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*Original Research*

# Biosynthesis of Silver Nanoparticles via the Fungus *Ophiostoma novo-ulmi*: A Green Approach

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## Abstract

The green synthesis of metal nanoparticles using non-toxic and biodegradable chemicals represents a sustainable, eco-friendly approach that enables the synthesis of nanoparticles with enhanced stability and specific bioactive properties. The synthesis of silver nanoparticles (AgNPs) using an aqueous extract of the fungus *Ophiostoma novo-ulmi* and an aqueous $AgNO_3$ solution is reported in this study. *Ophiostoma novo-ulmi* is a highly virulent ascomycete responsible for Dutch elm disease (DED), a destructive vascular wilt affecting elm trees (*Ulmus* spp.). It produces a diverse range of bioactive metabolites, enabling efficient reduction and stabilization of nanoparticles, while also representing a novel example of transforming a phytopathogenic organism into a valuable biotechnological resource. The biosynthesized AgNPs were characterized using ultraviolet-visible (UV-Vis) spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy, powder X-ray diffraction (PXRD), and transmission electron microscopy (TEM). The characterization results confirmed the successful synthesis of AgNPs mediated by the *O. novo-ulmi* extract, which acted as both the reducing and stabilizing agent, with a characteristic UV-Vis absorption maximum at ≈ 402 nm. FT-IR analysis identified key bioactive metabolites that are potentially responsible for bio-reductive activity. PXRD results revealed a crystallite size of ≈15 nm, calculated using the Scherrer equation, with a face-centered cubic (FCC) structure. Evaluation of antibacterial properties by the broth microdilution method demonstrated inhibitory effects of the biosynthesized AgNPs against both Gram-positive and Gram-negative bacteria. The synthesized AgNPs exhibited antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, with MIC values ranging from 0.01563 to 0.500 mg $mL^{-1}$. Overall, the findings highlight the high potential and effectiveness of *O. novo-ulmi* as a biological agent for fungal-mediated green synthesis of silver nanoparticles.

**Keywords:** AgNPs, fungi, green synthesis, Gram-negative bacteria, Gram-positive bacteria

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## Introduction

Nanoparticles range in size from one to 100 nanometers (nm), making them highly applicable in various fields of technology and science. Their properties depend on their shape and size [1]. Various methodologies exist for synthesizing nanoparticles, including chemical, physical, and biological approaches [1, 2]. However, physical and chemical nanoparticle synthesis methods are often time-consuming, potentially hazardous, and costly. The use of potentially unsafe chemicals with cytotoxic, carcinogenic, or genotoxic effects, as well as the generation of waste that can pollute the environment, raises concerns for health and environmental safety. Furthermore, the instability and toxicity of nanoparticles synthesized using such approaches limit their biomedical applications [3]. Therefore, the focus has recently shifted toward developing green chemistry approaches to produce nanoparticles without using hazardous substances. The ability of numerous organisms and bio-based materials to generate nanoparticles through biosynthesis has been discovered, providing a safer and more environmentally friendly method for producing nanoparticles with desirable size and morphology, enhanced stability, greater biocompatibility, and reduced toxicity [3-6].

Numerous previous studies have reported on the biosynthesis of various nanoparticles using extracts from plants, fungi, lichens, bacteria, and algae, as well as agricultural waste, eggshells, and even cow urine [2, 7]. Among biological methods, the use of fungal extracts represents a highly efficient strategy for producing a variety of metallic nanoparticles [8]. Compared to other biological materials used in green nanoparticle synthesis, fungi offer several advantages, including relatively easy cultivation and rapid growth, as well as the secretion of significant amounts of bioactive substances, such as enzymes, proteins, peptides, polysaccharides, and other macromolecules. These characteristics make fungi more suitable for large-scale production [4, 9]. Particular interest has been paid to filamentous fungi due to their ability to grow on readily available and inexpensive substrates and their capability to produce various economically valuable metabolites [10]. Despite the substantial progress in recent years and the reported success of synthesizing metal nanoparticles using various fungal species, the enormous potential of fungi to synthesize different types of metal nanoparticles and the underlying mechanisms are still not fully understood [4, 8].

