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Research Article

Green Synthesis and Bioactive Potential of Silver Nanoparticles: Antimicrobial and Anticancer Evaluation

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Abstract

The current work describes the green synthesis and characterization of silver nanoparticles (AgNPs) and their assessment with antimicrobial, bioassay, and anticancer properties. UV-vis spectroscopy was used to verify the formation of nanoparticles by a distinct plasmon resonance point at 440 nm, which corresponds to the average plasmon resonance of silver nanoparticles (400-460 nm). FTIR analysis identified the presence of functional groups of hydroxyl, carbonyl and ether/ alcohol, which is the evidence of the use of phytochemicals in stabilizing the nanoparticles. SEM-imaging revealed spherical-to-quasi-spherical sized AgNPs with diameter between 52 and 84 nm, with low agglomeration and uniform distribution reflecting stability and biomedical application. Antibacterial tests revealed strong anti-microbial effects against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Klebsiella pneumoniae* with an anti-microbial zone of 14-26 mm, which were more potent against antibiotics. Antifungal testing showed activity against *Aspergillus flavus*, but only slight inhibition of *Aspergillus terreus* and *Aspergillus niger*. The presence of dose-dependent cytotoxicity was shown in studies on bioassay against *K. pneumoniae*, where cell death was up to 73.61 per cent at the highest concentrations. Evaluation of anticancer activity with MCF-7 breast cancer cell line demonstrated that there was minimum concentration-dependent cell viability reduction, and the greatest reduction (21.8% viability) was at 2 µL concentration. In general, the prepared silver nanoparticles have positive multifunctional bioactivities, suggesting their potential in therapeutic and biomedical applications.

Keywords: Silver nanoparticles, Green synthesis, Antibacterial activity, Bioassay, MCF-7 cell line

1. INTRODUCTION

Nanotechnology is a fast-growing area in contemporary science that has cut across the borders of chemistry, biology, materials science, and medicine. Nanomaterials have provided new opportunities in biomedical applications particularly in drug delivery, diagnostics, and therapeutic interventions. Silver nanoparticles (AgNPs) are one of the most commonly studied nanomaterials thanks to their optical, catalytic, and antimicrobial properties that cannot be found in the bulk forms [1,2]. Their low size and large surface volume ratio give them increased reactivity and are therefore good subjects of biomedical study. The attraction of AgNPs is also justified by their past application in wound healing, disinfection and antimicrobial therapy of silver into a nanoscale system with enhanced effectiveness [3].

Although effective, traditional chemical and physical nanoparticle synthesis techniques typically use dangerous reagents, lots of energy, and produce toxic by-products, which restrict their biomedical application. Consequently, there is a growing interest in researchers adopting the concept of green synthesis which involves the use of biological systems including plants, bacteria, fungi and algae as reducing and stabilizing agents[4]. Recently, plant-mediated synthesis, especially, is considered a sustainable and environmentally friendly option as it uses phytochemicals (polyphenols, terpenoids, flavonoids, and alkaloids) to reduce silver ions into stable nanoparticles [5, 6]. These biomolecules which are naturally derived not only promote synthesis, but also add functional groups to promote stability and biological activity, and provide the twin benefits of green chemistry and biomedical functionality[7]. These strategies are in line with increased focus on eco-friendly and economical nanotechnology.

The synthesized AgNPs have to be characterized to ensure that they are formed, sized and morphologically, and to determine the effects of phytochemicals in stabilization. To confirm the synthesis, UV-Visible (UV-Vis) spectroscopy, Fourier-Transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM) are common techniques used to validate synthesis. A

characteristic of the nanoparticle formation with silver to be used is the emergence of a clear surface plasmon resonance (SPR) peak between 400 and 460 nm on the UV-Vis spectrum[8]. FTIR analysis allows to determine functional groups related to capping nanoparticles and identify which plant extracts contain hydroxyl, carbonyl, and aromatic moieties[9]. SEM also enables the visualization of morphology and size distribution of nanoparticles, which are important characteristics defining the way nanoparticles interact with biological systems[10]. Such characterization guarantees that the synthesized AgNPs are both well characterized and ready to conduct downstream biomedical research.

The biomedical significance of AgNPs has been reported well in the recent years. Their wide-spectrum antimicrobial activity has been validated in several studies against the pathogenic bacteria and fungi including multi-drug resistance[11, 12]. AgNPs have antimicrobial effects that are mainly mediated by destabilizing of microbial membranes, production of reactive oxygen species (ROS), and destabilization of DNA and protein synthesis that cause cell death[13]. In addition to antimicrobial activity, AgNPs have shown good anticancer effects and there is evidence that AgNPs induce oxidative stress, apoptosis and dysfunction of the mitochondrion in cancer cells [14,15]. These versatile bioactivity properties point to their potential in the clinical practice that might be used as a substitute or complement to traditional treatment methods. Furthermore, they are applicable to bioassays and cytotoxicity research with concentration-related effects as the reflection of their safety and efficacy profiles[16].Considering these opinions, the current research was aimed to synthesize silver nanoparticles in a green method, characterize them by spectroscopic and microscopic methods and analyse their biological activities such as antibacterial, antifungal, bioassay, and anticancer potential. This work will illustrate the versatility of AgNPs to be used in physical, chemical, and biological analyses and give reasons as to why this material can be used in the future in therapeutic and biomedical purposes.

