Research Article

Green Synthesis of Silver Nanoparticles from Endophytic Fungus *Alternaria carthami*-KUMBMDBT-30

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ABSTRACT

In this study, an extract from the *Alternaria carthami*-KUMBMDBT-30 strain was used to make silver nanoparticles in a way that was both cheap and good for the environment. Among other spectroscopic tools, the Bio-spectrophotometer, FTIR, SEM-EDAX, XRD, and DLS were used to look at how Alt-AgNPs form. Fourier Transform Infrared Spectroscopy (FTIR) analysis showed that there were peaks at 3739˚, 3260˚, 2903˚, 2845˚, 2067˚, 1620˚, 1524˚, 1219˚, and 1025˚ cm^{-1} that were related to different functional groups. SEM (Scanning Electron Microscopy) analysis showed that Alt-AgNPs (*Alternaria carthami*- synthesized silver nanoparticles) formed in a shape that was almost spherical and uniform. Energy Dispersive Analysis of X-ray (EDAX) was used to figure out what the Alt-AgNPs were made of. X-ray Diffraction (XRD) analysis at 2Ø degree values can be linked to the (111), (200), (220), (311), and (222). The distributed Alt-AgNPs sizes were measured with Dynamic Light Scattering (DLS), which showed that they were 97.15 nm in size. The studies results show that Alt-AgNPs might be useful in biomedical applications.

Keywords: Endophytic fungus, *Alternaria carthami,* Alt-AgNPs.

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INTRODUCTION

Nanotechnology in material science creates, synthesizes, and manipulates mass matter, molecules, and particles. NPs have 20–15000 atoms and are less than 100 nm.[1] Nanotechnology uses have grown in significance since nanoscale materials advanced science and humanity. Nanomaterials have unique mechanical, thermal, physiochemical, and biological qualities due to their large surface area-to-size ratio and quantum effect.[2] There are several chemical and physical methods for synthesizing AgNPs. (Laser ablations, gamma radiation assisted, polyol assisted, chemical reduction, thermal decomposition, etc.). Physical and other methods harm humans and the ecosystem. Biological NPs synthesis is fast, cheap, safe, and consistent with life. Endophytic fungus-mediated NPs synthesis is unique due to its fast growth, non-toxic biological components, ease of cultivation, upkeep, and handling. Fungus phytochemicals inhibit NPs clustering, capping, and stabilization.[3]

In "nanofactories," fungi can easily produce huge amounts of biomass and extracellular metabolites. These proteins reduce metal ions and cap AgNPs, increasing particle size and stability.[4] Endophytic bacteria, fungi, and actinomycetes produce lots of active compounds. Endophytic microbes can create AgNPs of various sizes, morphologies, and stabilities. Endophytic fungi produce more compounds and have more active metabolites than non-endophytes. This may

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be true for both compound secretion and metabolite action. Thus, these fungal species and their metabolites must be studied for medical and biotechnological uses.[5] Recent studies show that fungi can synthesize metal NPs intracellularly or extracellularly using reducing enzymes. The described fungal species created large, shaped NPs from fungal biomass or cell free extract, and many fungus-coated metal NPs have been synthesized. The endophytic fungus *Penicillium oxalicum* strain LA-1 culture filtrate produces AgNPs with antimicrobial, cytotoxic, and larvicidal properties.[6] The endophytic fungus *Lasiodiplodia pseudotheobromae* from *Eupatorium triplinerve* was used to synthesize extracellularly fungal extract-mediated AgNPs, which have substantial antibacterial and toxicological activities.[7] The endophytic fungus *Colletotrichum incarnatum* DM16.3 from *Datura metel* was used to test AgNP cytotoxicity and phytotoxicity.[8] Two endophytic fungi from *Gloriosa superba* L, *Alteraria solani* GS1 and *Penicillium funiculusum* GS2, produce AgNPs with average diameters of 5-20 and 5-10 nm and show significant antibacterial sensitivity in an antimicrobial susceptibility test.[9] According to the literature, various endophytic fungi (*Botryodiplodia theobromae*, *Alternaria alternata*, *Phoma* sp., *Trichoderma atroviride*, *Aspergillus flavus*, *Aspergillus niger*, *Trichoderma reesei*, and *Penicillium crustosum*) isolated from plants have various biological activities such as antimicrobial, antioxidant, anticancer, acaricides, photocatalytic, anti-candida, mosquitocidal activity has been reported.[3-5,10-13] The current study used *Phoenix sylvestris* endophytic fungus *Alternaria carthami* to synthesize silver nanoparticles (Alt-AgNPs) in an environmentally friendly way. Bio-spectrophotometer, FTIR, SEM, EDAX, XRD, and DLS were used to characterize synthesized Alt-AgNPs.