*Ophiostoma novo-ulmi* Brasier is an ascomycetous phytopathogenic fungus, the causal agent of the ongoing second pandemic of Dutch elm disease (DED) [11]. This pathogen is widespread throughout elm-inhabiting regions of the Northern Hemisphere in the form of two subspecies (subsp. *novo-ulmi* and subsp. *americana*) and their hybrids [12, 13]. Transmission mainly occurs during the breeding and feeding activities of elm bark beetle vectors, though it can also occur via root grafts of neighbouring trees. The pathogenic phase of *O. novo-ulmi* occurs within a tree's vascular system, particularly the xylem, causing vascular wilt and ultimately tree death. The saprotrophic phase occurs in the breeding galleries of the vector under elm bark. Initially, *O. novo-ulmi* was considered to be a necrotrophic pathogen. However, new evidence suggests that it is a hemibiotroph that first invades live cells before transitioning to a necrotrophic lifestyle to acquire nutrients [14]. It exhibits yeast-mycelium dimorphism and is capable of growing in the unicellular yeast stage and in the multicellular hyphal form, and both forms are assumed to be involved in pathogenesis [15]. Sequencing and functional annotation of the genome of the *O. novo-ulmi* strain H327 revealed a total of 8,640 protein-coding genes. Approximately 76% of these genes were assigned functions, including 311 carbohydrate-active enzymes and 48 cytochrome P450s, as well as 1,731 proteins that are potentially involved in pathogen-host interactions. Additionally, seven fungal secondary metabolite clusters were identified [16, 17].

The use of *O. novo-ulmi* in nanoparticle synthesis represents a sustainable and innovative approach. This green bioprocess converts a harmful organism into a valuable biological resource, while minimizing environmental impact and reducing the need for toxic chemicals. Fungi such as *O. novo-ulmi* secrete significant amounts of bioactive compounds, which can serve as natural reducing and stabilizing agents, making them particularly suitable for large-scale, eco-friendly nanoparticle production with potential industrial applications in antimicrobial, catalytic, and biomedical fields. This approach aligns with the principles of sustainability by transforming a problematic pathogen into a renewable resource, exemplifying a circular economy strategy in biotechnology that reduces chemical waste, energy consumption, and environmental hazards while generating high-value materials [18].

Among the various metallic nanoparticles, silver nanoparticles (AgNPs) are recognized for their broad-spectrum antimicrobial potential, which makes them applicable in medicine and agriculture, as well as for other purposes [1, 4, 19]. To the best of our knowledge, the synthesis of silver nanoparticles using the fungus *O. novo-ulmi* has not previously been reported. This study aimed to assess the potential of using the fungus *O. novo-ulmi* for the green synthesis of AgNPs. The successful synthesis and characterization of the resulting nanoparticles were conducted using UV-Vis spectroscopy (Ultraviolet-Visible Spectroscopy), Fourier Transform Infrared Spectroscopy (FT-IR), Transmission Electron Microscopy (TEM), and Powder X-ray Diffraction (PXRD), followed by the evaluation of their antibacterial properties.

## Materials and Methods

### Cultivation of *O. novo-ulmi* and Extract Preparation

The *O. novo-ulmi* isolate K3, obtained from an infected European white elm (*Ulmus laevis*) in the area of Kopački Rit, was cultivated on solid media (Oxoid Malt Extract, MEA, Thermo Scientific) covered with sterile cellophane (Cellophane Membrane Backing, Bio-Rad) in ø 90-mm Petri plates [20]. To prepare the extract, mycelia were stripped off the cellophane after growing for ten days in the dark at 22°C, and 23.7 g was mixed with 50 mL of demineralized water in a 200 mL beaker. The mixture was placed in an ultrasonic bath for 30 min, after which it was left for 7 days in the dark at room temperature. The mixture was filtered twice using Whatman No. 1 filter paper.

### AgNPs Synthesis

A total of 20 mL of the obtained fungal filtrate was mixed with 20 mL of 5 mM $AgNO_3$ in a 1:1 ratio. The pH of the prepared mixture was adjusted from the initial value of 7.3 to 8. After the formation of nanoparticles, centrifugation was performed for 35 min at 6000 rpm (Centrifuge Tehtnica Centric 322A, Domel d.o.o., Slovenia). The resulting nanoparticles were air-dried in the dark at room temperature and used for subsequent characterization and antibacterial testing. Control experiments were performed using (i) $AgNO_3$ solution without fungal extract and (ii) fungal extract without $AgNO_3$ under identical conditions to confirm that nanoparticle formation occurred exclusively due to fungal-mediated reduction.

### Characterization of AgNPs

The UV-Vis spectrophotometer Shimadzu UV-1900 was used to monitor the formation of silver nanoparticles. For spectra on the 20$^{th}$ day that showed increased noise, smoothing was performed using the Savitzky–Golay filter in OriginPro software (OriginLab Corporation). In order to identify functional groups involved in AgNP synthesis, FT-IR spectra of the prepared samples were recorded in the range of 400-4000 $cm^{-1}$ using a Shimadzu FT-IR-8400S spectrophotometer. The average crystallite size was calculated using Scherrer's equation:

$$D = \frac{K\lambda}{\beta cos\theta}$$

where D is the crystallite size (nm), $K$ is the shape factor, λ is the wavelength, $\beta$-full is the width at half maximum (FWHM) of the peak, and $\theta$ is the diffraction angle. X-ray diffraction patterns were recorded using an AERIS PAN analytical X-ray diffractometer. Transmission electron microscopy (TEM) with a JEOL JEM 1200 EX II (Jeol Ltd., Tokyo, Japan) was used to determine the size and morphology of the nanoparticles. Particle size and size distribution were determined from TEM micrographs using ImageJ software.

