

Harnessing *Passiflora Caerulea* L. as reducing agent for silver nanoparticle synthesis: exploring antimicrobial and catalytic potentials

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Abstract

A major step in the development of nanotechnology is the development of a reliable and environmentally-friendly method for synthesizing nanoparticles. Among all inorganic nanoparticles, silver nanoparticles have unique chemical, physical, and biological properties, and thus are extremely important. In present study, silver nanoparticles (Ag NPs) were prepared via an inexpensive and eco-friendly approach from silver nitrate salt and deploying *Passiflora caerulea* L., plant extract as the precursor and reducing agent, respectively. Herein, an expeditious, green, facile, and eco-friendly synthesis approach is introduced and the synthesized nanoparticles' antibacterial and catalytic activity was investigated, besides UV-Visible spectroscopy, XRD, SEM, and FT-IR were used to characterize silver nanoparticles. The characteristic absorption peak were exhibited by ensued Ag NPs under UV-Visible spectroscopy as a result of SPR (surface plasmonic resonance) band in 419nm wavelength. The FTIR spectrum of the aqueous extract and the nanoparticles indicated the presence of some important functional groups such as amines, carbonyl compounds, and phenols that are vital in facilitating the process of capping and bioreduction by the plant extract. Nanoparticles were roughly rod-shaped and ranged in size between 25 and 40nm as seen on SEM images analysis, and the XRD analysis confirmed that Ag was present in the nanoparticles. Ag NPs antimicrobial activity was examined against *Bacillus subtilis*, *Shigella dysenteriae*, and *Streptococcus pyogenes*. The antibacterial efficiency was evaluated using the disk diffusion assay, where zones of inhibition were measured to determine the effectiveness of Ag NPs against microbial strains. The results demonstrated significant antimicrobial activity, confirming the potential of these nanoparticles for biomedical applications. In addition, these photo-nanocatalysts were able to degrade Methylene Blue (MB) within 180 seconds.

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1. Introduction

Nanotechnology exploits the physical, chemical, and biological features of the materials in size ranging from 1 to 100 nanometers (nm), in various fields of sciences and industries. Today,

nanotechnology is widely researched area chiefly deployed in the design, synthesis, and distinction of nanomaterials, with a significant attempt to replace microstructure system procedures with nanostructure systems [1]. The nanostructure provides solutions to environmental, technological, and medical challenges embracing catalysis, textiles, solar energy conversion, paints, water purification, medicine, cosmetics, electronic devices, photonics, biosensors, air filters, storage containers, conductive inks, food, and food processing [2]. One of the main areas in nanotechnology is the synthesis of assorted metal nanoparticles (MNPs) as they have garnered significant consideration for their high reactivity, adsorption ability, surface area, and conductivity [3,4]. Synthesized MNPs based on morphology, size, and distribution nanoparticles present completely novel and improved properties. Usually, MNPs can be obtained by different approaches such as electrochemical technique [5], thermal decomposition synthesis [6], microwave irradiation method [7], laser ablation technique [8], and chemical methods [9]. Although these methods produce well-defined and pure nanoparticles but these approaches are expensive and result in potential environmental pollution due to the use of dangerous chemicals. Also, MNPs can absorb certain noxious chemicals on their surface; and lead to negative efficacy on health and medical applications. Therefore, there is a necessity for the development of safe, non-toxic, and inexpensive reagents and environmentally and trustworthy technique, without the employment of high energy, temperature, and pressure which actuate scientists towards bio-based greener processes [10-12]. Recently, greener synthesis of MNPs such as Au [13], Ag [14], TiO₂ [15], Cu₂O [16], ZnO [17] and MgO [18] is attracting attention. In these new approaches, diverse natural reducing agents such as live organisms (fungi, bacteria, yeast) [19], plant extracts (fruits, leaves, roots, seeds, and peels), and microbial enzymes [13, 20] have been employed for the greener biosynthesis of nanoparticles. Adjusting the reaction parameters, such as reactant stoichiometry, time, pH of the solution, concentration, temperature, surfactant, and capping agent, enable control of the size and shape of the resulting MNPs. Ag NPs have efficient applications in catalysis, electronics, biomedical devices, biosensors, and pharmaceuticals. Silver NPs have been synthesized from assorted plants such as *Gongronema latifolium* leaf [21], *Bryonia alba* leaf [22], *Acalypha hispida* [23], Tamarind fruit [24], *Arbutus Unedo* leaf [25], Cinnamon zeylanicum bark [26], *Aegle marmelos* leaf [27], among others.

Passiflora caerulea is a member of the Passifloraceae family and occurs abundantly in Qom province center of Iran (Fig. 1). *Passiflora caerulea* has sedative, muscle relaxant, antidepressant, anxiolytic, antispasmodic, and anticonvulsant effects and is employed in the treatment of diseases such as the manic phase of bipolar disorder, tension-related asthma, the fretfulness of teething children, the symptoms related with neuralgia and shingles [28, 29].



Fig. 1. *Passiflora caerulea* plant

To the best of our knowledge, biosynthesis of Ag NPs, using *Passiflora caerulea* L. extract by easy and environmentally approaches for antibacterial and catalytic activity effects have not been reported. The procedure deployed is clean, simple, and free of toxic organic solvent, and required only non-hazardous reagents like water,

plant extract, and AgNO_3 and can be useful in the large-scale preparation of Ag NPs. The antimicrobial activities towards both gram-positive and gram-negative bacterial strains displayed the zones of inhibition wherein synthesized silver NPs exhibited good antibacterial activity against 3 microorganisms such as *Bacillus subtilis* (ATCC6633), *Shigella dysenteriae* (PTCC1188), and *Streptococcus pyogenes* (ATCC19615); bacterial strains were cultured overnight at 37 °C in nutrient agar (NA). Additionally, dye degradation assessment was done by utilizing NaBH_4 and standard Methylene blue.

2. Experimental

2.1. Materials

Silver nitrate (AgNO_3) and sodium hydroxide, obtained from Merck (Germany), served as key reagents in the synthesis process. Solvents utilized in the experiments were acquired from Merck Chemical Company and employed directly without additional purification.

2.2. Preparing plant extracts

Fresh *Passiflora caerulea L.* leaves were collected from Qom province in the center of Iran. To eliminate debris, they were washed with tap water and then deionized water and finally, air-dried at room temperature; 20 g of finely cut dried leaves were stored in a 250 ml Glass Erlenmeyer flask with 180 mL deionized water and heated (80°C) for about 30 min. The extract was cooled to room temperature, and was filtered using Whatman filter paper No.1, and the extracts were kept at 4°C for further utilization.

