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Research /Araştırma

Antibacterial and Antifungal Activity of Nanofungal Molecules Using *Pleurotus eryngii* Mushroom

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ABSTRACT

Molecular synthesis at nano level is becoming a growing field of science due to its treatment and solution-oriented applications. Plants and bacteria in particular have an important place in nanoparticle production. The positive results of nanofungal structures in scientific studies in recent years have led the scientific world to turn to fungal nanomolecule synthesis. Silver coated nanofungal structures are highly preferred in medical and industrial applications. In our study, the Antibacterial and Antifungal activity of nano molecules obtained by using *Pleurotus eryngii* (Heliz Mushroom) and $AgNO_3$ was investigated by using Disk diffusion method. Ten different clinical strains were used in the study. Looking at the results obtained, it was determined that nano molecules formed zones varying between 9.1-15.0 against pathogenic microorganisms used. In addition, it was observed that nanoparticles formed zones more effective against some pathogens than antibiotics used as positive control.

Keywords: Nanofungal, *Pleurotus eryngii*, Antibacterial, Antifungal, $AgNO_3$

Pleurotus eryngii Mantarı Kullanılarak Elde Edilen Nanofungal Moleküllerin Antibakteriyal ve Antifungal Aktivitesi

ÖZET

Nano düzeyde molekül sentezi, tedavi eksenli ve çözüm odaklı uygulamaları nedeniyle her geçen gün büyüyen bir bilim alanı haline gelmektedir. Özellikle bitkiler ve bakteriler nanoparçacık üretiminde önemli bir yere sahiptir. Son yıllarda nanofungal yapıların bilimsel çalışmalarda olumlu sonuçlar vermesi, bilim dünyasının mantar yapılı nanomolekül sentezine yönelmesine neden olmuştur. Gümüş kaplı nanofungal yapılar tıbbi ve endüstriyel uygulamalarda çok fazla tercih edilmektedir. Yapmış olduğumuz çalışmada *Pleurotus eryngii* (Heliz Mantarı) ve $AgNO_3$ kullanılarak elde edilen nano moleküllerin Disk difüzyon metodu kullanılarak Antibakteriyal ve Antifungal aktivitesi araştırıldı. Çalışmada 10 farklı klinik suş kullanıldı. Elde edilen sonuçlara bakıldığında nano moleküllerin kullanılan patojen mikroorganizmalara karşı 9.1-15.0 arasında değişen oranlarda zonlar oluşturduğu belirlendi. Ayrıca nanoparçacıkların bazı patojenlere karşı pozitif kontrol olarak kullanılan antibiyotiklerden daha etkili zonlar oluşturduğu görüldü.

Anahtar Kelimeler: Nanofungal, *Pleurotus eryngii*, Antibakterial, Antifungal, $AgNO_3$

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INTRODUCTION

Nanomolecules production technology has gained an increasing momentum in recent years. The scope of the study area has gradually expanded. Especially in terms of medicine and biotechnology, the use of nano materials has become widespread. Nanoparticles are known to have versatile research areas such as biomedical applications, drug discovery, and cosmetics (Chaudhuri & Paria, 2012). The most extensively used metals in nanoparticle production research are structures such as Ag, Au, Pt and Pd. Among these, silver-derived nanoparticles have shown good results due to their important application in biomedicine (Kumar et al., 2020). Silver-based nanomolecules have a significant antibacterial capacity (Mohanpuria et al., 2008). It has become important to use silver nanoparticles as antimicrobial agents against the ever-increasing threats created by antibiotic-resistant microorganisms compared to other metals (Parashar et al., 2009). The use of biological synthesis approaches (plants, fungi, bacteria, algae, and actinomycetes) in the production of nanoparticles provides more advantages over other methods, as they are simple, cost-effective, reliable and environmentally friendly (Kumar and Yadav, 2009). Mushrooms have been used by humans for nutritional and medicinal purposes, due to their many pharmacological properties (Muszyńska et al., 2018). *Pleurotus eryngii* mushroom, which grows mostly in the Eastern Anatolia Region in our country, is an important species that is consumed as food and has economic importance (Akyüz and Kırbağ, 2007). *Pleurotus eryngii* is a mushroom species whose cultivation is rapidly increasing in many countries due to its taste, high nutritional content, medicinal properties, aromatic structure and long shelf life (Rodriguez Estrada, 2008; Moonmoon et al., 2010). *Pleurotus eryngii* mushroom contains many compounds with antioxidant properties such as polysaccharides and polyphenols (Lin et al., 2014). In addition, *Pleurotus eryngii* mushroom is preferred by scientists in medical, pharmaceutical and biotechnological studies (Gregori et al., 2007). Among the medicinal effects of various biological compounds produced by *Pleurotus eryngii*, it has been determined that Eryngeolysin has an antibacterial effect and Eryngin has an antifungal effect (Staji et al., 2009). Because of the medical and biomedical applications of silver nanoparticles, the use of fungal-derived polysaccharides is an important step towards natural medicine development (Radhakrishnan and Peter, 2021).

