Biological Activities of Ethanol Extracts of Hericium erinaceus Obtained as a Result of Optimization Analysis

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Article

Biological Activities of Ethanol Extracts of *Hericium erinaceus* Obtained as a Result of Optimization Analysis

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Abstract: Mushrooms are one of the indispensable elements of human diets. Edible mushrooms stand out with their aroma and nutritional properties. In this study, some biological activities of the wild edible mushroom *Hericium erinaceus* were determined. In this context, firstly, the most suitable extraction conditions of the fungus in terms of biological activity were determined. First, 64 different experiments were performed in the soxhlet device under 40-70°C extraction temperature, 3-9 hours extraction time and 0.5-2 mg/mL extraction conditions. As a result, total antioxidant (TAS) analysis was performed and extraction conditions were optimized so that the objective function was the maximum tas value. The data obtained from the experimental study was modeled with artificial neural networks (ANN), one of the artificial intelligence methods, and optimized with a genetic algorithm (GA). All subsequent tests were performed using the extract obtained under optimum extraction conditions. The antioxidant potential of the mushroom was determined by Rel assay kits, DPPH and FRAP methods. Antimicrobial activity was measured by the agar dilution method. Antialzeihmer activity was determined by acetyl and butyrylcholinesterase activities. Antiproliferative activity was measured against the A549 cancer cell line. Determination of total phenolic content was determined using the Folin-Ciocalteu reagent. Total flavonoid quantification was performed using aluminum chloride assay. The presence of standard compounds was screened using the LC-MS/MS device. The optimum extraction conditions were found to be 60.667 °C temperature, 7.833 hours and 1.98 mg/mL. It was determined that the mushroom has high antioxidant potential. It was found to be effective against standard bacterial and fungal strains at concentrations of 25-200 µg/mL. It was determined to have strong antiproliferative activity due to increased concentration. It was determined that the Anti-AChE value was 13.85±0.94 and the Anti-BChE value was 28.00±0.89. As a result of the phenolic analysis of the mushroom, it was determined that it contained 13 compounds. As a result, in our study, it was observed that *Hericium erinaceus* has strong biological activities when extracted under optimal conditions.

Keywords: Antialzeihmer; anticancer; antimicrobial; antioxidant; *Hericium erinaceus*; medicinal mushroom

1. Introduction

Mushrooms are natural products that stand out with their different features. It is known that mushrooms, which occupy an important place in people's diets, also have high medical potential.

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Many studies have shown that different mushroom species have different biological activities such as anticancer, antiproliferative, antioxidant, antimicrobial, and DNA protective [1–3].

Hericium erinaceus (lion's mane mushroom, mountain priest mushroom, bearded tooth mushroom) is cosmopolitanly distributed, found on hard trees, and is in the form of a cluster of hanging thorns arising from a single root. It is especially common in the autumn months. It is known that it is widely used in Chinese medicine. In this context, it is produced on logs in Far East Asian countries. Fruiting bodies have been reported to contain 57% carbohydrates (8% as dietary fiber), 4% fat and 22% protein [4]. In this study, it was aimed to determine the biological activities of extracts of *Hericium erinaceus* mushroom obtained under optimum conditions.

2. Material and Method

The mushroom samples used in the study were collected from Trabzon/Turkey. Following the identification procedures, the most suitable extraction conditions for the mushroom samples were determined.

2.1. Extraction Procedure Method

Firstly, 64 different experiments were carried out in the soxhlet device, including 40, 50, 60 and 70°C extraction temperature, 3, 5, 7 and 9 hours extraction time and 0.5, 1.0, 1.5 and 2 mg/mL extraction conditions. The obtained data was modeled with ANN and optimized with GA.

2.2. Modelling

Modeling was done with the ANN method. While the inputs of the model were extraction temperature, extraction time and extract concentration, the output was used as the TAS value. The layers of the ANN used in the study are given in Figure 1.

