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Amanita Muscaria Etanol Ekstraktının Antimikrobiyal ve Antioksidan Aktivitelerinin İncelenmesi

Investigation of the Antimicrobial and Antioxidant Activities of the Ethanol Extract of *Amanita muscaria*

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Öz

Kırmızı şapkası ve beyaz benekleriyle doğada kolayca tanınan *Amanita muscaria*, yalnızca görsel olarak değil, içerdiği biyolojik olarak aktif bileşikler sayesinde farmasötik açıdan da dikkat çeken bir mantar türüdür. *Muscimol*, ibotenik asit, fenolik bileşikler ve polisakkaritler gibi metabolitler bakımından zengin olan bu tür, potansiyel antioksidan ve antimikrobiyal ajan kaynağı olarak değerlendirilmektedir. Günümüzde artan antibiyotik direnci sorununa karşı doğal kaynakların araştırılması önem kazanmış; mantar türleri bu kapsamda öne çıkan alternatifler arasında yer almıştır. Etanol ile elde edilen *A. muscaria* ekstraktının farklı konsantrasyonları 12 referans, 11 çoklu ilaç dirençli (MDR), 12 klinik ve 6 gıda kaynaklı mikroorganizmaya karşı çalışılmıştır. Disk difüzyon analizleri, ekstraktın özellikle *Listeria monocytogenes, Pseudomonas fluorescens, Enterococcus faecium ve Staphylococcus aureus* suşlarında 7–10 mm aralığında inhibisyon zonları oluşturduğunu göstermiştir. Minimum inhibitör konsantrasyon değerlerinin 310–5.080 µg/mL aralığında, minimum bakterisidal konsantrasyonların ise genellikle 10.000 µg/mL'nin üzerinde bulunması, ekstraktın bakteriyostatik etkiye sahip olduğunu ortaya koymuştur. Gram-negatif bakterilerde etkinliğin zayıf olması, lipopolisakkarit tabakasının difüzyon engelleyici özelliğiyle ilişkilendirilmiştir. Antioksidan kapasite belirlemesinde, ekstraktın DPPH radikal giderme aktivitesi askorbik asit ile kıyaslanmıştır. 1000 µg/mL konsantrasyonda A. muscaria %97,05 inhibisyon ile askorbik aside (%94,67) eşdeğer düzeyde etki göstermiş; düşük konsantrasyonlarda ise daha güçlü radikal süpürme potansiyeli sergilemiştir (EC_{50} = 33,5 µg/mL). Elde edilen sonuçlar, A. muscaria'nın antimikrobiyal yönünün sınırlı, ancak antioksidan etkinliğinin oldukça yüksek olduğunu göstermektedir. Elde edilen bulgular, bu mantarın doğal antioksidan bileşiklerin önemli bir kaynağı olabileceğini, ayrıca antimikrobiyal etkinliğinin saf bileşiklerin ayrıştırılması, birlikte kullanım stratejileri veya nanoparçacık temelli yöntemlerle geliştirilebileceğini göstermektedir.

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Abstract

Characterized by its bright red cap adorned with white spots, Amanita muscaria is not only visually distinctive but also of considerable pharmaceutical interest due to its diverse array of biologically active compounds. Rich in metabolites such as muscimol, ibotenic acid, phenolic compounds, and polysaccharides, this species has been regarded as a potential source of antioxidant and antimicrobial agents. In light of the growing global challenge of antibiotic resistance, the exploration of natural sources has gained increasing significance, with fungal species emerging as promising alternatives. Different concentrations of the A. muscaria extract were tested against 12 reference, 11 multidrug-resistant (MDR), 12 clinical, and 6 food-isolated microorganisms. Disk diffusion assays revealed that the extract produced inhibition zones ranging from 7 to 10 mm, particularly against Listeria monocytogenes, Pseudomonas fluorescens, Enterococcus faecium, and Staphylococcus aureus. The minimum inhibitory concentration (MIC) values ranged between 310 and 5,080 µg/mL, while the minimum bactericidal concentrations (MBCs) were generally above 10,000 µg/mL, indicating a predominantly bacteriostatic effect. The relatively weak activity observed against Gram-negative bacteria was attributed to the diffusion-limiting properties of their lipopolysaccharide outer layer. Antioxidant capacity was assessed using the DPPH radical scavenging assay, in which the extract was compared with ascorbic acid. At 1000 µg/mL, A. muscaria exhibited 97.05% inhibition, comparable to that of ascorbic acid (94.67%), and demonstrated stronger radical scavenging potential at lower concentrations (EC_{50} = 33.5 µg/mL). These findings suggest that while the antimicrobial potential of A. muscaria is limited, its antioxidant activity is remarkably high. The results highlight the potential of this species as a valuable natural source of antioxidant compounds and indicate that its antimicrobial efficacy may be enhanced through the isolation of active constituents, combination strategies, or nanoparticle-based approaches.

