

Article

Green Synthesis of Silver-Decorated Zinc-Based Nanostructures Mediated by *Russula sanguinea* and Their Biofunctional Properties

Mustafa Emre Akçay

Department of Biology, Faculty of Science, Van Yüzüncü Yıl University, Van 65090, Türkiye; memreakcay@yyu.edu.tr

Abstract

The green synthesis of nanomaterials using biological resources has emerged as a sustainable alternative to conventional chemical routes. In this study, the wild ectomycorrhizal mushroom *Russula sanguinea* (Rs) was employed as a natural reducing and stabilizing agent for the biosynthesis of silver-decorated zinc-based nanostructures (Ag–ZnNSs/Rs). The formation and physicochemical properties of the nanostructures were systematically characterized using UV–Vis spectroscopy, FT-IR spectroscopy, SEM, TEM, and EDX analysis. Transmission electron microscopy revealed predominantly spherical nanoparticles with good dispersion, and quantitative analysis of 227 individual particles demonstrated an average diameter of 19.36 ± 7.89 nm (range: 10.92–61.00 nm). FT-IR analysis confirmed the involvement of fungal biomolecules in metal ion reduction and surface stabilization, indicating effective bio-capping of the nanostructures. The biofunctional performance of the biosynthesized Ag–ZnNSs/Rs was evaluated through antioxidant and antimicrobial assays. Compared to the crude mushroom extract, the nanostructures exhibited significantly enhanced 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity with an IC_{50} value of 7.29 ± 0.10 mg mL^{−1} compared to 13.66 ± 0.15 mg mL^{−1} for the crude extract. In addition, notable antimicrobial activity was observed against representative Gram-positive and Gram-negative bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*) as well as the yeast *Candida albicans*. Overall, this study demonstrates that *Russula sanguinea* is an effective biological platform for the green synthesis of silver-decorated zinc-based nanostructures with improved biofunctional properties, highlighting the potential of wild mushrooms as underexplored resources in sustainable nanomaterial development.

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1. Introduction

Nanomaterials have attracted increasing scientific interest owing to their size-dependent physicochemical properties and their broad applicability in biomedical, environmental, and technological fields. In recent years, the development of green synthesis strategies has emerged as a sustainable alternative to conventional chemical and physical approaches, which often require hazardous reagents, extreme reaction conditions, and high energy input [1,2]. Green nanotechnology emphasizes the use of biological systems as eco-

friendly platforms for nanomaterial production, enabling reduced environmental impact while enhancing material functionality.

Among biological resources, fungi represent particularly effective biofactories for nanomaterial synthesis due to their metabolic diversity and ability to secrete a wide range of secondary metabolites, including phenolics, flavonoids, polysaccharides, proteins, and organic acids [3,4]. These biomolecules can simultaneously act as reducing, capping, and stabilizing agents, allowing nanoparticle formation under mild conditions while imparting biological functionality to the material surface. Consequently, fungal-mediated green synthesis has become a promising strategy for producing stable and bioactive nanostructures within sustainable nanotechnology frameworks [5].

Silver-based nanomaterials have been extensively investigated due to their broad-spectrum antimicrobial properties and relevance in biomedical applications [6,7]. However, monometallic silver systems often face limitations related to particle aggregation, stability, and uncontrolled ion release. To address these challenges, recent research has increasingly focused on hybrid and decorated nanostructures, where silver is combined with other metal or metal-oxide components to achieve synergistic physicochemical and biological effects [8,9]. In this context, zinc-based nanostructures are particularly attractive owing to their structural versatility, biocompatibility, and ability to serve as effective scaffolds for silver deposition.

Silver–zinc hybrid systems, especially silver-decorated zinc-based nanostructures, have demonstrated enhanced stability and improved biological performance compared to their monometallic counterparts [10,11]. Importantly, such systems do not necessarily represent substitutional alloy formation at the atomic level but rather heterogeneous surface-decorated architectures in which silver nanoparticles are surface-associated with zinc-based matrices. This distinction is critical, as surface decoration enables controlled silver exposure while preserving the functional advantages of the zinc support, resulting in multifunctional nanomaterials suitable for antimicrobial and antioxidant applications.

Despite the growing interest in fungal-mediated nanomaterial synthesis, studies focusing on ectomycorrhizal mushrooms remain limited. The genus *Russula*, comprising ecologically important ectomycorrhizal species, is widely distributed in temperate forest ecosystems and is known for its rich content of bioactive compounds, including phenolics and pigments with reported antioxidant and antimicrobial activities [12,13]. *Russula sanguinea*, in particular, is characterized by its distinctive pigmentation and metabolite profile; however, its potential as a biological platform for the synthesis of hybrid metal nanostructures has not yet been systematically explored.

Silver nanoparticles (AgNPs) are widely recognized for their strong antimicrobial activity; however, concerns regarding cytotoxicity, environmental accumulation, and the potential induction of adaptive resistance mechanisms have encouraged the exploration of hybrid or composite nanostructures. Incorporation of zinc-based components alongside silver offers several potential advantages. Zinc-containing phases are known to exhibit intrinsic antimicrobial and antioxidant properties, lower toxicity profiles compared to high-dose silver systems, and improved biocompatibility in certain biological contexts. Moreover, Ag–Zn hybrid systems may provide synergistic antimicrobial effects through multi-target mechanisms, including membrane interaction, reactive oxygen species generation, and metal ion release dynamics. Therefore, the development of silver-decorated zinc-based nanostructures is not only structurally relevant but also biologically motivated, aiming to balance antimicrobial efficacy with reduced metal burden and enhanced functional performance.

In this context, the present study reports the green synthesis of silver-decorated zinc-based nanostructures (Ag–ZnNSs/Rs) mediated by the aqueous extract of *Russula sanguinea* (Rs). An earlier preliminary version of this study was disseminated as a non-peer-

reviewed preprint; however, the present manuscript includes newly generated quantitative TEM data, revised graphical analyses, and expanded structural and functional interpretation prepared specifically for formal peer review.

Comprehensive physicochemical characterization was performed using UV–Vis spectroscopy, FT-IR spectroscopy, SEM, TEM, and EDX analysis, with particular emphasis on transmission electron microscopy for detailed evaluation of particle size, morphology, and dispersion. Furthermore, the biofunctional properties of the biosynthesized nanostructures were investigated through antioxidant and antimicrobial assays, highlighting the potential of *R. sanguinea* as an underexplored fungal resource for sustainable nanomaterial development.

2. Materials and Methods

2.1. Field and Laboratory Studies for Identification of *Russula sanguinea*

Fresh fruiting bodies (basidiomata) of *Russula sanguinea* (Bull.) Fr. were collected during the 2023 mushroom fruiting season from mixed coniferous forest ecosystems dominated by *Pinus sylvestris* L. and *Picea orientalis* (L.) Peterm. in the Sarıkamış district of Kars province, Türkiye. The specimens were transported to the laboratory under controlled conditions to preserve morphological integrity.

Morphological identification was performed based on standard taxonomic monographs [14,15]. Macroscopic characteristics, including pileus color, lamellae structure, and organoleptic properties, were recorded in situ. Microscopic features (e.g., basidiospores, basidia, cystidia, and pileipellis structure) were examined using a light microscope (Leica DM500, Wetzlar, Germany) following rehydration and staining with standard reagents [16] (5% KOH, Congo Red, and Melzer's reagent). Spore measurements were obtained from at least 30 mature basidiospores using Leica LAS EZ image analysis software (Leica Microsystems GmbH, Wetzlar, Germany, version 3.4.0).

Based on the diagnostic blood-red pileus, adnate lamellae, acrid taste, and distinct micromorphological features, the specimens were confirmed as *Russula sanguinea* (Figure 1a,b). A voucher specimen has been deposited in the Fungarium of Van Yüzüncü Yıl University (VANF) under the accession number VANF-7897.



Figure 1. Morphological characterization of *Russula sanguinea* used for biosynthesis. (a) Macroscopic field photograph of the collected fruiting bodies (Scale bar: 2 cm). (b) Microscopic view of spores showing characteristic ornamentation (Scale bar: 10 µm).

