J Nanostruct 16(1): 474-483, Winter 2026

RESEARCH PAPER

Green Synthesis of Triangular Silver Nanoparticles Using Garden Cress Seed Extract Based on Gallic Acid and Their Antifungal Activity

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ARTICLE INFO

Article History:
Received 01 September 2025
Accepted 22 October 2025
Published 01 January 2026

Keywords:
Antifungal
Gallic Acid
Green synthesis
Plant extract
Triangular silver nanoparticles

ABSTRACT

Nanotechnology is a developing discipline in pharmaceutical sciences, characterized by particles that exist at the nanoscale and have enhanced reactivity compared to their larger counterparts. This work discusses a green production technique to prepare triangle silver nanoparticles (AgTNPs) using garden cress seed extract. Whole garden cress seed, commonly referred to as Rashaad seeds in the Middle East and Arabic countries, were extracted using distilled water to obtain the essential extract. The chemical components of the aqueous extract were analyzed using Electron Ionization Gas Chromatography-Mass Spectrometry (EI-GC/MS). Subsequently, the extract was employed as a reducing agent for the synthesis of AgTNPs. After the synthesis and purification of nanoparticles, UV-Vis Spectroscopy, Fourier Transform Infrared (FTIR) spectroscopy, X-ray Diffraction (XRD), Energy Dispersive X-ray (EDX) analysis, and Scanning Electron Microscopy (SEM) were employed for comprehensive characterization. Additionally, Gallic Acid was then loaded on these nanoparticles, and their antifungal activities were investigated before and after modification. The results showed significant inhibition when (AgTNPs) were tested in comparison with plant extract, while they showed remarkable activity as antifungal when Gallic Acid was loaded compared to free (AgTNPs). This work presents the novel use of plant extract for preparing triangular silver nanoparticles .

How to cite this article
Mohsin A., Al-Refaia R., G. Hadi A. Green Synthesis of Triangular Silver Nanoparticles Using Garden Cress Seed Extract Based on Gallic Acid and Their Antifungal Activity. J Nanostruct, 2026; 16(1):474-483. DOI: 10.22052/JNS.2026.01.043

INTRODUCTION

Nanotechnology is the scientific discipline that focuses on analyzing materials at the nanoscale [1]. A nanoparticle is a microscopic substance generally measuring between 1 and 100 nanometers [2,3]. The nanoparticles have distinct physical, chemical, and biological features at the nanoscale in contrast to their respective particles at higher scales [4]. Nanoparticles (NPs) are considered to possess remarkable physical and chemical properties due to their elevated surface area-to-volume ratio relative to bulk materials. Biological, chemical, and physical techniques can all be employed to synthesize nanoparticles [5]. Due to their particular features, nanoparticles (NPs) are utilized in various applications; nevertheless, the presence of some harmful or possibly hazardous substances in their manufacture restricts the

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scope of nanotechnology [6]. Green synthesis, a sustainable methodology in nanotechnology, generates nanoparticles with natural resources like plant extracts, hence reducing reliance on harmful chemicals and mitigating environmental impacts [7]. Metallic nanoparticles of diverse shapes, sizes, compositions, and physicochemical properties have been actively synthesized utilizing biological methods in recent years [8]. The word "green" refers not to color, but to the concept of generating nanoparticles from metal salts by utilizing the reducing properties of biologically active substances [9]. The utilization of leaves, flowers, stems, roots, or entire plants for the synthesis of nanoparticles (NPs) is regarded as an environmentally friendly method [10-16]. Green synthesis offers several advantages over chemical production of nanoparticles, such as an extended half-life of the nanomaterial, higher efficiency, and consequently lower toxicity and more biocompatibility [17]. Lepidium sativum, generally referred to as garden cress, is a rapidly growing edible herb within the mustard family (Brassicaceae). It has been utilized for centuries in traditional medicine for its numerous health advantages, with its seeds, leaves, and sprouts esteemed for their therapeutic powers [18]. Silver (Ag) is a highly desirable metal for nanoparticle production due to its extensive variety of uses stemming from its characteristics. The main applications of Ag involve anti-cancer therapies, biomedical technology, drug-gene delivery, and clinical antibacterial uses [19]. The green production of silver nanoparticles can be accomplished using bacteria, plant extracts, and fungi, thereby minimizing the need on hazardous chemicals, elevated pressure, use of energy, and temperature [20]. Silver triangular nanoplates (AgTNPs) are distinguished among various silver nanomaterials due to their distinctive triangular geometric anisotropy, which provides it with unique optical properties [21] and antimicrobial properties [22] because of the high anisotropy of sharp vertices and edges [23]. Triangular nanoparticles (TNPs) of silver have significantly greater strength than spherical silver nanoparticles [24]. The modification of methods of the controlled synthesis of triangular silver nanoplates has attracted lots of attention in recent years, owing to the extensive applications of silver nanoplates in diverse domains, including surface-enhanced Raman scattering (SERS) detection and the production of antibacterial agents [25]. Antifungal drug combinations have been extensively utilized in biological research and clinical practice to enhance therapeutic efficacy [26]. The requirement for new antifungal medicines has consistently increased due to the rise of resistant fungal isolates and the necessity for antifungals that exhibit reduced toxicity and lower costs [27]. In this study, garden cress extract has been used to synthesis silver seeds, which is required to prepare AgTNPs. Additionally, gallic acid was loaded on these NPs and their ability as antifungal agent has been investigated.

