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Comparison of Antimicrobial and Antioxidant Capacities of *Hericium erinaceus* (Lion's Mane) Mushroom Extracts Using Ultrasound-Assisted Sonication Technique

Aysel Gülbandılar[1*] Nuray Çömlekçioğlu[2] Şeyda Mermer[1] Zennur Çiyanci[1] Mehmet Arici[1]

[1] *Eskişehir Osmangazi University, Faculty of Agriculture, Department of Food Engineering, Eskisehir, Turkey*
[2] *Eskişehir Osmangazi University, Faculty of Agriculture, Department of Horticulture, Eskişehir, Türkiye*

***Correspondence:** aysel.gulbandilar@ogu.edu.tr

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Abstract

Mushrooms are important organisms due to their ability to produce different biological and chemical compounds. *Hericium erinaceus* is widely produced and consumed in many countries as an edible and medicinal mushroom due to its high protein and rich vitamin content. It is reported that this mushroom has many biological activities such as antimicrobial, antiinflamatuar, and antioxidant due to its richness in bioactive compounds. Since traditional extraction techniques used to reveal these bioactive effects are time-consuming and costly, the use of new-generation techniques has been preferred recently. In this study, the effect of ultrasound-assisted sonication (sound waves), which is among the new generation extraction techniques, on the dried form of *H. erinaceus* mushroom samples was investigated. During the extraction of the samples, 80% ultrasonic amplitude degree and different durations (10 and 20 min) were selected as variable parameters. Using these parameters, the extracts obtained were evaluated for total phenolic content by the Folin-Ciocalteu method, antioxidant capacity by the DPPH method, and antimicrobial activity by the MIC (Minimal Inhibition Concentration) method. The effect of sonication time on MIC values of mushroom extracts was found to be insignificant. On the other hand, the difference between the MIC results of microorganism types was found to be significant ($p<0.01$). MIC values varied between 62.50 and 111.11 µg/mL. It was determined that *H. erinaceus* extracts had antimicrobial activity. It was found that total phenolic content varied between 39.5-45.5 mg GAE/100 g and % DPPH amounts varied between 0.510-0.574 %. The average dry matter content of the samples was determined as 99.96% and the average ash content as 5.62%. As a result of instrumental color analysis, the average was measured as L*=56.65, a*=9.31, b*=31.85. It was determined that the ultrasound-assisted sonication method was effective in the extraction of *H. erinaceus* mushroom samples.

Key words

Hericium erinaceus, antimicrobial activity, sonication, antioxidant effect

Introduction

In recent years, global consumer demand for protein-rich foods has been continuously increasing due to the growing population. Mushrooms are an important food source with high protein, carotenoids, phenolic substances, vitamins, minerals, enzymatic and non-enzymatic compounds, low calorie and fat content. In addition, they are considered a functional food that provides health benefits due to their high antioxidant compounds (Ramkumar et al., 2010). Most mushrooms contain multicellular and unicellular eukaryotic structures with distinct bodies that can be seen with the naked eye and whose developed types can be picked by hand. According to their characteristics, they are grouped as edible mushrooms, medicinal mushrooms, and poisonous mushroom species. The edible part of mushrooms is preserved and consumed as stem and cap structures, fresh, by drying, freezing, canning, or using different evaluation methods (Öztürk and Kaya, 2022).

There are many studies on mushroom mycelia, stems, and caps as sources of antibiotics with antimicrobial effects. Some phenolic compounds, purines, pyrimidines, quinones, terpenoids, and phenyl propanoid derivative

antagonistic substances synthesized by mushrooms and generally specific to their organisms cause antimicrobial effects (Öztürk and Çopur, 2009; Barros et al., 2007).
In addition to being consumed as food, studies have shown that they have an important effect in preventing or slowing down many diseases such as cancer, hypertension, hypercholesterolemia, insomnia, allergy, stress, asthma, diabetes, etc. It is reported that the active substances found in medicinal mushrooms may be useful in the treatment of diseases such as antibiotics, antiprotozoal, antiviral, repairing internal balance, antitumor, anti-inflammatory, bioregulation, stroke, heart disease, cancer, regulation of biorhythm, and acquired immune deficiency syndrome (AIDS) (Öztürk and Çopur, 2009).

