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Multifunctional Green Synthesized Silver Nanoparticles from *Typha angustifolia*: Antimicrobial, Antiparasitic, and Enzyme Inhibitory Effects

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Abstract

Nanotechnology is a rapidly evolving field of science with broad applications in medicine, agriculture, environmental remediation, and biotechnology. Among various nanomaterials, silver nanoparticles (AgNPs) have gained considerable attention owing to their remarkable antimicrobial efficacy and unique physicochemical properties. In the present study, phytochemical screening of *Typha angustifolia* leaf extract was conducted and utilized for the green synthesis of AgNPs. The biosynthesized nanoparticles were characterized using UV–Visible spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared (FTIR) spectroscopy. Furthermore, the synthesized AgNPs were evaluated for their antimicrobial and pharmacological activities. HPLC analysis of *T. angustifolia* extract revealed the presence of diverse phenolic constituents, with

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ferulic acid detected at the lowest concentration (10.3 ppm) and chlorogenic acid recorded as the most abundant compound (317.9 ppm). The synthesized AgNPs demonstrated significant antifungal activity against *Aspergillus niger*, exhibiting a maximum growth inhibition of 64.4%. Strong antibacterial activity was also observed against *Escherichia coli*, producing an inhibition zone of 14.3 mm at a concentration of 10 mg/mL. In antiparasitic assays, the nanoparticles showed substantial inhibitory effects, achieving 75.41 ± 1.16% inhibition against promastigotes and 71.13 ± 0.12% inhibition against amastigotes at 200 µg/mL. Additionally, considerable inhibition of α-glucosidase and α-amylase enzymes was observed, indicating promising antidiabetic potential. Hemolytic evaluation confirmed that the AgNPs were biocompatible and exhibited negligible toxicity toward healthy human red blood cells (RBCs). These findings suggest that *Typha angustifolia*-mediated silver nanoparticles possess significant potential for antimicrobial, antiparasitic, and antidiabetic applications, highlighting their value as eco-friendly nanomaterials for future biomedical and pharmaceutical use.

INTRODUCTION

Nanotechnology is a rapidly advancing scientific discipline that focuses on the design, synthesis, and application of materials with dimensions typically below 100 nm (Sikeyi et al., 2023). By integrating principles of chemistry and physics, nanotechnology enables the fabrication of nanoscale materials with distinctive physicochemical properties. Owing to their reduced size, unique morphology, and high surface-area-to-volume ratio, nanoparticles exhibit enhanced optical, electrical, magnetic, and catalytic characteristics compared with their bulk counterparts (Altammar et al., 2023). These remarkable properties have led to their widespread application in diverse fields, particularly in biomedicine, where nanoparticles are extensively employed for antimicrobial therapies and targeted drug delivery systems.

Nanoparticles (NPs) possess unique features such as controlled size distribution, morphology, and surface characteristics, making them highly attractive for numerous technological and biomedical applications (Yusuf et al., 2023). Among various metallic

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nanoparticles, silver nanoparticles (AgNPs) have attracted considerable attention because of their potent antimicrobial activity, relatively low toxicity, and broad applicability in both laboratory and industrial settings (Ye et al., 2022). AgNPs are widely utilized in wound healing, pharmaceutical formulations, cosmetic products, and environmental remediation processes, including the photocatalytic removal of pollutants and heavy metals (Khan et al., 2023). Various approaches have been developed for the synthesis of AgNPs, including physical, chemical, photochemical, irradiation-assisted, and biological methods (Bhakya et al., 2016). However, conventional physicochemical techniques often require hazardous chemicals, elevated temperatures and pressures, and may generate toxic by-products (Falke et al., 2024). In contrast, green synthesis methods offer an environmentally friendly alternative by employing natural reducing and stabilizing agents derived from biological sources such as plants, microorganisms, polysaccharides, and polyoxometalates.

Plant-mediated synthesis of AgNPs has emerged as an attractive and sustainable approach because phytochemicals present in plant extracts can simultaneously act as reducing and capping agents during nanoparticle formation (Xia et al., 2020). Currently, extensive research is focused on the biosynthesis of nanoparticles using metals such as silver, gold, platinum, zinc, and iron (Altaf et al., 2022). Among these, AgNPs are particularly favored because of their biocompatibility, stability, and diverse biological activities (Sarkar & Paul, 2017). Medicinal plants are rich sources of bioactive compounds and have long been utilized in traditional medicine for the treatment of various diseases (Ahmed et al., 2016). Consequently, the use of plant extracts for nanoparticle synthesis has gained significant attention as a cost-effective, eco-friendly, and sustainable alternative to conventional chemical methods (Rana et al., 2021). Furthermore, the phytochemical composition of plant extracts strongly influences the physicochemical characteristics and biological activities of the synthesized nanoparticles.

