RESEARCH PAPER

Utilization of Edible Mushroom for Nanomaterial-Based Bioactive Material Development

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Abstract: *Gold nanoparticles (AuNP) were synthesized using edible mushroom Russula delica (RD) in this study. Possibilities to evaluate these synthesized nanoparticles (RD-AuNPs) as bioactive substances were investigated. Characterization of synthesized RD-AuNPs were characterized via UV-vis, XRD, FTIR, EDX. In a spherical view, RD-AuNPs with a crystal size of 34.76 nm were synthesized. As a result, fungal systems used for nanomaterial biosynthesis as an effective alternative to chemical synthesis can be used in different biotechnological and medical applications. RD-AuNPs produced by green synthesis can be evaluated in this context.*

Keywords: *AuNPs, fungus, SEM, UV-Vis, XRD, Antimicrobial.*

1. INTRODUCTION

The biological synthesis of nanoparticles is low cost, non-toxic, and easy to use, unlike chemically synthesized nanoparticles. Furthermore, it is simple to control size, shape, and stability in biological synthesis.

Old nanoparticles, which have important potential applications, can be evaluated in biomedical fields such as nanomedicine, new category catalysis medicine, nano-optoelectronics, and drug delivery.

It has been stated that it can be used in the electronics, food, and cosmetics industries, textile industries, agricultural applications, and many other fields [14].

Biological approaches using microorganisms and plant or plant extracts for metal nanoparticle synthesis are emerging as valuable alternatives to chemical methods [5].

Because chemical methods lead to the presence of some toxic chemicals adsorbed on the surface, which can cause adverse effects in medical applications. It is stated that these toxic chemicals can pollute the environment and harm animals [6].

Fungi are more efficient and suitable for the synthesis of nanoscale metal particles compared to other naturally occurring biological resources. The use of mushrooms contributes to the high productivity and stability of easy-to-manufacture particles with large amounts of protein. It is also stated that fungal mycelium can withstand the environments in bioreactors. Gold nanoparticles (AuNPs) find wider application in life sciences compared to other noble metals due to their optoelectronic properties and biocompatibility. It is supported by the literature that AuNPs are nowadays widely used in chemical catalysis, nonlinear optics, surface-enhanced Raman scattering, nanoelectronics, gene expression, disease diagnostic drug delivery, and controlled release of a drug at the desired site [7]. The uniqueness and desired tunability of the surface plasmon resonances of AuNPs provides advantages in biological application areas. This can be explained by the fact that AuNPs synthesized using fungal extracts show bactericidal and fungicidal activity against many different microorganisms [8, 9]. However, few studies have been conducted on the production of AuNPs using extracts of different edible mushrooms such as Volvariella volvacea and Agaricus bisporus, Pleurotus Florida. [7, 10]. It is known that R. delica is used as food in Turkey. Local people state that it grows in wooded habitats under both coniferous and deciduous trees. It is stated that R. Delica is gathered by the villagers every year when the weather conditions are suitable [11]. Although R. delica, an important food source, has strong antimicrobial and antioxidant activity, there are no definitive reports

on the nanoparticle forming potential of this fungus. This study aimed to explore the possibilities of synthesizing gold nanoparticles (AuNPs) using the pharmaceutically important edible mushroom R. delica (RD) and evaluating these nanoparticles (RD-AuNPs) for biomedical applications.

2. EXPERIMENTAL PROCEDURE

2.1. Macrofungus and Microorganisms

The R. delica used in the research was collected during the field studies conducted in Mardin/Turkey in April-May 2018. Species identification of fungi was made by diagnostic studies in which macroscopic and ecological structural data were specified. [12]. Mushroom samples are preserved as 50 g dry samples in Mardin Artuklu University Microbiology-Biochemistry Research Laboratory.

In the study, for antimicrobial activity tests; E. coli ATCC 25922, S. aureus ATCC 25923, B. subtilis ATCC 11774 C. albicans ATCC 10231 species found in Mardin Artuklu University Microbiology-Biochemistry Research Laboratory were used.

