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CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF *PHOLIOTA AURIVELLA* (BATSCH) P. KUMM.

PHOLIOTA AURIVELLA (BATSCH) P. KUMM.'NİN KİMYASAL BİLEŞİMİ VE BİYOLOJİK AKTİVİTELERİ

Cenker YAMAN[1]*, Gizem GÜL[1], Atakan BENEK[4], Dilay TURU[1], Selime Deniz BOZKURT[1], Ilgaz AKATA[3], Kerem CANLI[2,4]

[1]Dokuz Eylül University, Graduate School of Natural and Applied Sciences, Departmant of Biology, 35390, İzmir, Türkiye
[2]Dokuz Eylül University, Faculty of Science, Departmant of Biology, 35390, İzmir, Türkiye
[3]Ankara University, Faculty of Science, Departmant of Biology, 06560 Ankara, Türkiye
[4]Dokuz Eylül University, Research and Application Center of Fauna and Flora (FAMER), 35390, İzmir, Türkiye

ABSTRACT

Objective: *This study aims to determine the chemical composition of the mushroom Pholiota aurivella by investigating its antimicrobial, antibiofilm, and antioxidant activities. The potential effects of the ethanol extract of the mushroom on a total of 27 different microorganisms, including strains with multidrug resistance (MDR), were examined. Additionally, the biofilm inhibition capacity and free radical scavenging activities of the mushroom extract were tested.*
Material and Method: *The ethanol extract obtained from the P. aurivella sample was subjected to various tests to evaluate its biological activities. Antimicrobial activity was analyzed using disk diffusion and Minimum Inhibitory Concentration (MIC) tests. Antibiofilm activity was assessed based on its capacity to inhibit biofilm formation, while antioxidant activity was tested using the DPPH method. The chemical composition was determined by Gas Chromatography-Mass Spectrometry (GC-MS) analysis.*
Result and Discussion: *Our study reveals that P. aurivella is a promising natural agent, especially in terms of its antimicrobial and antibiofilm activities. The extract exhibited antimicrobial activity against 16 different Gram-positive and Gram-negative microorganisms. Expanding zones of inhibition were observed with increasing extract amounts in strains such as Klebsiella pneumoniae and Enterobacter aerogenes, which show multidrug resistance (MDR). In biofilm inhibition tests, the strongest effects were observed in strains of Listeria innocua and Bacillus subtilis DSMZ 1971. On the other hand, the antioxidant activity tested by the DPPH method was quite low; this can be explained by the absence of phenolic compounds in the chemical composition of the extract. GC-MS analysis identified the major components as linoleic acid (59.20%) and ethyl linoleate (17.13%). These findings indicate that P. aurivella, with its antibiofilm and antimicrobial properties, has potential for pharmaceutical applications and may offer an important natural resource for developing treatment options even against MDR pathogens.*
Keywords: *Antibiofilm activity, antimicrobial activities, antioxidant activity, GC-MS, Pholiota aurivella*

* **Corresponding Author / Sorumlu Yazar:** Cenker Yaman
e-mail / e-posta: cenkeryaman@gmail.com, **Phone / Tel.:** +905419592277

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ÖZ

Amaç: *Bu çalışma, Pholiota aurivella mantarının antimikrobiyal, antibiyofilm ve antioksidan aktivitelerini araştırarak kimyasal kompozisyonunu belirlemeyi amaçlamaktadır. Çalışmada, mantarın etanol ekstraktının, çoklu ilaç direncine sahip suşlar da dahil olmak üzere toplam 27 farklı mikroorganizma üzerindeki potansiyel etkisi incelenmiştir. Ayrıca, mantar ekstraktının biyofilm inhibisyon kapasitesi ve serbest radikal süpürme aktiviteleri test edilmiştir.*

Gereç ve Yöntem: *P. aurivella örneğinden etanol ile elde edilen ekstrakt, biyolojik aktivitelerini değerlendirmek amacıyla çeşitli testlere tabi tutulmuştur. Antimikrobiyal etkinlik, disk difüzyon ve Minimum İnhibitör Konsantrasyon (MİK) testleri ile analiz edilmiştir. Antibiyofilm aktivitesi, biyofilm oluşumunu inhibe etme kapasitesiyle değerlendirilirken, antioksidan aktivite DPPH yöntemi ile test edilmiştir. Kimyasal kompozisyon ise Gaz Kromatografisi-Kütle Spektrometrisi (GC-MS) analiziyle belirlenmiştir.*

Sonuç ve Tartışma: *Çalışmamız, P. aurivella'nın özellikle antimikrobiyal ve antibiyofilm aktiviteleri açısından umut verici bir doğal ajan olduğunu ortaya koymaktadır. Ekstrakt, 16 farklı Gram-pozitif ve Gram-negatif mikroorganizmaya karşı antimikrobiyal etkinlik gösterirken, çoklu ilaç direnci (MDR) gösteren Klebsiella pneumoniae ve Enterobacter aerogenes gibi suşlarda da artan ekstrakt miktarı ile genişleyen inhibisyon alanları gözlemlenmiştir. Biyofilm inhibisyonu testlerinde de en güçlü etkiler, Listeria innocua ve Bacillus subtilis DSMZ 1971 suşlarında gözlemlenmiştir. Öte yandan, DPPH yöntemiyle test edilen antioksidan aktivite oldukça düşüktür; bu durum, ekstraktın kimyasal bileşiminde fenolik bileşiklerin bulunmamasıyla açıklanabilir. GC-MS analizinde, majör bileşenlerin linoleik asit (%59.20) ve etil linoleat (%17.13) olduğu tespit edilmiştir. Bu bulgular, P. aurivella'nın antibiyofilm ve antimikrobiyal özellikleriyle farmasötik uygulamalar açısından potansiyele sahip olduğunu ve MDR patojenlere karşı dahi tedavi seçenekleri geliştirilmesinde önemli bir doğal kaynak sunabileceğini göstermektedir.*

Anahtar Kelimeler: *Antibyiofilm aktivite, antimikrobiyal aktivite, antioksidan aktivite, GC-MS, Pholiota aurivella*

INTRODUCTION

The search for antimicrobial agents began with Alexander Fleming's discovery of penicillin in 1928, and the need for new antibiotics - and consequently, research in this area - has continued to grow exponentially. Despite the leaps made in medical science over the past decades, infectious diseases remain an uncontainable problem. The indiscriminate use of antibiotics has led bacteria to develop resistance to existing drugs, strengthening their resistance mechanisms. For example, before the antibiotic era, the mortality rate of infections caused by *Staphylococcus aureus* was around 80% [1]. With the introduction of penicillin into our lives in the early 1940s, patients' chances of recovery increased significantly; however, as early as 1942, penicillin-resistant staphylococci began to appear first in hospitals and then in the community [2]. By the late 1960s, 80% of staphylococcal strains obtained from both hospitals and the community were resistant to penicillin [3]. This rapid resistance pattern, which first emerged in hospitals and then spread to the community, is not limited to penicillin. Almost every antibiotic faces this model of resistance. Unfortunately, unless our habits regarding antibiotic use change, new drugs will meet the same fate as the existing ones [4]. A study reports that 700,000 people die annually due to antimicrobial resistance, and this number is projected to exceed 10 million by 2050 [5].

An examination of all drugs produced between 1981 and 2010 shows that 118 new antibacterial drugs were developed, and 77 of them were derived from natural compounds [6]. There are many reasons why naturally obtained compounds are used more frequently and effectively in antimicrobial studies. Firstly, the compounds used are isolated from organisms that have survived to the present day by exhibiting resistance to pathogens through natural selection. In other words, they are products of existing resistance mechanisms. Additionally, because they exhibit lower toxicity, they offer a safer route with fewer side effects compared to synthetic antibiotics.