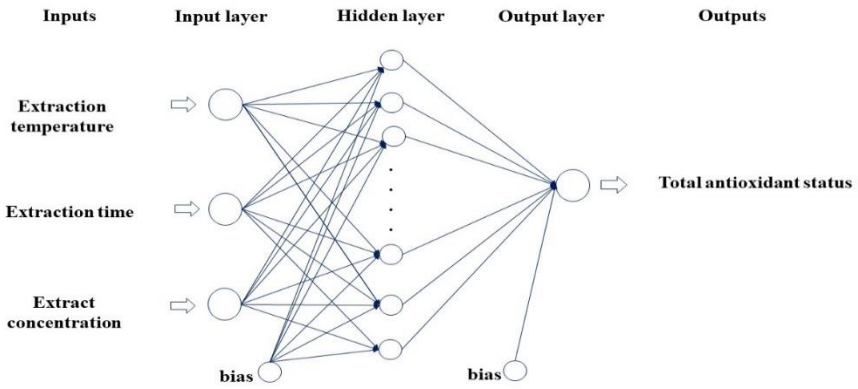

Figure 1. Layers of the ANN model.

80% of the data obtained from experimental studies was used for training, 10% for validation and 10% for testing. Levenberg-Marquardt (LM) algorithm was used for the learning process. The most appropriate network was tried to be determined by comparing the number of 20 different (1:20) hidden neurons. The learning coefficient and momentum coefficient were chosen as 0.5, the maximum number of iterations was 500, the number of verification checks was 50, and the error value was $1x10^{-5}$. Since there is a possibility of the performance surface getting stuck in a local minimum during the training of the network, a single training run may not produce optimum performance. Therefore, in order to approach a global minimum value, the networks must be retrained several times to obtain the optimal network. In this study, a total of 1000 training sessions were performed for each model. In the study, the mean square error (MSE) and mean absolute percentage error (MAPE) were used as performance indicators of the developed models. MSE and MAPE were calculated according to following Eq. 1 and 2 ;

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$$MSE = \frac{1}{n}\sum_{i=1}^{n}(e_i - p_i)^2 \quad (1)$$

$$MAPE = \frac{1}{n}\sum \left|\frac{e_i - p_i}{e_i}\right| * 100 \quad (2)$$

where, e is the experimental result, p is the prediction result, and n is the number of samples.

2.3. Optimization

The optimization procedure was done using GA. Studies have been carried out for different population numbers and the roulette wheel technique has been used for natural selection. For crossover, the single-point crossover method was used. The appropriate number of iterations was determined by analyzing the convergence graphs. Each optimization study was repeated at least 60 times, producing results very close to the global optimum.

2.4. Extraction Processes

In the optimization study conducted to determine the extraction conditions that maximize the biological activity of mushroom samples, optimum extraction conditions were determined (60.667 °C temperature, 7.833 hours and 1.98 mg/ml). Then, extracts were obtained by adjusting the optimum extraction conditions in the computer environment with the Gerhardt SOX-414 device. All analyzes in the study were made on the extract obtained from the optimum extraction condition. After the extraction process, ethanol used as solvent was evaporated using a Buchi R100 Rotary Evaporator and crude extracts were obtained.

2.5. MTT Analysis

The antiproliferative activity of the mushroom extract was determined by MTT assay against the A549 lung cancer cell line. Solutions were prepared from the extracts at concentrations of 25, 50, 100 and 200 µg/mL. Then, 70-80% confluence of the cells was achieved and separation was performed using 3.0 mL Trypsin-EDTA solution (Sigma-Aldrich, MO, USA). Then, the plates were seeded and incubated for 24 hours. Then, stock solutions were added and incubated for 24 hours. Supernatants were then dissolved in growth medium and replaced with 1 mg/mL MTT. It was incubated at 37 °C until a purple precipitate formed. Then, dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) was added to MTT and reading was performed at 570 nm using an Epoch spectrophotometer (BioTek Instruments, Winooska, VT) [5].

2.6. Anticholinesterase Analysis

Anticholinesterase activity of the mushroom extract was determined using the Ellman method. Galantamine was used as standard [6]. Stock solutions were prepared from mushroom extracts at concentrations between 200-3.125 µg/mL. Then, 130 µL of 0.1 M pH=8 phosphate buffer, 10 µL of stock solution, 20 µL of enzyme (AChE or BChE enzyme solution) were added to the microplate. Then, it was incubated for 10 minutes at 25 °C in the dark. Then, 20 µL of DTNB (5,5”-dithiobis-(2-nitrobenzoic acid)) solution and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added and reading was performed at 412 nm. The samples were repeated 3 times. IC50 values of percent inhibition of the samples were expressed as µg/mL.