2.2. Extract of R. delica and preparation of gold trichloride (HAuCl3.3H2O) solution

The preparation of the mushroom extract was made by modifying it according to the method of Acay and Baran [13]. Collected R. delica washed with distilled water and finally dried for 48 hours under room conditions. Then, 50 g dried sample was added 250 mL of distilled water and allowed to boil for 1 day. 5 minutes after boiling, cooled to room conditions and filtered by Whatman 1 filter paper and the extract was obtained for the synthesis of AuNP. Afterward, 1 mM solution was prepared using 49.0% purity gold trichloride ($HAuCl_3.3H_2O$).

2.3. Synthesis and Characterization of Gold Nanoparticle

50 mL of extract prepared from R. delica and 250 mL of $HAuCl_3.3H_2O$ solution were left to react in a 1000 mL flask at 45°C. Color change in solution was observed over time. The formation of AuNPs was observed by examining absorbance values for color change using Agilent Cary 60 UV-Vis spectrophotometer.

The characterization of gold nanoparticles was carried out as follows according to the methods described earlier [14].

Functional groups of bioorganic structures in mushroom responsible for reduction were analyzed using Fourier transforms infrared spectroscopy (FT-IR) analysis. After the reaction, the liquid suspension obtained on a large scale was centrifuged for 15 minutes at 6,000 rpm and the particles to be used for other characterization steps were precipitated. After centrifugation, the precipitated solid was washed 10 times with distilled water and allowed to dry in an oven at 75°C for 24 hours. These nanoparticles obtained were applied characterization tests. The elemental composition of the particles was evaluated with RadB-DMAX II computer-controlled energy dispersive X-ray spectrum (EDX), morphological images of EVO 40 LEQ (SEM) data were analyzed by scanning electron microscopy. The crystal structure of the particles was evaluated by RadB-DMAX II computer-controlled X-ray diffractometer (XRD) analysis.

Finally, the crystal particle size was calculated using the Debye-Scherrer equation (Eq: 1)

$$D = K\lambda / (\beta \cos\theta) \quad (1)$$

2.4. Antimicrobial activity of RD-AuNPs

The study was performed according to the method specified by Acay and Baran [13]. In summary, antimicrobial activities were determined by the microdilution method on minimum inhibition concentration (MIC) against standard bacterial strains and fungal strains. Mueller Hinton broth, Mc Farland 0.5 concentration adjusted microorganism solutions, and RD AuNPs solution were added to the microplate wells in appropriate amounts and the microplates were left to incubate at 37°C. The lowest non-growth concentration after incubation was considered the MIC [15, 16]. Vancomycin for Gram (+), colistin for Gram (-), and fluconazole for fungus were used as controls. In addition, the effect of the gold solution was also evaluated. The mean values of the study performed with 3 replications are given as MIC values.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of Gold Nanoparticle

Thanks to their intracellular and extracellular enzymes, fungi are excellent natural alternatives for producing singularly dispersed nanoparticles with well-defined geometries and sizes. Due to

their large biomass originating from mycelial forms, fungi are much more efficient in nanoparticle synthesis than bacteria. It has been reported in the literature that nanoparticles of various sizes and shapes were synthesized using various fungal species such as Colletotrichum sp., Fusarium oxysporum, Verticillium luteoalbum Asperigillus oryzae, Trichoderma viride, Alternata alternata, Trichothecium sp. [17, 18]. The fact that the fungal micelle structure is more resistant to agitation and other conditions in bioreactors compared to plant materials and bacteria has increased the interest in this group for nanomaterial synthesis [19].