Mushrooms have been part of the human diet since ancient times, both as a nutritious food and for medicinal purposes [7,8]. Examples of their medical use include their effects against diseases such as hypertension, hypercholesterolemia, and cancer [9]. It has been shown that a large portion of

mushrooms possess many important bioactivities, including antioxidant, antiviral, anti-angiogenic, anti-tumor, anti-inflammatory, anti-obesity, and immunomodulatory properties [10]. Furthermore, today mushrooms are used in various fields such as pharmacology, the textile industry, wastewater purification for ecosystem recovery, and the manufacture of cosmetic and hygiene products [11-14].

In this study, *Pholiota aurivella* (Batsch) P. Kumm., one of the mushrooms that have been part of the human diet for centuries, was selected. *P. aurivella* typically grows in clusters on live tree trunks during the summer and autumn months [15]. This mushroom, which has a sticky texture, golden yellow or orange color, and a spicy odor, has been reported in the literature as both edible and inedible [16,17].

Studies on *P. aurivella*, the subject of our research, are limited, and the disk diffusion, minimum inhibitory concentration (MIC), and antibiofilm tests conducted using its ethanol extract are pioneering. In this study, the antimicrobial activity of the mushroom was investigated using disk diffusion and MIC methods, along with its antibiofilm and antioxidant activities. Its chemical composition was determined through Gas Chromatography-Mass Spectrometry (GC-MS) analysis. This allowed us to gain insights into which compounds might play a role in the observed biological activities.

MATERIAL AND METHOD

Macrofungus Sample

P. aurivella specimens were collected from the Yomra region of Trabzon by Prof. Dr. Ilgaz Akata and, after being completely dried, were preserved under the herbarium code MA0015 at the Fauna and Flora Research and Application Center (FAMER) of Dokuz Eylül University.

Extraction Procedure

The extraction procedure was performed as described by Canlı et al. [18]. An ethanol extract containing 0.495 g of material in 15 ml was prepared for use in disk diffusion testing and GC-MS analysis. For the antioxidant test, the ethanol extract was diluted at a ratio of 1 mg/ml. For antibiofilm and MIC tests, the ethanol was removed from the extract, and the resulting residue was dissolved in 1% DMSO (dimethyl sulfoxide) to prepare a water-based extract.

Microorganisms

In this study, a total of 27 strains were analyzed, including 7 Food Isolates (FI), 12 Standard Isolates (ST), one of which is a yeast, 1 Clinical Isolate (CI), and 7 Multi-Drug Resistant (MDR) strains. The microorganisms were obtained from the microbiology laboratory of the Department of Biology, Faculty of Science, Dokuz Eylül University (Table1-4).

Preparation of Inocula

The bacteria were enriched by incubation at 37°C for 24 hours, while the yeast was incubated at 28°C for 48 hours. Subsequently, to standardize inoculum concentrations, each bacterial and yeast sample was adjusted to 0.5 McFarland standard using sterile 0.9% NaCl solution, corresponding to approximately 10^8 cfu/ml for bacteria and 10^7 cfu/ml for *Candida albicans*. The experiments were conducted with these standardized microorganism samples [19,20].

Disc Diffusion Method

The antimicrobial activity of the ethanol extract of *P. aurivella* was evaluated using the disk diffusion method as described by Andrews [20]. Three different volumes of the extract (50 µl, 100 µl, and 200 µl) were loaded onto 6 mm antimicrobial susceptibility test disks. Instead of applying the entire volume at once, these volumes were loaded incrementally in 10 µl steps. For the higher volumes, the disks were allowed to dry between applications to ensure complete evaporation of ethanol. The disks were left to dry overnight to evaporate the remaining ethanol, resulting in disks containing 2.88 mg, 5.77 mg, and 11.54 mg of the extract, respectively. Subsequently, pre-prepared microorganisms suspended in sterile saline solution were inoculated onto petri dish surfaces to ensure uniform coverage. Following inoculation, extract-loaded disks were placed on the agar surface, and the plates were

incubated. The diameters of the inhibition zones formed were measured in millimeters (mm) using a caliper and recorded.

Sterile blank disks and the extraction solvent (ethanol) were used as negative controls, while Gentamicin and Tobramycin antibiotic disks served as positive controls. All tests were conducted in triplicate, and the results are presented as means with standard errors [21].

Minimum Inhibitory Concentration Test

MIC is defined as the lowest extract concentration that inhibits visible bacterial growth. For the MIC test, the serial dilution method described by Baldas and Altuner [22] was applied, resulting in a concentration range of 0.055–7.067 mg/ml. For bacterial growth controls, wells without extract were used as positive controls, and wells without bacteria were used as negative controls. All tests were performed in triplicate.

Antibiofilm Activity

In this study, the antibiofilm test was adapted from [23]. The procedure consisted of two parts: determining biofilm formation conditions and evaluating antibiofilm activity.

Five strains were used in the study: *Bacillus subtilis* DSMZ 1971, *Listeria monocytogenes* ATCC 7644, *Escherichia coli* ATCC 25922, *Listeria innocua* (FI), and *Escherichia coli* (CI). The strains, adjusted to 0.5 McFarland standard, were transferred into microplates containing different concentrations of glucose monohydrate and incubated at 37°C for 24 and 48 hours. After incubation, the microplates were washed, stained with crystal violet, and allowed to sit. The biofilm stained with crystal violet was dissolved using an ethanol-acetone solution, and absorbance was measured at 550 nm using a microplate reader. The results showed that the optimal biofilm formation conditions for all tested strains were determined to be 48 hours of incubation with 1.5% glucose monohydrate concentration.

The *P. aurivella* DMSO-water extract, whose antibiofilm activity was to be assessed, was loaded into the microplates at a concentration range of 0.055–7.067 mg/ml and incubated under the previously determined optimal conditions. Negative controls, consisting of wells containing both extract and broth without microorganisms, were included to account for any background absorbance. Positive controls, consisting of wells containing both microorganisms and broth without extract, were used to ensure that biofilm formation occurred in the absence of the extract. Following incubation, the same procedures described in the biofilm formation stage were applied, and antibiofilm activities were determined by measuring absorbance at 550 nm with a microplate reader.

GC-MC Analysis

The analyses were performed with modifications based on the study by Benek et al. [24]. The experiments were conducted using an Agilent GC 8890-Agilent GC/MSD 5977B (Agilent Technologies Inc., Santa Clara, CA, USA) device equipped with an HP5-MS capillary column (30 m x 0.25 mm; film thickness 0.25 μm).

Analytical conditions were as follows: the injector temperature was set to 350°C, with helium used as the carrier gas at a flow rate of 1 ml/min. The injection mode was split, with a split ratio of 10:1, and the injection volume was 1 μl of the ethanol extract. The oven temperature was programmed to increase from 40°C to 350°C at a rate of 4°C/min, with a 10-minute hold at 350°C. MS scan conditions were set with a transfer line temperature of 280°C, interface temperature of 280°C, and ion source temperature of 230°C. Component identification was achieved by matching retention times with the Wiley-NIST MS libraries.

Antioxidant Activity

The extract's antioxidant activity was measured by observing its scavenging effects on the DPPH radical. The DPPH test was performed as described by Turu et al. [25]. The samples' absorbance was measured at 515 nm using a spectrophotometer. All tests were conducted in triplicate, with ascorbic acid prepared in ethanol at a concentration of 1 mg/ml used as the control.

Statistics

All tests were conducted in triplicate. One-way analysis of variance (ANOVA), a parametric method, was performed with a significance level of P = 0.05. The Pearson correlation coefficient was calculated to evaluate any potential correlation between antimicrobial activity intensity and concentration. Statistical analyses were performed using R Studio, version 2024.09.1 [26].

For antibiofilm results, the mean inhibition values were reported with ± SD based on nine replicates. The results obtained from each antioxidant assay were reported as mean ± SD, based on three independent experimental replicates. EC_{50} values were calculated and expressed as 95% confidence intervals using Four-Parameter Logistic Regression.