2.3. Green synthesis of silver NPs

Normally, an aqueous solution of the proper metal salt, silver nitrate, and a plant extract is utilized. Synthesizing nanoparticles occurs at room temperature, and is completed within 30 min. Silver nitrate was procured from Merck®, Germany. In a glass Erlenmeyer flask 90 mL, 0.005 mM solution of silver nitrate was made. Then, 10 mL of plant extract was added individually into silver nitrate solution (90 mL). The solution color change (from colorless to dark brown) proved the reduction of Ag^+ to Ag^0 (Fig. 2), as affirmed by the UV-Visible spectroscopy for its formation.

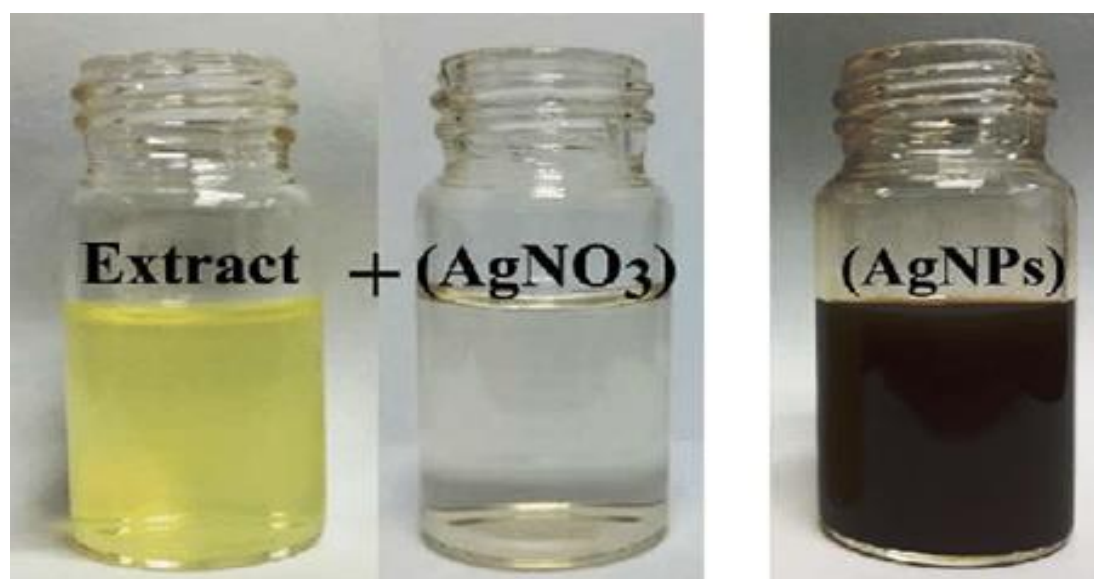


Fig. 2. The color change of the solution from colorless to black-brown during synthesis

2.4. Characterization

To perform UV-Vis spectral analysis, a Phystec-miniature UVS-2500 spectrophotometer was used. UV-Visible absorption spectrophotometer was used with a resolution of 1 nm within 190-1100 nm. Avatar Thermo Spectrophotometer system was used to record FT-IR spectra. The surface morphology and particle size were analyzed utilizing a Scanning Electron Microscope by SEM FEI Quanta 200. Spectral analysis was utilized for the XRD (Philips PW 1730). The synthesized silver nanoparticles amorphous nature is confirmed through XRD analysis within the angle range of 20°-80° and X-ray diffraction analysis.

2.5. Microbial strains

The Ag NPs were examined against a set of 3 microorganisms like *Streptococcus pyogenes* ATCC 19615 and *Bacillus subtilis* (ATCC 6633) *Shigella dysenteriae* (PTCC 1188). Bacterial strains were cultured in nutrient agar (NA) at 37 °C overnight.

2.6. Disk diffusion assay

The antimicrobial activity of Ag NPs was determined via the agar disc diffusion technique [30-32]. Dissolving the dried Ag NPs in DMSO, an ultimate concentration of 30 mg/ml was obtained, then it was filtered (0.45 µm Millipore filters) for sterilization. The discs (diameter of 6 mm) were impregnated with 10 µl of the Ag NPs. DMSO (as negative control) and 300 µg/disc were put over the inoculated agar. The plates were re-incubated at 37°C for bacterial strains for 24 h. Rifampin (5 µg /disc) and gentamicin (10 µg /disc) were utilized as positive controls for bacteria. The inhibition zones diameters were utilized as the measures of antimicrobial activity, then, each assay was performed two times. The inhibition zones diameters were utilized as an antimicrobial activity measure and each assay was conducted two times.

2.7. Dye reduction assay

Metal nanoparticles have the ability to catalyze chemical reactions that would otherwise not take place. Using NaBH₄ as a substrate, Ag NPs were assessed for their catalytic activity against MB. This basic aniline dye is also known as methylthioninium chloride, and it has many applications in both biology and chemistry, as a stain and in medicine [33, 34]. To evaluate organic dye reduction potency of green synthesized Ag NPs, a one-step process reaction was examined by utilizing NaBH₄ and Methylene blue purchased by Merck®. Typically, 10 mL of 10 mM MB stock solution is mixed with 3 mL of freshly prepared NaBH₄ (20 mM) solution. 3 mg Ag NPs was added to the prepared solution and the reduction of methylene blue by Ag NPs in solution was investigated by recording the decrease of absorbance with time. After each catalytic cycle, the AgNPs were recovered via centrifugation at 8000 rpm for 10 min. The precipitate was thoroughly washed with deionized water to remove residual reactants. The washed nanoparticles were then dried at 40°C under vacuum and stored under ambient conditions in a sealed vial for reuse in subsequent cycles. This recovery method ensured minimal catalyst loss and maintained efficiency over multiple cycles.

3. Results and Discussion

3.4. Characterization

Based on the studies, silver nanoparticle solution is in dark brown or dark red. The extract solution was light yellow; however, it changes to dark brown after treating with AgNO₃ indicating the formation of Ag NPs (Fig. 2). This color change is caused by the quantum confinement feature as a size-based property of nanoparticles affecting the nanoparticles' optical property.

3.4.1. UV-VIS spectroscopy

The silver nanoparticles' UV absorption peak appearing in the 400-450 nm range was examined [22–24]. The UV-Vis spectrum indicates the peak at 419 nm (Fig. 3), obviously revealing the spherical Ag NPs formation in all the plant extracts. The peak occurs at 419 nm by exciting the surface Plasmon over the silver nanoparticles' outer surface excited by the applied electromagnetic field.