2.2. Chemicals and Microbial Strains

Analytical grade reagents were used throughout the study without further purification. Silver nitrate (AgNO_3 , 99%) and zinc acetate dihydrate ($\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$) served

as the metal precursors for nanostructure synthesis. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) used for antioxidant assays, as well as microbiological media including Tryptic Soy Broth (TSB) and Mueller–Hinton Agar (MHA), were purchased from Sigma-Aldrich (St. Louis, MO, USA). For Transmission Electron Microscopy (TEM) preparations, carbon-coated copper grids (300 mesh) were employed. Deionized water was used for the preparation of all aqueous solutions and mushroom extracts.

The antimicrobial potential of the synthesized nanostructures was evaluated against a panel of standard reference strains, including Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*), Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*), and the yeast *Candida albicans*. These microbial strains were obtained from the Culture Collection of the Vocational School of Health Services, Van Yüzüncü Yıl University (Türkiye).

2.3. Preparation of Mushroom Extract and Nanostructure Synthesis

Collected *R. sanguinea* basidiomata were surface-cleaned with sterile distilled water, air-dried at room temperature for 21 days, and ground into a fine powder. A total of 50 g of the dried material was extracted with 250 mL of distilled water by maceration for 24 h. To enhance the extraction of bioactive compounds, microwave-assisted extraction was applied (400 W for 5 min) following previously reported protocols [17]. The extract was filtered and used as a natural reducing and stabilizing agent.

Green synthesis of silver-decorated zinc-based nanostructures was carried out by adding AgNO_3 and $\text{Zn}(\text{CH}_3\text{COO})_2 \cdot 2\text{H}_2\text{O}$ to the fungal extract at final concentrations of 1 mM each. The reaction mixture was magnetically stirred at ambient temperature ($25 \pm 2^\circ\text{C}$) in the dark for 48 h to prevent photoactivation, following mushroom-mediated synthesis approaches reported in the literature [18,19]. The resulting nanostructures were collected by centrifugation at 10,000 rpm for 15 min, washed three times with deionized water to remove residual organic components, and dried at 60°C for further analyses.

The bioreduction mechanism is attributed to fungal metabolites acting as electron donors for Ag^+ reduction and as stabilizing agents for zinc-based species, consistent with previously described fungal-mediated nanostructure formation mechanisms [1,3].

2.4. Determination of Antioxidant Activity (DPPH Assay)

The antioxidant activity of the crude *Russula sanguinea* extract and the biosynthesized Ag–Zn nanostructures was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay following established protocols [20,21]. Sample solutions at concentrations ranging from 1 to 20 mg mL^{-1} were mixed with freshly prepared DPPH solution and incubated in the dark at room temperature for 30 min.

After incubation, absorbance was measured at 517 nm using a UV–Vis spectrophotometer. The percentage of DPPH radical scavenging activity was calculated relative to a control according to standard equations. All measurements were performed in triplicate, and the results were expressed as mean $\pm$ standard deviation.

2.5. Antimicrobial Activity

The antimicrobial activity of the biosynthesized Ag–Zn nanostructures was evaluated using the disk diffusion method in accordance with the guidelines of the Clinical and Laboratory Standards Institute [22]. Briefly, overnight cultures of the test microorganisms were adjusted to 0.5 McFarland turbidity standard ($\approx 1 \times 10^8$ CFU mL^{-1} for bacteria) and uniformly spread onto the surface of Mueller–Hinton agar plates (for bacteria) or appropriate yeast agar medium (for *Candida albicans*).

Sterile paper disks (6 mm diameter) were impregnated with defined volumes of Ag–Zn nanostructure suspensions and placed onto the inoculated agar surfaces. Plates were

incubated at 37 °C for 24 h for bacterial strains and at 30 °C for 48 h for *C. albicans*. After incubation, antimicrobial efficacy was determined by measuring the diameters of the inhibition zones surrounding each disk. All experiments were performed in triplicate, and the results were expressed as mean inhibition zone diameters $\pm$ standard deviation.

2.6. Instrumental Characterization

Ultraviolet–visible (UV–Vis) spectroscopy was used to monitor the optical properties and formation of the biosynthesized nanostructures in the wavelength range of 200–800 nm, following standard nanoparticle characterization procedures [23]. Functional groups associated with the reduction and stabilization of metal species were identified by Fourier-transform infrared (FT-IR) spectroscopy in the range of 4000–800 cm^{-1} using the KBr pellet method, enabling the assessment of fungal biomolecules involved in surface capping [24].

Surface morphology and microstructural features were examined using a field-emission scanning electron microscope (FE-SEM, ZEISS Sigma 300, Carl Zeiss Microscopy GmbH, Oberkochen, Germany) equipped with SE2 and in-lens secondary electron detectors and a high-definition backscattered electron detector (HDBSD). Imaging was performed under high-vacuum conditions at an accelerating voltage of 15 kV. Elemental analysis was simultaneously conducted using an integrated energy-dispersive X-ray (EDX) detector to obtain qualitative and quantitative compositional information [25].

Transmission electron microscopy (TEM) imaging was performed at an accelerating voltage of 100 kV using a JEOL JEM-1010 transmission electron microscope (JEOL Ltd., Tokyo, Japan). TEM was used to determine particle size, morphology, and dispersion characteristics of the biosynthesized Ag–ZnNSs/Rs. For sample preparation, the nanostructures were dispersed in deionized water and ultrasonicated for 10 min to ensure homogeneous distribution. A drop of the diluted suspension was deposited onto a carbon-coated copper grid (300 mesh) and allowed to dry at room temperature under ambient conditions. Excess solution was carefully removed to minimize aggregation artifacts.

Quantitative size analysis was conducted by measuring 227 randomly selected individual nanoparticles from 23 independent TEM micrographs using ImageJ software (NIH, Bethesda, MD, USA, version 1.54). The extracted particle diameter data were further processed using Python-based data analysis tools, and the particle size distribution was expressed as a frequency histogram generated via custom Python scripts [26]. This combined image-based and computational approach ensured reliable statistical evaluation of nanoscale dimensions and minimized sampling bias.

2.7. Statistical Analysis

All experiments were conducted in triplicate unless otherwise stated, and the results are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using Python (Python Software Foundation, Wilmington, DE, USA, version 3.11.6) with the SciPy (version 1.11.4) library. Differences between two independent groups were evaluated using an independent samples t-test after verification of normal distribution assumptions. A p -value < 0.05 was considered statistically significant.

For antioxidant activity analysis, IC_{50} values were determined using nonlinear regression based on a sigmoidal dose–response model fitted to the experimental data. The goodness-of-fit was assessed using the coefficient of determination (R^2). Error bars shown in graphical representations correspond to standard deviation derived from three independent measurements.

3. Results and Discussion

3.1. Visual Observation and Color Change

The primary indication of the successful biosynthesis of silver-decorated zinc-based nanostructures (Ag–ZnNSs/Rs) was observed through visual monitoring of the reaction medium. Upon the addition of metal precursors (AgNO_3 and $\text{Zn}(\text{CH}_3\text{COO})_2$) to the cell-free aqueous extract of *Russula sanguinea*, the initially clear cherry-red solution gradually changed to a dark turbid brown within 48 h of incubation (Figure 2).

Such a color transformation is commonly associated with the formation of silver-based nanostructures and is attributed to surface plasmon resonance (SPR) phenomena arising from the collective oscillation of conduction electrons at the nanoparticle surface [1,27]. The emergence of brownish coloration provides qualitative evidence for the reduction of Ag^+ ions and the formation of nanoscale silver domains within the zinc-based matrix.

This visual change further suggests the involvement of bioactive fungal metabolites in the reduction and stabilization processes. Functional groups present in phenolics, flavonoids, and proteins are known to facilitate metal ion reduction while simultaneously acting as capping agents, thereby limiting nanoparticle aggregation [3,4]. Similar SPR-associated optical transitions have been widely reported in fungal-mediated synthesis of mono- and hybrid metal nanostructures [28,29].