RESULT AND DISCUSSION

Antimicrobial Activity

The diameters of the inhibition zones were measured in millimeters, and the results were presented in Table 1, 2, 3 and 4 alongside the positive controls, gentamicin and tobramycin. No growth was observed on the negative control discs.

Table 1. The antimicrobial activity of *P. aurivella* on standard microorganisms (Inhibition zones in mm)

Standard Isolated Microorganisms	50 µl	100 µl	200 µl	Gentamicin (10 µg)	Tobramycin (10 µg)
***Bacillus subtilis* DSMZ 1971**	8.00 ± 0.00	9.00 ± 0.00	9.33 ± 0.57	30	26
***Candida albicans* DSMZ 1386**	-	-	-	12	13
***Enterobacter aerogenes* ATCC 13048**	-	-	-	24	18
***Enterococcus faecalis* ATCC 29212**	-	7.00 ± 0.00	7.33 ± 0.57	12	8
***Escherichia coli* ATCC 25922**	-	-	-	22	20
***Listeria monocytogenes* ATCC 7644**	7.00 ± 0.00	9.00 ± 0.00	10.00 ± 0.00	28	24
***Pseudomonas aeruginosa* DSMZ 5071**	-	-	7.00 ± 0.00	15	22
***Pseudomonas fluorescens* P1**	-	-	-	13	12
***Salmonella enteritidis* ATCC 13076**	-	-	7.00 ± 0.00	21	-
***Salmonella typhimurium* SL 1344**	-	-	-	24	15
***Staphylococcus aureus* ATCC 25923**	7.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.00	21	14
***Staphylococcus epidermidis* DSMZ 20044**	8.00 ± 0.00	9.66 ± 0.57	11.00 ± 0.00	22	20

* Strains exhibiting no antimicrobial activity were indicated with a "–"

The only study investigating the antimicrobial activity of *P. aurivella* was conducted by Dyakov et al. (2011) [27], in which the antibiotic biosynthesis capacity of cultured fungi against various pathogens present in the same medium was examined. The study reported that *P. aurivella* did not

exhibit any antimicrobial activity against any strain. In the same study, other species such as *P. squarrosa* and *P. lenta* were also examined, and only *P. lenta* was observed to possess antibiotic biosynthesis capability.

Table 2. The antimicrobial activity of *P. aurivella* on food isolated microorganisms (Inhibition zones in mm)

Food Isolated Microorganisms	50 µl	100 µl	200 µl	Gentamicin (10 µg)	Tobramycin (10 µg)
Enterococcus durans	-	8.00 ± 0.00	8.33 ± 0.57	11	13
Enterococcus faecium	9.00 ± 0.00	10.00 ± 0.00	11.00 ± 0.00	28	15
Klebsiella pneumoniae	7.00 ± 0.00	7.33 ± 0.57	9.00 ± 0.00	19	23
Listeria innocua	8.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.00	13	15
Salmonella infantis	-	-	-	17	14
Salmonella kentucky	-	-	7.00 ± 0.00	12	16
Escherichia coli	-	-	7.00 ± 0.00	20	-

* Strains exhibiting no antimicrobial activity were indicated with a "–"

Table 3. The antimicrobial activity of *P. aurivella* on clinically ısolated microorganism (Inhibition zones in mm)

Clinically Isolated Microorganism	50 µl	100 µl	200 µl	Gentamicin (10 µg)	Tobramycin (10 µg)
Staphylococcus aureus	8.00 ± 0.00	9.00 ± 0.00	10.00 ± 0.00	22	18

* Strains exhibiting no antimicrobial activity were indicated with a "–"

Table 4. The antimicrobial activity of *P. aurivella* harboring multi-drug resistance microorganisms (Inhibition zones in mm)

Multi-Drug Resistance Microorganisms	50 µl	100 µl	200 µl	Gentamicin (10 µg)	Tobramycin (10 µg)
Escherichia coli	-	-	-	8	9
Klebsiella pneumoniae	7.00 ± 0.00	8.00 ± 0.00	9.00 ± 0.00	15	20
Acinetobacter baumannii	-	-	-	-	-
Enterobacter aerogenes	8.66 ± 0.57	9.00 ± 0.00	10.00 ± 0.00	16	18
Serratia odorifera	-	-	-	7	9
Streptococcus pneumoniae	-	-	-	10	8
Proteus vulgaris	-	-	-	11	11

* Strains exhibiting no antimicrobial activity were indicated with a "–"

Although different methods were used in the studies, a common finding was that no effect was observed for any *Pholiota*species, including *P. aurivella*, against *E. coli* ATCC 25922. In this regard, the two studies are consistent. However, while Dyakov et al. found no activity against numerous strains, our study clearly demonstrates that *P. aurivella* acquired antimicrobial activity against a total of 16 different strains, including multidrug-resistant ones, after undergoing the maceration process.

The observation of zone formation in all *Staphylococcus* strains studied is a noteworthy finding. In 2019, *S. aureus*, along with *E. coli*, *S. pneumoniae*, *K. pneumoniae*, and *P. aeruginosa*, accounted for 30.9% of the 7.7 million deaths related to infections, making it the leading bacterial cause of death in 135 countries. That same year, *S. aureus* was associated with 1.105.000 deaths, while methicillin-resistant strains (MRSA) were the most common cause of antimicrobial-resistant infection-related deaths in 27 countries [28]. Such data highlight the significant threat posed by staphylococci. The

effectiveness of *P. aurivella* against the *Staphylococcus* strains used in this study also offers promising potential for the treatment of other *Staphylococcus* strains.

As observed with many other strains, the inhibition zones for multidrug-resistant *K. pneumoniae* and *E. aerogenes* were found to expand with increasing extract concentrations. This suggests that higher extract quantities may yield greater efficacy against these pathogens. Considering the significant burden these pathogens, which do not respond to conventional antibiotic therapies, place on the healthcare sector, the importance of these findings becomes even more evident.

Table 5. Minimum inhibitory concentrations (MIC)

Microorganisms	MIC
***Listeria innocua* (FI)**	0.442 mg/ml
***Listeria monocytogenes* ATCC 7644**	1.767 mg/ml
***Enteroccus faecalis* ATCC 29212**	1.767 mg/ml
***Staphylococcus aureus* ATCC 25923**	3.533 mg/ml
***Staphylococcus aureus* (CI)**	3.533 mg/ml
***Escherichia coli* (FI)**	3.533 mg/ml
***Enterococcus durans* (FI)**	7.067 mg/ml
***Enterococcus faecium* (FI)**	>7.067 mg/ml
***Klebsiella pneumoniae* (FI)**	>7.067 mg/ml
***Salmonella kentucky* (FI)**	>7.067 mg/ml
***Pseudomonas aeruginosa* DSMZ 5071**	>7.067 mg/ml
***Salmonella enteritidis* ATCC13076**	>7.067 mg/ml
***Bacillus subtilis* DSMZ 1971**	>7.067 mg/ml
***Staphylococcus epidermidis* DSMZ 20044**	>7.067 mg/ml
***Enterobacter aerogenes* (MDR)**	>7.067 mg/ml
***Klebsiella pneumonnii* (MDR)**	>7.067 mg/ml

For *P. aurivella*, the MIC study was conducted for the first time, and the minimum inhibitory concentrations for seven strains were determined. Among these, the lowest inhibitory concentration was measured at 0.442 mg/ml for *L. innocua*. Conversely, the strain requiring the highest extract concentration for inhibition was *E. durans*, with a value 7.067 mg/ml (Table 5).