2. MATERIALS AND METHODS

Plant Collection and Extract Preparation

The *Parmotrema perlatum* was obtained in Eachanari, Coimbatore, Tamil Nadu, India and dried in sun during five days and then used. The sample was then ground into a fine powder and 10 g of the sample was weighed to make a 10% extract using distilled water as the solvent. The extract was poured into a conical flask and swirled in an orbital shaker at a temperature of 40°C at a stirring rate of 60-70 rpm over a time period of 24 hours to aid in the extraction of bioactive compounds. The extract was incubated and filtered using Whatman No.1 filter paper followed by collection in a sterilized container. The prepared extract was kept at 4 °C until futher analysis.

Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$) solution 1 mM in aqueous was prepared and combined with the *Parmotrema perlatum* extract in 1:1 ratio. Then, the reaction mixture was allowed to incubate at room temperature in dark chamber to avoid photo activation of silver nitrate. The formation of the nanoparticles was followed visually and it was observed that the solution changed color to brown, instead of light green, which confirmed the conversion of Ag+ ions to elemental silver (Ag^0). This change in color is due to the surface plasmon resonance (SPR) phenomenon, which is a typical marker of silver nanoparticle synthesis[17].

Characterization of the Synthesized Nanoparticle

UV-Visible Spectroscopy

UV-Visible (UV-Vis) spectroscopy was used to confirm the formation of the silver nanoparticles. The sample was scanned at the range of 200-700 nm following baseline correction using a blank. The formation of a surface plasmon resonance (SPR) peak at 400-460 nm was regarded as the evidence of AgNP formation[18].

FTIR Spectroscopy

The functional groups that exist in the extract and its contribution to the reduction and stabilization of silver nanoparticles were determined by FTIR analysis. The dried specimen of nanoparticles was examined within the 4000-400 cm-1 spectrum and the typical peaks of hydroxyl, carbonyl and ether groups were noted. These peaks revealed the appearance of bioactive compounds in the formation of nanoparticles and their stabilization[19].

Scanning Electron Microscopy (SEM)

Morphology and size distribution of the synthesized nanoparticles were studied by scanning electron microscopy (SEM). The samples were placed on aluminum stubs, covered with a thin shell of gold and placed under the instrument at 15 kV. SEM images presented data of particle shape, surface structure and size distribution and proved the nanoscale range of AgNPs[20].

Antibacterial Study

Agar well diffusion method was used to determine the antibacterial activity of the synthesized silver nanoparticles. Mueller-Hinton agar was prepared by dissolving 39 g of media in 1000 mL of distilled water which was then sterilized in an autoclave at 121°C in 15 minutes and poured into sterile Petri plates to solidify. After solidification, 70 µL of bacterial cultures (*Escherichia coli, Klebsiella pneumoniae, Bacillus subtilis,* and *Staphylococcus aureus*) were swabbed uniformly on to the agar surface with sterile cotton swabs. A sterile cork borer was used to create wells and 50 µL of the nanoparticle samplewas added to each well. The positive control was an antibiotic disc with cefazolin (CZ, 30 ug) and DMSO (Di- Methyl Sulfoxide) was the negative control. The plates were incubated at 37°C over 24 hours after which the antibacterial activity was determined by measuring the zone of inhibition in millimeters around each well[21].

Antifungal Activity

Agar well diffusion method was used to determine the antifungal activity of the silver nanoparticles produced. Malt extract agar media was prepared by vigorously mixing 35 g of medium in 1000 mL of distilled water, then autoclaving at 121°C for 15 minutes, then solidified by pouring into Petri plates. Upon solidification, 80 µL of fungal cultures (*Aspergillus flavus, Aspergillus niger*, and *Aspergillus terreus*) was swabbed uniformly to the agar surface. Wells were prepared and the samples of nanoparticles were added and 5 uL of fluconazole (10mg/mL) was employed as a standard antifungal control. Plates were incubated at 30 °C for 2-3 days and areas of inhibition were measured in millimeters at the end of the incubation period[21].