To quantitatively confirm this optical behavior and further elucidate the physico-chemical characteristics of the biosynthesized Ag–ZnNSs/Rs, UV–Vis spectroscopic analysis was subsequently performed.

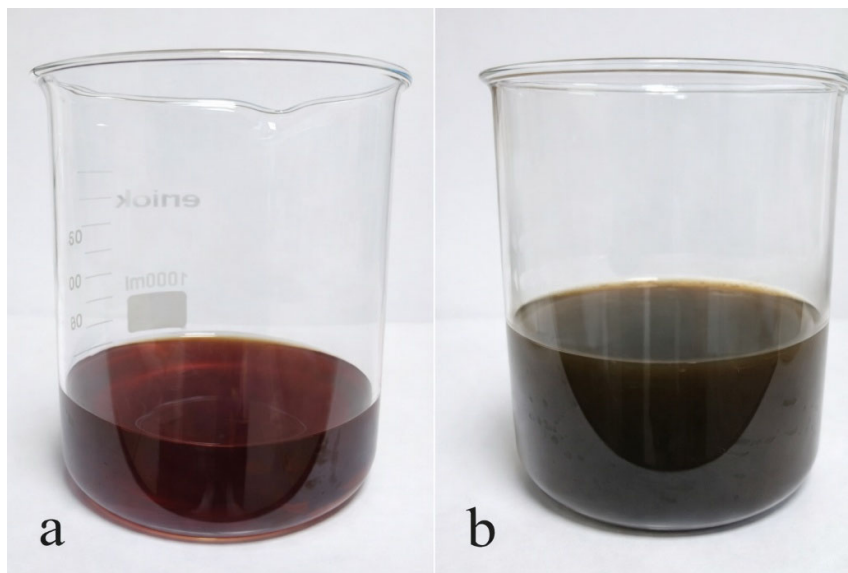


Figure 2. Visual observation of the reaction mixture during the green synthesis of silver-decorated zinc-based nanostructures (Ag–ZnNSs/Rs) mediated by *Russula sanguinea* extract: (a) cell-free aqueous mushroom extract prior to the addition of metal precursors, exhibiting a clear cherry-red coloration; (b) reaction mixture after 48 h of incubation following the addition of AgNO_3 and $\text{Zn}(\text{CH}_3\text{COO})_2$, showing a dark turbid brown color indicative of nanostructure formation.

3.2. Surface Morphology (FESEM Analysis)

The surface morphology and structural features of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs) were examined using field-emission scanning electron microscopy (FESEM) operated at an accelerating voltage of 15 kV. The FESEM

micrographs (Figure 3) revealed a heterogeneous and hierarchical architecture with a polydisperse morphological distribution.

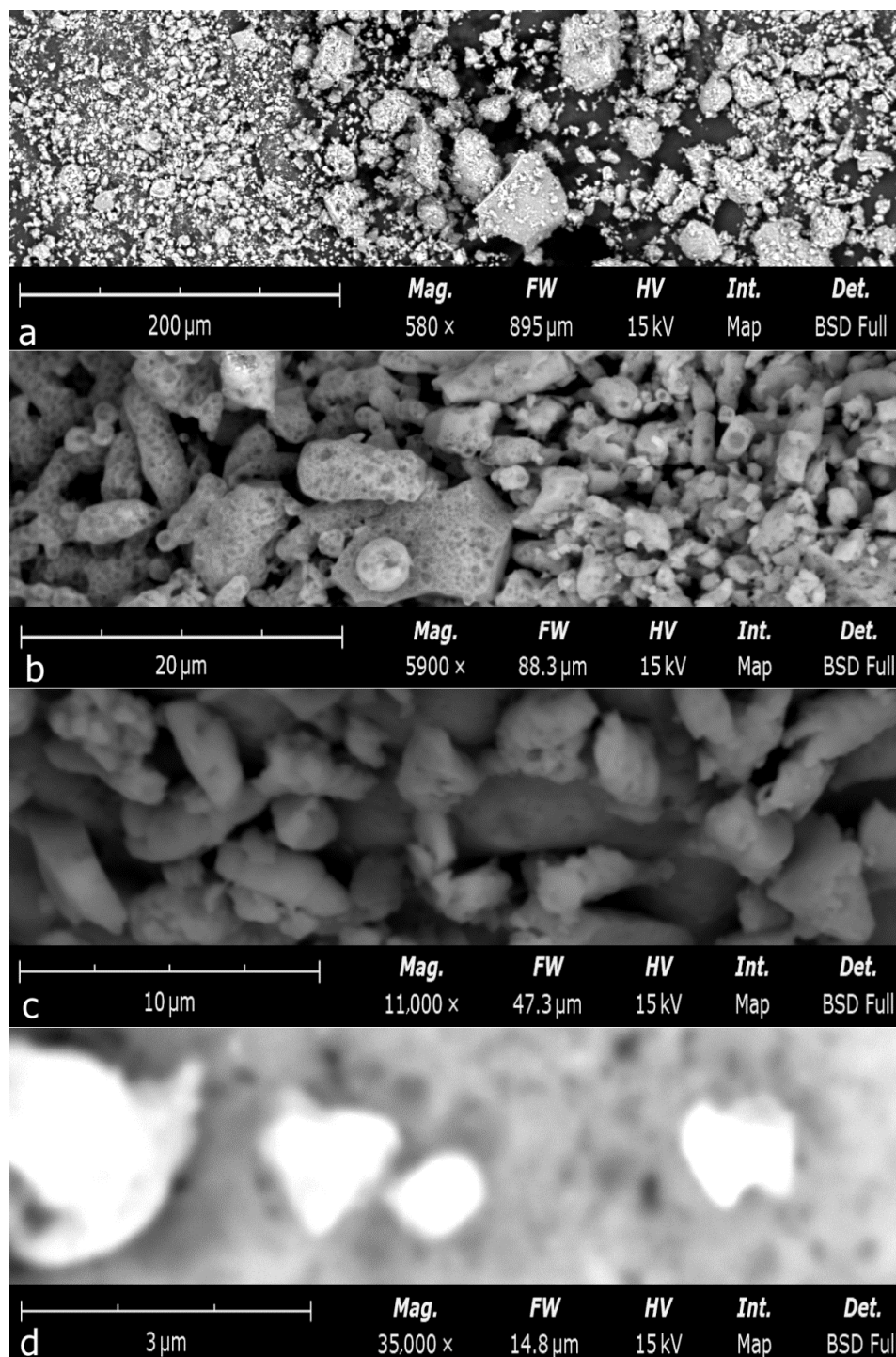


Figure 3. FESEM micrographs of the biosynthesized silver-decorated zinc-based nanostructures (Ag-ZnNSs/Rs) acquired using a backscattered electron detector at 15 kV, showing surface morphology at different magnifications: (a) low magnification with heterogeneous particle distribution and micro-agglomerates (200 μm); (b) intermediate magnification showing irregular laminar assemblies with granular features (20 μm); (c) higher magnification highlighting elongated and quasi-spherical domains (10 μm); and (d) high magnification revealing fine surface texturing of the composite structure (3 μm).

At lower magnifications, the material exhibited irregular laminar (plate-like) structures accompanied by compact micro-agglomerates. Such morphologies are commonly associated with zinc-based phases synthesized via green routes and may indicate the formation of a supporting inorganic matrix. At higher magnifications, fine quasi-spherical nanogranules were observed to be distributed across the surfaces of the larger laminar assemblies.

The coexistence of laminar structures and surface-deposited nanogranules suggests the formation of a hybrid or decorated nanostructural system, in which smaller nanoscale domains are associated with larger zinc-containing phases. The observed agglomeration behavior can be attributed to the presence of organic constituents derived from the *Russula sanguinea* extract, which likely contribute to particle stabilization and interparticle interactions during biosynthesis.

3.3. Elemental Composition (EDX Analysis)

The elemental composition of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs) was investigated using energy-dispersive X-ray (EDX) spectroscopy (Figure 4). The quantitative analysis revealed that silver (Ag) was the predominant metallic component, accounting for 48.5 wt%, while zinc (Zn) was present at 12.4 wt%, confirming the coexistence of both elements within the synthesized nanostructures.

