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Extract optimization and biological activities of *Otidea onotica* using Artificial Neural Network-Genetic Algorithm and response surface methodology techniques

Mustafa Sevindik[1], Celal Bal[2], Tetiana Krupodorova[3*], Ayşenur Gürgen[4] and Emre Cem Eraslan[1]

Abstract

In this study, the biological activities of *Otidea onotica* were investigated using two optimization methods, Response Surface Methodology (RSM) and Artificial Neural Network-Genetic Algorithm (ANN-GA). The extracts were tested for phenolic content, antioxidant potential, acetylcholinesterase and butyrylcholinesterase inhibitory activities and antiproliferative effects against A549 lung cancer cell line. The results show that the extracts obtained by ANN-GA optimization exhibited higher antioxidant activity compared to RSM extracts and had higher total antioxidant status (TAS), DPPH and FRAP values. Phenolic content analysis revealed eight phenolic compounds and the compounds with the highest concentrations were caffeic acid (in RSM extract) and gallic acid (in ANN-GA extract), respectively. Both extracts showed strong cytotoxic effects against A549 cells depending on the concentration, with ANN-GA extract showing higher antiproliferative activity. Our study provides important findings on the biological activities and therapeutic potential of *O. onotica* and particularly reveals that the ANN-GA optimization method plays an important role in increasing biological activity. The findings show that *O. onotica* extracts can be used in the treatment of cancer and neurodegenerative diseases in the future and that optimization techniques offer an effective strategy for enriching phenolic contents.

Keywords Antioxidant, Anticancer, Phenolic content, Medicinal mushroom, Optimization

Introduction

Mushrooms are ecologically and biologically important organisms that exist in a wide range of ecosystems. They contribute to the shaping of biodiversity by affecting plant health and ecosystem dynamics. Throughout history, people have used mushrooms not only as a food source, but also as poisons in hunting, in religious rituals and in traditional medicine to combat various diseases [1, 2]. They have high nutritional value thanks to the vitamins, minerals, proteins and bioactive compounds they contain, and they have an important place in worldwide culinary culture with their unique taste and aroma [2, 3]. In addition to their ecological roles, mushrooms

*Correspondence:
Tetiana Krupodorova
krupodorova@gmail.com
[1]Department of Biology, Faculty of Engineering and Natural Sciences, University of Osmaniye Korkut Ata, Osmaniye 80000, Turkey
[2]Gaziantep University, Oguzeli Vocational School, Gaziantep, Turkey
[3]Department of Plant Food Products and Biofortification, Institute of Food Biotechnology and Genomics of the National Academy of Sciences of Ukraine, Kyiv 04123, Ukraine
[4]Department of Industrial Engineering, Faculty of Engineering and Natural Sciences, University of Osmaniye Korkut Ata, Osmaniye 80000, Turkey

are of great interest in pharmaceutical and biotechnological fields due to their various bioactive compounds. Many species produce secondary metabolites such as polysaccharides, terpenoids, phenolic compounds and alkaloids, which have been shown to have antimicrobial, anticancer, anti-inflammatory and immunomodulatory effects [3–6]. For example, β-glucans found in many mushrooms strengthen the immune system, while some terpenoid compounds have been identified as potent anticancer agents. These findings highlight the importance of exploring the medical and industrial potential of fungal species. In this context, species belonging to the Pyronemataceae family are of scientific interest due to their short life cycle, irregular distribution, and rarity in natural environments [7]. Although monographic studies have been conducted on the genus *Otidea* in recent years [8], information on species diversity, phylogenetic relationships, and bioactive potential is still quite limited. Although there is some general information on the identification and taxonomy of *Otidea* species [9–12], there is insufficient data on their biological activities. According to the literature, six *Otidea* species have been identified in Turkey so far [13]. This study examines *Otidea onotica*, a relatively little-studied species. *O. onotica* (family: Otideaceae), also known as the "rabbit ear mushroom", is an apothecial mushroom that usually grows in deciduous forests with beech trees. This species, which can grow singly or in clusters between spring and autumn, attracts attention with its distinctive bowl-like structure and can reach a height of approximately 10 cm. Although it is not classified as poisonous, it is generally not considered edible due to its lack of flavor [14]. However, the medicinal and bioactive potential of *O. onotica* is largely unknown. Considering the increasing interest in the discovery of new bioactive compounds from mushrooms in recent years, this study aims to optimize the extraction conditions of *O. onotica* and elucidate the biological activities of optimized extracts. By determining the pharmacological and biological properties of this species, it aims to contribute to the use of mushrooms as a valuable resource for health and industry.

The aim of this study was to determine the optimum extraction conditions in order to reveal the biological potential of *Otidea onotica* species and to examine the pharmacological and biological activities of the obtained extracts in detail.

Materials and methods

The mushroom samples used in this study were obtained from the Trabzon (2024) (Türkiye). The mushroom samples collected during the field studies were brought to the laboratory environment, cut into thin slices and then dried at 40 °C for approximately 6 h (Drying device: Tribest-Sedona Supreme). After 6 h, the mushroom sample was completely hardened and free of moisture. The fruiting bodies were subsequently ground using a mechanical grinder until they passed through an 80-mesh sieve. The obtained powders were used for extraction processes. For the artificial intelligence-based optimization study, predetermined extraction parameters were applied in a computer-controlled environment in the Gerhardt SOX-414 device.

Extraction procedure

The extraction procedure was carried out based on a full factorial experimental design. Three distinct parameters: extraction temperature, extraction time, and ethanol/water ratio were chosen, and the extraction process was conducted using three levels for each of these parameters. This approach allows for a comprehensive understanding of the influence of extraction parameters on the biological activity of *O. onotica* extracts. A total of twenty-seven different experiments were performed in a Soxhlet apparatus, varying the extraction temperatures at 45, 55, and 65 °C, extraction times at 5, 10, and 15 h, and ethanol/water ratios at 0%, 50%, and 100%. The data obtained from these experiments were subsequently optimized using both Response Surface Methodology (RSM) and an artificial intelligence-based approach, specifically the integration of Artificial Neural Network (ANN) and Genetic Algorithm (GA).

Response Surface Methodology (RSM)

In this study, Response Surface Methodology (RSM) was utilized for optimization purposes. The independent variables selected were extraction temperature, extraction time, and ethanol/water ratio. The total antioxidant activity (TAS) value of the extract was designated as the response variable.

The optimization procedure was implemented using Design Expert 13 software, employing a second-order polynomial response model as described below Eq. 1:

$$Y_k = \beta_{k0} + \sum_{i=1}^{n} \beta_{ki} x_i + \sum_{i=1}^{n} \beta_{kii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{kij} x_i x_j \quad (1)$$

where Y_k was response variable (Y_i was TAS value of extract); x_i was coded process variables (x_1 was extraction temperature, x_2 was extraction time, and x_3 was ethanol/water ratio) and β_{k0} is the value of fitted response at the design center point, respectively.

The adequacy of the model was evaluated using the coefficient of determination (R^2), ANOVA analysis, and p-values. Critical points were determined by calculating the derivatives of the model to optimize the response variable. Additionally, three-dimensional surface plots were generated to visualize the effects of the independent variables. These plots were utilized to gain a

clearer understanding of how the variables influence the response.