Minimum Inhibitory Concentration

Microdilution bioassay was used to determine the antibacterial activity of the plant extracts in sterile 96-well microtiter plates, following the procedure of Linima *et al*. (2023)[22]. Each of the wells was inoculated with 0.1 mL of sterilized nutrient broth, and the extract of different concentrations (10, 20, 30, 40 and 50 uL) was added to the wells one by one. *Klebsiella pneumoniae* culture 10 uL was added to each well. The plates were left to incubate at 37^{0}C over a period of 24 hours and then growth of the bacteria was measured. Visual analysis was done on turbidity and Minimal Inhibitory Concentration (MIC) was determined as the minimum concentration of extract where no visible growth was exhibited. Quantitative confirmation of the results was done by measuring optical density (OD) at 600 nm with a 96-well ELISA plate reader (Robonik) and the percentage of cell death was calculated.

Anticancer Study

To assess the potential anticancer activity of the extract, the MCF-7 breast cancer cell line was used to measure cytotoxicity[23]. The cells were subcultured in Dulbecco Modified Eagle media (DMEM) with sodium carbonate, glucose and 10% bovine serum albumin (BSA). The cultures were cultivated in T-flasks and allowed to grow in a CO_2 incubator at 37°C, 70-80% relative humidity and a pH of 7.0-7.5 during 24-72 hours.

In the MTT assay, cells were grown in a 96-well plate and allowed 24 hours to adhere under normal culture conditions. The extract was then added to different concentrations to the cells, with the negative control being untreated cells, the blank being DMSO, and the positive control being 12.5 ug/mL of Doxorubicin. After 24 hours incubation, the cells were washed with DMSO and trypsinized. Then, 20 uL of MTT dye solution was placed into each well and left to incubate further after 24 hours at 37 °C in the CO_2 incubator to allow the formation of formazan crystals. To solubilize these crystals, 100 uL of DMSO was added to each well and mixed thoroughly. Absorbance was subsequently measured at 570nm on a 96 well ELISA plate reader (Robonik, India) and the percentage of cell death was calculated.

3. RESULT AND DISCUSSION

Synthesis of silver Nanoparticle from Plant Extract

The ability to successfully synthesize silver nanoparticles (AgNPs) with the help of *Parmotrema perlatum* extract was mainly demonstrated by the change of the color of the reaction mixture, which turned light green but changed to brown after incubating it with silver nitrate. This optical change shows the dissolution of silver ions (Ag^+) to metallic silver (Ag^0) nanoparticles, which is triggered by phytoconstituents in the extract. This type of color difference is a highly reported phenomenon of nanoparticle synthesis in plant-mediated synthesis, and is explained by the excitation of surface plasmon resonance (SPR)[6]. Phytochemicals in the lichens especially the polyphenols, flavonoids and terpenoids, were probably both reducing and stabilizing agents. These biomolecules provide electrons to silver ions and promote their reduction, and at the same time eliminate aggregation by capping processes[4]. The existing literature supports the fact that secondary metabolites in plant and microbial extracts could be used as environmentally friendly substitutes of dangerous chemical reducers, which highlights the sustainability of green nanotechnology[24].

Results are aligned with the previous studies in which natural extracts were effective mediators in the synthesis of AgNPs. Linima *et al.* (2023)[22] showed the biogenic synthesis of AgNPs with the help of the *Ricinus communis* leaf extract, where the nanoparticles reduce and stabilize effectively and can be used as antimicrobials. In a similar study, Nihala *et al.* (2025)[25] demonstrated that AgNPs synthesized through *Lactobacillus gasseri* supernatants presented significant biomedical properties, such as antimicrobial and antibiofilm effects, again confirming the success of biological systems in the synthesis of nanoparticles. These similarities also underscore the broad applicability of biological extracts as universal bio-tools in the manufacture of nanoparticles.

In addition to synthesis efficiency, the green method has considerable benefits compared to the traditional physical and chemical procedures. Conventional chemical synthesis normally uses toxic reagents such as sodium borohydride or hydrazine, which are hazardous to health and the environment. Plant-mediated and microbe-mediated approaches are, conversely, more compatible with biomedical uses, are safer by nature, and can be scaled[26]. Moreover, natural renewable sources, including lichens, also help to minimize environmental impact besides adding intrinsic bioactivities that can be used to increase the functional characteristics of the prepared nanoparticles. These encouraging findings in this paper show the potential of such *P. perlatum*-mediated AgNP synthesis to be sustainable, low-cost, and biologically relevant. The application of these nanoparticles in antimicrobial, antifungal, and anticancer treatment is likely to be extensive, and these findings are consistent with those of Rautela *et al.* (2019)[27], who highlighted the biomedical potential of green-synthesized nano silver.

To enhance the reproducibility and scalability of the technique to industrial and clinical uses, future research should be aimed at optimization of parameters like extract concentration, reaction time, and temperature.