### Antibacterial Properties of AgNPs

The minimum inhibitory concentrations (MICs) of the AgNPs synthesized using *O. novo-ulmi* extracts were determined using the broth microdilution method [21]. Antibacterial activity was evaluated against four standard bacterial strains obtained from MicroSwab (Italy): Gram-positive (*Bacillus subtilis* subsp. *spizizenii* WDCM 00003-ATCC® 6633™ and *Staphylococcus aureus* WDCM 00032, WDCM 00193-ATCC® 6538™) and Gram-negative (*Escherichia coli* ATCC® 35218™ and *Pseudomonas aeruginosa* WDCM 00025-ATCC® 27853™). Serial dilutions of AgNPs (ranging from 1.0 to 0.00024 mg $mL^{-1}$) were prepared and mixed with bacterial suspensions in the mid-logarithmic growth phase ($5\times10^5$ CFU $mL^{-1}$), which were cultivated in Mueller-Hinton broth. The assay was performed in sterile, flat-bottom 96-well polypropylene microtiter plates. Ciprofloxacin was used as a reference antibiotic and was diluted in the same way for comparison. Additional controls included bacterial growth control, as well as solvent control and medium sterility control, all of which were kept under identical conditions. After 24 h of incubation at 37ºC, a triphenyltetrazolium chloride (TTC) solution was added to each well, followed by an additional 3 h incubation at the same temperature. The MIC value was defined as the lowest concentration of AgNPs that completely inhibited visible bacterial growth, as indicated by the absence of color change. All experiments were performed in triplicate to ensure reproducibility.

## Results and Discussion

The advantages of using fungi to synthesize nanoparticles have been recognized, making mycosynthesis a major focus of numerous studies within the growing field of nanotechnology [6]. Fungi can produce various metal nanoparticles, with AgNPs being particularly promising [8, 22]. A wide variety of fungal enzymes, metabolites, and proteins can influence the formation of AgNPs, and the choice of fungal species significantly affects the properties of the synthesized nanoparticles [23]. Successful AgNP synthesis has been achieved using extracts from different fungal species, including several members of the genus *Fusarium* [24-26], *Aspergillus* [27-29], *Trichoderma* [30-32], and *Penicillium* [33, 34], as well as *Trametes versicolor* [35], *Pleurotus ostreatus* [36], and *Fomes fomentarius* [37], among others. This study demonstrated for the first time that *O. novo-ulmi*, a relatively easily cultivated pathogenic ascomycetous fungus [11], is an effective biological agent for the green synthesis of AgNPs mediated by fungi.

Fungal-mediated biosynthesis of AgNPs can occur through both intracellular and extracellular mechanisms. Intracellular synthesis involves adding the metal precursor to the mycelial culture. However, this method requires additional steps to release and recover the nanoparticles from the cells. The extracellular method, which involves adding the metal precursor to an aqueous filtrate containing only fungal biomolecules, is considered a more applicable route, resulting in the formation of free nanoparticles in the dispersion [4]. A wide range of fungal biomolecules can interact with silver ions and contribute to the reduction and stabilization of AgNPs; nevertheless, the precise mechanisms underlying extracellular synthesis remain incompletely understood [4, 38]. It is generally assumed that enzymes present in the fungal filtrate participate in the reduction of $Ag^+$ to $Ag^0$, leading to the formation of elemental silver nanoparticles [4]. Several studies have supported the role of NADH and NADH-dependent nitrate reductase in this process, although other reducing agents, such as quinones, sugars (e.g., glucose), and phenolic compounds, have also been implicated in AgNP formation [8, 39, 40]. The progression of the reaction is typically accompanied by a visible color change of the fungal filtrate to brown, which is commonly associated with the formation of silver nanoparticles. UV-Visible spectroscopy is routinely used to monitor the development of surface plasmon resonance (SPR) bands, reflecting alterations in the optical properties of the nanoparticles and providing confirmation of successful biogenic synthesis [4, 8, 41]. In this study, AgNPs synthesis was performed using an aqueous extract of *O. novo-ulmi*, which reduced $Ag^+$ and formed stable AgNPs. As expected, the color of the aqueous extract of *O. novo-ulmi* changed from light yellow to brown [42] within a few hours after adding the aqueous $AgNO_3$ solution. The initial characterization of the biologically synthesized silver nanoparticles was obtained using UV-Vis spectroscopy by measuring the absorbance in the range of 350-700 nm (Fig. 1). The UV–Visible spectrum of silver nanoparticles synthesized using fungal extracts exhibited a well-defined and symmetrical surface plasmon resonance (SPR) peak at 402 nm, confirming the successful formation of AgNPs. No color change or characteristic surface plasmon resonance (SPR) peak in the 400-450 nm region was observed in the control samples, confirming that the formation of AgNPs was solely mediated by the fungal extract. The shape and position of the SPR band suggest a relatively uniform size distribution, predominantly spherical morphology, and good colloidal stability. In addition to reflecting the optical properties of the nanoparticles, the SPR bands serve as a rapid and non-invasive indicator of biogenic nanoparticle formation, providing useful preliminary characterization before more detailed structural analyses [43]. As shown in Fig. 1, the SPR peak remained sharp and well defined over a period of three weeks, with no significant shift toward either lower or higher wavelengths. The stability of the SPR peak position over time suggests that the synthesized silver nanoparticles

