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Green Synthesis, Characterization of Silver Nanoparticles from Coriandrum Sativum Plant Extract and Its Antimicrobial Activities

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Abstract: *The Cissus quadrangularis is one of the most widely used medicinal plants in India and is distributed extensively throughout the country. The present study aimed to investigate the phytochemical constituents and antimicrobial activity of silver nanoparticles (AgNPs) synthesized using Cissus quadrangularis. The synthesized nanoparticles were characterized using UV–Visible spectrophotometry and Fourier-transform infrared (FTIR) spectroscopy. The antimicrobial activity was evaluated by the disc diffusion method.The biosynthesis of AgNPs was initially indicated by a visible color change in the reaction mixture from light yellow to honey brown. UV–Visible spectral analysis revealed a characteristic absorption band at approximately 430 nm, confirming the formation of silver nanoparticles. FTIR analysis of the C. quadrangularis-mediated AgNPs showed prominent peaks at 1631.52, 2147.12, and 3370.56 cm^{-1}, indicating the involvement of functional groups associated with primary amines. The absorption peak observed at approximately 2146 cm^{-1} was attributed to C≡C stretching vibrations of alkynes.Phytochemical screening of the synthesized C. quadrangularis AgNPs revealed the presence of flavonoids, proteins, alkaloids, phenolic compounds, carbohydrates, and terpenoids. The nanoparticles demonstrated notable antimicrobial activity, particularly against Staphylococcus aureus, which exhibited a maximum zone of inhibition of 26 mm. Overall, the silver nanoparticles synthesized using C. quadrangularis exhibited significant antimicrobial potential. The findings suggest that the bioactive compounds present in C. quadrangularis may contribute to the biological activity of the synthesized nanoparticles and could have potential applications in the development of antimicrobial agents.*

Key Words: Cissus quadrangularis, silver nanoparticles, FTIR, Petroleum ether and Chloroform extract, antimicrobial activity.

1. INTRODUCTION

Nanotechnology has emerged as a rapidly developing field with considerable potential for the fabrication of nanomaterials with controlled sizes and shapes for diverse applications, particularly in the medical and agricultural sectors [1]. The synthesis of nanoparticles has attracted considerable global attention because of their unique physicochemical properties. Various physical, chemical, and biological approaches have been developed for nanoparticle synthesis. However, conventional physical and chemical methods are often associated with high costs, toxicity, and adverse environmental impacts. These limitations have encouraged the development of greener approaches that are economical, non-toxic, and environmentally sustainable [2].

Green synthesis has therefore gained significant importance as an alternative approach for the production of nanomaterials using plants and microorganisms. Among biological sources, plants offer several advantages over microorganisms because they contain a wide range of bioactive compounds and biomolecules that can function as

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reducing, stabilizing, and capping agents during nanoparticle synthes [3]. Several studies have successfully demonstrated the green synthesis of silver nanoparticles (AgNPs) using plant extracts such as Ficus carica, Cocos nucifera, Moringa oleifera, Carica papaya, Eucalyptus hybrid, Allium cepa, Aloe vera, and Basella alba. These nanoparticles have also exhibited a range of significant biological activities.

Cissus quadrangularis is a perennial vining plant belonging to the family Vitaceae and is widely distributed in India and parts of Africa. It is also known as Vitis quadrangularis and has been traditionally used for medicinal purposes for centuries. The plant has been utilized in the management of various ailments, including helminthic infections, digestive disorders, ulcers, seizures, menstrual irregularities, and respiratory diseases. Owing to its rich phytochemical composition, C. quadrangularis has also attracted considerable interest in the field of green nanotechnology [4].