2.7. Antimicrobial Analysis

The effects of the mushroom extract against the test bacterial and fungal strains were determined by the agar dilution method. The test bacteria used were pre-cultured in Hinton Broth medium. Test fungi were pre-cultured in RPMI 1640 Broth medium. The findings were stated as the lowest extract concentration that stopped the growth of bacteria. Mushroom extract in the ranges of 12.5-800 µg/mL was tested against microorganisms [7–10]

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Test bacteria: Staphylococcus aureus ATCC 29213, S. aureus MRSA ATCC 43300, Enterococcus faecalis ATCC 29212, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Acinetobacter baumannii ATCC 19606

Test fungi: *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030

2.8. Total Phenolic Analysis

1 mL stock solutions were prepared from the mushroom extract, and 1 mL of Folin-Ciocalteu reagent (1:9, v/v) was added to these solutions. Then, it was vortexed and 0.75 mL of 1% Na_2CO_3 was added. It was then incubated at room temperature for 2 hours and measured at 760 nm. According to the calibration curve of the gallic acid standard solution, the total phenolic content was expressed as mg/g [11].

2.9. Antioxidant Analysis

2.9.1. Total Antioxidant and Oxidant Analysis

Total antioxidant status (TAS) and total oxidant status (TOS) of the mushroom extract were measured with Rel Assay TAS and TOS kits. The analysis was performed according to the protocol specified by the manufacturer. TAS values were expressed as mmol trolox equivalent/L, and TOS values were expressed as μmol hydrogen peroxide equivalent/L [12,13]. OSI value was determined by taking the percentage of TOS values compared to TAS values [14].

2.9.2. DPPH Free Radical Scavenging Activity

1 mg/mL stock solutions were prepared from the mushroom extract using DMSO. 1 mL of the solution was added to 160 μL DPPH solution (0.267 mM 4 mL, 0.004% methanol solution). Then, it was incubated for 30 minutes in a dark environment and at room temperature, and the absorbance was determined at 517 nm. Values are expressed as mg Trolox Equivalent/g extract [15].

2.9.3. Ferric Reducing Antioxidant Power Assay

100 μL stock solution was prepared from the mushroom extract. Then, this stock solution was mixed with 2 mL FRAP reagent, 300 mM acetate buffer (pH 3.6), 40 mM HCl and 20 mM FeCl3 6H2O solution, 10 mM 2,4,6-tris(2-pyridyl)-S-triazine solution. was added to the prepared FRAP solution (10:1:1). It was then incubated at 37°C for 4 min. Then, reading was taken at 593 nm. Values are expressed as mg Trolox Equivalent /g extract [15].

2.10. Phenolic Analysis

In our study, 24 standard compounds were screened within the fungus using the LC-MS/MS device. Analysis was performed using a C-18 Intersil ODS-4 (3.0mm x 100mm, 2μm) analytical column (column temperature 40 C°). The mobile phase is A (water, 0.1% formic acid), B (methanol, 0.1% formic acid) and the flow is 0.3mL/min and the injection volume is 2μL.

2.11. Statistical Analysis

'SPSS 21.0 for Windows' program was used in the statistical analysis of all analyzes carried out within the scope of this study. Simple Variance Analysis (SVA) was performed to determine the difference between the groups in the tests studied; Duncan test was applied at the confidence level ($\alpha = 0.05$) to determine the difference between groups.

3. Results and Discussions

3.1. Optimization of Extraction Conditions

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Optimization of mushroom extraction conditions was carried out in 3 stages. These are experimental study, modeling and optimization respectively. Components with antioxidant activity are known to have other medicinal properties [16]. Therefore, extraction conditions were optimized to maximize the total antioxidant potential. TAS values obtained after the experimental study are given in Table 1.

Table 1. TAS values of the extracts obtained in the study.

Extraction temperature °C	Extraction time (h)	Extract concentration (mg/mL)	TAS (mmol trolox equivalent/L)
40	3	0.25	2.799±0.233
		0.5	3.127±0.121
		1	3.556±0.074
		2	4.011±0.173
	5	0.25	3.297±0.106
		0.5	3.683±0.115
		1	4.149±0.119
		2	4.465±0.212
	7	0.25	4.159±0.186
		0.5	4.480±0.141
		1	4.965±0.076
		2	5.325±0.125
	9	0.25	3.355±0.139
		0.5	3.759±0.115
		1	3.979±0.086
		2	4.356±0.205
50	3	0.25	3.433±0.088
		0.5	3.612±0.059
		1	3.746±0.066
		2	3.803±0.080
	5	0.25	3.624±0.033
		0.5	3.782±0.073
		1	4.234±0.066
		2	4.535±0.070
	7	0.25	4.260±0.072
		0.5	4.530±0.119
		1	4.907±0.064
		2	5.254±0.122
	9	0.25	3.475±0.049
		0.5	3.846±0.103
		1	4.058±0.084
		2	4.374±0.063
60	3	0.25	3.651±0.118
		0.5	3.851±0.089
		1	4.086±0.142
		2	4.439±0.105
	5	0.25	3.882±0.098
		0.5	4.214±0.168
		1	4.725±0.158
		2	5.020±0.113
	7	0.25	4.731±0.091