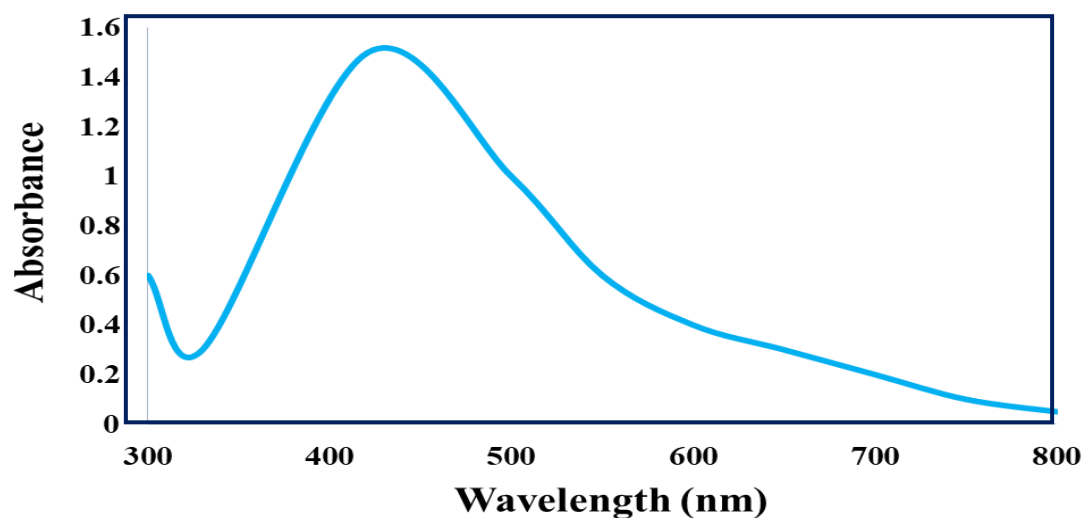


Fig. 3. The UV absorption peak of silver nanoparticles.

3.4.2. FT-IR spectroscopy

Through the Fourier transform infrared spectroscopy analysis, the possible biomolecule constituents of *Passiflora caerulea L.* was determined responsible for the reduction of silver ions and bio-reduced Ag NPs capping. Fig. 4 represent the FT-IR spectrum of synthesized Ag NPs with transmission peaks at 3436.78, 2921.80, 1626.65, 1384.39, 1081.85, 617.86, 559.10 and 472.01 cm^{-1} . The band at 3436.78 cm^{-1} in the spectrum is related to O-H stretching vibration revealing the existence of phenol and alcohol. The band at the 2921 cm^{-1} region is caused by C-H stretching for aromatic compound. The band at 1626 cm^{-1} in the spectra is related to C-C-C and N stretching revealing the existence of protein. The band at 1384 cm^{-1} was assigned for N-H stretch vibration in the proteins' amide linkage and contributing to the stability or capping of Ag NPs. The band at 1081 cm^{-1} was allocated for C-N (amines) and N-H stretch vibrations of the proteins. The band at 501, cm^{-1} , 1071, 1383, 1622, 2916, and 3432 cm^{-1} is related to C=N stretching of aliphatic amines of seed extracts, N -H, carboxylic acids derivative (C=O), stretching poly-phenols, respectively. Such compounds could be adsorbed over the Ag NPs surface by interaction via carbonyl groups or π -electrons. Nevertheless, other robust ligating agents must exist with adequate concentration, such as terpenoids and flavonoids. Thus, these biological molecules can perform dual functions of stabilization and formation of Ag NPs within the aqueous medium. The higher antioxidant and importance in the bioreduction of Ag NPs are the result from the presence of these compounds. For instance, terpenoids have a key role in metal ions reduction causing aldehyde groups in the molecules for oxidizing to the carboxylic acid. The FT-IR analysis confirmed the presence of various functional groups, including phenols and terpenoids, which play a crucial role in the formation and stabilization of silver nanoparticles. Phenolic compounds facilitate the reduction of Ag^+ ions, acting as reducing agents, while terpenoids contribute to the capping and stabilization of the synthesized nanoparticles by forming a protective

organic layer that prevents aggregation. Therefore, the created nanoparticles seemed to be attached to proteins and metabolites with functional groups as aldehydes, ketones, alcohols, carboxylic acids, and phenols. Phenols may act as the capping agent for the metal nanoparticles thus, they prevent agglomeration by stabilization of the medium.

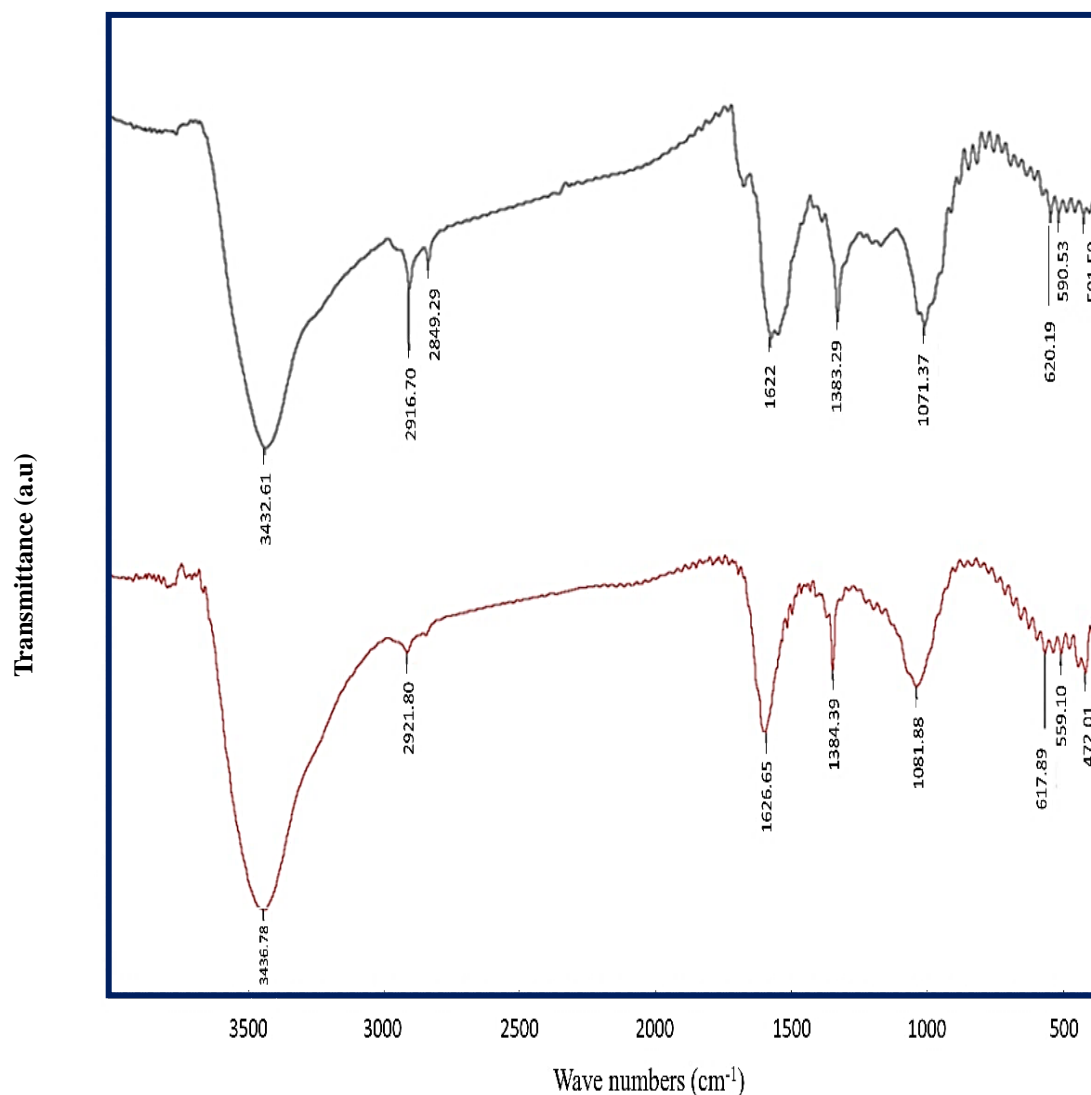


Fig. 4. The FTIR spectrum of *Passiflora caerulea* L. extract (black) and Ag NPs (red)

