BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING KING OYSTER (*PLEUROTUS ERYNGII*) EXTRACT: EFFECT ON SOME MICROORGANISMS

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Abstract. The integration of the principles of green chemistry into nanotechnology has become one of the key issues in nanotechnology research. Metal nanoparticle production, which does not contain toxic chemicals and does not harm the environment, needs to be developed to avoid adverse effects on medical applications. In this study, *Pleurotus eryngii* (PE) extract was used for preparation of silver nanoparticles (AgNPs). The presence of AgNP was understood that after adding 1 mM silver nitrate (AgNO3) to the fungus extract, the reaction turned from the open yellow to reddish brown. The analysis of samples taken at different times with the UV-Visible Spectrophotometer (UV-Vis) confirms the formation of PE-AgNPs. The Scanning electron microscopy-Energy Dispersive X-Ray Spectrum (SEM-EDX) analysis showed that spherical nanoparticles were formed. X-ray crystallography (XRD), analysis is calculated from Debye-Sherers inequality, in which PE-AgNP synthesized in the study was 18.45 nm in size. It has been demonstrated by using the minimum Inhibitory Concentration (MIC) method in which AgNPs have strong antimicrobial activity.
Keywords: *antimicrobial activity, XRD, SEM, TGA-DTA, AgNPs, Pleurotus eryngii*

Introduction

Nanoparticles (NPs) are particles having a size of 100 nm or less. Because they have a large surface area that allows them to be used in new applications, they tend to react differently from large substances containing the same composition particles (Abou El-Nour et al., 2010). The new features that NP has gained make them versatile in many research areas such as biomedical applications, drug exploration, luminescence, cosmetics and renewable energy technologies (Chaudhuri and Paria, 2012; Tran et al., 2013; Rauwel et al., 2015). Therefore many researchers have studied the synthesis of NP using various physical and chemical methods (Remya, et al., 2017). However, it is stated that the synthesis of nanoparticles with green chemistry approaches using biological entities such as plants, fungi, bacteria, algae and actinomycetes provides additional advantages over other methods because it is simple, cost-effective, reliable, and environment-friendly. For this purpose, the use of biological synthesis approaches for many researchers is increasingly important (Kumar and Yadav, 2009).

The fact that microbial infection is a serious problem in the agriculture and healthcare sector has made it mandatory to develop new antimicrobial agents with highly versatile properties such as antimicrobial effect strength, harmonious and low toxicity. In this context, the electrostatic attraction between the positively charged NPs and the negatively charged microbial cells has received worldwide attention due to the

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large surface/volume ratio resulting in improved physicochemical properties of NPs and increased antimicrobial activity (Lakshmeesha et al., 2014; Kavyashree et al., 2015). Antimicrobial properties of NP's, including Antibacterial and antifungal activities, have recently been reported extensively (Mallmann et al., 2015; Salem et al., 2015; Elgorban et al., 2016).

As reported, silver antimicrobial plays a unique role in catalytic and biological systems (Nadagouda et al., 2009). The synthesis of silver nanoparticles, compared to other metals, has become more important as an antimicrobial agent against the ever-increasing threats posed by antibiotic resistant microbes (Panaek, et al., 2006; Parashar et al., 2009).

Edible fungi, flavonoids, phenolic acids, tannins and oxidized polyphenols due to numerous reducing biomolecules has attracted great attention in NP synthesis (Philip, 2009; Palacios et al., 2011). In addition, mushroom extract has a high protein content and the carbonyl group of amino acids has the ability to prevent the aggregation of NP's and thus stabilize them in aqueous solution (Al-Batal et al., 2013). Numerous reducing biomolecules, micelles that offer a large surface area for interaction, because of the enzymes they secrete is thought to increase the effect of the functional groups of the nanoparticles and make the mushrooms advantageous compared to other organic sources.

In the literature, it is stated that mushroom extracts such as Volvariella volvacea, Pleurotus sajor Caju, Pleurotus Florida, Ganoderma lucidum, Agaricus bisporus, Inonotus Obliquius were used successfully in AgNP synthesis (Nagajyothi et al., 2014). However, there was no evidence that the *Pleurotus eryngii* extract was used. In the light of the above information, it is aimed to determine AgNP synthesis, characterization and antimicrobial activity of the obtained PE-AgNP's using Pleurotus eryngii which contains many active ingredients.