The formation of gold nanoparticles changes the color of the solution from light yellow to yellow due to the reduction of Au^{3+} ions while mixing the aqueous extract of the fungus with the aqueous chloroauric acid solution, and the formation of vibrations on the plasma surface indicates the formation of nanoparticles. UV-vis spectroscopy showed that the formation of gold nanoparticles was maximum at 398 nm (Figure 1). Similar studies have reported that the gold nanoparticles obtained via UV-vis analysis are formed at a maximum wavelength of 540 nm [20]. In another study, gold nanoparticles formed with the green synthesis showed at a wavelength of 547 nm [21]. The result shows that the protein and polysaccharides contained in the extract may be responsible for the biosynthesis of AuNPs, and the mushroom extract can be used as a reducing agent for AuNP synthesis. The AuNPs synthesized by R. delica mushroom extract were subjected to FT-IR analysis toidentify the biomolecules involved in stabilizing the nanoparticles in solution [22].

According to Figure 2A, the R. delica extract yielded strong bands at 3326 cm^{-1} (-OH, -NH), 2360 cm^{-1} and 2342 cm^{-1} (N≡C), 1636 cm^{-1} (C=O, C=C).

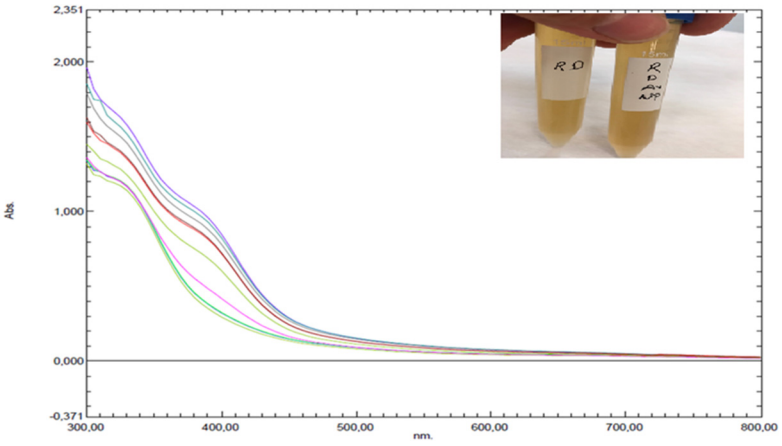

Fig. 1. UV–visible spectra of RD-AuNPs nanoparticles

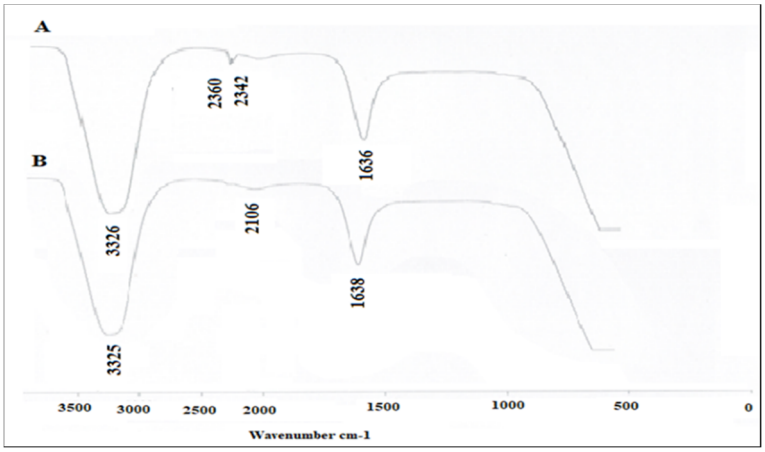

Fig. 2. A. FTIR Analysis of obtained extract from R. delica B. Changes of existing functional groups in R. delica after synthesis.

During the formation of gold nanomaterials (AuNP), when the functional groups involved in the reduction are examined, it is thought that the peak of 3325 cm^{-1} -OH stress, the peak at 1638 cm^{-1} may belong to -C=O or -C=C, and the peak at 2160 cm^{-1} may be C≡C stress (Figure 2B). The FT-IR results show that the surface capping of AuNPs synthesized by the mushroom extract is predominantly by proteins. Moreover, our results are consistent with those reported earlier for biosynthesized nanoparticles [22, 23].