3.4.3. Scanning Electron Microscopy (SEM)

SEM is an approach using electrons rather than light to create an output image. From the development of SEMs in the early 1950s, they have been central for numerous new areas of research such as nanotechnology and material science. Fig. 5a represents the roughly rod-shaped morphology and uniformly sized particles (25-40 nm) based on SEM analysis. The UV-Vis spectrum suggests spherical Ag nanoparticles formation; however, SEM analysis reveals rod-like structures. This discrepancy can be attributed to shape anisotropy, where elongated nanoparticles exhibit multiple plasmon resonance modes, but only the transverse mode (~400 nm) is prominently

detected. A mixture of shapes may also be present, with spherical nanoparticles dominating the optical response. Further characterization techniques, such as DLS or electron diffraction, could provide more insights into morphology distribution and its impact on plasmonic behavior.

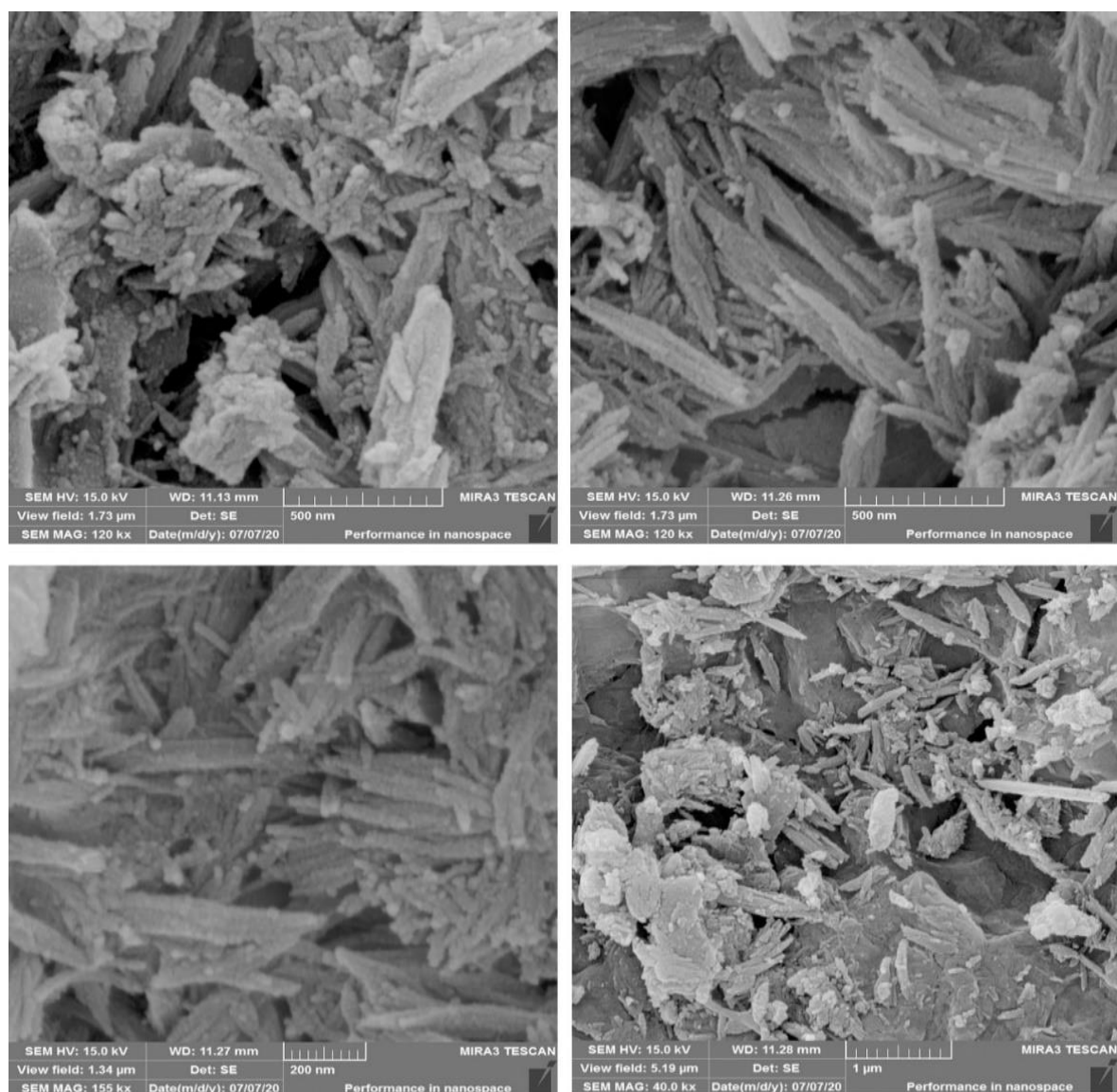


Fig. 5. SEM images of the biosynthesized Ag NPs

3.4.4. X-ray Diffraction (XRD)

The XRD method was utilized to recognize the unknown crystalline materials such as inorganic compounds and minerals; their identification is essential for study in material science, biology, and environmental sciences. Other important applications include the determination of the unit cell's dimensions, crystalline material characterization, and purity test of specimens. For these experiments, XRD helped to confirm the existence of silver NPs. According to the biosynthesized Ag NPs' XRD pattern, five clear peaks appeared at 38.09° , 44.1° , 64.3° , 77.5° , and 81.6° in line with (111), (200), (220), (311), and (222) reflections of the Ag NPs' face-centered cubic (fcc) phase (Fig. 6).