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MATERIALS AND METHODS

Reagents and Apparatus

Chemicals and reagents were purchased from Sigma-Aldrich, except the garden cress seed, which was provided from the Babylon governance market. Silver nitrate ($AgNO_3$), Hydrogen peroxide (H_2O_2), Trisodium citrate (TSC), Chitosan, Gelatin, and Sodium hydroxide (NaOH).

All UV–Vis absorption spectra were obtained using T80 UV\VIS Spectrometer PG Instruments Lt, Fourier Transform Infrared (FT-IR) analysis, X-ray Diffraction (XRD), Scanning Electron Microscope (SEM), Energy-Dispersive X-ray Spectroscopy (EDX), and Gas Chromatography-Mass Spectrometry (GC–MS) analysis.

Plant extract preparation

Garden cress was collected from the Babylon governance market, washed with water, and left to dry at room temperature for 4 days. Then, 20 g of plant seed was soaked in 100 mL of deionized water for 24h. The solution was heated to 100°C for 35 minutes, filtered, and maintained at 4°C until used.

Silver Seeds Preparation

Silver seeds were prepared environmentally friendly by using Garden cress seed extract according to a published paper [28] with significant modification. Briefly, 20 mL of an aqueous solution containing $AgNO_3$ (2.9-10.4 M) was mixed with 40 mL of plant extract and cooled in an ice bath. NaOH (0.1 M, 0.6 mL) was added dropwise to this aqueous solution while stirring strongly. The solution turned bright yellow rapidly. The seeds were then stored in a dark environment and allowed to age for two hours before their application.

Preparation of AgTNPs

The AgTNPs were synthesized following the method outlined in the literature, with some modifications [21]. Briefly, mix 5 mL silver seeds, 6 mL trisodium citrate, 6 mL Chitosan and Gelatin (1:1) (0.70 mM), and 240 µL H_2O_2 (30 W/W%) in 150.00 mL of ionized water. Move rapidly at room temperature for 10 minutes. Following 40 minutes of stirring, the solution exhibited red, green, and blue colors, indicating the synthesis of silver nanosheets.

Modification of AgTNPs with Gallic Acid

The interaction that occurs between AgTNPs and Gallic Acid, according to the method described in the literature [21]. Briefly, after adding 200 µL of AgTNPs to a centrifuge tube, Gallic Acid solutions (15 µM) or buffers were added for control. Add 0.05 mL of NaCl (4.00 mM) and achieve thorough mixing. The mixture was reacted for 7 minutes at ambient temperature. The color of the reaction tubes was immediately examined and analyzed utilizing a UV–Vis spectrophotometer within the wavelength spectrum of 400–800 nm.

Assessment of Antifungal Assay

The antifungal activity of the plant extract, Ag seeds, AgTNPs, and AgTNPs with Gallic Acid against *Fusarium spp* strain isolates from the cucumber strain was achieved by the agar dilution method [29]. The medium agar was augmented with various concentrations of plant extract, silver seeds, AgTNPs, and AgTNPs with Gallic Acid individually (0.5, 1, and 2 mg/mL). A disc measuring 1 cm of mycelial growth from the periphery of a 6-day-old fungal culture was positioned at the center of each plate. The inoculum plates were then incubated at 25°C. The treatment efficacy was evaluated after 7 days by quantifying the radial growth of the fungal colonies.

$$\text{Inhibition rate } (\%) = R - r \setminus \mathrm{R}$$

where (R) is the radial growth of fungal hyphae on the control

plate and (r) is the radial growth of fungal hyphae on the plate supplemented with AgTNPs or plant extract.