Materials and methods

Macrofungus

Pleurotus eryngii used in the study was obtained from Hakkari/Turkey in April-May 2018. Diagnostic macroscopic investigations were carried out using field study on ecological structural features and 500 g samples stored in the Microbiology-Biochemistry Research Laboratory of Mardin Artuklu University.

Microorganisms

For testing antimicrobial activity; *Escherichia coli* ATCC 25922, Staphylococcus *aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615 *Pseudomonas* aeruginosa ATCC 27853, standard bacterial strains with *Candida albicans* ATCC 10231 strains are available in the Microbiology-Biochemistry Research Laboratory of Mardin Artuklu University.

Preparation of mushroom extract

Pleurotus eryngii extract was used to prepare AgNP synthesis. The mushrooms collected from the field work, after washing with tap water, it was finally washed with distilled water. 200 g fresh sample, divided into small pieces, was boiled in 90 °C in distilled water 1000 ml. It was then cooled at room temperature and used in the AgNPs synthesis after filtration with Whatman No. 1 Filter paper (Acay et al., 2019).

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Synthesis of PE- AgNPs

Generally, the color change in the synthesis of AgNPs is determined by time-dependent UV-vis spectrophotometry measurements (Pugazhendhi et al., 2018). Therefore 1 mM AgNO3 solution is prepared. The Extract and solution were mixed at the rate of 1:5 and the colour change at room temperature was expected to occur. Reduction of the silver ions was observed to change the white colored solution to dark brown. The resulting dark-colored solution is centrifuged 5 min at 10000 rpm to remove the upper liquid phase and wash with distilled water until the remaining solid was clear. The obtained AgNPs were left to dry in the oven at 65 °C for 48 h and then allowed to dry and stored for characterization.

Characterization of silver nanoparticles

The characterization of silver nanoparticles was carried out as follows according to the methods described earlier (Baran, 2018). The UV-Vis Spectrophotometer was used which very useful technique for the primary characterization of prepared PE-AgNPs. In the study, the formation of silver nanoparticles was observed with measurements made at one hour intervals using the SHIMADZU UV-3600 Model UV-Vis Spectrophotometer in the reduction of Ag+ ions to Ago form in the solution. The AgilentCary 360 model FTIR device was used for the evaluation of the functional groups involved and responsible for the reduction. The X-ray refractivity pattern of the Obtained nanoparticles was done with the Crystal Dimension analysis RadB-DMAX II computer-controlled X-rays diffraction difractometry. The appearance of the nanoparticles and the silver element composition were analyzed by the EVO 40 LEQ Model device. In order to determine at what temperature the Nanoparticles were deteriorate, the AgNP was used to measure the flow rate of 20 mL/min in the N2 (g) atmosphere with a heating rate of 10 °C/min and the Shimadzu TGA-60 H Model device for TGA and DTA results at 25-900 °C.

Determination of antimicrobial effect

In the study, Antimicrobial activity *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Streptococcus pyogenes* ATCC 19615 *Pseudomonas aeruginosa* ATCC 27853, standard bacterial strains and *Candida albicans* ATCC 10231 strains were determined by using microdilusion method on the Minimum Inhibiting Concentration (MIC) In the study, microplate wells were left to incubate at 37 °C with the appropriate amounts of Mueller Hinton broth, Mc Farland 0.5 concentration adjusted microorganism solutions and PE-AgNP solution. The lowest concentration in the absence of reproduction after incubation was determined as the MIC value (Wang et al., 2017; Elshikh et al., 2016). Commercial antibiotics were used as control for Gram (+) (vancomycin), Gram (-) (colistin) bacteria and fungus (fluconazole). In addition, the effect of $AgNO^3$ solution was investigated. The study was performed with 3 repetitions and the mean values were determined as MIC value.

Results and discussion

AgNPs have free electrons that lead to surface plasmon resonance (SPR) absorption band due to the combined vibrations of the electrons of the metal nanoparticles with the light wave in the resonance (Nath et al., 2007). When the Mushroom extract was added

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to an aqueous silver nitrate solvent, the surface of the metal nanoparticles resulted in a colour change from yellow to dark orange (*Fig. 1*) in 5 days due to stimulation of plasmon vibrations. In the analysis of UV-vis spectroscopy, the formation of silver nanoparticles was observed with samples taken at one hour intervals. As shown in *Figure 1*, the absorption spectrum values of the samples taken without mixing for one hour at room temperature were measured and gave significant plasmon resonance at a maximum of 461 nm. Ramy and Hashem (2014) in their study using Pleurotus ostreatus extract obtained a maximum value of 450 Nm, while Ahmad et al. (2003) reported that the solution prepared using mushroom extract gave a maximum plasmon resonance of 436 Nm. Our results are consistent with previous studies (Huang et al., 2007; Verma et al., 2010).