Previous studies have demonstrated the potential of C. quadrangularis extracts in the synthesis of different metal nanoparticles. For instance, C. quadrangularis has been employed as a reducing agent for the synthesis of zinc oxide nanoparticles, which exhibited inhibitory activity against several microorganisms, including Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii, Bacillus cereus, and Mycobacterium smegmatis. Similarly, silver nanoparticles synthesized using C. quadrangularis stem extract have demonstrated considerable antibacterial activity. reported that C. quadrangularis-mediated AgNPs effectively inhibited the growth of both Gram-positive and Gram-negative bacteria at relatively low inhibitory concentrations of 10 µg/mL. Furthermore, calcium oxide nanoparticles synthesized using C. quadrangularis extract exhibited antibacterial activity against several pathogenic bacteria. The highest zone of inhibition was observed against Escherichia coli, followed by Vibrio cholerae, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Shigella dysenteriae, and Salmonella spp. [5-7].

Among the various metal nanoparticles, silver nanoparticles have received particular attention because of their broad range of biological activities, including antibacterial, antioxidant, antifungal, and cytotoxic effects. Their unique physicochemical and biological properties have led to extensive investigation of AgNPs for biomedical applications, including their use as photosensitizers, radiosensitizers, antiviral agents, and therapeutic materials. They have also been explored for potential applications in the treatment of several types of cancer, including skin, oral, breast, lung, and leukemia cancers [8].

Rheumatoid arthritis is a chronic autoimmune disorder characterized by persistent inflammation of the joints, infiltration of immune cells, and progressive destruction of cartilage and bone. Various immune cells, including macrophages, B cells, and T cells, contribute to the progression of the disease by releasing inflammatory mediators such as prostanoids and cytokines, which promote tissue damage and joint destruction. In recent years, considerable attention has been directed toward evaluating the anti-arthritic potential of biologically synthesized nanoparticles. For example, zinc oxide nanoparticles synthesized using Lagenaria siceraria extracts demonstrated approximately 82% inhibition in a bovine serum albumin (BSA)-based assay for anti-arthritic activity. Similarly, ZnO nanoparticles synthesized using Luffa acutangula peel extract exhibited significant anti-arthritic activity by effectively inhibiting protein denaturation of albumin and BSA [9.10] reported approximately 90% inhibition in the anti-arthritic activity of ZnO nanoparticles synthesized using Tectona grandis leaf extract.

Considering the medicinal importance of C. quadrangularis and the growing interest in plant-mediated nanoparticle synthesis, the present study focused on the green synthesis of silver nanoparticles using an aqueous extract of C. quadrangularis as a reducing and stabilizing agent. The synthesized AgNPs were characterized using X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, UV–Visible (UV–Vis) spectroscopy, and scanning electron microscopy (SEM). Furthermore, the anti-arthritic potential of the synthesized silver nanoparticles was evaluated using in vitro protein denaturation assays based on egg albumin and bovine serum albumin (BSA). The study was undertaken to assess the potential application of C. quadrangularis-mediated AgNPs as biologically active nanomaterials with promising anti-arthritic properties [11-13].

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II. MATERIALS AND METHODS

Collection and Preparation of Plant Material

The plant Cissus quadrangularis was collected from the Campus of Shivaji College Kannad. The stems of Cissus quadrangularis were thoroughly washed several times with distilled water to remove adhering dust and other impurities. The material was then shade-dried at room temperature. After complete drying, the stems were powdered using a domestic grinder. The resulting plant powder was stored in an airtight container until further use.

Preparation of Plant Extract

Ten grams of the dried Cissus quadrangularis stem powder were subjected to Soxhlet extraction using a solvent mixture consisting of 80 mL petroleum ether and 20 mL chloroform. The extraction temperature was maintained at 55 °C, and extraction was continued for approximately 3-4 h. The resulting extract was concentrated by transferring it into a clean round-bottom flask and evaporating the solvents using a distillation apparatus. The concentrated crude extract was stored in an airtight bottle at 4 °C until further experiments [14].

Preparation of Plant Extract Stock Solution

A 400 mg portion of the concentrated plant extract was accurately weighed and dissolved in 40 mL of double-distilled water. The solution was filtered through Whatman No. 1 filter paper to remove insoluble materials. The resulting stock solution was stored at room temperature and used for subsequent experiments.

