

# Bio-Fabrication of Silver Nanoparticles: *Murdannia pauciflora* (G. Brückn) Structural Analysis and Multifunctional Biological Properties

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## ABSTRACT

**Background:** *Murdannia pauciflora* is an ethnomedicinally important plant known for its diverse therapeutic properties. This study reports, for the first time, the green synthesis of Silver Nanoparticles (AgNPs) using aqueous extract of *M. pauciflora* and evaluates their phytochemical composition and biological activities. **Materials and Methods:** Preliminary phytochemical screening revealed the presence of phenols, alkaloids, tannins, glycosides, flavonoids, and saponins. GC-MS analysis identified 98 bioactive compounds, indicating the rich phytochemical profile of the plant extract and its suitability for nanoparticle synthesis. The synthesized AgNPs were characterized using UV-visible spectroscopy, FTIR, XRD, SEM, EDX, DLS, zeta potential analysis, and TEM. Characterization results confirmed the successful formation of stable nanoparticles with a Face-Centered Cubic (FCC) crystalline structure and predominantly rectangular morphology. **Results:** The AgNPs exhibited an average particle size of approximately 12 nm and a zeta potential value of -30 mV, suggesting good stability and uniform surface distribution. Biological studies demonstrated that AgNPs possessed notable pharmacological activities. Cytotoxicity evaluation against pancreatic cancer cells showed an  $IC_{50}$  value of 500  $\mu$ g/mL, whereas no inhibitory effect was observed against HepG2 liver cancer cells. **Conclusion:** The nanoparticles also exhibited significant  $\alpha$ -glucosidase inhibitory activity with an  $IC_{50}$  value of 78.44  $\mu$ g/mL, indicating potential antidiabetic properties. Furthermore, antioxidant activity was observed with a free radical scavenging  $IC_{50}$  value of 82.65288  $\mu$ g/mL. Overall, the green-synthesized *M. pauciflora* AgNPs demonstrated promising antioxidant, antidiabetic, and cytotoxic activities, highlighting their potential applications in biomedicine, cancer therapy, drug delivery, and antidiabetic formulations.

**Keywords:** AgNPs, Biomedical Activities, Characterization, *Murdannia pauciflora*, Phytochemical.

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## INTRODUCTION

The past few decades have seen a significant increase interest in nanomaterials (1-100 nm materials) across a wide range of industries, including biomedicine, energy storage, catalysis, and sensors (Xu *et al.*, 2020). Industry such as food, pharmaceuticals, and agriculture, due to their distinct physicochemical characteristics (Sheme *et al.*, 2023). The salts of certain metals, such as Copper (Cu), Iron (Fe), Gold (Au), Silver (Ag), Platinum (Pt), and Palladium (Pd), are used to create metal nanoparticles. AgNPs have several advantages over other metallic nanoparticles. Although many are classified as "AgNPs," some have a high concentration of Ag<sub>2</sub>O due to the large ratio of surface to bulk Ag

atoms. Over the past 10 years, AgNPs have found employment in a variety of medical and technological gadgets, surgical tools, bone cements, surgical masks, and other products. Additionally, wounds are treated with a specific quantity of ionic silver. Consequently, burns and wounds are treated using AgNPs. Molecular labeling uses AgNPs because of their large scattering cross sections and surface plasmon resonance. Thus, a large number of AgNPs are acknowledged for their numerous applications. Because of their remarkable catalytic attributes, it has drawn a lot of attention lately (Mallick *et al.*, 2024a; Mallick *et al.*, 2021). AgNPs are widely recognized for their potent and extensive antibacterial and cancer-fighting abilities. AgNPs' potential biological functions, such as enhancing wound and bone healing, promoting vaccine immunogenicity, and exhibiting antidiabetic properties, have also been investigated. Better medical applications of AgNPs will be made possible by understanding their biological mechanisms and possible cytotoxicity. Three distinct approaches are possible to create silver nanoparticles: physical, chemical, and biological synthesis. An intriguing substitute for physical and chemical creation of nanoparticles is biogenic synthesis (Gudikandula



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and Charya, 2016). Toxic stabilizing agents and capping agents are used in various physical and chemical processes to maximize reaction conditions, yet they are nevertheless costly and detrimental to humans and the environment. As a result, it is stressed to lower the risk of dangers by creating biosynthetic techniques that use natural items as substrates. Various biological precursors, including secondary metabolites and enzymes, are involved and facilitate the bioreduction reaction in a rapid and environmentally benign manner. Plant extracts from various plant parts, such as bark, peel, callus, leaves, flowers, fruit, stems, seeds, and rhizomes, can also yield nanoparticles of all sizes. According to (Kuppusamy *et al.*, 2015), these include spherical, triangular, pentagonal, hexagonal, nanorod, nanoprism, and irregular shapes. *Murdannia pauciflora* G. Brückn is a member of the Commelinaceae family, which has 650 species spread over 41 genera (Lee *et al.*, 2022). There are 55 species in the genus *Murdannia* worldwide. Though Asia has the greatest diversity, it is found widely throughout the warm temperate and tropical regions of the world (Nandikar and Gurav, 2015). Species of the genus *Murdannia* can be observed in grassy slopes and woods throughout Asia, including China and Thailand. The plant is traditionally used to treat skin conditions; the entire plant is indigenous to India and is known as a few-flowered dew flower (Malavika and Thenmozhi, 2023). Alkaloids, phenols, flavonoids, tannins, and saponins are chemicals found in *Commelina nudiflora* that may be a source of nutrition for both people and animals (Ujowundu *et al.*, 2007). In this study, we used whole plant extract of *M. pauciflora* (*M. pauciflora* AgNPs) to study the formation of AgNPs. UV-visible spectroscopy, Fourier-Transform Infrared (FTIR), X-ray Diffraction (XRD), Dynamic Light Scattering (DLS), zeta potential, Scanning Electron Microscopy (SEM) in conjunction with Energy Dispersive X-ray (EDX), Transmission Electron Microscopy (TEM), and other techniques were used to ascertain the size, shape, morphology, and stability of *M. pauciflora* AgNPs. The antioxidant activity of the *M. pauciflora* AgNPs was evaluated using the DPPH and ferric chloride method. The disc diffusion method for antimicrobial activity,  $\alpha$ -glucosidase inhibitory activity, and MTT assay (Panc-1 and HepG2) were used to assess the antimicrobial, antioxidant, antidiabetic and anticancer properties of the *M. pauciflora* AgNPs.

## MATERIALS AND METHODS

### Collection of *M. pauciflora* (G. Brückn.) Plant Material

*Murdannia pauciflora* (G. Brückner) whole plant (Figure 1) was collected from Sirsi, Uttara Kannada district of Karnataka, India. During January, the herbarium was identified by A. N. Sringeswara, curator at the Mahatma Gandhi Botanical Garden, University of Agricultural Sciences, GKVK, Bangalore, collected the specimen under no. C001, with Accession number UASB 5,774.

### Plant Extract Preparation

The plant surface was thoroughly washed with running tap water to eliminate any particulate matter and contaminants. The plants are shade-dried, and the dried samples were grounded by a mechanical grinder. About 25 g of plant powder were extracted using the Soxhlet method using several solvents at varying temperatures. Several iterations of the extraction procedure were conducted to obtain enough extract for more research. The semisolid extract was kept at 4°C until it was needed again after the crude extract was allowed to evaporate and then exposed to preliminary phytochemical analysis to determine its primary and secondary metabolites (Figure 2).

### Qualitative Phytochemical Analysis

The following conventional methods were carried out to test the extract for the presence of bioactive chemicals from the studies of (Banu and Cathrine, 2015; P M *et al.*, 2025; Praveen and Kakkalameli, 2025a).

### GC-MS Analysis of *M. pauciflora*

GC-MS Analysis of whole plant extract of *M. pauciflora* was performed using QP2010S, Shimadzu, Japan, Mass Spectrometer (GC) and Gas Chromatograph (MS) enhanced with an electron ionization detector. An EC-5 column (0.25  $\mu$ L, 30 m, 0.25 mm) and a 2-mm direct injector were included with the chromatograph. A 2-mm direct injector liner was used to inject a total of 2  $\mu$ L of sample. The temperature was set to begin at 60°C for 2 min and then increase to 450°C at a rate of 20°C/min (Chakraborty *et al.*, 2022). All specimens were injected straight into the capillary column using a 2-mm injector. The identification of the chromatographic peaks was achieved by their relative retention times, and the mass spectrometric analysis was conducted by precisely identifying the compounds through comparison of the spectral data with the National Institute of Standards and Technology library (RC & Kakkalameli, 2025; Vanajakshi *et al.*, 2025).

### Pilot Study

#### Concentration optimization

We evaluated the sample for three different concentrations of plant extract and 1 Mm AgNO<sub>3</sub> in the ratio of 1:9, 2:8, and 3:7 distinct concentrations for 10 mL of sample (plant extract + AgNO<sub>3</sub>) with the aim to find the optimal amount of extract required for the effective generation of AgNPs. And the 2:8 ratio concentration produced the best outcomes.

#### Optimization of pH

The ideal pH is necessary for the best synthesis of AgNPs. The samples (2:8) were treated with three different pH values: 3.3 (Acidic), 7 (Neutral), 10.1 (Basic) and. The best results were obtained in basic pH.

## Temperature optimization

The production of nanoparticles is strongly influenced by temperature. Therefore, 0.1 M AgNO<sub>3</sub> solution with a 2:8 ratio treated with three different temperatures -4, 40, and 60°C was used to identify the ideal temperature required for optimizing *M. pauciflora* AgNPs yield within the ideal time frame. At 60°C, the best synthesis was discovered.

## Bio-fabrication of Ag NPs from the aqueous extract of *M. pauciflora*

The entire plant extract of *M. pauciflora* was used to create silver-nitrate nanoparticles (AgNO<sub>3</sub> NPs). 25 g of plant extract and 5 Mm of AgNO<sub>3</sub> were produced as a stock solution. Additionally, 1 Mm of AgNO<sub>3</sub> was utilized for synthesis, and the solution was made up to the ratio of 2:8, 2 mL of plant extract and 8 mL of AgNO<sub>3</sub> solution. It is noted that the color of the reaction combination (leaf extract and silver-nitrate solution) changes with time. The creation of AgNPs is shown by the color changing from pale yellow to brown after 30 min of incubation. The yellow-colored solution gradually turned dark brown after 120 min, due to both the AgNPs' increasing concentration and particle size development (Bhavi *et al.*, 2024).