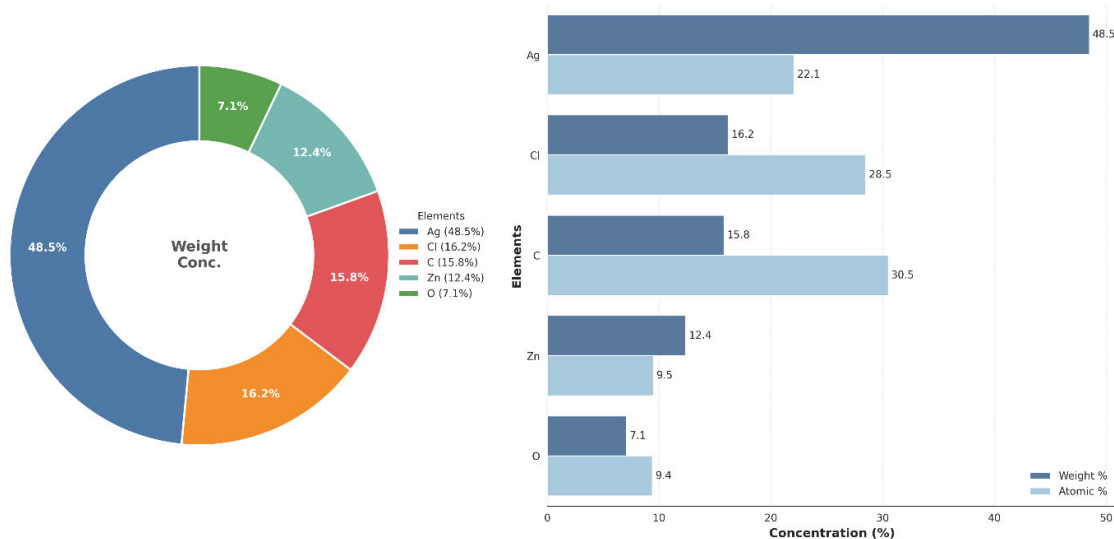


Figure 4. Quantitative elemental composition of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs) obtained from EDX analysis, presented as weight percentage (donut chart) and comparative weight and atomic percentages (bar chart) of the detected elements, including Ag, Zn, C, O, and Cl.

In addition to the metallic constituents, carbon (15.8 wt%) and oxygen (7.1 wt%) were detected. These elements are attributed to surface-associated organic biomolecules originating from the *R. sanguinea* extract, in agreement with FT-IR results confirming effective bio-capping of the nanostructures and the organic nature of the green synthesis process.

Chlorine (16.2 wt%) was detected in the EDX analysis. Since no chloride-containing precursors were used during synthesis, the Cl signal is attributed to surface-associated inorganic residues originating from the fungal extract or minor environmental contamination during sample preparation. It is therefore interpreted as a surface-related signal rather than evidence of structural incorporation into the Ag–Zn nanostructures. Minor

potassium (K) signals may originate from intrinsic mineral components of the fungal extract, as commonly reported in biosynthetic systems [10].

The strong Ag-L peaks (~3 keV) and the detectable Zn-L (~1 keV) and Zn-K α (~8.6 keV) signals in the EDX spectrum further confirm successful incorporation of both metals (Figure 5). It should be emphasized that EDX primarily reflects surface elemental composition rather than bulk stoichiometry. Therefore, the observed Ag/Zn ratio represents surface distribution characteristics and does not necessarily indicate alloy formation or exact bulk composition.

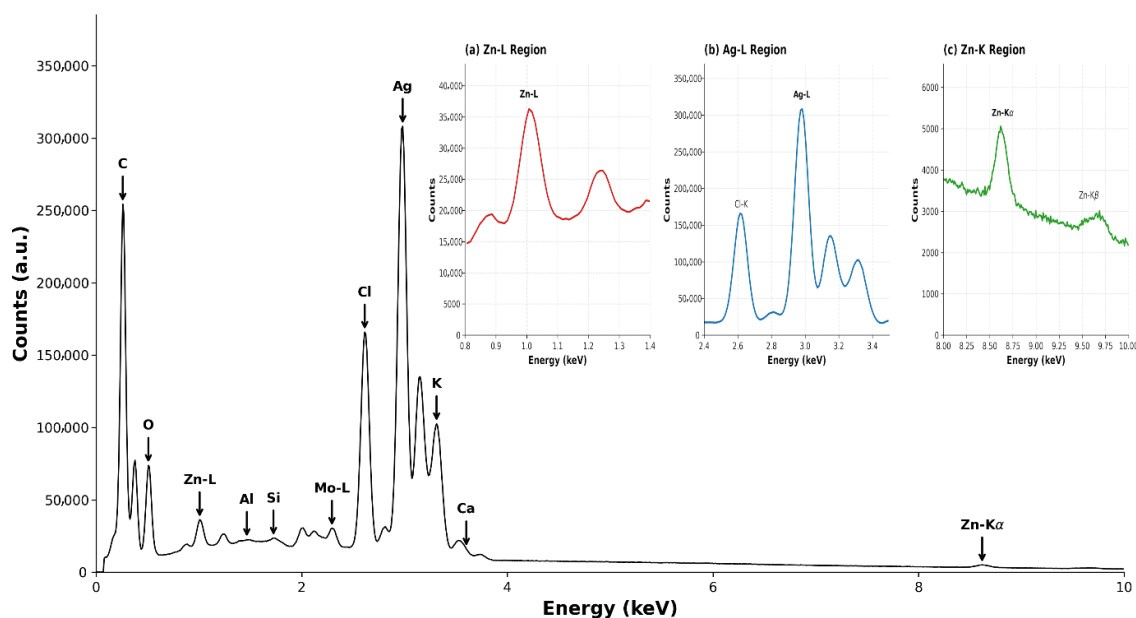


Figure 5. Energy-dispersive X-ray (EDX) spectrum of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs) showing the presence of C, O, Zn, Ag, Cl, K, and minor elements. Insets present magnified views of the (a) Zn L region (~1.0 keV), (b) Ag L region (2.0–3.3 keV), and (c) Zn K region (~8.6 keV) to highlight characteristic emission peaks and low-intensity features.

3.4. Nanostructural Morphology and Size Distribution (TEM Analysis)

Transmission electron microscopy (TEM) was employed to investigate the size, morphology, and dispersion characteristics of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs). Representative TEM micrographs (Figure 6a–c) revealed that the nanostructures exhibit a predominantly spherical morphology with a polydisperse size distribution. The particles were generally well dispersed, although localized agglomeration was observed, which is commonly reported in biologically mediated synthesis processes involving organic matrices.

Quantitative size analysis was carried out by measuring 227 randomly selected individual nanoparticles extracted from 23 independent TEM micrographs using image analysis tools, followed by Python-based statistical processing. The resulting particle size distribution histogram (Figure 6d) exhibited a unimodal distribution, with an average particle diameter of 19.36 ± 7.89 nm, ranging from 10.92 to 61.00 nm.

At higher magnifications, a faint light-contrast layer surrounding the darker metallic cores was observed. Such contrast features are frequently associated with surface-bound organic components originating from biological extracts and may indicate the presence of capping biomolecules derived from *Russula sanguinea*. This organic layer is expected to contribute to nanoparticle stabilization and reduced aggregation during biosynthesis.