In this study, the antibacterial and antifungal activity of nano molecules obtained by using *Pleurotus eryngii* (Heliz Mushroom) and Silver Nitrate grown in its natural environment in the province of Van was investigated.

MATERIALS AND METHODS

Preparation of mushroom extract

Pleurotus eryngii (Heliz) mushroom (Figure 1) collected from the province of Van was first described in accordance with systematic principles. The species determination of the collected mushrooms was made in Van Yüzüncü Yıl University, Faculty of Science, Department of Biology. After the appropriate sterile conditions were provided in the laboratory, the washing process was performed. Then it was dried at room temperature for two weeks. After this time was completed, the mushrooms were powdered with the help of a powerful grinder. Taking 50 g of the sample obtained, it was mixed with 250 ml of distilled water. This solution was kept at 300 rpm for 24 hours with the help of magnetic stirrer. Then it was subjected to boiling process at 80 °C for 15 minutes. The solution obtained was filtered using Whatmann No: 1 filter paper. The resulting aqueous extract was stored at +4 ° C to be used in nanoparticle production study (Selvi and Sivakumar, 2014; Meydan, 2021).

Figure 1. *Pleurotus eryngii* (Heliz) mushroom

Synthesis of $AgNO_3$ structured nano molecules

1 mM 500 ml AgNO3 solution and 100 ml *Pleurotus eryngii* aqueous extract prepared for nanomolecular synthesis were reacted in a flask. In this reaction that took place at room temperature, color change (yellow → brownish tone) occurred after 35-40 minutes (Figure-2). Centrifugation was used to separate the nanoparticles from the solution. The solution was centrifuged at 10,000 rpm for 8 minutes and the supernatant was removed with a micropipette. The pellet in the lower part was washed three times. The nano molecules obtained in this way were left to dry for four days at 40 °C. It was then kept at +4 ° C for Antibacterial and Antifungal activity (Prakash et al., 2013, Sun et al., 2014,).