MATERIALS AND METHODS

Isolation, identification and molecular characterization of endophytic fungus

Phoenix sylvestris healthy tissue (leaf) was collected in the Chitradurga region of Karnataka, India. To sterilize the tissue, the method described in[14,15] was used. The plant material was washed thoroughly under running water, then surface-sterilized by immersing in 70% ethanol for 3 min, then in sodium hypochlorite for 1 min, and lastly in 75% ethanol for 30 sec. After being washed three times with sterile distilled water, sterile samples were dried in a laminar air flow setting. The plant samples were cut into uniformly sized sections and spread out on agar plates containing streptomycin (1 mg/L, Himedia Pvt. Ltd., India) and potato dextrose agar (PDA, Himedia Pvt. Ltd., India). The purpose of this action was to prevent the growth of endophytic bacteria. Plant tissue samples cultured on potato dextrose agar and kept at 28 degrees Celsius. Endophytic fungus was cultured from hyphal tips that were transferred to new dishes of potato dextrose agar medium after incubation results were analyzed. It was necessary to do this so that the endophytic fungus could be cultivated in sterile conditions. The fungal isolates genomic DNA was extracted using a kit from Qiagen (USA), and the procedure followed the manufacturers guidelines.[7,9,16] Both ITS15'-CCGTAGGTGAACCTGCGG-3' and ITS4 5'-TCCTCCGCTTATTGATATGC-3' were used as primer pairs in the PCR. Purification of the PCR products was accomplished with the help of a gel extraction reagent. The obtained sequence was analyzed with nBLAST, and sequences that were very closely related to the one that was obtained were found in NCBI (http://www.ncbi.nlm.nih.gov) via a homology search. Multiple genome alignment was performed using MEGA-Version 7.0.14, and a phylogenetic tree was constructed using the Neighbor-Joining (NJ) method.

Extracellular green synthesis and characterization of synthesized Alt-AgNPs

The endophytic fungus *Alternaria carthami* was kept in Potato Dextrose Broth (PDB) at 28±2°C for 7–14 days. After incubation, the biomass was passed through Whatman no. 1 filter paper to make Alt-AgNPs and study them. Then, 1 mM of silver nitrate was added to a volume (1:1) of fungal filtrate of the same size and held in the dark at 28±4°C for 24 hr. After 24 hr, the cell filtrate solution with silver nitrate changed from light brown to dark brown in absorption spectrum. Alt-AgNPs were made. Written spectrum between 200 and 700 nm. The endophytic fungus synthesized Alt-AgNPs, which were cleaned by spinning a solution at 15,000 rpm for 15 min three times with a continuous washing pellet holding sterile distilled water. FTIR, SEM-EDAX, XRD, and DLS were performed on the pure powder after drying it in a hot air oven at 50°C for 24 hr.[17,18] FTIR investigated functional groups that could synthesize and stabilize Alt-AgNPs. The FTIR spectrum (Perkin-Elmer model) was recorded from 500 to 4000 cm^{-1} with 2 cm^{-1} precision. Synthesized Alt-AgNPs were examined using SEM-EDAX. Rapid diagnostic device cell measurements and crystalline structure were examined using X-ray diffraction. The size of spread-out Alt-AgNPs was measured using the Malvern Zeta Sizer Nano series compact scattering spectrometer and Dynamic Light Scattering (DLS) method.

RESULTS

Isolation, identification and molecular characterization of endophytic fungus

Phoenix sylvestris leaf samples used in traditional medicine yielded endophytic fungus. (Figure 1.a, b). Endophytic fungus was pale yellow color. (Figure 1.c,). Microscopic examination (Figure 1.d) and 18s rRNA gene PCR amplification with ITS1 and ITS4 primers confirmed this. Sanger dideoxy nucleotide sequencing of the expanded ITS region found the 18s rRNA gene. Multiple sequence alignment and pairwise nBLAST results showed identity with *Alternaria carthami*-KUMBMDBT-30, strain MW147606 in the NCBI Gen Bank. From the DNA data, MEGA-Version 7.0.14 constructed a neighbor-joining phylogenetic tree. (Figure 2).

