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Extract optimization of *Ulva lactuca* L. and biological activities of optimized extracts

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Abstract

Background Algae are microscopic or macroscopic organisms that can photosynthesize and are found in both freshwater and saltwater ecosystems and are considered one of the cornerstones of life. In our study, the biological activities of extracts produced under the extract conditions that provided the highest biological activity of *Ulva lactuca* L. were determined.

Methods Two different methods, Response Surface Method (RSM) and Artificial Neural Network-Genetic Algorithm (ANN-GA) integration were used for optimization. In this context, the optimum extract conditions were determined as 54.940 ˚C temperature, 6.513 h, 42.109 ethanol/water ratio according to the RSM method and 56.286 ˚C temperature, 7.2793 h, 36.8625 ethanol/water ratio according to ANN-GA. The antioxidant activities of the optimized extracts were evaluated by Rel Assay kits, DPPH and FRAP methods. Anticholinesterase activities were determined by testing against acetylcholinesterase and butyrylcholinesterase enzymes. In addition, antiproliferative effects were examined in A549 lung cancer cell line and phenolic compound contents were analyzed by LC-MS/MS.

Results As a result of the analyzes, it was seen that the extract obtained from the conditions recommended by ANN-GA exhibited higher activities. Optimized extracts of *U. lactuca* were found to have potential in terms of antioxidant activities. The highest total antioxidant value was determined as 6.272 ± 0.024 mmol/L. In addition, it was determined that extracts of *U. lactuca* produced under optimum conditions showed strong cytotoxic effects against A549 lung cancer cell line. In addition, gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid, quercetin and kaempferol were found in the extracts of the algae produced under optimum conditions. The highest detected compound was caffeic acid. In addition to all these properties, it was seen that *U. lactuca* has anticholinesterase potential in our study.

Conclusion In conclusion, optimized extracts of *U. lactuca* attract attention with their antioxidant and cytotoxic activities as well as anticholinesterase potential and can be considered as a potential source for cancer treatment and management of neurological diseases.

Keywords Ulva, Seaweed, Antioxidant activity, Anticholinesterase activity, Anticancer activity

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Introduction

In addition to being a rich source of nutrients for health, seaweeds are also of interest as an environmentally friendly and sustainable food alternative. In terms of nutrition, they are beneficial to human health due to their protein, dietary fiber, vitamins (especially B12, C and E) and minerals (iodine, magnesium, calcium) content. In addition, they have antioxidant and anti-inflammatory properties thanks to bioactive components such as polyphenols and flavonoids. These properties make seaweed not only a part of a healthy diet, but also a valuable source for functional foods and pharmaceutical products [1, 2]. In recent years, antimicrobial and anticancer effects of seaweeds have also been emphasized. For example, extracts of *Ulva lactuca* and *Gracilaria* spp. species have shown potential in the prevention and treatment of various diseases. In addition, the use of bioactive compounds obtained from algae in food supplements, pharmaceuticals and cosmetics is rapidly increasing [3, 4]. In addition, Nori (*Porphyra* spp.), Wakame (*Undaria pinnatifida*) and Kombu (*Laminaria* spp.) are among the species commonly consumed in Asian cuisine. These species are used in dishes such as soup, salad and sushi, and are also processed as gelling agents and stabilizers in the food industry [5, 6]. In this context, in our study, the antioxidant, anticholinesterase and antiproliferative activities of the extracts produced under optimum conditions that maximize the biological activities of *U. lactuca* and the phenolic contents of these extracts were determined.

Ulva lactuca is a type of green algae commonly found in marine ecosystems, known as "sea lettuce". This alga, which has a thin, wavy leaf-like structure, usually grows to 20–30 cm in length and is easily recognized by its bright green color. It grows on seashores, especially in tidal areas, clinging to stones and rocks. It is widely found in tropical and temperate climates and is important for ecological balance. This photosynthetic algae is an important part of the food chain in aquatic ecosystems and also attracts attention with its various biological activities [7, 8]. *U. lactuca*, which is very rich in terms of nutritional value, stands out as part of a healthy diet with its high protein and fiber content. It is especially rich in minerals such as vitamin C, vitamin B12, iodine, magnesium and calcium. With these properties, it offers health benefits such as strengthening the immune system, supporting digestion and regulating energy metabolism [9, 10]. This algae is used in both food and biotechnological applications. *U. lactuca*, which is frequently preferred in soups, salads and spices in Asian cuisine, is a sustainable food source. It also contributes to the agricultural sector by being used as organic fertilizer and animal feed. It is also evaluated in environmentally friendly applications such as biofuel production and wastewater treatment [11]. Recent studies have shown that this species contains bioactive substances such as phenolic compounds, polyphenols and flavonoids, and that these substances have antioxidant, antimicrobial and anticancer properties. Therefore, the importance of *U. lactuca* is increasing in terms of both health and industrial potential [10].

