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Optimization and *In Vitro* Evaluation of Silver Nanoparticles for Enhanced Antioxidant and Antimicrobial Activities

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Abstract: Biologically synthesized nanoparticles are becoming important due to their eco-friendly nature and realistic applications. In the present study, silver nanoparticles (AgNPs) were synthesized using the cell-free filtrate from fungus *Aspergillus niger* and were characterized using different studies. UV-visible spectrum confirmed the formation of AgNPs. SEM analysis detected spherical nanoparticles ranging in size from 10-20 nm. FT-IR analysis confirmed the presence of different functional groups and their possible partnership in nanoparticle synthesis and capping. X-ray diffraction studies revealed a crystalline structure consistent with the peaks observed in XRD pattern, corresponding to 111, 200, 220 and 311 planes. Optimization studies identified the ideal parameters for nanoparticle synthesis to be 10 g/100 ml of biomass, 1 mM $AgNO_3$, pH 7 and a temperature of 30°C. The AgNPs displayed antimicrobial activity against pathogenic strains of *Salmonella typhi* and *Enterococcus faecalis* with zones of inhibition measuring 11 mm and 14 mm respectively. The scavenging effect of the AgNPs examined using DPPH and ABTS assays revealed the maximum inhibition to be 92.16% and 90.34%, respectively.

Keywords: AgNPs, Characterization, Optimization, Antimicrobial, Antioxidant

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Introduction

Nanotechnology is experiencing rapid growth owing to its broad spectrum of applications across scientific domains. The advancements in technology at nanoscale, have led to the creation of functional structures ranging from single atoms to complex systems that can be employed in varied sectors owing to their dynamic properties. The necessity for precise manipulation of materials and the evolution of nanotechnology has led to development of different methods to fabricate and customize nanomaterials. These nanoparticles play a vital role in the field of agriculture, medicine, fuel, bioremediation and food products (Nasrollahzadeh *et al*., 2019).

Nanoparticle synthesis via physical methods employing top-down approach, minimizes chemical usage and offers numerous benefits such as scalability and precise control over stability. However, they can consume significant amounts of energy and generate harmful by-products. On the other hand, chemical synthesis methods produce nanoparticles with more homogeneous composition and uniformity, but they often conflict with modern principles of green chemistry (Niculescu *et al*., 2022).

Nanoparticles synthesized from plants and microorganisms are biocompatible, cost-effective, and can be customized for sustainable applications (Sajid *et al*., 2020). Biological methods of nanoparticle synthesis present a unique opportunity to employ natural agents like plant metabolites and biological molecules as reducing and stabilizing agents (Patil *et al*., 2020).

Among the various inorganic nanoparticles, silver nanoparticles are highly valued as they demonstrate favourable optical, catalytic, thermal, and electrical properties. Their extensive application in the field of electronics, medicine, diagnostics, textiles, food and cosmetics underscore their potential and versatility (Dhaka *et al.*, 2023). AgNPs are highly researched for treating melanoma due to their ability to combat inflammation, free oxygen radicals, tumours and biological carcinogens (Zhao *et al*., 2023).

Several studies have explored the biological synthesis of silver nanoparticles from plant extracts, highlighting their potent antimicrobial activities (Mustapha *et al*., 2022). Similarly, the production of AgNPs from bacterial sources (*Bacillus spp.)* has shown promising antimicrobial, cytotoxic and environmental remediation capabilities (Alsamhary, 2020; Samuel *et al.*, 2020).

Free radicals or reactive oxygen species (ROS) are recognized as the primary contributors to a range of degenerative disorders including mutations, cancer, heart diseases, and aging. Antioxidants, which act as scavengers of free radicals, neutralize them by donating electrons. Endogenous antioxidants are produced within the body, while exogenous antioxidants are acquired from external sources. Hulikere *et al.* (2019) and Mistry *et al.* (2021) investigated the antimicrobial and antioxidant properties of silver nanoparticles (AgNPs) synthesized from *Aspergillus* and *Cladosporium spp*. Additionally, Chi *et al.* (2022) demonstrated the promising anticancer effects of silver nanoparticles derived from *Aspergillus spp*. against the HeLa cell line.

Efficient biological synthesis of silver nanoparticles (AgNPs) depends on optimizing synthesis parameters to ensure consistent and reproducible results. This optimization also enables customization of nanoparticle properties, enhancing their performance and facilitating cost-effective scale-up (Khan *et al*., 2021).

Reproducibility of results can be confirmed with the help of characterization techniques encompassing spectroscopic, microscopic and physiochemical methods. They help determine the presence, size, shape, crystalline structure, surface topography, stability and chemical properties of nanoparticles. Therefore, the key lies in identifying characterization techniques that have the optimal capacity to study the characteristics of nanoparticles (Titus *et al*., 2019).

The present study focused on optimizing the reaction parameters for improved synthesis of AgNPs from fungus *Aspergillus niger*. The size, morphology, functional groups and crystalline nature of the AgNPs were characterized by ultraviolet–visible spectroscopy (UV–vis), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction *(*XRD). Antimicrobial activity of the AgNPs was tested using agar well diffusion method. The antioxidant properties were analysed using DPPH and ABTS assays.

Materials and Methods

Synthesis of AgNPs using Aspergillus niger:

Biological nanoparticle synthesis was conducted using the fungus (DGV01), which had been isolated from soil sample, screened for silver nanoparticle synthesis, molecularly identified and deposited in NCBI GenBank under Accession Number OR484843 (Sharma *et al.,* 2024). Initially, 1 g of fungal mat (wet weight) was introduced into 10 ml of sterile double distilled water. After 48 h of incubation on a rotary shaker, the mixture was filtered, and the filtrate was subsequently reacted with 1 mM concentration of silver nitrate. This reaction was carried out at room temperature in dark conditions.