Typha angustifolia L. (narrowleaf cattail), a perennial aquatic plant belonging to the family Typhaceae, is widely distributed in wetlands, marshes, and riparian ecosystems across many

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regions of the world (Bansal et al., 2019). The plant has attracted growing scientific interest because of its rich phytochemical composition and diverse medicinal properties. Previous studies have reported the presence of flavonoids, phenolic acids, tannins, alkaloids, and other bioactive metabolites that contribute to its antioxidant, antimicrobial, anti-inflammatory, and therapeutic activities. Traditionally, different parts of *T. angustifolia* have been used in folk medicine for the treatment of wounds, inflammation, gastrointestinal disorders, and various infections (Liu et al., 2025). The abundance of phytochemicals in *T. angustifolia* makes it a promising candidate for the green synthesis of metallic nanoparticles, where these compounds can facilitate the reduction of silver ions and stabilize the resulting nanoparticles. High-Performance Liquid Chromatography (HPLC) is a widely used analytical technique for the separation, identification, and quantification of bioactive compounds in complex plant matrices. Detailed phytochemical profiling through HPLC can provide valuable insights into the compounds responsible for nanoparticle synthesis and biological activity. With increasing interest in sustainable nanotechnology and plant-based therapeutics, the development of green synthetic routes for AgNP production has become an important area of research.

The present study aimed to synthesize silver nanoparticles using *Typha angustifolia* leaf extract and evaluate their antibacterial, antifungal, antiparasitic, and pharmacological potential. To the best of our knowledge, this is among the first comprehensive investigations exploring the green synthesis of AgNPs using *T. angustifolia*. The study also involved phytochemical characterization of the plant extract using HPLC to identify bioactive constituents involved in nanoparticle formation. The synthesized AgNPs were characterized using ultraviolet-visible (UV–Vis) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), Fourier-transform infrared (FTIR) spectroscopy, and transmission electron microscopy (TEM). Furthermore, the antimicrobial activity of the nanoparticles was evaluated against *Aspergillus niger* and *Escherichia coli*, while their antiparasitic efficacy was assessed against promastigote and amastigote forms of *Leishmania* species. In addition, the antidiabetic potential of the synthesized AgNPs was

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investigated through α-amylase and α-glucosidase inhibition assays, and their biocompatibility was examined using human red blood cells. Overall, this study sought to explore the therapeutic potential of *Typha angustifolia*-derived silver nanoparticles for a wide range of biomedical and pharmaceutical applications.

2. Materials and methods

2.1. Chemicals and apparatus

The reagents used in this experiment required additional purification; they were all analytical grade and obtained from reliable sources like Fluke and Merck. Purity was ensured by preparing solutions with deionized water. Silver nitrate ($AgNO_3$), methanol (CH_3OH), Petri plates, test tubes, Whatman filter paper (No. 1), nutritional agar broth, and sodium hydroxide were the main chemicals and materials used in the experiment.

2.2. Plant Collection and Extract Preparation

Fresh specimens of *Typha angustifolia* were collected during the flowering stage from local wetland habitats during March–April with the necessary permission from the landowner. Following collection, the plant samples were thoroughly cleaned and shade-dried under controlled conditions at approximately 33°C and 50% relative humidity to preserve their bioactive constituents. The dried plant material was subsequently ground into a fine powder using a laboratory grinder. To prepare the aqueous extract, the powdered material was mixed with deionized water and stirred thoroughly. The resulting mixture was filtered to remove insoluble residues, and the filtrate was collected. The aqueous extract was then concentrated by evaporating the solvent in a hot-air oven at 50°C overnight to obtain the crude extract. The dried crude extract was stored under appropriate conditions until further use in nanoparticle synthesis and subsequent experimental analyses.

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2.2. Phytochemical Analysis

For the qualitative analysis of the different bioactive components, the method by Prasad et al. (2024) was used. For example, alkaloids, saponins, flavonoids, phenols, the extract were investigated by using simple phytochemical tests.

2.3. High-performance liquid chromatography (HPLC) analysis

In short, 5 mL of 10% methanol was used to dissolve 1 mg of *Typha angustifolia* plant extract, followed by filtration through a 0.45 µm membrane filter. The Agilent 1260 HPLC system equipped with a reciprocating pump and a C18 column (Sorbex RXC-8, 4.6 × 100 mm, 18 µm) was operated at 30°C for the identification of phenolic acids. The system functions by drawing solvent into a cylinder and subsequently pushing it through the column via a back-and-forth moving piston. The mobile phase was also filtered using a 0.45 µm membrane filter and degassed in an ultrasonic bath before use.

A gradient elution system consisting of 0.2% H_3PO_4, methanol, and acetonitrile was applied at a flow rate of 1 mL/min. The gradient was increased stepwise to 5%, 50%, 70%, and 100% over 5, 15, 25, and 30 minutes, respectively, followed by isocratic conditions for an additional five minutes. Detection was carried out at 210 nm for 35 minutes, and 5 µL of both standards and *Typha angustifolia* extract samples were injected using an autosampler at different retention times. Finally, the K-factor was calculated from retention times, and calibration curves were constructed to quantify individual phenolic compounds such as chlorogenic acid, hydroxybutyric acid, sinapic acid, and ferulic acid present in the *Typha angustifolia* extract. The "K" value (retention factor) of the *Typha angustifolia* compounds was determined using the following formula:

$$K = (tR - tM)/tM \qquad (1)$$

where t_R denotes retention time, and t_M is the dead time.

2.4. Synthesis of the plant extract

For the preparation of the nanoparticles (NPs), 35 mg of dried ***Typha angustifolia*** powder was mixed and dissolved in 130 mL of deionized water, followed by incubation for 24 hours in a

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shaking water bath. The resulting solution was then filtered using Whatman filter paper No. 1. The obtained filtrate was subsequently left undisturbed for two days.