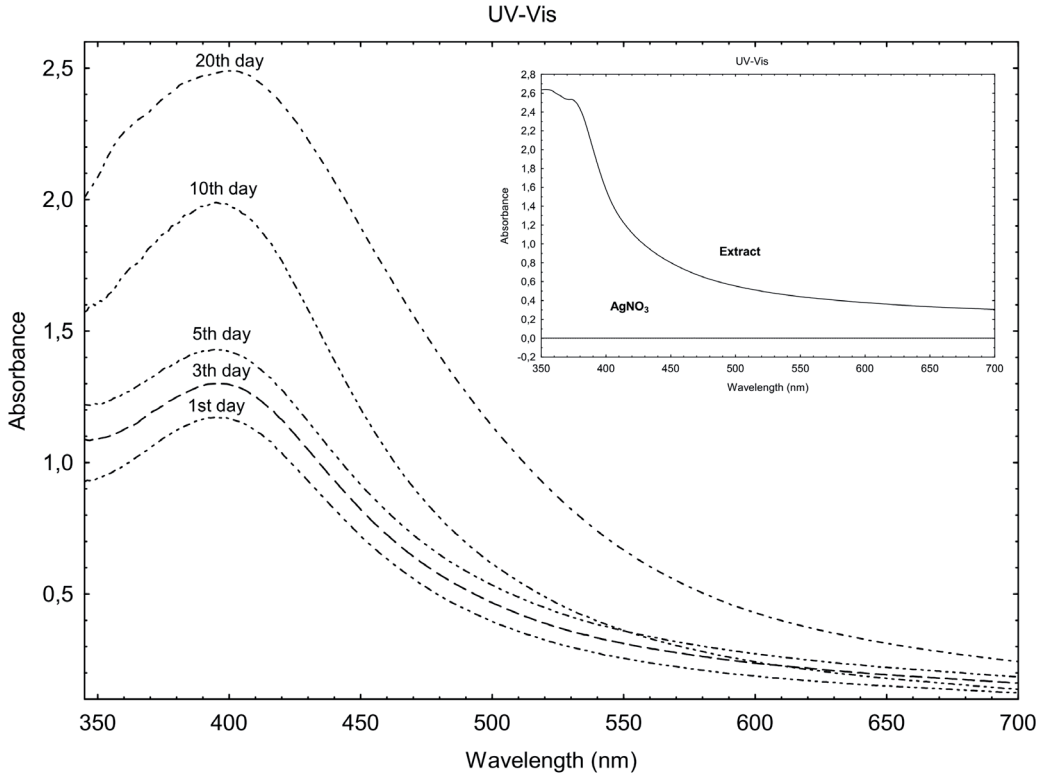


Fig. 1. UV-Vis spectra of silver nanoparticles synthesized using *Ophiostoma novo-ulmi* extract and $AgNO_3$. The inset shows the UV-Vis spectra of the fungal extract and 5 mM $AgNO_3$.

retain a relatively consistent size and shape, remain well-dispersed in the colloidal system, and do not undergo pronounced aggregation during the monitored period [44].

The FT-IR spectra of the aqueous extract of *O. novo-ulmi* and the silver nanoparticles were used for identifying the functional groups in the extract responsible for the reduction of $AgNO_3$ (Fig. 2). In general, the FT-IR spectra of the *O. novo-ulmi* extract (Fig. 2) reveal absorption bands located around 3300, 2932, 2853, 1654, 1554, 1410, 1250, 1030, 900, and 858 $cm^{-1}$. The FT-IR bands at 2850-3000 $cm^{-1}$, which correspond to the asymmetric and symmetric C–H stretching of $–CH_2–$ and $–CH_3–$ groups, are characteristic of the long aliphatic chains in lipids [45]. The wavenumber range between 1220 and 1700 $cm^{-1}$ in the FT-IR spectra is crucial for analysing the structure of proteins and peptides, as it encompasses several key modes associated with the amide group [45]. Within this region, three primary amide vibrations are particularly prominent: amide I, amide II, and amide III, all of which are important in characterizing the secondary structure of proteins. The band at 1030 $cm^{-1}$ is commonly associated with characteristic vibrations of starch and polysaccharides [46]. The bands between 800 and 900 $cm^{-1}$ are often associated with out-of-plane C–H bending in aromatic rings. Following the formation of AgNPs, shifts and/or decreases in the intensity of certain bands were observed, most notably in the regions around 2932 and 2853 $cm^{-1}$, as well as throughout the 800-1654 $cm^{-1}$ range. These changes allowed a comparative analysis between the spectra of the extract and AgNPs.