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		0.5	5.035±0.061
		1	5.221±0.093
		2	5.323±0.109
	9	0.25	3.950±0.102
		0.5	4.152±0.094
		1	4.404±0.046
		2	4.594±0.090
70	3	0.25	2.038±0.096
		0.5	2.301±0.107
		1	2.496±0.059
		2	2.623±0.118
	5	0.25	2.344±0.127
		0.5	2.619±0.089
		1	2.791±0.059
		2	3.010±0.119
	7	0.25	2.643±0.063
		0.5	2.906±0.095
		1	3.128±0.051
		2	3.282±0.074
	9	0.25	2.256±0.059
		0.5	2.407±0.064
		1	2.638±0.092
		2	2.717±0.110

When Table 1 is examined, it is seen that the extraction condition that gives the lowest TAS value (2.038±0.096) is 70˚C, 3 hours and 0.25 mg/mL concentration. Among the studied limits, it was observed that the highest TAS value was 5.323±0.109 at 70˚C, 3 hours and 0.25 mg/mL extraction concentration conditions. As the concentration increased at all temperature and time values, the TAS value also increased. TAS values decreased significantly when the extraction temperature increased to 70˚C. The data obtained was modeled with ANN in order to estimate intermediate values that were not made within the experimental limits and to carry out an optimization study. Among the established models, the architecture of the best prediction model was found to be 3-5-1. In other words, the prediction model produced using 5 hidden neurons was chosen as the best model. MSE, MAPE and R values of this model were calculated as 0.005, 1.499% and 0.996, respectively. Regression plot of TAS output was given in Figure 2.

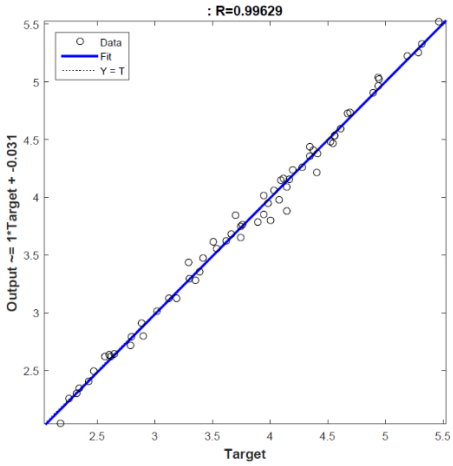

Figure 2. Regression plot of TAS output.

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The optimization process was carried out with the genetic algorithm method using the best selected ANN model. Among the different population numbers tried, the most appropriate population number was determined to be 20. After the optimization process of 60 iterations, the convergence curve of optimization process was drawn (Figure 3) and it was seen that the objective function value remained constant after the 9th iteration.

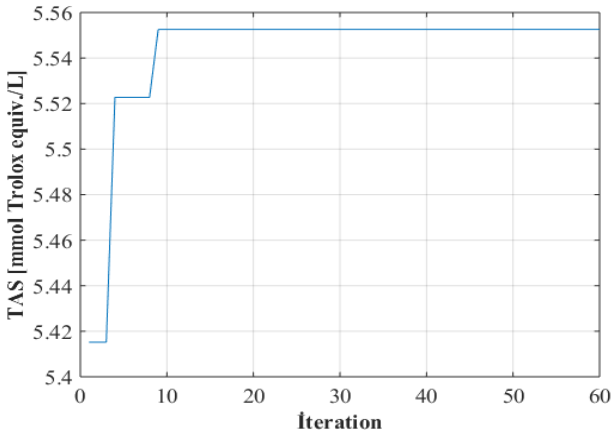

Figure 3. The convergence curve of optimization process.

After the optimization process, the optimum extraction conditions were found to be 60.667 °C temperature, 7.833 hours and 1.98 mg/mL. It was predicted that the maximum TAS value would be 5.5526 mmol/L under these extraction conditions.

