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OPTIMIZATION AND CHARACTERIZATION OF WOOD DECAY MUSHROOM *Ganoderma adspersum* EXTRACT: A COMPARISON BETWEEN RESPONSE SURFACE METHODOLOGY AND ARTIFICIAL NEURAL NETWORK-ANT LION ALGORITHM

***Ayşenur Gürgen*[1,♠]**

https://orcid.org/0000-0002-2263-7323

ABSTRACT

In this study, the bioactive properties of *Ganoderma adspersum*, a wood-decaying mushroom, were investigated. The study was designed in three steps: an experimental study, optimization of extraction conditions, and determination of bioactive properties of the optimum extracts. The main research problem was to determine the most effective extraction conditions to maximize the bioactive potential of *G. adspersum* using advanced optimization techniques. The extraction conditions were designed according to the I-optimal design and optimized using both the response surface method and the integration of artificial neural networks-ant lion algorithm. In the third step of the study, the bioactive properties of the two estimated extraction conditions and the extraction condition providing the highest total antioxidant status value obtained from the experimental studies were evaluated. Antioxidant activity, total phenolic and flavonoid content, antimicrobial properties, anticholinesterase activity, and phenolic content of three different optimum extracts were determined. As a result, the optimum extraction conditions suggested by artificial neural networks–ant lion algorithm optimization showed the best overall bioactive activity, highlighting the effectiveness of hybrid artificial intelligence-based models in bioactive compound extraction processes.

Keywords: Ant lion algorithm, artificial neural networks, bioactive compounds, extraction optimization, *Ganoderma adspersum,* optimization.

INTRODUCTION

The genus *Ganoderma* belongs to the family *Ganodermataceae* in the *Basidiomycota*, which are white rot fungi that can decompose cellulose as well as lignin (Aquino *et al.* 2022). It is known that *Ganoderma* and similar decaying species cause damage to plant stems and roots (Schwarze and Ferner 2003, Baby *et al.* 2015). *Ganoderma* species are not classified as edible mushrooms because they usually have a hard fruiting body and a thick, woody structure (Baby *et al.* 2015). However, *Ganoderma* mushrooms are one of the most important sources of medicinal mushrooms (Bulam *et al.* 2019). They have been used for medicinal purposes, as food additives, in fermentation, and in pharmaceuticals since ancient times, especially in countries such as China, Japan, and Korea (Kumar 2021). Currently, they are used as a preventive and therapeutic treatment against many diseases through the consumption of various preparations, teas, and coffees produced from *Ganoderma* mushrooms (Bajaj and Ballal 2021, Vamanu *et al.* 2021).

Previous studies on *Ganoderma* spp. have mostly focused on their nutritional properties or the determination of the medicinal activity of extracts obtained from them (Ferreira *et al.* 2015, Cör Andrejč *et al.* 2022). In the extracts of various *Ganoderma* species, polysaccharides, terpenoids, steroids, alkaloids, and phenolic compounds with anticancer, antioxidant, antitumor, antiviral, antibacterial, anticholinesterase, antifungal, anti-inflammatory, and immunomodulatory activities have been isolated so far (Ferreira *et al.* 2015, Ahmad *et al.* 2021, Rašeta *et al.* 2021, Łysakowska *et al.* 2023, Peng *et al*. 2024).

[1]Osmaniye Korkut Ata University. Faculty of Engineering and Natural Sciences. Department of Industrial Engineering. Osmaniye, Türkiye.

[♠]Corresponding author: aysenurgurgen@osmaniye.edu.tr

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As with all other natural resources, one of the most important steps in extracting bioactive components from *Ganoderma* mushrooms in the most efficient way is the extraction process (Zheng *et al.* 2020). It has been proven that the conditions of the *Ganoderma* extraction process affect the bioactivity of the extract (Raks *et al*. 2018, Chafouz *et al.* 2024, Zahmoul *et al.* 2024). Therefore, optimizing the extraction conditions and determining the bioactive activities of the extracts under these conditions are among the most important ways to achieve maximum efficiency from the extracts (Gil-Martín *et al.* 2022).