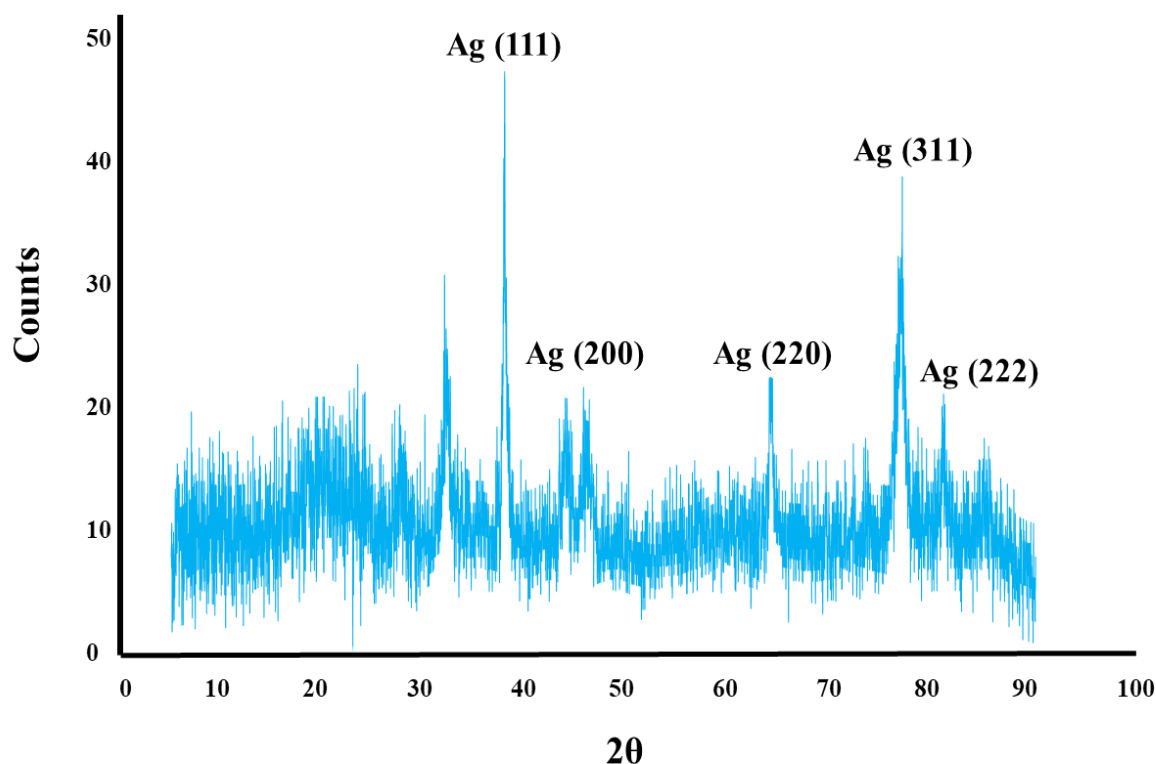


Fig. 6. The XRD experiment of the biosynthesized Ag NPs.

3.5. Antibacterial activity

Considering the antimicrobial properties of silver nanoparticles, they have extensive applications in the health industry, food storage, medicine, dye reduction, textile coatings, antiseptic creams, wound dressing, and numerous environmental applications. From many years ago, elemental silver and its compounds were utilized as antimicrobial agents to preserve water as silver coins /silver vessels. We assessed *Passiflora caerulea L.* extract mediated silver nanoparticles as the possible antibacterial agents. For respective antimicrobial activities, the plant extract and those mediated silver nanoparticles were immediately tested concerning both gram-positive and gram-negative bacterial strains representing the inhibition zones. Based on the inhibition zone, synthesized Ag NPs showed good antibacterial activity against 3 microorganisms including *Bacillus subtilis* (ATCC6633), *Streptococcus pyogenes* (ATCC 19615), *Shigella dysenteriae* (PTCC 1188) (Fig. 7). Bacterial strains were cultured in nutrient agar (NA) at 37 °C overnight and culturing fungi was performed in Sabouraud dextrose agar (SDA) at 30 °C overnight. Moreover, control and plant extract alone exhibited no antibacterial activity. The antimicrobial activity of silver nanoparticles is primarily attributed to multiple mechanisms, including the release of Ag⁺ ions, which interfere with bacterial metabolism, and direct interaction with microbial membranes, leading to structural disruption. Additionally, silver nanoparticles can induce oxidative stress in bacterial cells, generating reactive oxygen species (ROS) that cause protein, lipid, and DNA damage. These combined effects contribute to the potent antibacterial efficacy observed against *Shigella dysenteriae* and *Bacillus subtilis* in this study. Table 1 presents the antimicrobial activity results of the prepared silver nanoparticles assessed from the disc diffusion technique.

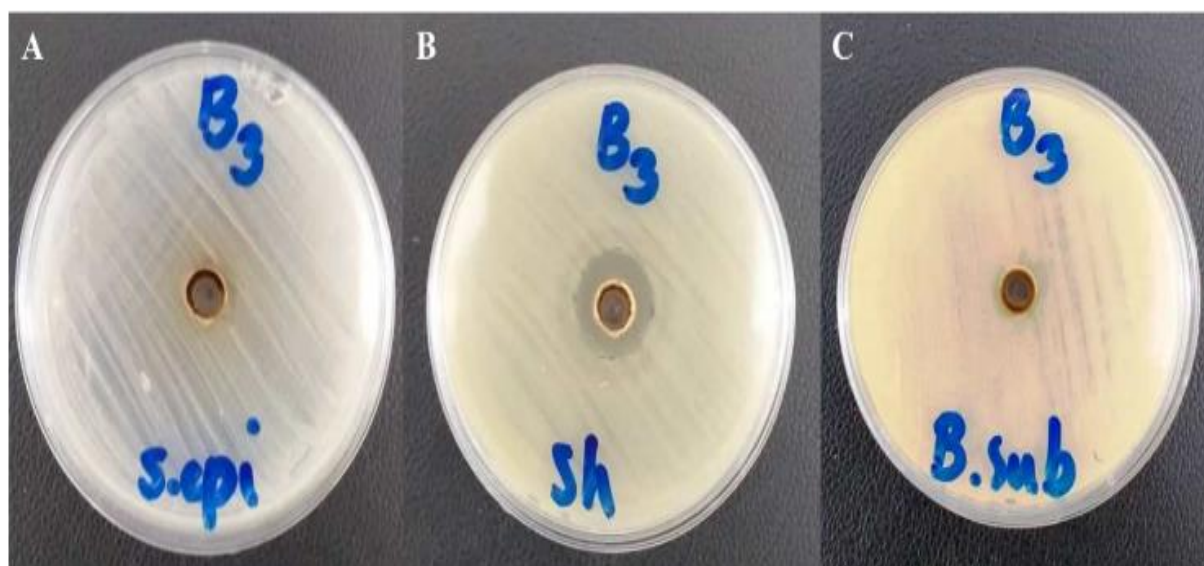


Fig. 6. The antimicrobial properties of silver nanoparticles on the A) *Streptococcus pyogenes*, B) *Shigella dysenteriae*, C) *Bacillus subtilis*.