Characterization of the synthesized AgNPs:

UV-Visible spectroscopy:

The optical properties exhibited by AgNPs facilitate their interaction with light at particular frequencies. The synthesized AgNPs were monitored using UV-visible spectrophotometer by analysing the absorption spectra of the solution in the range of 300-700 nm (Barabadi *et al*., 2021).

Scanning electron microscopy (*SEM*):

The sample preparation involved dispersing the supernatant comprising of the AgNPs onto the substrate. Subsequently, the prepared sample was transferred onto the stub for analysis (Akther *et al*., 2019).

Fourier Transform Infrared Spectroscopy (*FT-IR*):

The mycosynthesized silver nanoparticles were freeze-dried. Sample preparation was carried out upon dilution with potassium bromide (KBr) in the ratio of 1:100. Pellets were formed using deionised water at high pressure. The spectral bands were recorded using FTIR spectrometer (Nicolet 6700, Thermo Fisher Scientific) with a diffuse reflectance mode (DRS-8000) attachment. All measurements were carried out in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} (Win *et al*., 2020).

X-ray diffraction studies (*XRD*):

The XRD spectrum of the freeze dried AgNPs in pellet form was recorded with the help of an X-ray diffractometer. Operating parameters of CuK_{α} radiation at a wavelength of 1.5406 Å in the 2θ range with current of 100 mA and voltage of 30 kV were maintained (Mistry *et al.*, 2021).

Optimization studies for AgNP synthesis:

Optimization studies were conducted to explore the influence of pH, temperature conditions, fungal biomass quantity, and $AgNO_3$ concentration on nanoparticle synthesis. For optimization studies, the samples were incubated in darkness at room temperature for 48 h. After the incubation period, UV-visible spectroscopy was employed to identify the ideal conditions for synthesizing AgNPs (Shahzad *et al*., 2019).

Effect of biomass concentration on AgNP synthesis:

Varying concentrations of fungal biomass (10 g, 20 g and 30 g) along with different concentrations of silver nitrate was added to individual 250 ml Erlenmeyer flasks containing 100 ml of distilled water.

Effect of silver nitrate concentration on AgNP synthesis:

The effect of $AgNO_3$ concentration on AgNP synthesis was investigated by exposing the cell free filtrate (neutral pH) of optimized fungal biomass with $AgNO_3$ concentrations ranging from 1- 10 mM.

Effect of pH on AgNP synthesis:

To determine the optimal pH for synthesizing stable nanoparticles, the pH of the reaction mixture was adjusted across a range from pH 1 to pH 10 with the help of 0.1 N solutions of NaOH and 0.1 N HCl.

Effect of temperature on AgNP synthesis:

To identify the ideal temperature for nano synthesis, the reaction mixture (with biomass, $AgNO_3$ and pH maintained at optimum levels) was taken in different test tubes and incubated separately in water bath at temperatures of 35°C, 45 °C and 55 °C.

Antimicrobial activity of the AgNPs:

To determine the antimicrobial effect of the synthesized AgNPS, agar well-diffusion technique was performed using Mueller-Hinton plates (Alsamhary *et al*., 2020). Four wells loaded with 30 µl of 1 mM of silver nitrate solution, mycelial free extract, 1 mg/ml of streptomycin and the synthesized nanoparticles, respectively were tested for their activity against pathogenic *Salmonella typhi* ATCC 6539 and *Enterococcus faecalis* ATCC 29212 spread on the agar plate. The plates were incubated at 37℃ for 24 h and checked for the zone of inhibition.

Antioxidant activity of AgNPs:

For the preparation of sample for DPPH and ABTS assay, different concentrations of the mycosynthesized AgNPs (10 to 100 µg/ ml) prepared in methanol were used and tested individually for their antioxidant property by DPPH (1,1-diphenyl-2- picrylhydrazyl) and ABTS (2,2 -azino-bis-3- ethylbenzothiazoline-6-sulfonic acid) assay. The free radical scavenging activity, was used to determine the potency or the concentration (IC_{50} value) of the AgNPs required to inhibit 50% of the DPPH or ABTS molecules.

DPPH free radical scavenging activity of AgNPs:

The assay was carried out based on the methodology outlined by Keshari *et al.* (2020) with minor modifications. Gallic acid served as the standard. In a 96-well plate, to 100 µl of the individual test samples, 100 µl of DPPH (0.2 mM dissolved in methanol) was added. The contents were mixed well and incubated in dark conditions for 15-20 min at room temperature. The reaction was observed for colour change and absorbance was read at 517 nm. DPPH without any added sample served as control, methanol was used as blank.

ABTS assay:

For preparation of ABTS, 7 mM of ABTS was mixed with 15 ml of double distilled water and 264 µl of 140 mM of potassium persulphate and stored for 16 h in dark conditions. The mixture was then diluted with methanol and used further. To 50 µl of the test sample, 100 µl of ABTS working solution was added and stirred thoroughly. This was followed by incubation in dark for 10-15 min at room temperature and measured absorbance at 734 nm (Zhai *et al.*, 2017).

Free radical scavenging percentage was calculated as:

Free radical scavenging percentage = [(Ac-As) / Ac)] * 100

Where, Ac=absorption of control without test (i.e.) DPPH or ABTS alone, As= absorption of test samples (AgNPs).