Instrumentation (GC/MS)

Whole seeds of garden cress were milled into a fine powder. 0.5 g of the substance was dissolved in 5 mL of methanol using the orbital shaker at 300 rpm for 3 hours. After that, 1.5 mL of the diluted sample was taken and injected into the GC/MS analysis. A gas chromatograph (7890A GC) from (Agilent Technologies, USA) was used for identifying the chemical composition of water

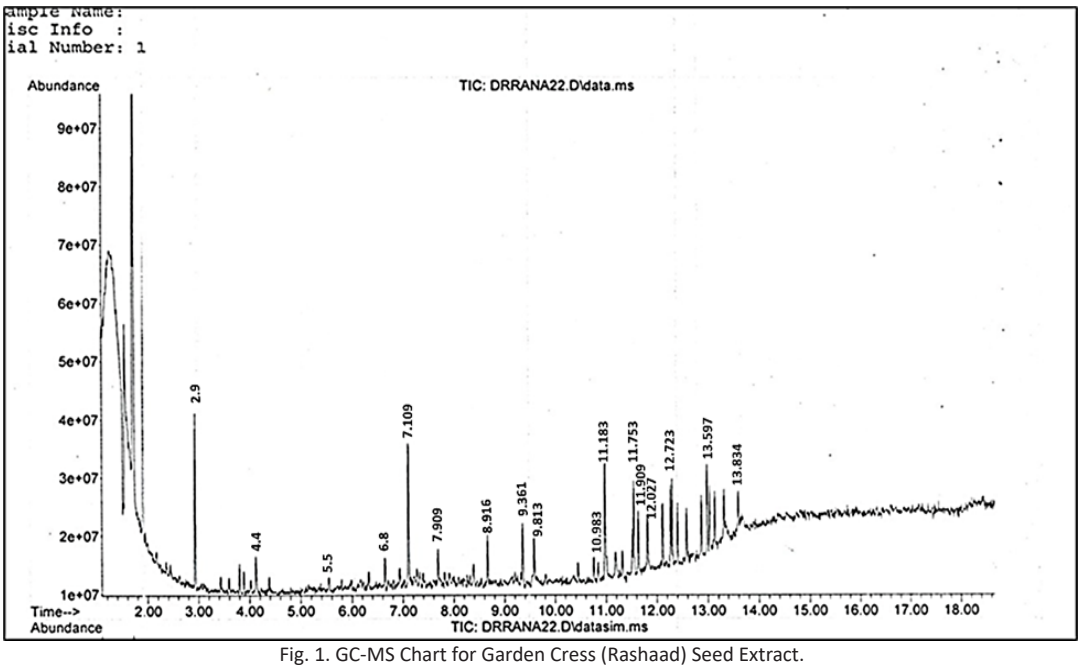


Fig. 1. GC-MS Chart for Garden Cress (Rashaad) Seed Extract.

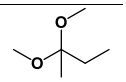
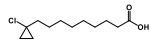
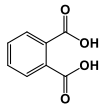
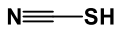
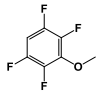
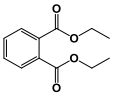
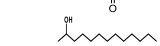
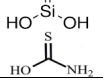
extract from garden cress seed (Lepidium sativum L.).

RESULTS AND DISCUSSION

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC/MS chromatograms was used to identify the structures of plant seed extract, the results showed different peaks for the plant extract as presented in Fig. 1, revealing various compounds. Chemical composition from the methanol solution of garden cress seed revealed 20 components present in the water residues of the extract. The various information results about each component in garden cress (Raahaad) seed are mentioned in Table1 such as retention time (tR), compound name, chemical formula, molecular weight (MW), peak area, percentage (%) area. The

Table 1. GC/MS Review of the Chemical Composition (%) from Garden Cress (Rashaad) Seed.