UV-visible spectrum analysis was used to track the Ag<sup>+</sup> ion reduction over time. The reaction mixture was centrifuged to extract the AgNPs. Before characterization, the nanoparticles were resuspended in 95% ethanol after being cleaned three times with deionized water. The green production of nanoparticles has been kept at a pH of 10.1 at temperature of 60°C. Following that, tiny precipitants were separated by centrifugation at 5,000 r.p.m. for 30 min. After the supernatants were removed, the precipitates were agitated with distilled water and ground with a mortar and pestle to achieve a homogeneous texture for further analysis and characterization.

## Characterization of *M. pauciflora* AgNPs

### UV-visible spectroscopy analysis

Using a dual-beam UV-9600 a spectrophotometer (Shanghai, China), the generated nanoparticles of *M. pauciflora* were exposed to UV-visible light. Followed by introduced to a cuvette, AgNPs nanostructures made from the entire plant extract were examined for optical absorbance over a 200-800 nm wavelength range. To provide a thorough assessment of the nanoparticles, the acquired spectrum data were then graphically depicted, with absorbance intensity mapped in the Y-axis and wavelength values plotted in the X-axis (Karadakatti *et al.*, 2024).

### FTIR

FTIR spectrum, which is made up of absorption peaks that match the frequency of vibrations between the atoms' bonds, stands in for the signature of the nanoparticles. Because every kind of nanoparticle has a different atom configuration, we

may use the FTIR spectra to determine if functional groups are present within the nanoparticles. It is possible to determine the functional groups in the nanoparticles by looking at the size of the peaks in the spectrum. AgNPs made from *M. pauciflora* the evaluation was conducted using a Thermo Fisher Scientific FTIR spectrophotometer (Waltham, MA, United States). To create a compacted pellet, Potassium Bromide (KBr) was mixed with the dried powder of manufactured nanoparticles. Identification of infrared absorption bands was made possible by the evaluation of the KBr pellets within the 400-4,000 cm<sup>-1</sup> spectral range. Using spectral data analysis in the 400-4,000 cm<sup>-1</sup> region, the existence of functional biomolecules in the sample was confirmed, allowing a thorough description of the chemical makeup of the nanoparticles (Jaast and Grewal, 2021; Praveen and Kakkalameeli, 2025b).

### XRD analysis

To create the powder XRD pattern, the dried powder of synthesized *M. pauciflora* AgNPs is exposed to an X-ray diffractometer (Rigaku Miniflex 600, SmartLab SE, Tokyo, Japan) at 40 kV and 30 mA, with a scanning rate of 40° min<sup>-1</sup>, at angles of 2θ that range from 20 to 80° (Thokchom *et al.*, 2023).

### SEM and EDX analysis

Topological structure and elemental composition of *M. pauciflora* AgNPs were detected by SEM paired with an EDX device (JEOL, JSM IT 500 LA, Peabody, MA, United States). Simply, *M. pauciflora* AgNPs were stuck to the stub using carbon tape, fixed, and flashed with gold. After that, the prepared stub was put in the instrument chamber for the analysis (Nayaka *et al.*, 2020).

### Zeta potential and DLS analysis

To evaluate the electrostatic charge and size distribution of *M. pauciflora* AgNPs. The samples are subjected to the zeta potential and DLS analysis using HORIBA SZ100, Kyoto, Japan. The uniform distribution of the *M. pauciflora* AgNPs is achieved by using ultrasonic agitation; the mixture was centrifuged for 20 min at 6,000 r.p.m. at 3.4 eV, which is essential for understanding behavior and possible applications (Bhavi *et al.*, 2024).

### TEM analysis

Using an FEI Tecnai G2 F30 system (Beijing, China), TEM was used to examine the detailed imagery of the *M. pauciflora* AgNPs, displaying their internal structure and morphology at an enhanced resolution. A 5 µL aliquot of the ZnO nanoparticle suspension was placed onto a copper grid and dried for 48 hr. Following the examination of the nanoparticles at an accelerating voltage of 300 keV, high-resolution pictures were obtained at a magnification range of 7,000×8,000, enabling accurate characterization of their morphological and structural characteristics (Abbigeri *et al.*, 2024).

## Biological activities

### Cytotoxicity

#### Cell culture

The National Centre for Cell Sciences in Pune, India provided the human pancreatic (PANC1) and liver cancer cell lines (HepG2). These cells were kept in Dulbecco's Modified Eagle Medium supplemented with 10 mm 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 0.1 mm nonessential amino acids, 1 mm sodium pyruvate, 1.5 g/L glucose, 2 mm l-glutamine, and a balanced salt solution containing 1.5 g/L Sodium Carbonate ( $\text{Na}_2\text{CO}_3$ ). To encourage cell proliferation, 10% fetal bovine serum (Gibco, United States) was also added. To avoid microbiological contamination, penicillin and streptomycin were added at a dosage of 100 international units/100  $\mu\text{g/mL}$ . The cells were kept in a humidified incubator with 5% Carbon Dioxide ( $\text{CO}_2$ ) at  $37^\circ\text{C}$  to provide ideal physiological conditions for growth and survival.

### Evaluation of cytotoxicity by MTT Assay

The inhibition percentage was calculated by MTT assay to assess the viability of cells. A 96-well microplate was seeded with cultured cells ( $1 \times 10^5$ ) and incubated for 48 hr at  $37^\circ\text{C}$  in a humidified 5%  $\text{CO}_2$  incubator. After incubation, 100  $\mu\text{L}$  of different concentrations (0-160  $\mu\text{g/mL}$ ) of AgNPs produced from *M. pauciflora* were added to each well after the monolayer was rinsed with fresh media. For additional exposure, the cells were kept under the same settings. After discarding the culture media, 100  $\mu\text{L}$  of MTT reagent was added to each well, and the mixture was then incubated for an additional 4 hr at  $37^\circ\text{C}$ . To ensure precise measurement, 100  $\mu\text{L}$  of Dimethyl Sulfoxide (DMSO) was added to dissolve the formazan crystals after the supernatant was removed. The cell viability percentage was computed using the optical absorbance measured at 590 nm. A dose-response curve was used to analyze the data and calculate the  $\text{IC}_{50}$  value,



**Figure 1:** Showing (A) Habitat, (B) Front view of the flower, (C) Side view of the flower.



**Figure 2:** Green synthesis of silver nanoparticles.

which represents the concentration needed to inhibit 50% of cell proliferation (Mandour *et al.*, 2023).

### Antidiabetic activity

#### *α*-glucosidase inhibition

The  $\alpha$ -glucosidase activity of *M. pauciflora* was determined by following the previously reported assays. Phosphate buffer (50 mM, pH 6.9) was combined with 50  $\mu$ L of  $\alpha$ -glucosidase (1 U/mL) from yeast (SRL, Bangalore, India) and subjected to different levels of concentration. AgNPs (0-125  $\mu$ g/mL) of *M. pauciflora* for 10 min at 37°C. 50  $\mu$ L of 5 mM p-nitrophenyl-D-glucopyranoside was added to the phosphate buffer to initiate the reaction. After 30 min at 37°C, the enzymatic process was stopped by adding 1 M  $\text{Na}_2\text{CO}_3$ . At 405 nm, absorbance was measured. Acarbose was used as a positive control, and the percentage of inhibition was computed and reported as  $\text{IC}_{50}$  values (Sukanya *et al.*, 2017).

### Antioxidant activity

#### DPPH assay

Different volumes of concentration of *M. pauciflora* AgNPs (0-250  $\mu$ g/mL) are mixed with 2 mL of DPPH (100  $\mu$ M), and the final volume is adjusted to 3 mL with methanol, and the reaction mixture is allowed to incubate in a dark chamber for 45 min. At the end of incubation period, absorbance was recorded using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) at 517 nm against the blank (Without sample/Standard). Free radical scavenging capacity of the samples were calculated and expressed in  $\text{IC}_{50}$  values in comparison with BHT as a standard antioxidant (Baliyan *et al.*, 2022).

## RESULTS AND DISCUSSION

### Qualitative Phytochemical Analysis

The phytochemical study reveals that the presence of phenols, flavonoids, alkaloids, tannins, saponins, and glycosides in *M. pauciflora*'s methanolic extract, whereas steroids are absent. The lack of steroids and alkaloids in the aqueous extract indicates that they are extracted using organic solvents, but the chloroform extract solely reveals the existence of saponins. These results highlight the importance of solvent choice in phytochemical research, which in turn affects *M. pauciflora*'s potential therapeutic uses. Additional studies may yield a more profound understanding of its pharmacological advantages (Table 1) (Huq, 2015).

### GC-MS Analysis

The GC-MS analysis of the aqueous extract shows approximately 98 bioactive compounds based on their retention time and area percentage, as shown in (Table 2). The compound bisabolol oxide b <math>\alpha</math> has a high area percentage of 20.16 and RT of 32.500, lactic acid RT 4.035, Pimelic ketone RT 5.859, cyclohexane <1,2-dioxo-> RT 7.144, Benzenecarboxylic acid RT 9.903, syringaldazine RT 11.583, Cineole <1,4-> RT 12.045, December 9-enoic acid RT 13.795, isoborneol <8-isobutyryloxy-> RT 16.151, Incensole RT 18.455, hexadecanoic acid <n-> RT 20.115, pyrazine <2-methoxy-> RT 25.725, valeranone, RT 26.900, dihydrotagetone RT 27.827, decamethylcyclopentasiloxane RT 28.039, Eicosene RT 30.190, Tricos-(9Z)-ene RT 33.667, Phytol RT 36.735, tetracontane <n-> RT 40.114. The GC-MS studies

on *Murdannia simplex* and *Murdannia lanuginosa* the root methanolic extract reveal that three compounds in six compounds in *M. simplex* and three compounds in *M. lanuginosa* and these plants have properties like an antimicrobial, antiproliferative and anti-inflammatory activities and also used as a fungicide due to the presence of compounds like H-Pyran-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl, salicylaldehyde and Azine (Nandikar *et al.*, 2018). The investigations by the researchers found that the methanolic and hexane extract of *M. bracteata*, *M. lanuginosa*, *M. simplex* the GCMS results shows bioactive compounds like stigmasterol,  $\alpha$ -tocopherol,  $\beta$ - $\beta$ -sitosterol in *Murdannia bracteata*, diphenyl-, 2-oxide 3,4-diphenylfurazan 2-oxide diphenylfuroxan and diphenylfurazan N-oxide furazan in *M. simplex* and *M. lanuginosa* shows the compounds like azine and salicylaldehyde (Figure 3) (Patrick and Bakhari, 2020).