ANN-GA

Modeling was conducted using the ANN method. The inputs of the model included extraction temperature, extraction time, and ethanol/water ratio, while the output was the TAS value. The data obtained from the experimental studies were divided, with 80% used for training, 10% for validation, and 10% for testing. The Levenberg-Marquardt (LM) algorithm was employed for the learning process. To determine the optimal network architecture, 20 different numbers of hidden neurons (ranging from 1 to 20) were compared. The learning coefficient and momentum coefficient were set to 0.5, the maximum number of iterations to 500, the number of validation checks to 50, and the error threshold to 1×10^{-5}. In this study, a total of 1000 training sessions were conducted 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 Eqs. 2 and 3:

$$\mathrm{MSE} = \frac{1}{\mathrm{n}} \sum_{\mathrm{i}=1}^{\mathrm{n}} \left(\mathrm{e_i} - \mathrm{p_i}\right)^2 \tag{2}$$

$$\mathrm{MAPE} = \frac{1}{\mathrm{n}} \sum \left| \frac{\mathrm{e_i} - \mathrm{p_i}}{\mathrm{e_i}} \right| * 100 \tag{3}$$

where, e is the experimental result, $\mathrm{p}$is the prediction result, and n is the number of samples.

The optimization process was carried out using the Genetic Algorithm (GA). Experiments were conducted for various population sizes, and the roulette wheel technique was applied for selection. The single-point crossover method was utilized for the crossover process. The optimal number of iterations was determined by examining the convergence graphs. To ensure results that were as close as possible to the global optimum, each optimization run was repeated at least 60 times.

Extraction stage for biological activity tests

After the extraction optimization of mushroom samples was completed, the optimal conditions obtained by RSM method were determined as 55.170 °C temperature, 6.434 h and 43.891 ethanol/water ratio. The most suitable conditions by ANN-GA method were found as 50.736 °C temperature, 11.741 h and 16.493 ethanol/water ratio. For biological activity analyses, the parameters closest to these conditions were adjusted with computer control in Gerhardt SOX-414 device and test extracts were obtained.

Phenolic analysis

The phenolic compound profile of the optimized mushroom extracts was analyzed using LC-MS/MS technique. In this analysis, 24 different standard compounds present in the extracts were examined. Phenolic compound analysis was performed using the Shimadzu LC-MS/MS-8030 system. For chromatographic separation, an Inertsil ODS 4 column (2 µm, 2.1 × 50 mm) was utilized. The process was conducted in a binary gradient mode with a flow rate of 0.4000 mL/min. The mobile phases consisted of water with 0.1% formic acid (Mobile Phase A) and methanol containing 0.1% formic acid (Mobile Phase B). Initially, the concentration of Mobile Phase B was set to 5.0%, with the B curve adjusted to 0. The system operated under a maximum pressure limit of 660 bar. Sample injections were handled automatically using the SIL-20ACXR autosampler. The column was maintained at a constant temperature of 40 °C using the CTO-10ASvp column oven, with an upper temperature limit set at 85 °C.

Anticholinesterase activity tests

Optimized extracts obtained from the mushroom were evaluated for anticholinesterase activity using the Ellman method [15]. In this study, galantamine was used as the standard reference compound. Stock solutions were prepared from the extracts at varying concentrations in the range of 200-3.125 µg/mL. In a microplate, 130 µL of 0.1 M phosphate buffer (pH 8) was added, followed by 10 µL of the stock solution and 20 µL of either AChE or BChE enzyme solution. The mixtures were incubated in the dark at 25 °C for 10 minutes. Subsequently, 20 µL of DTNB solution (5,5'-dithiobis-(2-nitrobenzoic acid)) and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added, and absorbance was measured at 412 nm. Enzyme inhibition percentages and IC50 values were calculated in µg/mL, and the results were analyzed accordingly.

Antiproliferative activity test

Mushroom-derived optimized extracts were assessed for their antiproliferative effects on the A549 lung cancer cell line. For this purpose, stock solutions were prepared at concentrations of 25, 50, 100, and 200 µg/mL. Once the cells reached 70–80% confluence, they were detached using 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA). The collected cells were plated and incubated for 24 h. After incubation, the stock solutions were added to the cells and incubated for an additional 24 h. Following this, the culture medium was removed and replaced with 1 mg/mL MTT solution. The cells were then incubated at 37 °C until a dark purple precipitate formed.

The precipitates were dissolved in dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA), and absorbance was measured at 570 nm using an Epoch spectrophotometer (BioTek Instruments, Winooska, VT) [16].

Antioxidant activity tests

DPPH free radical scavenging activity

The stock solutions of the optimized extracts obtained from the mushroom were prepared at a concentration of 1 mg/mL in DMSO. From this solution, 1 mL was mixed with 160 µL of a 0.267 mM DPPH solution (4 mL, 0.004% methanol) and incubated in the dark at room temperature for 30 m. After incubation, the absorbance of the mixture was measured at 517 nm. The resulting data were expressed as mg Trolox Equivalent per gram for each extract [17].

Total antioxidant and oxidant analysis

The total antioxidant capacity of the optimized extracts obtained from the mushroom was determined using the Rel Assay TAS kit. The results were reported as mmol Trolox Equivalent per liter. The total oxidant capacity was measured using the Rel Assay TOS kit, and the results were expressed as µmol hydrogen peroxide equivalent per liter [18, 19] The oxidative stress index (OSI) was calculated by taking the ratio of the TOS and TAS values and converting this ratio into a percentage [20].

Table 1 TAS values of *Otidea onotica* extracts

Experiment number	Extraction temperature (°C)	Extraction time (h)	Ethanol/water ratio (%)	TAS (mmol/L)
1	45	5	0	4.231 ± 0.050^{cd}
2	45	10	0	6.071 ± 0.042^{hi}
3	45	15	0	3.890 ± 0.023^{a}
4	45	5	0	4.245 ± 0.038^{d}
5	45	10	0	6.033 ± 0.054^{h}
6	45	15	0	3.910 ± 0.039^{a}
7	45	5	0	4.196 ± 0.061^{bcd}
8	45	10	0	6.108 ± 0.017^{i}
9	45	15	0	3.870 ± 0.039^{a}
10	55	5	50	6.574 ± 0.012^{j}
11	55	10	50	7.360 ± 0.024^{k}
12	55	15	50	5.840 ± 0.027^{g}
13	55	5	50	6.555 ± 0.029^{j}
14	55	10	50	7.344 ± 0.016^{k}
15	55	15	50	5.852 ± 0.009^{g}
16	55	5	50	6.553 ± 0.029^{j}
17	55	10	50	7.360 ± 0.029^{k}
18	55	15	50	5.854 ± 0.037^{g}
19	65	5	100	4.934 ± 0.019^{e}
20	65	10	100	5.345 ± 0.022^{f}
21	65	15	100	4.180 ± 0.019^{bc}
22	65	5	100	4.943 ± 0.027^{e}
23	65	10	100	5.346 ± 0.018^{f}
24	65	15	100	4.170 ± 0.013^{b}
25	65	5	100	4.958 ± 0.033^{e}
26	65	10	100	5.335 ± 0.019^{f}
27	65	15	100	4.162 ± 0.021^{b}

[a]Means with different superscript letters in the same column are significantly different ($p < 0.05$) according to Duncan's multiple range test

Ferric reducing antioxidant power assay

The optimized extracts obtained from the mushroom were used to prepare a 100 µL stock solution. A sample from this solution was then mixed with 2 mL of FRAP reagent. The FRAP reagent was prepared by combining 300 mM acetate buffer (pH 3.6), 40 mM HCl, and 20 mM $FeCl_3 \cdot 6\ H_2O$ solution with a 10 mM 2,4,6-tris(2-pyridyl)-S-triazine solution in a 10:1:1 ratio. The mixture was incubated at 37 °C for 4 m. Subsequently, the absorbance was measured at 593 nm, and the results were reported as mg Trolox Equivalent per gram [17].