There are different methods used in optimization processes. For example, Response Surface Methodology (RSM) is a statistical approach used to optimize the design of experiments and to maximize response variables such as extraction yield, component concentration, and others (Mohamad *et al.* 2020). The optimization process aims to determine the optimal values of experimental factors to achieve a desired response variable, such as maximum extraction yield (Mohamad *et al.* 2020). Artificial intelligence (AI) techniques are among the applications that are increasingly used in optimization processes. Artificial neural networks (ANNs) are mathematical structures inspired by the functioning of biological neural networks and can model complex relationships (Waltersmann *et al.* 2021). Both methods can be used to optimize the extraction process and aim to achieve the best results based on experimental data. Metaheuristic algorithms are effective, nature-inspired methods for solving complex optimization problems. The Ant Lion Optimizer (ALO) algorithm, based on predator-prey dynamics, demonstrates high performance in achieving global optima and avoiding local solutions. Due to these features, ALO is widely preferred in engineering and scientific studies (Abualigah *et al.* 2021).

The choice of optimization method can vary depending on the application requirements, data type, and optimization goals. The aim of this study was to optimize the extraction conditions of *G. adspersum* mushroom and to determine the bioactive properties of the optimized extract. For this purpose, this study was designed in three steps: first, the experimental design was prepared, and experimental studies were carried out; second, the obtained data were optimized separately using RSM and ANN-ALO integration; and finally, the bioactive properties of the extracts prepared from the optimization results were determined, revealing which optimization method is more suitable for *G. adspersum*.

MATERIAL AND METHODS

G. adspersum mushroom was collected from Konyaaltı/Antalya, Türkiye.The experimental design was based on the I-optimal design. The I-optimal design is a statistical approach that ensures experiments are planned and conducted in the most efficient way possible. The total antioxidant status (TAS) of the obtained mushroom extracts was measured using Rel Assay TAS kits. Trolox was used as the calibrator for the kit analysis. TAS values were expressed as mmol Trolox equivalent/L (Erel 2004, Erel 2005).

Optimization using response surface method (RSM)

RSM is widely used in all areas where various input variables affect the performance measures of a product or process. These performance measures are referred to as responses. The input variables are called independent variables and are determined based on the objective of the experiment (Khuri and Mukhopadhyay 2010). In this study, the independent variables were extraction temperature, extraction time, and methanol/water ratio. The response was the antioxidant activity of the extract. Design Expert 13 software was used for optimizing the extraction process using Equation 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 of mushroom extract); x_i was coded process variables (x_1 was extraction temperature, x_2 was extraction time, and x_3 was methanol/water ratio) and β_{k0} is the value of fitted response at the design center point, respectively.

Modelling using artificial neural network (ANN)

The modeling process with ANN was carried out using MATLAB software. The Levenberg-Marquardt algorithm was chosen as the learning algorithm in the training process of the models, which were created using a single hidden layer. In the study, 15 different hidden neuron numbers (1:15) were tested to determine the optimal number of neurons. The learning coefficient and momentum coefficient were set to 0,5 the maximum number of iterations to 100, the number of validation checks to 50, and the error value to 1×10^{-6}. Additionally, 1000 different models were created for each number of neurons, and the optimal network was selected.

Optimization ant lion algorithm (ALO)

The foraging behavior of ant lion larvae inspired the ALO algorithm, which mimics the interaction between ant lions and ants (Mirjalili 2015). In this study, the ALO algorithm was implemented using MATLAB software to optimize the extraction process of *G. adspersum*.

The ALO algorithm was configured with a population size of 30 individuals and a maximum of 100 iterations. Key parameters, such as the random walk intensity and trapping boundary adjustment, were tuned to ensure efficient convergence. The algorithm's performance was validated through multiple runs to guarantee robust results.