### Characterization of *M. pauciflora* AgNPs

#### Bio-fabrication of Ag NPs from the aqueous extract of *M. pauciflora*

*Murdannia pauciflora* AgNPs were synthesized by incubation the reaction mixture at 60°C for 30 min, allowing plant phytochemicals to reduce  $\text{Ag}^+$  ions (Figure 4a). The pH was adjusted to 10.1 with 1 N NaOH to enhance reduction efficiency and nanoparticle formation. After 10 min of stirring, the color changed from pale yellow to dark brown, confirming *M. pauciflora* AgNP formation. The proposed mechanism (Figure 4b) illustrates how the alkaline medium and phytochemicals drive  $\text{Ag}^+$  reduction and nanoparticle growth. The nanoparticles were purified by centrifugation at 5,000 r.p.m. for 30 min.

#### UV-visible spectroscopy analysis

The color shifts from pale yellow to brown, indicating the creation of nanoparticles. Additionally, the UV-visible spectrum provided spectroscopic confirmation. The intensity of *M. pauciflora* AgNPs ranged from approximately 350 to 485 nm, with a confirming spot at 405 nm (Figure 5). The findings of (Mukherji and Mondal, 2017), this peak results from the interaction between the Surface Plasmon Resonance (SPR) of metallic  $\text{Ag}^+$  and SPR, which is caused by the collective oscillation of conduction electrons on the NP surface when activated by light at a particular wavelength. The peak that emerged is consistent with earlier studies that discovered AgNPs' absorption at 406 nm (Bhavi *et al.*, 2025).

*Murdannia pauciflora* AgNPs' size has been connected to their intensity in the UV-visible spectrum. The presence of secondary metabolites in plants serves as a reducing agent and converts  $\text{Ag}^+$  to  $\text{Ag}^0$ . Similar SPR peaks are observed in other studies (Mohamad *et al.*, 2021).

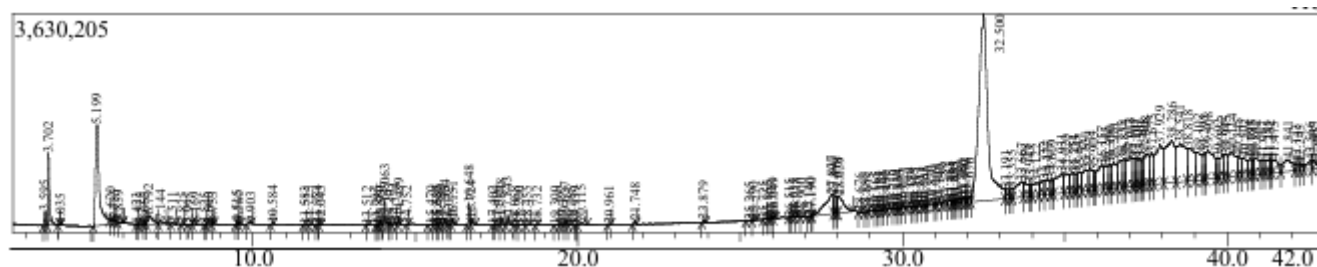
#### FTIR

FTIR spectrum of *M. pauciflora* AgNPs exhibited the characteristic peaks ranging from 4,000  $\text{cm}^{-1}$  to 600-1. The peaks 3,234.29, 3,138.63  $\text{cm}^{-1}$  attributed to O-H Stretching, the peaks 3,101.68, 3,005.20, 2,957.99, 2,713.71 attributed to C-H Stretching, 1,575.19 attributes to C=C Stretching, and the Peaks 2,251.84, 1,393.37, 1,011.64, 780.18, 665.65 are attributed to N = C = O Stretching, O-H. Bending, S = O Stretching, C=C Bending and C-Cl Stretching corresponding to alcohol, carboxylic acid, aromatic hydrocarbon. Alkene, alkene, alkyl halide, sulfoxide, aldehyde, isocyanate and cyclic alkane. These functional groups serve as a surface coating and promote in the efficient stabilization of nanoparticles and the plant's phytochemicals operate as a capping agent during synthesis (Akintelu *et al.*, 2020). These results are consistent with the discovery that AgNPs of *Commelina erecta* stretch vibrations of -OH groups in the peaks between 3,500 and 300  $\text{cm}^{-1}$ . These O-H groups are attributed to C-H stretching of aliphatic structures at 2,895 and 2,840  $\text{cm}^{-1}$ , xylan, cellulose, and hemicellulose, corresponding to vibrations of -CH and -CO at 1,400-900  $\text{cm}^{-1}$  (Table 3). *Commelina forskaolii* has disulfide amino and hydroxyl groups at peaks of 3,289.50, 1,617.18, 1377, 1,030.27, and 464.86 (Figure 6) (Vijay *et al.*, 2023).

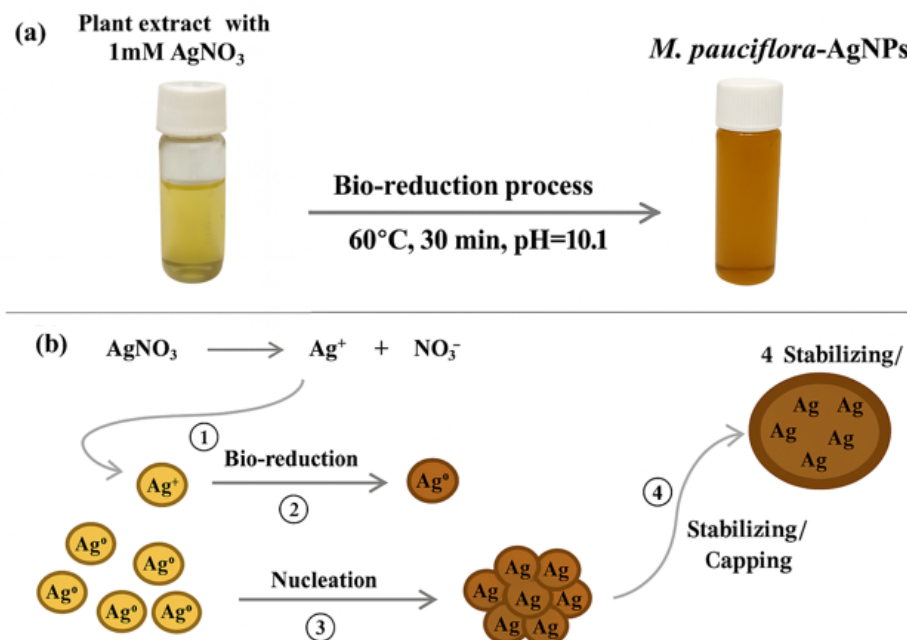
**Table 1: Preliminary Qualitative Phytochemical Analysis of *M. pauciflora* extracts.**

| Sl. No. | Phytochemical tests | MPM | MPA | MPC |
|---------|---------------------|-----|-----|-----|
| 1.      | phenols             | +   | +   | —   |
| 2.      | alkaloids           | +   | —   | —   |
| 3.      | flavonoids          | +   | +   | —   |
| 4.      | tannins             | +   | +   | —   |
| 5.      | saponins            | +   | +   | +   |
| 6.      | glycosides          | +   | +   | —   |
| 7.      | steroids            | —   | —   | —   |

Abbreviations: +=Present and —= Absent; A=Aqueous; C=Chloroform, MP = *M. pauciflora*; M=Methanol.



**Figure 3: Showing chromatogram of *M. pauciflora* aqueous extract.**



**Figure 4:** (A) Showing color shifting from pale yellow to brown color. (B) Mechanism of how plant phytochemical helps in synthesis of *M. pauciflora* AgNPs.

### XRD analysis

The XRD diffractogram displayed prominent peaks at  $2\theta = 28.28^\circ$ ,  $32.15^\circ$ ,  $38.16^\circ$ ,  $40.44^\circ$ ,  $44.16^\circ$ ,  $46.16^\circ$ ,  $50.15^\circ$ ,  $64.60^\circ$ ,  $77.46^\circ$ , and  $81.61^\circ$ , which correspond to the crystallographic planes (004), (101), (111), (112), (103), (104), (200), (220), (311) and (222), respectively (Figure 7). Among these, the characteristic reflections of metallic silver were clearly observed at  $38.16^\circ$  (111),  $44.16^\circ$  (200),  $64.60^\circ$  (220),  $77.46^\circ$  (311), and  $81.61^\circ$  (222). These values are in excellent matches with the standard diffraction data for face-centered cubic (FCC) silver reported in JCPDS File no. 00-004-0783 (Bhavi *et al.*, 2024). Using the Scherrer equation, the average crystallite size of the synthesized nanoparticles was estimated to be approximately 12.0 nm. Additionally, the diffraction peaks obtained at  $38.17^\circ$ ,  $44.36^\circ$ ,  $64.51^\circ$ ,  $77.43^\circ$ ,  $81.56^\circ$ , and  $98.06^\circ$  perfectly match the standard metallic Ag reflections of FCC Ag, further confirming the successful formation of phase-pure Silver Nanoparticles (AgNPs). The absence of additional peaks indicates that no secondary or impurity phases were present, supporting the high crystallinity and purity of the synthesized AgNPs.

The detailed XRD analysis in the present study offers meaningful insights into the crystallographic features and the potential applications of the synthesized nanoparticles. Similarly, our results are consistent with those documented for *Camellia sinensis* mediated silver nanoparticles by (Ali *et al.*, 2022; Mehta *et al.*, 2017).

### SEM analysis

The SEM investigation of *M. pauciflora* AgNPs reveals information on morphology and surface distribution. The synthesized AgNPs range in size from rod to rectangular, with aggregates containing clusters of nanoparticles. Examining the agglomerated masses revealed several nanoparticle aggregates. Nanoparticles could exist independently or in clusters. Some had a rod shape with an average size of 12.0 nm, as seen in (Figure 8). Some aggregation was observed, which is common in green synthesis methods because the bioactive molecules in the plant extract may not offer the same steric protection as synthetic capping agents (Johnson and Prabu, 2015; Oseghale *et al.*, 2025). found that *Commelina benghalensis* SEM investigation revealed the formation of reasonably spherical and homogenous Ag nanoparticles with diameters ranging from 13 to 51 nm.