Of the seven strains yielding results in MIC tests, six were gram-positive, clearly indicating that the extract is more effective against gram-positive bacteria compared to gram-negative ones. The thick peptidoglycan layer of gram-positive bacteria allows hydrophobic fatty acids and esters in the extract to easily penetrate the cell wall. In contrast, the outer membrane of gram-negative bacteria, containing a lipopolysaccharide (LPS) layer, restricts the passage of hydrophobic molecules, making them more resistant [29]. Therefore, the higher efficacy observed in gram-positive strains can be attributed to the composition of the extract. The extract was particularly effective against *L. innocua* and *L. monocytogenes* strains, even at very low concentrations. Bacteria of the genus *Listeria* pose a significant contamination risk in the food industry during both production and storage stages, as they can continue to proliferate at low temperatures, such as 4°C [30-32]. Even a minimal amount of the mushroom used in this study was capable of inhibiting the growth of *Listeria* species. While further studies are required, *P. aurivella* could potentially provide a novel solution to such challenges in this field.

In the study by Skalicka-Woźniak et al. (2010) [33], the MIC value of linoleic acid for *S. aureus* ATCC 25923 was reported as 500 µg/ml. In comparison, the MIC value of *P. aurivella* ethanol extract for the same strain was determined to be 3.533 µg/ml. Considering that 59.2% of the extract is composed of linoleic acid, the extract is believed to exhibit a strong synergistic antimicrobial effect.

Additionally, a study by Islek et al. (2021) [34] determined MIC values for *P. adiposa*, *P. lubrica*, and *P. squarrosa* species against nine different strains. Four microorganisms (*S. aureus, E. faecalis, E. coli, P. aeruginosa*) were common between the two studies and demonstrated antimicrobial activity in both cases, whereas *A. baumannii* and *C. albicans* showed no antimicrobial effect with *P. aurivella*.

Based on GC-MS analysis, approximately 88% of *P. aurivella* ethanol extract was found to consist of hydrophobic substances, such as linoleic acid esters, linoelaidic acid, palmitic acid, myristic acid, tetradecanal, 3-tetradecen (E)-, and other fatty acids and esters. The lack of effect observed in MIC tests against strains that showed inhibition in the disk diffusion test is thought to be due to the high content of hydrophobic compounds in the extract, which are less soluble in liquid media. Hydrophobic compounds may experience solubility issues in liquid environments, limiting the access of active compounds to bacterial cells and reducing or eliminating antimicrobial activity. In contrast, in disk diffusion tests, hydrophobic compounds can diffuse more effectively in agar, achieving locally high concentrations that inhibit bacterial growth. Previous studies in the literature support this possibility. Van Vuuren (2008) [35] noted that plant extracts may yield different results in disk diffusion and MIC tests, which can be attributed to the physicochemical properties of the extract.

Antioxidant Activity

The tests revealed that the antioxidant effect of the mushroom extract was quite limited. Even at the highest concentration, its effect was limited to just 20% in scavenging DPPH radicals, while the positive control, ascorbic acid, demonstrated a scavenging rate of 94.7% (Table 6).

Table 6. Antioxidant values

Concentration (µg/ml)	*P. aurivella* % scavenging	Ascorbic acid % scavenging
1000	20.80 ± 1.66	94.70 ± 0.002
500	16.90 ± 1.11	94.30 ± 0.059
250	14.80 ± 0.35	92.40 ± 0.010
125	13.20 ± 0.88	91.00 ± 0.017
62.5	10.50 ± 0.91	73.00 ± 0.041
31.25	9.00 ± 0.33	40.20 ± 0.071
15.62	8.26 ± 0.44	23.21 ± 0.273

Previously, linoleic acid was tested for antioxidant activity using the DPPH method, but no significant results were observed [36]. GC-MS analysis of the extract, which is composed of 59% linoleic acid, revealed that it predominantly consists of fatty acids and ethers, with no phenolic compounds detected. Considering that antioxidant activity is typically associated with phenolic compounds, this result is not unexpected.

In a study by Regeda et al. (2021) [37], the antioxidant activity of seven different *Pholiota* species was compared using the DPPH radical scavenging method. Methanol was used as the solvent to prepare biomass and culture liquid extracts, yielding DPPH scavenging rates ranging from 83.6% to 7.37%, with *P. aurivella* recording a value of 82.37%. Contrary to these findings, the low antioxidant activity observed in our study is thought to be due to the solvent used. Methanol, being more polar than ethanol, has a greater potential to effectively extract phenolic compounds.

When the statistical results were evaluated, the EC_{50} value of the mushroom extract was found to be 1.8160 ± 2.0329 mg/ml. However, since the highest observable scavenging activity in the test was around 20%, this value represents a statistical estimation. The EC_{50} value of ascorbic acid, used as the positive control, was determined to be 0.0400 ± 0.0005 mg/ml. Based on the overall results, the p-value of the ANOVA test comparing the extract and ascorbic acid was calculated as 1.094×10^{2}, indicating no statistically significant difference between the extract and the positive control. Furthermore, the Pearson correlation coefficient between the increasing dose of the extract and its effect was calculated as 0.8833, which indicates a strong positive correlation.

Since the primary focus of this study was not on antioxidant activity, other solvents were not considered for extraction. However, to comprehensively evaluate and understand the antioxidant capacity of *P. aurivella*, it is necessary to extract its phenolic compounds using solvents of varying polarities. This approach could contribute to a better understanding of the mushroom's potential antioxidant capacity.

Although mushrooms are known for their low-fat content, the fats they contain predominantly consist of polyunsaturated fatty acids [51]. This study aligns with that observation. The GC-MS analysis revealed that 59.2% of the extract consists of linoleic acid, an Omega-6 fatty acid; 17.13% consists of linoleic acid ethyl ester; 8.14% consists of hexadecanoic acid (palmitic acid), a common saturated fatty acid; and 2.75% consists of hexadecanoic acid ethyl ester. The extract was found to be predominantly composed of fatty acids. Notably, this is the first such analysis reported for *Pholiota aurivella*. Fatty acids are known to exhibit toxic effects on bacterial cells by destabilizing their membranes, disrupting respiratory processes, and ultimately leading to cell death [52]. The antimicrobial activity observed in this study is likely attributable to the high fatty acid content of the extract.

GC-MS Analysis

Linoleic acid has long been recognized for its antimicrobial and antifungal properties [53]. It has also been demonstrated through both *in vivo* and *in vitro* studies that it possesses anti-inflammatory and anti-atherogenic effects and may be effective against major health issues affecting a large portion of the population, such as cancer, diabetes, and obesity [54].

Hexadecanoic acid, also known as Palmitic acid, is recognized as a natural and essential component of human skin and has been shown to exhibit antimicrobial activity against *S. aureus* strains [55,56]. However, a study revealed that *S. aureus* strains can rapidly develop resistance to hexadecanoic acid within a few hours. While the mortality rate of colonies without resistance development was 99%, this rate dropped to only 12% in treated and resistant strains [57]. This finding highlights the remarkable ability of *S. aureus* strains to develop resistance mechanisms against external threats. In addition, Palmitic acid is also known to have hemolytic, hypocholesterolemic, and nematicidal effects [43].

The composition of 89.02% of the ethanol extract of *P. aurivella* was elucidated through GC-MS analysis (Table 7). This analysis revealed that the extract predominantly contains polyunsaturated and saturated fatty acids. The antimicrobial activity exhibited against Gram-positive bacteria is thought to result from the ability of these fatty acids to penetrate bacterial cell membranes and disrupt membrane stability. Major components of the extract, such as linoleic acid and palmitic acid, are known to exert antibacterial effects by altering bacterial membrane permeability and impairing cellular functions [58]. This analysis represents the first report on *P. aurivella* in the literature, providing a comprehensive foundation for exploring its biological activities. The chemical profile obtained suggests that this mushroom has the potential to be evaluated as a natural antimicrobial agent in future pharmacological applications.