The aim of this study was to optimize the extraction conditions of *U. lactuca* L. to determine the biological activities of its extracts, focusing on antioxidant, anticholinesterase, and antiproliferative effects, as well as identifying the phenolic compounds present, in order to assess its potential for cancer treatment and neurological disease management.

Materials and methods

The algae samples used in the study were collected from İskenderun (Turkey). The algae samples was identified by Dr. Nuh Korkmaz (N.K-227). The moss samples are kept in the Laboratory of the Biology Department of Osmaniye Korkut Ata University.

Extraction procedure method

The extraction process was designed following a full factorial experimental approach, with three parameters—extraction temperature, extraction time, and ethanol/water ratio—evaluated at three levels each. A total of 27 experiments were conducted using the Gerhardt SOX-414 device under varying conditions: extraction temperatures of 45 °C, 55 °C, and 65 °C; extraction times of 5, 10, and 15 h; and ethanol/water ratios of 0%, 50%, and 100%. The resulting data were optimized using both the Response Surface Method (RSM) and an artificial intelligence-based approach integrating Artificial Neural Networks and Genetic Algorithms (ANN-GA).

RSM

In this study, optimization was carried out using the Response Surface Method (RSM). The independent variables included extraction temperature, extraction time, and ethanol/water ratio, while the response variable was the total antioxidant activity (TAS) of the extract. The optimization process utilized Design Expert 13 software, employing a second-order polynomial response model.

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

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 model's adequacy was assessed through the coefficient of determination (R^2), ANOVA analysis, and *p*-values. Critical points were identified by analyzing the model's derivatives to optimize the response variable. Furthermore, three-dimensional surface plots were created to illustrate the impact of the independent variables, offering a clearer insight into their influence on the response.

ANN-GA

The Artificial Neural Network (ANN) method was employed for modeling, with extraction temperature, extraction time, and ethanol/water ratio as inputs, and the TAS value as the output. Experimental data were split into 80% for training, 10% for validation, and 10% for testing. The Levenberg-Marquardt (LM) algorithm was utilized for the training process. To identify the optimal network, 20 different hidden neuron configurations (1 to 20) were evaluated. The learning rate and momentum coefficient were set to 0.5, the maximum number of iterations was fixed at 500, validation checks were capped at 50, and the error threshold was defined as 1×10^{-5}. Each model underwent 1000 training sessions. Performance of the developed models was assessed using mean square error (MSE) and mean absolute percentage error (MAPE), calculated using Eq. 1 and Eq. 2.

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

$$\mathrm{MAPE} = \frac{1}{\mathrm{n}} \sum \left| \frac{\mathrm{e_i} - \mathrm{p_i}}{\mathrm{e_i}} \right| * 100 \tag{2}$$

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

Optimization was carried out using the Genetic Algorithm (GA). Various population sizes were tested, and the roulette wheel selection method was employed. Single-point crossover was used as the crossover technique. The optimal number of iterations was identified by examining the convergence graphs. To ensure results closely approximated the global optimum, each optimization run was repeated at least 60 times.

Extractions for bioactivity

Extracts providing the highest level of biological activity of algae samples were obtained by taking into account optimum production conditions. In this context, ideal conditions were determined as 54.940 ˚C temperature, 6.513 h and 42.109 ethanol/water ratio according to RSM method; optimum set was determined as 56.286 ˚C temperature, 7.2793 h and 36.8625 ethanol/water ratio according to ANN-GA integration. The conditions closest to these determined parameters were optimized using Gerhardt SOX-414 device (Germany) and in a computer-aided environment. The obtained extracts were used as the basis in carrying out biological activity tests.

Phenolic analysis

Phenolic content of optimized algae extracts was investigated using Shimadzu LC-MS/MS-8030. During the analysis, extracts were screened for 24 different standard compounds. Separation was carried out using C-18 Intersil ODS-4 analytical column (3.0 mm x 100 mm, 2 µm) kept at 40 °C. Water containing 0.1% formic acid (Phase A) and methanol containing 0.1% formic acid (Phase B) were preferred as mobile phases. The flow rate was determined as 0.3 mL/min and the injection volume of the samples was set as 2 µL.

DPPH free radical scavenger activity test

Stock solutions of algae extracts were prepared using DMSO at a concentration of 1 mg/mL. 1 mL of these solutions were mixed with 160 µL of 0.267 mM DPPH solution (4 mL in total, containing 0.004% methanol). The mixture was incubated at room temperature in a dark environment for 30 min. At the end of the period, the absorbance of the solution was measured at 517 nm using a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). The results were expressed as milligrams of Trolox Equivalent (mg TE/g) per gram of extract. Analyses were performed in three replicates [12].