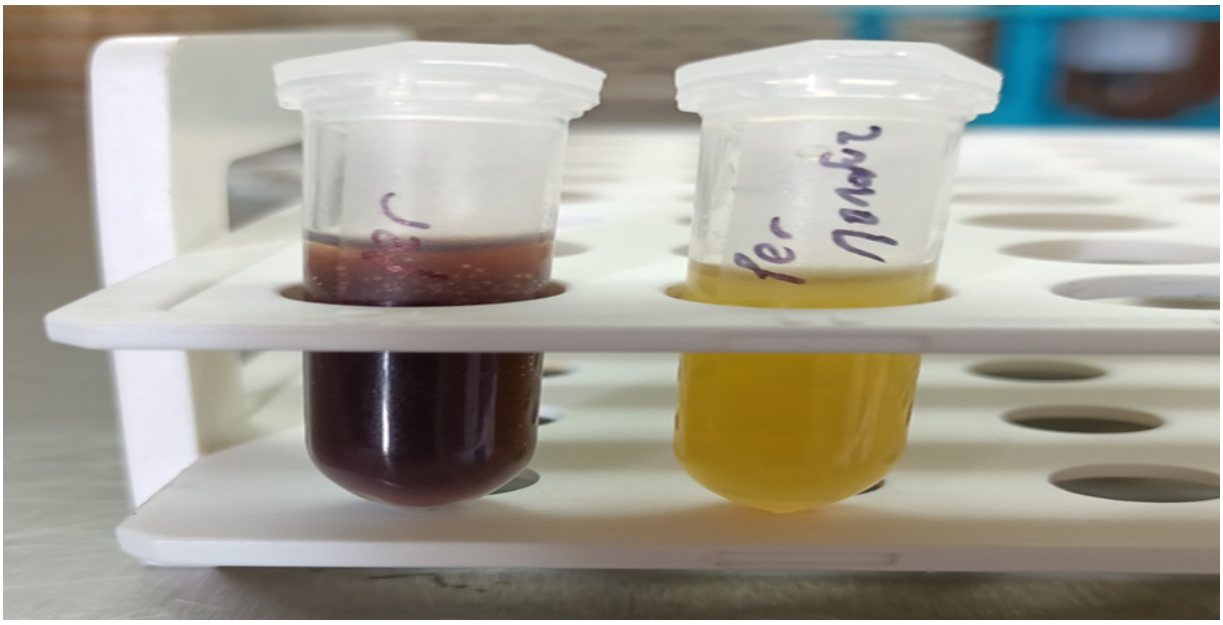

Figure 2. Color change during nanomolecule synthesis.

Antimicrobial activity

Antibacterial and Antifungal effects of nano molecules formed by using *Pleurotus eryngii* mushroom and $AgNO_3$ were investigated using disk diffusion method. Patented and clinical strains

used in the study (Table 1) were obtained from Van Yüzüncü Yıl University Research and Application Hospital. Pathogens were first allowed to grow in Tryptic Soy Broth broth (24 hours). Müller Hinton medium was used for the disk diffusion method. *Pleurotus eryngii* extract and AgNPs/Pe clusters were absorbed into 6 mm diameter blank discs. This process was carried out gradually with 25 µL on each disk (Figure 3). Rifampin and Oleandomycin antibiotics were used as positive controls for the reliability of the study. Discs prepared at room temperature, Rifampin (5 µg) and Oleandomycin (15 µg) antibiotic discs were placed in pathogen-planted media with the help of a sterile forceps. It was kept in the oven for 24 hours at 36.5-37 ^{o}C for incubation. According to the results obtained, zone measurements were made and images were recorded (Senthilkumar and Sivakumar, 2014; Meydan and Seçkin 2021).

Table 1. Strains used in the study

Pathogenic Microorganisms
Acinetobacter baumannii
Bacillus cereus ATCC 10876
Enterococcus faecalis ATCC 29212
Enterococcus faecium
Escherichia coli
Klebsiella pneumoniae
Pseudomonas aeruginosa ATCC 27853
Salmonella enterica
Staphylococcus aureus ATTC 29213
Candida albicans ATTC 90028 (Fungus)