Statistical analysis

The statistical analysis for all tests conducted within the scope of this study was carried out using the 'SPSS 21.0 for Windows' program. One-Way ANOVA was employed to assess the differences between groups, and Duncan's test was used at a significance level of $\alpha = 0.05$ to determine where these differences existed.

Results and discussions

The diversity of mushrooms, along with their chemical variety of useful compounds, offers ample opportunities for discovering new substances with potential pharmacological activities across different spectrums of action [2]. Therefore, in the current study we have studied biomedical potential of *O. onotica* extracts highlighting the potential of this mushroom as a source of therapeutic compounds. We focused our attention on studying the possible impact of mushroom extracts on some of the widespread diseases that prematurely claim many lives, cause massive health disorders and threaten human development in general.

Optimization of extraction conditions

In this study, total antioxidant levels (TAS) were investigated using different extraction temperatures (45 °C, 55 °C, 65 °C), durations (5, 10, 15 h), and ethanol/water ratios (0%, 50%, 100%). The TAS values obtained after the experimental study are presented in Table 1.

The highest TAS value (7.360 ± 0.029 mmol/L) was observed at 55 °C, 50% ethanol/water ratio, and 10 h of extraction, while the lowest value (3.870 ± 0.039 mmol/L) was obtained at 45 °C, 0% ethanol/water ratio, and 15 h. A 50% ethanol/water ratio optimized antioxidant extraction, and a 10-hour duration at 55 °C was associated with higher TAS values compared to other conditions. These

findings contribute to identifying optimal conditions for antioxidant extraction.

Two different optimization methods were applied using the data obtained from experimental studies. In the optimization process with RSM (Response Surface Methodology), linear, 2FI (two-factor interaction), quadratic, and cubic regression models were established, and the quadratic model was selected due to its highest R^2 (coefficient of determination) value. R^2 is a metric that indicates how well the model explains the response of the independent variables. A high R^2 value (e.g., ≥ 0.90) suggests that the model is suitable for explaining the data. In this study, the R^2 value of the model was found to be 0.933, demonstrating that the model can accurately explain the behavior of the independent variables and provide reliable results in the optimization process.

The quadratic polynomial equation created as a result of the multiple regression analysis to determine the TAS values of *O. onotica* is shown below.

$$\begin{aligned} TAS =& 7.46 + 0.045\,X_1 - 0.001\,X_2 \\ & - 0.303\,X_3 + 0.001\,X_1X_2 \\ & - 0.110\,X_1X_3 - 0.007\,X_2X_3 \\ & - 1.81\,X_1^2 + 0.004\,X_2^2 - 1.32\,X_3^2 \end{aligned}$$

In the equation X_1, X_2 ve X_3 correspond to the extraction temperature, extraction time, and ethanol/water ratio, respectively.

Response surface plots of TAS of *O. onotica* were shown at Fig. 1. In our study, the effect of different extraction parameters was investigated in order to determine the total antioxidant capacity (TAS, mmol/L) of *O. onotica*. The effects of variables such as extraction time,

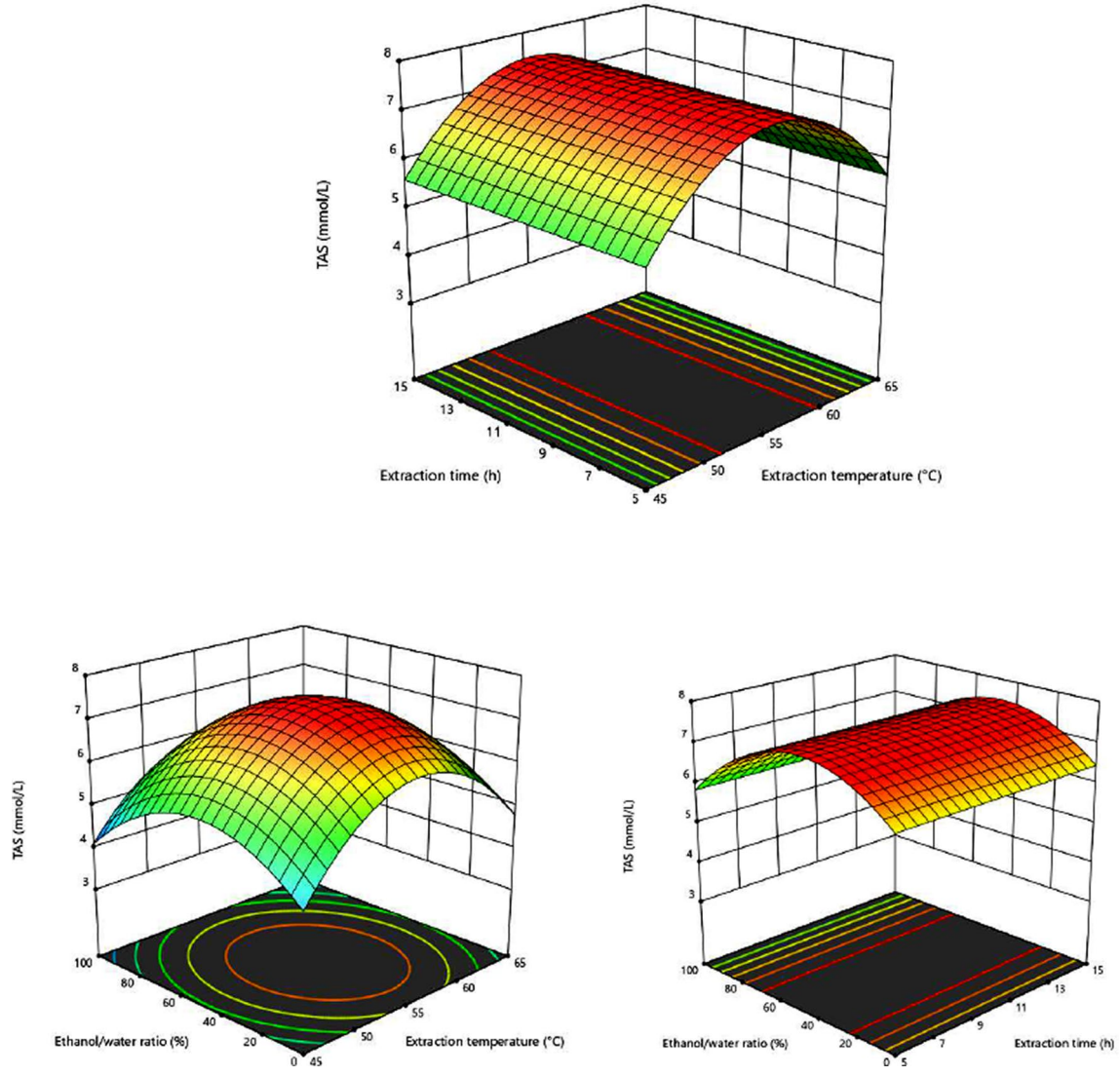

Fig. 1 Response surface plots of TAS of *Otidea onotica*

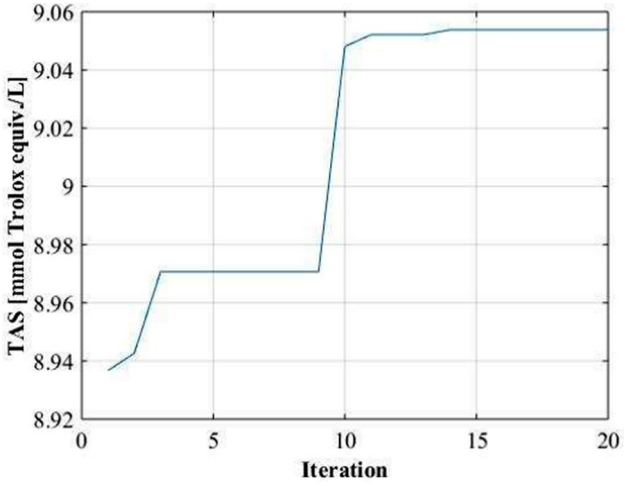

Fig. 2 Convergence graph

temperature and ethanol-water ratio on TAS were visualized with three-dimensional surface graphics (Fig. 1). The findings reveal that the antioxidant capacity reaches the maximum level at a certain temperature and solvent ratio, but when these parameters show excessive increase or decrease, the TAS value decreases.