No.	Retention time in min	Area of Peak %	Compound identified	Molecular formula	Molecular weight in (g/mol)	Structure
1	2.932	0.18	2,2-Dimethoxybutane	$C_6H_{14}O_2$	118.17	
2	.4134	0.01	1-chloro-cyclopropanenoanoic acid	$C_{12}H_{24}ClO_2$	232.12	
3	465.5	0.10	Phthalic acid	$C_8H_6O_4$	166.03	
4	6.894	0.11	1-Nanodecene	$C_{19}H_{38}$	266.51	
5	7.109	0.33	Benzyl nitrile	C_7H_5N		
6	7.909	0.20	Benzenemethanol	C_6H_8O	108.05	
7	8.916	0.17	2-piperidinone	C_5H_9NO	99.13	
8	9.361	0.28	Thiocyanic acid	CHNS	59.09	
9	9.813	0.20	1-Octadecene	$C_{18}H_{36}$	252.49	
10	10.983	1.07	2, 3, 5, 6, -Tetrafluoroanisole	$C_7H_4F_4O$	180.10	
11	11.183	0.60	Diethyl phthalate	$C_{12}H_{14}O_4$	222.24	
12	11.264	0.27	1,2-dibromo-oxalic acid	$C_2Br_2O_4$	247.82	
13	11.753	0.14	2-Tridecanol	$C_{13}H_{28}O$	200.37	
14	11.909	0.35	1-Hexadecanol	$C_{16}H_{34}O$	242.45	
15	12.027	0.70	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	280.41	
16	12.723	0.44	1, 2, 3-Triazol	$C_2H_3N_3$	69.07	
17	13.597	0.84	Diethyl benzamidomalonate	$C_{14}H_{17}NO_5$	279.29	
18	.59713	0.84	Acetic acid	$C_2H_4O_2$	60.05	
19	13.834	0.52	1, 2, 4-Benzenetri carboxylic acid	$C_9H_6O_6$	210.14	
20	15.787	0.85	Silicic acid	H_2SiO_3	78.11	
21	16.812	0.75	Thiocarbamic acid	CH_2NSOH	77.12	

relative percentage amount of each component was calculated by comparing its average peak area with the total area. The most main compounds found in water extract from garden cress seed, which play the majority roles in the bioreduction of Ag+ ions to Ag0 nanoparticles as a reducing agent are (2,3,5,6,-Tetrafluoroanisole (1.07%), Silicic acid (0.85%),Acetic acid (0.84%), Thiocarbamic acid (0.75%), 2-Myristynoyl-glycinamide (0.70%), Diethyl phthalate (0.60%),1,2,4-Benzenetri carboxylic acid (0.52%) and 1,2,3-Triazol (0.44%). Farther that, 0.18% of 2,2-Dimethoxybutane participated as a stabilizer to intermediate and facilatethe formation of Ag0. In the GC-MS analysis, 2-piperidinone and 1-Hexadecanol were identified as key contributors in nanoparticle formation. 2-piperidinone acted as a reducing agent, while 1-Hexadecanol (0.35%) served as a capping agent, stabilizing the nanoparticles synthesized using plant extract.

UV-Vis Spectrophotometry

The optical characteristics of AgTNPs were examined using (T80 UV/VIS Spectrometer PG Instruments Lt). As illustrated in Fig. 2. The absorption peak of the synthesized Ag TNPs occurred at 510 nm, and the solution exhibited a clear and transparent blue color. UV–Vis absorption spectra of the plant extract, Ag seed, and Ag TNPs with Gallic Acid were obtained at various time intervals within the range of 300 to 600 nm [30].