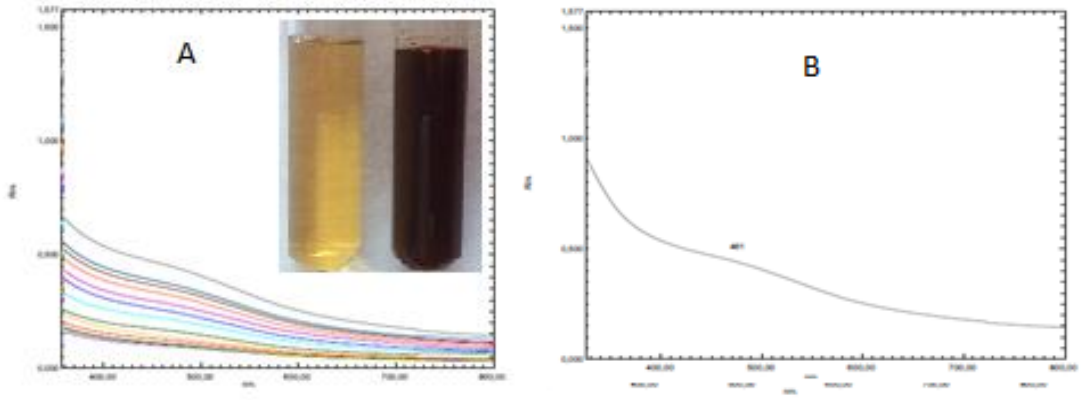

Figure 1. *A) Time dependent of PEAgNPs in UV visible spectroscopy. B) Synthesis of PEAgNP at UV spectrophotometry maximum absorbance value*

The XRD technique is a non-destructive technique and is used to describe the crystalline phase of nanoparticles. The X-ray diffraction pattern obtained for the PE-AgNP sample is shown in *Figure 2*. The PE-AgNP structure synthesized in the resulting diffraction pattern was obtained from the 111, 200, 220 and 311 characteristic pics ($2\theta = 32.41°$, 46.33°, 64.90° and 77.75° values). The crystal particle size of the obtained globally structured PE-AgNPs used Debye-Scherrer's inequality; the size of the synthesized nanoparticles was calculated to be 18.45 nm.

From Debye-Scherrer's equation (Baran, 2019):

$$D = K\lambda / (\beta \cos\theta) \qquad \text{(Eq.1)}$$

Inequality (*Eq. 1*): D = Particle size (nm), K = Fixed (0.90), λ = Wavelength X-ray (1.5406 ^{o}A), β = half of the highest peak value is specified in radians (FWHM), θ = fracture angle.

Owait and Ibraheem (2017) reported that mushroom metal-NP's are generally spherical and their size varies between 0.4 nm and $\geq$ 300 nm, but most are less than 75 nm. Considering the particle size, especially for biological applications, it is stated that the particle sizes between 30 and 150 nm are measurable, and a key used in the study of biological properties (Thorek and Tsourkas, 2008). The fact that the crystal grain size of the obtained global structure *PE*-AgNP is 18.45 nm suggests a new assessment.

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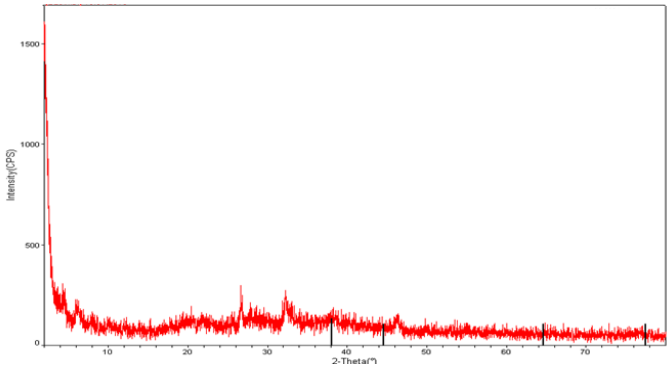
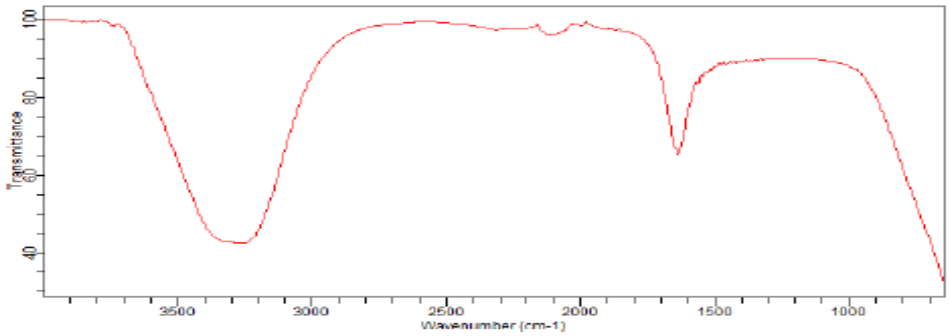