The surface morphology of AuNPs synthesized with extract of R. delica was analyzed by SEM. SEM analysis is used to explain the size and morphological properties of gold nanomaterials synthesized [24, 25]. When AuNPs were analyzed via SEM analysis data, it was found to have a spherical appearance (Figure 3). The data we obtain from SEM images are supported by the literature [22.23, 26, 27].

Results from EDX analysis (Figure 4) show strong gold. As indicated by Punuri et al., [28] the strong Au signal may have originated from biomolecules attached to the surface of the nanoparticles. Furthermore, it is seen in Figure 4, that EDX analysis of the gold nanomaterials obtained from having elemental structure and mushroom extract is a good reducing agent in the biosynthesis process.

The crystalline nature of as-prepared AuNPs was confirmed using XRD. When the XRD results of gold nanoparticles are examined, peaks situated in 111°, 200°, 220°, and 311° corresponding to 2θ show the spherical crystal structure of gold [29]. As seen in figure 5, the four dominant peaks seen in the XRD spectrum agree with Bragg's reflection of the AuNPs reported in previous studies [22.23, 26, 27]. The crystal size of the Au nanoparticles was calculated according to Debye-Scherrer's equation (Eq. 1) formula.

$$D = K\lambda / (\beta \cos\theta) \quad (1)$$

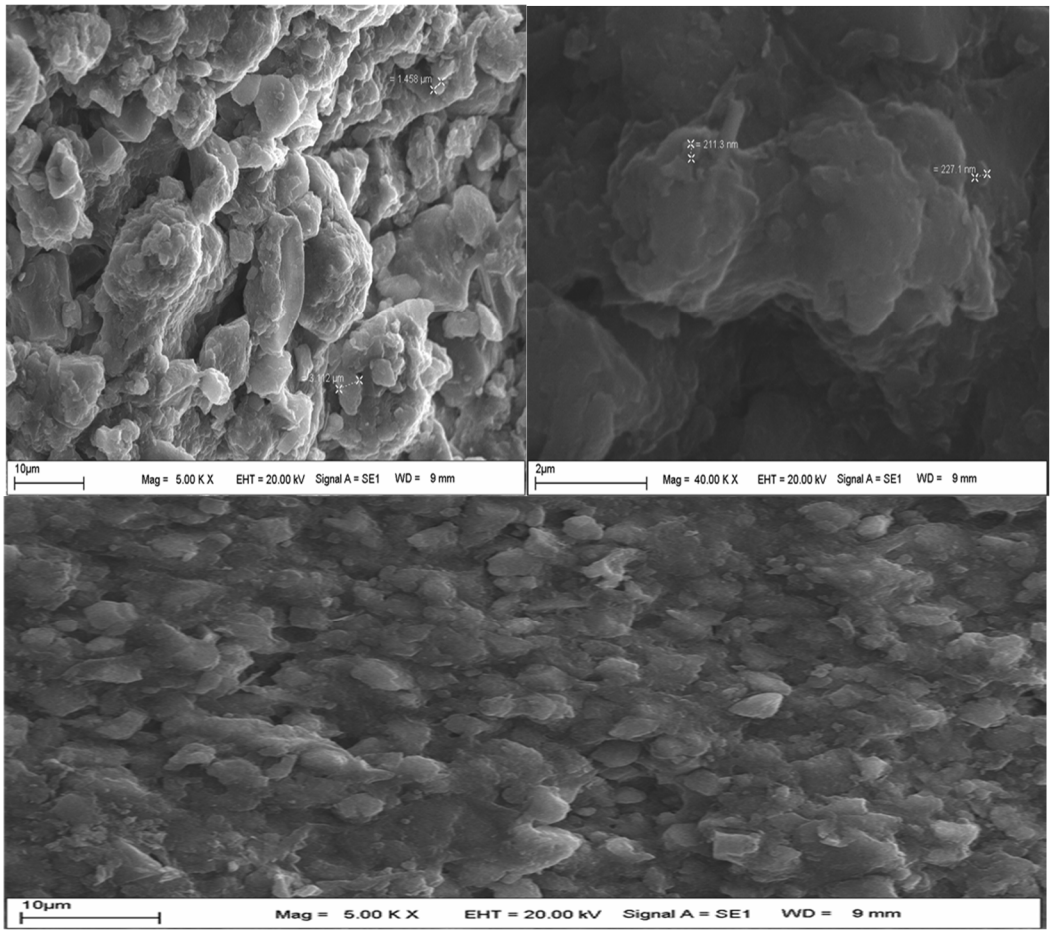

Fig. 3. SEM results of gold nanoparticles.

