver nanoparticles were synthesized by mixing 1 mL of *Equisetum* plant extracts with 20 mL of 1 mM aqueous silver nitrate solution. The reaction mixture was stirred around 1 h at room temperature. A reddish-brown color change was observed due to the formation of silver nanoparticles. The synthesized silver nanoparticles were purified by repeated centrifugation at 12,000 rpm in a refrigerated centrifuge. The supernatant liquid was decanted and the silver nanoparticles were collected. The purified nanoparticles were dried in a vacuum and used for analyses.

The *Equisetum* genus has been found to contain a variety of flavonoids, glucosides, phenolic acid derivatives, caffeic acid derivatives, terpenoids, and fatty acids[30]. Flavonoids rapidly reduce silver ions to silver nanoparticles at room temperature. Simultaneously, it also acts as a capping and stabilizing agent[8]. It has been also suggested that the tautomeric transformations of flavonoids from the enol form to the keto form may produce a reactive hydrogen atom that can reduce silver ions to form silver nanoparticles[31]. As a consequence, we have used *Equisetum* plant extracts to prepare silver nanoparticles via the green route.

2.3 *Physical characterization of silver nanoparticles*

The synthesized silver nanoparticles were initially characterized by UV-visible spectrophotometry (Perkin-Elmer 950 UV/VIS/NIR spectrophotometer). Infrared spectra were employed in a Nicolet Magna 750 FT-IR spectrometer, series II with samples prepared as KBr pellets. The crystallographic analysis of the silver nanoparticles was taken using Rigaku Ultima 4. SEM analysis was carried out to analyze the size and surface morphology of the silver nanoparticles using JEOL JSM IT 500 at a voltage of 15 KV.

2.4 *Antibacterial activity of silver nanoparticles*

The antibacterial activity of silver nanoparticles was monitored by the agar well diffusion method. Agar plates were prepared and the entire surface of the plates was inoculated by spreading several microbial inoculums. Then a hole of 6 to 8 mm in diameter was punched by using a sterile cork borer or a tip. In each plate, three wells were filled up with silver nanoparticle solution with different concentrations, one well was filled up with silver nitrate solution and the last one was filled up with control (Ampicillin). Zone of inhibition in agar plates were monitored and calculated after 24 h incubation at room temperature

3. Results and discussion

3.1 *Synthesis*

Silver nanoparticles are synthesized via green route synthesis. Here *Equisetum* plant extracts are mixed with stirred aqueous silver nitrate solution at room temperature. The plant is available in the eastern Himalayan ranges. Plant extracts act as a reducing agent as well as a capping agent. The synthetic route is a very simple, rapid, cost-effective and environment-friendly route for the synthesis of silver nanoparticles. In this synthetic procedure, silver nitrate solution was added to the *Equisetum* plant extracts and the resulting solution was stirred for a few minutes. It was noticed that the color of the solution changed to a dark brown color within the appropriate time (Figure 1). The brown color of the resulting solution indicates the formation of silver nanoparticles. Secondary metabolites present in the *Equisetum* leaf extract provide reducing agents as well as capping agents for the formation of silver nanoparticles.

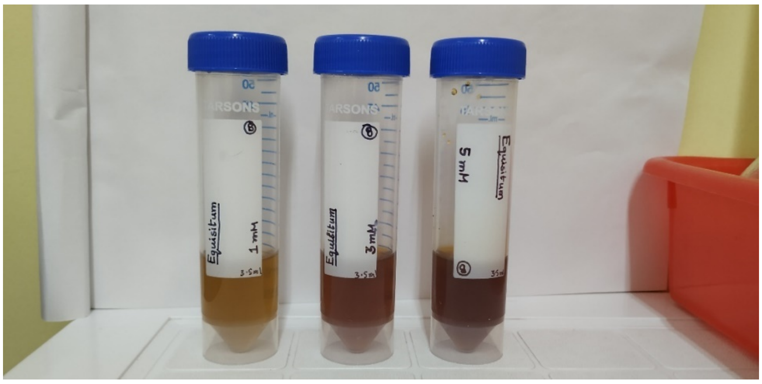


Figure 1. The aqueous solution of $AgNO_3$ with different concentrations (1 mM, 3 mM and 5 mM respectively) in the presence of *Equisetum* plant extract

3.2 *UV—visible spectra*

Initial characterization of the silver nanoparticles was carried out by taking UV—visible spectroscopy study. The UV–visible spectra of the silver nanoparticles are depicted in Figure 2. The silver nanoparticles showed an absorption band around 350 nm in the visible region[32]. The broadness of the peak suggests that the size of the silver nanoparticles is small. The broadening of the peak may also suggest that silver nanoparticles are polydisperse. The study confirms the formation of silver nanoparticles[24].