1. INTRODUCTION

Mushrooms have long been utilized not only for their nutritional value and gastronomic appeal but also for their beneficial effects on human health, making them valuable for both food and medicinal purposes (Singh et al., 2024). In recent years, wild mushroom species containing biologically active compounds have attracted significant interest from the pharmaceutical industry, particularly due to their anticancer, antioxidant, and immunomodulatory potential (Bentharavithana et al., 2025). Among these species, Amanita muscaria (L.) Lam. has drawn attention from both scientists and practitioners of traditional medicine. Commonly known as the fly agaric, A. muscaria is an iconic member of the phylum Basidiomycota, order Agaricales, and family Amanitaceae (Marchiori et al., 2024). It typically grows in podzolic soils of deciduous and coniferous forests (Falandysz & Treu, 2019). The cap of this mushroom, usually ranging from 4 to 21 cm in diameter, is bright red and covered with white warts, making it easily recognizable (Carboué & Lopez, 2021). Its distinctive appearance, wide distribution, and historical use in mystical rituals and folk medicine have made it a culturally significant species for generations (Grochowska & Weiner, 2025). Although A. muscaria contains numerous chemical compounds, its psychoactive

properties are primarily attributed to muscimol and ibotenic acid (Chrystofiak & Brohs, 2023, p. 213). The alkaloids found in mushrooms exhibit a variety of bioactivities, including antimicrobial, anticancer, and antidiabetic properties, which render them valuable resources for modern clinical and pharmacological research (Archana & Nagadesi, 2024). In addition, secondary metabolites such as phenolic compounds and terpenoids have attracted considerable interest for their potential antimicrobial and biological activities.

In recent decades, the rise in antibiotic resistance has become a global public health concern. According to the World Health Organization (WHO), approximately 1.27 million deaths per year are directly attributable to antibiotic resistance, with up to 5 million deaths linked indirectly to resistant infections (WHO, 2022). The widespread and often unnecessary use of antibiotics, particularly their application in livestock and agriculture to promote growth, has played a major role in the proliferation of antimicrobial resistance (Van Boeckel et al., 2022). The 2023 Global Antimicrobial Resistance and Use Surveillance System (GLASS) report highlighted the presence of multidrug resistance not only in Escherichia coli but also in Listeria innocua, a foodborne indicator species, and Enterococcus faecium, a common hospital-acquired pathogen. Notably, E. faecium has been listed by the WHO as a “critical priority” pathogen due to its resistance to vancomycin (WHO, 2017; WHO, 2024). Infections triggered by resistant E. faecium strains limit the success of conventional treatment protocols, extending clinical recovery times by 8 to 20 days and imposing additional costs of thousands of dollars per case on healthcare systems (Zhen et al., 2021).This alarming situation underscores the diminishing efficacy of existing antibiotics and the urgent need to identify new antimicrobial agents from natural sources such as plants, fungi, and microorganisms (Mookherjee et al., 2023; WHO, 2024).

Beyond their antimicrobial potential, mushrooms are also notable for their high antioxidant capacity, which, together with their multifaceted bioactivities, makes them valuable natural resources for pharmacological research (Kalač, 2013, p. 185; Yang et al., 2025). Oxidative stress caused by free radicals has been scientifically linked to cellular damage that contributes to chronic diseases such as cardiovascular disorders, cancer, diabetes, and aging (Liguori et al., 2018; Birben et al., 2012). Consequently, there is a growing interest in natural antioxidant sources. Mushrooms, in particular, stand out due to their rich content of phenolic compounds, flavonoids, ascorbic acid, and carotenoids (Liuzzi et al., 2023). Wild mushrooms are especially valuable, as environmental stress stimulates the production of

secondary metabolites associated with medicinal properties, resulting in a wide range of beneficial compounds (Martins et al., 2023). The phenolic compounds present in A. muscaria are among the natural molecules that may contribute to combating oxidative stress, highlighting the potential of this species for both medicinal and industrial applications.