Bioactivity of optimized extracts

Antimicrobial assays

The antimicrobial effects of the optimized *G. adspersum* extracts against the fungal and bacterial strains were determined using the agar dilution method. The minimum inhibitory concentration (MIC) values were defined as the lowest concentrations of the extracts that completely inhibited the growth of the standard bacterial and fungal strains. Muller Hinton Broth was used for culturing bacterial strains, while RPMI 1640 Broth was used for fungal strains. The mushroom extracts were tested at concentrations ranging from 12,5 to 800 µg/mL (Hindler *et al*. 1992, Bauer *et al.* 1966, Matuschek *et al.* 2014).

The standard bacterial strains used in the study included *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC 19606. The standard fungal strains included *Candida albicans* ATCC 10231, *Candida krusei* ATCC 34135, and *Candida glabrata* ATCC 90030.

Anticholinesterase activity test

The anticholinesterase activity (acetylcholinesterase and butyrylcholinesterase) of the optimized *G. adspersum* extracts was measured using the Ellman method (Ellman *et al.* 1961). Stock solutions of the mushroom extracts were prepared at concentrations ranging from 3,125 to 200 µg/mL.

For the assay, 130 µL of 0,1 M phosphate buffer (pH = 8), 10 µL of the stock solution, and 20 µL of enzyme solution (AChE or BChE) were added to the microplate. The mixture was incubated at 25 C in the dark for 10 minutes. After incubation, 20 µL of DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)) solution and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added. The absorbance was measured at 412 nm. The IC50 values were calculated from triplicate measurements and expressed in µg/mL.

Total phenolic and flavonoid assays

A stock solution of the optimized mushroom extracts was prepared at a concentration of 1 mg/mL. For the assay, 250 µL of this solution was mixed with 1 mL of Folin-Ciocalteu reagent (1:9, v/v). Subsequently, 0,75 mL of 1 % Na_2CO_3 was added, and the mixture was incubated at room temperature for 2 hours. The absorbance was measured at 760 nm. The total phenolic content (TPC) was calculated using a gallic acid standard calibration curve and expressed in mg/g (Bal *et al.* 2023).

The total flavonoid content (TFC) of the optimized mushroom extracts was determined using the aluminum chloride assay. For the assay, 0,1 mL of 10 % $Al(NO_3)_3$, 0,1 mL of 1 M NH_4CH_3COO, 4,3 mL of methanol, 0,5 mL of quercetin standard solution, and 0,5 mL of mushroom extract were mixed. The mixture was incubated for 40 minutes at room temperature, and the absorbance was measured at 415 nm. The total flavonoid content was calculated using a quercetin standard calibration curve and expressed in mg/g (Kormaz *et al.* 2023).

Phenolic analysis

The phenolic compound profile of the optimized *G. adspersum* extracts was analyzed using the LC-MS/MS technique. In this analysis, 24 different standard compounds present in the extracts were identified and quantified. The separation of the compounds was carried out at 40 C using a C-18 Intersil ODS-4 analytical column (3,0 mm × 100 mm, 2 µm). The mobile phase consisted of water containing 0,1% formic acid (Phase A) and methanol containing 0,1% formic acid (Phase B). The flow rate was set to 0,3 mL/min, and the injection volume was 2 µL for the analysis.

Statistical analysis

In this study, the SPSS 21 for Windows program was used for the statistical analysis of all data. Analysis of Variance (ANOVA) was performed to determine the differences between the groups in the studied tests. The Duncan test was applied at a confidence level of $\alpha = 0,05$ to identify significant differences between the groups.

RESULTS AND DISCUSSION

Experimental studies

TAS values of *G. adspersum* extracts were given in Table 1.

Table 1: TAS values of *G. adspersum* extracts.

Extraction temperature (°C)	Extraction time (h)	Methanol/water ratio (%)	TAS (mmol/L)
56,52	2,15	55	6,699fg*
56,80	5,30	71,88	6,466de
51,25	8	100	7,370k
56,50	5,30	2	6,306cd
40	2	0	5,426a
56,52	2,15	55	7,038ij
56,50	5,30	2	6,227c
40	8	0	7,198j
40	2	100	6,910hi
56,50	5,30	2	6,549ef
51,55	7,98	41,67	7,106j
70	4,36	40,40	6,909hi
70	2	0	6,663fg
70	7,99	100	6,709fg
41,50	2,30	50	5,921b
70	8	37,15	6,431de
56,80	5,30	71,88	6,495e
70	4,23	100	6,787gh
40,45	5,30	55	8,022l
40,45	5,30	55	8,081l

*Different letters indicate that the difference between the groups is statistically significant ($p<0,05$).