The TAS value of *O. onotica* extracts is mostly affected by the ethanol/water ratio among the extraction conditions studied (statistically $p < 0.05$), and less affected by the extraction time and extraction temperature. According to RSM optimization, the optimum conditions were predicted as 55.170 °C temperature, 6.434 h duration, and 43.891% ethanol/water ratio.

During the optimization phase utilizing artificial intelligence techniques, the data derived from experimental studies were modeled using an Artificial Neural Network (ANN). Following the selection of the best-performing model, optimization was carried out using a Genetic Algorithm (GA). Among the various models evaluated, the architecture of the most accurate prediction model was identified as 3-3-1. This indicates that the model with 3 hidden neurons was chosen as the optimal prediction model. The MSE, MAPE and R values of this model were calculated as 0.0006, 0.396% and 0.999 for all values, respectively.

The optimization process was conducted using a Genetic Algorithm (GA) applied to the best-selected Artificial Neural Network (ANN) model. Among the various population sizes tested, the most appropriate population size was identified as 10. Following 20 iterations, a convergence graph was generated (Fig. 2), revealing that the objective function value stabilized and remained constant after the 15th iteration. According to the integration of ANN- GA, the optimal conditions were predicted as a temperature of 50.736 °C, an extraction duration of 11.741 h, and an ethanol/water ratio of 16.493.

Table 2 Phenolic contents of *Otidea onotica*

Phenolic compounds	RSM extract (mg/kg)	ANN-GA extract (mg/kg)
Acetohydroxamic acid	927.63 ± 0.36	438.12 ± 0.65
Gallic acid	10276.91 ± 0.32	14335.66 ± 0.67
Protocatechuic acid	1283.17 ± 0.67	1465.90 ± 0.23
4-hydroxybenzoic acid	2445.88 ± 0.49	1621.07 ± 0.68
Caffeic acid	12144.02 ± 0.55	12361.55 ± 0.75
Quercetin	4205.66 ± 0.89	3781.69 ± 0.55
Catechinhydrate	351.10 ± 0.31	628.54 ± 0.68
2-hydoxycinamic acid	2584.17 ± 0.83	3691.58 ± 0.62

* RSM: Optimized extract for RSM analysis; ANN-GA: Optimized extract for Artificial Neural Network-Genetic Algorithm analysis ($p < 0.05$)

Phenolic contents

Mushrooms produce various bioactive compounds to provide defense against environmental factors. These compounds are an important part of the defense mechanisms that ensure the survival of fungi and may have a range of biological activities. This shows that mushrooms have potential not only for therapeutic purposes but also to support healthy life [21, 22]. In our study, phenolic compounds of extracts obtained from *O. onotica* were analyzed by LC-MS/MS technique under optimum conditions. The data obtained reveals the potential health benefits of these compounds and the findings are presented in detail in Table 2.

Although there is limited information in the literature on the phenolic content of *O. onotica*, the analyses performed in this study revealed the presence of various phenolic compounds that may increase the biological activity of the mushroom. Eight different phenolic compounds were detected in the optimized extracts by LC-MS/MS analysis: acetohydroxyamide acid, gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, quercetin, catechin hydrate and 2-hydroxycinnamic acid. These compounds are known as natural antioxidants that support various biological activities. Especially compounds such as caffeic acid and gallic acid provide significant contributions to the antioxidant, anti-inflammatory and antiproliferative properties of the mushroom [23, 24]. Phenolic compounds are important secondary metabolites that protect mushrooms against environmental stress factors. These compounds also offer significant benefits on human health. It has been reported in the literature that gallic acid has strong antioxidant, anticancer and anti-inflammatory properties [25]. Similarly, it has been emphasized that caffeic acid is known for its free radical neutralizing effects and may have antiproliferative effects especially on cancer cells [26]. In this study, it was determined that the highest concentration of caffeic acid was found in the extracts optimized with the RSM method, while gallic acid was found in the extracts optimized with the ANN-GA method. This shows that the optimization methods used are effective in enriching

phenolic compounds in the extracts. In studies on the biological activities of phenolic compounds, it has been reported that these compounds have cell damage prevention, DNA protection and immune system strengthening effects [27]. In our study, the higher gallic acid concentration in the extracts optimized with the ANN-GA method shows that this method is more effective in increasing biologically active compounds. This can be explained by the ability of the ANN-GA method to better model complex and nonlinear relationships. As stated in the literature, artificial intelligence-based methods such as artificial neural networks and genetic algorithms make it possible to obtain extracts with higher biological activity by optimizing extraction processes [28]. In addition, it has been shown in many studies that phenolic compounds found in mushrooms can exhibit antimicrobial and anticancer effects in addition to their antioxidant properties. For example, it has been shown that phenolic compounds found in medicinal mushrooms such as *Ganoderma lucidum* and *Lentinula edodes* inhibit the proliferation of cancer cells and strengthen the immune system [29, 30]. In this context, the high phenolic compound concentrations detected in *O. onotica* increase the potential of the mushroom to be used in pharmaceutical and therapeutic areas. Considering the role of optimization methods in the enrichment of phenolic compounds, it has been observed that the ANN-GA method is more effective in this process and makes significant contributions to increasing biological activity. While the RSM method is limited to simpler and linear models, the ANN-GA method was able to model the complex relationships between extraction parameters and biological activity more accurately. This resulted in higher biological activity in the extracts optimized with the ANN-GA method. In conclusion, this study has shown that the rich phenolic content of *O. onotica* contributes significantly to biological activity and that these compounds can be increased by optimization techniques. In future studies, the isolation of these phenolic compounds and detailed examination of their pharmacological properties and especially testing their antioxidant, anticancer and neuroprotective effects in in vivo models will contribute to a better understanding of the therapeutic potential of the mushroom. In addition, the development of new extraction techniques to increase the bioavailability levels of phenolic compounds may allow these compounds to be used more effectively in the pharmaceutical field.

Table 3 Antioxidant parameters of *Otidea onotica*

Parameters	RSM extract values	ANN-GA extract values
TAS (mmol/L)	6.423 ± 0.098	8.866 ± 0.051
DPPH (mg Trolox Equi/g)	95.68 ± 2.03	109.17 ± 2.62
FRAP (mg Trolox Equi/g)	117.41 ± 2.16	161.70 ± 3.51
TOS (µmol/L)	9.418 ± 0.108	14.724 ± 0.109
OSI (TOS/(TAS*10))	0.147 ± 0.001	0.166 ± 0.001

* RSM: Optimized extract for RSM analysis; ANN-GA: Optimized extract for Artificial Neural Network-Genetic Algorithm analysis, ($p < 0.05$)

Antioxidant activity

The antioxidant properties of mushrooms have attracted increasing attention in recent years due to their potential therapeutic effects on health. By scavenging free radicals, mushrooms can strengthen immune functions and support general health. In addition, biologically active compounds found in mushrooms are considered as a natural alternative to synthetic antioxidants widely used in the food and pharmaceutical industries [31, 32]. The antioxidant potential of optimized extracts obtained from *O. onotica* was evaluated (Table 3).

