Transaction on Biomedical Engineering Applications and Healthcare
Volume 6, Issue 1, April 2025
e-ISSN: 2582-7405

Green Synthesis of AgNPs Using Medicinal Himalaya Fern (*Thelypteris erubescens*): Structural Characterization, Effect on Maize Seed Germination and Antimicrobial Function

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Abstract

The green synthesis of silver nanoparticles (AgNPs) is a promising aspect of nanotechnology due to its stable, non-toxic, and eco-friendly approach. In this study, the medicinal Himalayan fern *Thelypteris erubescens* was used for the green synthesis of AgNPs (TeAgNPs), and its effects on seed germination of maize and antimicrobial properties were investigated. TeAgNPs were synthesized by adding silver nitrate to the plant methanolic extract. The synthesis of AgNPs was confirmed by a change in color from light yellow to dark brown, representing the actual reduction of silver ions. Scanning electron microscopy (SEM) images showed that the nanoparticles were mainly spherical, ranging in size from 30-40 nm, with some observed aggregation. X-ray (XRD) diffraction analysis indicated that the AgNPs displayed a crystalline structure with a particle size of 32.6 nm. The TeAgNPs application (concentrations: 25, 50, and 75 mg/L) significantly enhanced maize germination, root, and shoot growth, which was better than the control treatment. Moreover, TeAgNP concentrations (10, 15, and 20 µg/mL) displayed antibacterial and antifungal activity via agar well diffusion. The most potent inhibition was against *Klebsiella pneumonia* and *Aspergillus niger* (18.33 and 13.3 mm), respectively, highlighting TeAgNPs' potential anti-pathogenic use. TeAgNP application demonstrated superior antibacterial efficiency compared to the fern's methanolic extracts. Phytochemical analysis of the *T. erubescens* methanolic extracts identified various bioactive compounds, including alkaloids, tannins, and flavonoids, which might play a role in the stabilization and biological efficiency of its AgNPs. Our eco-friendly and cost-effective AgNP synthesis approach exploits the fern *Thelypteris erubescens* potential against microbial strains and its positive effects on plant growth for promising applications in agriculture.

Keywords

Thelypyteris erubescens, Silver nanoparticles, TeAgNPs, *Zea mays*, Seed Germination

INTRODUCTION

Nanotechnology has gained substantial consideration due to its essential role in a range of applications, such as nanomedicine, drug delivery, chemical sensors, medicine, and environment-friendly approaches [1–3]. According to the definition of nanoparticles (NPs), these are the preparation of substances with at least one dimension in nanometers. NPs have a size of 1–100 mm, which may be organic or inorganic and can be produced using chemical and physical methods.

Silver nanoparticles (AgNPs) have significant antibacterial, antifungal, and antiviral properties and can pass through bacterial cell walls, affecting cell membrane structure and potentially inducing cell death [4]. Among the other types of NPs, AgNPs can improve plant growth, photosynthetic pigments, and antioxidant ability of seeds [5]. Silver nanoparticles are used to generate antibacterial compounds [4]. Although there are few concerns for AgNP safety, no systemic harm from ingested silver nanoparticles has been recorded [4]. In case these AgNPs are realized in the environment, some potential harm is expected because the interaction of nanoparticles with hazardous chemicals and organic compounds can either increase or decrease their toxicity [4]. On the other hand, the use of nanoparticles has grown profoundly in recent years due to their wide range of applications in optics and electricity, and their physical and chemical properties make them unique [6]. Nanoparticles contain better and unique properties such as size, distribution, and morphology. The medical application of silver nanoparticles is very efficient in all areas of life where NPs are used, e.g., in industry, healthcare, the food industry, and cosmetics [7].

Using different living organisms or their parts, such as plants (traditional or endemic), algae, fungi, microbes, insects, and marine creatures to synthesize AgNPs is an innovative approach that has become one of the fastest-growing product categories in nanotechnology research [8]. Metals, including gold (Au), zinc (Zn), silver (Ag), iron (Fe), and copper (Cu), can synthesize NPs from different plant samples [9]. The natural synthesis of AgNPs is essential to developing eco-friendly and environmentally stable tools for crop growth improvements [9]. Green synthesis is used in

plants, fungi, bacteria, and algae. Nanoparticles produced by green synthesis are impurity-free, have better catalytic activity, have no side effects, and are more economical and environmentally stable [10]. In green technology, in-vivo and in-vitro productions of organic substances by microbes have similar properties to material prepared in the laboratory [7, 11].

Pteridophytes are spore-producing seedless plants, mostly found in moist, shady, and cold places from highlands to sea levels; some species are found in dry lands. The plant's height range is about 1–20 meters, depending on the environmental conditions. It has a diversity of bioactive compounds, e.g., saponins, tannins, and flavonoids, which can play an important part in the production of nanoparticles by reducing silver ions. Also, fern-meditated biofabrication of AgNPs recently gained attention [12].