### EDX analysis

EDX micro examination involves evaluating the energy and intensity distribution of X-ray signals generated by an electron beam on a specimen (Figure 9). Depicts the peaks inferred during EDX analysis. The spectra show that the synthesized AgNPs contain Ag, as well as additional elements like Manganese (Mn), Sodium (Na), Calcium, Potassium (K), Sulfur (S), and Silicon (Si). The other elements act as capping organic agents on the surface of the silver nanoparticles (Bhav *et al.*, 2024). The present study's EDX examination confirmed the presence of silver nanoparticles

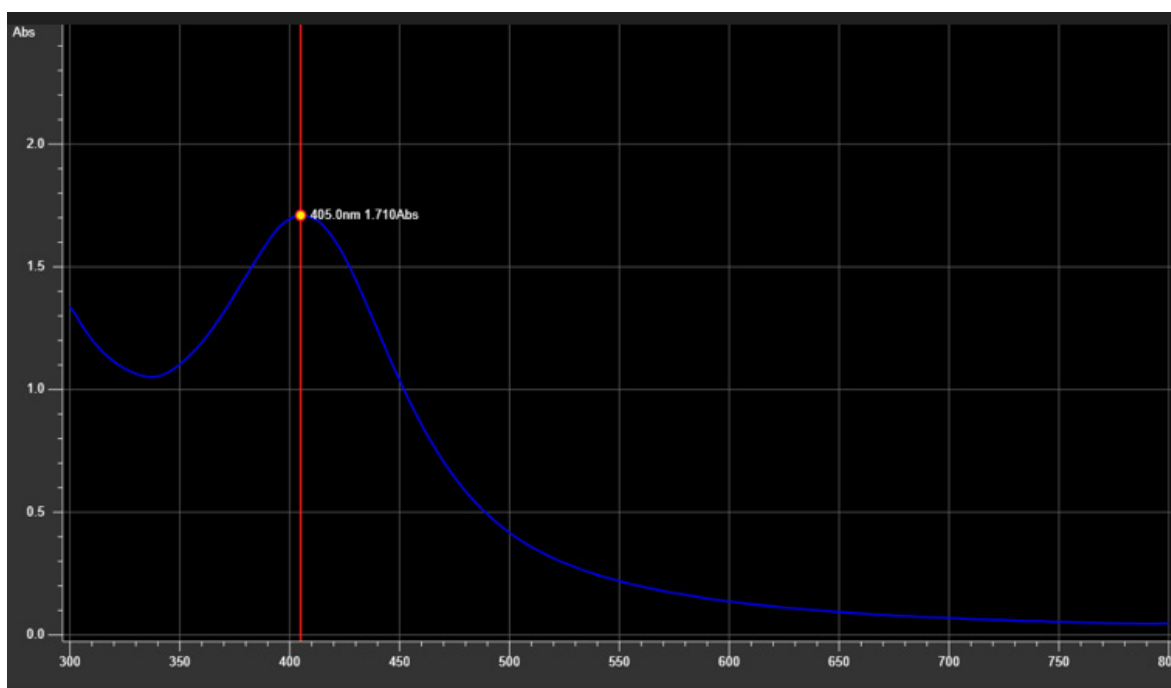


Figure 5: UV-vis Analysis of *M. pauciflora* AgNPs.

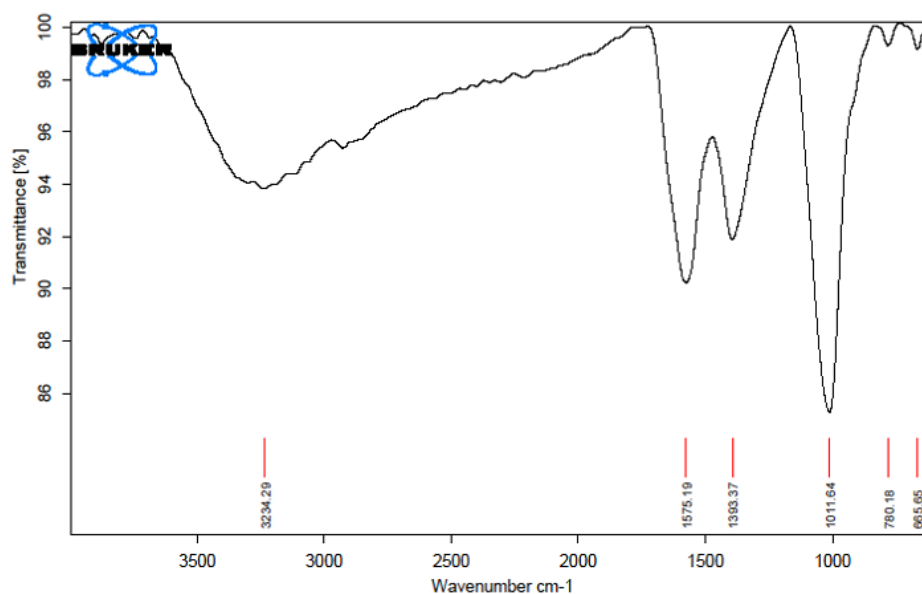


Figure 6: Fourier-Transform Infrared (FTIR) spectrum of *M. pauciflora* AgNPs.

from *M. pauciflora* and revealed high signal energy peaks for silver atoms.

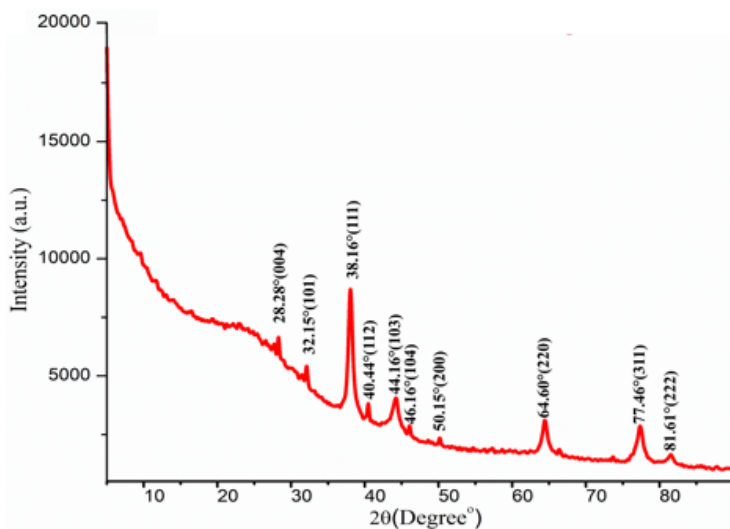
### Zeta potential and DLS analysis

The physicochemical characterization of the synthesized nanoparticles was evaluated through zeta potential and particle size distribution analyses, which are critical indicators of colloidal stability and uniformity. The zeta potential graph (Figure 10A) shows a sharp, symmetrical Gaussian-shaped peak centered near the negative potential region, indicating a highly charged particle surface. The relatively narrow and smooth peak suggests that the nanoparticles possess a consistent surface charge,

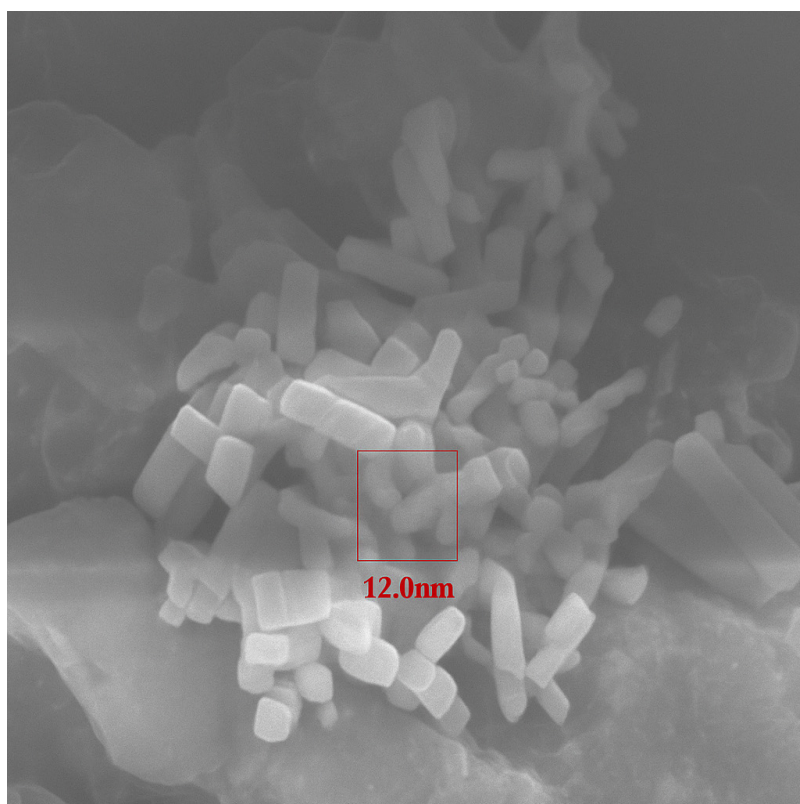
which supports the formation of a stable colloidal system. Zeta potential plays a crucial role in assessing the colloidal stability of nanoparticles and understanding their suitability for various applications (Vanlalveni *et al.*, 2021). A higher magnitude of zeta potential—typically more negative than  $-30$  mV or more positive than  $+30$  mV—is associated with strong electrostatic repulsion among particles, thereby minimizing aggregation. The peak pattern observed here confirms that the synthesized nanoparticles exhibit good dispersion stability, which is essential for their biological and catalytic applications. In the present study, the synthesized nanoparticles exhibited a distinctly negative zeta potential, which can be attributed to the presence of negatively

charged capping agents on their surface. These biomolecules provide electrostatic repulsion between particles, preventing agglomeration and thereby maintaining long-term colloidal stability (Khandel *et al.*, 2018). The particle size distribution profile (Figure 10B) further complements these findings by revealing a uniform and narrow size range, as evidenced by the compact histogram and the steep cumulative distribution curve. Most particles fall within a controlled nanoscale region,

demonstrating that the synthesis process successfully limited polydispersity. A narrow distribution is a hallmark of effective nucleation and growth mechanisms during nanoparticle formation. Such uniformity ensures reproducibility in functional properties, including surface reactivity, drug-loading behavior, and antimicrobial efficiency. Together, the zeta potential and DLS results confirm that the nanoparticles synthesized in this study are stable, uniformly dispersed, and structurally consistent. These