FTIR Spectral Analysis

Fig. 3-a illustrates the FT-IR spectra of the Lepidium sativum extract. The band at 3387 cm^{-1} is ascribed to O–H stretching vibrations. The absorption band at 2943 cm^{-1} was attributed to C–H vibrations, encompassing CH, CH_2, and CH_3 stretching and bending vibrations, both symmetric and asymmetric, and occasionally overlapping with O-H. The characteristic bands around 1604 and 1419 cm^{-1} were attributed to the asymmetrical and the symmetrical COO− stretching vibrations, respectively. The wave values ranging from 1700 to 1600 cm^{-1} and from 1600 to 1500 cm^{-1} were ascribed to Amide I (stretching vibrations of C–O and C–N groups) and Amide II (mostly due to N–H bending), indicating protein presence. The distinctive band at 1072 cm^{-1} was ascribed to the vibrations of C–O, C–O–C glycosidic, and C–O–H bonds[31]. The surface functional groups on AgTNPs were analyzed via FT-IR spectroscopy. Fig. 3-b illustrates that the peaks at around 1560, 1378, and 1281 cm^{-1} could be attributed to the C=O asymmetric stretching C-O stretching, and C–H stretching from the synthesis of AgTNP [32], also the peak at 2854 cm^{-1} refers to C_{sp3}-H [33]. On the other hand, the FT-IR data of AgTNPs containing Gallic Acid exhibited many absorption peaks, reflecting the diverse functional groups as seen in Fig. 3-c. A band between 3600 and 2500 cm^{-1} is ascribed to the stretching vibration of the OH group. The bands at 1617, 1540, and 1451 cm^{-1} correspond to the characteristic stretching vibrations of C–C bonds within the aromatic ring of Gallic Acid. The frequencies are similar to those reported elsewhere. Several additional peaks identified between 1000-1300 cm^{-1} are ascribed to the stretching vibrations of the C–O bond and

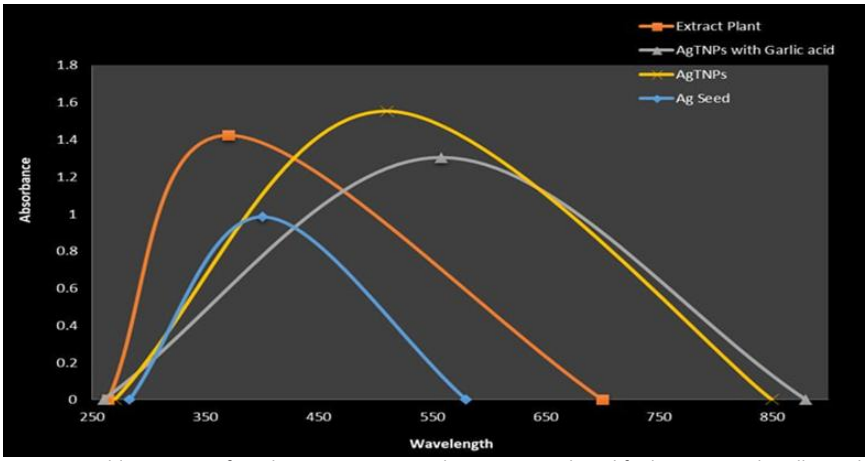


Fig. 2. UV-Visible spectrum for: plant extract, Ag Seeds, AgTNPs, and modified AgTNPs with Gallic Acid.

the bending vibrations of the O–H bond in Gallic Acid [34].

SEM, XRD, EDX

Initially, $AgNO_3$ is reduced to form AgTNPs by a chemical reduction process. The oxidative capacity of H_2O_2 is essential in the synthesis of AgTNPs [21]. It is evident from Fig. 4. SEM showed that the synthesized AgTNPs were triangular in shape of uniform dimensions, and spatially distinct from one another. Their average edge length was estimated to be approximately 130.57 nm, utilizing the Nano Measurer. While AgTNPs with Gallic Acid determined the average edge length to be approximately 77.7 nm.

The EDX spectrum for silver nanoparticles. The principal peaks of the elements Ag, C, and O were identified. No more peaks were observed, therefore confirming the purity of AgTNPs as illustrated in Fig. 5. Moreover, a few Ag atoms on the surface of AgTNPs are expected to undergo oxidation, resulting in negligible quantities of AgO and Ag_2O.

The biomolecules in the plant extract possess surface hydroxyl groups that convert Ag ions to AgTNPs. Confirm this with patterns from XRD analysis of AgTNPs, which are shown in Fig. 6. The observed peaks in patterns 2θ= 38.8°, 44.9°,