Results and Discussion

Characterization of nanoparticles at primary level can be attained using UV-Visible spectroscopy. In case of AgNPs, when the intrinsic frequency of free moving electrons in the closely spaced conduction and valence bands matches the frequency of incident light, unique absorption and scattering phenomena occurs. The free electrons oscillate on the nanoparticle surface, creating a resonance called surface plasmon or Mie resonance (Patil *et al.*, 2021).

A prominent plasmon band nearing 430 nm was detected by UV-visible spectroscopy (Fig. 1), indicating surface plasmon resonance and reduction of silver ions in the aqueous phase. Similar findings were reported by Win *et al.* (2020) and Sharma *et al.* (2022) in their study utilizing other fungi.

The SEM studies examined the surface morphology of the AgNPS (Fig. 2). This analysis involved focusing an electron beam onto the sample surface and detecting emitted signals, comprising secondary and backscattered electrons. The observations revealed spherical scattered nanoparticles in the range of 10-25 nm. Comparable results were also documented by Chi

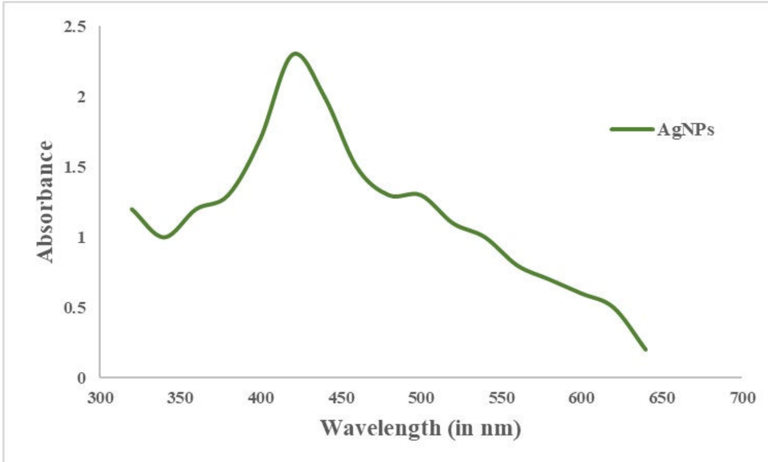

Fig. 1: UV Visible spectrum of AgNPs.

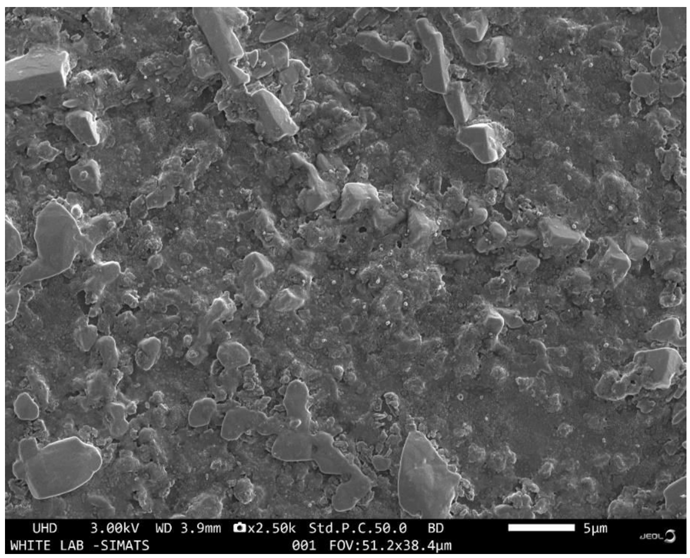

Fig. 2: SEM micrograph of the mycosynthesized AgNPs.

et al. (2022).

The investigation to understand the involvement of functional groups or biological molecules in the reduction of silver nitrate to silver was carried out using FT-IR analysis (Patil *et al*., 2021). The position and intensity of the peaks can differ based on the synthesis approach, nature of stabilizing agents, size and shape of nano-particles. As depicted in Figure 3, the presence of peaks at 3270 cm^{-1} represents a spectrum of hydroxyl groups with a O–H stretch. The vibrant stretches at 2933.61 cm^{-1} and 1456.87 cm^{-1} can be assigned to C-H stretch of aldehydes. Absorption bands at 1074.37 cm^{-1} and 1238.84 cm^{-1} signify C-N stretch of aliphatic amines while 1536.49 signal N-O asymmetric stretch with nitro compounds. The absorption peak at 1635.07 can be allotted to an amide as such peaks reflect N-H bend of primary amines (Elamawi *et al*., 2018).

This analytical method can help identify possible interactions between the AgNPs and bioactive compounds which can influence the production, stability of the nanoparticles as a capping agent. Overall, the FTIR analysis validated the presence of hydroxyl (-OH) and amine (-N-H) functional groups, which are responsible for the

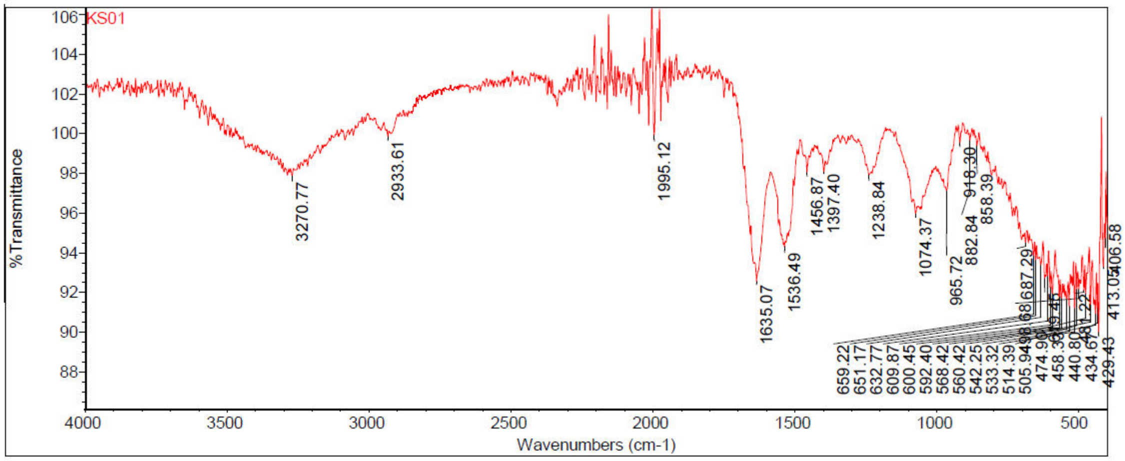

Fig. 3: FT-IR spectra of AgNPs.