Green Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using the prepared Cissus quadrangularis plant extract. Briefly, 40 mL of the plant extract stock solution was mixed with 10 mL of 10 mM aqueous silver nitrate ($AgNO_3$) solution. The reaction mixture was maintained at room temperature. The experiment was performed in triplicate to ensure reproducibility.After approximately 1 h of incubation, the reaction mixture exhibited a visible colour change from colourless to honey-brown. This colour change was considered an initial indication of the formation of silver nanoparticles and was subsequently confirmed by UV–Visible spectroscopic analysis [15,16].

Characterization

XRD analysis was performed using X-ray 7000, Shimadzu to confirm the amorphous/crystalline character of the materials. Diffraction patterns were measured using Cu, K-alpha radiation at 30 kV and 30 mA in, with 2h range of 0 to 80 . The synthesized nanoparticle was characterized using Shimadzu FTIR spectrometer. Diffuse reflectance FTIR spectrum for silver nanoparticles was measured from 4000 to 400 cm 1. Surface morphology of nanoparticles was obtained from scanning electron microscopy (SEM). SEM analysis was performed using JEOL JM 5600 equipped with 20 kV voltage and wavelength range of 5–6 nm. UV–Vis spectrum was
obtained from Jasco V650 Vis spectrophotometer with the wavelength ranging from 200 to 600 nm.

UV-Visible Spectroscopic Analysis

The bioreduction of Ag^+ ions during the formation of silver nanoparticles was monitored using UV–Visible spectroscopy. The absorption spectrum of the reaction mixture was recorded using a SYSTRONICS UV–Visible Spectrophotometer 119 with a spectral resolution of 1 nm over the wavelength range of 400–520 nm.

For spectral analysis, 1 mL of the synthesized nanoparticle suspension was diluted tenfold with Millipore water to minimize errors associated with high optical density. The characteristic surface plasmon resonance absorption band was recorded to confirm the formation of AgNPs [17].

Fourier Transform Infrared (FTIR) Spectroscopic Analysis

For FTIR analysis, the synthesized silver nanoparticle suspension was centrifuged at 10,000 rpm for 30 min. The resulting pellet was collected and washed three times with 20 mL of deionized water to remove unbound proteins, enzymes, and other soluble phytochemicals. The purified nanoparticle pellet was subsequently dried and thoroughly ground with potassium bromide (KBr).

The prepared samples were subjected to FTIR analysis, and the spectra were recorded over the range of 4000–400 cm^{-1}. The observed absorption bands were used to identify the functional groups associated with the plant-derived phytochemicals involved in the reduction and stabilization of the synthesized silver nanoparticles [18].

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Preliminary Phytochemical Analysis

Preliminary qualitative phytochemical analysis was performed to identify the major classes of phytoconstituents associated with the synthesized AgNPs. The analysis was carried out using standard qualitative phytochemical screening procedures as described [19].

Antimicrobial Activity

Microbial Strains

The antimicrobial activity of the synthesized silver nanoparticles was evaluated against five bacterial strains: Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Staphylococcus aureus, and Pseudomonas fluorescens. Authentic pure cultures of the bacterial strains were obtained from the Venture Institute of Biotechnology and Bioinformatics Research, Madurai, Tamil Nadu, India. Overnight cultures of the respective bacterial strains were used for the antimicrobial assays [20].

Agar Disc Diffusion Method

The antibacterial activity of the synthesized silver nanoparticles was evaluated against the selected bacterial pathogens using the agar disc diffusion method. The bacterial cultures were initially inoculated into nutrient broth and incubated for 24 h before performing the assay.

Sterile Mueller–Hinton Agar (HiMedia) plates were prepared and allowed to solidify. The respective bacterial cultures were uniformly inoculated onto the surface of the solidified agar using sterile swabs. Sterile discs impregnated with the synthesized silver nanoparticles were then carefully placed on the inoculated agar surface [21].

A mixture of petroleum ether and chloroform was used as the solvent control, while ampicillin was used as the standard antibiotic. The plates were incubated at 37 °C for 24 h. Following incubation, the antibacterial activity was assessed by measuring the diameter of the zones of inhibition surrounding the discs.