Pteridophyte-based green synthesis of silver nanoparticles could reduce antimicrobial resistance. Due to secondary metabolites conjugated to AgNPs, these are used for many biological activities, such as anti-cancer, anti-fungal, anti-bacterial, anti-diabetic, and anti-allergic, especially in pteridophytes [13, 14]. Humans have used pteridophytes as folk medicines for a long time (about 2000 years ago). Pteridophytes belonging to different families and genera showed high pharmacogenetic potential, which was explored through antimicrobial and antifungal activities [15]. Pteridophytes are also a source of medicines; some species of pteridophytes are used in the homeopathic treatment of many diseases like diarrhea and bladder irritability [15]. Furthermore, various ferns spp have been used in folk medicine for their therapeutic potential as an important source for the synthesis of nanoparticles.

Thelypteris erubescens (Wall. ex Hook.) Ching is the accepted name of Glaphyropteridopsis erubescens of the family Thelypteridaceae. It is widely distributed in different Himalayan regions of China, Pakistan, and India and is also found in Japan and the Philippines. It is a perennial fern and grows primarily in the temperate biome. It is one of the most essential ingredients in traditional medicine [16]. The fern plant's rhizomes are robust, decumbent, woody, and glabrous, and it stands between two and three meters tall. Stapes are 1–2 m thick, ribbed, glabrous, stramineous throughout, and frequently reddish. Fronds are grouped together. Each frond has 40–50 pairs of opposing, sessile, proximal pinnae, with some pairs being sharply inclined distally. Because of its therapeutic uses, this fern is typically utilized as a herbal plant. In order to prepare recipes, young leaves and stems are frequently eaten as wild vegetables [17, 18]. The root powder is used as an antidote for scorpion bites, the frond dough is applied externally for rheumatism, and its leaf decoction is used to treat gastritis [19]. Pharmacogenetic description of pteridophytes investigates their medical significance. Different parts (rhizome, leaves, and stems) of *T. erubescens* comprise numerous bioactive compounds [20] that might increase the biological potential and expedite the synthesis of AgNPs. The use of this fern for producing AgNPs is exciting because of its medicinal properties.

Our research aims to search the biological perspective of bio-fabricated AgNPs from Himalaya fern (*Thelypteris erubescens*) for the first time and evaluation of their antibacterial potential, explicitly focusing on their effect on seed germination of maize. This research provides insight into the benefits of nanoparticle synthesis from ferns and their potential application in microbial resistance and crop growth. The result of this study will provide sustainable and environmentally friendly solutions for medicine and agriculture.

MATERIAL AND METHODS

Sampling

Thelypteris erubescens plants were collected from district Sudhanoti, tehsil Trarkhal (Nerian) Azad Jammu and Kashmir (AJK, Pakistan) in December 2022, identified by taxonomists from the Department of Botany, Mohi-ud-Din Islamic University (MIU) AJK, and deposited in the herbarium of the same university (Figure 1). Plant samples were placed in labeled cotton bags and transported to the botanical laboratory, MIU, for processing. The selected samples were immediately transferred to the refrigerator at 4°C upon arrival to prevent degradation and were then shade-dried for 2-3 weeks at room temperature (22-26ºC). The plant samples were washed separately through tap and distilled water (DW) and then crushed into small pieces. The parts of the plants were placed in the fresh newspaper at room temperature (24 ± 1°C) for drying after shade drying; the plant samples were ground using a mechanical grinder into fine powder and stored at room temperature in airtight containers until use.

Figure 1: *Thelypteris erubescens* plants from district Sudhanoti, tehsil Trarkhal (Nerian) Azad Jammu and Kashmir (AJK, Pakistan)

Preparation of Plant Methanolic Extract

The powder (10 g) of plants was dissolved in 250 mL of methanol solvent and distilled water (dH2O) in separate 500 mL beakers. For uniform extraction, the temperature was maintained by continuously stirring at 80°C while boiling for 30 minutes. The plant mixture was filtered by Whatman filter paper 1 into flasks after cooling. This filtered liquid extract was concentrated with the help of a rotary evaporator and water bath. At 4°C, it was stored for the subsequent synthesis of nanoparticles [21, 22, 23].

Preparation of Nanoparticles

TeAgNPs were prepared by adding silver nitrate solution in 1:1, 1:2, and 1:3 to the methanolic plant extracts of *T. erubescens* and stirred for 24 hours at room temperature [24]. The reaction mixture's color changed (from pale yellow to brown), reducing silver ions to metallic silver, indicating the formation of nanoparticles. The reaction mixture was left for one day, and the nanoparticles were isolated and centrifuged for 15 minutes at 1500 rpm. Nanoparticles were separated, and after washing with distilled water, they were characterized through various techniques, including SEM and X-ray diffraction [25].

Characterizations of Nanoparticles

Scanning Electron Microscopy (SEM)

SEM was conducted to characterize the size and shape of the TeAgNPs and observe the structure of nanoparticles. A small sample was dropped on the carbon-coated copper grid and dried rapidly. The extra solution was removed using blotting paper to observe the sample's morphology using SEM (JEOL JSM-6490A). The JEOL JEM-Plus-1400 apparatus of Tokyo, Japan, photographed the microscopic AgNPs [26].

X-ray Diffraction (XRD) Analysis

The XRD technique was used to check the size and crystallographic structure of the TeAgNPs by using X-ray diffraction (XRD, PANalytical analyzers, Netherlands). In this technique, samples were prepared according to the X-ray diffraction method [21]. A thin coating of nanoparticles was created on a glass plate by dipping it in an AgNP solution for X-ray diffraction experiments. The crystalline structure of nanoparticles was obtained on the diffractogram of AgNPs, and the width of the XRD peak, and the size of nanoparticles was measured using the same diffractogram [27, 28].