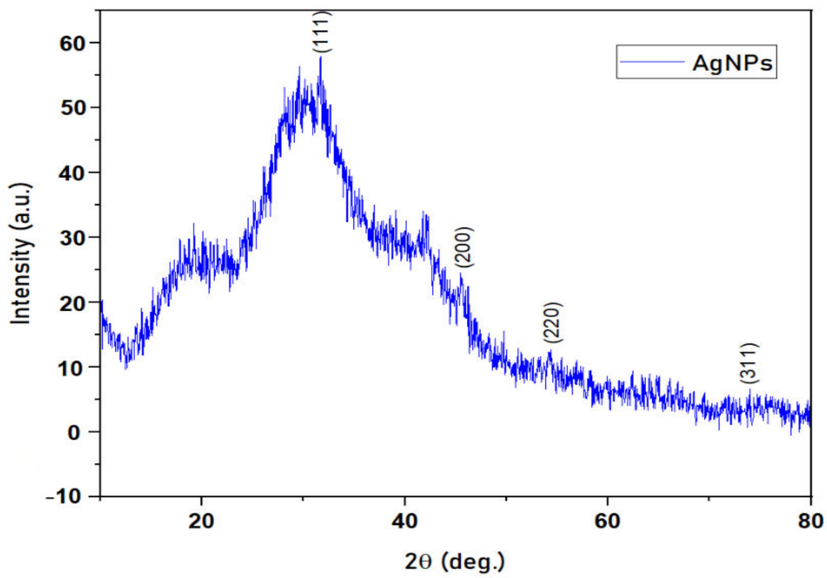

Fig. 4: XRD pattern of AgNPs.

reduction of Ag^{+} ions to Ag0. These groups also function as stabilizing agents or capping agents by preventing aggregation due to adsorption (Hamad, 2019; Ameen *et al*., 2023).

Figure 4 displays the distinctive XRD patterns of the mycosynthesized AgNPs. The peaks corresponding to 111, 200, 220, and 311 appearing at 2θ representing the value of 37.96°, 45.65°, 54.27° and 74. 35°, respectively. The broad pattern of the XRD diffraction peaks can be attributed to the nanocrystalline nature of the mycosynthesized AgNPs. These values of the XRD plot closely correspond to the data for pure silver nanoparticles in the JCPDS (Joint Committee on Powder Diffraction Standards) file no. 04-0783 database. This provides insight into the crystalline structure, enhancing understanding of the physical characteristics of the produced nanoparticles. Comparable results were reported earlier by Awad *et al.* (2022).

Parameters like nanoparticle morphology, size, stability and physiochemical properties require optimization as they can control the efficiency of the synthesized nanoparticles. In the course of optimization studies, the findings were established through the results of UV spectroscopy

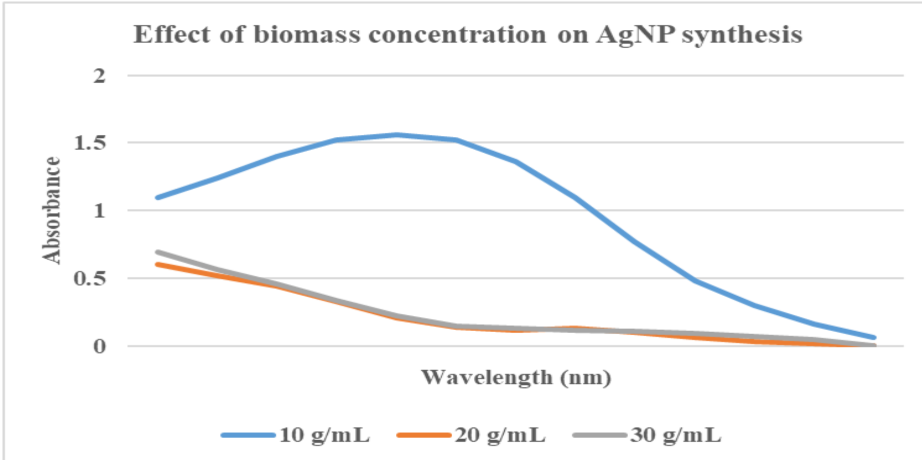

Fig. 5: Effect of biomass concentration on AgNP synthesis.

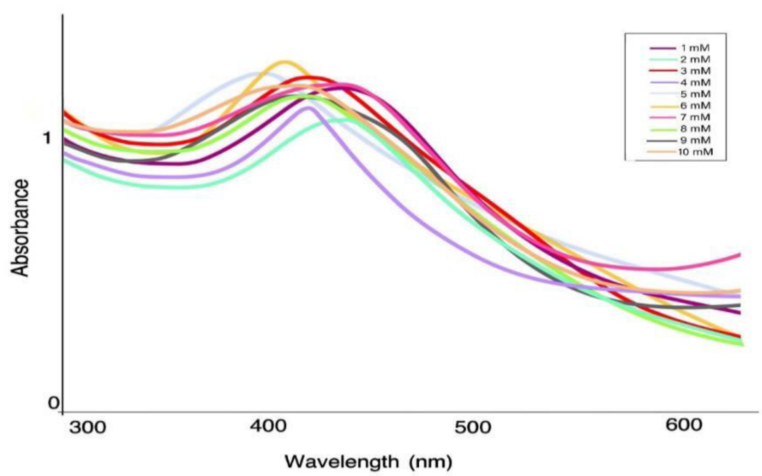

Fig. 6: Effect of silver nitrate concentration on AgNP synthesis.

analysis.