All assays were performed in triplicate, and appropriate control plates were maintained throughout the experiment. The zone of inhibition was measured in millimetres (mm) from the edge of the disc to the outer margin of the clear zone, and the results were recorded for comparison among the tested samples [22].

III. RESULTS AND DISCUSSION

3.1. X-ray Diffraction (XRD) Analysis

The crystalline nature of the synthesized silver nanoparticles was investigated by X-ray diffraction (XRD) analysis. The diffraction peaks observed at approximately 38°, 44.37°, 66.46°, and 77.44° were indexed to the (111), (200), (220), and (311) crystallographic planes, respectively. These characteristic diffraction peaks confirmed the crystalline nature of the synthesized silver nanoparticles and were consistent with previously reported results [23].

The obtained XRD pattern was compared with the standard Joint Committee on Powder Diffraction Standards (JCPDS) data, File No. 87-0720, and showed good agreement with the standard diffraction values for metallic silver. Among the observed reflections, the intense peak at approximately 38° corresponding to the (111) plane was characteristic of crystalline silver nanoparticles. Additional non-indexed peaks observed around 32° and 46° may be associated with the crystallization or deposition of bio-organic compounds derived from the Cissus quadrangularis extract on the nanoparticle surface, as previously reported [24,25].

The average crystallite size of the synthesized silver nanoparticles was estimated using the Debye–Scherrer equation:

$$D=0.9\lambda/\beta\cos\theta$$

where D is the crystallite size, λ represents the wavelength of the incident X-ray, β is the full width at half maximum (FWHM) of the diffraction peak, and θ is the Bragg diffraction angle. Based on the XRD data, the average crystallite size of the synthesized silver nanoparticles was found to be approximately 24 nm. The calculated particle-size data are presented in Table 1.

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Table 1 Calculated particle size of Silver nanoparticle.

Miller indices (hkl) Plane	Peak position 2h value	Height	width	Crystallite size
111	38.18	11564.25	0.37067	24.98
200	44.37	254.89	0.00339	28.65
220	66.46	19944.53	0.44756	22.21
311	77.48	1745.55	0.51389	2016

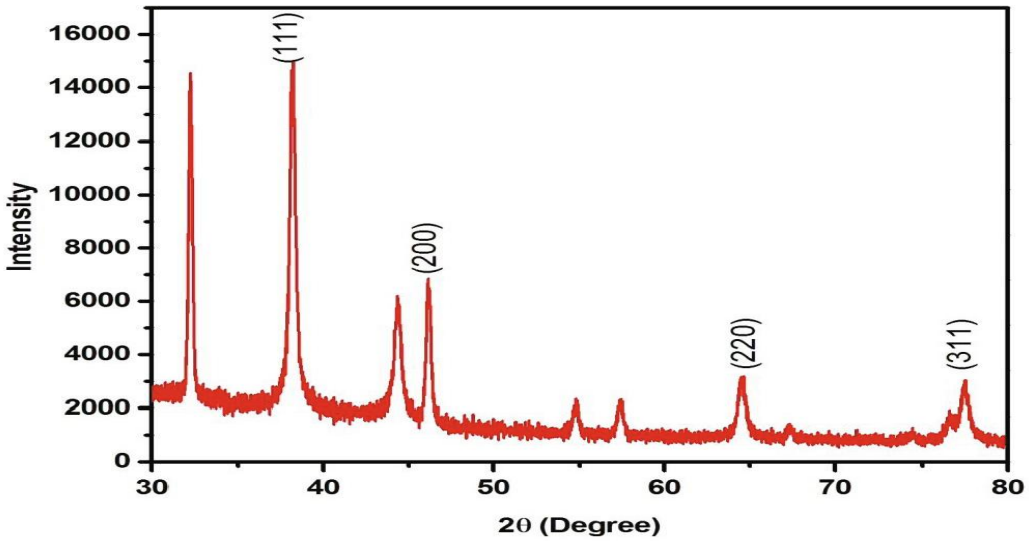


Fig. 1. XRD pattern of Silver Nanoparticle from Cissus Quadrangularis Extract.