FT-IR peaks observed at 900 $cm^{-1}$ and other signals below 1000 $cm^{-1}$ in the fungal extract disappeared in the spectrum of the biosynthesized AgNPs. This change suggests that the functional groups responsible for these vibrations, such as C–O–C ring vibrations and aromatic C–H stretches, are no longer freely present or have undergone chemical modifications during nanoparticle formation. The disappearance of these peaks does not indicate the absence of biomolecules; rather, it implies their involvement in the reduction and subsequent capping or stabilization of the AgNPs, contributing to nanoparticle stability and preventing aggregation [47, 48].

Overall, FT-IR analysis of fungal-mediated silver nanoparticles revealed noticeable changes in functional groups associated with fungal biomolecules, supporting their role in the reduction of $Ag^+$ ions and stabilization of the resulting nanoparticles. These observations generally indicate that fungal metabolites contribute to the biogenic formation and capping of AgNPs. The results of FT-IR analysis revealed the presence of diverse biomolecules, including proteins, lipids, and carbohydrates, within the aqueous fungal extract, which may play a key role in the biogenic synthesis and stabilization of AgNPs.

In the context of biogenically synthesized AgNPs, PXRD provides essential information regarding their crystalline nature and phase composition. The presence of characteristic diffraction peaks corresponding to

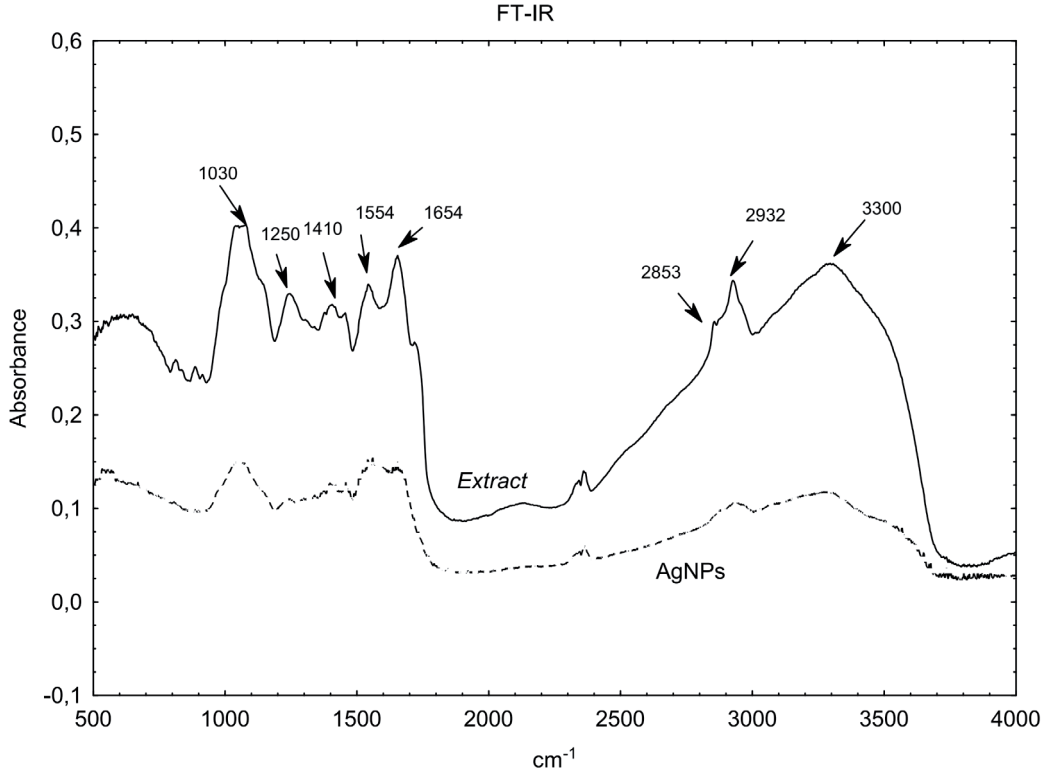


Fig. 2. FT-IR spectra of the aqueous extract of *Ophiostoma novo-ulmi* and the synthesized silver nanoparticles (AgNPs).

metallic silver confirms the formation of crystalline $Ag^0$ and enables its distinction from potential by-products such as unreacted silver ions, silver salts, organic residues, or amorphous phases. Furthermore, analysis of diffraction peak positions and widths allows for the estimation of average crystallite size and offers insight into the structural stability of the synthesized nanoparticles.