Maximum synthesis of AgNPs was supported at biomass concentration of 10 g/100 ml (Fig. 5). At concentrations higher than 10g/100 ml, synthesis failed to occur owing to excessive amount of reducing agents (Phanjom *et al*., 2017).

Nanoparticle synthesis was observed at all concentrations of silver nitrate (Fig. 6). Molar concentration of 1 mM is optimum as it is comparatively less toxic and supports synthesis and monodispersity of AgNPs. Comparable results were reported by Osorio *et al.* (2021).

The fungal cell-free filtrate when treated with 1 mM of $AgNO_3$ solution at different pH levels, it revealed that at very acidic pH values ranging between 1-3, no discernable colour change was observed. The colour change in the filtrate is due to surface plasmon resonance of the synthesized AgNPs. A very slight colour change was present at pH 4 and 5 which gradually subsided. At pH values above 6, a steady transition to brown colour occurred within 10-12 h of the reaction, indicative of nanoparticle synthesis. The colour intensity increased with increasing pH values (Fig. 7). Shift of absorption peaks towards the UV region further supported the theory of nanoparticle synthesis.

It can be interpreted that at alkaline pH, the hydroxide ions improve the reduction capacity of the proteins involved in the synthesis, enabling

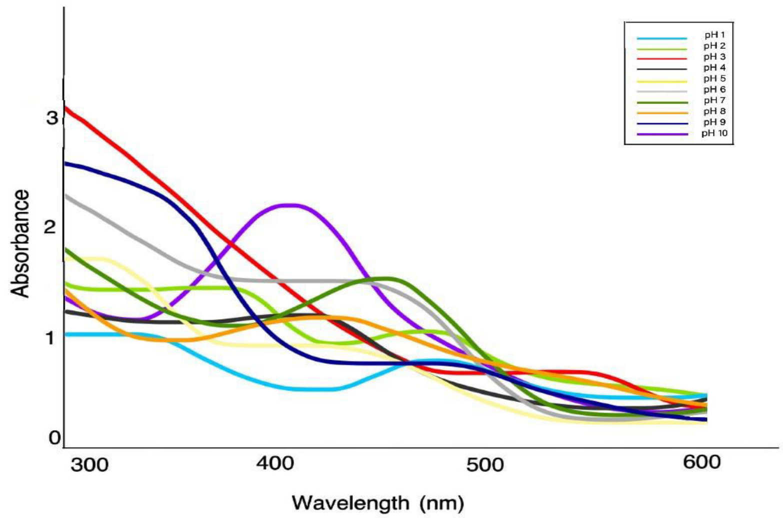

Fig. 7: Effect of pH on AgNP synthesis.

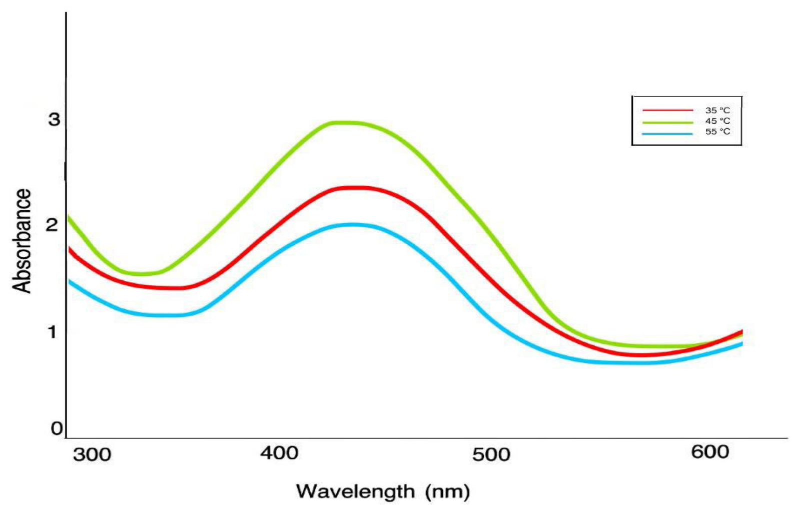

Fig. 8: Effect of temperature on AgNP synthesis.

them to function as reducing agents to facilitate the reduction reaction of metal ions. Similar findings were previously documented by Phanjom *et al.* (2017) and Othman *et al.* (2019).

Nanoparticle aggregation was observed between pH 4 and 5 indicating larger sized nanoparticles. At pH 7, nanoparticles exhibited good monodispersity and stability, as evidenced by a consistent absorbance peak. Prevention of aggregation at alkaline pH ranges is linked to the deprotonation of all functional groups on the nanoparticles, resulting in the nanoparticles carrying negative surface charges (OH^-) ions, which promote electrostatic repulsion between them. Previous studies reported similar results (Gontijo *et al.*, 2020; Alves *et al.*, 2022).