Medicinal mushrooms are rich sources of secondary metabolites that have activity against a wide range of microorganisms, including bacteria, yeasts and filamentous fungi. The *H. erinaceus* mushroom species is also reported to have significant antimicrobial properties. Phenol-like and fatty acid-like compounds obtained from extracts of this mushroom have been shown to have antifungal and antibacterial activity (Thongbai et al., 2015).
Nowadays, multidrug resistance in humans is on the rise due to the widespread and indiscriminate use of antimicrobial drugs. For this reason, fungi have attracted the attention of researchers in the search for new antimicrobial agents (Smania et al., 2001). *H. erinaceus* is one of the commercially cultivated edible and medicinal mushroom species. The main active constituents of *H. erinaceus* are polysaccharides, terpenoids (especially hericenones and erinacines, which are unique to this species), steroids, alkaloids, lactones and some phenolic compounds (Ma et al., 2010). In addition to its health-promoting properties, this mushroom species is also valued for its flavor, especially in Asian countries (Khan et al., 2013).

It is seen that fungi will have an important place in the search for new antimicrobial agents in the future. Thanks to the active compounds contained in macromolecules, phenolic compounds are reported to show antioxidant activity due to their hydroxyl groups, electron and free radical scavenging activity or hydrogen atom donating properties. Therefore, there is a positive relationship between phenolic compounds and antioxidant activity (Gupta, 2013).

Traditional extraction methods are more widely used in mushroom extraction. Recently, ultrasound-assisted sonication methods, which are new-generation extraction techniques, have also been used. In a study conducted by Mau et al. (2002), the researchers stated that the polyphenols passing into the methanolic extract of *H. erinaceus* mushroom are natural antioxidants with excellent reducing, scavenging and chelating effects on iron ions. In another study, it has been reported that ultrasonic extraction can increase the extraction rate of active biological heat-sensitive components under low-temperature processing conditions, which makes it more effective than traditional extraction techniques (Lianfu and Zelong, 2008).

The mechanical impact of ultrasonication can lead to the penetration of solvents into tissue cells, increased mass transfer, and cell wall disruption. This creates a suitable environment for the release of cell content and, compared to traditional extraction method, leads to a shorter processing time and a reduction in the amount of solvent required (Gallo, et al., 2018).
In our study, we aimed to investigate the antioxidant and antimicrobial properties of *H. erinaceus* extracts obtained by the innovative technology of ultrasonic extraction.

Material and Method

Material

Hericium erinaceus mushroom samples were used as material in this study. Dry mushroom samples were obtained from ESOGU, Faculty of Agriculture, Department of Horticulture, and analyses were carried out in the Food Engineering Department Laboratory. Mushroom samples were analyzed for microbiological and physicochemical properties.

In the investigation of the antimicrobial activity of H. erinaceus extracts, gram positive bacteria; *Bacillus subtilis*, *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (NRRL B-767), *Enterococcus faecalis* (ATCC 29212), Gram negative bacteria; *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* strains were used.In addition to the microbiological properties of the fungal species, total dry matter, ash, color (*L*, a*, b*)*, total phenolic contents and DPPH amount analyses were performed.

Methods

Preparation of Hericium erinaceus Extracts and Ultrasound-Assisted Sonication

The extraction method used by Chia et al. (2020) was modified and according to this, 5 g of powdered *H. erinaceus* sample was mixed in 500 mL of pure water (solid/liquid ratio was used as 1/100) for 2 hours on a magnetic stirrer to prepare the suspension.

Then, the Ultrasound-Assisted Sonication (Bandelin, Sonopuls HD 4000, Berlin Germany, TS413 probe) technique, which is among the non-thermal technologies, was used. Sonication amplitude and application duration were selected as 80% and 10 to 20 min as a result of preliminary studies. *H. erinaceus* samples dissolved in pure water were subjected to sonication at a single amplitude (80% amplitude) and 2 different duration (10 and 20 min). The sonication probe was immersed in the *H. erinaceus* suspension and this process was carried out under control in an ice water bath to prevent overheating. To compare the samples, the suspension without any treatment was accepted as the control (Tavakoli et al., 2021; Chia et al., 2020).