2.5. Nanoparticles synthesis

AgNPs were synthesized using a green synthesis approach. In a 10 mL graduated cylinder, 1 mL of $AgNO_3$ solution (99% purity, Shanghai Chemical Reagent Company) with a concentration of 0.01 M was first measured and then diluted to 10 mL using deionized water to obtain a final concentration of 0.001 M. Subsequently, NaOH (99.95% purity, Sigma-Aldrich) was added to adjust the pH to 10, creating an alkaline medium that facilitates the reduction of Ag^+ ions and enhances the stability and uniformity of the nanoparticles. Thereafter, 4 mL of the centrifuged extract of *Typha angustifolia* was introduced into the solution. A visible color change to pale brownish-yellow confirmed the formation of AgNPs. The phytochemicals present in *Typha angustifolia*, such as flavonoids and phenolic compounds, acted as reducing agents responsible for the conversion of silver ions into nanoparticles, while also serving as capping agents to prevent aggregation and improve stability. The resulting pale brownish suspension was then heated at 40 °C for 72 hours, leading to the formation of AgNP powder. Before their application in further studies, the synthesized nanoparticles were characterized using various spectroscopic and microscopic techniques.

2.6. Characterization of the prepared nanoparticles

To study the optical properties, assessment of functional groups and surface configuration of the prepared NPs was characterized via FTIR (SPECTRUM, 65), UV (SPECORD 200 Plus, Analytik Jena, Germany), SEM (SEM, JEOLJSM 25910), and XRD (XRD, Bruker, D8). The nanoparticle size was calculated via the Debye–Scherrer equation.

2.7. Antimicrobial activity

2.7.1. Antibacterial Assay

Pure preserved bacterial cultures were initially grown on nutrient media to obtain fresh, active cultures. The agar well diffusion method was employed to evaluate the antibacterial activity

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of the synthesized AgNPs. The assay was performed at different concentrations of AgNPs, including 2.5 mg/mL, 5 mg/mL, and 10 mg/mL. Carbapenem was used as the positive control, while DMSO served as the negative control.The antibacterial efficacy was tested against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa*. The assay was carried out following a previously reported protocol (Salas-Orozco et al., 2024). The antibacterial activity was determined using the following formula:

$$\%\text{Inhibition} = \text{Ti}/\text{Ci} \times 100 \qquad (2)$$

Where T_i = inhibition in the test and C_i = inhibition in the control.

2.7.2. Antifungal assay

The pure preserved cultures of fungal strains were grown on potato dextrose medium (PDA) to obtain the fresh cultures. The antifungal activity was assessed at various concentrations of AgNPs, including 2.5mg/mL, 5mg/mL and 10mg/mL, while Fluconazole and DMSO were used as a positive and negative control, respectively. To determine the antifungal potential, *Aspergillus niger*, *Verticillium dahliae*, *Candida albicans,* and *Alternaria alternata* were used by following the standard methodology (Zenat et al., 2024), and the antifungal activity was determined through the subsequent formula:

$$\text{Growth Inhibition}\% = \times\ (\text{C} - \text{T})/\text{C} \qquad (3)$$

Where C means the growth of the fungus in the control plate, and T shows the growth of fungi in an AgNPs-treated plate.

2.8. Pharmacological Applications

2.8.1. Antiparasitic assay

The antiparasitic activity of the synthesized AgNPs was evaluated against *Leishmania tropica* promastigotes following a previously reported protocol (Lima et al., 2024). In brief, different concentrations of AgNPs (25–200 µg/mL) were tested using the 3-(4,5-dimethylthiazol-

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2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, with slight modification based on earlier studies (Kumar et al., 2023). Amphotericin B was used as the positive control. After treatment, microplates were incubated for 48 h at 27 °C, followed by the addition of 10 µL MTT solution to each well and further incubation for 4 h at 27 °C. Cell viability was then measured using a microplate reader (Fluoroskan Ascent, Thermo Labsystems, Finland) at 500 nm, and percentage inhibition was calculated using the following formula:

$$\% \text{ Inhibition} = 100 \times \text{Asample/Bcontrol} \quad (4)$$

where A_{sample} represents the AgNPs-treated sample absorbance, and $B_{control}$ means the absorbance of the control sample.

2.8.2. Antidiabetic assay

The antidiabetic activity of bio-fabricated AgNPs was evaluated through α-amylase and α-glucosidase inhibition assays following a previously reported method (Al-Radadi et al., 2022) with slight modifications. AgNPs were tested at concentrations ranging from 20 to 320 µg/mL, with acarbose used as the positive control. For α-amylase, the reaction mixture containing phosphate buffer, enzyme, AgNPs, and starch substrate was incubated at 60 °C for 30 min, followed by addition of HCl and iodine solution, and absorbance was measured at 520 nm. For α-glucosidase, the reaction mixture was incubated at 35 °C using p-nitrophenyl-D-glucopyranoside as substrate, followed by enzyme addition and further incubation; absorbance was recorded at 400 nm using a UV–Vis spectrophotometer.