Characterization of the Synthesized Silver Nanoparticles

The effective production of silver nanoparticles (AgNPs) is mainly verified through UV-Visible spectroscopy, the most common characterization method because of being sensitive to the formation of nanoparticles. The current experiment showed that a dominant peak of absorption was recorded at approximately 430-440 nm, the typical surface plasmon resonance (SPR) of AgNPs. SPR is a collective wave of conduction electrons at the nanoparticle surface caused by incident light and is very sensitive to particle size, shape and the dielectric surrounding[28]. This finding is also supported by the separate brown coloration that appears visually in the solution because the change between light green and brown coloration is characteristic of AgNP formation. The form and width of the absorption peak give data over the distribution of particles. A fairly sharp peak would be associated with monodispersive nanoparticles, whereas wider peaks would indicate polydispersive[29].

The broadness of the peak, in this case, revealed a certain range of the particle sizes which is also in line with the SEM results. The fact that the SPR band lies at the 420-450 nm range highly supports the notion that the produced particles are silver nanoparticles as has been reported in earlier plant-mediated synthesis studies. Indicatively, when natural extracts were employed to synthesize nanoparticles, Santhiya *et al.* (2025)[30] obtained similar SPR peaks of about 430 nm, which confirms the current results.It is also important that no other complexes or by-products were formed during the reaction because the additional absorption bands are not present in the spectrum. This purity is also an important criterion regarding biomedical usage, where nanoparticles free of contaminants are necessary[4]. Similar results are reported in other green synthesis studies. Kumar *et al*. (2017)[31], who synthesized AgNPs with blackberry extract and recorded the presence of SPR peaks of about 425 nm, and Awwad and Salem (2012)[33] with carob leaf extract. Collectively, the UV-Vis analysis in the current work is evidence of successful synthesis of AgNPs. The feature SPR peak that the visual color change upheld and was in agreement with previous literature determines the efficiency of the green synthesis methodology that utilized *Parmotrema perlatum* extract as reducing and stabilizing agent.

The FTIR spectroscopic method is an important method of analyzing functional groups in the reduction and stabilization of silver nanoparticles (AgNPs). The FTIR spectrum in the current study showed highly dominant peaks of hydroxyl (O-H), carbonyl (C=O), and C-O, and other bands that represented aliphatic and aromatic functional groups. O-H stretching vibrations will give a broad band at about 3344 cm^{-1} and the vibration is normally associated with alcohols and phenolic compounds. Such entities have been repeatedly reported to serve as hydrogen donors and reduce the Ag^{+} ions to Ag^{0} nanoparticles[33].

Peaks of high absorption fall between 1800-1650 cm^{-1} and belong to carbonyl (C=O) stretching, which is a functional group prevalent in flavonoids, terpenoid, and other secondary metabolites. These biomolecules do not only reduce silver ions but also help in the capping and stabilizing of the prepared nanoparticles, thus avoiding agglomeration[34]. The clear band at 1055 cm^{-1} is related to C-O stretching vibrations of the ethers and alcohols, which also confirms the presence of phytochemicals extracted out of the plants working with AgNPs. These functional groups prove the existence of compounds found in the *Parmotrema perlatum* extract to be directly engaged in nanoparticle production. The results are consistent with previous ones. Santhiya *et al.* (2025)[30] showed that phenolics and flavonoids in lichen extracts are very important in stabilizing nanoparticles. On the same note, Ahmed *et al.* (2016)[4] indicated that the O-H and C=O groups of neem leaf extract reduced and capped AgNPs. The interlacing between these functional groups is a good indication that the primary and secondary metabolites in the extract are synergistic in the mediation of AgNP synthesis.

In general, the FTIR examination does not only reveal that the formation of nanoparticles involves biomolecular processes and mechanisms, but also highlights the fact that natural extracts are an effective alternative to synthetic reducing agents (that are environmentally friendly). This is the green chemistry benefit of utilizing *Parmotrema perlatum* extract, in which phytochemicals play a dual purpose as reducing and stabilizing agents, to stabilize nanoparticles and maintain their functionality.

The morphology, distribution, and size of the synthesised silver nanoparticles (AgNPs) were examined by Scanning Electron Microscopy (SEM). The SEM micrographs indicated that the size of the nanoparticles was between 52 and 84 nm, and spherical to quasi-spherical. This morphology is in line with the overall result of green synthesis methods, in which phytochemicals of plant or lichen extracts serve as reducing and stabilizing agents[4]. The compared homogeneous distribution of a small degree of agglomeration in the images implies that the biomolecules in *Parmotrema perlatum* extract were highly effective in capping the nanoparticles and hence increasing stability. The size range of particle distribution lies within the nanoscale, an important parameter that determines the physical and biological properties of AgNPs. Smaller-sized nanoparticles, especially less than 100-nm in diameter, have been observed to exhibit high surface plasmon resonance (SPR) and enhanced antimicrobial action through high surface area-to-volume[1]. The analysis of the SEM in this experiment correlates with the UV-Vis spectral analysis that has shown an SPR maximum at approximately 440 nm again proving the dimensions of the nanoparticles.