Ferric Reducing Antioxidant Power (FRAP) test

A 100 µL stock solution was prepared from the optimized algae extracts. This solution was mixed with 2 mL of FRAP reagent. FRAP reagent was obtained by mixing 20 mM $FeCl_3$ 6 H_2O solution prepared with 300 mM acetate buffer (pH 3.6), 40 mM HCl, and 10 mM 2,4,6-tris(2-pyridyl)-S-triazine (TPTZ) solution at a ratio of 10:1:1. The mixture was incubated at 37 °C for 4 min. After the process, absorbance measurements were carried out at 593 nm using a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan). The results were expressed in milligrams of Trolox Equivalent per extract (mg TE/g). Analyses were performed in three replicates [12].

Total antioxidant and oxidant status analysis

The total antioxidant status of optimized algae extracts was determined using the Rel Assay TAS kit (Rel Assay Diagnostics, Megatıp, Gaziantep, Turkey). The measurement results were expressed in mmol Trolox equivalent/L. Similarly, the total oxidant status was analyzed with the Rel Assay TOS kit and the results were recorded as µmol hydrogen peroxide equivalent/L. The

oxidative stress index (OSI) value was calculated by dividing the TOS values by the TAS values and converting them into percentages. Analyses were performed in three replicates [13–15].

Antiproliferative activity tests

The antiproliferative effect of optimized algae extracts was evaluated in A549 lung cancer cell line. For this purpose, stock solutions were prepared at concentrations of 25, 50, 100 and 200 µg/mL. After 70–80% confluence of the cells was achieved, the cells were dissociated with 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) and transferred to culture plates. The plates were incubated for 24 h to settle the cells. Then, the prepared extract solutions were applied to the cells and incubated once more for 24 h. After the process, the supernatants were replaced with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (1 mg/mL) and incubated at 37 °C until purple precipitate formation was observed. The MTT solution was then dissolved with dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) and absorbance values were measured at 570 nm wavelength using Epoch spectrophotometer (BioTek Instruments, Winooska, VT). Analyses were performed in three replicates [16].

Anticholinesterase activity test

Anticholinesterase activities of optimized algae extracts were analyzed based on the Ellman method [17]. In this study, galantamine was used as a reference substance. Various stock solutions were prepared from algae extracts with concentrations ranging from 200 to 3.125 µg/mL. For the test, 130 µL of 0.1 M phosphate buffer (pH = 8) was added to the microplate, followed by 10 µL of stock solution and 20 µL of AChE or BChE enzyme solution, respectively. The mixture was incubated at 25 °C in the dark for 10 min. Then, 20 µL of DTNB solution (5,5′-dithiobis-(2-nitrobenzoic acid)) and 20 µL of substrate solution (acetylcholine iodide or butyrylcholine iodide) were added. Absorbance measurements were carried out at a wavelength of 412 nm. Using the obtained data, IC50 values of enzyme inhibition rates were calculated and expressed in µg/mL units. Analyses were performed in three replicates.

Statistical analysis

Analyses were performed in three replicates. All statistical analyses in this study were performed using the 'SPSS 21.0 for Windows' software. For comparisons involving multiple groups, a One-Way Analysis of Variance (ANOVA) was used to assess differences among them. The Duncan test was then applied at a significance level of $\alpha = 0.05$ to identify specific group differences.

Results and discussions

Optimization of extraction conditions

TAS values obtained after the experimental study are given in Table 1.

This study investigated the impact of extraction temperature, time, and ethanol/water ratio on the total antioxidant activity (TAS) of *U. lactuca* kernel extracts. Extraction temperature had a significant effect on TAS values, with the lowest values observed at 45 °C, the highest at 55 °C, and lower values again at 65 °C. In terms of extraction time, 10 h generally yielded the highest TAS values, while 15 h resulted in a decrease in TAS. The ethanol/water ratio was also a crucial factor, with the highest TAS values obtained at a 50% ethanol ratio, while pure ethanol and pure water produced lower TAS values. In conclusion, the optimal conditions were found to be 55 °C temperature, 10 h extraction time, and a 50% ethanol ratio, suggesting that these parameters provide the best optimization for the antioxidant capacity of *U. lactuca* extracts.