The PXRD pattern of the synthesized nanoparticles shows a crystalline structure with several main characteristic signals at 2θ values of 38.08°, 44.35°, 64.31°, and 77.33°, which were indexed as the (111), (200), (220), and (311) crystallographic planes of the face-centered cubic (*fcc*) lattice of silver (Fig. 3). The presence of additional, less intense peaks may be attributed to residual organic components from the extract, i.e., biomolecules that participated in the reduction and stabilization of the silver nanoparticles during the synthesis [37]. Using the Scherrer equation, the average crystallite size was calculated to be approximately 15 nm.

Transmission Electron Microscopy (TEM) was employed to obtain direct high-resolution images of the synthesized silver nanoparticles, enabling accurate determination of their particle size, morphology, and degree of aggregation, thereby complementing the data obtained from UV-Vis, FT-IR, and PXRD analyses. TEM analysis revealed predominantly spherical silver nanoparticles that were well dispersed, with no evidence of pronounced aggregation [49]. Based on the TEM image, a particle size distribution histogram was constructed, showing that most nanoparticles ranged from 18 to 27 nm, with an average size of 22 nm. The histogram provides a quantitative assessment of the particle size distribution, while the TEM images offer visual confirmation of the particle morphology and uniformity (Fig. 4).

Nanoparticles with sizes below 50 nm are generally considered more effective, as smaller diameters are frequently associated with enhanced antimicrobial activity [50-52]. Numerous studies have reported that silver nanoparticles biosynthesized by different fungal species exhibit a range of particle sizes depending on the fungal strain and synthesis conditions. For example, AgNPs synthesized using *Aspergillus sydowii* had an average size of 12 nm [53]. *Cladosporium cladosporoides*, *Penicillium chrysogenum*, and *Purpureocillium lilacinum* produced AgNPs predominantly below 20 nm [54], while different *Trichoderma* species enabled the synthesis of nanoparticles of 8-60 nm in size [55]. *Fusarium oxysporum* produced AgNPs ranging from 21.3 to 37.3 nm in the study by Ahmed et al. [56] and from 20 to 50 nm in the study by Durán et al. [39]. The size range of AgNPs obtained in this study demonstrated that *O. novo-ulmi* is capable of generating nanoscale silver particles within a similar size range as other fungal species, which may have significantly contributed to the observed antibacterial efficacy.

The ability of green-synthesized AgNPs to act as antimicrobial agents, particularly as antibacterial agents, is a primary basis for their many potential applications [23]. Consequently, evaluation of antibacterial activity is one of the most commonly employed parameters for assessing the biological activity of biosynthesized AgNPs [57-61]. The AgNPs synthesized using the aqueous extract of *O. novo-ulmi* were shown to exhibit

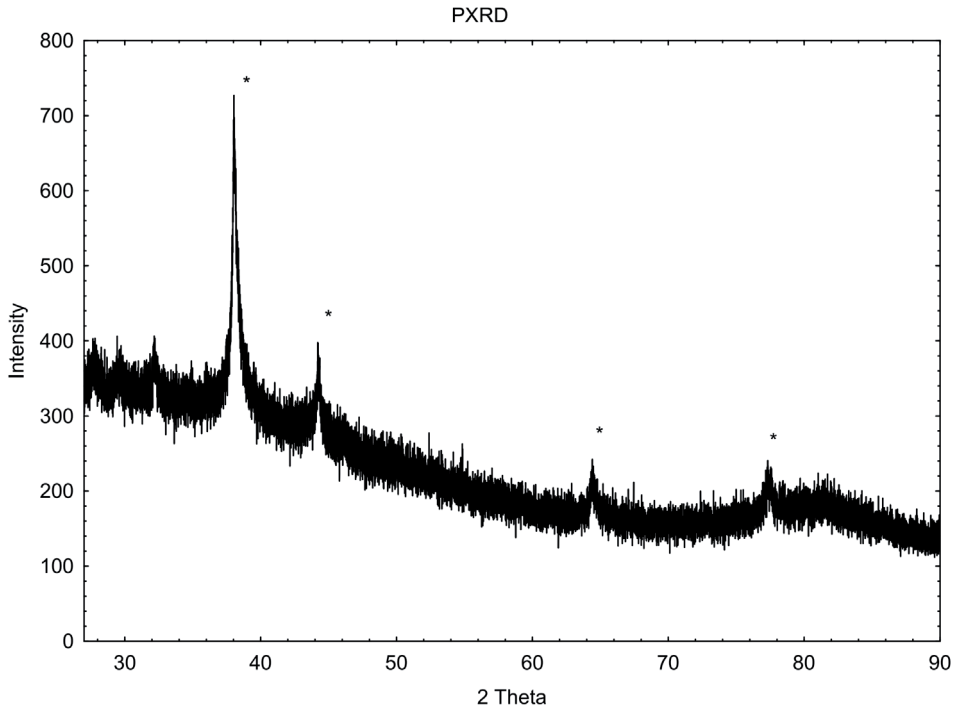


Fig. 3. PXRD pattern of the synthesized silver nanoparticles.