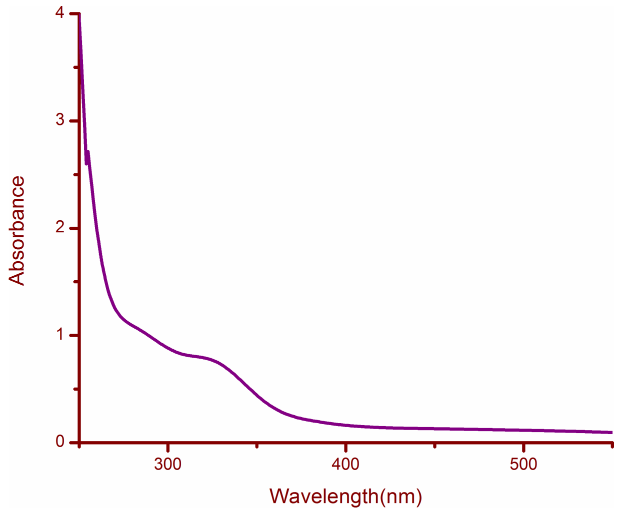


Figure 2. UV-visible spectra of Ag nanoparticles synthesized from *Equisetum* leaf extract

3.3 *IR spectra*

Figure 3 depicts the FT-IR spectra of silver nanoparticles synthesized from *Equisetum* plant extracts. The peak around 3400 cm^{-1} arises due to the presence of O–H stretching of alcohol and phenol. It may also occur due to the presence of N– H stretching. The peak around 1600 cm^{-1} corresponds to the C=O stretching of enolic ketones and aromatic ester as well as the C=C stretching of alkene. It is worth mentioning that the IR spectrum of the sample also contained bands around 1066 cm^{-1}, indicating the presence of aromatic C-H bending mode. From the IR spectra, we may conclude that the characteristic peaks are obtained due to the presence of flavonoids, terpenoids, and fatty acids in the plant extracts[21]. At room temperature, silver ions are generally reduced by flavonoids to form silver nanoparticles.

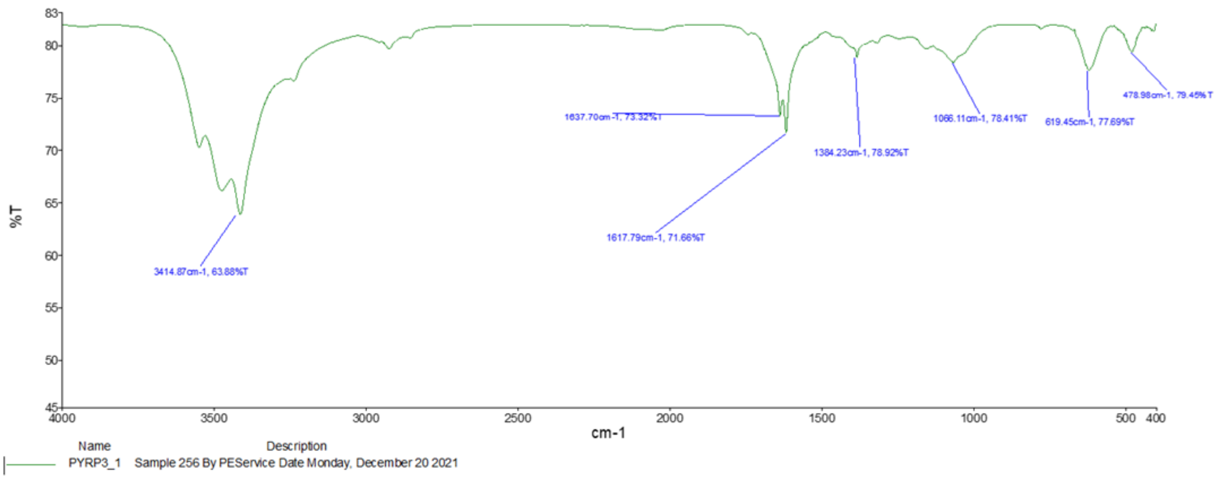


Figure 3. IR spectra of Ag nanoparticles using *Equisetum* plant extract

3.4 *Phase and micro-structural characteristics of silver nanoparticles*

The crystalline nature of the synthesized silver nanoparticles was monitored by a powder X-ray diffraction study. The X-ray diffraction pattern of silver nanoparticles obtained by *Equisetum* plant extracts is depicted in Figure 4. By examining the X-ray diffraction pattern obtained using Cu Kα radiation, the phase formation behavior of the produced silver nanoparticles was investigated. The XRD pattern for pure silver nanoparticles agrees well with JCPDS data (card no. 04-0783). The pattern demonstrates a single phase with a face-centered cubic structure and a very small amount of unreacted $AgNO_3$ as a secondary phase. The grain size of the prepared material is estimated using Scherrer's formula (Equation (1)). For the assessment of average crystallite size, the four most noticeable peaks (111), (200), (220), and (311) at 2θ values 38.25, 46.15, 64.43, and 77.4 correspondingly have been taken into consideration (Table 1). The estimated crystallite size is found to be around 10.28 nm.