Among the extracts obtained, the highest total antioxidant status (TAS) value was observed in the extracts obtained at 40 °C, 45 °C; 5 h,30 h, and 55 % methanol/water ratio, with a value of 8,081 mmol/L. Conversely,

the lowest TAS value was found in the extracts obtained at 40 °C; 2 h, and 0 % methanol/water ratio, with a value of 5,426 mmol/L. Statistical analysis of all results revealed a statistically significant difference between the groups ($p < 0,05$). Therefore, it was concluded that the extraction conditions significantly affected the antioxidant activity of the extracts.

Optimization

RSM

In the I-optimal design applied for experimental studies to determine the total antioxidant activity value of *G. adspersum* mushroom, linear, quadratic, cubic, and two-factor interaction models were examined. The summary of the fit is presented in Table 2.

Table 2: Fit summary.

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0,1641	0,0018	0,1291	-0,1551	
2FI	0,0229	0,0051	0,4724	0,2586	Suggested
Quadratic	0,2148	0,0057	0,5527	-0,0159	
Cubic	0,0057		0,9408		

As a result of the analysis, the two-factor interaction (2FI) model was identified as the best model for explaining the extraction conditions. The proposed model is provided in Equation 2.

$$R = 6,8518 - 0,2726A + 0,2278B + 0,4250C - 0,5851AB - 0,2708AC - 0,0487BC \quad (2)$$

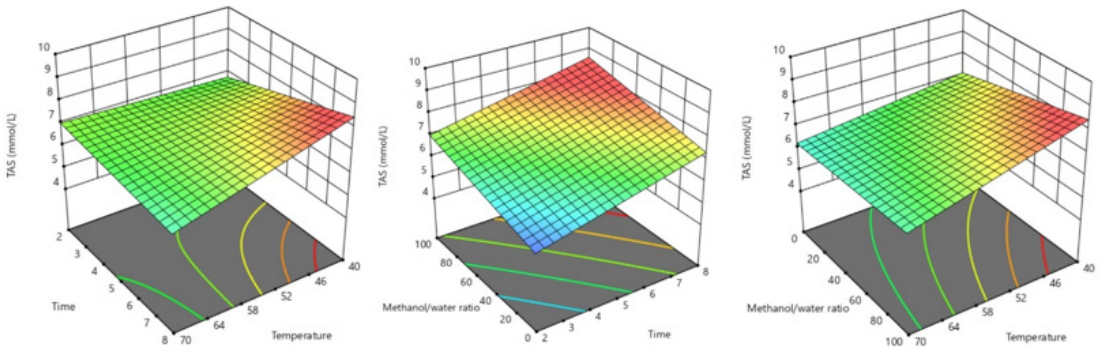

Figure 1: Response surface plots of total antioxidant status of *G. adspersum* mushroom

The response surface graphs obtained from the analysis are presented in Figure 1. In this study, which investigated the effect of extraction conditions on the total antioxidant activity of *G. adspersum* mushroom, the methanol/water ratio was found to have the highest effect, and this effect was statistically significant ($p < 0,05$). The optimum extraction conditions estimated by the RSM method were 41,671 °C, 7,309 h and 87,163 % methanol/water ratio, with a predicted total antioxidant activity of 8,175 mmol/L.

ANN-ALO

Among the models established, the architecture of the best ANN model was determined to consist of 3 input neurons, 10 hidden neurons, and 1 output neuron. The performance of the best model is presented in Table 3. The mean squared error (MSE) values were calculated as 0,0087; 0,018 and 0,020 for the training, validation, and test datasets, respectively. For the entire dataset, the MSE value was 0,010.

Table 3: Performance of optimum model.