**Figure 7:** X-ray Diffraction (XRD) Analysis of *M. pauciflora* AgNPs.



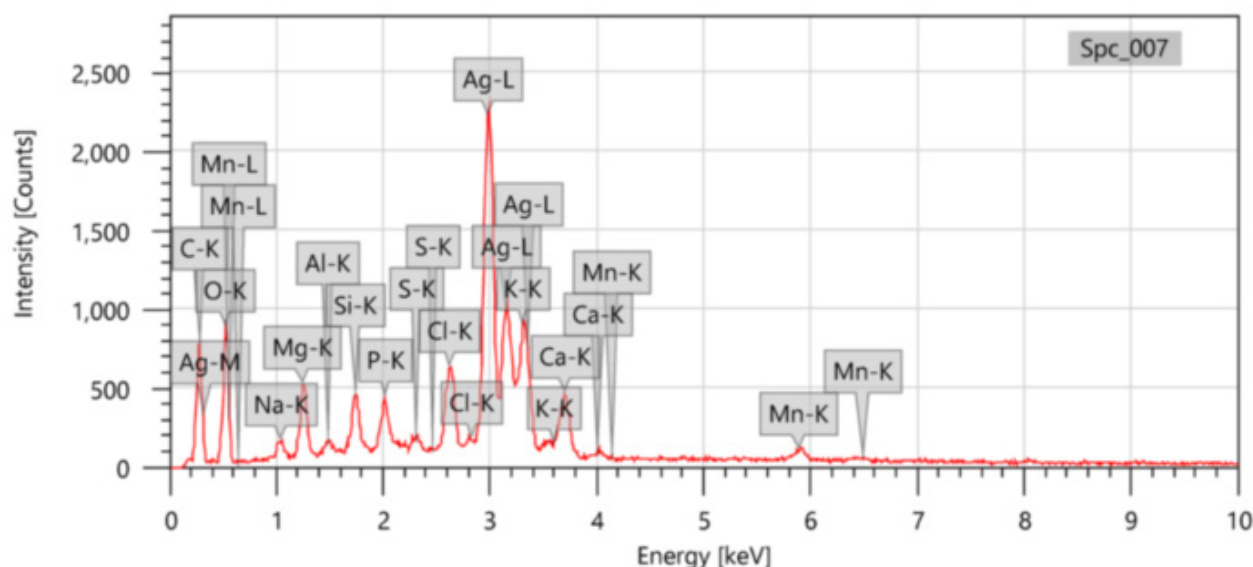
**Figure 8:** Scanning Electron Microscopy (SEM) image of *M. pauciflora* AgNPs.

**Table 2: GC-MS Analysis of Bioactive compounds from *M. pauciflora*.**

| Peak# | R. Time | Area       | Area% | Similarity | Base m/z | Compound name                                    |
|-------|---------|------------|-------|------------|----------|--------------------------------------------------|
| 1     | 3.595   | 828,568    | 0.28  | 96         | 45.00    | Butadienol <2,3->                                |
| 2     | 4.035   | 2106       | 0.00  | 42         | 43.95    | geranyl isovalerate                              |
| 3     | 4.035   | 14,762,377 | 4.95  | 98         | 43.95    | lactic acid                                      |
| 4     | 5.620   | 85,565     | 0.03  | 95         | 42.00    | butyrolactone <gamma->                           |
| 5     | 5.735   | 15,240     | 0.01  | 76         | 108.05   | pyrazine <2,6-dimethyl->                         |
| 6     | 5.859   | 53,930     | 0.02  | 81         | 98.00    | pimelic ketone                                   |
| 7     | 6.423   | 9,885      | 0.00  | 62         | 43.00    | isobutyrate <heptyl->                            |
| 8     | 6.525   | 37,122     | 0.01  | 64         | 45.00    | ethanoic acid                                    |
| 9     | 6.665   | 60,428     | 0.02  | 66         | 117.00   | malate <diethyl->                                |
| 10    | 6.792   | 1,592,782  | 0.53  | 84         | 43.00    | Butan-2-one <3-mercapto->                        |
| 11    | 7.144   | 377,836    | 0.13  | 85         | 70.00    | cyclohexane <1,2-dioxo->                         |
| 12    | 7.511   | 66,331     | 0.02  | 75         | 68.00    | Cyclohexen-1-one, <2->                           |
| 13    | 7.825   | 23,163     | 0.01  | 79         | 71.05    | tetrahydrofurfuryl alcohol                       |
| 14    | 7.995   | 21,410     | 0.01  | 53         | 115.05   | hexanal <dimethyl-> acetal                       |
| 15    | 8.169   | 42,994     | 0.01  | 70         | 43.00    | furanone <4-acetoxy-, 2,5-dimethyl-, 3(2H)->     |
| 16    | 8.525   | 11,881     | 0.00  | 57         | 43.95    | butyrolin                                        |
| 17    | 8.600   | 54,170     | 0.02  | 56         | 85.00    | ipsdienol                                        |
| 18    | 8.753   | 30,459     | 0.01  | 83         | 57.05    | undecane <n->                                    |
| 19    | 9.515   | 114,434    | 0.04  | 74         | 43.00    | acetate <2,4-dimethyl-, ethyl-, 1,3-dioxolane>   |
| 20    | 9.903   | 76,453     | 0.03  | 61         | 105.05   | Benzenecarboxylic acid                           |
| 21    | 10.584  | 26,207     | 0.01  | 69         | 86.00    | triethylamine                                    |
| 22    | 11.583  | 22,451     | 0.01  | 30         | 281.00   | syringaldazine                                   |
| 23    | 11.725  | 23,819     | 0.01  | 43         | 73.05    | Acsantol isomer I                                |
| 24    | 11.984  | 74,675     | 0.03  | 89         | 150.05   | guaiacol <4-vinyl->                              |
| 25    | 12.045  | 28,248     | 0.01  | 65         | 43.00    | cineole <1.4->                                   |
| 26    | 13.512  | 19,202     | 0.01  | 84         | 41.00    | caryophyllene <(E)->                             |
| 27    | 13.795  | 10,318     | 0.00  | 49         | 45.05    | Decembar 9-enoic acid                            |
| 28    | 13.850  | 28,716     | 0.01  | 44         | 69.05    | cinnamaldehyde <alpha-hexyl->                    |
| 29    | 13.936  | 170,365    | 0.06  | 36         | 73.00    | pentanol, <3-Methyl-3->                          |
| 30    | 14.063  | 1,031,212  | 0.35  | 97         | 55.00    | tridecanol <n->                                  |
| 31    | 14.203  | 145,803    | 0.05  | 88         | 132.05   | curcumene <alpha->                               |
| 32    | 14.374  | 143,996    | 0.05  | 91         | 119.10   | Funebrene <alpha->                               |
| 33    | 14.489  | 293,214    | 0.10  | 71         | 191.10   | phenylacetate <methyl-, para-tert-butyl->        |
| 34    | 14.752  | 20,026     | 0.01  | 84         | 69.05    | Sesquisabinene                                   |
| 35    | 15.470  | 73,232     | 0.02  | 53         | 148.95   | phthalic acid, bis(2-ethylhexyl) ester (6CI,8CI) |
| 36    | 15.635  | 66,877     | 0.02  | 69         | 151.05   | dihydrojasmone                                   |
| 37    | 15.680  | 36,423     | 0.01  | 62         | 57.00    | valerate <ethyl->                                |
| 38    | 15.780  | 95,535     | 0.03  | 72         | 68.95    | callicarpenal                                    |
| 39    | 16.032  | 26,081     | 0.01  | 64         | 71.05    | linalyl anthranilate                             |
| 40    | 16.151  | 234,578    | 0.08  | 79         | 108.05   | isoborneol <8-isobutyryloxy->                    |
| 41    | 16.648  | 906,597    | 0.30  | 86         | 55.00    | Tetradec-1-ene                                   |
| 42    | 16.724  | 284,948    | 0.10  | 93         | 57.00    | propanoate <decyl->                              |

|    |        |            |       |    |        |                                             |
|----|--------|------------|-------|----|--------|---------------------------------------------|
| 43 | 17.402 | 15,383     | 0.01  | 59 | 43.95  | decanal <n->                                |
| 44 | 17.490 | 27,956     | 0.01  | 51 | 39.95  | vinylcyclohexane <1-acetoxy-, 2 s-butyl->   |
| 45 | 17.843 | 524,709    | 0.18  | 74 | 124.05 | pyrazine <2-isobutyl-, 3-methoxy->          |
| 46 | 18.068 | 11,400     | 0.00  | 59 | 45.00  | Tridec-(2E)-en-1-ol                         |
| 47 | 18.200 | 58,467     | 0.02  | 58 | 70.05  | isophorone <2-hydroxy->                     |
| 48 | 18.455 | 51,173     | 0.02  | 68 | 43.00  | incensole                                   |
| 49 | 18.732 | 16,466     | 0.01  | 59 | 149.05 | Rosifoliol                                  |
| 50 | 19.300 | 30,306     | 0.01  | 49 | 39.95  | cyclohexanemethanol <2,4-dimethyl-> acetate |
| 52 | 19.939 | 28,198     | 0.01  | 58 | 70.05  | tiglate <isoamyl->                          |
| 53 | 20.115 | 185,576    | 0.06  | 90 | 73.00  | hexadecanoic acid <n->                      |
| 54 | 20.961 | 33,092     | 0.01  | 70 | 57.05  | butyrate <decyl->                           |
| 56 | 25.725 | 7,593      | 0.00  | 36 | 39.95  | pyrazine <2-methoxy->                       |
| 57 | 25.855 | 2159       | 0.00  | 50 | 39.95  | menthol                                     |
| 58 | 26.049 | 107,712    | 0.04  | 34 | 339.15 | Purolan                                     |
| 59 | 26.515 | 6,086      | 0.00  | 52 | 57.05  | octadecyl chloride                          |
| 60 | 26.635 | 38,449     | 0.01  | 50 | 81.05  | Tetrahydroionol isomer II                   |
| 61 | 26.900 | 14,566     | 0.00  | 49 | 125.15 | valeranone                                  |
| 62 | 27.140 | 40,118     | 0.01  | 50 | 99.10  | pulegol <trans->                            |
| 64 | 27.827 | 5,842,843  | 1.96  | 41 | 57.05  | dihydrotagetone                             |
| 65 | 27.890 | 1,327,616  | 0.45  | 40 | 57.05  | Ambercore isomer I                          |
| 66 | 28.039 | 3,397,594  | 1.14  | 39 | 73.00  | decamethylcyclopentasiloxane                |
| 67 | 28.676 | 24,541     | 0.01  | 49 | 81.05  | ethyl menthone <2->                         |
| 68 | 28.975 | 91,572     | 0.03  | 53 | 43.95  | n-Tetradecanonitrile                        |
| 69 | 29.165 | 61,444     | 0.02  | 58 | 57.05  | citronellyl propionate                      |
| 70 | 29.285 | 66,919     | 0.02  | 50 | 81.05  | Palustrol                                   |
| 71 | 29.378 | 84,215     | 0.03  | 49 | 95.10  | menthyl isovalerianate                      |
| 72 | 29.490 | 60,826     | 0.02  | 38 | 96.05  | 2-tert-butyl-4-methyl cyclohexanol isomer I |
| 73 | 29.575 | 129,964    | 0.04  | 57 | 83.05  | octadecanol <n->                            |
| 74 | 29.710 | 90,730     | 0.03  | 54 | 97.05  | Pseudodiosphenol                            |
| 75 | 29.895 | 33,996     | 0.01  | 46 | 96.05  | Eremophilone <8-hydroxy-dihydro->           |
| 76 | 30.190 | 143,528    | 0.05  | 62 | 97.05  | eicosene                                    |
| 77 | 30.315 | 74,374     | 0.02  | 57 | 57.05  | tridecanal <n->                             |
| 78 | 30.511 | 166,877    | 0.06  | 67 | 57.05  | Octadec-1-ene                               |
| 79 | 30.706 | 55,014     | 0.02  | 48 | 67.00  | Oct-2-ene <8-ethoxy-, 2,6-dimethyl->        |
| 80 | 30.870 | 394,533    | 0.13  | 56 | 69.05  | phytol acetate                              |
| 82 | 31.555 | 206,879    | 0.07  | 49 | 69.05  | Applelide                                   |
| 83 | 31.605 | 198,737    | 0.07  | 55 | 69.00  | Timberol, cis-                              |
| 84 | 31.655 | 352,067    | 0.12  | 54 | 95.10  | Tetrahydroionol isomer II                   |
| 85 | 31.902 | 555,900    | 0.19  | 45 | 354.95 | heptadecanol <n->                           |
| 86 | 31.970 | 248,541    | 0.08  | 50 | 57.05  | octadecyl chloride                          |
| 87 | 32.070 | 520,972    | 0.17  | 47 | 39.95  | sclareol                                    |
| 88 | 32.500 | 60,141,722 | 20.16 | 56 | 178.00 | bisabolol oxide b <alpha->                  |
| 89 | 33.191 | 1,181,379  | 0.40  | 61 | 69.00  | Hex-2-enal <2-isopropyl-, 5-methyl->        |
| 90 | 33.355 | 908,647    | 0.30  | 55 | 111.05 | ethyl menthone <2->                         |