This study aims to investigate, for the first time, the antimicrobial activity of the ethanol extract of A. muscaria and to evaluate its antioxidant capacity, contributing to the growing body of research on natural bioactive compounds from fungal sources.

2. MATERIALS AND METHODS

2.1. Mushroom Sample

The Amanita muscaria (L.) Lam. mushroom used in this study was collected and taxonomically identified by Prof. Dr. Ilgaz AKATA. For reference purposes, the specimens were deposited at the Dokuz Eylül University Fauna and Flora Research and Application Center (FAMER), İzmir, Türkiye.

2.2. Extraction

The collected mushroom samples were completely dried and finely ground using a household grinder. The powdered material was extracted with ethanol (Sigma-Aldrich, St. Louis, MO, USA) at room temperature under constant shaking at 160 rpm for 48 hours. After extraction, the mixture was filtered through Whatman No. 1 filter paper into glass flasks (Benek et al., 2021). The ethanol extract was evaporated using a rotary evaporator at 40 °C. The obtained dry extracts were weighed, and a stock solution of 15 mL was prepared. To remove particulate matter, the stock solution was passed through a 0.45 µm syringe filter.

2.3. Inoculum Preparation

All bacterial strains were incubated at 37 °C for 24 hours, whereas Candida albicans DSM 1386, C. glabrata, and C. tropicalis were incubated at 28 °C for 48 hours (Canlı et al., 2023). Each inoculum was prepared by transferring morphologically identical colonies grown on solid media into sterile 0.9% saline solution using a sterile loop to form a suspension. The turbidity of each suspension was adjusted to match the 0.5 McFarland standard. After standardization, bacterial inocula were estimated to contain approximately 10^8 CFU·mL^{-1}, while yeast inocula contained approximately 10^7 CFU·mL^{-1} (Tunca-Pınarlı et al., 2023). To determine the antimicrobial activity of A. muscaria, a total of 12 standard strains, 11 multidrug-resistant (MDR) strains, 12 clinical isolates, and 6 foodborne isolates were used.

2.4. Antimicrobial Activity Assays

2.4.1. Disk Diffusion Assay

The antimicrobial activity of *A. Muscaria* was evaluated using the disk diffusion method based on the procedure described by Andrews (2001) and modified by Munteanu et al. (2021). Mueller-Hinton Agar (MHA) was poured into sterile 90 mm Petri dishes to a depth of 4.0 ± 0.5 mm. Ethanol extracts of *A. muscaria* were impregnated into 6 mm Oxoid antimicrobial susceptibility test disks in three different volumes: 30 µL, 60 µL, and 150 µL, corresponding to extract amounts of 1.83 mg, 3.66 mg, and 9.15 mg, respectively. After inoculating the surface of the agar plates with the test microorganisms, the prepared disks were placed on the plates and incubated under suitable conditions. Upon completion of incubation, the diameters of inhibition zones were measured in millimeters using a ruler (Ersin, 2025). To ensure reliability, sterile blank disks and ethanol were used as negative controls, while gentamicin and tobramycin antibiotic disks served as positive controls.

2.4.2. Broth Microdilution Assay (MIC Determination)

The minimum inhibitory concentration (MIC) values of *A. muscaria* ethanol extract were determined using the broth microdilution method. Bacterial suspensions were adjusted to a 0.5 McFarland standard prior to testing. The mushroom extract was serially diluted, and 100 µL (equivalent to 6.10 mg) from each dilution was transferred to sterile 96-well microplates. Subsequently, 50 µL of microbial inoculum was added to each well to reach a total volume of 150 µL. After incubation for 24 hours, the lowest concentration of the extract that completely inhibited visible microbial growth was recorded as the MIC (Canlı et al., 2023). All experiments were performed in triplicate, and results were expressed in µg/mL. Microbial growth was assessed visually. The positive control consisted of inoculated LB broth, while the negative control contained the extract without microorganisms.