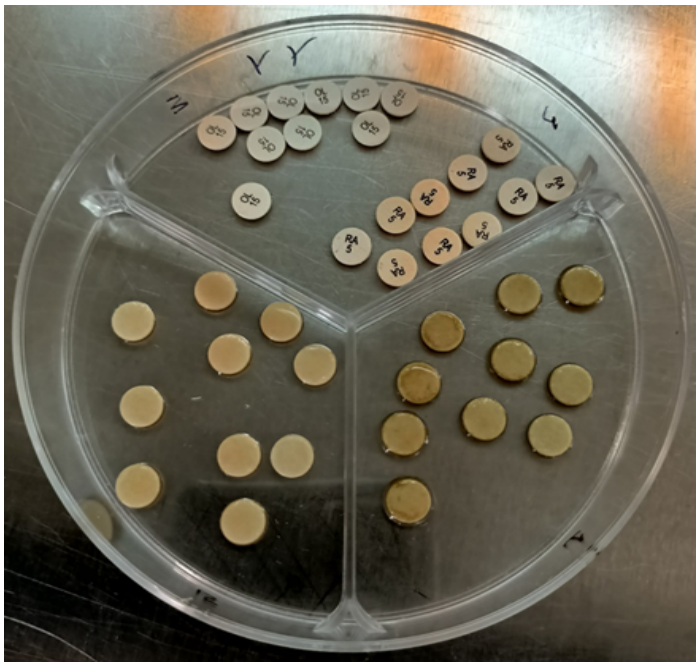

Figure 3. Absorption of samples on blank discs

RESULTS AND DISCUSSION

Many studies have been conducted on the antimicrobial activities of nanoparticles in recent years. Silver nanoparticles (AgNPs) obtained using *Pleurotus citrinopileatus* extract were found to have a bactericidal effect against pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* (Maurya et al., 2016). In our study, Antimicrobial activity of AgNPs/Pe, mushroom extract and antibiotics used as positive control, obtained by using *Pleurotus eryngii* mushroom and $AgNO_3$, were investigated using disk diffusion method. It was observed that Ag-NPs obtained using *Calotropis procera* leaf and fruit extract caused a significant decrease in the colonization levels of *Vibrio cholera* and enterotoxic *Escherichia coli* bacteria (Salem et al., 2015). According to a study in rabbits, silver-coated nanoparticular implants were determined to inhibit *Staphylococcus aureus* biofilm formation (Gupta et al., 2014). Silver nanoparticles tend to affect more than one site in bacterial cells as biocides and therefore have a broad spectrum (Markowska et al., 2013). It has been determined that Ag NPs/Vb obtained by using *Veronica beccabunga* plant has antibacterial effect against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* microorganisms (Seçkin and Meydan, 2021). Syed et. al., (2019) found that silver nanoparticles synthesized using the root extract of *Cassia toral L.* showed antibacterial action against *P. seudomonas* and *S. aureus* pathogens. According to the data obtained as a result of the study, it was determined that AgNPs/Pe formed zones varying between 9.1-12.2 mm against nine different pathogenic bacteria. It was seen that the nano molecules used had antibacterial activity. *C. albicans* and *C. tropicalis* showed high sensitivity to AgNPs (Mallmann et al., 2015). *Candida albicans* mushroom was used to determine the antifungal effect. It was determined that AgNPs formed an inhibition zone of 15 mm diameter against *Candida albicans* pathogen and thus showed antifungal effect. The zone diameters obtained as a result of the disk diffusion method are given in Table 2. Looking at the results, it was seen that Silver nanoparticles were more effective against *Bacillus cereus* ATCC 10876 bacteria than Rifampin antibiotics. In addition, AgNPs were determined to have a much better activity than Oleandomycin and Rifampin antibiotics against *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATTC 90028 pathogens. Some images obtained in the study are given in Figure 4.

Table 2. Zone diameters obtained as a result of the disk diffusion method.

Pathogenic Microorganisms	**AgNPs/Pe (mm)**	**Pe Extract (mm)**	**Oleandomycin (mm)**	**Rifampin (mm)**
Acinetobacter baumannii	9.4	8.0	9.5	10
Bacillus cereus ATCC 10876	12.1	-	21.6	11.3
Enterococcus faecalis ATCC 29212	11.0	9.2	13.5	15.3
Enterococcus faecium	10.1	8.2	14.7	-
Escherichia coli	9.2	8.3	-	9.4
Klebsiella pneumoniae	12.2	-	13.3	-
Pseudomonas aeruginosa ATCC 27853	11.0	-	9.0	9.1
Salmonella enterica	9.1	8.1	11.3	12.2
Staphylococcus aureus ATTC 29213	11.2	-	20.5	11.4
Candida albicans ATTC 90028 (Fungus)	15.0	-	-	-