Effect of TeAgNPs on Maize Seed Germination

Seeds of maize (*Zea mays*, cultivar "Narc F1") were immersed in a 5% sodium hypochlorite solution (w/v) for 15 minutes, then soaked in distilled water for 2 hours. Afterward, the seeds were dipped into two TeAgNPs solutions (prepared in DW) with three concentrations of 25, 50, and 75 mg/L for two hours, while the seeds of the control group were soaked only in DW using a previously established method [29]. 5 mL TeAgNPs of each concentration or DW for the control group were pipetted onto the filter papers in Petri plates, each containing ten maize seeds placed 1 cm apart. Petri dishes were then covered with parafilm and incubated in the growth chamber at 28°C for 10 days under controlled conditions. The seed germination rates and percentages were monitored daily, and the data were recorded. The germination rate and percentage were calculated using the formula and equation mentioned in the previous protocols [30].

In another experimental setup, TeAgNPs and dH2O-treated seeds were prepared with the concentrations stated above to be grown in soil pots using a modified method [21]. Subsequently, germinated seeds after 4 days were directly transferred to the prepared soil pots and grown for two weeks, and data were recorded from the maize plants. After 14 days of growth, the maize seedlings were removed from soil pots carefully, and the lengths and fresh weight of the root and shoot of seedlings were measured and photographed accordingly. The length of the seedlings was measured with the help of a ruler, and the fresh weight of the seedlings was determined in grams using the standard protocol [21].

Antibacterial Activity

Fresh bacterial cultures of pathogenic bacterial strains: *Bacillus mycoides*, *Salmonella enterica*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* were grown by the selected strains and mixed into a 12 mL sterile nutrient broth medium, and the level of turbidity was set to 0.5, equivalent to a cell density of 10^8 CFU/mL. An autoclave was used to sterilize Petri plates and media at 121°C. Nutrient agar media (50 mL) was poured into Petri plates at 50 to 70°C and allowed to solidify in laminar flow only for 20 minutes. One loop of culture was used to streak and prepare agar Petri media. A cork borer was used to prepare two holes in a round shape for streaked bacteria culture. 50 µL of plant methanolic extract and TeAgNPs (concentrations 10, 15, and 20 µg/mL) were poured into the wells via a micropipette, and then the prepared plates were incubated for 24 hours in the dark at 28°C. After 24 hours of incubation, the inhibition zones were measured to sort out the antibacterial activity. Ciprofloxacin (50 µg/mL) was used as a positive control, while DMSO (50 µL) was used as a negative control. The zones of inhibition were measured with a ruler in millimeters (mm). This antibacterial experiment was repeated three times, and the average mean values of the zone of inhibition were measured [31].

Antifungal Activity

The test fungal organisms used in this study, *Aspergillus niger* and *Botrytis cinerea*, were obtained from the Department of Plant Breeding and Plant Pathology, University of Poonch Rawlakot, Pakistan. The antifungal activity of the TeAgNPs was evaluated using molten Potato Dextrose Nutrient Broth (PDB) media. The fungal cultures were inoculated in the PDB media in 20 mm Petri dishes following a thorough hand-to-hand mixing process. The culture plates were placed in a laminar airflow chamber to firm up before being divided into wells on the agar plate using a typical 5 mm cork borer. A volume of 100 µL of different concentrations of TeAgNPs (15, 20, and 25 µg/mL) was added to each well. 50 µL of itraconazole (0.5 mg/mL in DW) was used as the positive antifungal control. After that, the plates were sealed and kept at 25 ± 2°C for five days. The antifungal activity was determined by measuring the zone of inhibition using a standard scale. The readings were shown in mm to calculate the average of three replicates [32].

Phytochemical Analysis

Invitro qualitative phytochemical tests were done for *Thelypteris erubescens* extracts, as elucidated subsequently.

Determination of flavonoid

1 mg plant extract in a test tube was taken, and a few drops of NaOH and dilute HCl were added to each plant sample. The yellow color was an indication of the existence of flavonoids [33].

Determination of alkaloid

A sample of about 1.5 mg of plant extract was placed in a test tube, and a few drops of NaOH and diluted HCl were added. The color progressively changed to yellow, indicating the presence of an alkaloid [34].

Determination of Saponin

We added 2 mg of plant extract in a test tube with 2 ml of water, then shook it in the test tube until the foams formed, shown in the upper meniscus of the solution. These foams' stability indicated saponin's existence [35, 36].

Determination of Tannins

Exact 2 mg of each plant sample was mixed in 3 ml of 5 % ferric chloride solution. The presence of a dark blue color indicated the presence of tannins [33].

Determination of Terpenoid

2 mg of each plant extract was added with 3 ml Sulphuric acid and 2 ml chloroform in a test tube. The presence of a layer and radish brown color provided evidence of terpenoids [37].

Determination of Glycoside

For the indication of glycoside, 2 mg of each plant sample was mixed with 2 ml of acetic acid, 2 ml of chloroform, and 2 ml of sulphuric acid were added to each test tube. After the reaction, the green color indicated the existence of glycoside [38].