Determination of Minimum Inhibitory Concentration (MIC)

The microtube dilution technique was applied to determine the minimum concentration value (MIC) of *H. erinaceus* samples that inhibits the growth of the tested microorganisms (Büyükkıdan et al. 2022). Each microorganism culture was incubated in Mueller Hinton Broth (MHB) at 37 °C overnight and adjusted to a concentration of approximately 10^8 cfu/mL (with 0.5 McFarland) in tubes containing 15 mL of double-strength MHB. Dilutions of the compounds were prepared with 1000 µL of sample and 1000 µL of sterile distilled water at a ratio of 1:1, and 11 dilutions were made. Then, 100 µl of sterile distilled water was added to the 12th row of horizontal wells 1 to 12 of sterile 96-well microplates from top to bottom. After that, from top to bottom, 100 µL each of the previously prepared dilutions were added to wells 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 and 100 µL each of the single-strength MHB medium to the last wells of the horizontal row, respectively. Then, it was kept in an oven at 37 °C for 24 hours and the reading was done (Büyükkıdan et al. 2022).

Determination of Total Dry Matter

Sterile Petri dishes were dried in an incubator at 105 °C for 1 hour, then placed in a dessicator and cooled for half an hour. Then, approximately 7-8 ml of *H. erinaceus* suspension (solid/liquid ratio 1/100) was added to the Petri dishes, weighed again and placed in an incubator at 65 °C for 24 hours for drying. The samples that were dried in the incubator and reached constant weight were taken to the desiccator, waited for half an hour and weighed on a precision balance. Total dry matter values were calculated with the value obtained from the weighing of the dried samples (Doğan and Doğan 2022).

Determination of Ash Amount

For the determination of ash in the samples, approximately 1 g of the sample was weighed and dried in the incubator at approximately 105 °C. It was burned in the ash oven at 550 °C until the ash color turned to white. Ash content was calculated as percentages. (Doğan and Doğan 2022).

Determination of Color Values

The color values (*L**, *a** and *b**) of *H. erinaceus* extracts were obtained from the samples mixed in the magnetic stirrer. The color was measured using a Minolta (CR-400, Minolta Co, Osaka, Japan) colorimeter device on a white background from 5 different points in the liquid sample added to a small plastic container. Color intensities were determined according to the criteria given by the International Commission on Illumination CIELAB (Commision Internationele de I'e Clairage), which is based on three-dimensional color measurement. According to these criteria; *L**=0; black, *L**=100; white (darkness/lightness), +*a**=red, -*a**=green and +*b**=yellow, -*b**=blue show the color intensities (Sulejmani and Hayaloğlu 2016).

Total Phenolic Content Analysis

The modified Folin-Ciocaltaeu spectrophotometric method was used to determine the total phenolic content. Summarily, 0.4 mL of the extract solution prepared at 5 mg/mL concentration was taken, 2 mL of Folin reagent diluted 1:10 and 1.6 mL of 10% Na_2CO_3 solution were added to the test tube and incubated for two hours. The absorbance values of the samples incubated in the dark were measured against ethanol at 765 nm. Total phenolic content was determined by using the standard curve of Methyl Alcohol - Phenoline - Na_2CO_3 to determine the Methyl Alcohol - Phenoline - Na_2CO_3 value in 1 g extract. Results are expressed as mg gallic acid equivalent (GAE)/100 g (Kaya et al., 2021).

Determination of the Scavenging Effect of DPPH (1,1-Diphenyl-2-picrylhydrazyl) Radicals

The radical scavenging activity of the sample extracts was determined according to the method of Brand et al. (1995). In the DPPH radical scavenging activity method, mushroom extracts extracted in water were used and the results were given as % inhibition value. The concentration of 0.4 mg/ml sample and synthetic antioxidants were diluted to 1:1 and 5 different concentrations of solutions were prepared. In test tubes, 1.25 mL of these samples were taken and 3.75 mL of 6.10-5 M DPPH solution was added. After mixing, the tubes were kept in the dark and at room temperature for half an hour, and the absorbance values were measured against the methanol blind.
Antiradical activity (%)= [(A control- Asample)/ Acontrol)] x 100

Here Acontrol is the absorbance of the control and Asample is the absorbance of the sample at 517 nm wavelength. Using these values, the sample concentration (IC_{50}) values at the time when half of the DPPH free radical was

scavenged were calculated. Results are expressed as mg trolox equivalents (TE)/100 g (Kaya et al., 2021).