Table 1. Inhibition zone test result of Ag NPs against microbes

Microorganisms	Inhibition zone (mm)		
	Ag NPs	Rifampin	Gentamicin
<i>Bacillus subtilis</i>	8	10	9
<i>Shigella dysenteriae</i>	11	30	17
<i>Streptococcus pyogenes</i>	11	21	31

3.6. Catalytic activity

By employing NaBH_4 as a reducing agent and Ag NPs as catalyst, as well as a UV-Vis spectrophotometer to monitor the reduction, methylene blue was reduced to leuco-methylene blue. The absorption peak of methylene blue lies at 664 nm, known as saturated MB. The maximum absorption band of MB in aqueous solution occurs at 664 nm due to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions [35]. Analyzing the decrease of absorbance over time enabled us to determine how catalytically active Ag NPs are in the reduction of methylene blue. The oxidized state of MB exhibits a blue hue, which subsequently becomes colorless when reduced into leuco-methylene blue. Fig. 8a indicate that reduction of MB by NaBH_4 in the absence of catalyst 'Ag NPs' is impossible. To investigate the best reaction conditions, various pH values and temperature were applied in to the reaction. In order to assess the catalytic performance of green synthesized Ag NPs at acidic and basic PH values, we studied the dye reduction reaction from 3 to pH=12. According to the UV-VIS spectrum analysis, it was observed that MB reduces faster in acidic pH (Fig. 8b). Contrary to this, Ag NPs catalyze only 60 percent of the MB dye in 3 minutes when the pH is higher than 8, showing that Ag NPs are PH-sensitive nanocatalysts. Moreover, it was investigated that when temperature rises, these nanocatalyst are able to degrade MB yet (Fig. 8a). Room temperature, however, proved to be the optimal temperature.

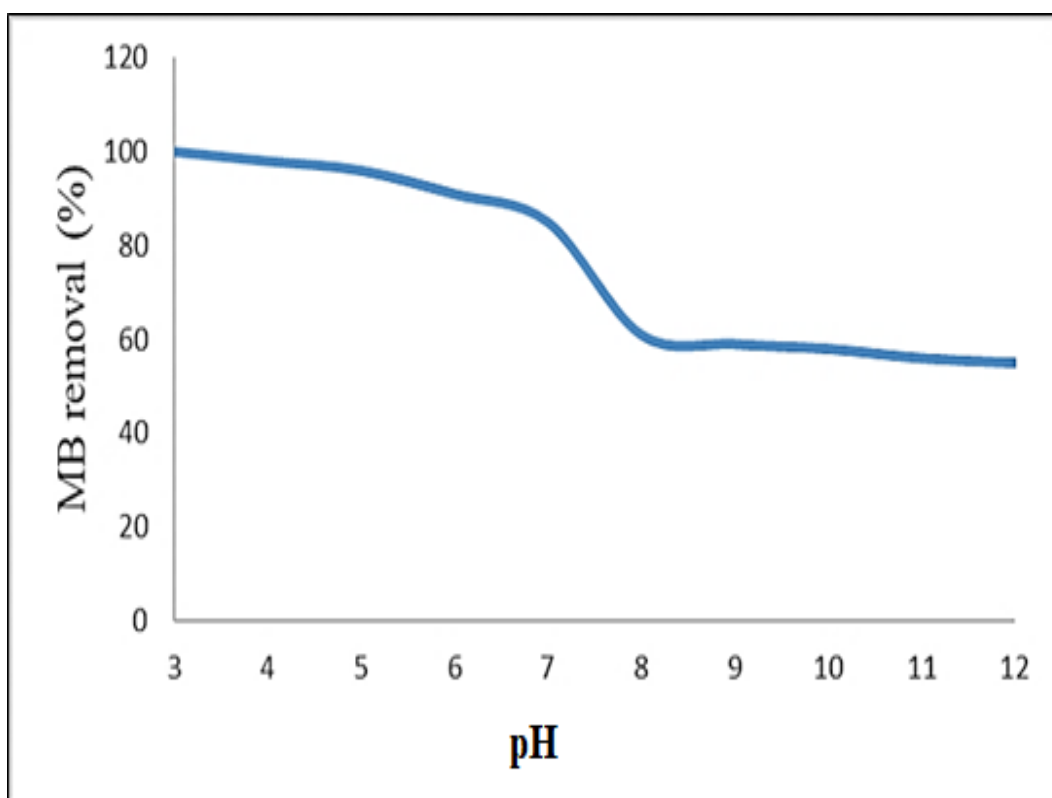
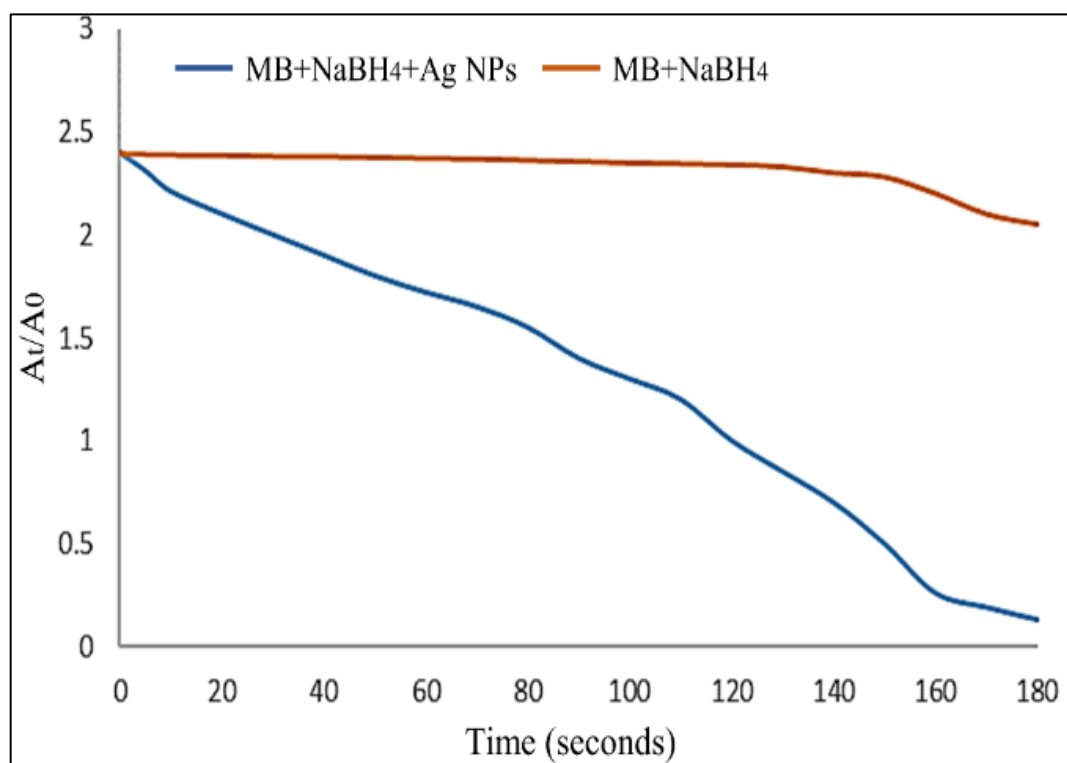