Determination of Steroid

For the evidence of steroid, 1.5 mg of each plant sample was mixed with 2 ml chloroform and Sulphuric acid in a test tube. In low amounts of chloroform, the red color indicated the existence of steroids [38].

Determination of Quinones

About 1 mg of each plant extract was mixed in a test tube with concentrated sulphuric acid, and the red color indicated the existence of quinones [39].

Determination of Coumarin

1 mg of each plant extract was mixed with 10% NaOH. The solution's yellow color showed coumarin's presence [40].

Statistical analysis

All experiments in this investigation were run in triplicate, and statistical software was used to determine the mean. To quantify dispersion, the standard deviation was calculated. ANOVA and Tukey's post hoc test were used to statistically assess all of the data. Statistics 8.1 was used to calculate the significant differences at a significance level of $p<0.05$.

RESULTS

Synthesis and Characterization of AgNPs

In this study, green synthesis of TeAgNPs was done. These compounds create metal particles that are widely employed in research and nanotechnologies. The principles of green chemistry are used in the biological process of metal nanoparticle synthesis, and most plants are used for AgNP biosynthesis [41]. The fern was selected based on their nutritional and medicinal potential [42]. Many fern species have been utilized in traditional medicine by local and Indigenous peoples throughout history [43-45]. Some ferns can decrease stomach pain, bowel problems, toothache, cramps, nausea, and vomiting. Many species of ferns are used against many infections, diarrhea, weakness, stomach cramps, and headaches [46, 47].

This study used the previously mentioned methods to synthesize AgNPs from AgNO3 salt using the methanolic and aqueous crude extract Himalaya fern (Thelypteris erubescens). The formation of NPs was indicated by a color change from light yellow to dark brown, as previously described by Mehrotra et al. [48], followed by further characterization. The size and shape of the resulting NPs are affected by the reaction temperature, time, and concentration of salt.

XRD Analysis

The XRD evaluation has been widely employed in numerous nanoparticle studies to determine the crystal type and crystalline purity of biosynthesized AgNPs. XRD examined the crystallinity of AgNPs in this study. The size of AgNPs was calculated using the Debye-Scherrer equation, described in the protocol of Trieu et al. [49], which states that particle size reduction causes the broadening of diffraction peaks. The XRD pattern of TeAgNPs indicated octahedral crystalline structure planes with the XRD spectrum. The XRD peaks revealed a significant crystalline structure of the biosynthesized AgNPs (Figure 2).

The observed peaks at 2θ° angle reflected at position 24.6, 29.1, 32.2, 39.6, 48.3, and 78.6 reflecting (101), (111), (204), (313), (210), (110), and (401) XRD planes respectively. The crystal structure observed in the peaks revealed a crystal lattice of 3.8 nm of the TeAgNPs. The XRD pattern was well matched with the joint committee for powder diffraction studies (JCPDS) card No. 43-2176, suggesting that the prepared NPs are AgNPs. The average crystalline structure examined via full-width half maximum (FWHM) revealed that the particle crystalline size was 32.6 nm.

T. erubescens played a role in the fabrication of silver ions in the solution, resulting in the synthesis of AgNPs. Studies found that the biosynthesized AgNPs play a role in microbial activity against pathogenic strains [50, 51]. Our study is also consistent with previous studies that indicate the presence of plant secondary compounds plays a role in reducing the crystalline size of the NPs. The secondary metabolites attach to the surface of the AgNPs, further helping in their antimicrobial activities.

Transaction on Biomedical Engineering Applications and Healthcare
Volume 6, Issue 1, April 2025
e-ISSN: 2582-7405

These results revealed that the plant extract of T. erubescens played a role in one way: the crystalline structure helps enter the microbial cell. In contrast, in another way, due to secondary metabolites, the NPs damage the cell metabolism and result in cell death. The morphology of the TeAgNPs was determined via SEM micrographs at different resolutions. The particles revealed a spherical structure and cloudy agglomeration, confirming that the particles are spherical within the 30-40 nm size range (Figure 2). This spherical symmetry of the AgNPs would be helpful for biological applications. The spherical shape of the NPs enhances their potential to attach to bacterial cell membranes and leads to damage to the nucleic acids and essential proteins.

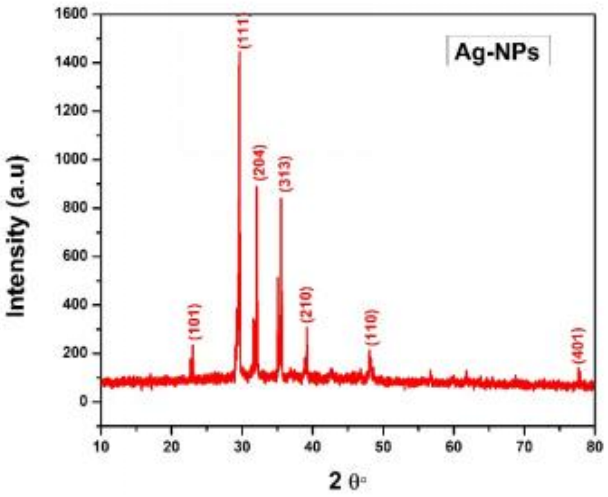

Figure 2: X-ray diffraction (XRD) diffractogram patterns of the *T. erubesense-mediated* AgNPs