The following formula was used to get the % inhibition (Abs = absorbance):

$$\% \text{ Enzyme inhibition} = \left(\frac{\text{Abs Sample} - \text{Abs negative control}}{\text{Abs blank} - \text{Abs negative control}}\right) \times 100 \quad (5)$$

2.8.3. Biocompatibility assay

A hemolytic assay was performed to evaluate the biocompatibility of the synthesized AgNPs using freshly isolated human red blood cells, following a previously reported protocol (Govindappa et al., 2020). Blood (2 mL) was collected from a healthy volunteer in an EDTA tube and centrifuged at 1200 rpm for 15 min to isolate erythrocytes, which were washed three times

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with PBS (pH 7.3). The RBC suspension was prepared by mixing 200 µL erythrocytes with 8.9 mL PBS. For the assay, 100 µL of RBC suspension was incubated with 100 µL of AgNPs (20–160 µg/mL) at 30 °C for 60 min. Dimethyl sulfoxide was used as the positive control. Hemoglobin release was measured at 520 nm using a microplate reader, and percent hemolysis was calculated using the standard formula.

$$\%\ Hemolysis = (sampleAbs - neg.control\ Abs)/(positve\ control\ Abs - neg.control\ Abs) \times 100 \quad (6)$$

2.9. Statistical assessment

All the activities were performed in triplicates and SPSS, version 16.0, and OriginPro9 were applied to analyzed it statistically.

3. Results and Discussions

3.1. Phytochemical Analysis

The qualitative phytochemical analysis revealed that the deionized water extract of *Typha angustifolia* contains a variety of secondary metabolites, including phenols, flavonoids, alkaloids, saponins, and tannins in varying amounts (Table 1). Among these, phenolic compounds were found to be the most abundant, followed by flavonoids (Ahmed et al., 2022).

Table 1. Qualitative Phytochemical Analysis of *Typha angustifolia* using distilled water (TADW)

Sr. No	Metabolites
1	Phenols
2	Flavonoids
3	Alkaloids
4	Saponins
5	Tannins

Key: +++: shows the presence of abundance, ++: shows the presence of moderate quantity, +: shows existence but in small concentrations.

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3.2. Identification of fractions via HPLC

HPLC plays a crucial role in analyzing the phytochemical composition of natural products. The presence of selected compounds in *Typha angustifolia* is illustrated in Fig. 1 and Fig. 2. The extract of *Typha angustifolia*, prepared in 10% aqueous methanol (water:methanol = 90:10 v/v), showed the highest concentration of chlorogenic acid (317.9 ppm), followed by hydroxybutyric acid (283.7 ppm), sinapic acid (45.3 ppm), vanillic acid (14.2 ppm), and ferulic acid (10.3 ppm) (Table 2). Comprehensive metabolic profiling of bioactive constituents is essential to evaluate the biomedical potential of individual phenolic compounds and their associated biological activities. Several studies have reported efficient separation and identification of phenolic acids using HPLC techniques (Kumar et al., 2017).

Medicinal plants are rich sources of bioactive compounds provided by nature (Halder et al., 2023). Phenolic acids are known to play a significant role in reducing aging-related disorders such as diabetes and cancer (Mishra et al., 2021). Chlorogenic acid exhibits antioxidant, anti-ulcer, anti-pyretic, and neuroprotective activities and has also been reported to regulate glucose and lipid metabolism, contributing to the management of diabetes and cardiovascular diseases (Hamed et al., 2024). Similarly, sinapic acid possesses antibacterial, antioxidant, anti-cancer, anti-inflammatory, and anxiolytic properties. Its derivative, 4-vinylsyringol, is a potent antimutagenic and antioxidant agent that inhibits inflammatory cytokines and carcinogenesis. Additionally, sinapine (sinapoyl choline) has been identified as a potential acetylcholinesterase inhibitor with therapeutic relevance for various diseases (Yates et al., 2019). Overall, these bioactive constituents enhance the medicinal significance of *Typha angustifolia*, and further isolation and characterization of these compounds are recommended.