|    |        |           |      |    |        |                                |
|----|--------|-----------|------|----|--------|--------------------------------|
| 91 | 33.667 | 4,192,656 | 1.41 | 67 | 57.05  | tricos-(9Z)-ene                |
| 92 | 33.943 | 931,845   | 0.31 | 64 | 57.05  | Hexadec-(8Z)-enal <14-methyl-> |
| 93 | 35.325 | 1,418,120 | 0.48 | 35 | 112.10 | Labd-(13E)-8,15-diol           |
| 94 | 36.500 | 4,044,496 | 1.36 | 65 | 111.10 | Tetrahydroionol isomer I       |
| 95 | 36.735 | 4,425,729 | 1.48 | 66 | 71.05  | phytol                         |
| 96 | 40.114 | 6,394,222 | 2.14 | 69 | 57.05  | tetracontane <n->              |
| 97 | 42.566 | 1,813,978 | 0.61 | 45 | 85.05  | Ambercore isomer III           |
| 98 | 42.885 | 218,262   | 0.07 | 54 | 83.05  | Tetradec-2-enal <trans->       |



**Figure 9:** Energy Dispersive X-ray (EDX) image of *M. pauciflora* AgNPs.

features enhance their potential applicability in biomedical, environmental, and nanotechnological fields, where stability and monodispersity are crucial for predictable performance. DLS analysis further supported these observations. The measurement of hydrodynamic diameter and Polydispersity Index is widely recognized as a reliable method for characterizing plant-mediated silver nanoparticles.

### TEM analysis

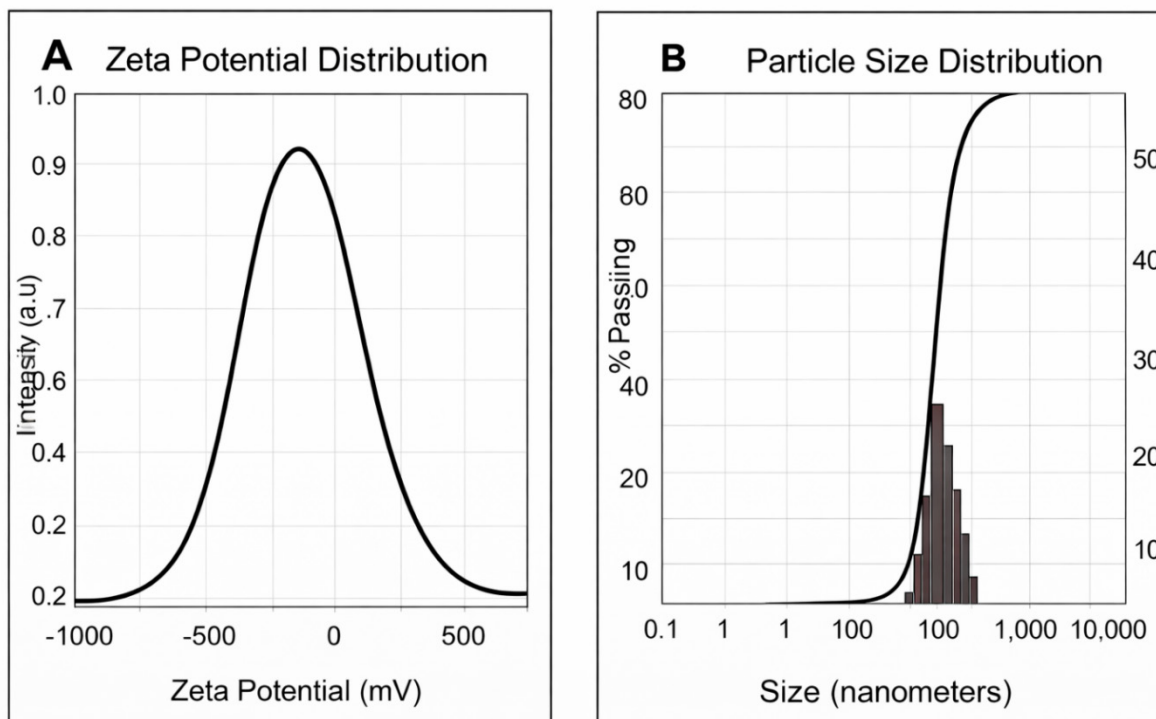
TEM was employed to examine the morphology, size, and dispersion of the synthesized Ag nanoparticles. TEM is widely regarded as a powerful tool for confirming nanoparticle dimensions and shapes with high accuracy (Dudkiewicz *et al.*, 2011). In this study, TEM analysis showed that the *M. pauciflora* derived AgNPs were predominantly rectangular. Figures 11A-11C present both low- and high-magnification views of the nanoparticles. The high-magnification image clearly illustrates well-defined rectangular AgNPs with a uniform size distribution. As shown in Figure 11B, the particles measured approximately 12 nm, aligning closely with the size estimated from the XRD analysis. The consistency in particle size and shape is particularly important; as such, uniformity can significantly enhance the nanoparticles' electrical and optical characteristics. Similar findings are found in other studies. *Murdannia loriformis*

synthesized AgNPs with a particle of size of  $12.60 \pm 2.83$  nm reported by Isa *et al.* (2023). This, in turn, makes them highly suitable for applications in biomedical, environmental, and nanotechnology fields.

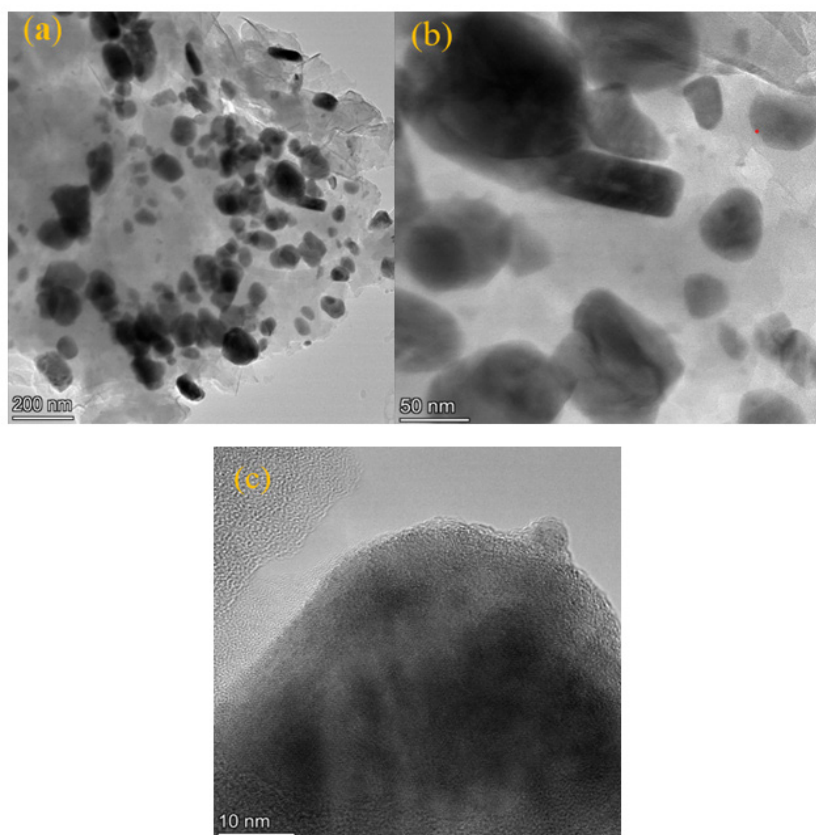
### Biological activities

#### Evaluation of cytotoxicity

The cytotoxic activity of *M. pauciflora*-derived AgNPs was evaluated against PANC-1 and HepG2 cancer cell lines at concentrations ranging from 100 to 500  $\mu\text{g/mL}$ . The nanoparticles showed a mild, concentration-dependent inhibition toward the PANC-1 cell line, producing 20, 19, 17, 15, and 13% inhibition at the respective concentrations. From this trend, the  $\text{IC}_{50}$  value was calculated to be approximately 500  $\mu\text{g/mL}$ , indicating relatively low cytotoxic potency. In contrast, no measurable cytotoxic effect was observed against the HepG2 liver cancer cell line at any of the tested concentrations, suggesting that the biosynthesized AgNPs from *M. pauciflora* may exhibit cell-line-specific activity, with a weak response limited to PANC-1 cells the cisplatin used as a standard drug. Previous studies reported that silver nanoparticles synthesized from *C. nudiflora* exhibited an  $\text{IC}_{50}$  value of 100  $\mu\text{g/mL}$  against the HCT-116 colon cancer cell line (Kuppusamy *et al.*, 2016). Additionally, various extracts of *C. benghalensis* including



**Figure 10:** Dynamic Light Scattering (DLS) graph of *M. pauciflora* AgNPs.



**Figure 11:** High Resolution Transmission Electron Microscopy (HR-TEM) of *M. pauciflora* AgNPs  
(A) Nanoparticles with a scale bar of 200 nm. (B) Close-up view of nanoparticles with a scale bar of 50 nm. (C) Detailed view showing a scale bar of 10 nm.

chloroform, methanol, n-hexane, benzene, and ethanol were tested against Hep-2 and MDA-MB-231 cancer cell lines. Among these, the chloroform extract showed the highest cytotoxicity against the breast cancer cell line MDA-MB-231 (Figures 12-18) (Batoool *et al.*, 2020).