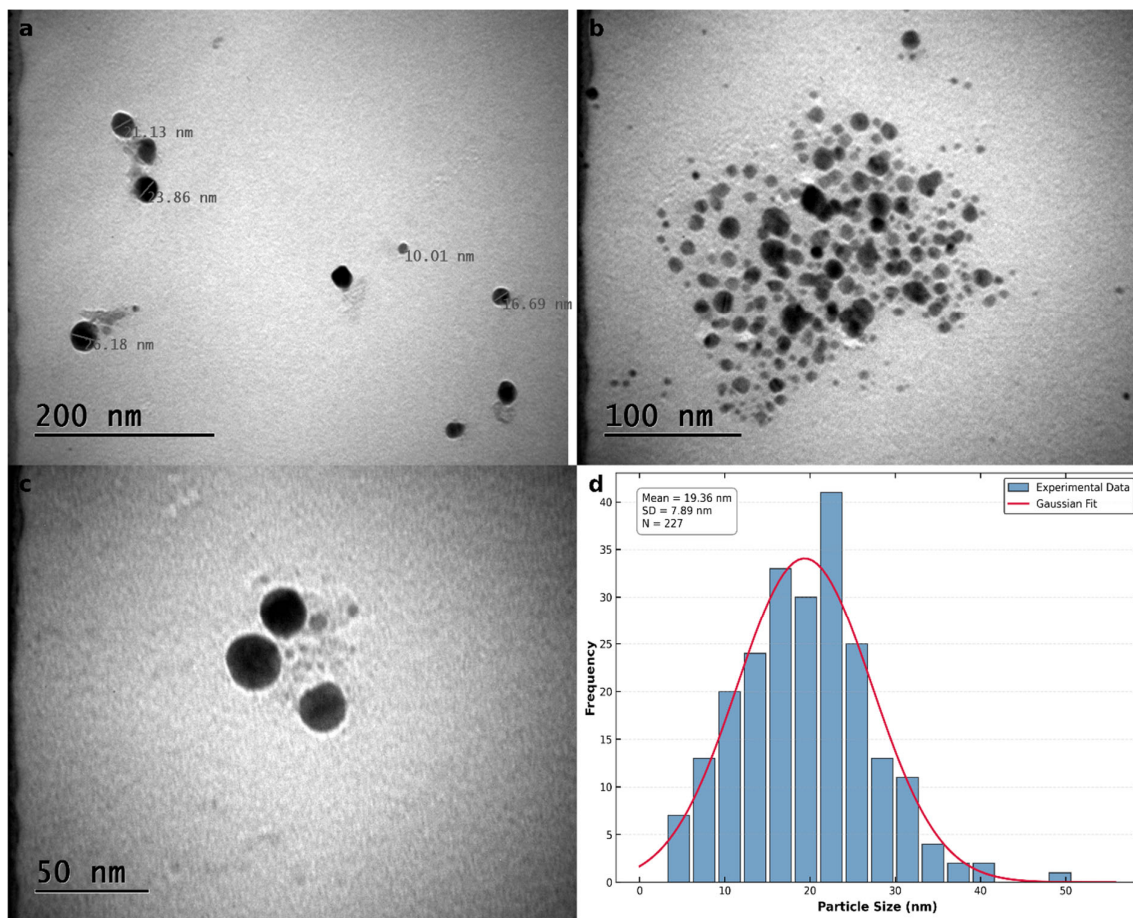


Figure 6. Transmission electron microscopy (TEM) analysis of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs): (a–c) representative TEM micrographs at increasing magnifications showing predominantly spherical nanoparticles with localized agglomeration (scale bars: 200 nm (a), 100 nm (b), and 50 nm (c)); (d) particle size distribution histogram derived from measurements of 227 randomly selected nanoparticles, showing an average particle diameter of 19.36 ± 7.89 nm with a Gaussian fit.

3.5. Optical Properties and UV–Vis Spectroscopy

Preliminary evidence for the biogenic synthesis of Ag–Zn nanostructures was obtained through visual observation of the reaction medium. Upon the addition of the *Russula sanguinea* extract to the metal precursor solution, the initially clear cherry-red solution gradually changed to a dark turbid brown within 48 h of incubation, indicating the reduction of metal ions and the formation of colloidal nanostructures.

The UV–Vis absorption spectra (Figure 7) provided further support for this transformation. The native *R. sanguinea* aqueous extract exhibited characteristic absorption features in the UV region below 300 nm, which are commonly associated with fungal secondary metabolites, including phenolic and aromatic compounds [2,30]. Following the green synthesis process, a pronounced change in the absorption profile was observed, most notably the emergence of a broad absorption band centered at approximately 423 nm. This feature is widely attributed to the surface plasmon resonance (SPR) of silver-based nanostructures synthesized via biological routes [1,27,31,32].

In the lower UV region, additional spectral variations were detected, which may be related to zinc-containing phases or ZnO-associated electronic transitions. Although ZnO typically exhibits absorption in the 300–380 nm range, these features appeared broadened or less distinct in the present system. Such behavior can be associated with the dominance

of the Ag-related SPR band, as well as the influence of surface-bound biomolecules derived from the fungal extract, which are known to modify optical responses in biologically synthesized nanostructures [4,33].

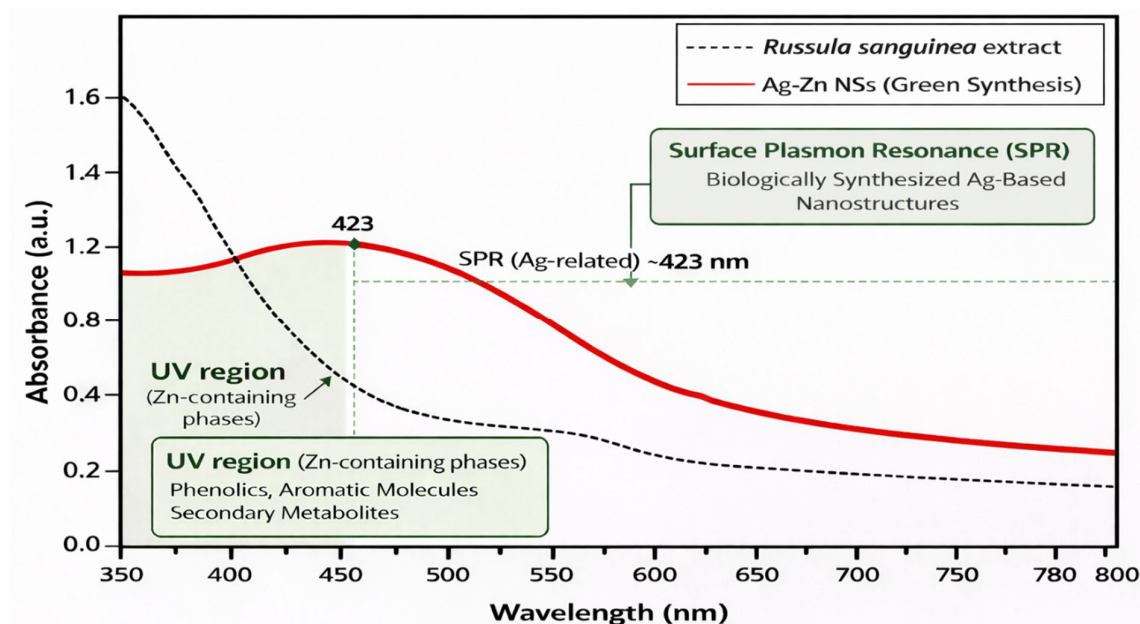


Figure 7. UV–Vis absorption spectra of the native *Russula sanguinea* aqueous extract and the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs), showing the emergence of a broad absorption band centered at ~423 nm after the green synthesis process, attributed to the surface plasmon resonance (SPR) of silver-based nanostructures.

Overall, the observed spectral changes, in combination with visual color transformation and complementary characterization results, provide supportive evidence for the successful formation of biogenically synthesized Ag–Zn-based nanostructures. Similar UV–Vis characteristics have been reported in other mushroom-mediated syntheses of metal and hybrid nanostructures, where fungal metabolites significantly influence optical properties and colloidal stability [29,34].

3.6. Chemical Functionality and Interaction Mechanism (FT-IR Analysis)

Fourier transform infrared (FT-IR) spectroscopy was employed to investigate the chemical functionalities involved in the biogenic reduction of metal ions and the stabilization of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs). The FT-IR spectrum of the native *Russula sanguinea* aqueous extract (Figure 8) exhibited several characteristic absorption bands associated with fungal secondary metabolites.