3.2. FTIR Spectroscopic Analysis

The FTIR spectrum of the silver nanoparticles synthesized using Cissus quadrangularis extract is presented in Figure 2. FTIR analysis was performed to identify the functional groups associated with the synthesized nanoparticles and to determine the possible involvement of plant-derived phytochemicals in nanoparticle formation and stabilization [26].

A broad absorption band at 3378 cm^{-1} was attributed to O–H stretching vibrations, which may be associated with hydroxyl-containing compounds such as phenols and alcohols present in the plant extract. The absorption band observed at 2923 cm^{-1} was assigned to C–H stretching vibrations, possibly originating from organic compounds present in the extract. The band at 1735 cm^{-1} was attributed to C=O stretching vibrations associated with carbonyl-containing groups, such as carboxylic acids or esters [27].

A strong absorption band at 1622 cm^{-1} was assigned mainly to amide-related vibrations, suggesting the possible involvement of proteins or other biomolecules in the stabilization of the nanoparticles. The bands observed at 1365 cm^{-1} and 1054 cm^{-1} were associated with CH_2-related vibrations and C–O stretching vibrations of ester or ether groups, respectively. These findings suggest that phytochemicals present in Cissus quadrangularis extract may have contributed to the reduction of silver ions and stabilization of the synthesized silver nanoparticles [28].

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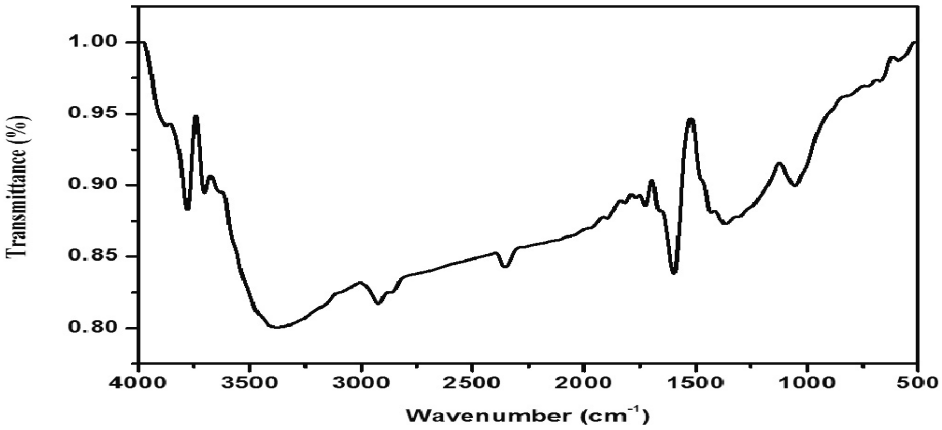


Fig. 2. FTIR spectrum of Silver nanoparticle from Cissus Quadrangularis plant extract.

3.3. UV–Visible Spectroscopic Analysis

The UV–Visible absorption spectrum of the Cissus quadrangularis-mediated silver nanoparticles is shown in Figure 3. The formation of silver nanoparticles was associated with the surface plasmon resonance (SPR) phenomenon, which results from the collective oscillation of conduction electrons in metallic nanoparticles upon interaction with incident light.

A visible colour change from colourless to yellowish-brown was observed during the reaction, indicating the reduction of silver ions (Ag^+) to metallic silver nanoparticles (Ag^0). The synthesized AgNPs exhibited a characteristic SPR absorption band at approximately 410 nm, confirming the successful formation of silver nanoparticles [29].