Table 1 TAS values of the extracts obtained in the study

Experiment number	Extraction temperature (°C)	Extraction time (h)	Ethanol/water ratio (%)	TAS (mmol/L)
1	45	5	0	3.723 ± 0.029^{c}
2	45	10	0	5.038 ± 0.057^{g}
3	45	15	0	3.184 ± 0.021^{a}
4	45	5	0	3.739 ± 0.023^{c}
5	45	10	0	5.051 ± 0.033^{g}
6	45	15	0	3.173 ± 0.036^{a}
7	45	5	0	3.730 ± 0.038^{c}
8	45	10	0	5.053 ± 0.040^{g}
9	45	15	0	3.163 ± 0.031^{a}
10	55	5	50	5.642 ± 0.022^{h}
11	55	10	50	6.256 ± 0.029^{j}
12	55	15	50	4.854 ± 0.026^{f}
13	55	5	50	5.653 ± 0.026^{h}
14	55	10	50	6.272 ± 0.024^{j}
15	55	15	50	4.836 ± 0.015^{f}
16	55	5	50	5.646 ± 0.032^{h}
17	55	10	50	6.240 ± 0.026^{j}
18	55	15	50	4.858 ± 0.034^{f}
19	65	5	100	4.028 ± 0.018^{d}
20	65	10	100	4.267 ± 0.013^{e}
21	65	15	100	3.579 ± 0.017^{b}
22	65	5	100	4.053 ± 0.035^{d}
23	65	10	100	4.235 ± 0.022^{e}
24	65	15	100	3.557 ± 0.040^{b}
25	65	5	100	4.040 ± 0.025^{d}
26	65	10	100	4.237 ± 0.016^{e}
27	65	15	100	3.552 ± 0.019^{b}

[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

In this study, two distinct optimization methods were employed using data obtained from experimental studies. For the optimization conducted with the Response Surface Method (RSM), linear, 2FI, quadratic, and cubic regression models were developed, with the quadratic model being selected due to its highest R^2 value. The R^2 value indicates the model's effectiveness in explaining the dependent variable's response based on the independent variables. A high R^2 value demonstrates the model's suitability. In this study, the R^2 of the model was found to be 0.853.

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

$$\begin{aligned} TAS =& 6.26 - 0.017\,X_1 - 0.002\,X_2 \\ & - 0.305\,X_3 - 0.003\,X_1X_2 + 0.019\,X_1X_3 \\ & - 0.005\,X_2X_3 - 1.62\,X_1^2 - 0.002\,X_2^2 - 1.02\,X_3^2 \end{aligned}$$

In the equation X_1, X_2 ve X_3 represent extraction temperature, extraction time and ethanol/water ratio, respectively. Response surface plots of TAS of *U. lactuca* were shown at Fig. 1.

Among the extraction conditions investigated, the TAS value of *U. lactuca* extracts was most significantly affected by the extraction temperature and ethanol/water ratio (statistically significant at $p < 0.05$), while the extraction time had a lesser effect. According to RSM optimization, the optimal conditions were predicted to be a