Fig. 7. (a) spectral analysis of presence and absence of Ag NPs in MB reduction reaction and, (b) pH effect on MB reduction by Ag nanoparticles

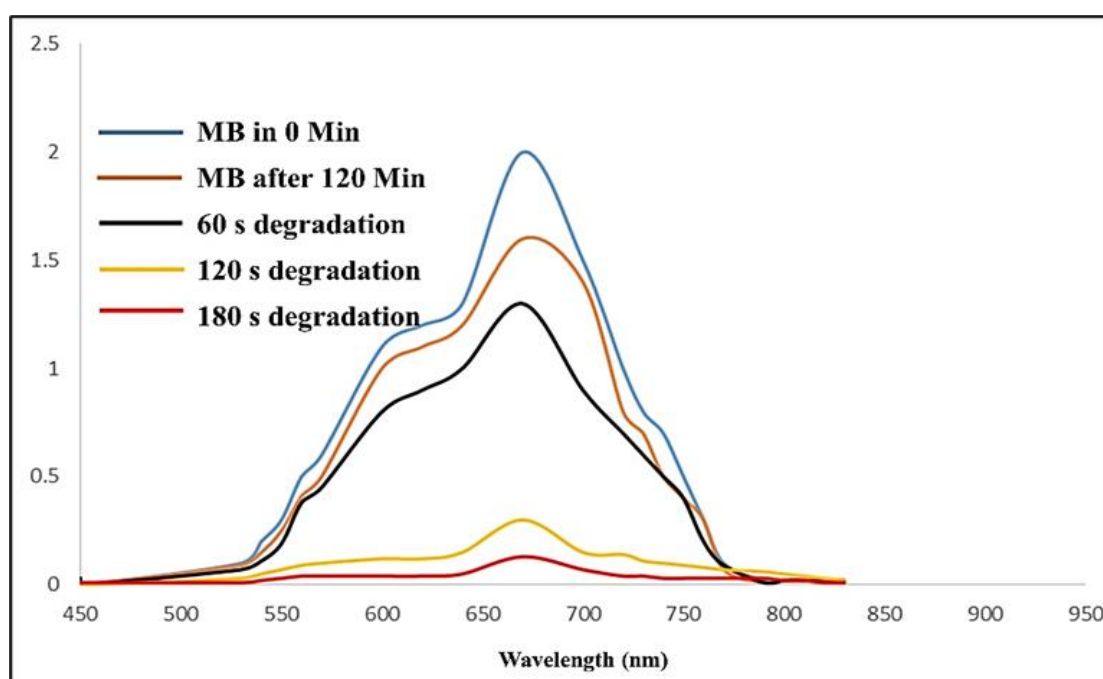
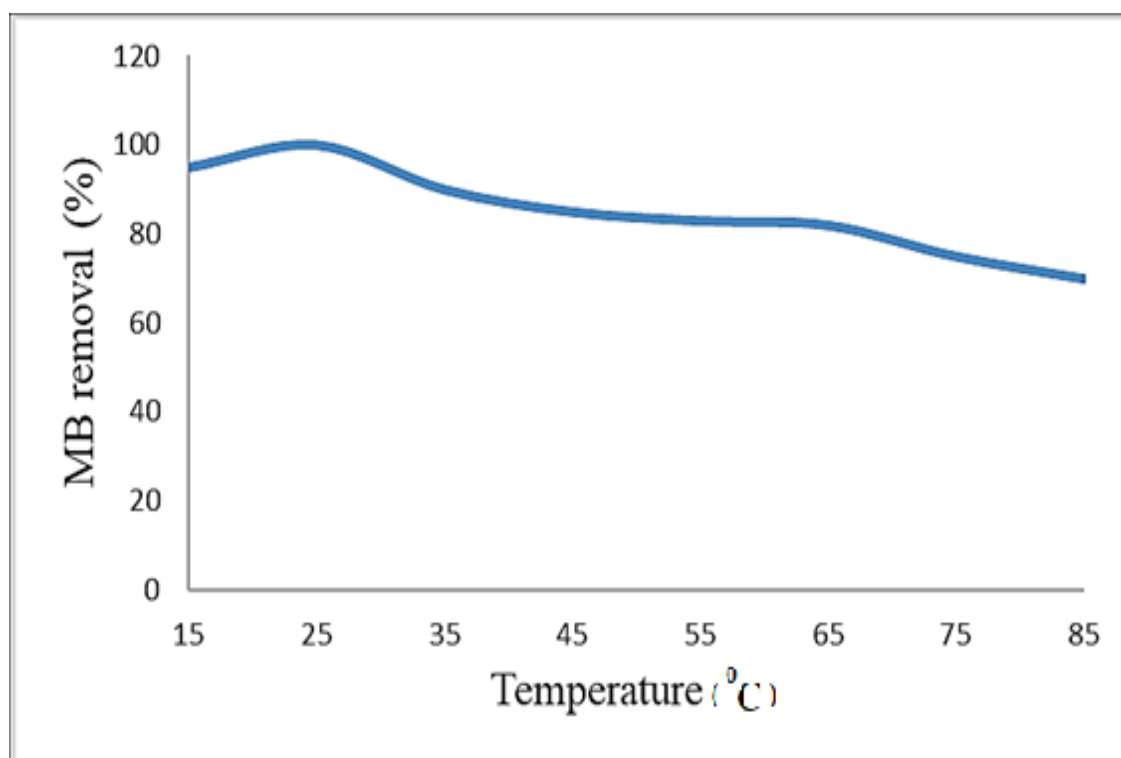


Fig. 8. (a) Temperature effect on MB reduction by Ag nanoparticles (b) MB degradation in successive reduction by Ag nanocatalyst

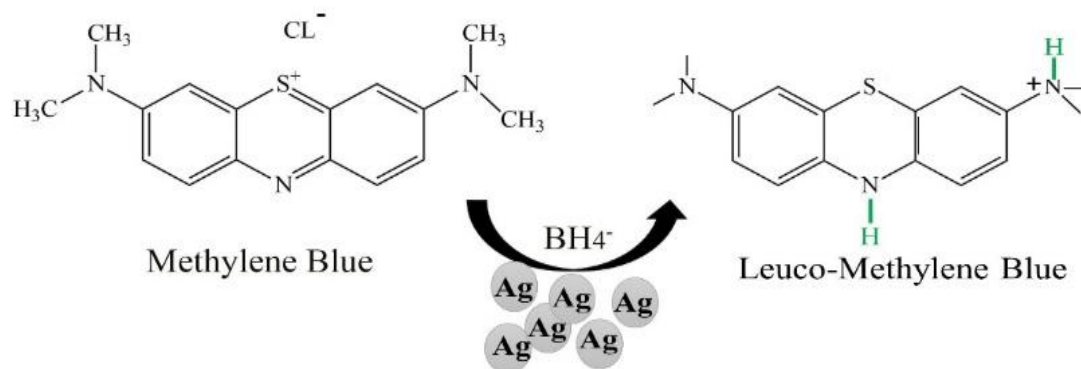
Thus, the catalytic reaction was done in acidic PH and room temperature as optimum reaction conditions. Ag NPs markedly reduced MB in 3 minutes by 100%, indicating the efficiency of catalytic reduction of MB with Ag NPs (Fig. 8b). Table 2 compares our results with previously reported data of degradation times for 100% MB dye reduction in the presence of Ag NPs synthesized with plant extracts.