Table 7. Biochemical screening of *Pholiota aurivella*

Retention Time	Area%	Compound Name	Formula	Molecular Weight (g/mol)	Known Activity
25.868	0.15	3-Tetradecene, (E)-	$C_{14}H_{28}$	196.37	Antimicrobial and antifungal activity [38]
35.464	0.28	Tetradecanal	$C_{14}H_{28}O$	212.37	Bioluminescence reactions [39]
37.227	0.56	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	Antiurease, antielastase and antioxidant [40], insecticide [41, 42]
42.410	8.14	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Antimicrobial activity [42] and nematicide [43]
42.517	2.75	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.5	Antioxidant, hemolytic, hypocholesterolemic, anti-androgenic activity, nematicide and flavor [43]
44.924	0.24	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294.5	Antioxidant [44] and anticancer activity [45]
46.590	17.13	Ethyl Linoleate	$C_{20}H_{36}O_2$	308.5	Anti-inflammatory [46] and antioxidant activity [47]

Table 7 (*continue*). Biochemical screening of *Pholiota aurivella*

46.736	59.20	Linoleic acid	$C_{18}H_{32}O_2$	280.4	Anticancer [48], antioxidant [49] and anti-inflammatory activity [50]
62.576	0.32	2-Methyl-3-(3-Methyl-2-Butenyl)-2-(4-Methyl-3-Pentenyl)oxetane	$C_{15}H_{26}O$	222.37	-
67.122	0.25	Fumaric acid, pent-4-en-2-yl tridecyl ester	$C_{22}H_{38}O_4$	366.5	-

* Compounds with no known activity in the literature are indicated with "-"

Antibiofilm Activity

This study represents the first investigation into the antibiofilm activity of *P. aurivella*. A significant increase in biofilm formation was observed in *B. subtilis* DSMZ 1971, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 7644, and *L. innocua* strains, which correlated positively with the increase in extract concentration. In contrast, a positive but weak correlation was observed in the *E. coli* strain, known to possess an efflux pump system (Table 8).

Table 8. Correlation results of mushroom extract and biofilm effect

Microorganisms	Correlation (R^2)
***Bacillus subtilis* DSMZ 1971**	0.942559
***Escherichia coli* ATCC 25922**	0.921367
***Listeria monocytogenes* ATCC 7644**	0.916541
***Listeria innocua* (FI)**	0.956585
***Escherichia coli* (CI)**	0.693696

Although the extract maintained its ability to reduce biofilm formation, its effect was weaker compared to the other strains. This is thought to be due to the intracellular efflux pump system. While the exact mechanism is not fully understood, it is known that these pump systems are specialized to protect bacterial cells from toxic substances, such as antibiotics, that could potentially damage the cell, and they are highly effective in this role [59].

The absence of antimicrobial activity of *P. aurivella* against *E. coli* ATCC 25922, along with the strong biofilm activity enhancement, supports this hypothesis. The concentrations at which the strongest biofilm inhibition was observed are presented in Table 9. For each microorganism, the value of 0.220 mg/ml is indicated as the concentration where the highest inhibition was achieved.

Table 9. The most effective biofilm inhibition values found against microorganisms

Microorganisms	Biofilm Inhibition Concentration (mg/ml)	Biofilm Inhibition (%)	OD 550
***Bacillus subtilis* DSMZ 1971**	0.220	20.97 ± 1.29	0.304 ± 0.03
***Escherichia coli* ATCC 25922**	-	-	-
***Listeria monocytogenes* ATCC 7644**	0.220	50.65 ± 2.57	0.131 ± 0.06
***Listeria innocua* (FI)**	0.220	52.75 ± 1.36	0.135 ± 0.04
***Escherichia coli* (CI)**	0.220	46.27 ± 3.22	0.112 ± 0.02

B. subtilis exhibited moderate biofilm inhibition rates (approximately 21%), while *E. coli* showed no biofilm inhibition under the tested conditions. These findings highlight the potential of the extract to address biofilm formation in certain strains, such as *B. subtilis*, while suggesting strain-specific variability in its activity against *E. coli*. Taken together, these results demonstrate the extract's selective

antimicrobial and antibiofilm activity, emphasizing its potential for applications targeting specific bacterial pathogens. (Figure 1-3)

Biofilm inhibition tests demonstrated strong effects with inhibition rates exceeding 50% against biofilm-forming microorganisms, including *L. innocua* and *L. monocytogenes* (Figure 4 and Figure 5). Additionally, antibiofilm activity was observed at sub-MIC concentrations below the MIC value of 0.442 mg/ml against the food isolate *L. innocua*. The observation of antibiofilm activity at sub-MIC values, where antimicrobial effects were not present, highlights the true antibiofilm potential of the extract. Furthermore, it is believed that this activity is attributed to linoleic acid, the major component of the extract. As previously demonstrated by Kim et al. (2019) [60] in a study on *P. aeruginosa*, linoleic acid can inhibit biofilm formation without affecting bacterial cell growth. These findings, consistent with our study, highlight the potential applications of linoleic acid in biofilm inhibition.

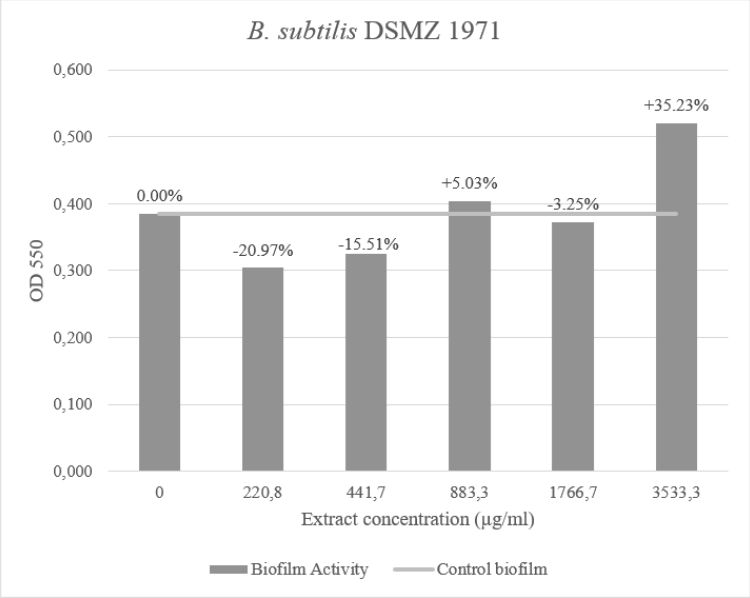

Figure 1. Antibiofilm effect of mushroom extract on *Bacillus subtilis* DSMZ 1971

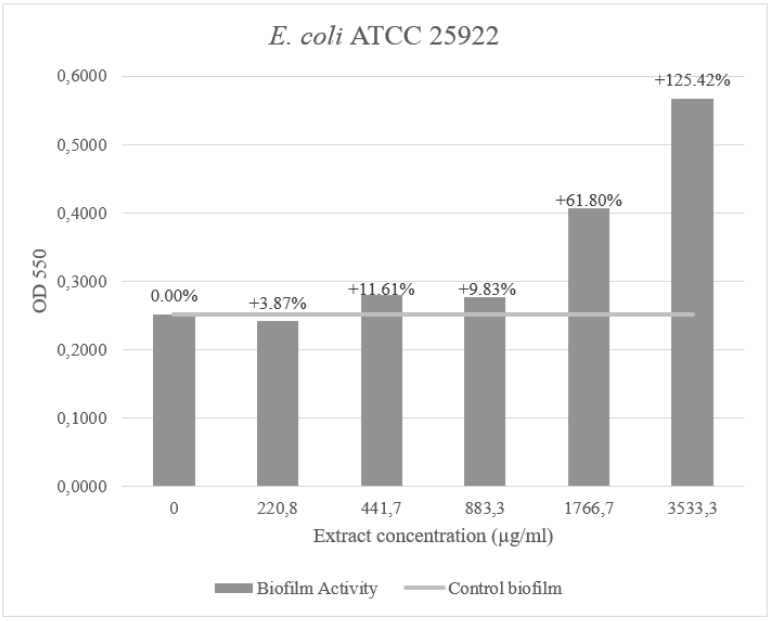

Figure 2. Antibiofilm effect of mushroom extract on *Escherichia coli* ATCC 25922