2.4.3. Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The minimum bactericidal concentration (MBC) was defined as the lowest concentration of the extract capable of completely killing the tested microorganisms. The MBC test was conducted following the MIC assay (Benek, 2024, p. 68). From wells showing no visible growth in the MIC test, 10 µL aliquots were spread on solid Mueller-Hinton Agar plates. After incubation, the lowest concentration that produced no visible microbial colonies was considered the MBC (Tazehkand & Yılmaz, 2019). The minimum fungicidal concentration

(MFC) test followed the same procedure, except that incubation for fungal strains was performed at 27 °C for 48 hours.

2.5. Antioxidant Activity Assay

2.5.1. DPPH (2,2-Diphenyl-1-picrylhydrazyl) Radical Scavenging Assay

The antioxidant capacity of the extract was evaluated using the DPPH radical scavenging assay. A DPPH stock solution was prepared by dissolving 3.9432 mg of DPPH in 50 mL of ethanol (Mensor et al., 2001). All plate loading procedures were conducted under minimal light exposure due to the photosensitivity of DPPH. The mushroom extract was mixed with the DPPH solution and allowed to react for 30 minutes in the dark at room temperature. After incubation, absorbance was measured at 515 nm using a UV–Vis spectrophotometer. Ascorbic acid was used as the positive control (Tunç et al., 2020).

2.6. Statistical Analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD) to ensure accuracy and reproducibility. Statistical analyses were performed using RStudio (version 2025.05.1 + 513) employing one-way ANOVA and Pearson correlation tests

3. RESULTS

The antibacterial activity of the ethanol extract of Amanita muscaria against various microorganisms is presented in Table 1 as inhibition zone diameters. The extract was tested at three different concentrations (30 µL, 60 µL, and 150 µL) against a wide range of bacterial and fungal strains. The results revealed that the antimicrobial activity of A. muscaria was concentration-dependent, exhibiting varying levels of inhibition across different microbial species.

Table 1: Antimicrobial activity of *Amanita muscaria* against tested microorganisms (inhibition zone diameters in mm)

Microorganisms	**30µL***	**60µL***	**150µL***	**Gentamicin**	**Tobramycin**
Bacillus subtilis DSM 1971	8± 0,00	8± 0,00	8± 0,00	30,00± 0,00	26,00± 0,00
Candida albicans DSM 1386	-	-	-	12,00± 0,00	13,00± 0,00
Enterobacter aerogenes ATCC 13048(Reference isolate)	-	-	-	24,00± 0,00	18,00± 0,00
Enterococcus faecalis ATCC 29212(Reference isolate)	-	-	-	12,00± 0,00	8,00± 0,00
Escherichia coli ATCC 25922(Reference isolate)	-	-	-	22,00± 0,00	20,00± 0,00