Scanning Electron Microscopy (SEM)

Green synthesized AgNPs obtained from T. erubescens extract were analyzed through SEM to study the morphology of the TeAgNPs at 1, 5, and 10 µm. The detailed size of TeAgNPs is displayed in Figure 3. The majority of the nanoparticles examined were monodispersed and polydispersed, with some aggregated forms of the particles present. Silver's destabilizing effect commonly causes AgNP agglomeration [52]. The SEM revealed that the particles possess electrostatic attraction due to aggregation in the particles. This electrostatic attraction might be helpful in the attachment with microbial cells. Moreover, it also indicates that the particle size is very narrow, and these results are consistent with previous studies. Thus, it is recommended that the prepared AgNPs be suitable for biological applications (Figure 3).

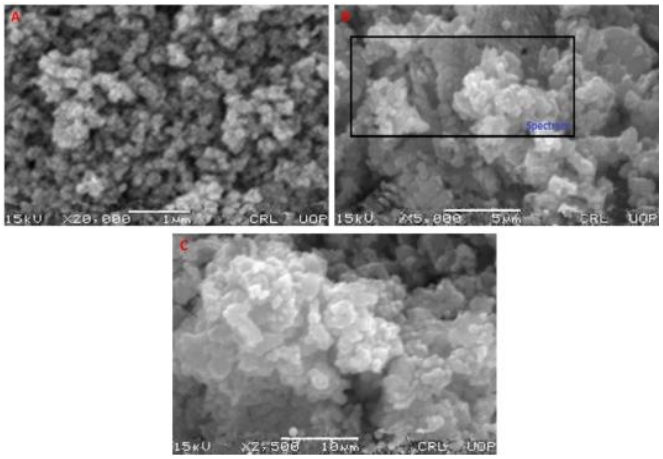

Figure 3: (a, b, c) Morphology of the *Thelypteris erubescens* mediated Ag-NPs

Seed Germination and Pot Experiment Analysis

The effect of treatments on germination percentage and rate is summarized in Table 1. The control group exhibited a germination percentage of 80 ± 3.33% and a germination rate of 0.75±0.05, significantly lower than those of the treated groups. Treatment with TeAgNPs at 25 mg/L significantly increased germination percentage (86.66±5%) and germination rate (0.88±0.11). Further increases in TeAgNP concentration to 50 mg/L led to a 95±5% germination percentage, while the germination rate increased to 0.90±0.05. The highest concentration of TeAgNPs, 75 mg/L, achieved the maximum germination percentage (96.66±3.33%) and germination rate (0.95±0.01), significantly higher than the control and lower concentration treatments. Statistical analysis revealed significant differences among the treatments, with higher concentrations of TeAgNPs showing superior performance ($p < 0.05$) (Figure 4,5).

The findings show that higher concentrations of TeAgNPs significantly improve root dry weight, with apparent statistical differences as indicated by the grouping letters ($a > b > c$). The lowest root dry weight was observed in the control group and with TeAgNPs at 25 mg/L, at about 0.8 g (group "c"). Treatment with TeAgNPs at 50 mg/L resulted in a significant increase to about 1.2 g (group "b"), while the highest root dry weight was observed with TeAgNPs at 75 mg/L, reaching approximately 1.6 g (group "a").

The results of shoot dry weight rose considerably with greater concentrations of TeAgNPs compared to the control. After receiving 25 mg/L of TeAgNPs, the shoot dry weight increased to 2.7 g (group "c"), while the control group showed the lowest shoot dry weight at about 2.2 g (group "d"). TeAgNPs at 50 mg/L caused further increases, reaching about 3.8 g (group "b"), whereas TeAgNPs at 75 mg/L produced the highest shoot dry weight, 4.5 g (group "a") (Figure 6). According to the grouping letters ($a > b > c > d$), all treatments exhibited statistically significant differences, demonstrating that increasing TeAgNP concentrations significantly increase shoot dry weight.

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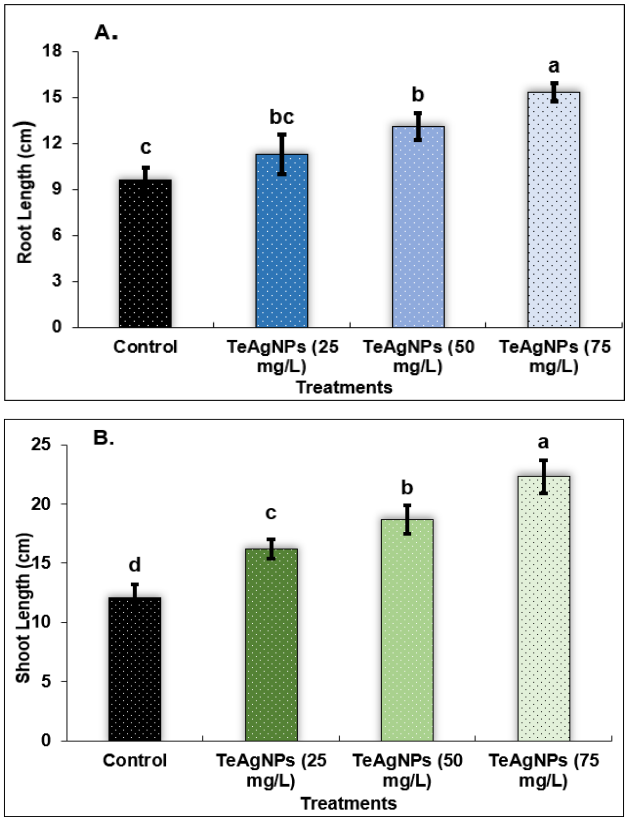