	Training	Validation	Test	All
MSE	0,0087	0,018	0,020	0,010
R	0,990	1	1	0,986
MAPE	0,990	1,855	1,628	1,140

The MSE values approaching 0 and R values approaching 1indicate the most successful model parameters among the established models. The R value of the selected model was calculated as 0,986 for the entire dataset and is presented in Figure 2.

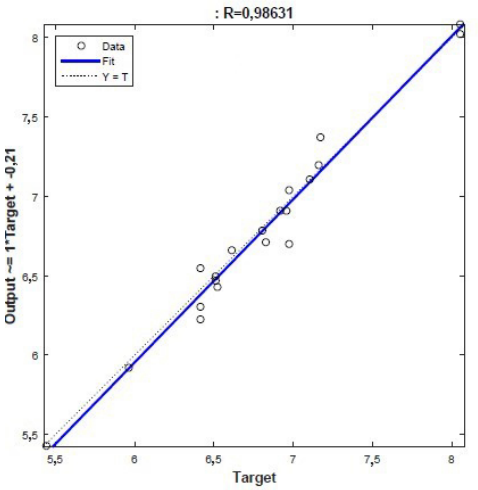

Figure 2: Regression plot.

The MAPE value is a metric that reflects the prediction accuracy of the model. A MAPE value of 5 indicates that the model predicts with a 5 % error. The MAPE value of the selected model was calculated as 1,140 for the entire dataset, demonstrating that the model has the ability to predict with a very low percentage of error.

The selected model was used for the optimization process. The objective function in this study was to maximize the TAS value. The optimization process was performed using the ALO algorithm, and the optimization course is presented in Figure 3. In the 1st iteration, the model predicted the TAS value as 7,851 and after subsequent improvements, it consistently provided the same value starting from the 5th iteration. This indicates that the number of iterations chosen for the model was sufficient.

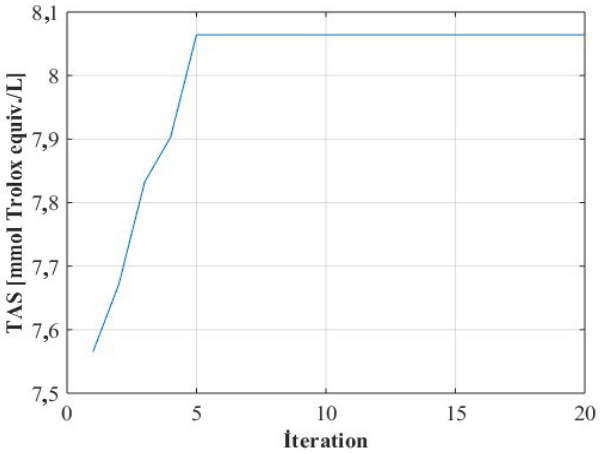

Figure 3: Optimization course.

As a result of the ANN-ALO integration, the optimum extraction conditions were determined to be 42,207 °C; 5,680 h and 53,634 % methanol/water ratio, with a predicted TAS value of 8,089 mmol/L.

Bioactivity of optimized extracts

Antioxidant activity

Oxidative stress occurs when the antioxidant defense system in the human body is insufficient. As a result of oxidative stress, serious diseases such as cancer, diabetes, obesity, respiratory diseases, cardiovascular diseases, and neurodegenerative disorders can develop (Raut and Khullar 2023). Supplemental antioxidants can be used to mitigate the effects of oxidative stress (Sevindik *et al.* 2020). In this study, the antioxidant potential of *G. adspersum* was determined, and the optimum extract concentrations were analyzed. The findings are presented in Table 4.

Bioactivity tests were conducted on three different optimized extracts. These samples were obtained from:

1. The extraction conditions that provided the highest TAS value in the experimental study,
2. The optimum extraction conditions predicted by the RSM method, and
3. The extraction conditions predicted by the ANN-ALO integration.

The TAS, total oxidant status (TOS), and oxidative stress index (OSI) values of the optimized *G. adspersum* extracts are presented in Table 4.