Distinct bands at 2975 and 2904 cm^{-1} were assigned to C–H stretching vibrations of aliphatic groups present in lipids and protein side chains. Additional absorption peaks observed at 1387 cm^{-1} correspond to N–H bending vibrations of amine groups, while bands at 1081 and 1046 cm^{-1} are attributed to C–O stretching vibrations of carbohydrates and ether groups. A weaker band around 881 cm^{-1} can be attributed to aromatic C–H out-of-plane bending vibrations typically associated with substituted benzene rings, indicating the presence of phenolic-type compounds commonly reported in fungal extracts [35,36]. The characteristic Zn–O stretching vibrations, generally reported in the 400–600 cm^{-1} region, were not captured in the present spectrum, as the recorded wavenumber range was limited to 4000–800 cm^{-1} . Therefore, the presence of zinc-containing phases was confirmed by EDX analysis rather than by direct FT-IR detection of Zn–O modes.

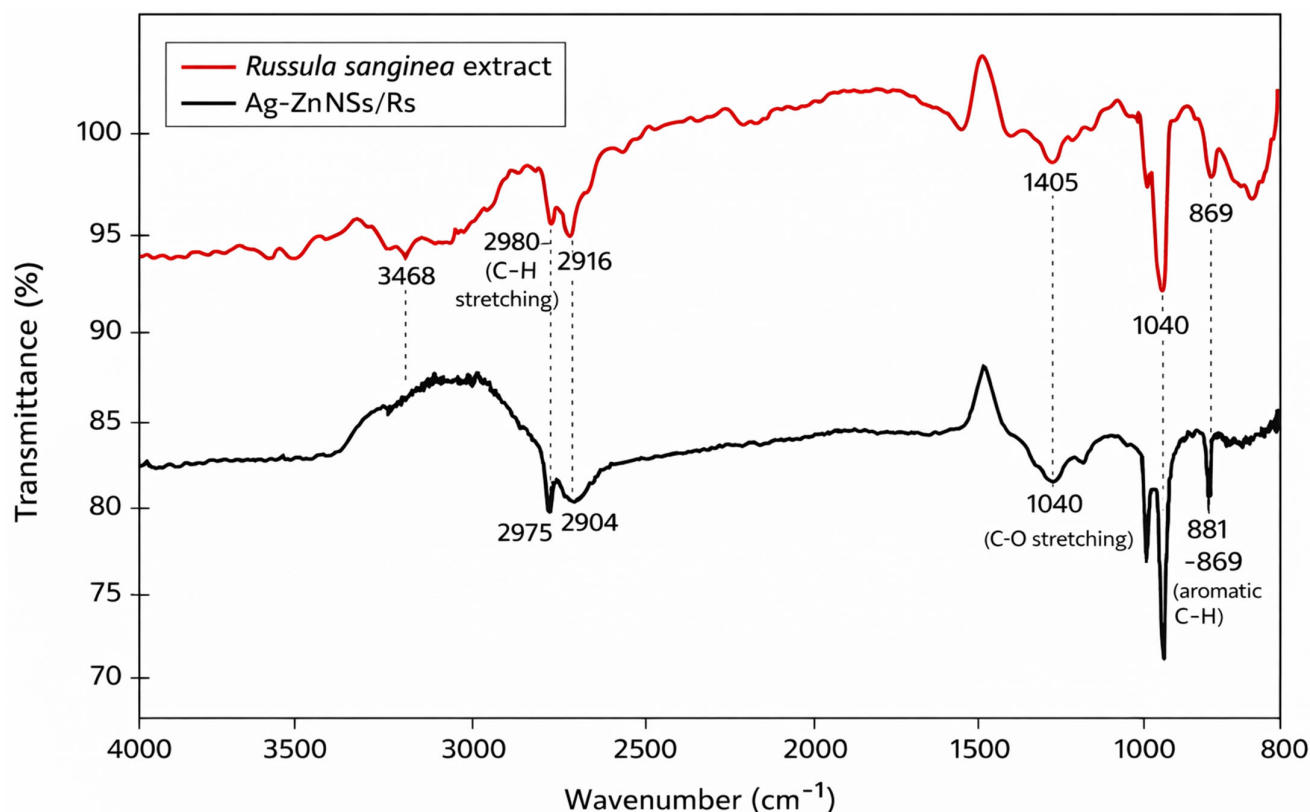


Figure 8. FT-IR spectra of the native *Russula sanguinea* aqueous extract (black line) and the biosynthesized Ag-Zn nanostructures (Ag-ZnNSs/Rs, red line). Characteristic absorption bands corresponding to O-H/N-H stretching, C-H stretching, and fingerprint region vibrations are indicated. The spectra are vertically offset for clarity.

After the green synthesis of Ag-ZnNSs/Rs, noticeable changes in the FT-IR spectrum were observed. A broad absorption band centered at 3468 cm^{-1} , corresponding to O-H and N-H stretching vibrations, became more pronounced, suggesting enhanced hydrogen-bonding interactions between hydroxyl/amine-containing biomolecules and the nanoparticle surface. In addition, slight shifts in the amine-related band from 1387 to 1405 cm^{-1} and in the C-O stretching region toward $\sim 1040 \text{ cm}^{-1}$ were detected. The absorption bands observed at 2980–2916 cm^{-1} are attributed to C-H stretching vibrations of aliphatic groups, while the band centered at $\sim 1040 \text{ cm}^{-1}$ corresponds to C-O stretching vibrations associated with alcohols, polysaccharides, and other oxygen-containing biomolecules. Similar vibrational assignments have been reported for biologically synthesized metal and metal oxide nanostructures stabilized by organic capping agents [37,38], supporting the interpretation that fungal-derived biomolecules actively participate in surface stabilization.

Such spectral shifts and intensity variations are frequently reported in biologically mediated nanoparticle synthesis and are generally associated with the interaction of organic functional groups with metal surfaces [8,39]. These observations suggest that residual fungal biomolecules remain associated with the Ag-Zn nanostructures and may contribute to surface stabilization and reduced particle agglomeration.

3.7. Antioxidant Potential (DPPH Scavenging Assay)

The antioxidant potential of the *Russula sanguinea* extract and the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs) was quantitatively evaluated using the DPPH radical scavenging assay. IC₅₀ values were calculated using nonlinear regression analysis of concentration–response curves. The crude fungal extract exhibited moderate antioxidant activity, with an IC₅₀ value of 13.66 ± 0.15 mg mL^{−1}. In contrast, the biosynthesized Ag–ZnNSs/Rs showed significantly enhanced radical scavenging activity, yielding a substantially lower IC₅₀ value of 7.29 ± 0.10 mg mL^{−1} ($p < 0.05$), corresponding to an approximately 1.87-fold improvement in antioxidant efficiency.

The comparative DPPH scavenging performance of the extract and the nanostructures is presented in Figure 9a, clearly demonstrating the superior antioxidant capacity of Ag–ZnNSs/Rs. Such enhancement is commonly reported in mycogenic nanomaterials and is generally associated with the increased surface area of nanoscale systems combined with the presence of surface-associated bioactive compounds [19,40,41].

The improved radical scavenging behavior may be related to the presence of surface-bound fungal biomolecules, such as phenolic and proteinaceous constituents, which can facilitate electron or hydrogen transfer to neutralize DPPH radicals. A schematic representation of this proposed antioxidant mechanism is illustrated in Figure 9b. Similar enhancement mechanisms have been described in previous studies on biologically synthesized metal-based nanostructures [42].