Figure 4: Effect of different concentrations of *Thelypteris erubescens*-mediated AgNPs on the growth of Maize seedlings. (A) Root Length (B) Shoot Length

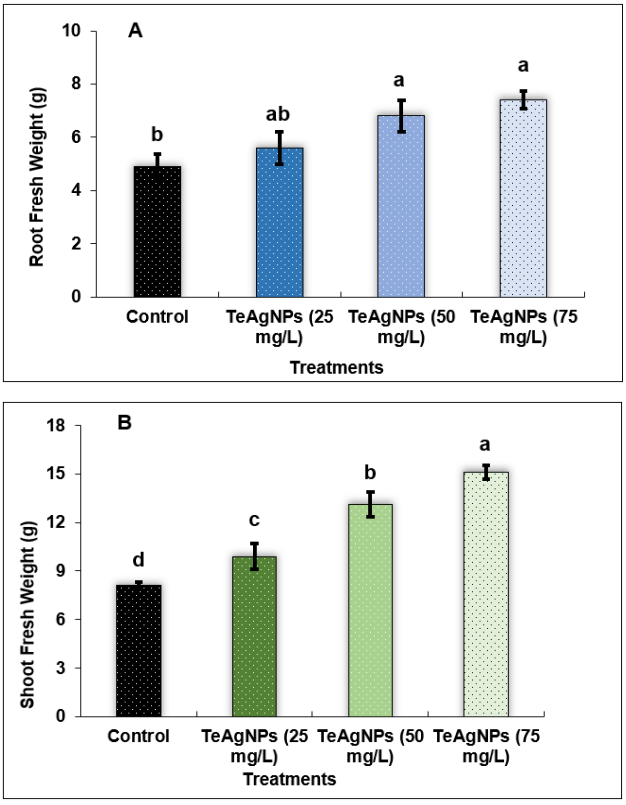
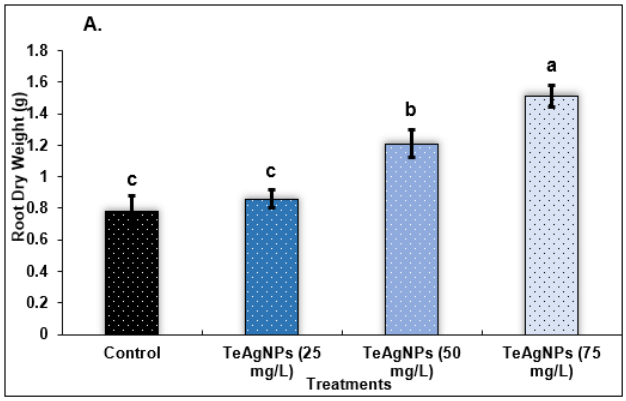


Figure 5: Effect of different concentrations of *Thelypteris erubescens*-mediated AgNPs on the growth of Maize seedlings. (A) Root Fresh Weight (B) Shoot Fresh Weight.



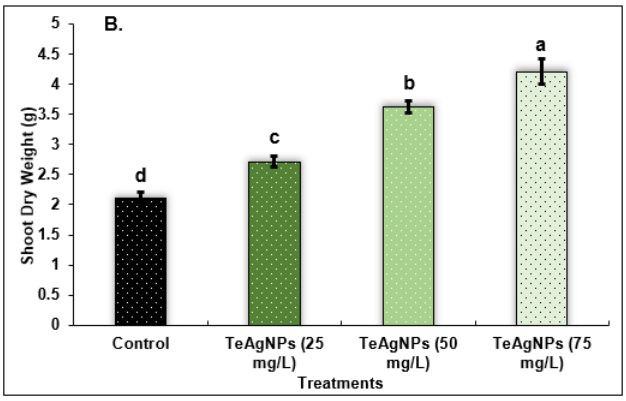


Figure 6: Effect of different concentrations of *Thelypteris erubescens*-mediated AgNPs on the growth of Maize seedlings. (A) Root Dry Weight (B) Shoot Dry Weight.

Figure 7: Maize Seedling Morphology is grown in pots (14 days) under TeAgNPs Treatments (Scale: 10 cm)

Table 1: Evaluation of seed germination and rate under different concentrations of TeAgNPs after 10 days in petri plates.