Characterization of synthesized Alt-AgNPs

The reduction of silver ions to AgNPs in the reaction mixture from light brown to dark brown showed the efficacy of Alt-AgNPs produced by the endophytic fungus *Alternaria carthami*. Reducing silver ions to Alt-AgNPs caused this color change. (Figure 3.a and b). Synthesized Alt-AgNPs were analyzed using the bio-spectrophotometer. The endophytic fungus *Alternaria carthami* produced Alt-AgNPs with a Surface Plasmon Resonance (SPR) peak near 415 nm. (Figure 3.c). Silver nitrate has been reduced to create Alt-AgNPs. The bioreduction of silver ions into Alt-AgNPs demonstrated that external proteins released into the colloidal solution may be involved in the process. Silver ions and bioactive components in endophytic fungi *Alternaria carthami* extracts were studied using FTIR. These silver-interacting bioactive components stabilize Alt-AgNPs. Figure 4 displays the FTIR spectra of synthesized Alt-AgNPs. The peak at 2903, 2845 cm^{-1} is associated with the presence of stretching vibrations in the methyl C-H asym group, and the band at 3260 cm^{-1} is ascribed to the hydroxy group, H-bonded OH stretch. In this case, the NH length of aromatic primary amines was responsible for the 3739 cm^{-1} peak. The transition metal carbonyl peak is at 2067 cm^{-1}, the main amine, NH bend is at 1620, 1524, cm^{-1} and the aromatic

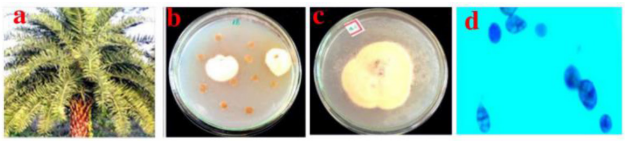

Figure 1: (a) *Phoenix sylvestris* medicinal plant, (b) Endophytic fungus grown from surface sterilized leaf segment of *Phoenix sylvestris* on PDA media after 4 to 7 days, (c) Colony morphology of *Alternaria carthami*, (d) Microscopic view of endophytic fungus *Alternaria carthami*.

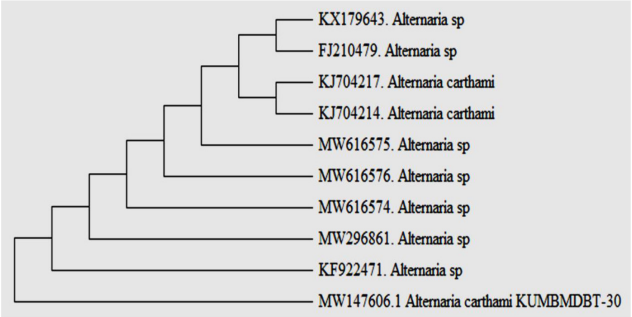

Figure 2: Phylogenetic analysis of *Alternaria carthami* strains with ITS sequences of closely related fungal strains.

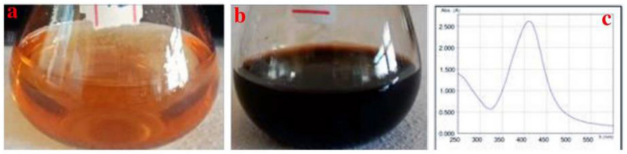

Figure 3: (a) Cell filtrate of endophytic fungus *Alternaria carthami*, (b) Color change to reddish brown after treating with 1mM $AgNO_3$. (c) Bio-spectrophotometer of green synthesized Alt-AgNPs.

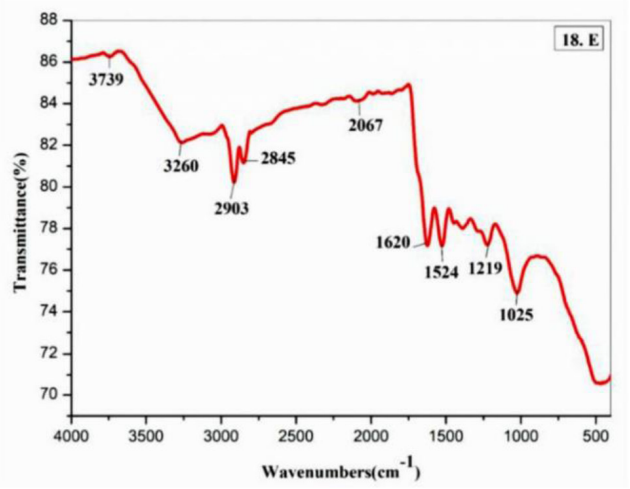

Figure 4: FTIR pattern of green synthesized Alt-AgNPs.