Listeria monocytogenes ATCC 7644	-	8,00± 1,41	10,00± 1,41	28,00± 0,00	24,00± 0,00
Pseudomonas aeruginosa DSM 50071	-	-	-	15,00± 0,00	22,00± 0,00
Pseudomonas fluorescens P1	-	7,00± 4,35	8,00± 4,35	13,00± 0,00	12,00± 0,00
Salmonella enteritidis ATCC 13076	-	-	-	21,00± 0,00	15,00± 0,00
Salmonella typhimurium SL 1344	-	-	-	24,00± 0,00	15,00± 0,00
Staphylococcus aureus ATCC 25923(Reference isolate)	8,00± 0,57	9,00± 0,57	8,00± 0,57	21,00± 0,00	14,00± 0,00
Staphylococcus epidermidis DSM 20044	-	-	-	22,00± 0,00	20,00± 0,00
Enterococcus durans	-	-	7,00±4,04	11,00± 0,00	13,00± 0,00
Enterococcus faecium	8,00± 4,04	9,00± 4,04	9,00± 4,04	28,00± 0,00	15,00± 0,00
Klebsiella pneumoniae (Food isolate)	-	-	-	19,00± 0,00	23,00± 0,00
Listeria innocua	-	-	7,00±4,04	13,00± 0,00	15,00± 0,00
Salmonella infantis	-	-	-	17,00± 0,00	14,00± 0,00
Salmonella kentucky	-	-	-	12,00± 0,00	16,00± 0,00
Staphylococcus aureus (Clinical isolate)	-	-	-	22,00± 0,00	18,00± 0,00
Streptococcus mutans	-	-	-	22,00± 0,00	24,00± 0,00
Staphylococcus hominis	-	-	-	9,00± 0,00	11,00± 0,00
Staphylococcus haemolyticus	-	-	-	10,00± 0,00	10,00± 0,00
Staphylococcus lugdunensis	-	-	-	17,00± 0,00	18,00± 0,00
Shigella boydi	-	-	-	20,00± 0,00	18,00± 0,00
Acinetobacter baumannii	-	-	-	18,00± 0,00	16,00± 0,00
Shigella flexneri	-	-	-	16,00± 0,00	14,00± 0,00
Enterococcus faecalis (Clinical isolate)	-	-	7,00±4,04	12,00± 0,00	10,00± 0,00
Klebsiella pneumoniae (Clinical isolate)	-	-	-	18,00± 0,00	18,00± 0,00
Candida tropicalis	-	-	-	-	-
Candida glabrata	-	-	-	7,00± 0,00	8,00± 0,00
Escherichia coli (Multidrug-resistant isolate)	-	-	-	8,00± 0,00	9,00± 0,00
Klebsiella pneumonia	7,00±0,70	-	8,00±0,70	15,00± 0,00	20,00± 0,00
Acinetobacter baumannii	-	-	-	-	-
Enterobacter aerogenes (Multidrug-resistant isolate)	-	-	7,00± 4,04	16,00± 0,00	18,00± 0,00
Serratia odorifera	-	-	-	7,00± 0,00	9,00± 0,00
Proteus vulgaris	-	-	-	11,00± 0,00	11,00± 0,00
Streptococcus pneumonia	-	-	-	10,00± 0,00	8,00± 0,00
Staphylococcus aureus MRSA (Multidrug-resistant isolate)	-	-	8,00± 4,61	-	7,00± 0,00
Staphylococcus aureus MRSA + MDR (Multidrug-resistant isolate)	-	-	-	22,00± 0,00	21,00± 0,00
Providencia rustigianii	-	-	-	16,00± 0,00	19,00± 0,00
Achromobacter sp.	-	8,00± 1,41	10,00± 1,41	9,00± 0,00	-

The antimicrobial potential of the ethanol extract of Amanita muscaria was evaluated against various bacterial and yeast strains using the disk diffusion technique. The extract exhibited an inhibition zone of 8 ± 0.00 mm against Bacillus subtilis DSM 1971 at all tested concentrations

(30, 60, and 150 µL). For Listeria monocytogenes ATCC 7644, inhibition zones of 8.00 ± 1.41 mm and 10.00 ± 1.41 mm were observed at concentrations of 60 µL and 150 µL, respectively. In the case of Pseudomonas fluorescens P1, inhibition zones measured 7.00 ± 4.35 mm and 8.00 ± 4.35 mm at 60 µL and 150 µL concentrations, respectively.For Staphylococcus aureus ATCC 25923, the extract produced inhibition zones of 8.00 ± 0.57 mm, 9.00 ± 0.57 mm, and 8.00 ± 0.57 mm at concentrations of 30, 60, and 150 µL, respectively. In Enterococcus durans, E. faecalis, Listeria innocua, Enterobacter aerogenes, and S. aureus MRSA strains, inhibition zones were observed only at the highest concentration (150 µL), measuring 7.00 ± 4.04 mm, 7.00 ± 4.04 mm, 7.00 ± 4.04 mm, 7.00 ± 4.04 mm, and 8.00 ± 4.61 mm, respectively. Against Enterococcus faecium, inhibition zones of 8.00 ± 4.04 mm, 9.00 ± 4.04 mm, and 9.00 ± 4.04 mm were recorded at 30, 60, and 150 µL concentrations, respectively. For Klebsiella pneumoniae, the extract produced inhibition zones of 7.00 ± 0.70 mm at 30 µL and 8.00 ± 0.70 mm at 150 µL, whereas for Achromobacter sp., zones of 8.00 ± 1.41 mm and 10.00 ± 1.41 mm were observed at 60 µL and 150 µL, respectively. The extract did not exhibit any inhibitory effect against the other tested microorganisms. Gentamicin and Tobramycin were used as positive controls in the experiment.

Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) values of the ethanol extract of Amanita muscaria against standard microorganisms

Microorganisms	**MIC(µg/mL)**	**MBC/MFC(µg/mL)**
Bacillus subtilis DSM 1971	>5.083	-
Listeria monocytogenes ATCC 7644	>2.541	>10.166
Pseudomonas fluorescens P1	>1.270	>10.166
Staphylococcus aureus ATCC 25923	-	-
Enterococcus durans	>1.270	-
Enterococcus faecium	-	-
Listeria innocua	>317	>5.083
Enterobacter faecalis	>5.083	>10.166
Klebsiella pneumonia	-	-
Enterobacter aerogenes	-	-
Staphylococcus aureus MRSA	-	-
Achromobacter sp.	-	-

The minimum inhibitory concentration (MIC) values of the ethanol extract of Amanita muscaria (L.) Lam. were evaluated against different microorganisms. The MIC value for Bacillus subtilis DSM 1971 was determined to be >5,083 µg/mL, while for Listeria

monocytogenes ATCC 7644 and Pseudomonas fluorescens P1, the MIC values were >2,541 µg/mL and >1,270 µg/mL, respectively. No measurable MIC value was observed for Staphylococcus aureus ATCC 25923. For Enterococcus durans, the MIC value was recorded as >1,270 µg/mL. Furthermore, the minimum bactericidal concentration (MBC) values for L. monocytogenes and P. fluorescens were determined to be >10,166 µg/mL. MBC/MFC values for the remaining tested microorganisms could not be determined.

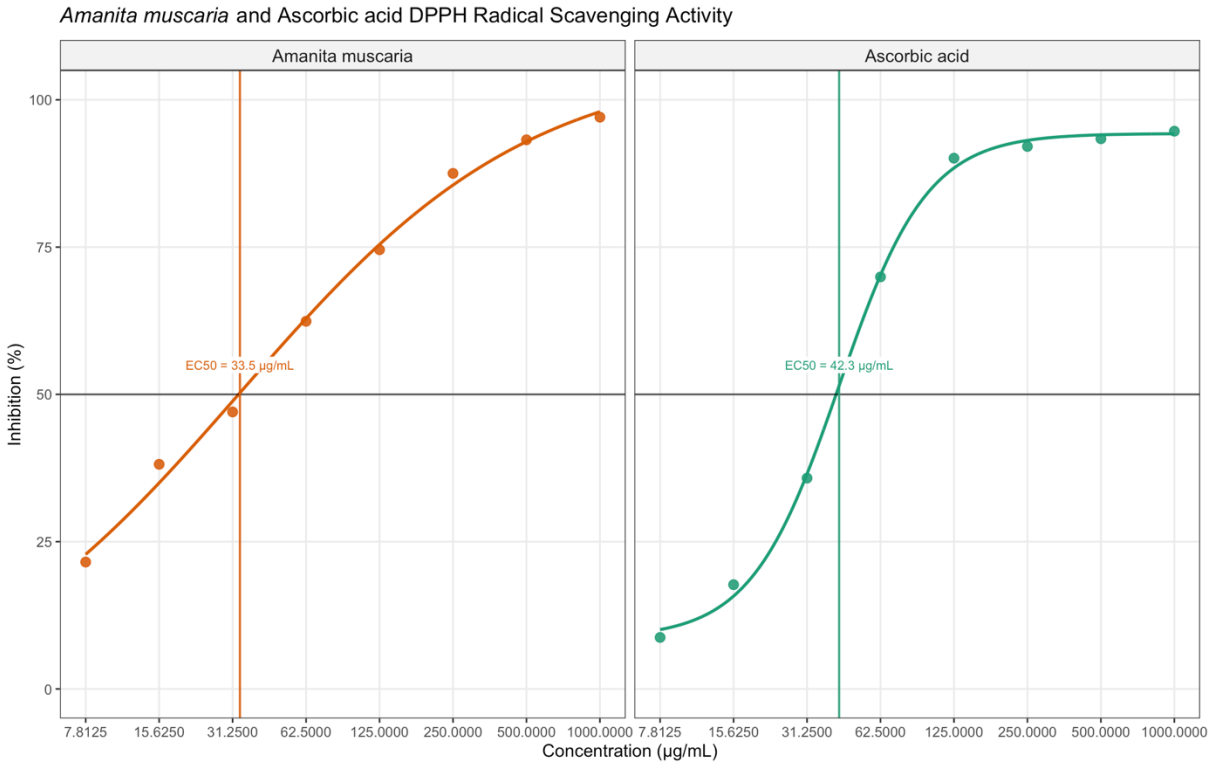

Figure 1. DPPH radical scavenging activity of *Amanita muscaria* and ascorbic acid