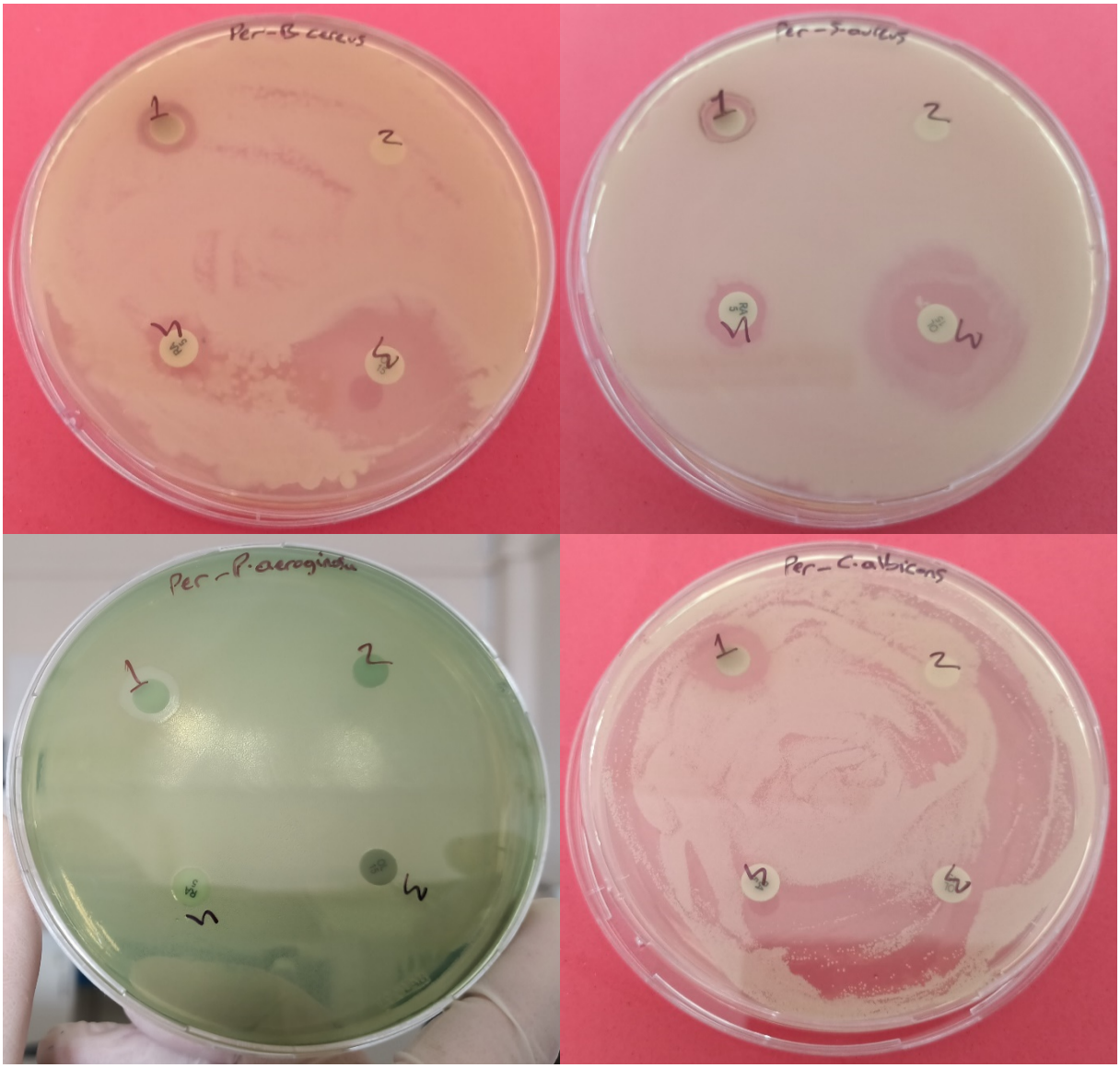

Figure 4. Some images obtained as a result of the Disk Diffusion method

CONCLUSION

Bacterial and fungal infections have become a serious health problem. The resistance of pathogenic microorganisms to existing antibiotics has become an urgent issue in the medical world. For this reason, developing new antibacterial and antifungal agents with antimicrobial activity and easily accessible is a must in terms of health. It is thought that the nano molecules obtained by using the *Pleurotus eryngii* mushroom have a high antimicrobial effect and can be used in the production of active ingredients, especially in the field of pharmacology.

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