There is no evidence in the literature about the antioxidant activities of *O. onotica*. There are many studies on the antioxidant activities of different mushroom species [33–35]. In our study, the antioxidant potential of the extracts of *O. onotica* produced under optimum conditions was determined. In this context, it was observed that the ANN-GA extracts used in the study exhibited higher antioxidant potential compared to the RSM extract. In addition, the TAS, TOS and OSI values of *O. onotica* were determined for the first time in our study. There are studies on different wild mushrooms in literature. TAS values of *Lactarius deliciosus*, *Hericium erinaceus*, *Cantharellus cibarius*, *Hebeloma sinapizans*, and *Candolleomyces candolleanus* were reported as 7.468, 5.426, 5.511, 4.540, and 5.547 mmol/L, respectively. TOS values were reported as 13.161, 6.621, 7.289, 10.303, and 8.572 µmol/L, respectively. OSI values were reported as 0.176, 0.122, 0.132, 0.227, and 0.155, respectively [17, 36–39]. Compared to these studies, it was observed that the RSM extract of *O. onotica* used in our study had lower TAS values than *L. deliciosus* and higher TAS values than *H. erinaceus*, *C. cibarius*, *H. sinapizans*, and *C. candolleanus*. ANN-GA extracts of *O. onotica* were determined to have higher TAS values than *L. deliciosus*, *H. erinaceus*, *C. cibarius*, *H. sinapizans*, and *C. candolleanus*. TAS value is an indicator of the totality of antioxidant compounds produced in the body of the mushroom. In our study, it was determined that the extracts of *O. onotica* produced under optimum conditions had high antioxidant potential.

The TOS value is an indicator of oxidant compounds produced in the body of the mushroom. The TOS value of the RSM extract of *O. onotica* used in our study was found to be lower than *L. deliciosus* and *H. sinapizans*, and higher than *H. erinaceus*, *C. cibarius* and *C. candolleanus*. It was determined that the ANN-GA extract of *O. onotica* had higher TOS values than *L. deliciosus*, *H. erinaceus*, *C. cibarius*, *H. sinapizans*, and *C. candolleanus*.

In this context, it was observed that the optimized extracts of the mushroom had high TOS values.

The OSI value shows how much the mushroom suppresses oxidant compounds with antioxidant compounds. As the OSI value increases, it is seen that the oxidant-antioxidant balance of mushrooms are dominant in favor of oxidant compounds. The OSI value of the RSM extract of *O. onotica* used in our study was determined to be higher than *H. erinaceus* and *C. cibarius*, and lower than *L. deliciosus*, *H. sinapizans* and *C. candolleanus*. The OSI value of the ANN-GA extract of *O. onotica* was determined to be higher than *H. erinaceus*, *C. cibarius* and *C. candolleanus*, and lower than *L. deliciosus* and *H. sinapizans*. In this context, it was observed that *O. onotica* used in our study has a normal level of potential in suppressing oxidant compounds. In addition, in our study, it was determined that the ANN-GA extract of *O. onotica* had higher DPPH and FRAP values compared to RSM extracts. In this context, it was determined that the extracts of *O. onotica* produced under optimum conditions that provided the highest biological activity had high antioxidant potential. The results obtained revealed that the extracts produced with the ANN-GA optimization method had higher total antioxidant levels (TAS) compared to extracts obtained from other mushroom species. The fact that ANN-GA extracts exhibited higher DPPH and FRAP values compared to extracts optimized with the RSM method showed that this method was more effective in increasing antioxidant capacity. TOS and OSI analyses showed that the extracts optimized with the ANN-GA method exhibited moderate activity in suppressing oxidant compounds. The fact that RSM extracts had lower OSI values suggests that this method may control the oxidant-antioxidant balance more effectively in some cases. However, the fact that extracts optimized with the ANN-GA method show higher values in terms of total antioxidant capacity makes this method superior in terms of biological activity.

O. onotica, unlike other mushroom species, stands out with its high TAS, especially in extracts optimized with the ANN-GA method. This reveals that *O. onotica* is a rich source of biologically active components. In addition, the high DPPH and FRAP values of the extracts obtained with the ANN-GA method show that it has the potential to effectively neutralize free radicals. This strong antioxidant capacity of *O. onotica* suggests that it may be a potential natural source that can be used in the prevention and treatment of diseases associated with oxidative stress. Therefore, the medical importance of *O. onotica* is increasing and it becomes possible to evaluate it in pharmaceutical and nutraceutical applications in the future.

Table 4 Anticholinesterase activity of *Otidea onotica*

Sample	AChE (μg/mL)	BChE (μg/mL)
RSM extract	36.59 ± 1.39^{c}	52.95 ± 1.22^{c}
ANN-GA extract	30.89 ± 0.91^{b}	45.28 ± 0.65^{b}
Galantamine	8.06 ± 0.30^{a}	15.27 ± 0.19^{a}

[a]Means having the different superscript letter(s) in the same column are significantly different ($p < 0.05$) according to Duncan's multiple range test, RSM: Optimized extract for RSM analysis; ANN-GA: Optimized extract for Artificial Neural Network-Genetic Algorithm analysis

Anticholinesterase activity

Alzheimer's disease is a progressive neurological disorder that manifests itself with symptoms such as memory loss, cognitive impairment, and personality changes. An important factor in the development of the disease is damage to brain cells and a decrease in neurotransmitters, especially acetylcholine [40]. Acetylcholine is a chemical transmitter that plays a critical role in cognitive functions such as learning and memory. Cholinesterase enzymes control the levels of this neurotransmitter by terminating the effect of acetylcholine. However, in Alzheimer's disease, increased cholinesterase activity can lead to the rapid breakdown of acetylcholine, further impairing neurological functions. Therefore, cholinesterase inhibitors are drugs commonly used in the treatment of Alzheimer's, and these drugs work to block the action of the enzymes to increase acetylcholine levels [41]. In this study, anticholinesterase activity of RSM and ANN-GA extracts obtained from *O. onotica* was investigated. The IC_{50} values obtained are important to evaluate the potential effects of these extracts. These data are presented in Table 4.