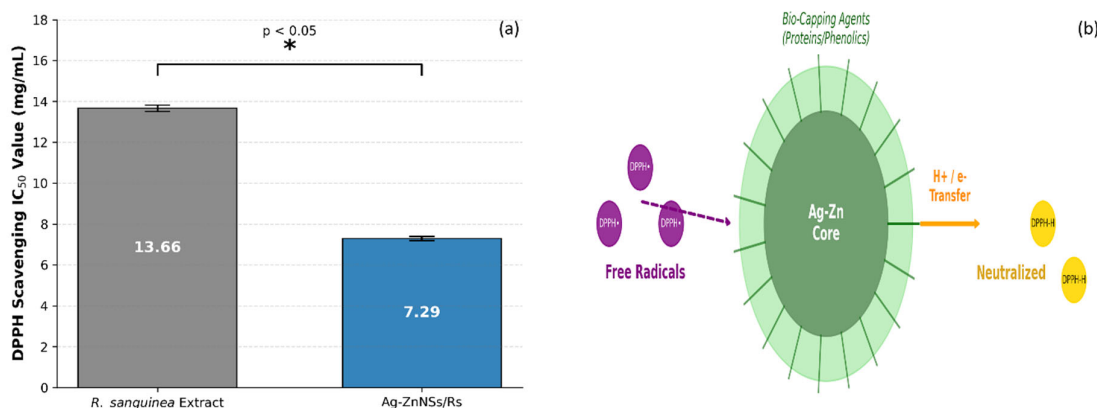


Figure 9. Antioxidant activity and proposed radical scavenging mechanism of the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs): (a) comparison of DPPH radical scavenging IC₅₀ values of the *Russula sanguinea* extract and Ag–ZnNSs/Rs, showing significantly enhanced antioxidant activity of the nanostructures (* $p < 0.05$), values represent mean $\pm$ standard deviation ($n = 3$), error bars indicate SD; (b) schematic illustration of the proposed antioxidant mechanism, in which bio-capping agents derived from fungal biomolecules facilitate electron or hydrogen transfer, leading to neutralization of free radicals.

To contextualize the antioxidant performance, previous studies on biologically synthesized silver- and zinc-based nanostructures have reported DPPH IC₅₀ values spanning a broad range depending on the fungal source and synthesis conditions. IC₅₀ values typically within the range of 5–20 mg mL^{−1} have been reported for mushroom-mediated silver nanostructures [29,42]. Antioxidant efficiency has been shown to be strongly influenced by particle size and surface-bound biomolecules [1,6], and more recent analyses have confirmed comparable activity profiles for mycogenic nanomaterials [34]. In comparison, the Ag–ZnNSs/Rs synthesized in the present study exhibited an IC₅₀ value of 7.29 ± 0.10 mg mL^{−1}, which falls within the reported performance range and reflects effective radical scavenging capability consistent with biologically capped nanostructures.

Overall, the DPPH assay results indicate that nanoscale structuring of the *R. sanguinea* extract into Ag–Zn-based nanostructures significantly improved its antioxidant performance, highlighting the potential of mycogenic nanomaterials as functional antioxidant systems.

3.8. Antimicrobial Activity (Qualitative Screening)

The antimicrobial activity of the *Russula sanguinea* crude extract and the biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs) was qualitatively evaluated against a panel of Gram-positive bacteria, Gram-negative bacteria, and yeast using the disk diffusion assay. The inhibition zone diameters are summarized in Table 1, while representative graphical and photographic results are presented in Figures 10 and 11.

Table 1. Antimicrobial activity of *Russula sanguinea* crude extract and biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs) evaluated by the disk diffusion assay against selected bacterial and fungal strains.

Microorganism	Strain	Crude Extract	Ag–Zn NSs/Rs	Positive Control
Gram-Positive Bacteria				
<i>Bacillus cereus</i>	NCTC 9946	–	11 ± 0.2	14 ± 0.3
<i>Bacillus subtilis</i>	ATCC 6633	8 ± 0.5	16 ± 0.3	13 ± 0.5
<i>Staphylococcus aureus</i>	ATCC 6538	9 ± 0.8	12 ± 0.1	34 ± 0.1
Gram-Negative Bacteria				
<i>Escherichia coli</i>	ATCC 25922	9 ± 0.1	10 ± 0.5	10 ± 0.1
<i>Pseudomonas aeruginosa</i>	ATCC 27853	8 ± 0.1	12 ± 0.4	–
Fungi (Yeast)				
<i>Candida albicans</i>	ATCC 10231	8 ± 0.3	13 ± 0.2	30 ± 0.2

Values represent mean inhibition zone diameters (mm) ± standard deviation (n = 3). “–” indicates no detectable inhibition under the tested conditions. Rifampin (5 µg disk^{−1}) was used as the positive control for Gram-positive and Gram-negative bacterial strains, while fluconazole (25 µg disk^{−1}) was used as the positive control for *Candida albicans*.

Overall, the crude mushroom extract exhibited limited antimicrobial activity, producing inhibition zones ranging from 8.0 to 9.8 mm, with no detectable effect against *Bacillus cereus*. The differential susceptibility observed among Gram-positive strains may be attributed to species-specific variations in cell wall structure, membrane permeability, and sporulation capacity. *Bacillus cereus* is known to exhibit higher tolerance to certain antimicrobial agents, particularly due to its spore-forming ability and stress-response mechanisms, which may reduce sensitivity to bioactive compounds present in the crude extract. *Bacillus subtilis* and *Staphylococcus aureus*, however, may be comparatively more susceptible to phenolic and proteinaceous constituents derived from *R. sanguinea*.

In comparison, the biosynthesized Ag–ZnNSs/Rs demonstrated consistently enhanced antimicrobial activity, yielding inhibition zones between 10.5 and 16.0 mm across all tested microorganisms.

Among the tested strains, the strongest inhibitory effects were observed against the Gram-positive bacterium *Bacillus subtilis* (16 ± 0.3 mm) and the yeast *Candida albicans* (13 ± 0.2 mm). This susceptibility trend is consistent with previous reports indicating that Gram-positive bacteria and yeasts are generally more sensitive to metal-based nanostructures, likely due to the absence of an outer lipopolysaccharide membrane that characterizes Gram-negative bacteria [29]. Nevertheless, Ag–ZnNSs/Rs also exhibited notable activity against *Pseudomonas aeruginosa* (12 ± 0.4 mm), a strain that showed no detectable inhibition with the rifampin control, reflecting its known intrinsic resistance profile. *Pseudomonas aeruginosa* is widely recognized for its intrinsic resistance to rifampin, primarily

due to the low permeability of its outer membrane and the presence of efficient multidrug efflux systems. Therefore, the absence of an inhibition zone for rifampin in the present assay is consistent with previously reported antimicrobial susceptibility patterns rather than indicating experimental limitations.

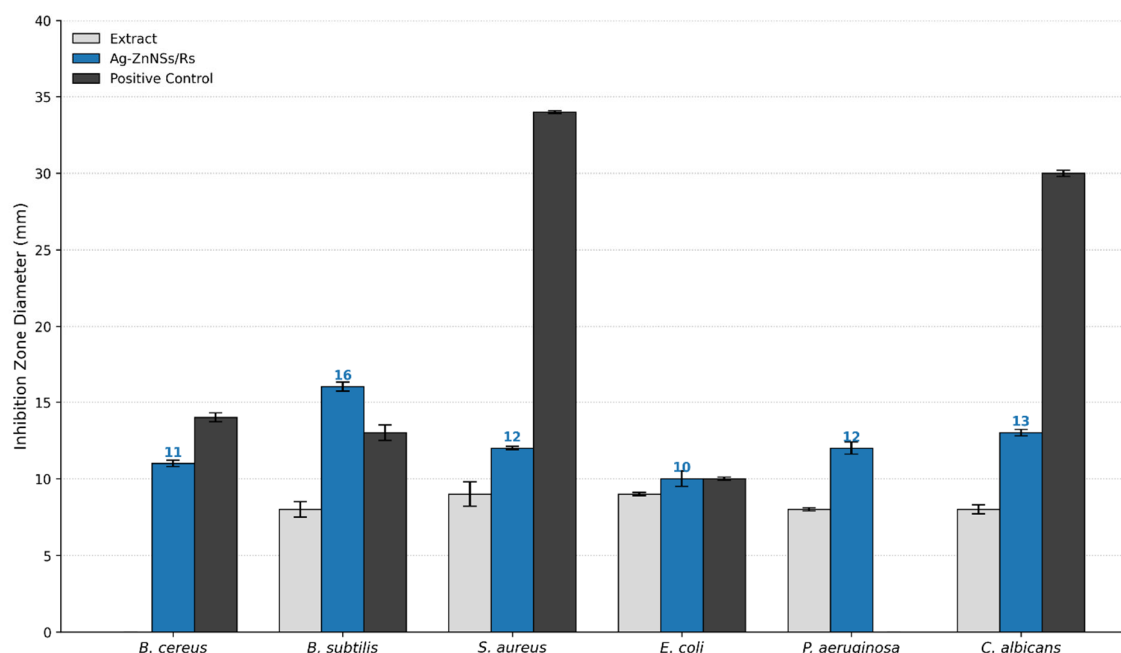


Figure 10. Comparison of inhibition zone diameters produced by *Russula sanguinea* crude extract, biosynthesized Ag–Zn nanostructures (Ag–ZnNSs/Rs), and positive controls against selected bacterial and yeast strains, as determined by the disk diffusion assay. Values represent mean inhibition zone diameters (mm) $\pm$ standard deviation ($n = 3$). Error bars indicate SD.