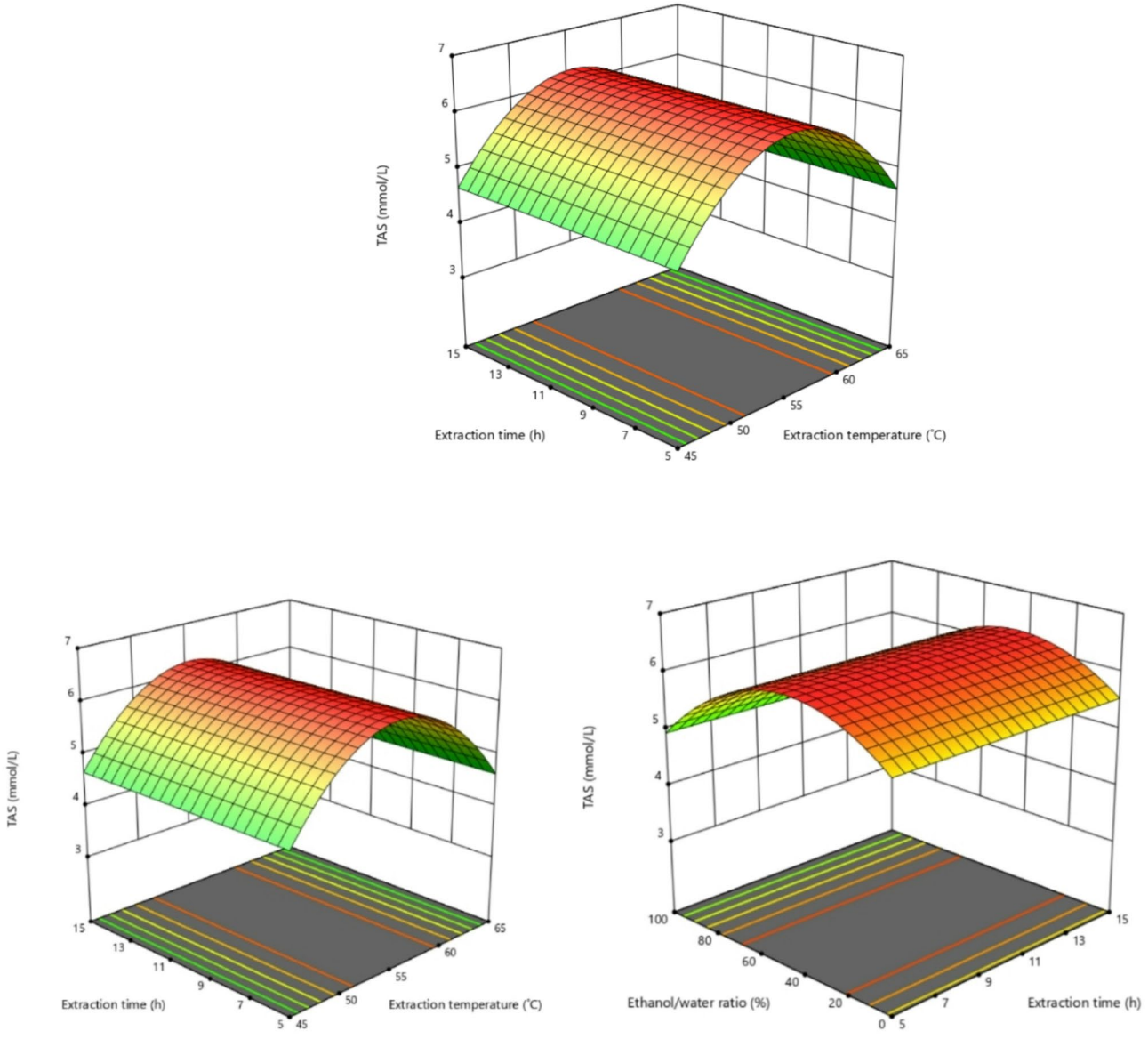

Fig. 1 Response surface plots of TAS of *Ulva lactuca*

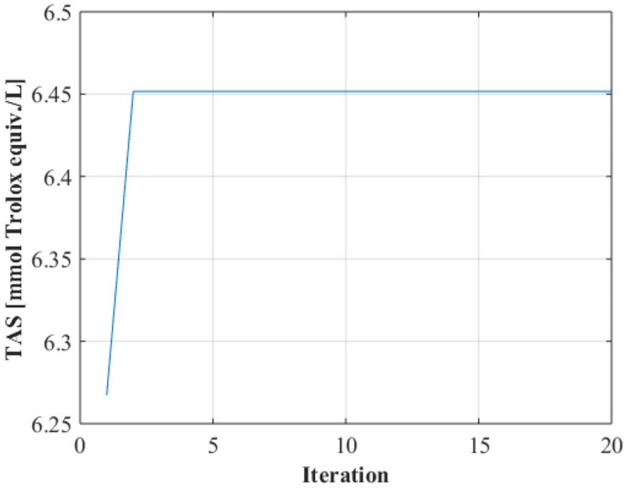

Fig. 2 Convergence graph

temperature of 54.009 °C, an extraction time of 13.560 h, and an ethanol/water ratio of 41.917%.

During the optimization process using artificial intelligence techniques, the experimental data were modeled using an artificial neural network (ANN), followed by optimization with a genetic algorithm (GA). The optimal prediction model was identified as having a 3-3-1 architecture, indicating that the model with three hidden neurons was found to be the most effective. The MSE, MAPE, and R values for this model were calculated as 0.0001, 0.130%, and 0.999, respectively, demonstrating a strong agreement between the model's predictions and the actual data.

The optimization process was performed using the genetic algorithm (GA) with the best-selected ANN model. Among the different population sizes evaluated, a population size of 10 was identified as optimal. Following 20 iterations, the convergence graph (Fig. 2) revealed that the objective function value stabilized after the second iteration.

Using the ANN-GA integration, the predicted optimal conditions were determined to be a temperature of 56.286 °C, a duration of 7.279 h, and an ethanol-to-water ratio of 36.862%.

Phenolic contents

Phenolic compounds are important components that help protect algae against environmental stress factors and show biological activity. Flavonoids, phenolic acids, tannins and their derivatives included in this group support the biological functions of algae. Phlorotannins, which are densely found in algae, attract attention with their antioxidant, anticarcinogenic and anti-inflammatory properties [18, 19]. Detailed analyzes of these compounds are usually performed with advanced technology devices such as LC-MS/MS. Thanks to these analyzes, the phenolic profile of algae is revealed comprehensively and their usability in both food and health fields is increased [20]. In our study, the phenolic contents of the optimized extract of *U. lactuca* were scanned on the LC-MS/MS device. The findings are shown in Table 2.