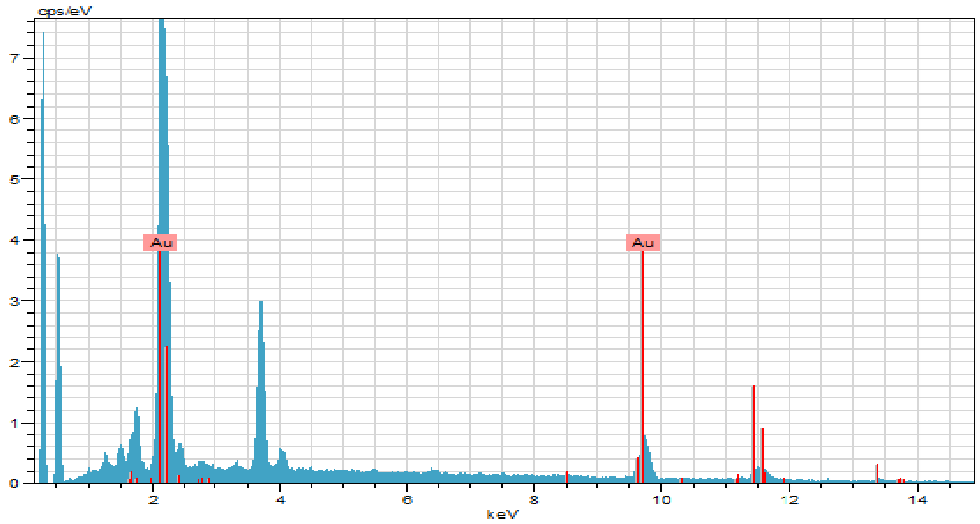

Fig. 4. EDX profile of green synthesized AuNPs

The crystal size of RD-AuNPs was calculated as 34.76 nm.

Furthermore, as shown in Figure 5, it is understood that the gold nanomaterials obtained are elemental structures via the phase diagram.

3.2. Antimicrobial activity of RD-AuNPs

The antimicrobial activity of RD-AuNPs was determined using the MIC method using gram-positive (B. subtilis and S. aureus) and gram-negative (E. coli) and C. albicans, a fungus (Table 1). The optimal level of inhibition were 0.153 mg/mL for E. coli, 0.306 mg/mL for S. aureus, 0.306 mg/mL for B. subtilis, and 0.016 mg/mL for C. albicans. Compared with antibiotic and gold solution, it was important to obtain lower concentrations of inhibition (Table 1).

Türkoglu et al. [11] tested the antimicrobial effect of R. delica's ethanol extracts against Gram-positive and Gram-negative bacteria and a yeast species and stated that R. delica extract had a narrow antibacterial spectrum against microorganisms. In addition, it was determined that the extract used showed stronger activity against Gram-positive bacteria than Gram-negative bacteria.

Eskandari-Nojehdehi et al. [8] stated that AuNPs have the potential to kill cells by entering the bacterial cell wall and that AuNPs can interact with anionic groups of cell wall components and structurally alter the cell surface.