Although Gram-positive bacteria possess a relatively thick peptidoglycan layer that may limit passive nanoparticle internalization, antimicrobial efficacy does not necessarily depend on cytoplasmic penetration. The small average particle size (~19 nm) and high surface-to-volume ratio of Ag–ZnNSs/Rs likely promote adhesion to the bacterial cell wall. Such surface interactions can induce localized membrane destabilization, increased permeability, and oxidative stress through reactive oxygen species (ROS) generation. These contact-mediated mechanisms have been widely reported for silver- and zinc-based nanostructures and may explain the observed susceptibility of *Bacillus subtilis* and *Staphylococcus aureus* in the present study.

The observed enhancement in antimicrobial performance following nanoscale structuring may be related to the combined effects of the Ag–Zn inorganic core and the presence of surface-associated fungal biomolecules derived from *R. sanguinea*. Such biomolecules, as indicated by FT-IR analysis, may facilitate interactions between the nanostructures and microbial cell envelopes. Similar synergistic effects have been widely reported for biologically synthesized silver- and zinc-based nanomaterials, which are known to exert antimicrobial activity through multiple non-specific mechanisms, including membrane perturbation and oxidative stress generation [1,6].

It should be noted that disk diffusion assays provide comparative and qualitative information rather than direct quantification of antimicrobial potency. Nevertheless, the consistently larger inhibition zones produced by Ag–ZnNSs/Rs compared to the crude extract across all tested strains provide clear evidence of enhanced antimicrobial performance following green synthesis.

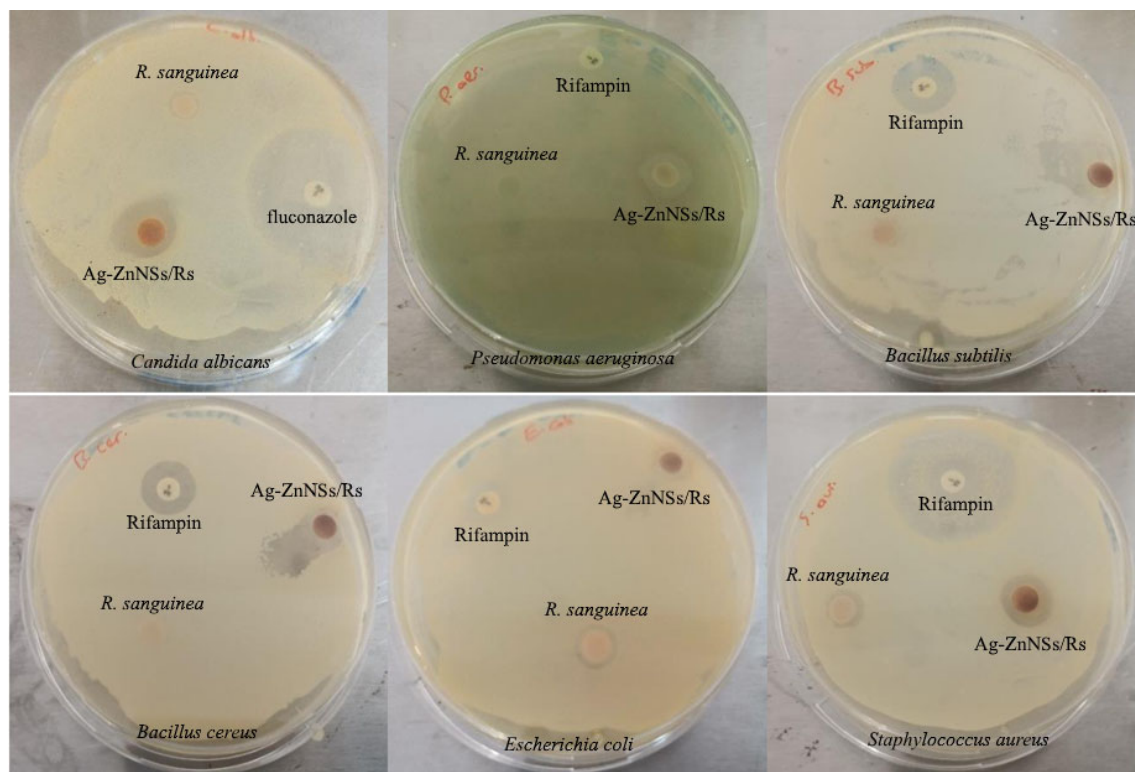


Figure 11. Representative photographs of the agar well diffusion assay demonstrating the inhibition zones produced by Ag–ZnNSs/Rs. The clear zones surrounding the wells visually confirm the bactericidal and fungicidal activity of the nanostructures, with maximum inhibition observed against *Bacillus subtilis* and *Candida albicans*.

Overall, these results highlight *R. sanguinea* as a promising biological resource for the development of mycogenic nanostructures with improved antimicrobial activity and support further quantitative investigations, such as MIC/MBC determination and contact-based antimicrobial assessments.

3.9. Comprehensive Structural and Functional Evaluation of Ag–ZnNSs/Rs

The structural and functional properties of the biosynthesized Ag–ZnNSs/Rs were interpreted through integrated physicochemical and biological analyses. TEM confirmed predominantly spherical nanoparticles with a mean diameter of 19.36 ± 7.89 nm, while SEM revealed heterogeneous surface architecture typical of biologically synthesized systems. EDX analysis verified the coexistence of silver-rich surface domains and zinc-containing phases, and FT-IR spectral shifts indicated effective bio-capping by fungal-derived biomolecules. These structural features—nanoscale size distribution, increased surface-to-volume ratio, and surface-associated organic functionalities—provide a coherent explanation for the enhanced antioxidant and antimicrobial activity observed relative to the crude extract. Collectively, the data support a structure–function relationship within the Ag–ZnNSs/Rs system without extending beyond the experimental evidence.

4. Conclusions

This study presents a green and sustainable approach for the synthesis of silver-decorated zinc-based nanostructures (Ag–ZnNSs/Rs) using the *Russula sanguinea* aqueous extract. The biosynthetic route eliminates the need for toxic reagents and highlights the value of wild mushrooms as renewable biological platforms for nanomaterial fabrication.

Rather than focusing on individual characterization outcomes, the findings collectively demonstrate that biologically mediated synthesis enables the formation of multi-functional nanostructures, where metal species coexist with surface-associated fungal biomolecules. This hybrid nature appears to play a key role in enhancing biological performance, as reflected by improved antioxidant and antimicrobial activities relative to the crude extract.

Importantly, the study does not suggest the formation of a fully alloyed Ag–Zn system, but instead supports a silver-decorated zinc-based architecture stabilized by fungal metabolites. Such systems may offer advantages in terms of surface reactivity and biological compatibility.

Overall, this work underscores the potential of *Russula sanguinea* and similar wild mushrooms as underexplored resources in green nanotechnology. The results provide a foundation for future studies aimed at quantitative antimicrobial evaluation, mechanistic investigations, and application-driven optimization of biologically synthesized metal-based nanostructures.

Future studies involving quantitative ion-release profiling and mechanistic ROS analysis would further elucidate the functional behavior of this system.

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