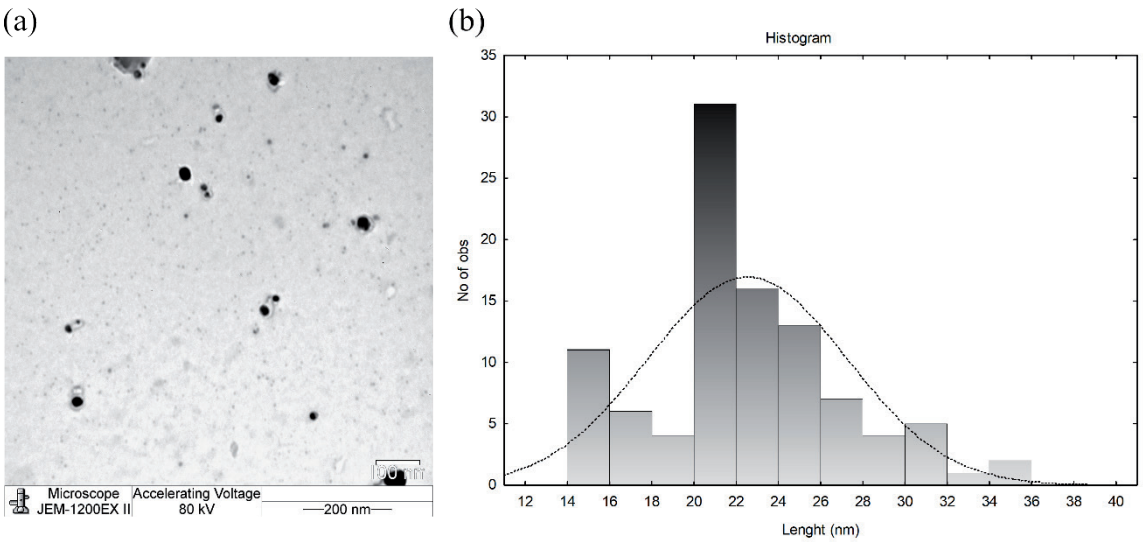


Fig. 4. a) TEM image of the silver nanoparticles synthesized using *Ophiostoma novo-ulmi* and b) the corresponding particle size distribution histogram.

inhibitory effects against all tested bacterial strains (Table 1). The strongest bioactivity was detected against Gram-positive *B. subtilis*, as indicated by the lowest MIC value of 0.01563 mg/mL, while the MIC value against *S. aureus* was 0.125 mg/mL. Among the two Gram-negative bacterial strains used in this study, stronger bioactivity of AgNPs was observed against *P. aeruginosa*, with an MIC value of 0.03125 mg/mL, than against *E. coli*, for which the MIC value was 0.500 mg/mL. The observed MIC values of the AgNPs synthesized using the fungus *O. novo-ulmi* were generally within the range observed for AgNPs synthesized using other green synthesis approaches [59]. The antimicrobial activity of AgNPs is associated with various mechanisms, which start with the adhesion to and penetration of cells, accompanied by damage to intracellular structures and biomolecules, the induction of oxidative stress, and the modification of microbial signal transduction pathways [50, 62]. The antibacterial effects of AgNPs may vary between Gram-positive bacteria and Gram-negative bacteria, which is associated with differences in the cell wall; however, many studies have shown the potency of AgNPs against both [52, 62, 63]. Gharieb et al. [64] demonstrated that AgNPs synthesized using the endophytic fungus *Fusarium proliferatum* have an antibacterial effect on multidrug-resistant bacteria, with a stronger effect on Gram-negative bacteria than on Gram-positive bacteria. The higher sensitivity of Gram-negative bacteria has been attributed to the stronger affinity of AgNPs for their surfaces, promoting membrane damage and subsequent penetration into the cell [52]. However, AgNPs synthesized using the endophytic fungus *Talaromyces funiculosus* exhibited the strongest antibacterial activity against *E. coli*, while their activity against *P. aeruginosa* was lower compared to that observed for Gram-positive bacteria [48]. Similarly, AgNPs biosynthesized using *Chaetomium globosum*, an endophytic fungus isolated from *Panax notoginseng*, showed inhibitory effects against all tested bacterial strains, with the most pronounced antibacterial effect observed against *S. aureus*, whereas the effects on *E. coli* and *B. subtilis* were comparable [65]. Furthermore, AgNPs synthesized using *Fusarium acuminatum* and tested against multidrug-resistant *S. aureus*, *Salmonella typhi*, *Staphylococcus epidermidis*, and *E. coli* showed higher sensitivity against *S. aureus* than against *E. coli* [40], which is consistent with the results obtained in this study. Also, Vijayakumar et al.