Table 2 Phenolic contents of *Ulva lactuca* (dry weight)

Phenolic compounds	RSM extract (mg/kg)	ANN-GA extract (mg/kg)
Gallic acid	2846.61	2924.88
4-hydroxybenzoic acid	629.11	1142.73
Caffeic acid	14557.06	19346.25
Vanillic acid	4862.69	7538.14
Syringic acid	976.45	1247.08
Quercetin	10934.52	12746.93
Kaempferol	7335.51	7961.07

U. lactuca is an important source of phenolic compounds among green algae. This algae is rich in phenolic compounds such as p-quercetin, kaempferol, and apigenin. These phenolic compounds exhibit strong antioxidant properties due to their free radical scavenging activities [10]. In addition, compounds such as naringin and catechin are also found in *U. lactuca* among flavonoids, and these compounds have inflammation-reducing and cell-protective effects [21]. In addition, tannins are also present in the phenolic content of *U. lactuca* and attract attention with their antibacterial activities [22]. In our study, the phenolic contents of the extracts of *U. lactuca* produced under optimum conditions that provide the highest biological activities were screened. As a result of the analyzes, 7 phenolic compounds were detected in both extracts of the algae. In addition, all of the compounds detected in the ANN-GA extract were determined to be higher. In our study, the phenolic compounds of *U. lactuca* were examined in detail and the presence of several important compounds was determined. These compounds include gallic acid, 4-hydroxybenzoic acid, caffeic acid, vanillic acid, syringic acid, quercetin and kaempferol. The analyses show that these phenolic compounds are found in different concentrations in both extracts. In particular, caffeic acid stands out as the highest detected compound in both extracts. Caffeic acid is an important phenolic acid, especially known for its antioxidant properties and widely found in plant-based products. It is found in high concentrations in plants and is known for its potential to reduce oxidative stress. This compound can help prevent cell damage by neutralizing free radicals in the body. Studies have also shown that caffeic acid has anti-inflammatory and anticarcinogenic effects [23–25]. This study shows that *U. lactuca* may be a rich source of phenolic compounds and has significant potential in the field of health, especially in terms of its caffeic acid content. It is known that phenolic compounds have various positive effects on human

health as well as the capacity to increase the resistance of algae to environmental stress factors. In this context, it is anticipated that *U. lactuca* may find wider use in the food, cosmetics and pharmaceutical industries.

Antioxidant activity

Oxidative stress is a condition that occurs as a result of excessive accumulation of free radicals in cells and can cause damage to biological systems. Free radicals occur as a result of normal metabolic processes, but environmental factors, stress, poor nutrition and habits such as smoking can increase the severity of this condition. Long-term effects of oxidative stress have been associated with various diseases such as cancer, heart disease, diabetes and neurological diseases [26–28]. Antioxidants come into play to neutralize excessive free radicals in the body. Antioxidants protect cells from oxidative damage by neutralizing free radicals. Natural antioxidants such as vitamin C, vitamin E, selenium and phenolic compounds fulfill this protective function. Phenolic compounds, especially those obtained from plant sources, stand out with their strong antioxidant properties and therefore their positive effects on health are being investigated [29, 30]. In our study, the antioxidant potentials of optimized extracts of *U. lactuca* were determined. The findings are shown in Table 3.

U. lactuca (green algae) attracts attention with its various biological activities and is an important source of natural compounds, especially with its antioxidant properties. This algae species contains many active compounds that show protective effects against oxidative damage in the body [31]. In the literature, the antioxidant activities of *U. lactuca* extracts tested with DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) methods were found to be high. It is emphasized that the antioxidant capacity of algae can strengthen defense mechanisms against environmental stress factors and also has the potential to be beneficial to human health [32, 33]. In our study, the antioxidant activities of optimized extracts of *U. lactuca* were determined with DPPH and FRAP methods and it was seen that they had antioxidant potential. In addition, TAS, TOS and OSI values were determined for the first time. There are TAS, TOS and OSI studies on plant species in the literature. In this context, the TAS value of *Anthemis cotula* was reported as 7.625 mmol/L, TOS value as 11.247 µmol/L and OSI value as 0.148 [34]. (Sabik et al., 2024). The TAS value of *Hypericum spectabile* was reported as 9.306 mmol/L, TOS value as 13.065 µmol/L and OSI value as 0.140 [35]. The TAS value of *Arum dioscoridis* was reported as 6.486 mmol/L, TOS value as 13.578 µmol/L and OSI value as 0.209 [36]. The TAS value of *Alcea kurdica* was reported as 3.298 mmol/L, TOS value as 8.312 µmol/L and OSI value as 0.252 [37]. It was observed that the ANN-GA extract of *U. lactuca* used in our study had higher TAS value compared to the RSM extract. It was determined that both extracts of *U. lactuca* had lower TAS values than *Anthemis cotula*, *Hypericum spectabile* and *Arum dioscoridis* and higher TAS values than *Alcea kurdica*. The TAS value is an indicator of the totality of antioxidant compounds found in natural products. In our study, it was determined that the optimized extracts of *U. lactuca* had antioxidant potential, and this finding shows that the seaweed has protective properties against environmental stress factors and may offer potential benefits in the field of health. This result reveals the importance of evaluating *U. lactuca* as a natural antioxidant source.