Treatments	Germination Percentage %	Germination Rate
Control (DW)	80 ± 3.33 ^{c}	0.75 ± 0.05 ^{c}
TeAgNPs (25 mg/L)	86.66 ± 5 ^{b}	0.88 ± 0.09 ^{b}
TeAgNPs (50 mg/L)	95 ± 5 ^{a}	0.90 ± 0.05 ^{ab}
TeAgNPs (75 mg/L)	96.66 ± 3.33 ^{a}	0.95 ± 0.01 ^{a}

Antibacterial and Anti-Fungal Activity

The produced AgNPs were tested for antibacterial activity against Gram-positive Bacillus mycoides and Gram-negative *Salmonella enterica, Klebsiella pneumoniae, and Acinetobacter* at varying doses (10, 15, and 20 µg/µL) using the agar well diffusion method. Ciprofloxacin (50 µg/µL), a common antibiotic, was utilized as a positive control to compare the results to, while DMSO (50 µl) was used as a negative control. The highest significance inhibition zone was recorded against *Klebsiella pneumoniae* (18.33) at 20 µg/µL concentration, followed by *Salmonella enterica* (14.5) at 20 µg/µL. AgNPs' bactericidal properties are influenced by their size, shape, surface charge ratio, dose, and particle dispersion state. Furthermore, AgNPs' toxicity to bacteria rises with particle size. Because the current size of our isolated nanoparticles is relatively small, it has a minimal antibacterial effect.

Anti-fungal activities of TeAgNPs were evaluated for three different concentrations (15, 20, and 25 µg/mL) against two pathogenic fungal strains, namely *A. niger* and *B. cinerea*. The results were compared to the typical fungicides, and Itraconazole was used as a positive control, while DMSO (50 µl) was used as a negative control. The highest significance inhibition zone was recorded against *A. niger* for TeAgNPs at 25 µg/mL concentration (13.3±0.6 mm). However, for *B. cinerea*, it was 11.35±3.52 mm at 25 µg/mL concentration. TeAgNPs against the *A. niger* for the same concentration and volume showed a more significant zone of inhibitions (Table 3). In this study, plant extract and DMSO showed no inhibition activities. Overall, the TeAgNPs showed prominent antifungal potential against both pathogenic strains, revealing that the presence of pteridophyte compounds enhanced the antifungal potential of the NPs (Table 3).

Table 2: Antibacterial activity of TeAgNPs against different pathogenic bacterial strains.

Pathogenic Bacteria	Treatments	Mean Zone of Inhibition (mm ± S.D.)
Bacillus mycoides	Plant Methanolic Extract (PEM)	4.3±1.6
	TeAgNPs-10 µg/mL	12.67±0.94
	TeAgNPs-15 µg/mL	11.67±0.47
	TeAgNPs-20 µg/mL	12.33±2.05
	Ciprofloxacin	20.1±2.2
Salmonella enterica	PEM	2.6±0.66
	TeAgNPs-10 µg/mL	13.67±0.47
	TeAgNPs-15 µg/mL	13.2±0.81
	TeAgNPs-20 µg/mL	14.5±3.31
	Ciprofloxacin	19.81±0.97
Klebsiella pneumoniae	PEM	6.33±1.25
	TeAgNPs-10 µg/mL	13.67±2,06
	TeAgNPs-15 µg/mL	15.0±1.63
	TeAgNPs-20 µg/mL	18.33±1.25
	Ciprofloxacin	19.3±0.51
Acinetobacter baumanni	PEM	5.4±0.94
	TeAgNPs-10 µg/mL	15.0±3.87
	TeAgNPs-15 µg/mL	15.3±3.91
	TeAgNPs-20 µg/mL	11.0±0.81
	Ciprofloxacin	21.22±1.57

Table 3: Anti-fungal activity of TeAgNPS against *Botrytis cinerea* and *Aspergillus niger* (Zone of inhibition measured in mm)

TeAgNPs Concentration	*Botrytis cinerea*	*Aspergillus niger*
15 µg/mL	6.14 ± 0.95 ^{c}	8.41 ± 0.47 ^{d}
20 µg/mL	9.70 ± 0.81 ^{bc}	11.18 ± 0.28 ^{c}
25 µg/mL	11.35 ± 3.52 ^{b}	13.3 ± 0.63 ^{b}
Itraconazole (Positive control)	17.3 ± 3.1 ^{a}	24.3 ± 1.11 ^{a}

Phytochemical Analysis

The quantitative phytochemical analysis of *T. erubescens* was carried out for aqueous and methanolic extracts using the method described by T. erubescens has medicinal properties due to the presence of most phytochemical compounds, such as flavonoids, alkaloids, saponins, coumarins, tannins, and terpenoids (Table 4). All these phytochemicals were present in plant extracts, while steroids, quinines, and glycosides were absent. In this research, neither quantitative analysis of total phenolic content nor total flavonoid content was studied. So, other phytochemicals must be explored to isolate new phytochemical compounds[53].

Table 4: Qualitative phytochemical screening of *Thelypteris erubescens* extract (+ sign mean detected; - sign mean not detected.