Temperature is another crucial factor that can affect the formation and stability of nanoparticles. When reaction was performed at different temperatures, it was noticed that synthesis occurred at all temperature ranges (Fig. 8). Post 3-4 h of synthesis, agglomeration occurred in the

Table 1: Antimicrobial activity of the of the biologically synthesized AgNPs against the test strains

Organism	Mycelial free extract	$AgNO_3$ solution (1 mM)	Ag Nps (1mg/ml)	Standard antibiotic (1mg/ml)
	Zone of inhibition (in mm)			
Salmonella typhi ATCC 6539	-	9	11	15
Enterococcus faecalis ATCC 29212	-	8	14	17

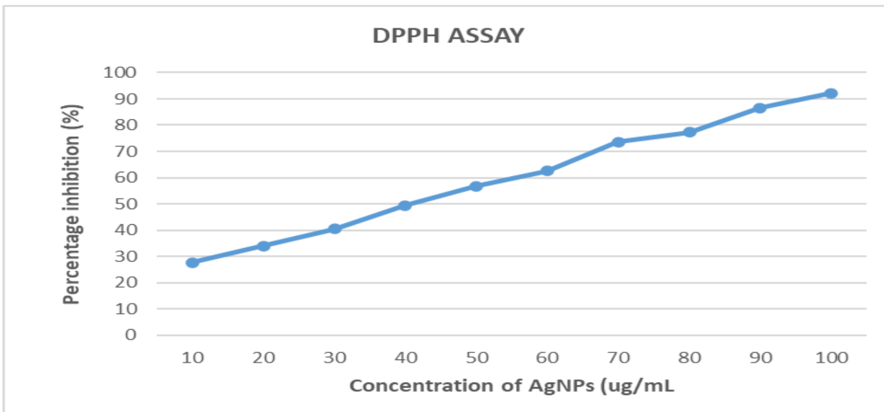

Fig. 9: DPPH free radical scavenging activity.

nanoparticles synthesized at higher temperatures. Temperature of 35 °C was correlated to be optimum for stable AgNPs formation. The obtained results can be correlated to the results of Feroze *et al.* (2020) and Al-Soub *et al.* (2022), who concluded that a temperature of 30 °C and 33 °C resulted in optimum yield of AgNPs from *Penicillium* and *Aspergillus* strains. In contrast, Soleimani *et al.* (2022) reported that a temperature of 60°C at pH 7 supported nanoparticle synthesis from entomopathogenic fungi.

Antimicrobial Activity:

The surface properties of silver nanoparticles enhance their ability to interact with pathogens, thereby facilitating antimicrobial activities. The biologically synthesized AgNPs showed potent antimicrobial activity against the gram-negative *Salmonella typhi* ATCC 6539 and gram-positive *Enterococcus faecalis* ATCC 29212 at a test concentration of 1mg/ml (Table 1). These findings align with the earlier studies demonstrating the antimicrobial activity of AgNPs against *Enterococcus* isolates (Syame *et al*., 2020) and *Salmonella* isolates (Farouk *et al.*, 2020). The inhibitory effect of $AgNO_3$ on the pathogens is neglected due to its possible harmful effects on human cells while the mycelial cell-free extract showed no activity.

Antioxidant activity of the AgNPs was assessed using both DDPH and ABTS assays. The stable DPPH radical can react with hydrogen to show absorbance at 517 nm. Upon scavenging action by antioxidants, the DPPH methanol is reduced to DPPH-H (hydrazine) form and as a result, its colour changes from violet to yellow. 100 µg/ml of the AgNPs were able to show a maximum inhibition of 92.16% (Fig. 9) whereas the IC_{50} value of AgNP was found to be 38 µg/ml. The results were comparable with other antioxidant studies of nanoparticles from fungi (Govindappa *et al.*, 2020; Ameen *et al.*, 2023). It was also evident

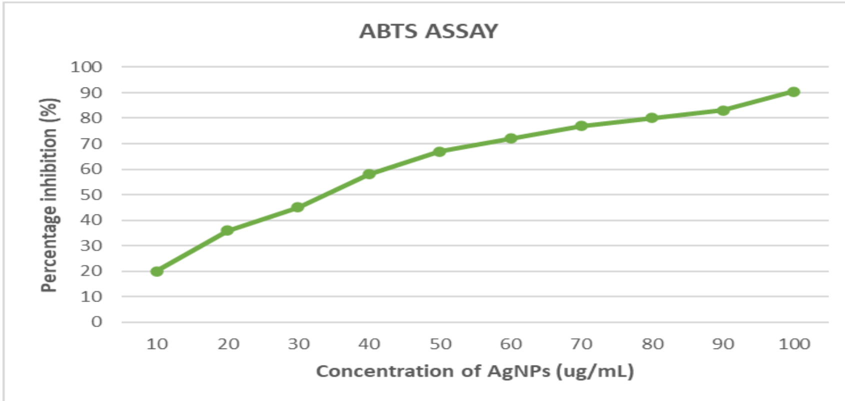

Fig. 10: ABTS radical scavenging activity.

that percentage increase in the AgNP concentration increased the antioxidant attack by inhibiting the free radicals in a dose dependent manner (Qiao *et al*., 2022). This activity can also be linked to the diverse functional groups identified in the FT-IR studies. These groups have the capacity to donate hydrogen from their hydroxyl group (-OH) to free radicals, thereby forming stable phenoxyl radical (Yousaf *et al*., 2020).

When ABTS radical is reacted with potassium persulfate, oxidation reaction results in the formation of greenish-blue chromophore. The neutralization or reduction of ABTS can occur via electron donation by antioxidants and is characterized by discoloration of the chromophore which is proportional to the percentage inhibition. The reaction was monitored at 734 nm and the AgNPs indicated a maximum inhibition of 90.34% at a concentration of 100µg/ml (Fig. 10). The IC_{50} values for AgNPs was estimated to be 40 µg/ml, aligning with findings with comparable values in antioxidant studies involving nanoparticles from fungi (Madhanraj *et al*., 2017).