## Antidiabetic activity

### $\alpha$ -glucosidase inhibition

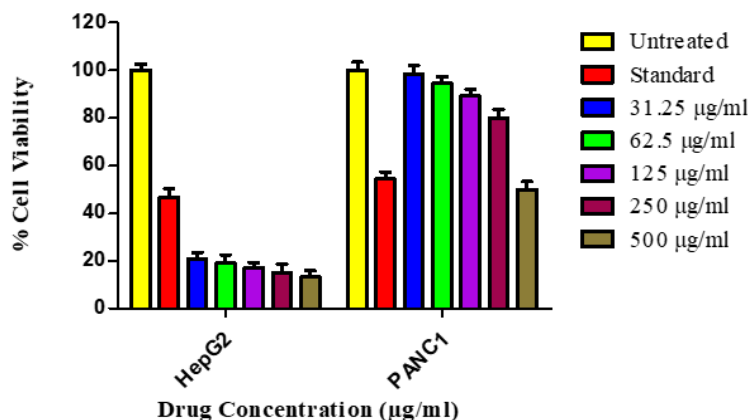
The  $\alpha$ -glucosidase assay showed that *M. pauciflora* AgNPs inhibit the enzyme in a clear concentration-dependent manner, with activity increasing from 8 to 66% between 20 and 100  $\mu$ L. The  $IC_{50}$  value of 78.44  $\mu$ g/mL indicates moderate inhibitory potential. In comparison, acarbose displayed stronger inhibition across all concentrations and with an  $IC_{50}$  of 30.57  $\mu$ g/mL, confirming its higher potency. Despite this difference, the results suggest that biosynthesized AgNPs can interact with and suppress  $\alpha$ -glucosidase activity, likely due to the combined effects of silver

nanoparticles and the bioactive constituents of *M. pauciflora*. This indicates potential antidiabetic relevance. *Murdannia bracteata* showed a clear dose-dependent  $\alpha$ -glucosidase inhibition, with an  $IC_{50}$  value of  $117.04 \pm 2.34$   $\mu$ g/mL (Ooi *et al.*, 2015). Similarly, the whole plant of *C. benghalensis* demonstrated notable antidiabetic effects in alloxan-induced diabetic rats ("Antidiabetic activity and phytochemical investigation on the whole plant of *Commelina benghalensis* Linn. in male albino rat", 2016).

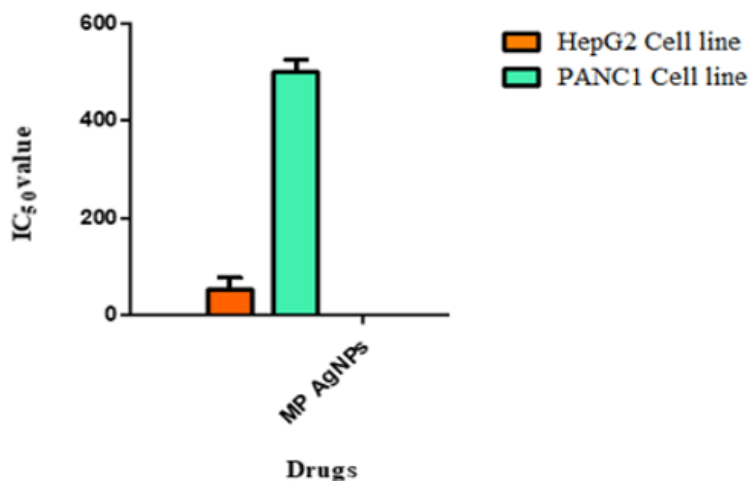
## Antioxidant activity

### DPPH assay

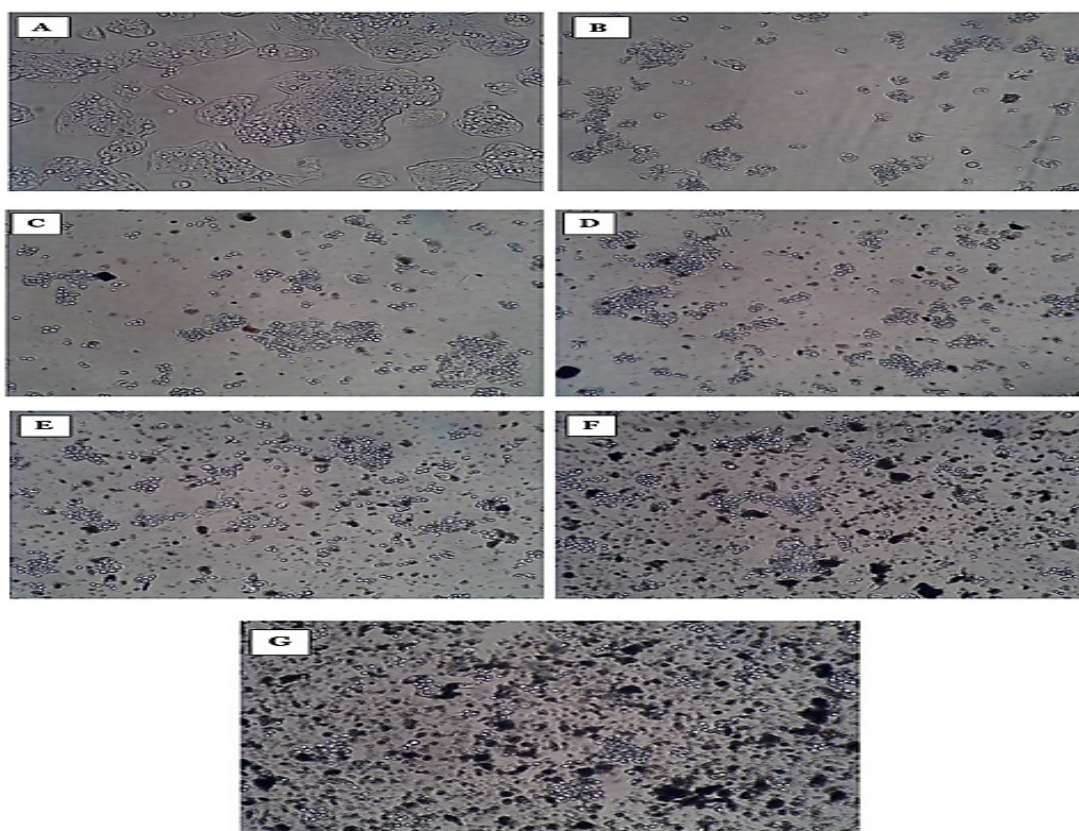
The free radical scavenging activity plays a vital role in protecting cells from oxidative damage. In this study, the antioxidant potential of biosynthesized *M. pauciflora* AgNPs was assessed using the DPPH assay. The nanoparticles showed a gradual, dose-dependent increase in scavenging activity, with inhibition values of 6, 20, 35, 47, and 61% at concentrations of 20, 40, 60, 80, 100, and  $IC_{50}$  value



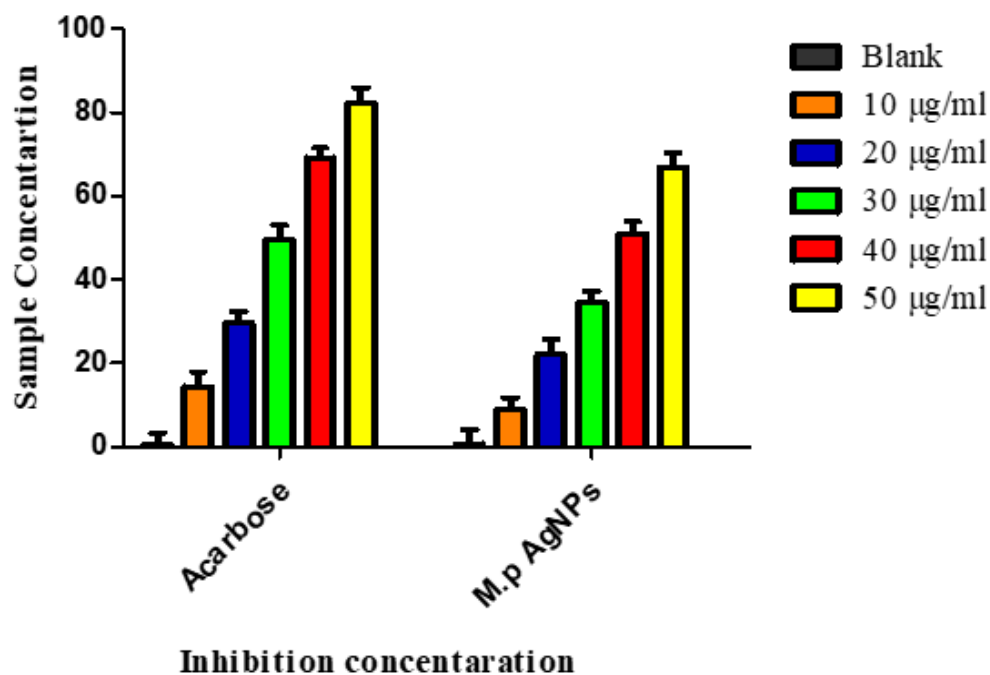
**Figure 12:** Cytotoxic assay showing different concentrations of *M. pauciflora* AgNPs against HepG2 and PANC1 cell line.