Although there is no evidence in the literature regarding the anticholinesterase activity of *O. onotica*, studies on the anticholinesterase activities of different mushroom species provide important clues in this area. Studies on various medicinal mushroom species have shown that these mushrooms have the potential to inhibit acetylcholinesterase and butyrylcholinesterase enzymes. For example, it has been reported that *Pleurotus florida* extracts are effective as acetylcholinesterase inhibitors and have potential in the treatment of cognitive dysfunctions [42]. Similarly, it has been reported that mushroom species such as *Clavariadelphus truncatus*, *Craterellus tubaeformis* and *Hygrophorus pudorinus* also exhibit strong anticholinerase activities and offer natural alternatives, especially in the treatment of neurodegenerative diseases such as Alzheimer's [43]. The role of enzymes in the treatment of diseases is considered one of the cornerstones of modern medicine. Controlling enzyme activities can make treatment processes more targeted and effective and reduce the side effects of therapeutic agents. In this context, a detailed study of enzyme inhibition mechanisms can increase treatment efficacy, especially in the treatment of chronic and complex diseases, while minimizing undesirable side effects [44]. Neurodegenerative

diseases, especially Alzheimer's disease, are associated with decreased acetylcholine levels and imbalances in the cholinergic system. Therefore, cholinesterase inhibitors are one of the basic drug classes frequently used in the treatment of Alzheimer's. In this study, acetylcholinesterase activities of extracts obtained from *O. onotica* were evaluated using two different optimization methods, RSM and ANN-GA. The results revealed that extracts optimized with the ANN-GA method showed higher inhibitory effects on AChE and BChE enzymes. This shows that the ANN-GA method offers a more effective strategy in increasing biological activity. The ANN-GA method contributed to obtaining stronger inhibitory properties by optimizing the concentrations of biologically active compounds in the extracts. However, when the obtained results were compared with strong and specific cholinesterase inhibitors such as galantamine, it was observed that the inhibitory effects of *O. onotica* extracts were lower. Galantamine is a reference compound widely used in the treatment of Alzheimer's disease and strongly inhibits both AChE and BChE enzymes [45]. Despite this, the moderate inhibitory capacity of *O. onotica* extracts as a natural cholinesterase inhibitor indicates their potential to be used especially in the prevention of mild cognitive impairment or in complementary treatment strategies.

Cholinesterase inhibitory activities of mushrooms are generally attributed to the phenolic compounds, terpenoids and polysaccharides they contain. In particular, it has been widely documented in the literature that phenolic compounds such as gallic acid, caffeic acid and quercetin exhibit inhibitory effects on cholinesterase enzymes [46, 47]. In this study, the presence of these compounds at higher concentrations in the extracts optimized by the ANN-GA method may have played an important role in the increase in anticholinerase activity. In addition, the known strong antioxidant properties of phenolic compounds may provide neuroprotective effects by creating a synergistic effect on the nervous system together with cholinesterase inhibition. As a result, the anticholinerase activity shown by *O. onotica* extracts indicates that this mushroom may be a potential natural agent in the treatment of neurological diseases. Extracts optimized with the ANN-GA method provide advantages in terms of producing more effective cholinesterase inhibitors. In future studies, isolating active compounds and examining their neuroprotective effects and supporting them with in vivo studies may reveal the potential of the mushroom in the pharmaceutical field and may offer new therapeutic options in the treatment of neurodegenerative diseases.

Antiproliferative activity

Mushrooms attract attention as a potential treatment source in the treatment of serious diseases such as cancer with the various bioactive compounds they contain.

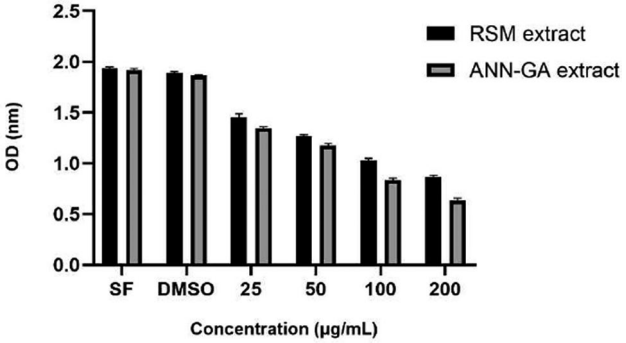

Fig. 3 Antiproliferative activity of *O. onotica* extracts. SF: Group kept only in the medium without applying any chemical substance. DMSO: Group to which only DMSO was applied together with the medium. Extract Application: Groups to which the extract was applied at concentrations of 25, 50, 100 and 200 µg/mL), RSM: Optimized extract for RSM analysis; ANN-GA: Optimized extract for Artificial Neural Network-Genetic Algorithm analysis

The compounds found in these mushrooms are known for their ability to inhibit the growth of tumors and stop the proliferation of cancer cells. In addition, some types of mushrooms specifically affect cancer cells while not harming healthy cells. These features may provide selective toxicity in cancer treatment, allowing more targeted and effective results to be obtained in the treatment processes [48, 49]. However, a more detailed examination of the biological action mechanisms of these mushroom-derived compounds is of great importance for their wider use in clinical applications. In this study, the antiproliferative effects of optimized extracts obtained from *O. onotica* on the A549 lung cancer cell line were investigated. The results obtained provided important findings showing the potential of this mushroom in cancer treatment, and the details are presented in Fig. 3.

Although there is no evidence in the literature regarding the antiproliferative activity of *O. onotica*, various studies have shown that some mushroom species are effective against the A549 lung cancer cell line. For example, *Lignosus rhinocerus* sclerotia extracts were determined to have antiproliferative effects on A549 cells [50]. Similarly, *Calvatia gigantea* extracts were reported to inhibit cell proliferation in the A549 cell line [51]. In addition, some medicinal mushrooms such as *Taiwanofungus camphoratus* were found to have cytotoxic effects by activating both apoptosis and autoptosis mechanisms on A549 cells through the compound antrodin C [52]. In this study, the effects of *O. onotica* extracts optimized by RSM and ANN-GA methods on the A549 lung cancer cell line were evaluated. The results obtained showed that the extracts optimized with the ANN-GA method had stronger cytotoxic effects compared to those obtained with the RSM method. Both extracts showed significant cytotoxic effects on A549 cells in a dose-dependent manner; however, the extract optimized with the ANN-GA method was determined to have higher antiproliferative

activity. This supports that the ANN-GA optimization method is more effective in increasing biological activity. These findings reveal that *O. onotica* exhibits significant biological activity against cancer cells and that the ANN-GA method is an effective tool in increasing this activity. In addition, the fact that the extracts exhibit strong effects depending on the concentration suggests that dosage adjustments are of critical importance in the treatment processes. This situation emphasizes the necessity of careful dose optimization for the effective use of mushroom extracts in the treatment process. The antiproliferative effects of mushrooms are generally attributed to the biologically active compounds they contain, such as phenolic compounds, polysaccharides, terpenoids and sterols [53]. The strong antioxidant and antiproliferative effects of phenolic compounds, especially gallic acid and caffeic acid, are well known in the literature [54, 55]. In this study, the presence of these compounds in higher concentrations in the extracts optimized with the ANN-GA method may be an important factor that increases the effect of the extracts on cancer cells. Although there is no direct data in the literature on the antiproliferative effects of *O. onotica*, the findings obtained in this study indicate that this mushroom may be a potential natural resource in the field of cancer treatment. The higher biological activity obtained with the ANN-GA optimization method revealed that this method is effective in increasing the concentration of biologically active compounds in the extracts. In conclusion, the cytotoxic effect of *O. onotica* on the A549 lung cancer cell line is an important finding supporting the potential of this mushroom to be used in cancer treatment. In future studies, detailed studies on different cancer cell lines and in vivo models will contribute to our better understanding of the therapeutic properties of this mushroom. In addition, isolating the active compounds found in *O. onotica* and examining their biological activities in more detail will be an important step in revealing the potential of the mushroom in pharmaceutical applications. Such studies may contribute to the development of new anticancer agents obtained from natural sources and may provide valuable information especially for clinical applications seeking natural treatment alternatives.