Statistical Analysis

The study was conducted according to the randomized plots experimental design with 3 replications. The analysis of variance (ANOVA) was performed using the Tarist Statistics Program (Açıkgöz et al., 2004). Means were compared with Least Significant Different (LSD).

Results and Discussion

In the study, the physicochemical, antioxidant content and antimicrobial properties against some pathogenic microorganisms were determined in the extracts obtained from *H. erinaceus* mushroom by Ultrasound-Assisted Sonication Technique.

Results of Minimum Inhibitory Concentration (MIC)

Dilution series prepared at a ratio of 1 to 2 of *H. erinaceus* samples were mixed and incubated with test microorganisms in equal proportions and the lowest dilution ratio that prevented microorganism growth was determined as the minimum inhibitory concentration. It was determined that all of the *H. erinaceus* extracts had antimicrobial activity, while some of them showed similar effects to the control compounds (Table. 1).

Table 1. MIC (µg/mL) results of commercial antibacterials and antifungals on pathogens

	MIC, µg/mL						
Test Material	*E. faecalis*	*E. coli*	*L. monocytogenes*	*S. aureus*	*B. subtilis*	*P. aeruginosa*	*C. albicans*
Vankomycin	62.50	31.25	125	31.25	250	62.50	-
Levoflaxacin	62.50	31.25	31.25	31.25	62.50	31.25	-
Sefepim	31.25	62.50	31.25	62.50	62.50	31.25	-
Chloramphenicol	62.50	62.50	62.50	62.50	62.50	125	-
Fluconazole	-	-	-	-	-	-	62.50
Ketoconazole	-	-	-	-	-	-	62.50

The analysis results of the MIC results in *H. erinaceus* samples are given in Table 2. According to the variance analysis, the effect of sonication time was found to be insignificant. On the other hand, the differences between the MIC results of the microorganisms was found to be significant ($p<0.01$). *H. erinaceus* was found to have antimicrobial effect. It was determined that *H. erinaceus* samples showed the highest activity against *P.aeruginosa*, *L. monocytegenes*, *S. aureus*, *B. subtilis*, *E. faecalis*, *C. albicans* and *E. coli* microorganisms with ultrasound-assisted extraction, respectively.

Table 2. MIC of *H. erinaceus* Extracts on Test Microorganisms (µg/mL)

	L. monocytegenes	***E. faecalis***	***S.aureus***	***C. albicans***	***E. coli***	***B. subtilis***	***P. aeruginosa***
Control	52.08	52.08	62.50	83.33	125.00	62.50	72.92
10 min	62.50	125.00	104.17	125.00	125.00	104.17	52.08
20 min	83.33	104.17	83.33	83.33	83.33	104.17	62.50
Mean	65.97^{ab}	93.75^{abc}	83.34^{abc}	97.22^{bc}	111.11^{c}	90.28^{abc}	62.50^{a}
LSD *p<0,01*				32.967			

Different letters in the same row mean significant difference

The effect of mushroom extracts against *P. aeruginosa* bacteria was similar to that of Vancomycin (62.50 µg/mL), while Chloramphenicol had a higher MIC value (125 µg/mL) compared to the control compound. It was determined that the average MIC value of mushroom extracts against *L. monocytegenes* bacteria was 65.97 µg/mL and this value was more effective than Vancomycin antibiotic.

The MIC value of the mushroom extracts against *E. coli* bacteria was 111.11 µg/mL, but it was lower than the antibacterial compounds tested.

The MIC values of Fluconazole and Ketoconazole on *C. albicans* yeast were 62.50 µg/mL and the average MIC value of mushroom extracts was 97.22. The effect of mushroom extracts was lower than the control compounds.
Nowadays, the search for new antimicrobial agents against multidrug resistance continues rapidly. It has been determined that fungi, especially among natural products, are very effective in the discovery of new drugs and fungi may be the source of natural antimicrobials. Antimicrobial agents are very important in modern medicine for disease treatment, infection prevention and food storage. They are also used to destroy pathogenic microorganisms or prevent their development, effectively treating diseases caused by microorganisms. Fungi have been proven to be a rich source of new antibacterial and antifungal agents. Various antibacterial chemicals, including illudin, colibrial, pleurotin, drosophilin A and frustulosin, have been identified in basidiomycetes. Many fungi are used as antimicrobial and antifungal agents because they are capable of producing toxic metabolites (Azevedo et al., 2022).