$$d = \frac{K\lambda}{\beta \, Cos \, 2\theta} \quad (1)$$

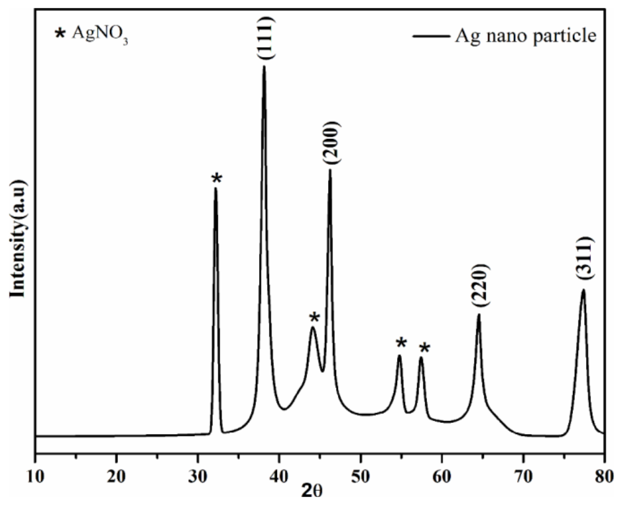


Figure 4. X-ray diffractogram patterns of silver nanoparticles by using *Equisetum* plant extract

Table 1. XRD results of silver nanoparticles from *Equisetum* plant extract

Planes	2θ	β	d (nm)	Avg. particle size (nm)
111	38.45	0.98	8.490	10.286
200	46.15	0.7	12.199	
220	64.43	0.7	13.265	
311	77.4	1.4	7.190	

Similar results were also previously reported for silver nanoparticles by using the green route synthetic procedure [16,33].

The morphology of the silver nanoparticles was obtained by scanning electron microscopy. The SEM image is shown in Figure 5. The shape of the biosynthesized silver nanoparticles was spherical in nature with an average diameter of 10–20 nm. Similar types of SEM results have been documented by some research groups [8,34]. The presence of silver nanoparticles was also supported by the EDX experiments (Figure S1).

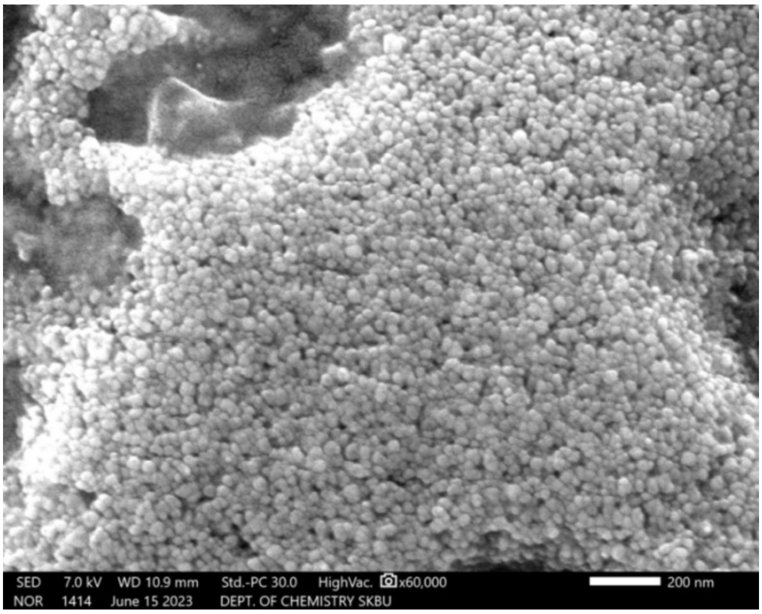


Figure 5. SEM image of the of Silver nanoparticles by using *Equisetum* plant extract

3.5 *Antimicrobial activity*

Antimicrobial activity of the silver nanoparticles was performed by agar well diffusion method. Inhibition zone photographs of *Chromobacterium violaceum* with silver nanoparticles as a representative example are presented in Figure S2. The results are tabulated in Table 2. The reported silver nanoparticles exhibit antibacterial activities as well as anti-fungal activities. From the table, it was shown that *Chromobacterium violaceum, Escherichia coli, Enterobacter cloacae, Pseudomonas aeruginosa, Escherichia fergusonii*, *Salmonella enterica* and gram-positive *Enterococcus faecalis* are sensitive to silver nanoparticles. The reported silver nanoparticles also showed antifungal activity against the fungus *Aspergillus niger*. The antimicrobial activity of silver nanoparticles reveals that the silver nanoparticles obtained from *Equisetum* extract show very good antibacterial potential against human pathogens.