Compound	Observation	Methanolic Extract of Selected Plant	Aqueous Extract of selected Plant
Alkaloid	No ppt	+	+
Glycoside	Light Yellow colour	-	-
Steroid	Reddish brown	+	+
Flavonoid	White Yellow	+	+
Saponin	Vapor formation	+	+
Terpenoid	Brown	+	+
Quinine	Reddish	-	-
Coumarin	Deep yellow	-	-

DISCUSSION

The use of *Thelypteris erubescens* as a reducing and stabilizing agent for AgNP synthesis is an eco-friendly

alternative to chemical methods, emphasizing sustainability and reduced toxicity. The current study showed the green synthesis of silver nanoparticles (AgNPs) using methanolic extracts of *Thelypteris erubescens*. The synthesis of AgNPs was confirmed by a characteristic color change from light yellow to dark brown. Characterization techniques, including XRD and SEM, revealed significant insights into the structural and morphological properties of the nanoparticles. The XRD analysis showed that the biosynthesized AgNPs possess a crystalline octahedral structure with peaks corresponding to AgNPs based on the JCPDS reference (43-2176). The average crystalline size was calculated to be 32.6 nm using the Debye-Scherrer equation. SEM analysis revealed that the nanoparticles were spherical, with a 30–40 nm size range. The particles' morphology and narrow size distribution suggest their suitability for biological applications, particularly in antimicrobial activities [49, 50].

The biosynthesized AgNPs demonstrated significant biological activities. Seed germination analysis showed that increasing concentrations of TeAgNPs enhanced germination percentage and rate compared to the control. The highest concentration (75 mg/L) yielded a germination percentage of 96.66% and a germination rate of 0.95, indicating the potential of TeAgNPs as bio-enhancers in plant development. The findings reveal that higher concentrations of TeAgNPs significantly improve root dry weight, with clear statistical differences as indicated by the grouping letters (a > b > c). The lowest root dry weight was recorded in the control group and with TeAgNPs at 25 mg/L, both at approximately 0.8 g (group "c"). Treatment with TeAgNPs at 50 mg/L led to a significant increase to about 1.2 g (group "b"), while the highest root dry weight was observed at 75 mg/L, reaching approximately 1.6 g (group "a"). Similarly, the results of shoot dry weight increased significantly with greater concentrations of TeAgNPs compared to the control. The control group exhibited the lowest shoot dry weight at about 2.2 g (group "d"), followed by an increase to 2.7 g with 25 mg/L of TeAgNPs (group "c"). Further increases were seen with 50 mg/L of TeAgNPs, resulting in 3.8 g (group "b"), while the highest shoot dry weight of 4.5 g was recorded with TeAgNPs at 75 mg/L (group "a"). According to the grouping letters (a > b > c > d), all treatments showed statistically significant differences, confirming that increasing TeAgNP concentrations significantly enhance both root and shoot dry weights [51].

Antimicrobial evaluations further highlighted the efficacy of TeAgNPs. Against bacterial strains, TeAgNPs showed the highest zone of inhibition against *Klebsiella pneumoniae* at 20 µg/µL, followed by *Salmonella enterica*. The results indicated that smaller-sized nanoparticles exhibit higher bactericidal activity due to increased surface area and enhanced interaction with microbial membranes. In antifungal studies, TeAgNPs demonstrated significant inhibitory effects against *Aspergillus niger* and *Botrytis cinerea*, with maximum activity at 25 µg/mL. The presence of pteridophyte compounds in the plant extract likely contributed to the enhanced antifungal activity of the nanoparticles.

Phytochemical analysis of *T. erubescens* extracts revealed the presence of several bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and terpenoids, which play a critical role in AgNP synthesis and biological activities. These secondary metabolites facilitated the reduction and stabilization of AgNPs and enhanced their antimicrobial potential. The absence of steroids, coumarins, glycosides, and quinones in the extract underscores the need to explore further other phytochemicals for novel bioactive compounds [52].

The study demonstrates that fern-mediated AgNPs offer significant advantages as eco-friendly and cost-effective agents for diverse applications. Their antimicrobial activity and role in enhancing seed germination highlight their potential use in agriculture and medicine. Future studies should explore optimizing AgNP synthesis conditions and isolating specific phytochemical compounds to enhance their biological applications further.

CONCLUSION

Green-synthesised T. erubescens nanoparticles are good for the environment and protect against pollution. AgNPs have numerous safe uses in the domains of cosmetics, medicine, and defense. Researchers have looked into using the plant to make folk remedies to cure antimicrobial diseases, and biosynthesized AgNPs contribute to microbial activity against pathogenic strains. T. erubescens produces silver AgNPs that enhance maize seed germination. Thus, without having any adverse consequences, the synthesis of AgNPs from T. erubescens increases maize crop productivity. Although chemical fertilizers increase crop yields as well, they may have different negative impacts on the environment, soil health, and human health than AgNPs.

Acknowledgments

All the authors are grateful to The Islamic Development Bank, Suleyman Demirel University, Isprta, Turkey, and Mohi-ud-Din Islamic University Nerian Sharif for providing the necessary facilities for the research work and research writing. We are also very thankful to Shameen Arif, who helped with lab experiments.

Author Contributions

S.K conducted a lab experiment. S.K wrote the first draft and S.A. helped in the lab work K.I.S helped in validation and reviewed and edited the first draft of the manuscript. T.D. S and S.K. finalized the proofreading and final draft.

Statement of data availability

The corresponding author will disclose the data that support the study's findings upon reasonable request.

Statement of Interest

The authors declare no conflicts of interest.

Funding

Islamic Development Bank (IsDB), which provided the funds for the publication of this research work.

Access to data statement

The authors affirm that the publication contains the data that supports the study's findings.

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