Antimicrobial activity varies depending on the species, strain, cultivation conditions, and extraction methods.

Some compounds exhibit narrow antimicrobial spectra and carry the risk of developing resistance over time. This limited spectrum of activity may limit the usefulness of these fungi as broad-spectrum antimicrobials (Wong et al., 2009).

It was found that extracts from *H. erinaceus* showed antimicrobial activity against *E. coli, B. subtilis, S. aureus* and *C. albicans* (Kim et al. 2000) and MRSA and *Streptococcus mutans, Enterobacter cloaca, Salmonella typhimurium* and *Candida lipolytica* microorganisms (Suleiman et al. 2022). In contrast, Wong et al. (2009) reported that *H. erinaceus* inhibited the growth of various bacteria such as *Bacillus cereus, B. subtilis, Enterococcus faecalis*, and *Salmonella* sp. However, they stated that no antifungal activity was found. Water and 50:50 water - methanol extracts of *Lactarius deliciosus, A. bisporus, L. edodes, P. ostreatus, G. lucidum, H. erinaceus, Morchella* spp., *B. edulis* and *C. cibarius* macrofungi were more effective than methanol extracts in inhibiting *E. coli, P. aeruginosa* and *S. aureus* (Alkın et al., 2021).

In a study conducted to determine the antibiofilm activity of *H. erinaceus* and to define its phenolic compound profile, inhibitory activities of this fungal species against bacterial growth and biofilm formation were performed against *P. aeruginosa, S. typhimurium, P. mirabilis* and *S. aureus*. The highest antimicrobial activity was found against *S. aureus* (11.7 mm), followed by *P. mirabilis* (6 mm). The study revealed that *H. erinaceus* did not show sufficient antibacterial activity to inhibit the growth or kill the tested pathogens compared to the positive control (Darmasiwi et al., 2022). Narmuratova et al. (2023) reported that the culture fluid and mycelial mass of *Hericium* spp. showed high antimicrobial activity against *S. aureus*, but no effect against *A niger, P. polonicum* and *M. globosus*.

Physicochemical Analysis Results

Dry Matter, Ash and Color Values (CieLAB)

Mushroom samples were evaluated in terms of physical and chemical analysis results and dry matter, ash and color matter were determined. It was determined that *H. erinaceus* mushroom differed in terms of composition. The average dry matter was 99.96% (Table 3). Cohen et al. (2014) stated in their study that the total dry matter amount of this mushroom was 42.9-83.6%. It is seen that the dry matter values we found in our study are compatible with this study.

Ash content in foodstuffs is one of the parameters determining food quality. The average ash content in this mushroom was found to be 5.62% (Table 3). Abdulla et al. (2008) determined the ash content as 4%.
In order to obtain objective results in color measurement, instrumental devices are used and quantitative results are obtained by determining the *L**, *a**, *b** values. If the color L* values are high, the color is stated as more luminous. The color *a** value expresses the spectrum ranging from green (-) to red (+). The color *b** values indicate blue (-) and yellow (+) colors (Sulejmani and Hayaloğlu 2016).
In this study, the *L**, *a**, *b** values were determined as 56.65, 9.31 and 31.85, respectively. The results are shown in Table 3.

Table 3. Mean of Dry Matter, Ash and Color (CieLAB) of *H. erinaceus*

		Mean	Minimum	Maximum
Dry matter, %		99.96	99.94	99.98
Raw ash, %		5.62	4.99	6.48
CieLAB	*L**	56.65	51.36	60.13
	*a**	9.31	8.65	9.70
	*b**	31.85	30.77	32.69