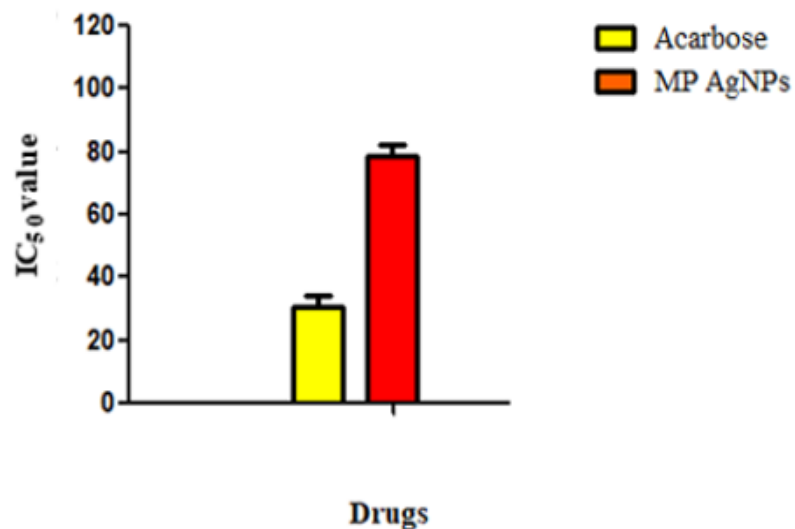
**Figure 13:** Showing cytotoxic assay of  $IC_{50}$  value of *M. pauciflora* against HepG2 and PANC1 cell line.



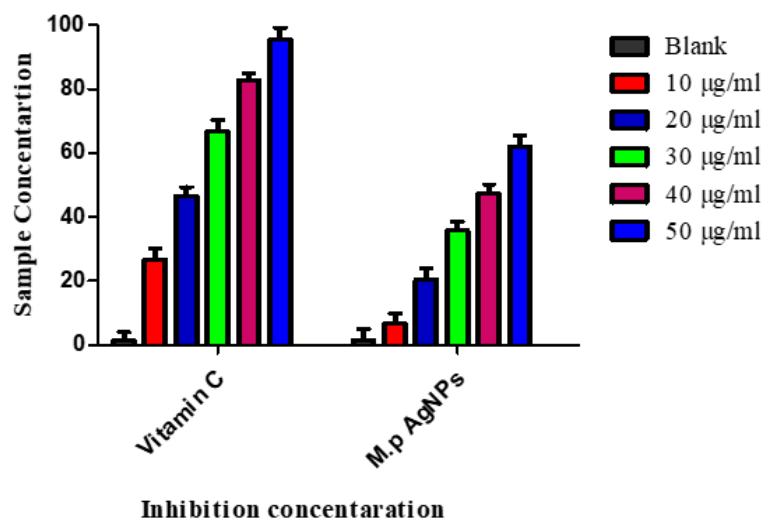
**Figure 14:** Microscopic images showing the cytotoxic effects (% cell viability) at different concentration of *M. pauciflora* AgNPs (A) Untreated (B) Standard (C) 31.25 µg/mL (D) 62.5 µg/mL (E) 125 µg/mL (F) 250 µg/mL (G) 500 µg/mL.



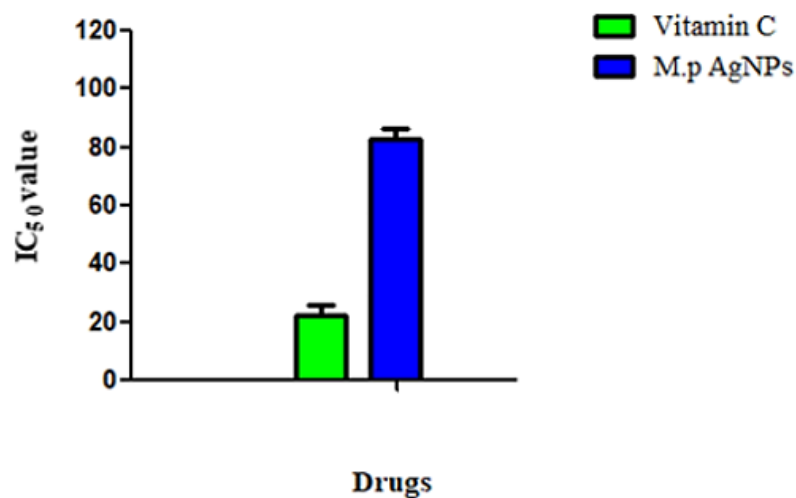
**Figure 15:** Antidiabetic activity showing different Concentrations of *M. pauciflora* AgNPs against SD Acarbose.



**Figure 16:** Showing  $\alpha$ -glucosidase activity of IC<sub>50</sub> value of *M. pauciflora* against SD. Acarbose.



**Figure 17:** Antioxidant activity showing different Concentrations of *M. pauciflora* AgNPs against SD. Vitamin C.



**Figure 18:** Antioxidant activity of IC<sub>50</sub> value of *M. pauciflora* AgNPs against SD. Acarbose.

**Table 3: Fourier-Transform Infrared (FTIR) Peak Values interpretation functional groups of *M. pauciflora* AgNPs.**

| Absorption (cm <sup>-1</sup> ) | Intensities of functional group | Functional Group     | Compound class       |
|--------------------------------|---------------------------------|----------------------|----------------------|
| 3,234.29                       | Strong, Sharp                   | O-H stretching       | alcohol              |
| 3,138.63                       | Very Strong, Very Broad         | O-H stretching       | carboxylic acid      |
| 3,101.68                       | Medium to Weak                  | C-H stretching       | aromatic hydrocarbon |
| 3,005.20                       | Medium to weak                  | C-H stretching       | aromatic hydrocarbon |
| 2,957.99                       | Medium                          | C-H stretching       | alkane               |
| 2,713.71                       | Medium                          | C-H stretching       | aldehyde             |
| 2,251.84                       | Weak to Medium Sharp            | N = C = O stretching | isocyanate           |
| 1,575.19                       | Medium                          | C = C stretching     | cyclic alkane        |
| 1,393.37                       | Medium                          | O.-H. Bending        | carboxylic acid      |
| 1,011.64                       | Strong, Sharp                   | S = O stretching     | sulfoxide            |
| 780.18                         | Strong, Sharp                   | C-Cl stretching      | alkyl halide         |
| 665.65                         | Strong                          | C = C. Bending       | alkene               |

Abbreviations: C C. Bending.

of 82.65288 µg/mL, respectively. This increasing trend suggests that the AgNPs efficiently donate electrons to neutralize DPPH free radicals. Interestingly, their activity surpassed that of ascorbic acid, the positive control, recorded at IC<sub>50</sub> value of 22.15 µg/mL (Graph 5). The strong antioxidant response may be attributed to phenolic and flavonoid compounds present in the plant extract, which likely act as capping agents on the nanoparticle surface. These phytochemicals facilitate the conversion of DPPH from purple to yellow, indicating effective radical quenching. Together, these findings highlight the potential of *M. pauciflora* AgNPs as promising natural antioxidants (Naaz *et al.*, 2024). Reported that AgNPs synthesized from *Tradescantia pallida* showed a free radical scavenging activity with an IC<sub>50</sub> value of 91.87 µg/mL. Similarly, AgNPs from *C. erecta* demonstrated strong antioxidant potential, with IC<sub>50</sub> values of 69.92±1.01 µg/mL and 108.21 ±3.34 µg/mL (Mallick and Pradhan, 2024).

## CONCLUSION

The present study successfully demonstrates, for the first time, the green synthesis of Silver Nanoparticles (AgNPs) using the aqueous extract of the ethnomedicinal plant *M. pauciflora*. The eco-friendly synthesis approach highlights the role of plant-derived phytochemicals as effective reducing and stabilizing agents, offering a sustainable alternative to conventional chemical methods. Phytochemical screening confirmed the presence of biologically active constituents such as phenols, flavonoids, alkaloids, tannins, glycosides, and saponins, while GC-MS analysis revealed a rich profile of 98 bioactive compounds that likely contributed to nanoparticle formation and biological activity. Comprehensive characterization using UV-visible spectroscopy, FTIR, XRD, SEM, EDX, DLS, zeta potential, and TEM confirmed the successful formation of stable and uniformly distributed AgNPs. The nanoparticles exhibited a face-centered cubic crystalline structure with a rectangular morphology, an average particle size of approximately 12 nm, and a surface

charge of -30 mV, indicating good colloidal stability and reduced aggregation. These physicochemical properties are crucial for enhancing biological interactions and potential biomedical performance. Biological evaluations revealed that *M. pauciflora* AgNPs possess notable antioxidant and antidiabetic activities, as evidenced by their free radical scavenging and α-glucosidase inhibitory effects. The observed α-glucosidase inhibition, with a lower IC<sub>50</sub> value compared to the standard, suggests their potential usefulness in managing postprandial hyperglycemia. Cytotoxicity studies against a pancreatic cancer cell line further demonstrated moderate anticancer potential, indicating that these nanoparticles may serve as promising candidates for future cancer-related applications. Overall, the findings of this study highlight the biomedical relevance of *M. pauciflora* mediated AgNPs and support their potential applications in antioxidant therapy, antidiabetic formulations, and targeted cancer treatment. Further *in vivo* studies and mechanistic investigations are warranted to validate their safety, efficacy, and clinical applicability.

## ACKNOWLEDGEMENT

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## ABBREVIATIONS

**AgNPs:** Silver Nanoparticles; **BHT:** Butylated Hydroxytoluene; **BSS:** Balanced Salt Solution; **DLS:** Dynamic Light Scattering; **DMEM:** Dulbecco's Modified Eagle Medium; **DMSO:** Dimethyl Sulfoxide; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EDX:** Energy Dispersive X-ray; **FCC:** Face-Centered Cubic; **FTIR:** Fourier-Transform Infrared; **GC-MS:** Gas Chromatography-Mass Spectrometry; **HEPES:** 4-(2-hydroxyethyl)-1-p

iperazineethanesulfonic acid; IC<sub>50</sub>: Half Maximal Inhibitory Concentration; JCPDS: Joint Committee on Powder Diffraction Standards; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PDI: Polydispersity Index; SEM: Scanning Electron Microscopy; SPR: Surface Plasmon Resonance; TEM: Transmission Electron Microscopy; XRD: X-ray Diffraction; ZnO: Zinc Oxide.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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