Conclusion

This study highlights the biological potential of *O. onotica* mushroom and highlights the effect of different optimization methods in increasing biological activity. When the RSM and ANN-GA techniques used in the study were compared, it was seen that the ANN-GA method was more successful in increasing the biological activity of mushroom extracts. The research results showed that the extracts optimized with the ANN-GA method had higher antioxidant capacity. These extracts also exhibited superior performance in terms of effective elimination and reduction power of free radicals. In addition, it was determined that the extracts obtained with the ANN-GA method contained higher concentrations of phenolic compounds that support biological activity. Especially, the high amount of phenolic compounds with strong antioxidant properties such as gallic acid and caffeic acid increases the therapeutic potential of the mushroom. In the study, the anticholinesterase activities of the extracts were also examined and it was determined that the extracts optimized with the ANN-GA method had higher capacity to inhibit acetylcholinesterase and butyrylcholinesterase enzymes. Although they have lower inhibitory power compared to reference inhibitors, these natural extracts can be evaluated as supportive agents in the management of neurodegenerative diseases. However, it was determined that extracts optimized with ANN-GA method exhibited stronger antiproliferative effects on lung cancer cells. Increasing cytotoxic effects depending on concentration indicate that *O. onotica* may be a potential natural source in cancer treatment. In particular, while RSM method is suitable for modeling more linear or slightly nonlinear relationships based on polynomial equations, ANN-GA method has the ability to capture complex and nonlinear dependencies. This feature allows ANN-GA to model complex relationships between extraction parameters and biological activity more accurately, which allows obtaining extracts with higher biological activity. As a result, antioxidant, anticholinesterase and antiproliferative activities of *O. onotica* extracts were significantly increased thanks to ANN-GA optimization. These findings support the usability of the mushroom in cancer treatment, management of neurological diseases and antioxidant therapies. In future studies, it is recommended that the pharmacological properties of this mushroom be examined in more detail and its potential for medical applications be investigated.

Abbreviations

AChE	Acetylcholinesterase
ANN	Artificial Neural Network
BChE	Butyrylcholinesterase
DMSO	Dimethyl Sulfoxide
DPPH	1,1-Diphenyl-2-Picrylhydrazil
FRAP	Ferric Reducing Antioxidant Power
GA	Genetic Algorithm
MAPE	Mean Absolute Percentage Error
MSE	Mean Squared Error
RSM	Response Surface Methodology
TAS	Total Antioxidant Status
TOS	Total Oxidant Status

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Author contributions

M.S. and C.B. supervised the research. A.G., T.K. and M.S. performed the experiments, analyzed the data, and wrote the manuscript. A.G., E.C.E. and T.K.

designed the experiments. M.S. provided assistance during the experiments. A.G., M.S., T.K., C.B. and E.C.E. revised the manuscript. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This research does not involve any ethical issues.

Consent for publication
Not applicable.

Clinical trial number
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Competing interests
The authors declare no competing interests.

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References

1. Mushtaq W, Hayri B, Akata İ, Sevindik M. Antioxidant potential and element contents of wild edible mushroom suillus granulatus. Kahramanmaraş Sütçü İmam Üniversitesi Tarım Ve Doğa Dergisi. 2020;23(3):592–5.
2. Venturella G, Ferraro V, Cirlincione F, Gargano ML. Medicinal mushrooms: bioactive compounds, use, and clinical trials. Int J Mol Sci. 2021;22(2):634.
3. Shaffique S, Kang S-M, Kim A-Y, Imran M, Aaqil Khan M, Lee I-J. Current knowledge of medicinal mushrooms related to anti-oxidant properties. Sustainability. 2021;13(14):7948.
4. Mohan K, Karthick Rajan D, Muralisankar T, Ramu Ganesan A, Marimuthu K, Sathishkumar P. The potential role of medicinal mushrooms as prebiotics in aquaculture: A review. Reviews Aquaculture. 2022;14(3):1300–32.
5. Łysakowska P, Sobota A, Wirkijowska A. Medicinal mushrooms: their bioactive components, nutritional value and application in functional food production—a review. Molecules. 2023;28(14):5393.
6. Sevindik M, Bal C, Eraslan EC, Uysal I, Mohammed FS. Medicinal mushrooms: a comprehensive study on their antiviral potential. Prospects Pharm Sci. 2023;21(2):42–56.
7. Dzhagan V, Krupodorova T, Atamanchuk A, Lytvynenko Y. Growth and morphological characteristics of some pyrophilous discomycetes in culture. Biosystems Divers. 2023;31(3):282–9.
8. Olariaga I, Vooren NV, Carbone M, Hansen K. A monograph of otidea (Pyronemataceae, Pezizomycetes). Persoonia-Molecular Phylogeny Evol Fungi. 2015;35(1):166–229.
9. Xu F, Ge Z-W, LoBuglio KF, Pfister DH. Otidea species from China, three new species with comments on some previously described species. Mycological Progress. 2018;17:77–88.
10. Carbone M, Agnello C, Kautmanová C, Ge Z, Alvarado P. Phylogenetic and morphological studies in otidea alutacea and O. bufonia clades (Pezizales), with the new species otidea adorniae. Ascomycete Org. 2019;11(4):117–26.
11. Parslow M, Suz LM, Spooner B. The British species of Otidea (3): taxa present in Britain. [Ascomycete org] 2019;11(6).
12. Xu Y-Y, Mao N, Yang J-J, Fan L. New species and new records of otidea from China based on molecular and morphological data. J Fungi. 2022;8(3):272.
13. Akata İ, Doğan HH, Çetin B. A new record for otidea genus from Turkey. Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi. 2009;2(1):21–4.
14. Jordan M. The encyclopedia of fungi of Britain and Europe. 1995.
15. Ellman GL, Courtney KD, Andres V Jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7(2):88–95.
16. Bal C, Akgul H, Sevindik M, Akata I, Yumrutas O. Determination of the anti-oxidative activities of six mushrooms. Fresenius Envir Bull. 2017;26(10):6246–52.
17. Sevindik M, Gürgen A, Khassanov VT, Bal C. Biological activities of ethanol extracts of hericium Erinaceus obtained as a result of optimization analysis. Foods. 2024;13(10):1560.
18. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004;37(4):277–85.
19. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005;38(12):1103–11.
20. Sevindik M. The novel biological tests on various extracts of cerioporus varius. Fresenius Environ Bull. 2019;28(5):3713–7.
21. Bills GF, Gloer JB. Biologically active secondary metabolites from the fungi. Microbiol Spectr. 2016;4(6). https://doi.org/10.1128/microbiolspec.funk-0009-2016.
22. Comlekcioglu U, Ozkose E, Akyol İ, Ekinci M. Fatty acid analysis of anaerobic ruminal fungi neocallimastix, caecomyces and Orpinomyces. Int J Agric Biology. 2010;12(4):635–7.
23. Aijaz M, Keserwani N, Yusuf M, Ansari NH, Ushal R, Kalia P. Chemical, biological, and Pharmacological prospects of caffeic acid. Biointerface Res Appl Chem. 2022;13:324.
24. Sabik AE, Sevindik M, Mohammed FS, Dogan M. A new natural source against A549 lung cancer cells anthemis Cotula and its biological activities and phenolic contents. Pharm Chem J. 2024;58(7):1134–40.
25. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines. 2018;5(3):93.
26. Abdelshafy AM, Belwal T, Liang Z, Wang L, Li D, Luo Z, Li L. A comprehensive review on phenolic compounds from edible mushrooms: occurrence, biological activity, application and future prospective. Crit Rev Food Sci Nutr. 2022;62(22):6204–24.
27. Sun W, Shahrajabian MH. Therapeutic potential of phenolic compounds in medicinal plants—Natural health products for human health. Molecules. 2023;28(4):1845.
28. Gürgen A, Sevindik M. Application of artificial neural network coupling multiobjective particle swarm optimization algorithm to optimize pleurotus ostreatus extraction parameters. J Food Process Preserv. 2022;46(11):e16949.
29. Shafiq MA, Imene HH, Tasneem H, Muntaha ST, Almu MA, Nasir S. The role of mushrooms and their bioactive compounds in cancer prevention and treatment. Insights-Journal Health Rehabilitation. 2025;3(3):291–304. Health & Allied)).
30. Kurl S, Kaur S, Mittal N, Kaur G. Mushrooms and colorectal cancer: unveiling mechanistic insights and therapeutic innovations. Phytother Res. 2025;39(1):480–93.
31. Wasser SP. Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med Mushrooms. 2010;12(1).
32. Chaturvedi VK, Agarwal S, Gupta KK, Ramteke PW, Singh M. Medicinal mushroom: Boon for therapeutic applications. 3 Biotech. 2018;8:1–20.
33. Bermúdez-Gómez P, Fernández-López J, Pérez-Clavijo M, Viuda-Martos M. Evaluation of sample size influence on chemical characterization and in vitro antioxidant properties of flours obtained from mushroom stems coproducts. Antioxidants. 2024;13(3):349.
34. Effiong ME, Umeokwochi CP, Afolabi IS, Chinedu SN. Comparative antioxidant activity and phytochemical content of five extracts of pleurotus ostreatus (oyster mushroom). Sci Rep. 2024;14(1):3794.
35. Nuinamwong S, Sermwittayawong D, Sukpondma Y, Tansakul C, Purintrapibal Y, Decha N. Extraction, characterization, antioxidant, and antidiabetic activities of ethanolic extracts from the split gill mushroom (Schizophyllum commune). Food Bioscience. 2024;61:104857.
36. Karalti İ, Eraslan EC, Sarıdoğan BGÖ, Akata I, Sevindik M. Total antioxidant, antimicrobial, antiproliferative potentials and element contents of wild mushroom Candolleomyces candolleanus (Agaricomycetes) from Turkey. Int J Med Mushrooms. 2022;24(12).
37. Ahmad Z, Özdemir B, Sevindik M, Eraslan Ec, Selamoglu Z, Celal B. Phenolic compound and antioxidant potential of Hebeloma sinapizans mushroom. AgroLife Sci J. 2023;12(2):12–7.
38. Gürgen A, Unal O, Sevindik M. Biological activities of the golden Chantarelle mushroom Cantharellus cibarius (Agaricomycetes) extracts obtained as a result of single and multi-objective optimization studies. Int J Med Mushrooms. 2024;26(12):63–74.
39. Gürgen A, Sevindik M. Single and Multi-Objective optimization of the red pine mushroom lactarius deliciosus (Agaricomycetes) extraction conditions