3.2. Antiproliferative Activity

The increase in cancer cases in recent years has led researchers to investigate new treatment methods. The use of food supplements is recommended in cancer treatments, especially to strengthen the immune system [17]. In our study, the effect of *H. erinaceus* mushroom against A549 cancer cell line was investigated. The findings obtained are shown in Figure 3.

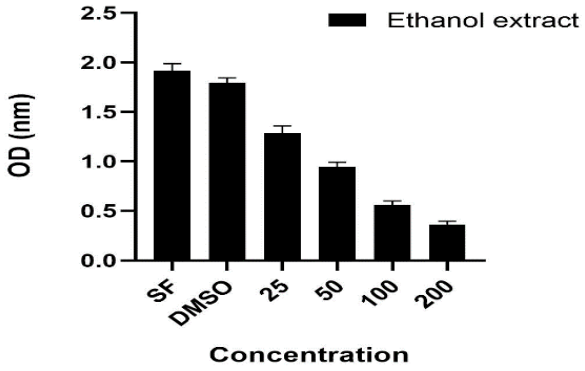

Figure 3. Antiproliferative activity of *H. erinaceus* extract.

In a previous study conducted in China, it was reported that water and ethanol extracts of *H. erinaceus* had antiproliferative activity on HepG2, Huh-7, HT-29 and NCI-87 cell lines [18]. In a different study conducted in Turkey, it was reported that *H. erinaceus*' ethanol-water, ethanol, methanol-water, water, ethyl acetate, and ether extracts had antiproliferative activity against MCF-7 cells [19]. In our study, ethanol extracts of *H. erinaceus* were used and their activity against the A549 cancer cell line was tested. According to the findings, it was determined that the mushroom extract

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exhibited strong activities depending on the increase in concentration. It was determined that it exhibited the highest activity at a concentration of 200 µg/mL. In this context, it has been determined that mushrooms may be an important anticancer agent.

3.3. Antialzeihmer Activity

There are many diseases caused by oxidative stress. Among these diseases, Alzheimer's disease, which shows a significant increase with age, is seen to be especially common in people over the age of 65. It is thought that the number of cases may exceed 80 million in the near future. There is currently no known treatment for this disease, which occurs for different reasons. However, the use of food supplements that reduce the effect of oxidative stress may play a role in reducing the disease [20]. In this study, anticholinesterase activity was detected using ethanol extracts of *H. erinaceus*. IC50 values of the findings are shown in Table 2.

Table 2. Anti-AChE and anti-BChE values of *H. erinaceus.*

	AChE (µg/mL)	BChE (µg/mL)
Ethanol	13.85±0.94^{b}	28.00±0.89^{b}
Galantamine	6.21±0.62^{a}	25.01±0.43^{a}

* Means followed by different letter(s) differ significantly at $p<0.05$ (Duncan's multiple range test).

Anticholinesterase activity of *H. erinaceus* has not been previously reported in the literature. Acetyl and butyrylcholinesterase activities of different fungal species have been reported [21–23]. In our study, acetyl and butyrylcholinesterase activities of *H. erinaceus* were determined. It was determined that both acetyl and butyrylcholinesterase activities were higher than the standard study galantamine. The presence of enzymes that cause the etiology of human diseases and the inhibition of these enzymes can be very important in the fight against diseases [24]. In our study, it was determined that the mushroom extract used in this context could be used in the treatment of neurodegenerative diseases by helping to suppress cholinesterase enzymes.

3.4. Antimicrobial Potential

Today, many antimicrobial drugs used against microbial diseases are inadequate. The main reason for this is seen as the increase in the number of resistant microorganisms due to unconscious drug use. In addition, factors such as possible side effects of synthetic drugs have led researchers to discover new antimicrobial drugs [25]. In this study, the antimicrobial potential of *H. erinaceus* was determined. The findings obtained are shown in Table 3.

Table 3. MIC values of *H. erinaceus.*

	S. aureus	***S. aureus* MRSA**	***E. faecalis***	***E. coli***	***P. aeruginosa***	***A. baumannii***	***C. glabrata***	***C. albicans***	***C. krusei***
Ethanol extract	25	25	50	100	100	100	100	50	100
Ampicillin	1.56	3.12	1.56	3.12	3.12	-	-	-	-
Amikacin	-	-	-	1.56	3.12	3.12	-	-	-
Ciprofloksasin	1.56	3.12	1.56	1.56	3.12	3.12	-	-	-
Flukanazol	-	-	-	-	-	-	3.12	3.12	-
Amfoterisin B	-	-	-	-	-	-	3.12	3.12	3.12

*25, 50, 100 µg/mL represents the lowest concentration that inhibits the growth of microorganisms.