Total Phenolic Content Results

The total phenolic content and antioxidant activity results of different extraction methods applied to *H. erinaceus* are given in Table 4. The effect of the extraction method on total phenolic content was statistically insignificant ($p<0.05$). However, the highest total phenolic content was 47.5 mg GAE/100 g in the control (water bath) group extract. The total phenolic contents obtained from the ultrasonic extraction were 43.6 mg GAE/100 g in 20 min and 39.5 mg GAE/100 g in 10 min, respectively. It was thought that the decrease in the amount of total phenolic matter in 10 min may be related to the deterioration of the structure, and the increase in the phenolic matter value with the increase in time may be due to the release of protein and phenolic bonds. These results show that *H. erinaceus* mushroom is rich in total phenolic substances. Phenolic compounds are compounds that have one or more hydroxyl groups in common and an aromatic ring. Phenolic acids, tannins, lignans and coumarins are naturally occurring compounds found in fruits, vegetables, grains, roots and leaves. *H. erinaceus* is rich in polysaccharides, terpenoids, steroids, alkaloids, lactones and some phenolic compounds specific to this species (Ma et al., 2010). These components are considered a good choice against chronic degenerative diseases. Recent studies reveal the potential health benefits of phenolic compounds as antioxidants against oxidative stress diseases. The total amount of phenolic substances in the *H. erinaceus* mushroom was reported as 0.52 mg GAE/100 g (Kaya et al. 2021). Altıntaş (2023) reported that *H. erinaceus* showed a high nutritional value with protein, carbohydrate,

dietary fiber and glucan content. Total phenolic and total flavonoid contents of water, ethanol and methanol extracts of this mushroom were determined. Methanol extract was found to have the highest phenolic content (27.12 mg GAE/g extract) among the three extract types.

DPPH (1,1-Diphenyl-2-picrylhydrazyl) Radical Scavenging Effect

The difference between the DPPH (%) of mushroom samples extracted in a water bath with the new generation method ultrasonic-assisted extraction was found to be significant.
As seen in Table 4, the water bath treatment resulted in the highest DPPH content. As a result of the sonication treatment, a significant difference was determined between the samples treated for 10 and 20 min. The mean DPPH values were 0.528 % and 0.510 %, respectively.

It was determined that ultrasonic-assisted extraction and time were effective on DPPH amounts. When the statistical analysis results were evaluated, the highest DPPH amount was determined in the control group application (0.574 %). In the extractions performed using an ultrasonic device; it was determined that the sonication process applied for 10 minutes was more effective than 20 min (0.528 %) (Table. 4). When the results of the statistical analysis were evaluated, the highest amount of DPPH was determined in the control group application (0.574 %). In the extractions performed by ultrasound-assisted sonication, it was determined that the sonication process applied for 10 min was more effective than 20 min (0.528 %).
Kaya et al. (2021) determined the DPPH value as 82.78 mg TE/100 g in H. *erinaceus* mushroom in their studies.

Table 4. Total Phenolic Substances and DPPH (%) values in Ultrasound Assisted Sonication Extracts

	Total Phenolic Content (mg GAE/ 100 g)	**DPPH %**
Control	45.5	0.571^{a}
10 min	39.5	0.528^{ab}
20 min	43.6	0.510^{b}
LSD p<0.05	ns	0.046

Different letters in the same column mean significant difference, ns: non-significant

Darmasiwi et al. (2022), determined the total phenolic content of *H. erinaceus* as 1652 µg/ml. It was stated that this fungus is a medicinal fungus with strong antioxidant activity, producing active biological metabolite ester, and may be an antibiofilm agent that can be developed as a nutraceutical and natural food preservative.

The classical extraction techniques used to obtain metabolites from fungi require high resources and energy consumption, and the techniques have proven to be expensive and ineffective, especially on a biomedical scale. Valu et al. (2020), used the ultrasonic extraction method to identify biological compounds with high antioxidant activity from the mycelium of *H. erinaceus*. The extraction process was studied with varying parameters to determine the best extraction efficiency of metabolites involved in such antioxidant activity. In the study, it was determined that it is an effective and qualitative method to obtain natural antioxidants from *H. erinaceus* mushroom. This biomass could be used both as a food source and as a possible phytotherapeutic tool in the prevention or treatment of various neurodegenerative disorders that require drugs with strong antioxidant activity. In the study comparing the antioxidant capacity of water, ethanol and methanol extracts of *H. erinaceus* by different methods (DPPH, ABTS, FRAP and CUPRAC); methanol extract showed the highest DPPH (38. 88 µM TE/g extract), FRAP (21.44 µM TE/g extract) and CUPRAC (30.05 µM TE/g extract) activities, while ABTS (24.44 µM TE/g extract) activity was the highest for ethanol extract (Altıntaş, 2023).