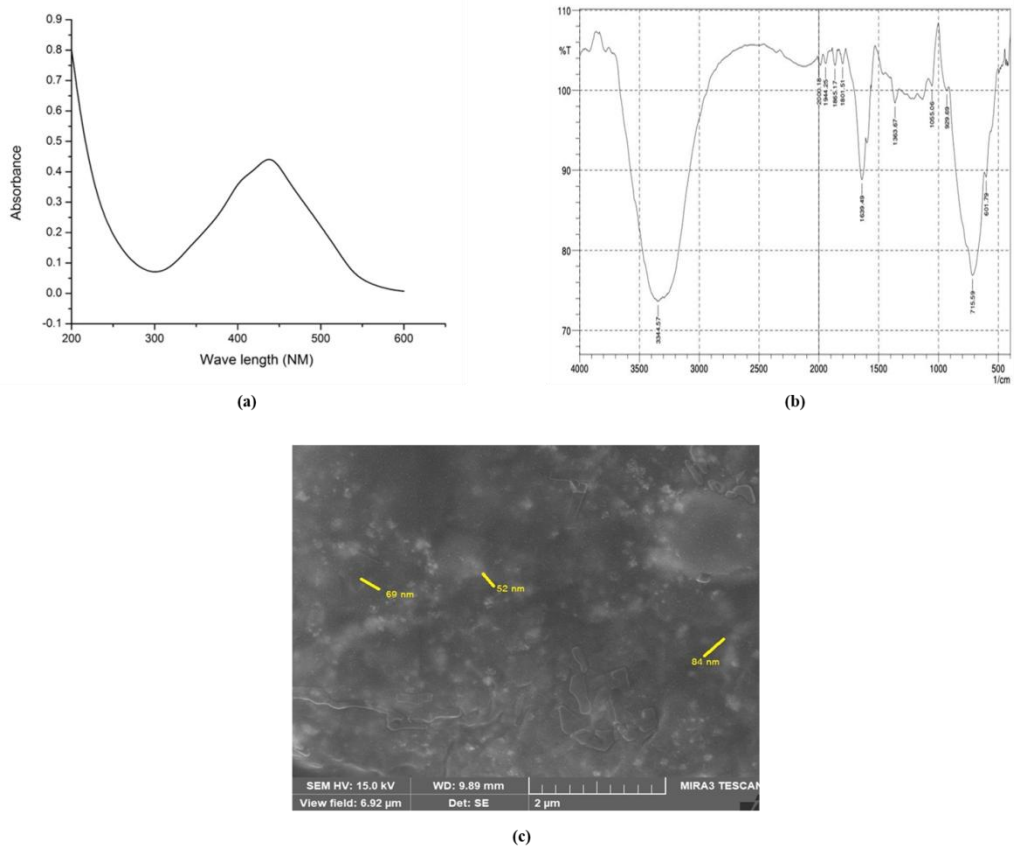

Figure 1: Characterization Studies (a) UV-Vis Spectrum; (b) FTIR Spectrum and; (c) SEM image of Silver Nanoparticle Synthesized using *Parmotrema perlatum* Extract

These outcomes can be compared with previous research. Spherical (50-80 nm) AgNPs were synthesized with lichen extracts[30]and *Acalyfa indica* leaf extracts reported similar morphology[29]. In both studies, it was stressed that natural biomolecules do not only guide the creation of spherical nanoparticles, but also minimize the possibility of aggregation, thereby forming more stable colloidal suspensions. Moderate polydispersity was also characteristic of the biogenic synthesis in the current study since several phytochemicals can undergo reduction and capping processes at once[34].SEM analysis confirms the efficient biosynthesis of AgNPs, its morphology of a sphere, nanoscale size, as well as its stable distribution. The data confirms the efficiency of *Parmotrema perlatum* extract as a bio-reductant and stabilizer, which should be used to produce eco-friendly nanomaterials in biomedical and environmental biomaterials.

Antimicrobial Activity of the Silver Nanoparticle

The activity of the biosynthesized silver nanoparticles (AgNPs) against Gram-positive and Gram-negative bacteria was tested against a control antibiotic disc (Cefazolin) and the results were compared. Figure 2 (a) indicated that the AgNPs had a strong inhibitory effect, but the effect was normally smaller than the zones that were produced by the antibiotic disc. The most sensitive organisms tested were *Bacillus subtilis* and *Klebsiella pneumoniae*, and their *Bacillus subtilis* and *Klebsiella pneumoniae* showed the largest inhibition zones of 26 mm and 25 mm, respectively. Conversely, *Staphylococcus aureus*, and *Escherichia coli* were less susceptible with the 14 mm, and 14 mm, respective inhibition zones. As estimated, the standard antibiotic disc showed greater inhibition in all the strains with a range of 29 mm to 33 mm on *S. aureus* and *B. subtilis* respectively.