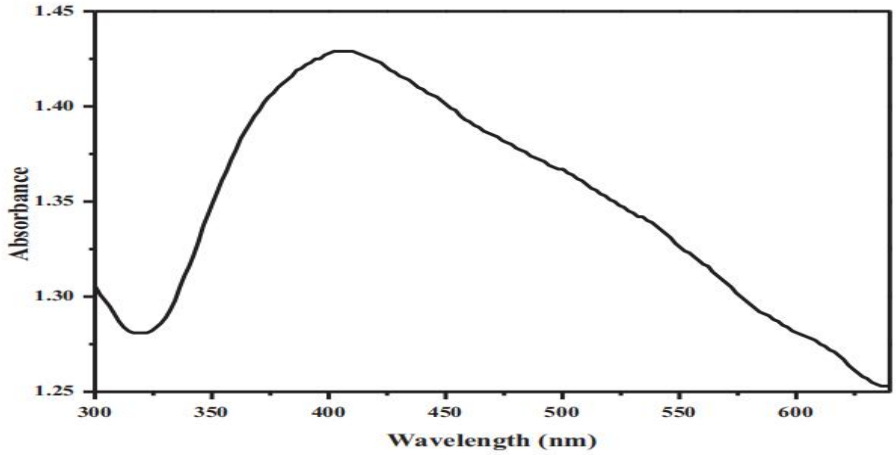


Fig. 3. UV-Vsible spectrum of Silver nanoparticle from Cissus Quadrangularis plant extract.

3.4. Scanning Electron Microscopy (SEM) Analysis:

The surface morphology and particle characteristics of the synthesized silver nanoparticles were examined using scanning electron microscopy (SEM), as shown in Figure 4. The SEM images revealed that the synthesized nanoparticles were predominantly spherical in shape and exhibited a polydisperse distribution.

Some degree of particle agglomeration was also observed. Such aggregation may be attributed to interactions between nanoparticles and biomolecules or phytochemical constituents present in the Cissus quadrangularis extract, which may act as reducing and stabilizing agents during nanoparticle synthesis [30].

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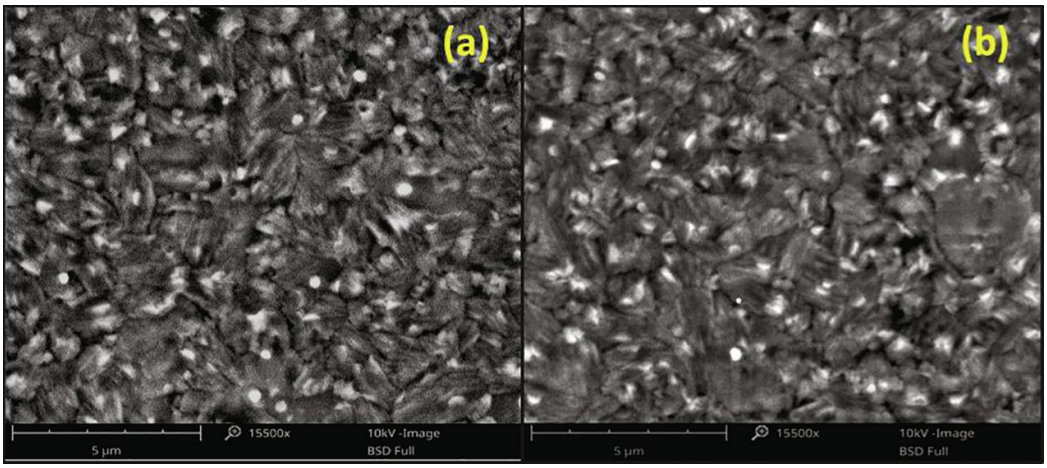


Fig. 4. SEM images of Silver nanoparticle from Cissus Quadrangularis plant extract

Antimicrobial Activity:

The antimicrobial activity of the synthesized silver nanoparticles was evaluated against five bacterial pathogens, namely Escherichia coli, Pseudomonas aeruginosa, Shigella flexneri, Staphylococcus aureus, and Pseudomonas fluorescens, using the well diffusion method (Fig. 4 and Table 2).

The results demonstrated that the synthesized silver nanoparticles exhibited considerable antibacterial activity against the tested bacterial strains. Among the pathogens investigated, Staphylococcus aureus showed the highest susceptibility, producing a maximum zone of inhibition of 28 mm in diameter. Both Pseudomonas aeruginosa and Pseudomonas fluorescens exhibited inhibition zones of 26 mm in diameter. These findings indicate that the synthesized silver nanoparticles possess promising antibacterial activity against both Gram-positive and Gram-negative bacterial pathogens [30-33].