Antioxidants prevent free radical reactions and cellular damage occurring in living organisms (Nimse and Pal, 2015). As a result of some studies on *H. erinaceus*, which has a strong antioxidant effect in this aspect of mushrooms, it was reported that fresh and dry cap extracts showed high phenolic content and high ferric-reducing antioxidant power (FRAP) (Wong et al., 2009; Atila et al., 2018). Antioxidant activity varies according to different components found in mushroom species. *H. erinaceus* is a medicinal mushroom that produces the active biological metabolite erinacin A with strong antioxidant activity. The classical extraction techniques used to date to obtain metabolites from fungi are expensive techniques on a biomedical scale, requiring high resources and energy consumption. Extraction is recognized as an important step in the development of essential traditional fungal-based medicines. Researchers are making special efforts to find the most efficient extraction methods to obtain extracts with better yields and high bioactivity (Valu et al., 2020).

Hot water, 0.9% NaCl, citric acid and 1.25 M NaOH/0.05% $NaBH_4$ were used separately for the extraction of soluble H. erinaceus polysaccharides (HEP). Then, their physicochemical properties and biological activities were analyzed and compared. It was reported that extraction methods had significant effects on extraction yields and physicochemical properties. The lowest extraction yield of HEPs was obtained during water extraction (Yan et al. 2018).

Several techniques can be used to extract primary and secondary metabolites from plants. For non-volatile compounds, the traditional method involves immersing the plant in a solvent (water, alcohol, oil), known as solid-

liquid extraction or maceration. In recent years, modern extraction techniques have been developed using "assisted" technologies such as ultrasound, pressure and microwaves. These methods aim to improve performance by reducing process time and conserving energy and solvents through various intensification mechanisms (Lefebvre et al. 2021).

Five variables (MeOH in % solvent, extraction temperature, extraction temperature, amplitude, cycle, and sample:solvent ratio) were selected for the determination of total phenolic compounds and antioxidant activity in wild and commercial mushrooms based on ultrasound-assisted techniques. Total phenolic compounds content and antioxidant activity have been considered as response variables in the study. With the multi-response optimization method, 0.2 g of sample extracted with 15.3 mL of solvent (93.6% MeOH) at 60 °C for 5 min and using 16.86% amplitude and 0.71 s^{-1} cycle were determined as optimum conditions. They stated the optimized method has been performed with deviations lower than 5%, which proved the reproducibility and sensitivity of the extraction method. It was also determined that the extraction method provided maximum antioxidant activity and was a suitable method for the extraction of phenolic compounds from mushroom samples (Aliaño-González et al., 2022).

Conclusion

In this study, the physicochemical properties, antioxidant content and antimicrobial properties of *H. erinaceus* mushroom against some pathogenic microorganisms were determined in methanol extracts obtained by applying the ultrasound-assisted sonication method, which is among the new generation techniques, at 80% ultrasonic amplitude and different durations (10 and 20 min). It was determined that the ultrasound-assisted sonication method is an effective method in the extraction of *H. erinaceus* mushroom samples.

Recently, the demand for natural products has been increasing. Mushrooms, which have an important place among these, are among the preferred and widely consumed products due to their rich content. Different extraction solutions are used to extract the bioactive components of mushrooms. These extracts are effective on total phenolic matter, antioxidant capacity and antimicrobial activity. According to the results of the study, it is thought that *H. erinaceus* mushroom can be utilized in the treatment of diseases due to its antimicrobial activity properties. It has been determined that extraction applications utilized on edible and medicinal mushrooms using new technologies may have increasing effects on total phenolic substance contents and antioxidant activity amounts. It will be possible to create basic data for industrial/technological research by using new generation techniques, different solvents and test microorganisms to expand the areas of use, and by conducting more detailed studies.

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Author Contributions: AG and NC; planned of overall research, statistical analyses, writing-review and editing; SM, ZC and MA; prepared of samples and laboratory analysis.

Conflict of Interest

The authors declare that they have no conflict of interest.

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