The claimed antibacterial activity of AgNPs may be explained by the fact that small particles and great surface area of such nanoparticles increase their capacity to interact with bacteria walls and membranes. AgNPs are also reported to release Ag^+ ions, which bind to thiol groups of proteins, disrupt cell membrane permeability, and produce reactive oxygen species (ROS), which ultimately causes cell death[1, 36]. Higher sensitivity of *B. subtilis* and *K. pneumoniae* implies that structural variations in bacterial cell walls (the peptidoglycan thickness of Gram-positive bacteria and the outer membrane of Gram-negative bacteria cells) might contribute to AgNP susceptibility. The same has been observed in other studies, where biosynthesized AgNPs were found to be highly active as antibacterial against a variety of pathogens but with some differences based on bacterial species[37]. This decreased inhibition in relation to antibiotics is not surprising, since AgNPs do not follow a specific pathway of action but can be considered through non-specific action. Their wide-spectrum activity, however, points to their possible use as substitute or complementary antimicrobial agents, in particular when dealing with the problem of antibiotic resistance[38]

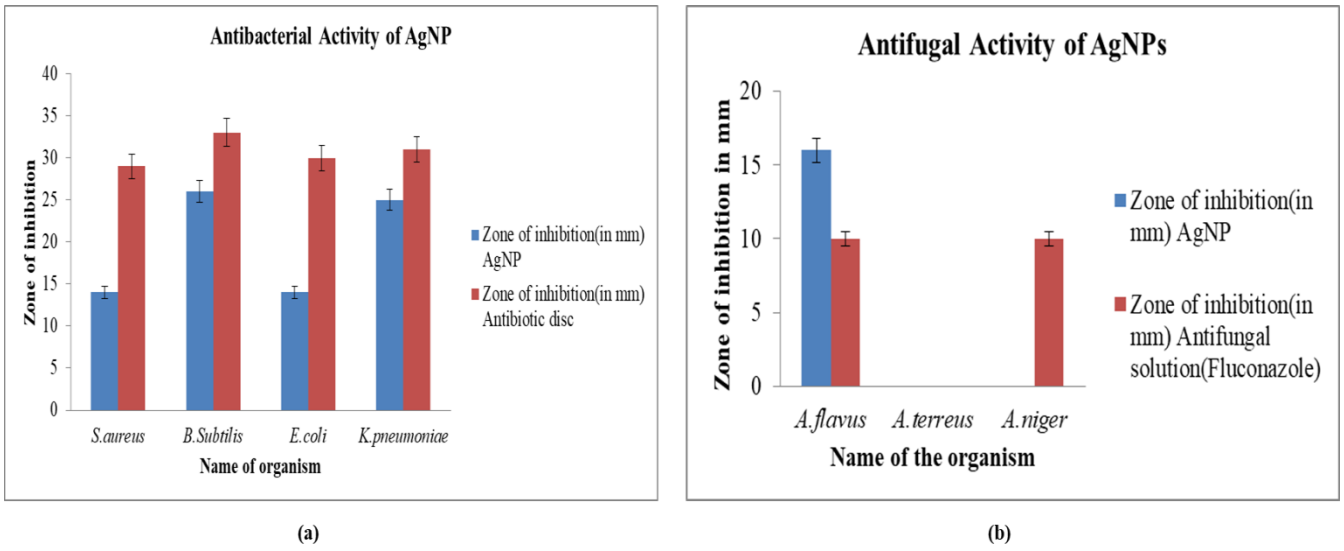

Figure 2: Antimicrobial activity of the AgNP; (a) Antibacterial activity; (b) Antifungal activity

The antifungal properties of biosynthesized silver nanoparticles (AgNPs) against three fungal strains, *Aspergillus flavus, Aspergillus terreus* and *Aspergillus niger*, were evaluated and compared with a conventional antifungal reference, fluconazole. The findings showed that AgNPs were selective antifungal agents and different sensitivity was found among the fungal species used. In the case of *A. flavus*, the AgNPs had a strong zone of inhibition of 16 mm that was considerably greater compared to the inhibition zone of fluconazole of 10mm. This observation indicates that AgNPs have great antifungal potentials against *A. flavus*, which may be attributed to the capability of agitating fungal cell wall integrity, membrane destruction, and causing oxidative stress by generating reactive oxygen species (ROS). The high antifungal activity of AgNPs as compared to the traditional medications has also been previously noted, especially against the *Aspergillus* species[37].