The DPPH radical scavenging activity of the *Amanita muscaria* extract was evaluated at various concentrations and compared with that of ascorbic acid (Figure 1). At the highest concentration (1000 µg/mL), *A. muscaria* exhibited 97.05% inhibition, which was very close to that of ascorbic acid (94.67%). At 500 µg/mL, both samples showed similarly high inhibition rates (*A. muscaria*: 93.21%; ascorbic acid: 93.39%). At intermediate concentrations (250 and 125 µg/mL), ascorbic acid displayed slightly higher activity, whereas at lower concentrations (≤31.25 µg/mL), *A. muscaria* demonstrated stronger radical scavenging potential. In particular, at 15.625 µg/mL, *A. muscaria* showed 38.1% inhibition, compared to 17.7% for ascorbic acid; at the lowest concentration (7.8125 µg/mL), *A. muscaria* achieved 21.54%

inhibition, approximately 2.5 times higher than ascorbic acid (8.74%). The EC_{50} value of the *A. Muscaria* extract was calculated as 33.5 µg/mL, whereas that of ascorbic acid was 42.3 µg/mL.

4. RESULTS AND DISCUSSION

The antimicrobial potential of the ethanol extract of Amanita muscaria was evaluated against various bacterial and yeast strains using the disk diffusion technique. The results revealed that the extract exhibited a limited inhibitory effect on certain microorganisms. For Listeria monocytogenes, inhibition zones of 8.00 ± 1.41 mm and 10.00 ± 1.41 mm were observed at concentrations of 60 µL and 150 µL, respectively, whereas Pseudomonas fluorescens P1 showed inhibition zones ranging between 7.00 and 8.00 mm. Moreover, Enterococcus faecium demonstrated consistent inhibition zones of approximately 8–9 mm at all tested concentrations (30–60–150 µL). These findings suggest that A. muscaria exhibits more pronounced activity against Gram-positive bacteria. Similarly, Chelela et al. (2014) reported that this mushroom possesses a narrow antimicrobial spectrum and is particularly ineffective against Gram-negative bacteria. The absence of inhibition zones for Klebsiella pneumoniae, Escherichia coli, and Salmonella species in the present study supports these findings.

This difference can be attributed to structural variations in bacterial cell walls. The outer membrane of Gram-negative bacteria contains a lipopolysaccharide (LPS) layer that restricts the diffusion of phenolic and terpenoid compounds present in extracts, thereby diminishing antimicrobial efficacy (Nikaido, 2003; Delcour, 2009). Conversely, the thick yet more permeable peptidoglycan layer in Gram-positive bacteria facilitates easier penetration of these compounds, contributing to the inhibition of bacterial growth (Silhavy et al., 2010). Consistent with this, L. monocytogenes, a Gram-positive and foodborne pathogen, exhibited one of the largest inhibition zones, likely due to the permeability of its cell wall structure, which makes it more susceptible to such compounds.

When the MIC and MBC values are considered together, it is clearly observed that the tested extract exhibits limited antimicrobial activity and predominantly demonstrates a bacteriostatic profile. The determination of MIC values of > 5,083 µg/mL for Bacillus subtilis, > 1,270 µg/mL for Pseudomonas fluorescens, and > 2,541 µg/mL for Listeria monocytogenes indicates that bacterial growth can only be inhibited at relatively high concentrations. In contrast, MBC values of > 10,166 µg/mL for all tested microorganisms reveal that the extract

is insufficient to exert a bactericidal effect. These findings suggest that the extract acts primarily by suppressing bacterial proliferation rather than inducing cellular death, confirming the predominance of a bacteriostatic mode of action. Similarly, Kosanić et al. (2012) reported that many fungal extracts exhibit low antimicrobial activity, which is mainly associated with growth-inhibitory (static) effects.

The biochemical profile of A. muscaria provides insight into its limited antimicrobial effect. This species contains secondary metabolites such as muscimol, ibotenic acid, and various phenolic compounds (Michelot & Melendez-Howell, 2003; Wagner et al., 2023). Muscimol primarily acts as a GABA_A receptor agonist with neuropharmacological effects, but there is limited evidence of its direct antibacterial mechanism (Voynova et al., 2020). Therefore, the extract alone may show weak antimicrobial potential. On the other hand, phenolic and antioxidant compounds in the extract may indirectly suppress microbial growth by reducing oxidative stress and limiting cellular damage (Bhambri et al., 2022).