using artificial intelligence methods and biological activities of optimized extracts. Int J Med Mushrooms. 2025;27(2):59–73.
40. Gürgen A, Sevindik M, Krupodorova T, Uysal I, Unal O. Biological activities of hypericum spectabile extract optimized using artificial neural network combined with genetic algorithm application. BMC Biotechnol. 2024;24(1):83.
41. Blandina P, Efoudebe M, Cenni G, Mannaioni P, Passani MB. Acetylcholine, Histamine, and cognition: two sides of the same coin. Learn Mem. 2004;11(1):1–8.
42. Randhawa K, Singh V, Kaur S, Kaur R, Kumar S, Shri R. Isolation of pleurotus Florida derived acetylcholinesterase inhibitor for the treatment of cognitive dysfunction in mice. Food Sci Hum Wellness. 2021;10(4):490–6.
43. Deveci E, Tel-Çayan G, Çayan F, Yılmaz Altınok B, Aktaş S. Chemical characterization, in vitro antioxidant, anti-cancer and enzyme Inhibition activities of three edible mushroom species. J Food Meas Charact. 2023;17(5):5518–33.
44. Świątek Ł, Sieniawska E, Sinan KI, Maciejewska-Turska M, Boguszewska A, Polz-Dacewicz M, Senkardes I, Guler GO, Bibi Sadeer N, Mahomoodally MF. LC-ESI-QTOF-MS/MS analysis, cytotoxic, antiviral, antioxidant, and enzyme inhibitory properties of four extracts of geranium pyrenaicum burm. F.: A good gift from the natural treasure. Int J Mol Sci. 2021;22(14):7621.
45. Adarvez-Feresin CW, Ortiz JE, Piñeiro MD, Parravicini O, Enriz RD, Garro AD, Feresin GE. Inhibitory effect of galantamine and donepezil combination against cholinesterase: an in Silico and in vitro study. Arch Pharm. 2024;357(4):2300581.
46. Mišković J, Rašeta M, Čapelja E, Krsmanović N, Novaković A, Janjusevi L, Karaman M. Mushroom species stereum hirsutum as natural source of phenolics and fatty acids as antioxidants and acetylcholinesterase inhibitors. Chem Biodivers. 2021;18(11):e2100409.
47. Salawu SO, Alao OF, Faloye OF, Akindahunsi AA, Boligon AA, Athayde ML. Antioxidant potential of phenolic-rich two varieties of Nigerian local rice and their anti-cholinesterase activities after in vitro digestion. Nutr Food Sci. 2016;46(2):171–89.
48. Park H-J. Current uses of mushrooms in cancer treatment and their anticancer mechanisms. Int J Mol Sci. 2022;23(18):10502.
49. Nandi S, Sikder R, Rapior S, Arnould S, Simal-Gandara J, Acharya K. A review for cancer treatment with mushroom metabolites through targeting mitochondrial signaling pathway: in vitro and in vivo evaluations, clinical studies and future prospects for mycomedicine. Fitoterapia. 2024;172:105681.
50. Lee M, Tan N, Fung S, Tan C, Ng S. The antiproliferative activity of sclerotia of lignosus rhinocerus (Tiger milk Mushroom). Evidence-Based Complement Altern Med. 2012;2012(1):697603.
51. Eroğlu C, Seçme M, Atmaca P, Kaygusuz O, Gezer K, Bağcı G, Dodurga Y. Extract of Calvatia gigantea inhibits proliferation of A549 human lung cancer cells. Cytotechnology. 2016;68:2075–81.
52. Wang W, Yang H, Deng J, Zhu L, Yang Y, Liu Z, Zhang J, Tang C, Zhang Z, Zhuang H. Increased Inhibition effect of Antrodin C from the stout Camphor medicinal mushroom, Taiwanofungus Camphoratus (Agaricomycetes), on A549 through crosstalk between apoptosis and autophagy. Int J Med Mushrooms. 2019;21(6):595–610.
53. Kumar K, Mehra R, Guiné RP, Lima MJ, Kumar N, Kaushik R, Ahmed N, Yadav AN, Kumar H. Edible mushrooms: A comprehensive review on bioactive compounds with health benefits and processing aspects. Foods. 2021;10(12):2996.
54. Şabik AE, Mohammed FS, Sevindik M, Uysal I, Bal C. Gallic acid: derivatives and biosynthesis, Pharmacological and therapeutic effect, biological activity. Bulletin of university of agricultural sciences and veterinary medicine Cluj-Napoca. Food Sci Technol. 2024;81(1):18–27.
55. Alam M, Ahmed S, Elasbali AM, Adnan M, Alam S, Hassan MI, Pasupuleti VR. Therapeutic implications of caffeic acid in cancer and neurological diseases. Front Oncol. 2022;12:860508.

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