In a study reported from Korea, the ethanol extract of H. erinaceus was reported to be effective against Listeria monocytogenes, Staphylococcus aureus, Pseudomonas fluorescens, Salmonella enteritidis, Vibrio parahaemolyticus and Escherichia coli at concentrations between 1-10 mg/mL [26]. In a different study, it was reported that water, methanol and water/methanol extracts of H. erinaceus

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were effective against Pseudomonas aeruginosa and L. monocytogenes [27]. Compared to these studies, in our study, it was determined that the extracts of H. erinaceus obtained under optimum conditions exhibited the highest activity against S. aureus and S. aureus MRSA at a concentration of 25 µg/mL. It was also determined that it inhibited the growth of E. faecalis and C. albicans at a concentration of 50 µg/mL. In addition, it was found to be effective against E. coli, P. aeruginosa, A. baumannii, C. glabrata and C. krusei at a concentration of 100 µg/mL. In this context, it was determined that the mushroom extract used in our study had a very effective antimicrobial potential in the concentration range of 25-100 µg/mL. As a result, it was determined that the mushroom extract had high antimicrobial potential.

3.5. Total Phenolic Contents

Different bioactive compounds are responsible for many biological activities. In our study, the total phenolic content of the ethanol extract of *H. erinaceus* was determined. The findings obtained are shown in Table 4.

Table 4. Total antioxidant, total oxidant, oxidative stres index, total phenolic values of *Hericium erinaceus.*

TAS (mmol/L)	5.426±0.123
TOS (µmol/L)	6.621±0.197
OSI (TOS/(TAS*10))	0.122±0.003
TPC (mg/g)	59.75±1.82
DPPH (mg Trolox Equi/g)	73.36±2.04
FRAP (mg Trolox Equi/g)	107.66±2.41

Values are given as mean ± standard deviation. (n=3).

It was previously reported that the total phenolic contents of water, methanol and water/methanol extracts of *H. erinaceus* ranged between 1.96-6.31 mg/g [27]. In a different study, it was reported that the n-hexane, chloroform, ethyl acetate, n-butanol and water extracts of *H. erinaceus* varied between 4.36-35.18 mg/g [28]. In a different study, the total phenolic content of the ethanol extract of *H. erinaceus* was reported as 41.28 mg/g [29] In our study, ethanol extract of *H. erinaceus* was used and it was determined that the extracts produced under optimum conditions were 59.75±1.82 mg/g. It was determined to have a higher total phenolic content than different extracts reported in the literature. In this context, it has been determined that it can be an important source in terms of total phenolic content.

3.6. Antioxidant Activity

Free radicals are oxidant compounds routinely produced as a result of metabolic activities. Increased levels of these compounds can cause cellular damage. Antioxidants function to reduce or suppress the effects of these compounds [30]. Oxidative stress occurs as a result of the imbalance between antioxidants and oxidant compounds. As a result of oxidative stress, serious diseases such as cancer, cardiological disorders, and neurodegenerative diseases can occur in humans. Supplementary antioxidants may serve to prevent these diseases [31]. In this context, the antioxidant potential of *H. erinaceus* used in our study was determined. The findings obtained are shown in Table 4.

TAS, TOS and OSI values of *H. erinaceus* have not been reported in the literature. It was detected for the first time in this study. Additionally, the FRAP activities of n-hexane, chloroform, n-butanol and water extracts of *H. erinaceus* were previously reported as 10.66-174.82 µmol/g. In the same study, it was reported that the DPPH activities of the extracts had 16.83-71.70% inhibition at 1.5 mg/mL [28]. In our study, the DPPH value of the ethanol extract of *H. erinaceus* was determined as 73.36±2.04 and the FRAP value was 107.66±2.41. There are TAS, TOS and OSI studies on different wild mushrooms in the literature. The TAS value of the *Agrocybe praecox* mushroom was reported as 2.97, TOS value 7.63 and OSI value 0.26 [32]. The TAS value of *Candolleomyces candolleanus* was reported as 5.547, TOS