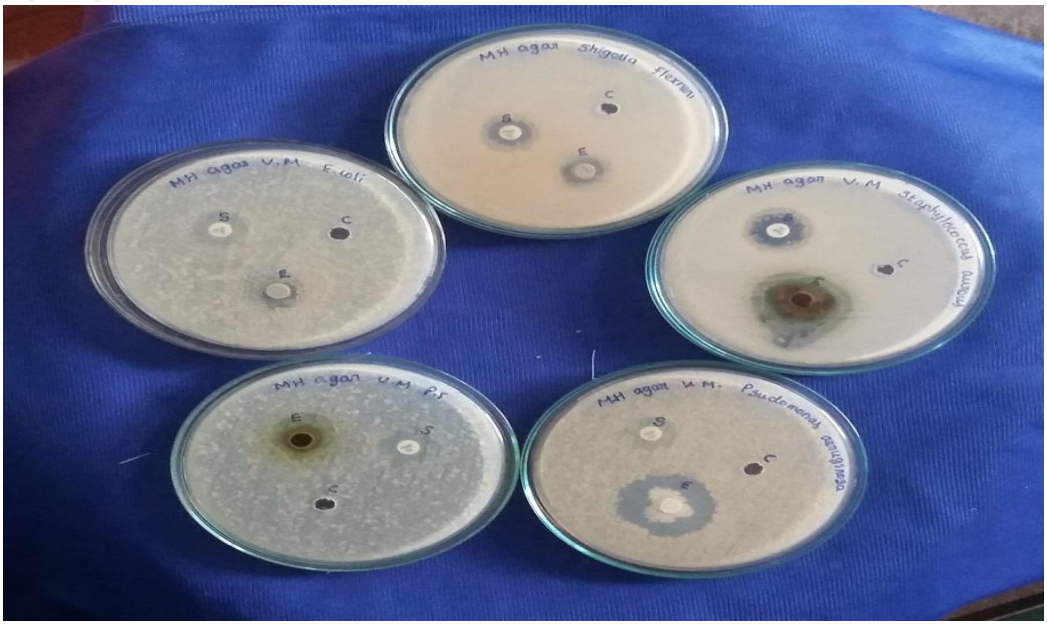


Figure 5: Antimicrobial activity of synthesized Silver nanoparticle against selected pathogens.

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Table 2:Antimicrobial Activity of Cissus quadrangularis Silver Nanoparticles Against Selected Pathogens

Microorganism	Zone of Inhibition In Diameter (in mm)		
	Control	Standard	Extract
Escherichia coli	0	16	12
Pseudomonas aeruginosa	0	15	26
Shigella flexneri	0	12	12
Staphylococcus aureus	0	18	28
Pseudomonas fluorescence	0	15	24

IV. CONCLUSION

A simple, eco-friendly, cost-effective, and non-toxic green synthesis approach was employed in the present study for the preparation of silver nanoparticles (AgNPs). Aqueous extract of Cissus quadrangularis served as both a reducing and stabilizing agent during the synthesis process. The synthesized AgNPs exhibited an average particle size of approximately 24 nm. The involvement of phytochemicals present in the plant extract in the reduction of silver ions (Ag^+) to silver nanoparticles was supported by the functional groups identified through FTIR analysis. The UV–Visible spectrum showed a prominent absorption band at 410 nm, confirming the formation of AgNPs. SEM analysis revealed that the synthesized nanoparticles were predominantly spherical in morphology. Furthermore, the synthesized silver nanoparticles demonstrated significant anti-arthritic activity. Overall, the findings indicate that Cissus quadrangularis-mediated green synthesis provides a promising and sustainable approach for producing AgNPs and may contribute to further investigations into their potential applications in arthritis management and the development of novel therapeutic agents.

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Author Contributions:

D.T. Sakhare is the sole author. The author read and approved the final manuscript.

Conflicts of Interest:

The authors declare no conflicts of interest.

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