Table 4: TAS, TOS and OSI values of optimized extracts *G. adspersum.*

Extract number	Source	TAS (mmol/L)	TOS (mmol/L)	OSI	TPC (mg/g)	TFC (mg/g)
1	Experimental study	8,150 ± 0,063b*	8,308 ± 0,036a	0,098 ± 0,001a	92,19 ± 1,45b	64,87 ± 1,26b
2	RSM	8,017 ± 0,074a	10,230 ± 0,090c	0,122 ± 0,001c	78,79 ± 0,81a	53,38 ± 0,91a
3	ANN-ALO	8,199 ± 0,054c	8,632 ± 0,030b	0,102 ± 0,001b	95,37 ± 1,00c	68,75 ± 0,36c

*Different letters indicate that the difference between the groups is statistically significant ($p<0,05$).

It has been reported that *G. adspersum* exhibits antioxidant activity using different methods in previous studies (Raks *et al.* 2018, Chafouz *et al.* 2024). In this study, the TAS, TOS, and OSI values of *G. adspersum* were determined for the first time using Rel Assay kits. The results showed that the highest TAS value was detected in Extract 3, while the highest TOS and OSI values were detected in Extract 2.

In previous studies investigating the antioxidant activity of wild mushrooms, the TAS values of *Hericium erinaceus*, *Hebeloma sinapizans*, *Candolleomyces candolleanus*, and *Octaviania asterosperma* were reported as 5,426; 4,540; 5,547 and 3,410 mmol/L, respectively. Their TOS values were reported as 6,621; 10,303; 8,752 and 7,548 µmol/L, and their OSI values were reported as 0,122; 0,227; 0,155 and 0,221 respectively (Sevindik *et al.* 2021, Karaltı *et al.* 2022, Ahmad *et al.* 2023, Sevindik *et al.* 2024).

The three different extracts of *G. adspersum* used in this study exhibited higher TAS values compared to *H. erinaceus*, *H. sinapizans*, *C. candolleanus*, and *O. asterosperma*. The TAS value is an indicator of the totality of antioxidant compounds within natural products (Mohammed *et al.* 2020). The antioxidant potential of the extracts obtained in this study was found to be high.

The TOS value reflects the totality of oxidizing compounds produced in natural products (Mohammed 2020). The TOS values of Extract 1 and Extract 3 were determined to be higher than those of *H. erinaceus* and *O. asterosperma* but lower than those of *H. sinapizans* and *C. candolleanus*. This indicates that the oxidant levels of the different *G. adspersum* extracts used in this study were within normal ranges.

The OSI value represents the percentage dominance of oxidant-effective compounds over antioxidant effective compounds in natural products (Mohammed 2020). Both Extract 1 and Extract 3 exhibited lower OSI values compared to *H. erinaceus*, *H. sinapizans*, *C. candolleanus*, and *O. asterosperma*.

It has been observed that the *G. adspersum* extracts produced as a result of optimization exhibit significant potential in scavenging oxidant compounds.

Total phenolic contents

Phenolic compounds and flavonoids are responsible for many biological activities. In this study, the total phenolic and flavonoid contents of the three optimized extracts of *G. adspersum* were determined and are presented in Table 4. The highest total phenolic and flavonoid contents were found in Extract 3, followed by Extract 1 and Extract 2, respectively.

In previous studies, the TPC of petroleum ether, dichloromethane, and methanol extracts of *G. adspersum* was reported to range from 38,06 to 67,87 mg/g, while the TFC ranged from 20,79 to 43,41 mg/g (Chafouz *et al.* 2024). Compared to these findings, the extracts in the present study exhibited higher total phenolic and flavonoid contents. This difference is attributed to the fact that the extracts in this study were produced under optimized conditions that maximize biological activities.

Anticholinesterase activity

There has been a significant increase in the number of oxidative stress-induced diseases. Alzheimer's disease, which is particularly common in individuals over the age of 65, is becoming increasingly prevalent. It is estimated that more than 80 million people may be affected by Alzheimer's disease soon (Reitz *et al.* 2023). Dietary supplements play a crucial role in mitigating the effects of oxidative stress. In this study, the anticholinesterase activity of *G. adspersum* extracts obtained under different conditions was determined. The findings are presented in Table 5.