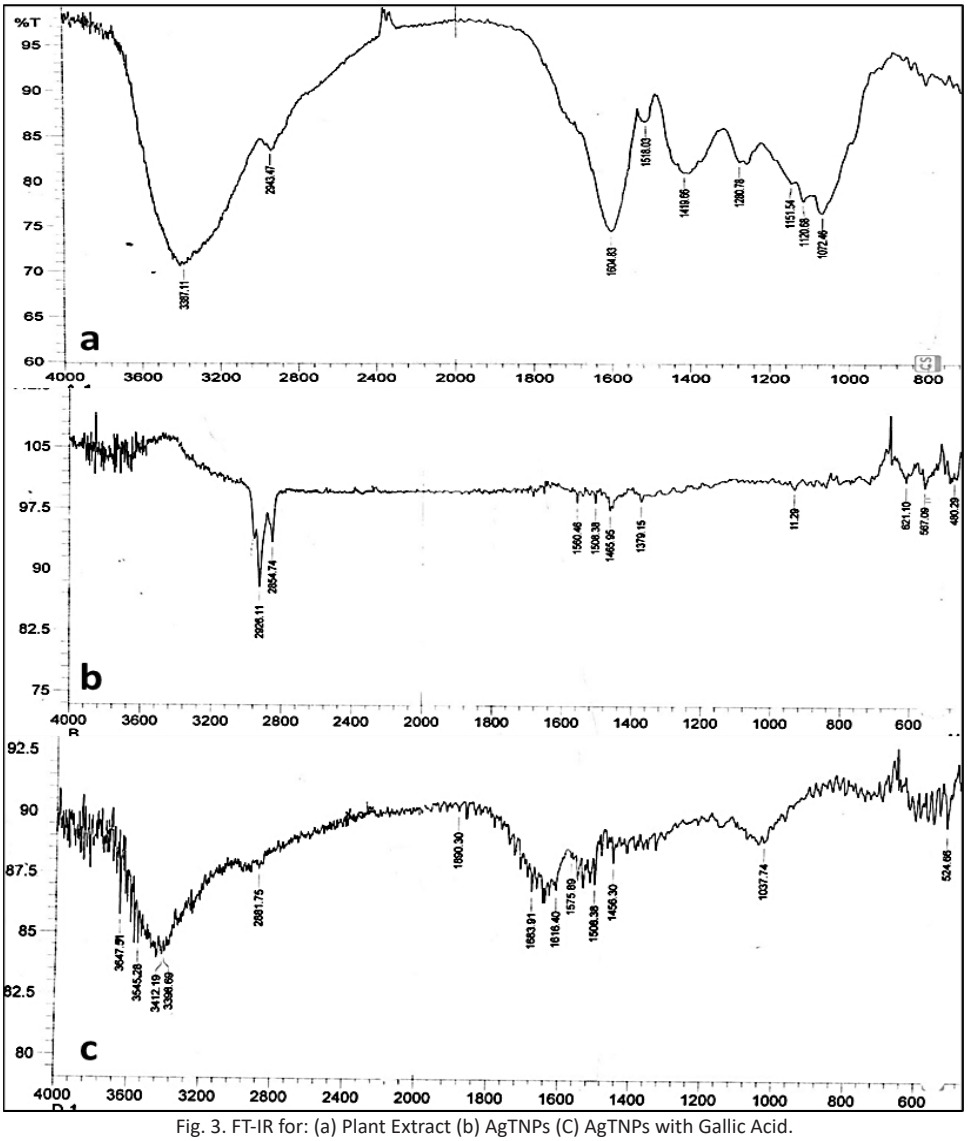


Fig. 3. FT-IR for: (a) Plant Extract (b) AgTNPs (C) AgTNPs with Gallic Acid.

64.89°, and 77.7°, corresponding to 111, 200, 220, and 311, respectively, with a big agreement with previous studies [23]. While XRD analysis for AgTNPs loaded with Gallic Acid, presented the peaks in patterns 2θ=38.03°,44.21°,64.38°, 77.33°, and 81.46° corresponding to 111, 200, 220, 311, and 222 may improve the modification of AgTNPs with Gallic Acid [35].