Fig. 1. Chromatogram of HPLC for *Typha angustifolia* extract

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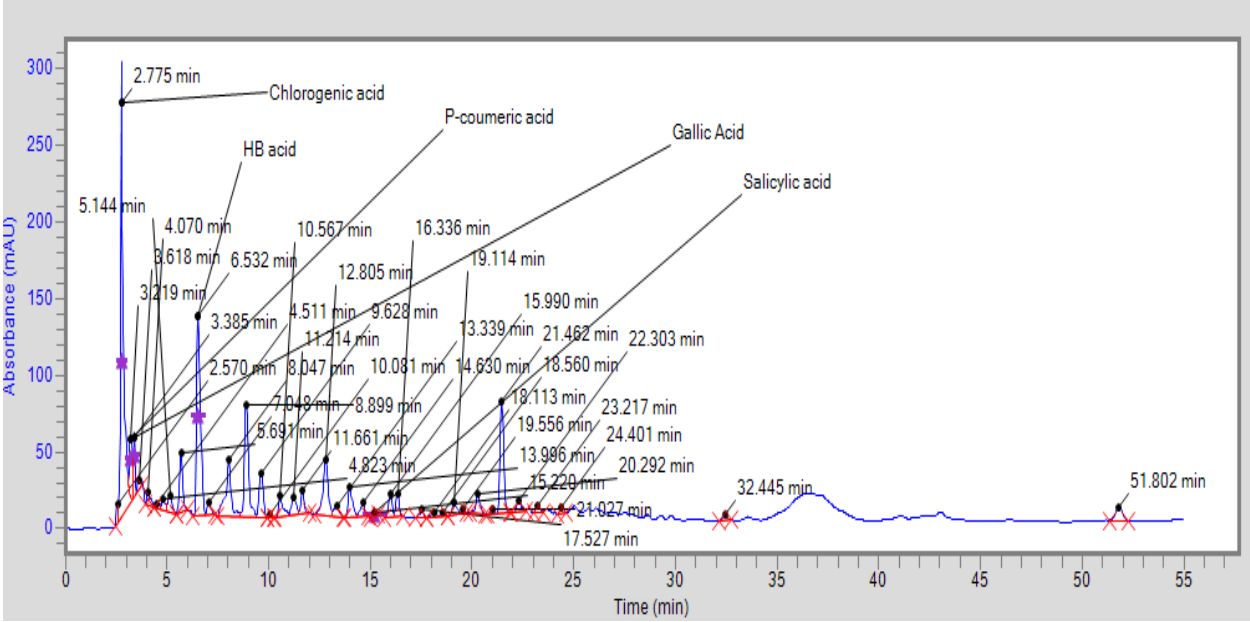

Fig. 2. Peak allocation in the HPLC *Typha angustifolia* extract spectrum. HB = hydroxybutyric.

Table 2. Concentration of five phenolic compounds observed in *Typha angustifolia* using HPLC.

Compounds	RT when single standard was run	RT when a mixture of standards was run	K-factor	Concentration (ppm)
Chlorogenic acid	2.775	2.880	0.00013	317.9
Hydroxybutyric acid	6.532	6.759	0.00016	283.7
Sinapic acid	12.029	12.238	0.0000567	45.3
Vanillic acid	8.055	7.899	0.0000777	14.2
Ferulic acid	12.871	12.512	0.0000767	10.3

*RT: Retention time; ppm: parts per million

3.3. Characterization of Bio-fabricated AgNPs

3.3.1. UV-Vis Spectrophotometry of AgNPs

The optical behavior of the prepared AgNPs was scrutinized via UV–Vis spectroscopy in a range of 200 to 800nm. The peak was developed at a wavelength of 410 nm (Fig. 3). The obtained

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peak value was closely aligned to previous work (Narayanan et al., 2021). It has previously been proven that AgNPs show absorption at a comparable peak 400 nm (Tamilselvan et al., 2022). This little variation may be due to the botanic source that has been used in the preparation of the NPs.

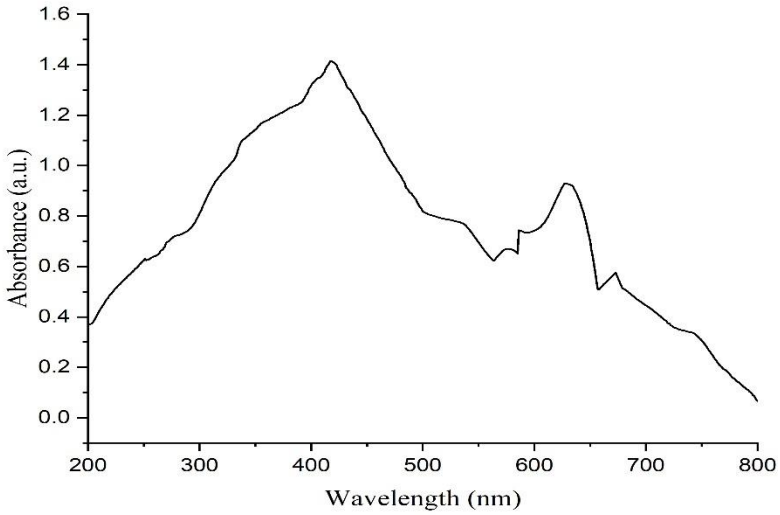

Fig. 3. UV spectrum of plant-mediated AgNPs, showing a maximum at 410 nm.

3.3.2. XRD study of AgNPs

The XRD was executed to explore the crystalline behavior of the prepared AgNPs. The crystalline properties of the NPs contribute vitally to their performance (Recio-Poo et al., 2023). The plant-mediated NPs proved a crystalline nature and showed characteristic peaks at 2θ. The XRD analysis revealed peaks at 2 θ values of 37.2 θ, 44.3 θ, 63.2θ, and 77.2 θ, which were indexed to (63), (130), (145), (160), and (165) planes of a cubic structure (Fig. 4), following the JCPD card no. 04-0783. The strongest peak of AgNPs establishes their crystalline nature. The XRD pattern of the AgNPs is aligned with former studies (Jaiswal et al., 2023). The particle size was calculated as 55 nm. The crystalline sizes of the prepared NPs were calculated with the help of the Debye–Scherrer equation as follows:

$$D = k\lambda/(\beta cos\theta) \qquad (7)$$

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where D shows the crystalline size (nm), k is a constant, λ denotes the wavelength of the X-ray radiation, β represents the full width at half maximum (FWHM) of the intensity and broad peaks, and θ is the Bragg's or diffraction angle.