Table 2 comparison of the catalytic activity of green synthesized Ag NPs mediated by different plants

Plant name	Target pollutant	Degradation time	Ref.
Gmelina arborea	Methylene blue	10 min	[36]
Biophytum sensitivum	Methylene blue	9 min	[37]
Convolvulus arvensis	Methylene blue	20 min	[38]
Passiflora caerulea	Methylene blue	3 min	Present study

3.6.1. Mechanism of MB dye degradation

When a chemical reaction takes place, bond dissociation energy (BDE) plays an important role in breaking and/or forming new bonds. While MB dye and NaBH_4 are reacting, electrons are transferred. In the aforesaid reaction, NaBH_4 served as a donor, and the dye served as an acceptor [39]. The silver nanocatalyst in this mixture served as an intermediate between the MB dye and BH_4^- ions. In the beginning, it lowered the bond dissociation energy by improving the electron transfer between them. Consequently, NaBH_4 reduced MB more quickly in the presence of Ag NPs. Clearly, the green synthesized Ag NPs by *Passiflora caerulea L.* extract act as an efficient phyto-nanocatalyst in organic dye reduction (Fig. 9). *Passiflora caerulea* contains a rich composition of phytochemicals such as flavonoids, terpenoids, and phenolic compounds, which play a significant role in the enhanced catalytic activity of Ag NPs. These biomolecules act as reducing agents and stabilizers, influencing the size, morphology, and surface properties of the nanoparticles. The presence of specific antioxidant-rich compounds may also contribute to increased electron transfer rates, facilitating a more efficient degradation of MB compared to Ag NPs synthesized from other plant extracts.

**Fig. 9.** Mechanism of dye reduction by Ag nanoparticles and NaBH_4

3.6.2. Recyclability of nanocatalysts

Catalysts used in large-scale applications must exhibit high activity and stability. Considering the high catalytic activity of the Ag NPs, the degrading efficiency of the nanoparticles was tested by reusing them on MB for five consecutive cycles. As shown in figure 10, after 5 rounds of degradation, no significant loss in catalytic activity was detected.

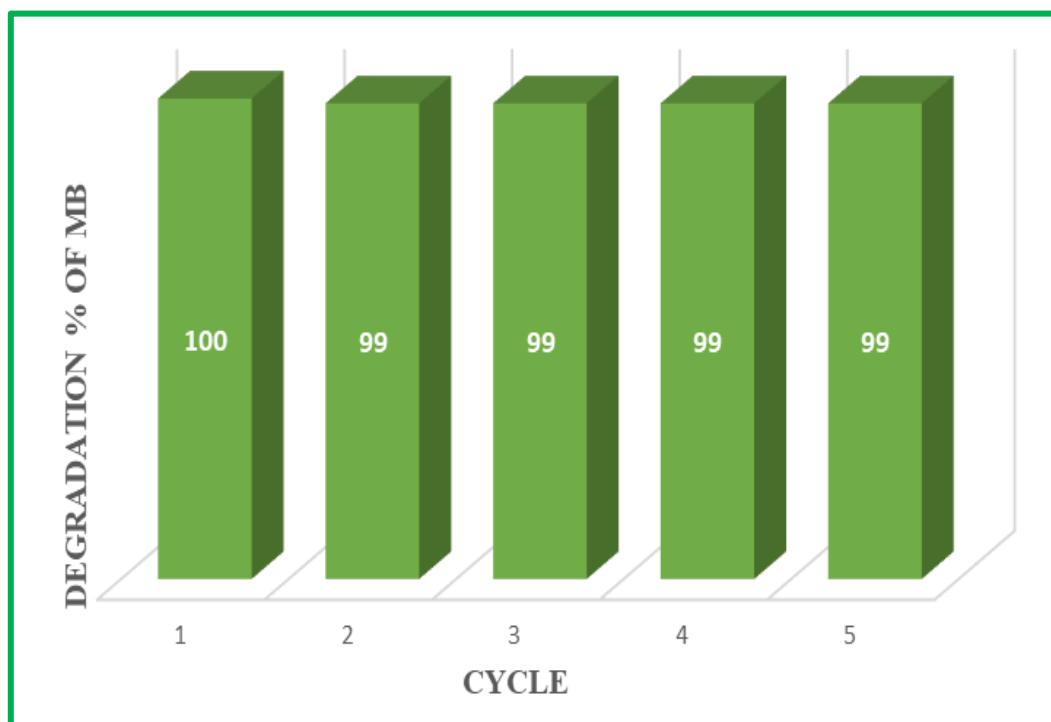


Fig. 10. Degradation cycle efficiency of MB dye

4. Conclusions

Green synthesis of silver nanoparticles utilizing plants is safe, cost-effective, non-toxic, and eco-friendly pathway which is amenable for synthesis on a larger scale. *Passiflora caerulea* is a member of the Passifloraceae family representing the great ability to synthesis Ag NPs at optimal temperature circumstances. The UV absorption peak at 419 nm indicates the Ag NPs synthesis and SEM studies helped decode their distribution and morphology. The biofabrication of the Ag NPs was confirmed by FTIR studies through the action of various phytochemicals with their various functional groups. The synthesized nanoparticles' purity, nature, and phase composition were affirmed by XRD patterns. The Ag NPs display antimicrobial activity against *Shigella dysenteriae*, *Bacillus subtilis*. In addition, these phyto-nanocatalysts exhibit remarkable dye degradation activity that in one-step 180 seconds reaction can remove and discolour methylene blue. Utilizing plant extract is beneficial for the synthesis since it is energy-efficient and cost-effective and protects the environment and human health resulting in safer products with minimum generation of waste. This eco-friendly technique can be an economical substitute to the conventional chemical or physical approaches utilized to synthesize silver nanoparticles. While the green synthesis method minimizes chemical waste and promotes sustainability, it is crucial to consider the potential environmental implications of silver nanoparticles. Studies suggest that AgNPs may exhibit toxicity in aquatic ecosystems, impacting microbial communities and higher organisms. Further research is needed to understand their long-term ecological effects and develop strategies for mitigating potential risks. Consequently, it possesses the potential to be utilized in biomedical and environmental applications and has a key role in future medical devices and optoelectronics.

Conflict of Interests

The authors have no conflicts of interest to declare.

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