Table 1. Minimum inhibitory concentrations (MICs) of silver nanoparticles synthesized using *O. novo-ulmi* extracts against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* (mg $mL^{-1}$).

| | MIC (mg $mL^{-1}$) | | | |
|---|---|---|---|---|
| | Gram-positive | | Gram-negative | |
| | *B. subtilis* | *S. aureus* | *E. coli* | *P. aeruginosa* |
| AgNPs | 0.01563 | 0.125 | 0.500 | 0.03125 |
| C * | 0.244 | 1.953 | 1.953 | 0.488 |

* C-ciprofloxacin in µg $mL^{-1}$.

[66] demonstrated that *E. coli* was the least affected by AgNPs synthesized using the macrofungus *Phellinus adamantinus*, compared to *S. aureus*, *B. subtilis*, and *P. aeruginosa*. In this study, *B. subtilis* was shown to be the most sensitive to AgNPs synthesized using *O. novo-ulmi* extract, which is in agreement with the findings of Li et al. [51], who demonstrated the notable antibacterial performance of AgNPs against *B. subtilis*. The AgNPs were found to have a stronger effect on membrane permeability and accelerate the leakage of reducing sugars from the cytoplasm of *B. subtilis* compared to *E. coli* and *S. aureus,* with the effect being dependent on particle size [63]. Variability in the sensitivity of bacterial strains to biosynthesized AgNPs is consistently reported [67, 68]. Although AgNPs are generally reported to have broad-spectrum antimicrobial activity, including antibacterial and antifungal properties [53, 54, 69], significant differences in antimicrobial properties can be observed even when using closely related fungal strains for nanoparticle synthesis [69]. Understanding the species-specific efficiency of nanoparticles requires a deeper understanding of their antibacterial mechanisms [52]. The size and shape of AgNPs can vary depending on the fungus used and the specific biomolecules involved in the synthesis [8]. Fungal biomolecules, such as proteins, enzymes, amino acids, polysaccharides, and secondary metabolites, act as reducing and stabilizing agents during AgNP synthesis and are also crucial in determining their unique physicochemical properties and biological activity [38, 70, 71]. The specific coating of the nanoparticles not only contributes to their size and stability, but also to their biocompatibility, surface charge, and reactivity [6], which can affect their applicability against specific human or agriculturally important microorganisms [70, 72]. Obtaining the desired properties of biologically synthesized nanoparticles depends on numerous factors, ranging from the choice of the organism used as a biological agent for green synthesis and its cultivation protocols to the optimization of the various parameters important for the biosynthesis process, such as the metal precursor and its concentration, agitation speed, incubation time, temperature, and pH [54, 72]. Modifying the growth conditions of fungi, such as by exposing them to suboptimal or stressful conditions, can increase the efficiency with which green AgNPs are synthesized [73].

In this study, *O. novo-ulmi* was cultivated at its optimal growth temperature [11], which enabled the production of silver nanoparticles with broad antibacterial potential and demonstrated the suitability of this plant pathogenic fungus for nanoparticle biosynthesis. However, genotype-dependent variability in responses to growth temperature has been reported for this species [13, 20]. Therefore, further studies investigating the influence of different growth temperatures and other potential stress conditions may enhance the applicability of *O. novo-ulmi* in myconanotechnology. In addition, to fully explore the biomedical potential of the synthesized nanoparticles, future research should also evaluate their antifungal and antitumor activities.

## Conclusions

This study presents a green, environmentally sustainable, and efficient approach to synthesizing silver nanoparticles (AgNPs) using an aqueous extract of *Ophiostoma novo-ulmi*, a highly aggressive phytopathogen that causes Dutch elm disease. The use of this fungus, which naturally causes considerable ecological and economic damage, adds substantial value to the proposed approach by repurposing harmful, non-beneficial biological material into a process that yields high-value nanomaterials. The synthesis method is rapid, cost-effective, and does not require additional stabilizing agents, further reinforcing its green character. Characterization confirmed the successful synthesis of AgNPs of an appropriate size and morphology, which exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. In summary, these findings demonstrate the considerable potential of *O. novo-ulmi* as an effective biological agent for the green biosynthesis of silver nanoparticles. Future research should focus on optimizing synthesis parameters for large-scale production, evaluating long-term stability and toxicity, and exploring broader biomedical applications such as anticancer, antifungal, and drug delivery systems. Additionally, the environmental applications of these nanoparticles, including water purification and antimicrobial coatings, warrant further investigation.

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## AI Usage Disclosure Statement

During the preparation of this manuscript, the authors used InstaText and ChatGPT (OpenAI) solely for language editing, including spelling, grammar, word choice, readability, and clarity. These tools were not used for data analysis, interpretation of results, or generation of scientific conclusions. The authors reviewed and revised all outputs generated by these tools and take full responsibility for the content of the published article.

## Conflict of Interest

The authors declare no conflict of interest.

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