Additionally, studies suggest that A. muscaria extracts may display enhanced efficacy under certain conditions. Borlaza (2019) demonstrated that combining mushroom extracts with silver nanoparticles significantly increases antibacterial activity, implying that fungal metabolites can interact synergistically with metal ions, improving cell permeability and extending activity even against Gram-negative bacteria.

The antioxidant (DPPH) analysis revealed that A. muscaria possesses strong free radical scavenging potential. At 1000 µg/mL, the extract achieved 97.05% inhibition, comparable to ascorbic acid (94.67%). Notably, at lower concentrations, A. muscaria exhibited superior scavenging ability: at 15.625 µg/mL, the extract showed 38.1% inhibition compared with 17.79% for ascorbic acid; at the lowest concentration (7.8125 µg/mL), it exhibited 21.54% inhibition, approximately 2.5 times higher than ascorbic acid (8.74%). These results align with previous reports by Ferreira et al. (2009) and Kalač (2013), who observed strong antioxidant capacities in wild mushrooms due to their high phenolic content. Furthermore, Chun et al. (2021) highlighted the critical role of mushroom polysaccharides in radical scavenging mechanisms. The EC_{50} value of A. muscaria extract (33.5 µg/mL) was close to that of ascorbic acid (42.3 µg/mL), indicating a comparable free radical neutralizing potential.

The present findings are also consistent with the study by Tapan et al. (2024), which examined the antioxidant properties of different Amanita species in Türkiye. Despite A. muscaria exhibiting relatively low total phenolic content (2.44 mg GAE/g dw), it showed

significant radical scavenging activity in both DPPH and ABTS assays. This suggests that its antioxidant efficiency may not solely depend on phenolic content but also on other bioactive constituents such as polysaccharides acting synergistically. Moreover, Tapan et al. reported that polyphenolic compounds in Amanita species are strongly associated with antioxidant molecules capable of reacting with both DPPH and ABTS radicals, supporting the high inhibition rates observed in this study.

However, when evaluating the antioxidant potential of Amanita muscaria, it is essential to consider that this species contains neurotoxic and psychoactive compounds such as muscimol and ibotenic acid. Previous studies have clearly demonstrated that these compounds exert effects on the central nervous system, with ibotenic acid exhibiting excitatory neurotoxic properties and muscimol acting as a potent GABA_A receptor agonist capable of inducing sedative and hallucinogenic effects (Michelot and Melendez-Howell, 2003; Feeney et al., 2010). Therefore, although A. muscaria-derived extracts may exhibit promising antioxidant activity in in vitro systems, the direct translation of this activity into food, nutraceutical, or pharmaceutical applications remains constrained by safety and toxicity concerns. The literature further emphasizes that the biological activities of A. muscaria are largely influenced by the extraction methodology, fractionation processes, and the extent to which toxic constituents are removed (Satora et al., 2006; Corker et al., 2021). In this context, targeting purified fractions or specific bioactive compounds devoid of toxic components, rather than crude extracts, represents a more appropriate approach for the biologically meaningful and safe evaluation of antioxidant activity. Consequently, the antioxidant capacity of A. muscaria should be assessed alongside its potential toxicological risks and supported by comprehensive safety evaluations.

Overall, the present study demonstrates that A. muscaria exhibits limited antimicrobial activity but strong antioxidant potential. While its antimicrobial effect may restrict its standalone therapeutic application, enhanced efficacy could be achieved through the isolation of active compounds, combination therapies, or nanoparticle-based formulations. Conversely, its potent antioxidant properties position A. muscaria as a promising natural source for combating oxidative stress and preventing chronic diseases.

In conclusion, A. muscaria extract stands out primarily for its antioxidant potential, whereas its antimicrobial effect remains supplementary. This research provides valuable evidence supporting the potential of A. muscaria as a natural antioxidant source and

establishes a scientific foundation for future studies on compound purification, toxicity assessment, and synergistic applications. Nevertheless, in future studies, elucidating the compositional profile of the extract using advanced analytical techniques such as HPLC and/or GC–MS and correlating these chemical data with antimicrobial findings will enable a clearer understanding of the underlying sources of the observed biological activity.

Conflict Of Interest

There is no conflict of interest between the authors, there is no person, institution or organization providing financial support.

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Author Contributions

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