TOS value is an indicator of the totality of oxidant compounds produced in natural products. In our study, it was observed that *U. lactuca*'s ANN-GA extract had a higher TOS value compared to RSM extract. It was determined that both extracts of *U. lactuca* had lower TOS values than *A. cotula*, *H. spectabile* and *A. dioscoridis*. In addition, it was observed that *U. lactuca*'s ANN-GA extract had a higher TOS value than *A. kurdica*, while RSM extract had a lower TOS value.

OSI value shows how much oxidant compounds are suppressed by antioxidant compounds. The OSI value of Ulva lactuca's ANN-GA extract used in our study was determined to be higher than *A. cotula* and *H. spectabile*, while RSM extract was lower. In addition, it was determined that both extracts of *U. lactuca* had lower OSI values than *A. dioscoridis* and *A. kurdica*. As a result, it was seen that the optimized extracts of *U. lactuca* had the potential to suppress oxidant compounds.

In our study, the biological activities of the optimized extracts of *U. lactuca* were examined in detail. As a result of the analyzes, it was determined that both extracts of *U. lactuca* had high antioxidant potential. In addition to the antioxidant activities tested especially with DPPH and FRAP methods, TAS, TOS and OSI values were also determined for the first time. It was seen that ANN-GA extract had higher TAS and TOS values compared to RSM extract and was also lower than some plant species in terms of OSI value. These findings show that *U. lactuca* has protective properties against environmental stress factors and offers potential benefits in the field of health. This finding raises the potential of optimized extracts of

Table 3 Antioxidant parameters of *Ulva lactuca*

Parameters	RSM extract values	ANN-GA extract values
TAS (mmol/L)	5.680 ± 0.050	6.135 ± 0.074
TOS (µmol/L)	6.304 ± 0.079	9.761 ± 0.084
OSI (TOS/(TAS*10))	0.111 ± 0.002	0.159 ± 0.001
FRAP (mg Trolox Equi/g)	95.36 ± 1.55	106.14 ± 0.82
DPPH (mg Trolox Equi/g)	73.11 ± 1.08	87.16 ± 0.65

U. lactuca to be used as natural antioxidant sources, and encourages further investigation of this seaweed.

Antiproliferative activity

Algae are organisms with nutritional and medicinal properties that are widely found in marine and freshwater ecosystems. In recent years, research on the anticancer effects of algae has increased. Thanks to the biologically active compounds they contain, algae can prevent the growth of cancer cells, promote apoptosis (programmed cell death) and inhibit metastasis. The anticancer potential of algae attracts attention as an important area for researchers looking for new, natural treatment methods in cancer treatment [10, 38]. In our study, the activity of the optimized extract of *U. lactuca* against the A549 lung cancer cell line was determined. The findings are shown in Fig. 3.

Many studies have examined the antiproliferative (cell proliferation inhibition) effects of *U. lactuca*. It is stated that these effects are associated with the potential to inhibit the growth of cancer cells. The compounds contained in *U. lactuca*, especially polysaccharides, phenolic compounds and flavonoids, can reduce oxidative stress by neutralizing free radicals, which can prevent the proliferation of cancer cells. In addition, *U. lactuca* has been shown to inhibit the G1 phase of the cell cycle and prevent cell division and proliferation [39–41]. In our study, the effects of optimized extracts of *U. lactuca* against the A549 lung cancer cell line were investigated. Studies have shown that ANN-GA extract of seaweed is more effective against A549 lung cancer cell line and both extracts show strong cytotoxic effects depending on the concentration, and these findings support the investigation of seaweed as a potential cancer treatment agent. In our study, antiproliferative effects of optimized extracts of *U. lactuca* on A549 lung cancer cell line were investigated. The analyses showed that the extract of *U. lactuca* obtained by the ANN-GA method showed stronger cytotoxic effects against A549 lung cancer cell line compared to the RSM extract. Both extracts exhibited significant antiproliferative activity depending on the concentration, and these findings support the use of *U. lactuca* as a potential cancer treatment agent. It was determined that extract optimization played an important role in increasing biological activity, and it was seen that the second extract in particular provided a more effective formulation in inhibiting the growth of cancer cells. The obtained results indicate that *U. lactuca* should be subjected to more detailed studies as a source of biological activity in cancer treatment.