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value as 8.572 and OSI value as 0.155 [33]. The TAS value of *Entoloma sinuatum* was reported as 2.64, TOS value 6.58 and OSI value 0.25 [34]. Compared to these studies, the TAS value of *H. erinaceus* used in our study was Found to be higher than *Agrocybe praecox* and *Entoloma sinuatum*, but lower than *Candolleomyces candolleanus*. TAS value represents the totality of antioxidant compounds produced within the mushroom. High TAS values indicate that the mushroom has high antioxidant potential. According to the results obtained from *H. erinaceus* used in our study, its extracts produced under optimum conditions were found to have high antioxidant potential. TOS value is an indicator of the totality of oxidant compounds produced within the mushroom. The TOS value of *H. erinaceus* used in our study was determined to be lower than *Agrocybe praecox* and *Candolleomyces candolleanus* and higher than *Entoloma sinuatum*. In this context, according to the results obtained from *H. erinaceus* used in our study, it was determined that the oxidant compound levels of the extracts produced under optimum conditions were at normal levels. OSI value is determined by taking the percentage of the mushroom's TOS value compared to its TAS value. OSI value shows the percentage of antioxidant compounds that suppress oxidant compounds. It was determined that *H. erinaceus* used in our study had lower OSI values than *Agrocybe praecox*, *Candolleomyces candolleanus* and *Entoloma sinuatum*. In this context, it has been observed that *H. erinaceus* has a significant potential in suppressing oxidant compounds. As a result, it appears that extracts of *H. erinaceus* obtained under optimum conditions have high antioxidant activity.

3.7. Phenolic Contents

Fungi produce many biologically active secondary metabolites. While these compounds are not nutritionally important, they have very important medical potential. In our study, standard compounds found in *H. erinaceus* were scanned on the LC-MS/MS device. The findings obtained are shown in Table 5.

Table 5. Phenolic contents of *H. erinaceus.*

Phenolic compounds	**Values (ppb)**
Acetohydroxamic acid	2464.15
Catechinhyrate	17546.53
Vanillic acid	none
Syringic acid	None
Thymoquinone	None
Resveratrol	9459.3
Myricetin	1361
Kaempferol	None
Fumaric acid	9393.41
Gallic acid	92003.93
Protocatechuic acid	3848.8
4-hydroxybenzoic acid	408.74
Caffeic acid	None
Salicylic acid	None
Phloridzindyhrate	230.83
2-hydoxycinamic acid	940.18
Oleuropein	None
2-hyroxy1,4 naphthaquinone	None
Naringenin	426.01
Silymarin	None
Quercetin	2342.81
Luteolin	444.83
Alizarin	None
Curmin	None

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As a result of the analysis, the presence of acetohydroxamic acid, catechinhyrate, resveratrol, myricetin, fumaric acid, gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, phloridzindyhrate, 2-hydoxycinamic acid, naringenin, quercetin and luteolin was found in *H. erinaceus*. It has been reported in the literature that the presence of 3,4 dihydroxy-benzoic acid, caffeic acid, syringic acid, routine, ellagic acid, p-coumaric acid, salicylic acid, vanillin, ferulic acid, sinapic acid, rosmarinic acid and t-cinnamic acid is found in *H. erinaceus* [35]. In a different study, the presence of 4-hydroxybenzoic acid, α-resorcylic acid, 4-coumaric acid, ferulic acid and syringic acid in *H. erinaceus* was reported [28]. In another study, the presence of protocatechuic acid, p-coumaric acid, succinic acid, catechin, 2-hydroxybenzoic acid and ferulic acid was reported in *H. erinaceus* [36]. In this context, in addition to these studies, it was seen in our study that *H. erinaceus* could also be an important source of phenolic compounds determined within it. In addition, it is thought that the differences in these mushrooms reported in the literature vary due to the stress factors encountered by the mushroom in its environment and the differences in the regions where it is collected. As a result, it is thought that *H. erinaceus* may be a natural source in pharmacological designs for the compounds determined within it.

4. Conclusion

In this study, the biological activity of the extract obtained from the optimum extraction conditions of the wild edible mushroom *H. erinaceus* was determined. According to the findings, it has been determined that the extracts obtained in this way have high antioxidant, antiproliferative and antimicrobial potential. In addition, 13 phenolic compounds were detected in the mushroom. By comparing with literature data, it was determined that optimally produced extracts showed higher activities As a result, it has been seen that *H. erinaceus* is an important natural resource for pharmacological designs.

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