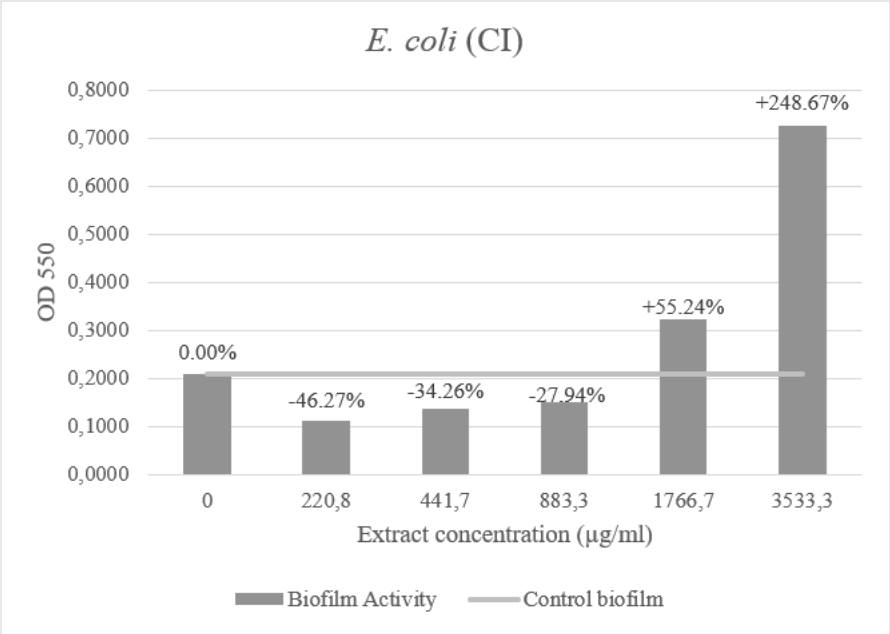

Figure 3. Antibiofilm effect of mushroom extract on *Escherichia coli* (CI)

When compared to a study [61] in which the natural compounds cinnamaldehyde (160 µg/ml) and resveratrol (100 µg/ml) were found to be effective against *L. monocytogenes* biofilm, the result of the mushroom extract, composed of complex compounds, showing activity at a concentration of 220.8 µg/ml is notable. The study concludes that the sub-MICs of these natural antimicrobial compounds reduce biofilm formation by suppressing the quorum sensing system rather than inhibiting flagellum formation. Further studies need to be conducted to investigate the mechanism of action of *P. aurivella*.

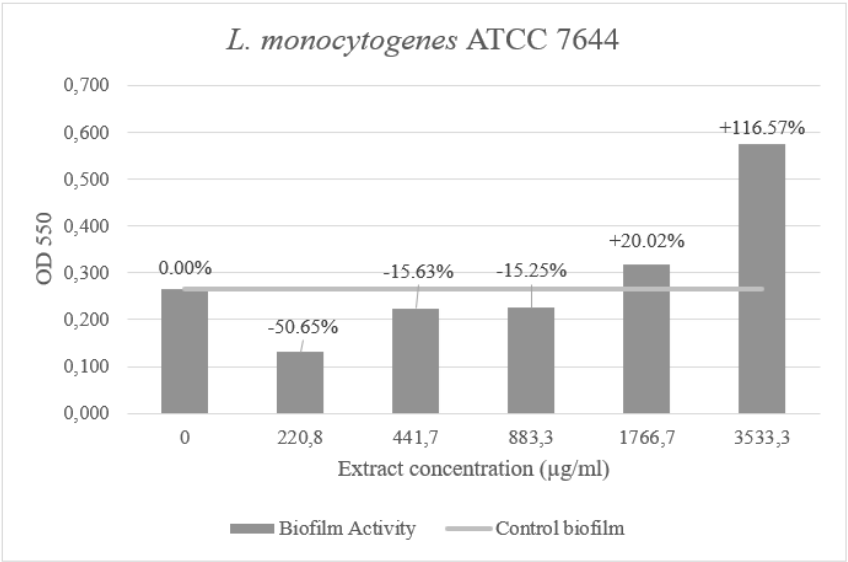

Figure 4. Antibioiofilm effect of mushroom extract on *Listeria monocytogenes* ATCC 7644

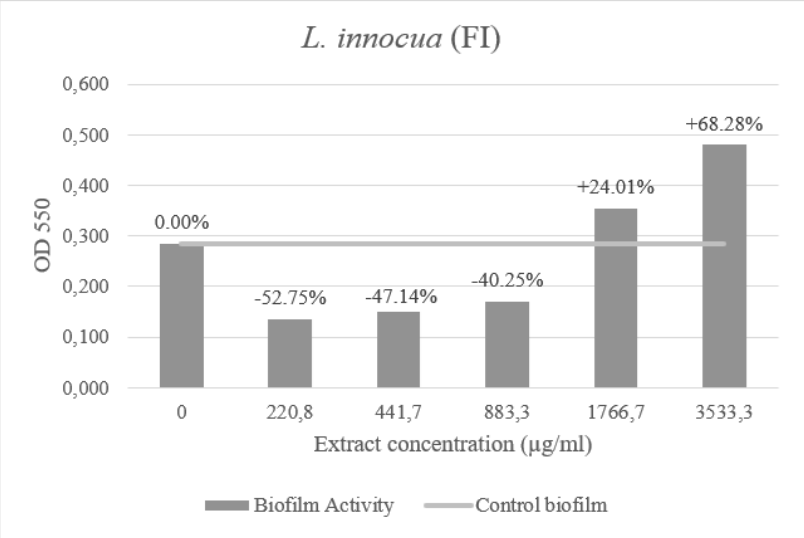

Figure 5. Antibiofilm effect of mushroom extract on *Listeria innocua* (FI)

Listeriosis, caused by *L. monocytogenes*, is a serious foodborne infection primarily affecting vulnerable groups. The bacterium's ability to form biofilms enables environmental persistence, making disinfection in the food industry challenging. This increases the need for advanced prevention strategies against biofilm formation and cell communication [62]. Particularly, the consistency of antimicrobial and antibiofilm activities in *Listeria* species highlights the dual functionality of the extract, not only against planktonic cells but also in combating biofilm-related challenges, making it a promising candidate for pharmaceutical applications targeting multidrug-resistant pathogens.

The results obtained demonstrate that the extract possesses antibiofilm activity against both Gram-positive and Gram-negative bacteria. Consistent with the MIC test, these findings indicate that the extract not only inhibits bacterial growth but also prevents biofilm formation, suggesting a promising potential for controlling persistent infections. These results emphasize that *P. aurivella* could be utilized as a potential antimicrobial agent in pharmaceutical applications and should be further supported by comprehensive research in the future.

AUTHOR CONTRABIBUTIONS

Concept: C.Y., G.G., K.C.; Design: C.Y., G.G., A.B.; Control: A.B., D.T., I.A., K.C.; Sources: A.B., I.A.; Materials: S.D.B., I.A., K.C.; Data Collection and/or Processing: C.Y., G.G., A.B., D.T.; Analysis and/or Interpretation: C.Y., A.B., D.T.; Literature Review: C.Y., G.G., S.D.B.; Manuscript Writing: C.Y., G.G., D.T., S.D.B.; Critical Review: A.B., D.T., K.C.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Archer, G. L., Climo, M.W. (2001). *Staphylococcus aureus* bacteremia-consider the source. New England Journal of Medicine, 344(1), 55-56.
2. Rammelkamp, C.H., Maxon, T. (1942). Resistance of *Staphylococcus aureus* to the Action of Penicillin. Proceedings of the Society for Experimental Biology and Medicine, 51(3), 386-389.