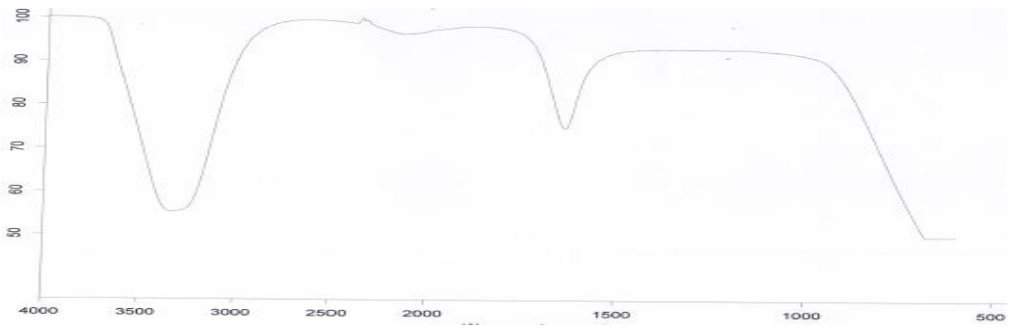
Figure 2. *XRD measurements of AgNPs confirming the spherical crystalline structure of PE-AgNPs*

FT-IR identifies functional groups connected to the surface of metal nanoparticles, since identical absorption patterns are different from those corresponding to free groups, and gives information about the surface chemistry of nanoparticles.

As shown in *Figure 3*, the results of P. eryngy extract and PE-AgNPs were compared as a result of the FT-IR. It is thought that; the peak at 3529 cm^{-1} stems from NH; the peak at 3270 cm^{-1} stems from OH conjugated to free water, alcohol, and phenol groups; the shift at 1672-1635 cm^{-1} stems from carbonyl or primary amide band; and the reason for the peak at 2114 cm^{-1} is C≡C alkyne or C-N group. These functional groups play a role in the reduction (*Fig. 3*). Mandal et al. (2005) stated that proteins in P. ostreatus fungi could bind to nanoparticles by free amine or cysteine groups in proteins and thus stabilize AgNPs.



A



B

Figure 3. *A. FT-IR spectrum of P eryngii extract spectrum. B. FT-IR spectrum of synthesised PE-AgNPs*

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Scanning Electron Microscope (SEM) is a tool that scans the surface of the sample and records the return of the beam. Since metal nanoparticles are electrically conductive, they are easy to scan with SEM. SEM analyses of spherical PE-AgNP obtained in 18.45 NM size are shown in *Figure 4*. So far, the researchers have identified a wide range of shapes, such as spherical, diamond, rod-like, and cube, whose diameters are smaller than 1 to 100 nm, as shown in their characterization patterns (Banerjee and Rai, 2017).