The details mechanism of antibacterial activity of silver nanoparticles is not well established. Although the mechanism of the antibacterial activity of the silver nanoparticles may affect the cell membrane generation, inhibits protein synthesis, and metabolic processes by comparison with other reported work [35,36]. Silver nanoparticles probably penetrate the cells of microorganisms after adhering to their surface. Silver nanoparticles have a high tendency to attack sulphur and phosphorus-containing bio-molecules inside the wall [37]. Consequently, it mutilates processes like the replication of DNA, the structure of proteins etc. Le et al. reported that silver nanoparticles bind with the cell surface of *E. Coli* and *V. cholerae* and then penetrate the cell with simultaneous damage to the cell cytoplasm[38]. Le. et al. also show that the reported silver nanoparticles increase the cell permeability. Consequently, it affects the regulation of transport through the plasma membrane and leads to cell death.

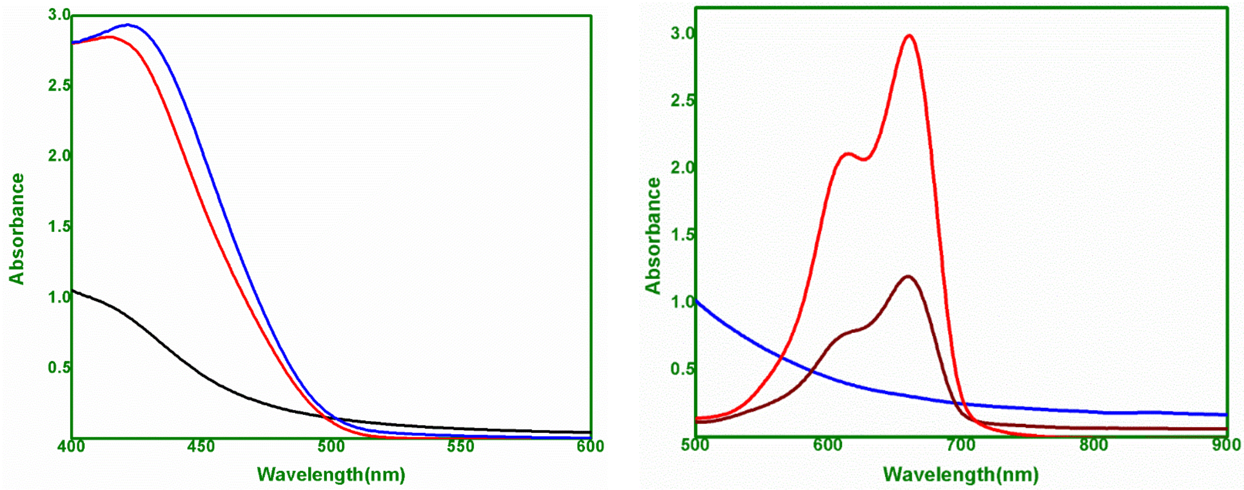
Small-size silver nanoparticles show high antimicrobial activity due to their large surface area[39]. The small size of nanoparticles suggests that nanoparticles come into contact with the bacterial cell with a higher percentage compared to large particles[40,41]. Baker et al. describes that the penetration of silver nanoparticles depends upon the size of the nanoparticles[42]. It is shown that higher antibacterial activity against human pathogens will occur when the size of the silver nanoparticles ranges from 10 nm to 20 nm. Such small-sized nanoparticles have a larger surface area to come into contact with bacterial walls. Small-size particles have higher antibacterial activity than large particles.