AgNPs were unable to demonstrate any antifungal effect in the case of *A. niger*, whereas fluconazole revealed a moderate size of the inhibition zone of 10 mm. Likewise, *A. terreus* was not inhibited by AgNPs or fluconazole. The lack of antifungal effect on these species could be explained by intrinsic resistance mechanisms such as changes in cell wall composition, efflux pump, and the capacity of fungal spores to endure nanoparticle-induced stress[39]. These measurements suggest that AgNPs antifungal activity is species-specific and relies on structural and biochemical characteristics of the fungal pathogens. This increased activity of AgNPs over *A. flavus* relative to fluconazole underscores the potential of nanoparticles as alternative antifungal agents particularly to deal with drug resistant strains of fungi. Research has indicated that AgNPs react with fungal hyphae and spores leading to morphological abnormalities and dysfunction of the cellular organelles and this inhibits growth[40]. The absence of action against *A. terreus* and *A. niger*, however, indicate that their antifungal range should be extended through additional optimization of the synthesis parameters, particle size, and concentration. The biosynthesized AgNPs proved to have high antifungal effect on *A. flavus* which was much more efficient than the conventional antifungal fluconazole, but none in *A. terreus* and *A. niger.* These findings emphasize the promise and the constraints of AgNPs as antifungal agents and the need to evaluate them strain-by-strain prior to clinical or agricultural implementation.

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) test was performed to ascertain the antibacterial property of biosynthesized silver nanoparticles (AgNPs) against the *Klebsiella pneumoniae*. This finding showed that the nanoparticles had a high inhibitory tendency, and a distinct concentration-response tendency in the bacterial mortality. In the lowest concentration of 10 µl the nanoparticles caused cell death to the extent of 47.23%, which demonstrates the strong antibacterial effects caused by the nanoparticles even at low doses. With the concentration raised up to 20 µl, the percentage of cell death was improved to 57.05, which indicated a great improvement in bactericidal effect. Interestingly, at 30 µl the percentage of cell death marginally dropped to 54.60. This slight deviation may be explained by aggregation of nanoparticles that decreases the effective surface area on which bacteria can interact, or differences in bacteria growth conditions during the assay. This exception notwithstanding, an increasing trend in activity was noted at higher concentrations with 63.19% and 73.61% cell death observed at 40 µl and 50 µl, respectively. These results have a strong indication that AgNPs behave in a dose-dependent way with the highest degree of bacterial inhibition being attained at the higher concentrations.

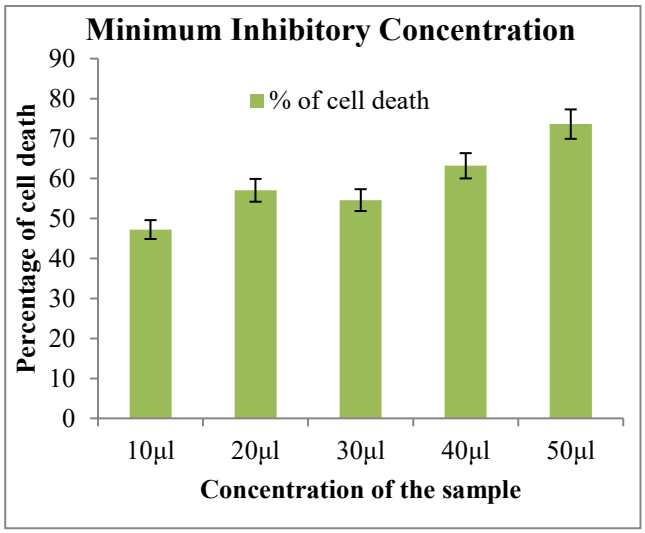

Figure 3: Minimum Inhibitory Concentration (MIC) assay of silver nanoparticles against *Klebsiella pneumoniae* showing percentage of cell death at different concentrations.

AgNPs antibacterial activity under concentration dependence is consistent with the body of literature regarding nanoparticles-induced oxidative stress, cell membrane disruption, and DNA replication interference as important pathways[41]. Nanoparticles have extraordinary physicochemical characteristics such as small size and a high ratio of surface to volume that facilitate their entry into microbial cell walls, which leads to destabilization of the membrane and cell lysis[42]. Results of the SEM of the present study also confirmed the presence of the nanoparticles within the effective size range of 52-84 nm as well. The same outcome is identified in the case of Swarnalatha *et al.* (2024)[17], who also noted the potential potent antibacterial action of plant-mediated AgNPs against *E. coli* and *K. pneumoniae*, where the absence of a zone of inhibition and the MIC values demonstrated the results. Mosselhy *et al.* (2015) also added that the stability that is provided by phytochemicals in the green-synthesized nanoparticles boosts their antimicrobial capacity and reduces toxicity.In general, the results prove that the biosynthesized *Parmotrema perlatum* extract-generated AgNPs are efficient against *K. pneumoniae*. The inhibitory effect that is marked is a strong one especially at high concentration, which explains their ability to be used as an alternative to traditional antibiotics in treating the multidrug-resistant pathogens. Nevertheless, additional research on cytotoxicity, biocompatibility, and in vivo efficacy is necessary prior to the actualization of clinical applications.