Table 5: Anti-AChE and anti-BChE values of optimized extracts *G. adspersum*

Extract number	Source	AChE µg/mL	BChE µg/mL
1	Experimental study	28,59 ± 0,80c	39,52 ± 0,56c
2	RSM	34,11 ± 0,60d	46,83 ± 0,41d
3	ANN-ALO	25,93 ± 0,62b	32,29 ± 0,76b
-	Galantamine	5,62 ± 0,18a	16,76 ± 0,32a

*Different letters indicate that the difference between the groups is statistically significant ($p<0,05$).

Extract 3 exhibited the highest anticholinesterase activity, followed by Extract 1 and Extract 2, respectively. Compared to galantamine, which was used as a standard, the three different extracts in this study demonstrated lower anticholinesterase activity. In previous studies, *G. adspersum* was reported to have acetylcholinesterase activity ranging from 15,29 to 430,93 µg/mL and butyrylcholinesterase activity ranging from 19,97 to 75,30 µg/mL (Tel-Çayan *et al.* 2015). The anticholinesterase activities of all three extracts used in this study were found to be consistent with the data reported in the literature.

Determining the presence of enzymes involved in the etiology of diseases is crucial. By suppressing these disease-causing enzymes, it may be possible to develop effective strategies for combating diseases (Świątek *et al.* 2021). Acetylcholinesterase and butyrylcholinesterase activities of the studied mushroom extracts were determined to be high, indicating that *G. adspersum* can be utilized as a natural product with potential therapeutic applications.

Antimicrobial activity

In recent years, the increase in the number of diseases caused by microorganisms has become a growing concern for humanity. The rise in antibiotic-resistant microorganisms, particularly due to the irresponsible use of antibiotics, has prompted researchers to explore new antimicrobial agents. In this context, researchers have turned their attention to natural antimicrobial compounds due to the potential side effects of synthetic drugs (Ayon 2023).

Mushrooms are among the most important natural products in this regard. In this study, the antimicrobial activity of three different extracts of *G. adspersum* was determined. The minimum inhibitory concentration (MIC) values obtained are presented in Table 6.

Table 6: MIC values of *Ganoderma adspersum's* optimized extracts.

Extract number	Source	*S. aureus*	*S. aureus* MRSA	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A. baumannii*	*C. glabrata*	*C. albicans*	*C. krusei*
1	ES*	25**	25	100	50	100	100	50	50	100
2	RSM	50	50	100	50	200	100	100	200	200
3	ANN-ALO	25	25	100	50	100	100	50	100	100

*ES: Experimental study **25, 50, 100, 200 µg/mL represents the lowest concentration that inhibits the growth of microorganisms.

In this study, the antimicrobial effects of three different *G. adspersum* extracts against standard bacterial and fungal strains were investigated. According to the findings, extract 1 and extract 3 exhibited almost similar activity, while extract 2 was found to be less effective. The antimicrobial activity findings can be summarized as follows:

Extracts 1 and 3 were effective against *S. aureus* and *S. aureus MRSA* at a concentration of 25 µg/mL, while extract 2 was effective at 50 µg/mL. All extracts were effective against *E. faecalis* and *A. baumannii* at a concentration of 100 µg/mL. Against *E. coli*, all extracts were effective at a concentration of 50 µg/mL. Against *P. aeruginosa* and *C. krusei*, extracts 1 and 3 were effective at a concentration of 100 µg/mL, while extract 2 was effective at 200 µg/mL. Against *C. glabrata*, extracts 1 and 3 were effective at a concentration of 50 µg/mL, while extract 2 was effective at 100 µg/mL. Against *C. albicans*, extract 1 was effective at a concentration of 50 µg/mL, extract 2 at 200 µg/mL, and extract 3 at 100 µg/mL.