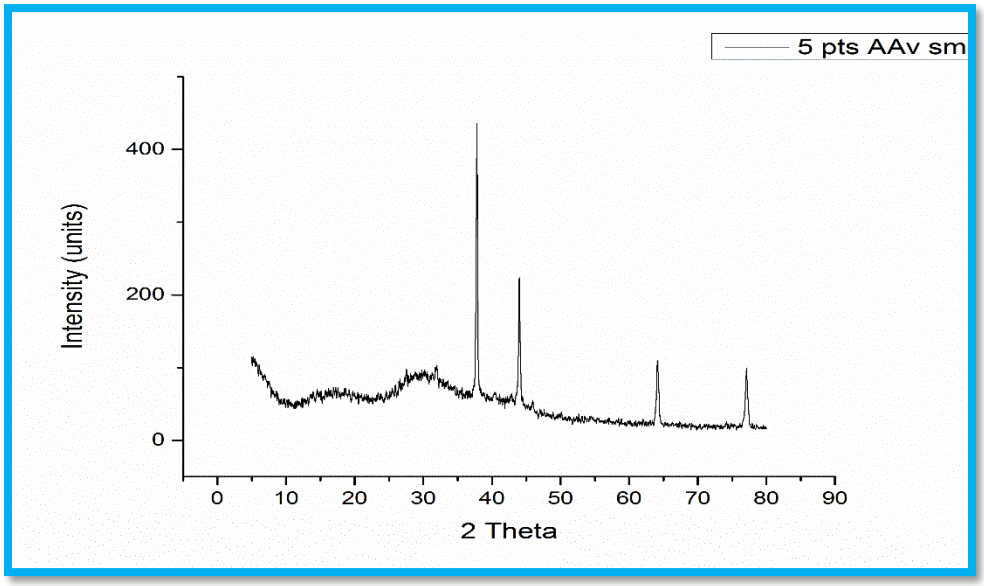

Fig. 4. XRD spectrum of AgNPs

3.3.3. FTIR Study

The FTIR spectrum of the plant extract (Fig. 5) revealed a broad peak at 3600 cm^{-1} corresponding to O–H stretching vibrations. The band observed at 3450 cm^{-1} was attributed to C–H stretching of aromatic compounds, while the absorption at 2200 cm^{-1} indicated N–H stretching vibrations. In addition, the peak at 1000 cm^{-1} was associated with alkyl amine stretching, whereas the band near 500 cm^{-1} was assigned to Ag–O vibrations, which is comparable to the value of 519.92 cm^{-1} reported by Haiying et al. (2023). Minor variations may be due to differences in plant source and experimental conditions. These results suggest that phytochemicals present in the aqueous extract likely played a key role in both the reduction and stabilization of AgNPs (Huq et al., 2022). Overall, the findings are consistent with previous studies reporting similar FTIR signatures for green-synthesized AgNPs (Hashem et al., 2025).

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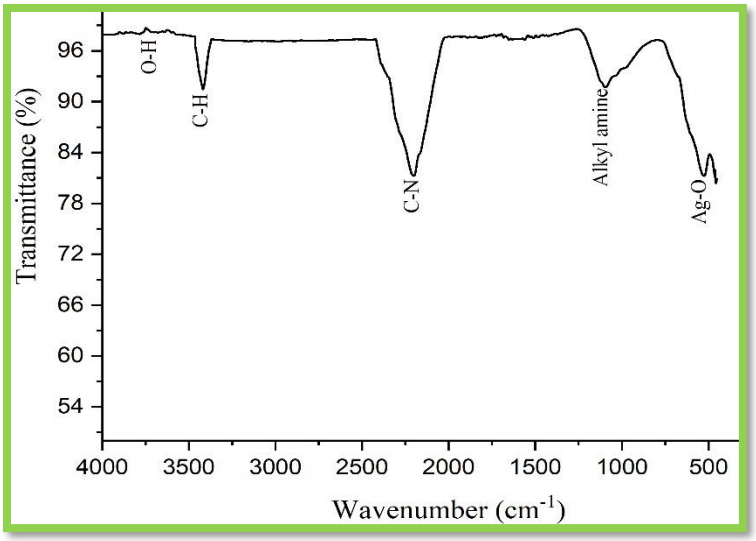

Fig. 5. FTIR spectrum of phyto-fabricated AgNPs.

3.3.4. SEM analysis

Surface configuration of the prepared AgNPs was assessed via SEM. SEM investigation revealed the spherical shape of the NPs having a size from 55-60nm in a dispersed manner at 10,000X magnification (Fig. 6). These results are aligned with previously published work (Zaman et al., 2023; Kobayashi et al., 2025).