Table 2. Results of antimicrobial activities of silver nanoparticles using *Equisetum* plant extract

Sl. No.	Microorganism name	Zone of inhibition (mm)				
		AgNPs solution			$AgNO_3$ solution	Control (Ampicillin)
		1 mM	3 mM	5 mM		
1.	***Chromobacterium violaceum*** [MCC 2216]	13	15	14	12	9
2.	***Enterococcus faecalis*** Gram (+) [MCC 3037]	14	16	15	12	22
3.	***Escherichia coli*** [MCC 3099]	15	17	18	12	0
4.	***Enterobacter cloacae*** [MCC 3111]	15	17	16	13	0
6.	***Pseudomonas aeruginosa*** [MCC 4242]	14	16	19	13	0
7.	***Escherichia fergusonii*** [MCC 4329]	14	17	16	11	0
8.	***Salmonella enterica*** [MCC 4378]	13	16	17	16	0
9.	***Aspergillus niger*** (Fungus)	12	13	15	8	0

3.6 *Catalytic activity of silver nanoparticles*

UV–visible spectrometry has been used to observe the degradation behavior of titan yellow and methylene blue dye. The degradation studies were observed at their maximum absorbance. Figure 6A,B depicted the dye degradation of titan yellow and methylene blue in the presence of silver nanoparticles and $NaBH_4$ respectively. The characteristic absorption peaks of titan yellow and methylene blue solution were noticed at 425 and 660 nm respectively. With both dyes, it was noticed that the absorbance peak decreases in the presence of silver nanoparticles and $NaBH_4$ with time. It was shown from Figure 6A,B that the degradation of dyes occurred in the presence of silver nanoparticles and $NaBH_4$. $NaBH_4$ was able to degrade both the dyes alone. However, the degradation rate of both dyes changed significantly when silver nanoparticles were used as the catalytic agent along with $NaBH_4$. The increase in dye removal in the presence of silver nanoparticles was due to the higher existence of active sites on nanoparticles that lead to greater dye removal. The silver nanoparticles synthesized using leaf extracts of *Equisetum* exhibit promising catalytic degradation of methylene blue and titan yellow dyes.



(A) (B)

Figure 6. (**A**) Simultaneous degradation of titan yellow in the presence of $NaBH_4$ and silver nanoparticles [(i) Blue color: absorbance of titan yellow (ii) Red color: absorbance of titan yellow in the presence of $NaBH_4$ (iii) Green color: absorbance of titan yellow in presence of $NaBH_4$ and silver nanoparticles; (**B**) Simultaneous degradation of methylene blue in the presence of $NaBH_4$ and silver nanoparticles [(i) Red color: absorbance of methylene blue (ii) Brown color: absorbance of methylene blue in the presence of $NaBH_4$ (iii) Blue color: absorbance of methylene blue in presence of $NaBH_4$ and silver nanoparticles

4. Conclusions

In this paper, we have synthesized silver nanoparticles via an environmentally and cost-effective green route synthetic pathway by using *Equisetum* plant extracts. Reducing agents and capping agents were provided by secondary metabolites present in the *Equisetum* plant extracts. The resulting silver nanoparticles were characterized by UV-visible spectroscopy, IR spectroscopy, SEM and XRD studies. The X-ray diffraction pattern shows the four most noticeable peaks (111), (200), (220), and (311) at 2θ values 38.25, 46.15, 64.43, and 77.4 degrees. The particle size of the silver nanoparticles falls in the range of 10–20 nm. An absorption band around 350 nm indicates the formation of silver nanoparticles. IR spectra suggest that the presence of secondary metabolites from plant extracts may be responsible for the formation of silver nanoparticles. The reported silver nanoparticles show antibacterial activity against gram-positive *Enterococcus faecalis,* and gram-negative *Chromobacterium violaceum, E. coli, Enterobacter cloacae, Pseudomonas aeruginosa, Escherichia fergusonii* and *Salmonella enterica* human pathogenic bacteria as well as also exhibit antifungal activity against the fungus *Aspergillus niger*. The reported silver nanoparticles synthesized using leaf extracts of *Equisetum* show catalytic degradation of methylene blue and titan yellow dyes.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

Supplementary Materials

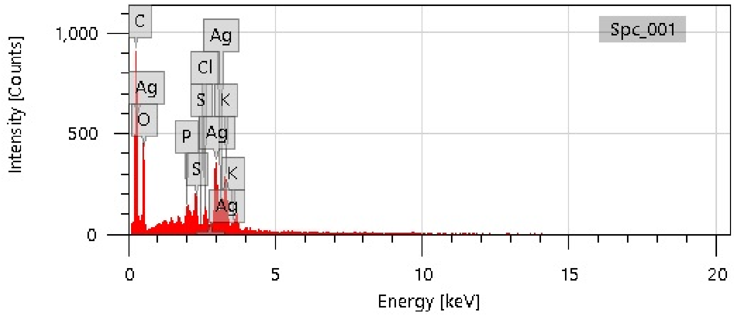

Figure S1. EDX image of Ag nano particles using Equisetum plant extract

Figure S2. Inhibition zone photographs of Chromobacterium violaceum with silver nanoparticles

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