3. Chambers, H.F. (2001). The changing epidemiology of *Staphylococcus aureus*. Emerging Infectious Diseases, 7(2), 178. [CrossRef]
4. World Health Organization Web site. (1996). Antimicrobial resistance. Retrieved December 24, 2024, from https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance. Erişim tarihi: 24.12.2024.
5. Jim O'Neill (2016). Tackling drug-resistant infections globally: Final report and recommendations. Analysis & Policy Observatory. Retrieved August 12, 2005, from ttp://www.bmj.com/cgi/content/full/317/7150/. Erişim tarihi: 24.12.2024.
6. Newman, D.J., Cragg, G.M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products, 75(3), 311-335. [CrossRef]
7. Wasser, S.P., Weis, A.L. (1999). Medicinal properties of substances occurring in higher basidiomycetes mushrooms: Current perspectives. International Journal of Medicinal Mushrooms, 1(1), 31-62. [CrossRef]
8. Imtiaj, A., Lee, T.S. (2007). Screening of antibacterial and antifungal activities from Korean wild mushrooms. World Journal of Agricultural Sciences, 3(3), 316-321.
9. Altuner, E.M., Akata, I. (2000). Antimicrobial activity of some macrofungi extracts. Sakarya Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 14, 1(2010).
10. Martinez-Medina, G.A., Chávez-González, M.L., Verma, D.K., Prado-Barragán, L.A., Martínez-Hernández, J.L., Flores-Gallegos, A.C., Aguilar, C.N. (2021). Bio-funcional components in mushrooms, a health opportunity: Ergothionine and huitlacohe as recent trends. Journal of Functional Foods, 77, 104326. [CrossRef]
11. Ahmadi, M., Vahabzadeh, F., Bonakdarpour, B., Mehranian, M. (2006). Empirical modeling of olive oil mill wastewater treatment using loofa-immobilized Phanerochaete chrysosporium. Process Biochemistry, 41(5), 1148-1154. [CrossRef]
12. Pazarlıoğlu, N. K., Sariişik, M., Telefoncu, A. (2005). Laccase: Production by Trametes versicolor and application to denim washing. Process Biochemistry, 40(5), 1673-1678. [CrossRef]
13. Hyde, K., Hyde, K., Bahkali, A., Moslem, M. (2010). Fungi-an unusual source for cosmetics. Fungal Diversity, 43, 1-9. [CrossRef]
14. Dyakov, M.Y., Kamzolkina, O.V., Shtaer, O.V., Bis'ko, N.A., Poedinok, N.L., Mikhailova, O.B., Efremenkova, O.V. (2011). Morphological characteristics of natural strains of certain species of basidiomycetes and biological analysis of antimicrobial activity under submerged cultural conditions. Microbiology, 80(2), 274-285. [CrossRef]
15. Doğan, A. (2018). CCI4 İle oksidatif stres oluşturulan sıçanlarda Pholiota aurivella liyofilize ekstrenin bazı biyokimyasal ve hematolojik parametrelere etkisi. Mantar Dergisi, 9 (1), 58-66. [CrossRef]
16. Lee, J., Park, M., Park, J., Cho, Y., Kim, C., Kim, C., Jo, J., Lim, Y. (2020). Taxonomic study of the *Genus pholiota* (Strophariaceae, Basidiomycota) in Korea. Mycobiology, 48, 476-483. [CrossRef]
17. Solak M.H., Gücin F. (1992). New records of macrofungi for Turkey from Bursa District and other macrofungi found in the district. Turkish Journal of Botany, 16 (1): 335-346.
18. Canlı, K., Akata, I., Altuner, E. (2016). *In vitro* antimicrobial activity screening of *Xylaria hypoxylon*. African Journal of Traditional, Complementary, and Alternative Medicines. 13, 42-46. [CrossRef]
19. Canlı, K., Altuner, E., Akata, I. (2015). Antimicrobial screening of *Mnium stellare*. Bangladesh Journal of Pharmacology, 10, 321-325. [CrossRef]
20. Andrews, J.M. (2003). BSAC standardized disk susceptibility testing method (version 6). Journal of Antimicrobial Chemotherapy, 60, 20-41.
21. Benek, A., Canlı, K., Altuner, E.M. (2022). Antimicrobial and antioxidant activities of some mosses. Anatolian Bryology, 9(1), 42-49.
22. Baldas, B., Altuner, E.M. (2018). The antimicrobial activity of apple cider vinegar and grape vinegar, which are used as a traditional surface disinfectant for fruits and vegetables. Communications Faculty of Sciences University of Ankara Series C Biology, 27(1), 1-10. [CrossRef]
23. Tunca-Pinarli, Y., Benek, A., Turu, D., Bozyel, M.E., Canli, K., Altuner, E.M. (2023). Biological activities and biochemical composition of endemic *Achillea fraasii*. Microorganisms, 11(4), 978.
24. Benek, A., Turu, D., Canli, K. (2024). Determination of biological activity and biochemical content of ethanol extract from fruiting body of *Tricholoma bufonium* (Pers.) Gillet. Journal of Fungi, 10(11), 761. [CrossRef]
25. Turu, D., Bozkurt, S.D., Yaman, C., Gül, G., Benek, A., Canlı, K. (2024). Determination of biochemical content and antioxidant activity of *Calliergonella cuspidata* (Hedw.) Loeske. Anatolian Bryology, 10(1), 25-33. [CrossRef]