Conclusion

This study aimed to optimize the synthesis conditions for formation of stable, monodispersed nanoparticles using *Aspergillus niger* with focus on characterization, antimicrobial and antioxidant studies. The peak value at 430 nm in the UV-visible spectrum range confirmed nanoparticle synthesis. The SEM images helped visualize their spherical morphology and nanoscale size. FT-IR peaks aided in identifying functional groups and chemicals bonds which can be associated with formation and stability of the nanoparticles. The antimicrobial findings of the biologically synthesized AgNPs have revealed promising outcomes against the tested strains. XRD analysis helped understand the crystalline structure. Optimization of synthesis parameters helped maximize synthesis with consistent and enhanced efficiency. DPPH and ABTS radical scavenging assays confirmed the substantial antioxidant potential of the synthesized AgNPs, suggesting their efficacy in combating disorders caused by free radicals.

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References

Akther T and Hemalatha S. (2019) Mycosilver nanoparticles: Synthesis, characterization and its efficacy against plant pathogenic fungi. BioNanoScience 9(2): 296-301.

Alsamhary KI. (2020) Eco-friendly synthesis of silver nanoparticles by *Bacillus subtilis* and their antibacterial activity. Saudi J Biol Sci. 27(8): 2185-2191.

Al-Soub A, Khleifat K, Al-Tarawneh A, Al-Limoun M, Alfarrayeh I, Al Sarayreh A and Albashaireh A. (2022) Silver nanoparticles biosynthesis using an airborne fungal isolate, *Aspergillus flavus*: optimization, characterization and antibacterial activity. Iranian J Microbiol. 14(4): 518.

Alves MF and Murray PG. (2022) Biological synthesis of monodisperse uniform-size silver nanoparticles (AgNPs) by fungal cell-free extracts at elevated temperature and pH. J Fungi 8(5): 439.

Ameen F, Al-Homaidan AA, Al-Sabri A, Almansob A and AlNAdhari S. (2023) Anti-oxidant, anti-fungal and cytotoxic effects of silver nanoparticles synthesized using marine fungus Cladosporium halotolerans. Appl Nanosci. 13(1): 623-631.

Awad MA, Eid AM, Elsheikh TMY, Al-Faifi ZE, Saad N, Sultan MH, Selim S, Al-Khalaf AA and Fouda A. (2022) Mycosynthesis, characterization, and mosquitocidal activity of silver nanoparticles fabricated by *Aspergillus niger* Strain. J Fungi (Basel) 8(4): 396.

Barabadi H, Mojab F, Vahidi H, Marashi B, Talank N, Hosseini O and Saravanan M. (2021) Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles. Inorganic Chem Commun. 129: 108647.

Chi NTL, Veeraragavan GR, Brindhadevi K, Chinnathambi A, Salmen SH, Alharbi SA, Krishnan R and Pugazhendhi A. (2022) Fungi fabrication, characterization, and anticancer activity of silver nanoparticles using metals resistant *Aspergillus niger.* Environ Res. 208: 112721.

Dhaka A, Mali SC, Sharma S and Trivedi R. (2023) A review on biological synthesis of silver nanoparticles and their potential applications. Results Chem. 6(1): 101108.

Elamawi RM, Al-Harbi RE and Hendi AA. (2018) Biosynthesis and characterization of silver nanoparticles using *Trichoderma longibrachiatum* and their effect on phytopathogenic fungi. Egyptian J Biol Pest Control 28(1): 1-11.

Farouk MM, El-Molla A, Salib FA, Soliman YA and Shaalan M. (2020) The role of silver nanoparticles in a treatment approach for multidrug-resistant Salmonella species isolates. Int J Nanomed. 15: 6993-7011.

Feroze N, ArshadB, Younas M, Afridi MI, Saqib S and Ayaz A. (2020) Fungal mediated synthesis of silver nanoparticles and evaluation of antibacterial activity. Microsc Res Technique 83(1): 72-80.

Gontijo LAP, Raphael E, Ferrari DPS, Ferrari JL, Lyon JP and Schiavon MA. (2020) pH effect on the synthesis of different size silver nanoparticles evaluated by DLS and their size-dependent antimicrobial activity. Matéria (Rio de Janeiro) 25(4): e-12845.

Govindappa M, Lavanya M, Aishwarya P, Pai K, Lunked P, Hemashekhar B and Raghavendra VB. (2020) Synthesis and characterization of endophytic fungi, *Cladosporium perangustum* mediated silver nanoparticles and their antioxidant, anticancer and nano-toxicological study. BioNanoScience 10: 928-941.

Hamad MT. (2019) Biosynthesis of silver nanoparticles by fungi and their antibacterial activity. Int J Environ Sci Technol. 16: 1015-1024.

Hulikere MM and Joshi CG. (2019) Characterization, antioxidant and antimicrobial activity of silver nanoparticles synthesized using marine endophytic fungus- *Cladosporium cladosporioides*. Process Biochem. 82: 199-204.

Keshari AK, Srivastava R, Singh P, Yadav VB and Nath G. (2020) Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. J Ayurveda Integrative Med. 11(1): 37-44.

Khan S, Naushad M, Al-Gheethi A and Iqbal J. (2021) Engineered nanoparticles for removal of pollutants from wastewater: Current status and future prospects of nanotechnology for remediation strategies. J Environ Chem Engineer 9(5): 106160.

Madhanraj R, Eyini M and Balaji P. (2017) Antioxidant assay of gold and silver nanoparticles from edible basidiomycetes mushroom fungi. Free Radicals Antioxidants 7(2): 137-142.