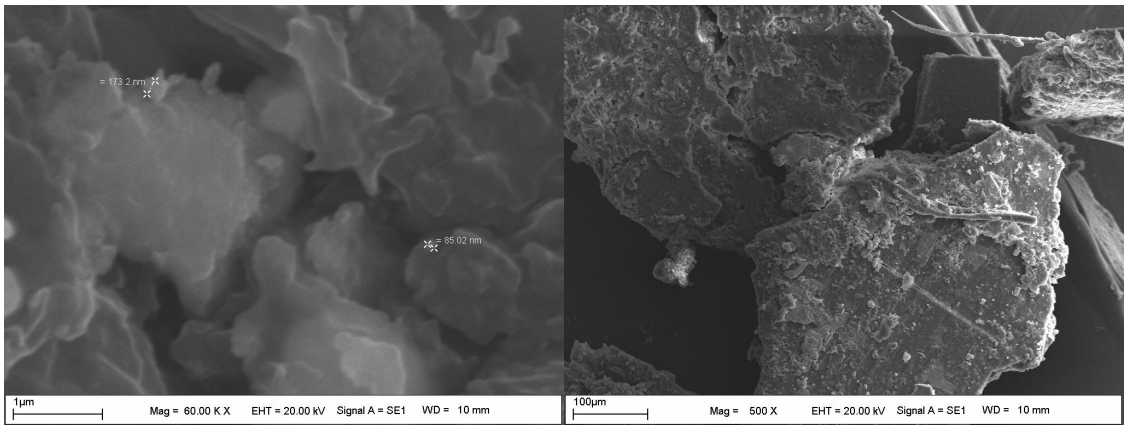

Figure 4. *Images of synthesised PE-AgNP of SEM analyses*

In addition, it was observed that the particles that were synthesized by EDX analysis were in elemental form (*Fig. 5*).

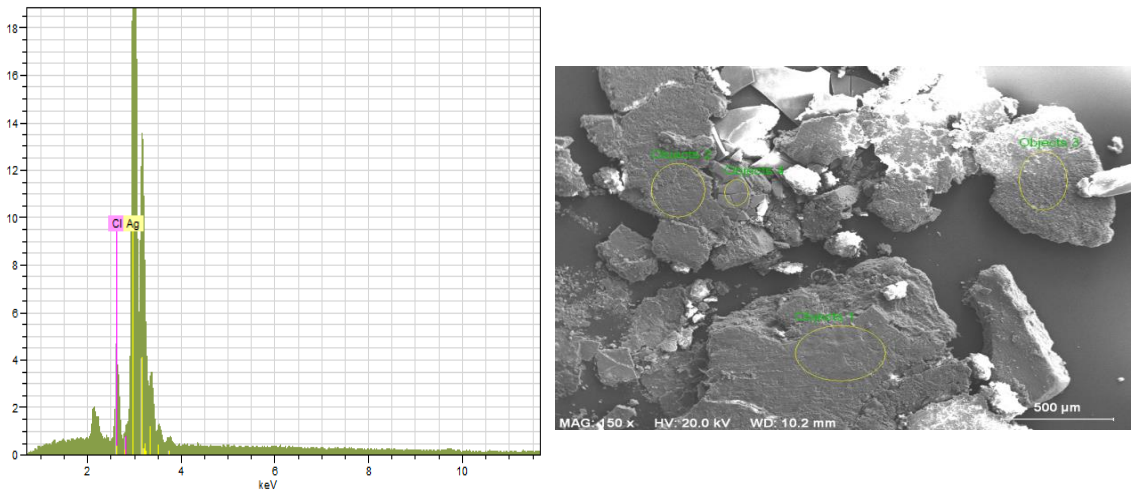

Figure 5. *EDX profile of PE-AgNPs showing its elemental composition*

Nanoparticles prepared with mushroom extract (PE-AgNPs) between 30-900 °C TGA and DTA data were analyzed at a flow rate of 20 mL min^{-1} in N2 (g) atmosphere with a heating rate of 10 °C min^{-1}. The TGA curve shows a loss of sample mass due to thermal degradation and the DTA curve indicates the maximum temperature of decomposition at each stage of the degradation (Baran, 2019, 2018). It is thought that

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the 3% mass loss of the synthesized nanoparticule at 11-102 °C is due to moisture, 2.5% loss of the 102-179 °C is due to H2O formation of OH groups side by side, 33.5% loss of the 179-390 °C is due to phenolicity or ring groups, and the 27% loss of the 390-881 °C is due to the degradation of the substance. In this study, PE-AgNPs synthesized up to 881 °C were also shown in *Figure 6*. Although TGA-DTA analysis of plant-derived AgNPs was performed in the literature, no relevant TGA-DTA data on mushroom-based AgNP were found.