C-H in-plane bend is at 1219,1025 cm^{-1}. FTIR showed multiple secondary metabolites, compounds, and proteins are likely involved in Alt-AgNP formation. The experiment also showed that proteins cap Alt-AgNPs, preventing aggregation and formulation stabilization. SEM was used to analyze Alt-AgNPs created specially. The endophytic fungus *Alternaria carthami* synthesized high-density Alt-AgNPs to show surface morphology and form characteristics using SEM. Alt-AgNPs made nearly spherical and uniformly. (Figure 5.a, b, c). The endophytic fungus *Alternaria carthami* nanoparticles energy dispersive analysis of X-ray implies silver is the main element. Surface plasmon resonance gives

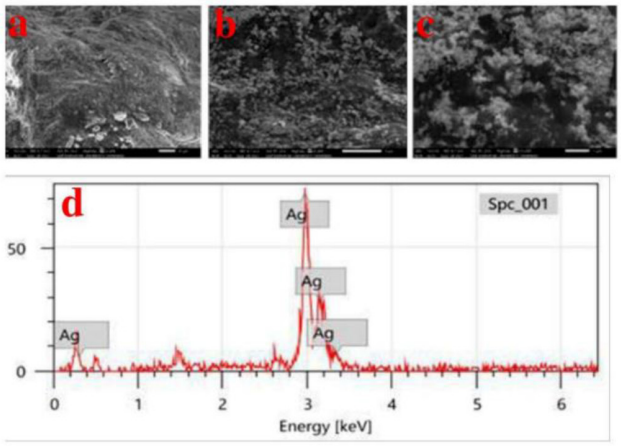

Figure 5: (a), (b) and (c) SEM images of green synthesized Alt-AgNPs. (d) EDX pattern of green synthesized Alt-AgNPs.

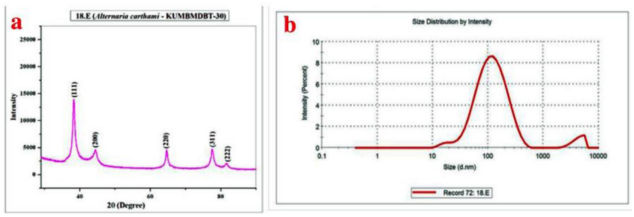

Figure 6: (a). XRD pattern of green synthesized Alt-AgNPs. (b). DLS pattern of green synthesized Alt-AgNPs.

metallic Alt-AgNPs a strong 3 keV signal peak. Silver atoms emit strong signals, while carbon and oxygen atoms emit lesser signals (Figure 5.d). XRD analysis indicated synthesized Alt-AgNP crystallinity. The two-angled planes (111), (200), (220), (311), and (222) had X-ray diffraction peaks at 38.33°, 38.34°, 76.67°, 78.30°, and 89.69°, respectively. (Figure 6a). All silver Face-Centered Cubic (FCC) lattice phase peaks were in the usual JCPDS data (File No 87-0719). Alt-AgNPs have strong peaks at 2 Ø angles because they are made with silver nitrate. Nanoparticle generation amplifies Bragg's peaks. X-ray diffraction shows bioorganic phase crystallization in Alt-AgNPs. As a result of particle size analysis, the hydrodynamic radii or sizes of the synthesized Alt-AgNPs was determined. Dynamic light scattering was used to determine that the average size of the synthesized Alt-AgNPs was 97.15 nm. A single peak was found, indicating that the synthesized Alt-AgNPs are of high quality (Figure 6b).