Mistry H, Thakor R, Patil C, Trivedi J and Bariya H. (2021) Biogenically proficient synthesis and characterization of silver nanoparticles employing marine procured fungi *Aspergillus brunneoviolaceus* along with their antibacterial and antioxidative potency. Biotechnol Letts. 43: 307-316.

Mustapha T, Misni N, Ithnin NR, Daskum AM and Unyah NZ. (2022) A review on plants and microorganisms mediated synthesis of silver nanoparticles, role of plants metabolites and applications. Int J Environ Res Public Hlth. 19(2): 674.

Nasrollahzadeh M, Atarod M, Sajjadi M, Sajadi SM and Issaabadi Z. (2019) Plant-mediated green synthesis of nanostructures: mechanisms, characterization, and applications. Interface Sci Technol. 28: 199-322.

Niculescu AG and Grumezescu AM. (2022) Applications of chitosan-alginate-based nanoparticles—An up-to-date review. Nanomaterials 12(2): 186.

Osorio-Echavarría J, Osorio-Echavarría J, Ossa-Orozco CP and Gómez-Vanegas NA. (2021) Synthesis of silver nanoparticles using white-rot fungus

Anamorphous *Bjerkandera* sp. R1: Influence of silver nitrate concentration and fungus growth time. Sci Rep. 11(1): 3842.

Othman AM, Elsayed MA, Al-Balakocy NG, Hassan MM and Elshafei AM. (2019) Biosynthesis and characterization of silver nanoparticles induced by fungal proteins and its application in different biological activities. J Genetic Engineer Biotechnol. 17: 1-13.

Patil RB and Chougale AD. (2021) Analytical methods for the identification and characterization of silver nanoparticles: A brief review. Materials Today Proc. 47: 5520-5532.

Patil S and Chandrasekaran R. (2020) Biogenic nanoparticles: a comprehensive perspective in synthesis, characterization, application and its challenges. J Genetic Engineer Biotechnol. 18(1): 67.

Phanjom P and Ahmed G. (2017) Effect of different physicochemical conditions on the synthesis of silver nanoparticles using fungal cell filtrate of *Aspergillus oryzae* (MTCC No. 1846) and their antibacterial effect. Adv Natural Sci Nanosci Nanotechnol. 8(4): 045016.

Qiao ZP, Wang MY, Liu JF and Wang QZ. (2022) Green synthesis of silver nanoparticles using a novel endophytic fungus *Letendraea sp.* WZ07: Characterization and evaluation of antioxidant, antibacterial and catalytic activities (3-in-1 system). Inorganic Chem Commun. 138: 109301.

Sajid M and Płotka-Wasylka J. (2020) Nanoparticles: Synthesis, characteristics, and applications in analytical and other sciences. Microchem J. 154: 104623.

Samuel MS, Jose S, Selvarajan E, Mathimani T and Pugazhendhi A. (2020) Biosynthesized silver nanoparticles using *Bacillus amyloliquefaciens*; Application for cytotoxicity effect on A549 cell line and photocatalytic degradation of p-nitrophenol. J Photochem Photobiol B Biol. 202: 111642.

Shahzad A, Saeed H, Iqtedar M, Hussain SZ, Kaleem A, Abdullah R, Sharif S, Naz S, Saleem F, Alhetasham A and Chaudhary A. (2019) Size-controlled production of silver nanoparticles by *Aspergillus fumigatus* BTCB10: likely antibacterial and cytotoxic effects. J Nanomaterials 2019(1): 5168698.

Sharma A, Sagar A, Rana J and Rani R. (2022) Green synthesis of silver nanoparticles and its antibacterial activity using fungus *Talaromyces purpureogenus* isolated from *Taxus baccata* Linn. Micro Nano Systems Letts. 10(1): 2.

Sharma K, Dakshinamoorthi BM and Jagadeeswari S. (2024) Characterization studies and antimicrobial efficacy of silver nanoparticles synthesized using *Aspergillus niger*: A biological driven approach. Uttar Pradesh J Zool. 45(13): 100-112.

Soleimani P, Mehrvar A, Michaud JP and Vaez N. (2022) Optimization of silver nanoparticle biosynthesis by entomopathogenic fungi and assays of their antimicrobial and antifungal properties. J Invertebrate Pathol. 190: 107749.

Syame SM, Mansour AS, Khalaf DD, Ibrahim ES and Gaber ES. (2020) Green synthesis of silver nanoparticles using lactic acid bacteria: assessment of antimicrobial activity. World's Vet J. 4: 625-633.

Titus D, Samuel EJJ and Roopan SM. (2019) Nanoparticle characterization techniques. Green Synthesis, Characterization Applications Nanoparticles 2019: 303-319.

Win TT, Khan S and Fu P. (2020) Fungus-(*Alternaria sp.*) mediated silver nanoparticles synthesis, characterization, and screening of antifungal activity against some phytopathogens. J Nanotechnol. 2020(1): 8828878.

Yousaf H, Mehmood A, Ahmad KS and Raffi M. (2020) Green synthesis of silver nanoparticles and their applications as an alternative antibacterial and antioxidant agent. Materials Sci Engineer C 112: 110901.

Zhai X, Zhang C, Zhao G, Stoll S, Ren F and Leng X. (2017) Antioxidant capacities of the selenium nanoparticles stabilized by chitosan. J Nanobiotechnol. 15: 1-12.

Zhao J, Guo F, Hou L, Zhao Y and Sun P. (2023) Electron transfer-based antioxidant nanozymes: Emerging therapeutics for inflammatory diseases. J Controlled Release 355: 273-291.