In a previous study, the petroleum ether, dichloromethane, and methanol extracts of *G. adspersum* were reported to be effective against *S. aureus, K. pneumoniae, E. coli, Proteus vulgaris, Proteus mirabilis, Bacillus subtilis, C. albicans,* and *Aspergillus niger* (Chafouz *et al.* 2024). In this study, the effects of *G. adspersum* extracts produced under optimum conditions were investigated against test microorganisms. In addition to the microorganisms reported in the literature, the extracts were found to be effective against the bacterial and fungal strains used in this study. As a result, it was determined that *G. adspersum* extracts produced under optimum conditions exhibit strong antimicrobial activities.

Phenolic contents

Secondary metabolites are recognized as medically important bioactive compounds that do not possess nutritional properties. Mushrooms produce a wide range of secondary metabolites in their structures (Łysakowska *et al.* 2023). In this study, the phenolic contents of *G. adspersum* extracts produced under optimum conditions that exhibit the highest biological activity were determined. The findings are presented in Table 7.

Table 7: Phenolic contents of optimized extracts *G. adspersum.*

Phenolic compounds (mg/kg)			
Extract number	1	2	3
Source	Experimental study	RSM	ANN-ALO
Vanillic acid	2339,10	1968,73	3164,92
Syringic acid	782,06	316,41	645,87
Kaempferol	None	None	96,73
Fumaric acid	13954,91	13421,62	13714,54
Gallic acid	10287,42	8145,75	11950,42
Protocatechuic acid	5389,54	5391,66	5469,57
4-hydroxybenzoic acid	1001,25	209,84	1647,15
Caffeic acid	373,28	None	200,19
2-hydoxycinamic acid	None	None	599,61
Quercetin	3496,57	4019,55	3628,62

In this study, the phenolic contents of *G. adspersum* extracts produced under optimum conditions were determined. The presence of vanillic acid, syringic acid, fumaric acid, gallic acid, kaempferol, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, 2-hydroxycinnamic acid, and quercetin was identified in these mushroom extracts. Kaempferol was detected only in Extract 3, while caffeic acid was found in extract 1 and extract 3. Additionally, 2-hydroxycinnamic acid was detected only in extract 3. As a result of the analysis, the highest concentration of fumaric acid was identified in *G. adspersum*.

Previous studies have reported that *G. adspersum* contains fumaric acid, caffeic acid, 2,4-dihydroxybenzoic acid, ferulic acid, coumarin, ellagic acid, and rosmarinic acid (Tel-Çayan *et al.* 2015). In another study, it was reported that *G. adspersum* contains gallic acid, protocatechuic acid, 3,4-dihydroxyphenylacetic acid, and p-hydroxybenzoic acid (Sułkowska-Ziaja *et al.* 2022). The phenolic contents of *G. adspersum* extracts produced under optimum conditions that exhibit the highest biological activity were determined. In this context, it was concluded that *G. adspersum* may serve as a natural source of the detected compounds, and its strong biological activities are likely due to the presence of these compounds in its structure.

CONCLUSIONS

This study clearly demonstrates the significant impact of extraction conditions on the bioactive properties of the extracts. One of the key findings is that the optimization method employed also plays a critical role in determining these properties. Through the optimization of *G. adspersum* under the studied conditions, the extraction conditions suggested by the ANN-ALO integration exhibited superior bioactive properties compared to those suggested by the RSM method. These results highlight the importance of advanced optimization techniques in enhancing the extraction efficiency and bioactivity of natural compounds.

Practically, the findings have promising implications for industries focused on pharmaceuticals, functional foods, and natural preservatives. The superior bioactive properties-particularly antioxidant, antimicrobial, and anticholinesterase activities-of the extracts optimized using the hybrid AI model underscore the potential of *G. adspersum* as a valuable source of natural bioactive compounds. These results suggest that applying advanced AI-based optimization techniques can significantly improve the efficiency and effectiveness of bioactive compound extraction, facilitating the development of high-value natural health products and contributing to the broader use of sustainable and eco-friendly extraction processes.

Authorship contributions

A. G.: Investigation, methodology, formal analysis, writing - review & editing

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