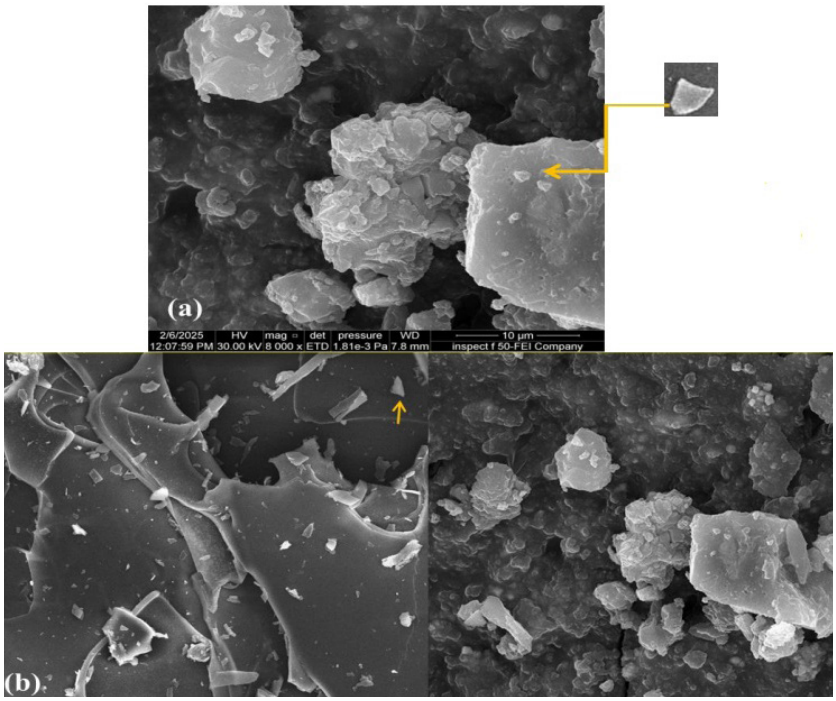


Fig. 4. SEM imaging of: (a) AgTNPs (b) AgTNPs with Gallic Acid.

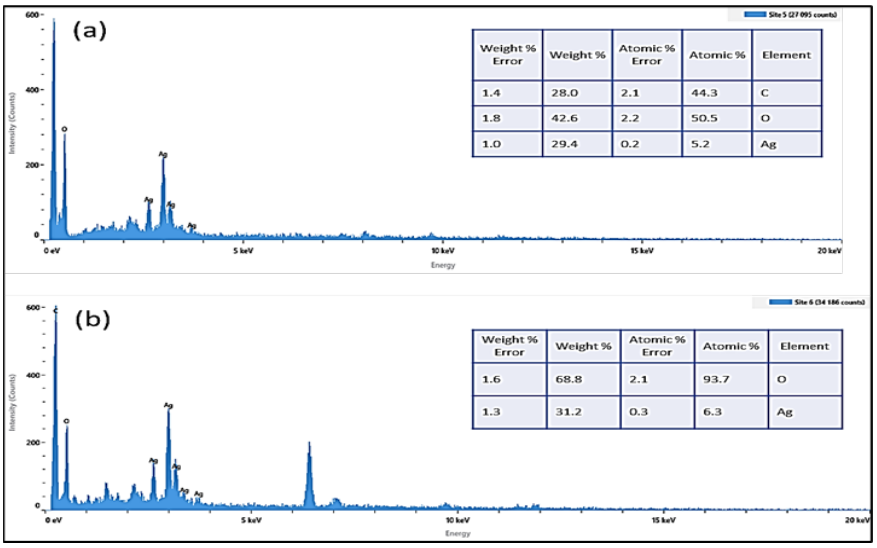


Fig. 5. EDX of: (a) AgTNPs (b) AgTNPs with Gallic Acid.

Antifungal Activity

Triangle silver nanoparticles AgTNPs could be an excellent candidate for clinical antifungal agents due to their extensive antimicrobial efficacy. The agar-well diffusion technique was used to assess the activity of AgTNPs on *Fusarium spp* strain, and the results showed that synthesized AgTNPs had potential antifungal action against the tested

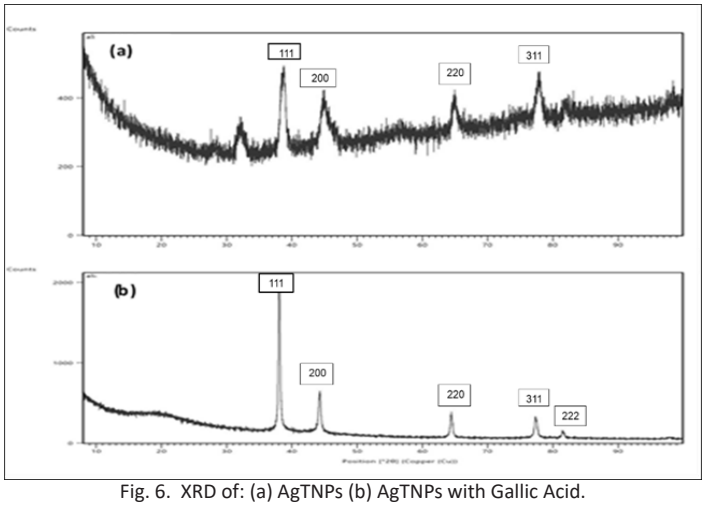


Fig. 6. XRD of: (a) AgTNPs (b) AgTNPs with Gallic Acid.