26. Silge, J., Robinson, D. (2016). Tidytext: Text mining and analysis using tidy data principles in R. Journal of OpenSource Software, 1(3), 37. [CrossRef]
27. Dyakov, M.Y., Kamzolkina, O.V., Shtaer, O.V., Bis' ko, N.A., Poedinok, N.L., Mikhailova, O.B., Efremenkova, O.V. (2011). Morphological characteristics of natural strains of certain species of basidiomycetes and biological analysis of antimicrobial activity under submerged cultural conditions. Microbiology, 80, 274-285. [CrossRef]
28. Linz, M.S., Mattappallil, A., Finkel, D., Parker, D. (2023). Clinical impact of *Staphylococcus aureus* skin and soft tissue infections. Antibiotics, 12(3), 557. [CrossRef]
29. Sheu, C.W., Freese, E. (1973). Lipopolysaccharide layer protection of gram-negative bacteria against inhibition by long-chain fatty acids. Journal of Bacteriology, 115(3), 869-875. [CrossRef]
30. Borucki, M.K., Reynolds, J., Wiedmann, M., Boor, K.J. (2003). Tracking and source attribution of *Listeria monocytogenes* in ready-to-eat products and processing environment. Emerging Infectious Diseases, 9(3), 267-274.
31. Oliver, S.P., Jayarao, B.M., Almeida, R.A. (2005). Foodborne pathogens in milk and the dairy farm environment: Food safety and public health implications. Foodborne Pathogens and Disease, 2(2), 115-129. [CrossRef]
32. Buchanan, R.L., Klawitter, L.A. (1992). Effect of temperature and salinity on the survival of *Listeria monocytogenes* in marine species. Journal of Food Protection, 55(8), 668-672.
33. Skalicka-Woźniak, K., Los, R., Głowniak, K., Malm, A. (2010). Antimicrobial activity of fatty acids from fruits of *Peucedanum cervaria* and *P. alsaticum*. Chemistry & Biodiversity, 7(11), 2748-2754. [CrossRef]
34. Islek, C., Saridogan, B.G.O., Sevindik, M., Akata, I. (2021). Biological activities and heavy metal contents of some Pholiota species. Fresenius Environmental Bulletin, 30(6), 6109-6114.
35. Van Vuuren, S.F. (2008). Antimicrobial activity of South African medicinal plants. Journal of Ethnopharmacology, 119(3), 462-472. [CrossRef]
36. Fagali, N., Catala, Á. (2008). Antioxidant activity of conjugated linoleic acid isomers, linoleic acid and its methyl ester determined by photoemission and DPPH techniques. Biophysical Chemistry, 137 1, 56-62. [CrossRef]
37. Regeda, L., Bisko, N., Gurinovych, N. (2021). The antioxidant activity of extracts of the mycelium and the culture fluid of medicinal macromycetes of Pholiota (Fr.) P. Kumm. genus. Journal of Organic and Pharmaceutical Chemistry. [CrossRef]
38. Wood, W., Kubo, A., Shaffer, T. (2010). Antimicrobial activity of long-chain (*E*)-3-alken-2-ones. Bioorganic & Medicinal Chemistry letters, 20(6), 1819-1820. [CrossRef]
39. Ulitzur, S., Hastings, J.W. (1979). Evidence for tetradecanal as the natural aldehyde in bacterial bioluminescence. Proceedings of the National Academy of Sciences, 76(1), 265-267.
40. Sokmen, B., Hasdemir, B., Yusufoglu, A., Yanardag, R. (2014). Some monohydroxy tetradecanoic acid isomers as novel urease and elastase inhibitors and as new antioxidants. Applied Biochemistry and Biotechnology, 172, 1358-1364. [CrossRef]
41. Sivakumar, R., Jebanesan, A., Govindarajan, M., Rajasekar, P. (2011). Larvicidal and repellent activity of tetradecanoic acid against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say.) (Diptera:Culicidae). Asian Pacific Journal of Tropical Medicine, 4(9), 706-710. [CrossRef]
42. Javid, S., Purohit, M., Kumar, Y., Ramya, K., Mithuna, N., Salahuddin, M., Kumar, B. (2020). Semisynthesis of myristic acid derivatives and their biological activities: A critical insight. Journal of Biologically Active Products from Nature, 10, 455-472. [CrossRef]
43. Tyagi, T., Agarwal, M. (2017). Phytochemical screening and GC-MS analysis of bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and *Eichhornia crassipes* (Mart.) solms. Journal of Pharmacognosy and Phytochemistry, 6(1), 195-206.
44. Masuda, T., Yamada, K., Maekawa, T., Takeda, Y., Yamaguchi, H. (2006). Antioxidant mechanism studies on ferulic acid: Identification of oxidative coupling products from methyl ferulate and linoleate. Journal of Agricultural and Food Chemistry, 54(16), 6069-6074.
45. Zhu, Y.P., Su, Z.W., Li, C.H. (1989). Growth-inhibition effects of oleic acid, linoleic acid, and their methyl esters on transplanted tumors in mice. JNCI: Journal of the National Cancer Institute, 81(17), 1302-1306.
46. Park, S., Seetharaman, R., Ko, M., Kim, D., Kim, T., Yoon, M., Kwak, J., Lee, S., Bae, Y., Choi, Y. (2014). Ethyl linoleate from garlic attenuates lipopolysaccharide-induced pro-inflammatory cytokine production by inducing heme oxygenase-1 in RAW264.7 cells. International Immunopharmacology, 19(2), 253-261. [CrossRef]

47. Gorkum, R., Bouwman, E., Reedijk, J. (2004). Fast autoxidation of ethyl linoleate catalyzed by [Mn(acac)3] and bipyridine: A possible drying catalyst for alkyd paints. Inorganic chemistry, 43(8), 2456-2458. [CrossRef]
48. Devery, R., Miller, A., Stanton, C. (2001). Conjugated linoleic acid and oxidative behaviour in cancer cells. Biochemical Society Transactions, 29(2), 341-344. [CrossRef]
49. Basiricò, L., Morera, P., Dipasquale, D., Tröscher, A., Bernabucci, U. (2017). Comparison between conjugated linoleic acid and essential fatty acids in preventing oxidative stress in bovine mammary epithelial cells. Journal of Dairy Science, 100(3), 2299-2309. [CrossRef]
50. Vangaveti, V., Jansen, H., Kennedy, R., Malabu, U. (2016). Hydroxyoctadecadienoic acids: Oxidised derivatives of linoleic acid and their role in inflammation associated with metabolic syndrome and cancer. European Journal of Pharmacology, 785, 70-76. [CrossRef]
51. Sande, D., de Oliveira, G.P., Moura, M.A.F., de Almeida Martins, B., Lima, M.T.N.S., Takahashi, J.A. (2019). Edible mushrooms as a ubiquitous source of essential fatty acids. Food Research International, 108524. [CrossRef]
52. DeMars, Z.R., Krute, C.N., Ridder, M.J., Gilchrist, A.K., Menjivar, C., Bose, J.L. (2021). Fatty acids can inhibit *Staphylococcus aureus* SaeS activity at the membrane independent of alterations in respiration. Molecular Microbiology, 116(5), 1378-1391. [CrossRef]
53. Kabara, J.J., Swieczkowski, D.M., Conley, A.J., Truant, J.P. (1972). Fatty acids and derivatives as antimicrobial agents. Antimicrobial Agents and Chemotherapy, 2(1), 23-28. [CrossRef]
54. Hartigh, L. (2019). Conjugated linoleic acid effects on cancer, obesity, and atherosclerosis: A review of pre-clinical and human trials with current perspectives. Nutrients, 11. [CrossRef]
55. Takigawa, H., Nakagawa, H., Kuzukawa, M., Mori, H., Imokawa, G. (2005). Deficient production of hexadecenoic acid in the skin is associated in part with the vulnerability of atopic dermatitis patients to colonization by *Staphylococcus aureus*. Dermatology, 211(3), 240-248. [CrossRef]
56. Cartron, M.L., England, S.R., Chiriac, A.I., Josten, M., Turner, R., Rauter, Y., Foster, S.J. (2014). Bactericidal activity of the human skin fatty acid cis-6-hexadecanoic acid on *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy, 58(7), 3599-3609. [CrossRef]
57. Neumann, Y., Ohlsen, K., Donat, S., Engelmann, S., Kusch, H., Albrecht, D., Foster, S.J. (2015). The effect of skin fatty acids on *Staphylococcus aureus*. Archives of Microbiology, 197, 245-267. [CrossRef]
58. Neumann, Y., Ohlsen, K., Donat, S., Engelmann, S., Kusch, H., Albrecht, D., Foster, S.J. (2015). The effect of skin fatty acids on *Staphylococcus aureus*. Archives of Microbiology, 197, 245-267. [CrossRef]
59. Alav, I., Sutton, J., Rahman, K. (2018). Role of bacterial efflux pumps in biofilm formation. Journal of Antimicrobial Chemotherapy, 73, 2003-2020. [CrossRef]
60. Kim, H.S., Ham, S.Y., Jang, Y., Sun, P.F., Park, J.H., Lee, J.H., Park, H.D. (2019). Linoleic acid, a plant fatty acid, controls membrane biofouling via inhibition of biofilm formation. Fuel, 253, 754-761. [CrossRef]
61. Liu, Y., Wu, L., Han, J., Dong, P., Luo, X., Zhang, Y., Zhu, L. (2021). Inhibition of biofilm formation and related gene expression of *Listeria monocytogenes* in response to four natural antimicrobial compounds and sodium hypochlorite. Frontiers in Microbiology, 11, 617473. [CrossRef]
62. Da Silva, E. P., De Martinis, E.C.P. (2013). Current knowledge and perspectives on biofilm formation: The case of *Listeria monocytogenes*. Applied Microbiology and Biotechnology, 97, 957-968. [CrossRef]