Cytotoxicity Assay

Biosynthesized AgNPs of *Parmotrema perlatum* extract also exhibited a high level of cytotoxicity against the MCF-7 breast cancer cell line, which further supported their anticancer potential. The fact that cell viability was significantly suppressed in the lowest concentrations used (2-4 µl) is an indication that the nanoparticles are very powerful even at low dosage. This implies that the presence of the bioactive compounds in the lichen extract does not only serve as reducing and stabilizing agents but the effects could be synergistic with the cytotoxic effects of AgNPs. Anticancer properties of plant-derived metabolites, including flavonoids, phenols, and terpenoids, are quite famous, and their appearance in the capping layer of AgNPs could increase ROS formation and apoptotic signaling[34]. The significant decrease in viability at 2 µl indicates a fast nanoparticle-cell interaction to the extent of disrupting vital cellular activities such as mitochondrial respiration, resulting in apoptotic or necrotic cell death.

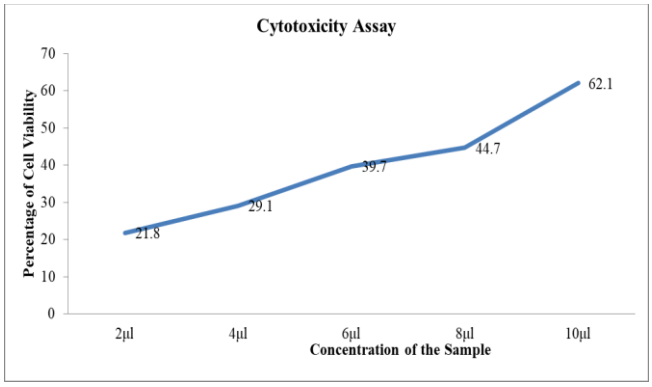

Figure 4: Cytotoxic effect of silver nanoparticles synthesized from *Parmotrema perlatum* extract against MCF-7 breast cancer cell line showing percentage cell viability at different concentrations

This mildly positive shift in cell viability with increased concentrations (6-10 µl) can be counter-intuitive but has been reported in other nanoparticle-based cytotoxicity studies[12, 42]. This could be explained by the aggregation of nanoparticles at high concentrations thereby decreasing the effective surface area and bioavailability to cellular absorption. Also, too high nanoparticle concentration can cause adaptive stress, such as activation of antioxidant enzymes, efflux pumps, and DNA repair, which are counter-cytotoxic to a certain degree. Irrespective of this variability, the net overall results still suggest that there is considerable anticancer potential, at lower effective concentrations.

Combined, the existing results indicate that AgNPs produced through *Parmotrema perlatum* could become a viable alternative to traditional chemotherapeutics, particularly in breast cancer. Biosynthesized AgNPs might offer a safer and plant-based nanomedicine system where systemic toxicity is less than that of standard drugs, such as doxorubicin, which has dose-limiting cardiotoxicity[15]. The encouraging activity in vitro needs additional investigation into their mechanism of action on a molecular scale, including apoptosis biomarkers like caspase activation, Bax/Bcl-2 and ROS quantification. Furthermore, in vivo experiments are required to verify their biodistribution, pharmacokinetics, and therapeutic effect.

4. CONCLUSION

The current research was able to prove the green synthesis of silver nanoparticles (AgNPs) using extract of *Parmotrema perlatum* as a reducing and stabilizing agent. UV-Vis spectroscopy, FTIR and SEM characterization showed the formation of stable, well

dispersed, spherical nanoparticles with bioactive functional groups capping the surfaces. The AgNPs produced had high antimicrobial effects against both Gram-positive and Gram-negative bacteria, *Bacillus subtilis* and *Klebsiella pneumoniae* being most susceptible. Moreover, antifungal culture showed encouraging inhibition of *Aspergillus flavus* and the nanoparticles showed a broad spectrum antimicrobial activity. Their effective bactericidal activity was further confirmed by the MIC and bioassay results in a concentration-dependent way. Notably, anticancer assessment of MCF-7 breast cancer cells revealed that they have strong cytotoxic effects, especially at low concentrations, which implies their potential as new anticancer agents. Altogether, the research highlights the efficacy of *P. perlatum*-mediated AgNPs as environmentally safe, cost-efficient, and versatile nanomaterials with important biomedical potentials. They have antimicrobial and anticancer action, which makes them good research candidates in the pharmaceutical and therapeutic arena. Further studies are necessary on the detailed molecular mechanisms, cytotoxicity and in vivo research to ascertain that they are safe and effective in transplantation to the clinic.

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Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Ethical Approval

Not Applicable

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