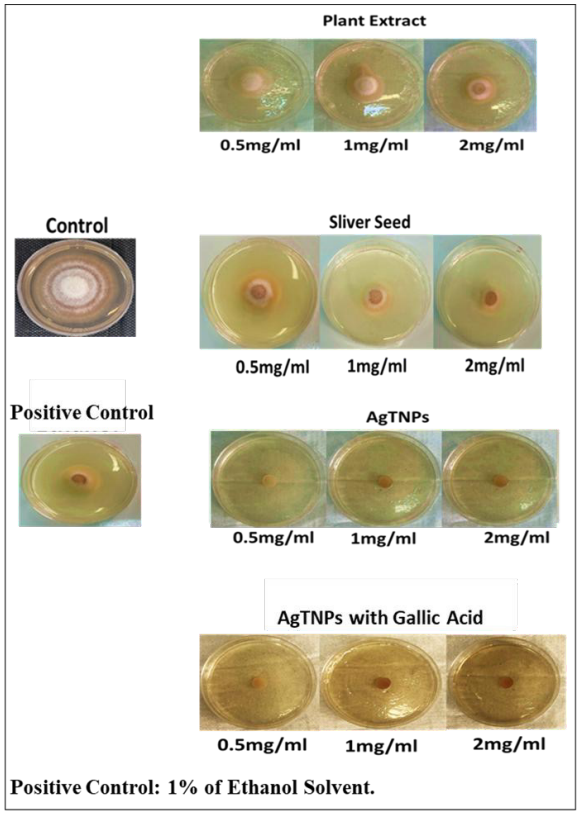


Fig. 7. Antifungal activity for plant extract, Ag seeds, AgTNPs, and AgTNPs with Gallic Acid

fungal strains. This may be due to their ability to limit fungal development by separating the cell membrane from the cell wall and intruding into the cytoplasm, resulting in the loss of cell wall integrity and homogeneity [36].

The results of this study indicated that the mycelial growth of *Fusarium spp*. The strain in PDA medium was significantly inhibited by AgTNPs with Gallic Acid, with an inhibition rate of 97% regardless of the highest concentration, as illustrated in Fig. 6. Also, AgTNPs at (0.5, 1, and 2 mg/mL) caused an inhibition rate of 95% at each concentration. Whereas plant extract at concentrations (0.5, 1, and 2 mg/mL) inhibited 43%, 47.5%, and 55% respectively. Also, Silver Seeds at concentrations (0.5, 1, and 2mg/mL) caused 52.5%, 65%, and 66% respectively. Mycelial growth was inhibited in the PDA medium. The inhibition of mycelium growth in the PDA medium escalated with rising concentrations of AgTNP. According to the results of this study, previous research has indicated that AgTNPs can function as antifungal agents for reducing several fungal plant diseases [37].

CONCLUSION

The development of a highly efficient, environmentally sustainable, and cost-effective biosynthetic method for nanoparticles is becoming essential for nanotechnology research and applications. This study presents a promising green synthesis technique for AgTNPs with notable antifungal activity. Garden cress seed extract was used to synthesize AgTNPs with improved functionality. The synthesized AgTNPs have a triangular structure and uniform dimensions. The nanoparticles undergo comprehensive characterization by UV-Vis and Fourier transform infrared spectroscopy, confirming their stability and identifying their functional groups. The average size of the prepared AgTNPs and the crystallinity were evaluated using XRD, SEM, and EDX. Based on the findings, the results suggest that AgTNPs are an excellent option for viral disease treatment. This work may hold a significant potential for further exploration in agricultural or biomedical antifungal treatments. Further studies may be required to confirm these findings and explore other potential treatment options for different diseases.

ACKNOWLEDGMENTS

The authors express thanks to the administrators of Babylon University, College of Science, Chemistry Department, for supplying the necessary facilities and equipment.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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