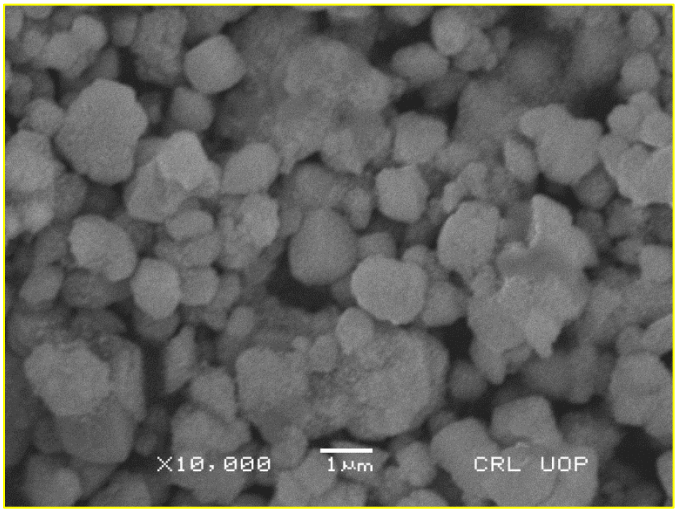

Fig. 6. SEM image of bio-fabricated AgNPs

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3.4. Antimicrobial assays

3.4.1. Antibacterial activity

In the present study, *Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli,* and *Staphylococcus aureus* were utilized for antibacterial activity testing. The antibacterial potential of the prepared NPs is shown in Table 3. 100 µl of each AgNPs concentration (2.5, 5, and 10 mg/mL) was used to determine the inhibition zone (ZOI). The highest ZOI was 14.3 mm at 10 mg/mL, trailed by 13.1 mm at 5 mg/mL and 11.1 mm at 2.5 mg/mL against *E. coli* (Table 3), while the lowest ZOI was 14.0mm at 10 mg/mL, followed by 11.6 mm at 5 mg/mL and 10 mm at 2.5 mg/mL against *S. aureus* (Table 3). In an antibacterial trial, the cell membrane is the first line of defense to protect the bacteria from the foreign agent. The larger surface-to-volume ratio of AgNPs causes the production of more reactive ions. These reactive ions lead to DNA damage, lysis of proteins, and breakdown of enzymes, and finally cause bacterial death (Ohiduzzaman et al., 2024). Our findings are in good agreement with earlier related studies (Menichetti et al., 2023; Zaman et al., 2023).

Table 3. Antibacterial activity of prepared AgNPs (2.5 mg/mL, 5mg/mL & 10mg/mL), as zones of inhibition (ZOI) for 100 µl samples.

S. No.	Bacterial Species	Positive control (in mm) (Carbapenems)	Negative control (DMSO)	Deionized water Extract Zone of Inhibition (mm)		
				2.5 mg/mL	5mg/mL	10mg/mL
1	*Escherichia coli*	20mm	0mm	11.1mm	13.1	14.3mm
2	*Pseudomonas aeruginosa*	21mm	0mm	10.2 mm	12.9	13.8mm
3	*Salmonella typhi*	20mm	0mm	10.1mm	12.4	13.5mm
4	*Staphylococcus aureus*	19mm	0mm	10mm	11.6	14.0mm

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3.4.2. Antifungal activity

The antifungal activity of biosynthesized AgNPs was evaluated using PDA-based Petri plates. The maximum inhibition of 64.4% was observed against *Aspergillus niger* at a concentration of 10 mg/mL, followed by 61.1% at 5 mg/mL and 55.0% at 2.5 mg/mL, as shown in Table 4. The strong antifungal performance of AgNPs can be attributed to their small size and large surface area, which enhance their reactivity and stability compared to bulk silver particles (Sharma et al., 2014). Over the past decade, AgNPs have been widely reported to inhibit the growth of fungal pathogens such as *Aspergillus niger*, *Candida albicans*, and *Fusarium graminearum* (Nsengumuremyi et al., 2020). Their antifungal mechanism is mainly associated with the generation of reactive oxygen species (ROS) and strong interactions with fungal cell walls (Xie et al., 2023). AgNPs disrupt fungal cells by damaging the cell wall and plasma membrane, leading to changes in membrane structure, permeability, and fluidity, ultimately causing cellular dysfunction (Alavi et al., 2019). This disruption results in depolarization, oxidative stress, and leakage of cellular components (Vela-Corcia et al., 2024). Additionally, released Ag^+ ions penetrate the cytoplasm, interfering with signaling pathways and damaging DNA, RNA, and enzymatic systems by disrupting ribosome assembly (Do et al., 2025).

Table 4. Antifungal activity of bio-manufactured AgNPs (2.5 mg/mL, 5 mg/mL, & 10mg/mL)

S. No.	Fungal species	Positive control (in mm) (Fluconazole)	Negative control (DMSO)	AgNPs		
				Zone of Inhibition (%)		
				2.5 mg/mL	5 mg/mL	10 mg/mL
1	*A. niger*	70	0	55	60.1	64.4
2	*A. alternata*	69	0	50	55.4	61.2
3	*C. albicans*	69	0	50	54.4	61

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4	*V. dahlia*	65	0	48.3	53	60.1

3.5. Pharmacological activities

3.5.1. Anti-diabetic Assay

Diabetes mellitus (DM) is a metabolic disorder characterized by elevated blood glucose levels resulting from insufficient insulin production by pancreatic cells. One effective strategy for managing postprandial hyperglycemia involves the inhibition of key carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase, in the gastrointestinal tract (Barman et al., 2024). In the present study, AgNPs were evaluated for their inhibitory effects against these enzymes at concentrations ranging from 10 to 320 µg/mL (Table 5). The highest inhibition was observed at 320 µg/mL, showing 61.24 ± 0.35% for α-amylase and 59.56 ± 1.34% for α-glucosidase. These notable antidiabetic effects suggest that AgNPs may offer promising potential for the development of new therapeutic approaches for diabetes management (Hassan et al., 2023).