Anticholinesterase activity

Cholinesterase inhibitors are compounds that inhibit enzymes and prevent the destruction of neurotransmitters. Thanks to these properties, cholinesterase inhibitors play an important role in the treatment of neurological diseases, especially Alzheimer's disease (AD). Inhibition of acetylcholinesterase may help improve brain functions, since increasing acetylcholine levels can improve cognitive functions such as memory and learning [42, 43]. In our study, the anticholinesterase activity of the optimized extract of *U. lactuca* was determined. The IC50 values of the obtained findings are shown in Table 4.

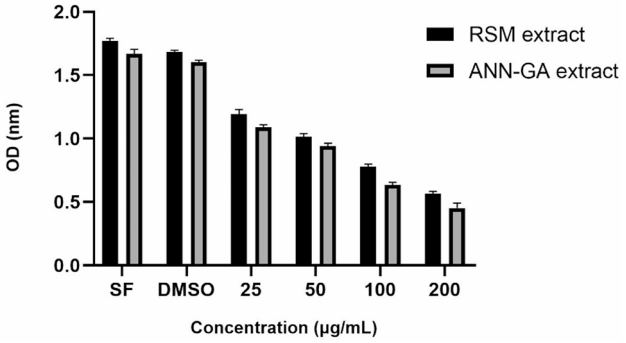

Fig. 3 Antiproliferative activity of *Ulva lactuca* extracts

Table 4 Anticholinesterase activity of *Ulva lactuca*

Sample	AChE (µg/mL)	BChE (µg/mL)
RSM extract	43.82 ± 1.12^{c}	58.17 ± 1.14^{c}
ANN-GA extract	37.66 ± 0.82^{b}	48.04 ± 0.93^{b}
Galantamine	7.54 ± 0.22^{a}	18.09 ± 0.09^{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

In our study, anticholinesterase activities of *U. lactuca* extracts produced under optimum conditions that provide the highest biological activities were investigated. In the literature, the activity of sulfated polysaccharides obtained from *U. lactuca* in inhibiting acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) was reported with IC50 values of 106.93 µg/mL and 93.45 µg/mL, respectively [44]. However, it was found that both extracts used in our study showed higher anticholinesterase activities. Especially, *Ulva lactuca* extract obtained by ANN-GA method exhibited stronger anticholineresterase activity compared to RSM extract, indicating that extract optimization plays an important role in increasing biological activity. However, both extracts were determined to have lower efficacy compared to standard galantamine. These findings reveal that *U. lactuca* has a strong potential in terms of anticholineresterase activity but needs further optimization for pharmaceutical applications. In addition, they show that *U. lactuca* can be an important source for the development of enzyme inhibition-based treatment strategies in biotechnology and pharmaceutical fields. These results emphasize that extract optimization is a critical step in increasing the

biological activities of natural compounds and finding more effective treatment methods.

Conclusion

In our study, extraction conditions were determined to optimize the biological activity of *U. lactuca* and various biological activities of the extracts obtained under these conditions were evaluated. The extracts exhibiting the highest biological activity obtained with artificial intelligence applications stand out as a potential antioxidant source by exhibiting strong antioxidant activities. In addition, in antiproliferative tests performed on the A549 lung cancer cell line, it was determined that the optimized extracts showed strong cytotoxic effects. As a result of phenolic component analyses, the compound detected in the highest amount was caffeic acid. In addition, anticholinesterase activities of *U. lactuca* were also determined, which suggests it as a potential treatment source for neurological diseases. As a result, optimized extracts of *U. lactuca* have great potential for use in health areas such as cancer treatment and management of neurological diseases. These findings encourage further investigation of *U. lactuca* as a natural source of biological activity.

Abbreviations

AChE	Acetylcholinesterase
ANN	Artificial Neural Network
ANN-GA	Artificial Neural Network-Genetic Algorithm
BChE	Butyrylcholinesterase
DMSO	Dimethyl Sulfoxide
DPPH 2	2-Diphenyl-1-Picrylhydrazyl
DTNB 5	5′-Dithiobis-(2-Nitrobenzoic Acid)
FRAP	Ferric Reducing Antioxidant Power
GA	Genetic Algorithm
LC-MS/MS	Liquid Chromatography-Mass Spectrometry/Mass Spectrometry
MAPE	Mean Absolute Percentage Error
MSE	Mean Squared Error
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
OSI	Oxidative Stress Index
OSI	Oxidative stress index
R	Correlation Coefficient
RSM	Response Surface Methodology
TAS	Total Antioxidant Status
TOS	Total Oxidant Status
TPTZ	2,4,6-Tris(2-Pyridyl)-S-Triazine

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The experimental design, studies, analysis, writing and revisions of this manuscript were made by N.K.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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