DISCUSSION

Phoenix sylvestris, commonly called Indian date, is a date palm native to India and southern Pakistan. "Indian date" describes it. Its history and dietary benefits are known worldwide. This plant is rich in carbohydrates, phenols, amino acids, flavonoids, tannins, alkaloids, terpenoids, food fibers, vitamins, and minerals. The plants components have antipyretic, cardiotonic, laxative, diuretic, and antioxidant actions.[19] *Alternaria carthami* is found in *Phoenix sylvestris* fronds. Morphological and ITS rDNA studies identified the endophyte as *Alternaria carthami*. Most endophytic fungi are characterized molecularly using ITS regions.[20] The endophytic *Alternaria carthami* was confirmed by phylogenetic analysis and the similarity between the reference sequence and the strain isolated from leaf tissue. Endophytic fungi are renewable, easily accessible, economical, and ecologically sound sources of physiologically active natural products, which is one of their main benefits.[21] Fungi are potent biological agents for mycogenic metal nanoparticle synthesis, according to a review of related research. Fungal filtrate enzymes and proteins that change Ag to Ag0 are needed for mycogenic AgNP synthesis. Most fungal filtrates contain reducing agents like proteins, alkaloids, and phenolic compounds, which may promote mycogenic AgNP synthesis.[22] *Alternaria* sp. reduces silver more effectively than NPs. The spectral studys standardization part included periodic spectroscopy. The standard surface plasmon resonance peak of mycosynthesized AgNPs had maximum absorption at 450 nm. The absorbance wavelengths range from 400 to 450 nm, with a specific absorbance peak exhibiting an increased nanoparticle concentration.[23] This work investigates synthesized Alt-AgNPs using bio-spectrophotometer. Alt-AgNP characterization. Silver nitrate has been converted to Alt-AgNPs because their SPR peak was near 400 nm. FTIR spectroscopy helps stabilize gold and silver nanoparticles by identifying and classifying potential capping proteins.[24] FTIR investigation examined the interaction of silver ions with bioactive components in *Alternaria carthami* fungal preparations that stabilize Alt-AgNP. Because it is easy to prepare samples and record images, Scanning Electron Microscopy (SEM) may help determine nanoparticle dimensions and shape. SEM images are two-dimensional (2D) representations of three-dimensional (3D) objects from a certain viewing point, but they contain 3D information that can be used with model-based measurement to reconstruct a basic structure with sub-nm accuracy.[25] SEM frequently employs EDAX. The conducting sample is hit by a 10–20 keV electron beam. This emits X-rays whose energy depends on the substance being examined. EDAX can detect the composition or quantity of nanoparticles near and at the surface because they contain heavy metal ions. EDAX very quickly and accurately detects silver, gold, and palladium nanoparticles.[26] XRD may assess single crystals and

numerous elements. X-rays are reflected into the sample and watched as they scatter atoms. The trial sample is removed. The dispersed X-rays interfere. Bragg's Law shows this interaction in crystals and polycrystalline substances. The law determines material qualities.[27] Dynamic light dispersion precisely determines suspension particle sizes. It requires high particle densities but is fast and simple.[28] The current study's documented findings provide focus on the specific features of synthesized Alt-AgNPs.

CONCLUSION

Biological green Alt-AgNP synthesis is effective and safe. *Alternaria carthami* was found in *Phoenix sylvestris*. The fungus converted silver nitrate to high-quality crystalline Alt-AgNPs, which were characterized using a bio-spectrophotometer, FTIR, SEM-EDAX, XRD, and DLS. FTIR functional groups are capping agents. The Alt-AgNPs were 97.15 nm in size, nearly spherical, and extremely strong. EDAX determined Alt-AgNPs elemental compositions. Animal models validate biomedical uses in future research.

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CONFLICT OF INTEREST

The authors confirm that they have no known conflicts of interest or competing financial interests that could have influenced the work produced.

ABBREVIATIONS

FTIR: Fourier Transform Infrared Spectroscopy; **SEM:** Scanning Electron Microscopy; **EDAX:** Energy Dispersive Analysis of X-ray; **XRD:** X-ray diffraction DLS- Dynamic Light Scattering; **Alt-AgNPs:** *Alternaria carthami* synthesized silver nanoparticles.

SUMMARY

Phoenix sylvestris leaves were used to isolate a promising endophytic strain of *Alternaria carthami* (KUMBMDBT-30), which was then deposited in the gene bank with the accession number MW147606. It is simple to cultivate the endophytic fungus *Alternaria brassicae* in a lab setting for the fermentation-based generation of secondary metabolites. Alt-AgNPs were created using culture filtrate that had been harvested. The Alt-AgNPs were an excellent natural source of bioactive compounds that were thought to be a plentiful supply for drugs and as bio protectants. Because of these properties, silver nanoparticles are an excellent substitute for a variety of medical applications.

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