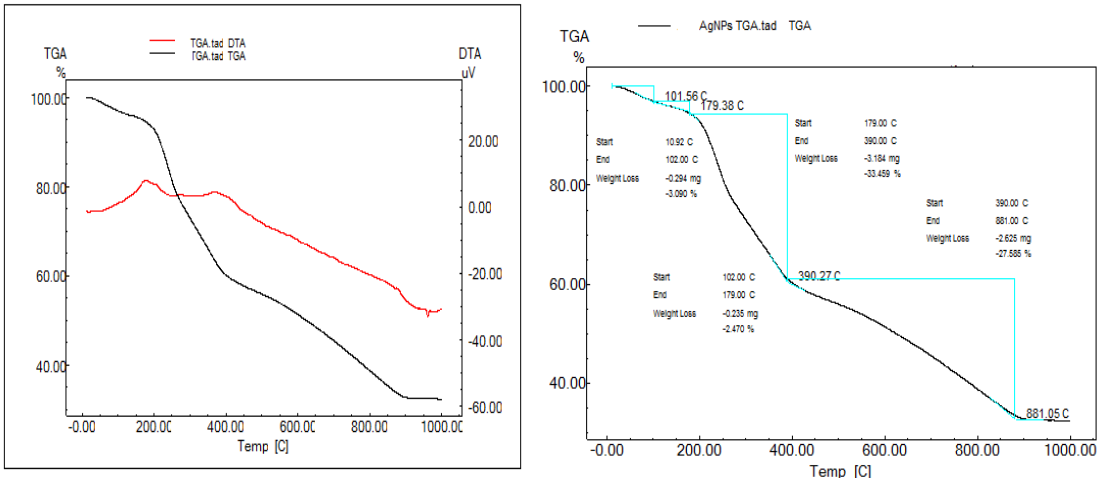

Figure 6. *TGA-DTA analysis result of synthesised PE-AgNPs*

It is widely known that resistance to antibiotics in microbes is a global problem that worsens at an alarming rate. It has become a major problem in many infectious diseases such as multiple antibiotic resistant bacteria and tuberculosis. Some studies in the past have shown that biologically synthesized metal nanoparticles have more biological activity than physically and chemically synthesized nanoparticles (Antony et al. 2011; Manikprabhu et al., 2013). Gopinath et al. (2015) reported that AgNPs synthesized from Fusarium oxysporum and antibiotics showed antimicrobial activity both individually and in combination against these bacteria the results of the same study confirmed that all resistant bacteria that are normally resistant to antibiotics are sensitive to the presence of AgNPs. In another study, Haq et al. (2015) showed that AgNPs synthesized from five fungal species (*Agaricus bisporus, Helvella lacunosa, Ganoderma applanatum, Pleurotus florida and Fomes fomentarius*) showed antimicrobial activity against methicillin-resistant *S. aureus*. However, AgNPs synthesized from *Agaricus bisporus* have been reported to be more powerful than the other four AgNPs. The antimicrobial activity of PE-AgNPs used in the study against *E. coli, S. aureus, S. pyogenes and P. aeruginosa* with standard bacterial strains and *C. albicans* strain is shown in *Table 1*. MIC values of PE-AgNP and silver nitrate were examined and compared. The study was performed with 3 repetitions and the mean values were determined as MIC value.

The MIC values of *S. aureus, S. pyogenes, E. coli, P. aeruginosa* and *C. albicans*, respectively, 0.07, 0035, 0.018, 0035 and 0.07 mg L^{-1} results were obtained (*Table 1*). Compared with Silver nitrates and antibiotics, AgNP can be used as an alternative to existing antibiotics as a result.

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Table 1. *MIC values of synthesized PE-AgNPs (mg l^{-1})*

Microorganisms	AgNPs	Silver nitrat	Antibiotic
S. aureus ATCC 25923	0.07	2.65	1
S. pyogenes ATCC 19615	0.035	1.32	1
E. coli ATCC25922	0.018	0.66	2
P. aeruginosa ATCC 27853	0.035	0.66	2
C. albicans ATCC 10231	0.07	0.66	

Conclusions

It is clear that *P. eryngii*, a renewable fungus, can be effectively used to synthesize bioactive nanoparticles using cheap substances in an environmentally friendly and non-toxic environment. The synthesis of PE-AgNPs was carried out at room temperature for 5 days by adding the fungus extract to an aqueous silver nitrate solvent. It was observed that PE-AgNPs with a crystal size of 18.45 nm and a spherical appearance showed a strong antimicrobial activity against human pathogen microorganisms. In this study, we believe that synthesized PE-AgNPs can contribute greatly to the search for antimicrobial agents. At the same time, these biosynthesized AgNPs can be used as effective growth inhibitors in various microorganisms; this makes them applicable to various medical devices, industrial, food, agricultural, biotechnological and environmental applications.

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