Lee et al. [30], in their study using Inonotus obliquus (Chaga mushroom) mushrooms, reported that the highest antibacterial activity was

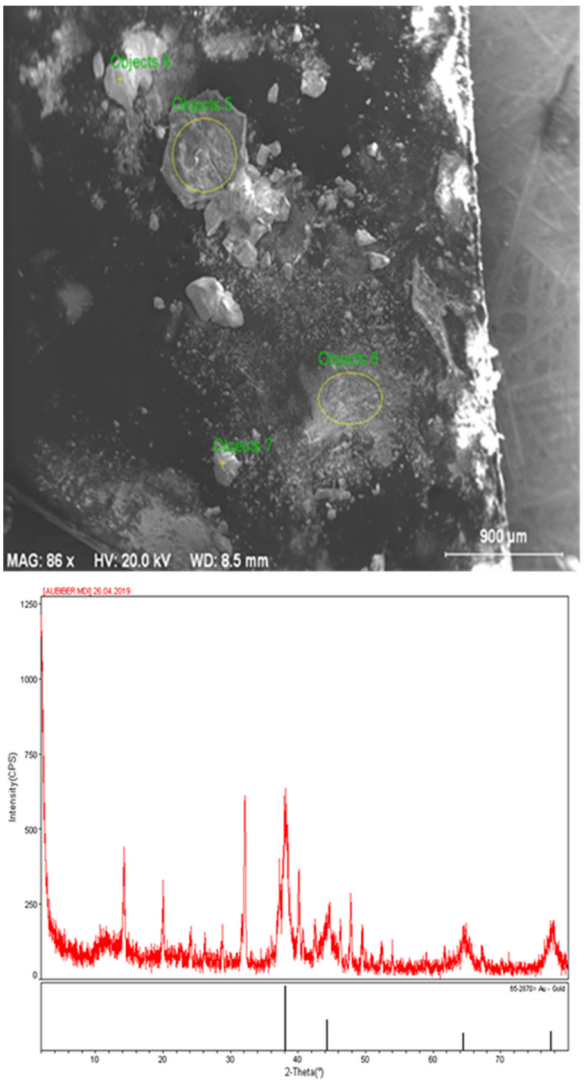

Fig. 5. Diagram of XRD and phase analysis of AuNPs

Table 1. MIC values of Synthesized R. delica Gold nanoparticles (RD-AuNPs) (mg /mL) on $HAuCl_4.3H_2O$ solution and Gram (+) (vancomycin), Gram (-) (colistin) Antibiotic and fungi (fluconazole).

	ORGANISM	RD-AuNPs (mg /mL)	$HAuCl_4.3H_2O$ (mg /mL)	Antibiotics (mg /mL)
Gram Positive	S. aureus ATCC 29213	0.306	0.5	1
	B. subtilis ATCC 11774	0.306	0.10	2
Gram Negative	E. coli ATCC 25922	0.153	0.25	2
Fungi	C. albicans ATCC 10231	0.016	0.25	2

Lee et al. [30], in their study using Inonotus obliquus (Chaga mushroom) mushrooms, reported that the highest antibacterial activity was observed against S.aureus (16 nm) followed by E.coli (14 nm) and B.substilis (12 nm).
Literature studies have shown that AuNPs are more effective on Gram-negative bacteria than Gram-positive bacteria, unlike fungal extracts [31]. El-Sayed et al. [32] reported that AuNPs of 20-50 nm size show the most efficient cellular uptake and specific cell toxicity is associated with AuNPs with a particle size of 40-50 nm. The effective antimicrobial activity seen in the study can be attributed to this reason.

4. CONCLUSION

RD-AuNPs synthesized to have a crystal size of 34.76 nm in the spherical appearance. It has been determined that RD-AuNPs synthesized by the green method have a good antimicrobial effect and are effective in lower concentrations compared to commercial antibiotics. As a result, the fungal extract used a simple, rapid, clean, efficient, cost-effective, and green method for nanomaterial biosynthesis as an effective alternative to chemical synthesis that can be used in different biotechnological and biomedical applications. Also, with the increase of nanotechnological research, synthesized AuNPs will open new fields in the production of pharmaceutical products in the future. RD-AuNPs produced by green synthesis can be evaluated in this context. Finally, the environmentally friendly method using mushroom extract, large-scale production of biocompatible AuNPs, and produced AuNPs can provide an alternative route for biomedical applications.

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