Table 5. Alpha-amylase and α–glucosidase assay of plant-mediated AgNPs

Conc. µg/mL	Alpha-amylase		α–glucosidase	
	AgNPs (%)	Acarbose (%)	AgNPs (%)	Acarbose (%)
320	61.24±0.35	79.32±1.33	59.56±1.34	80.44±1.83
160	45.14±0.52	70.61±1.62	45.27±0.29	60.50±1.76
80	35.41±0.38	60.54±1.18	34.59±0.35	55.31±1.22
40	25.13±0.36	51.44±0.84	22.44±0.23	45.33±1.34
20	20.13±0.25	30.38±0.77	18.42±0.34	40.44±0.68

3.5.2. Antileishmanial activity

Leishmaniasis is a rare but highly transmissible tropical and subtropical infectious parasitic disease. According to the WHO, it is endemic in 87 countries, with approximately 1.6 to 3 million new cases reported annually worldwide. The disease is transmitted through the bite of infected *Phlebotomus*

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and *Lutzomyia* sandflies, which introduce an intracellular parasite into the human host. Its persistent spread is largely attributed to inefficient vector control and suboptimal therapeutic strategies. In the present study (Table 6), the promastigote and amastigote forms of *Leishmania tropica* were evaluated using the MTT assay in the presence of AgNPs at concentrations ranging from 25 to 200 µg/mL. A significant dose-dependent cytotoxic effect was observed, with maximum parasite mortality recorded at 200 µg/mL, showing 75.41 ± 1.16% inhibition for promastigotes and 71.13 ± 0.12% for amastigotes. Similar dose-dependent antileishmanial effects have also been reported in previous studies (das Neves et al., 2024).

Table 6. Antileishmanial Activity of bio-inspired AgNPs.

Concentration (µg/m)	Promastigote inhibition	Amastigote	IC50 (µg/ml)	IC50 (µg/ml)
200	75.41±1.16	71.13±0.12	77.43 Amastigote	68.33 Promastigote
100	65.44±0.23	61.11±1.09		
50	55.14±1.20	50.33±1.14		
25	39.22±0.26	38.39±0.10		

3.5.3. Biocompatibility assay

The hemolytic assay was performed to analyze the toxic nature of the phyto-bombarded NPs against the RBCs. RBC and AgNP concentrations from 20 to 160 µg/mL were combinedly cultured in a buffer solution prepared to imitate an extracellular environment. The hemolytic assay is based on the release of hemoglobin due to the lysis of the RBCs when AgNPs are applied. The findings of the hemolytic activity are presented in **Table 7.** The American Society for Testing and Materials documented that substances with hemolysis >2% are non-hemolytic (Carpenter et al., 2024). It can be examined from the findings (Table 7) that *biofabricated AgNPs* revealed biocompatibility even at the highest dose. These results make them suitable for biomedical usage. Our results are aligned with the previous work, and the prepared *AgNPs* can be subjected to beneficial applications (Alonso-Montemayor et al., 2023).

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Table 7. % Hemolytic activity of bio-fabricated AgNPs

S.NO	Concentration (µg/mL)	Hemolysis %	Positive control (Dimethyl sulfoxide)
1	160	1.33±0.11	4.33±0.12
2	80	0.86±0.14	4.33±0.12
3	40	0.69±0.10	4.33±0.12
4	20	0.50±0.13	4.33±0.12

4. Conclusion

This study highlights the promising potential of silver nanoparticles (AgNPs) synthesized using *Typha angustifolia* plant extract, demonstrating notable antimicrobial, antiparasitic, and antidiabetic activities. Comprehensive characterization through UV–Vis spectroscopy, XRD, FTIR, and SEM confirmed the successful synthesis of AgNPs and provided detailed insights into their structural and physicochemical properties. The synthesized AgNPs exhibited strong antibacterial activity, particularly against *Escherichia coli*, along with significant antifungal effects. They also showed potent antiparasitic activity against both promastigote and amastigote forms, in addition to marked inhibitory effects on α-glucosidase and α-amylase enzymes, indicating their potential as antidiabetic agents. Importantly, the nanoparticles demonstrated good biocompatibility with human red blood cells, supporting their safety for biomedical applications.

Overall, these findings emphasize the therapeutic potential of *Typha angustifolia*-derived AgNPs and encourage further research into their clinical applications as effective antimicrobial, antiparasitic, and antidiabetic agents.

Supplementary Information

Not applicable.

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Data Availability

Data is made available on the request.

Competing interest

There are no competing interests among the authors.

Ethical responsibilities of authors

All authors have read and agreed to the final version of this paper. The manuscript is not submitted to any other journal nor under consideration. All the data and results produced have been prepared in this study.

Ethics approval

Not applicable.

Authors Contributions

Arooj James and Yasmeen Kausar contributed equally to the study by performing the experiments, data collection, and initial manuscript drafting. Anwar Kamal assisted in the data analysis and interpretation of results. Nasreen Ashraf supervised the overall research work, contributed to the study design, critical revision of the manuscript, and provided intellectual guidance. Amina Bibi contributed to the conceptualization of the study, supervision, validation of results, and final approval of the manuscript. All authors reviewed and approved the final version of the manuscript.

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