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Determination of optimum extract conditions and evaluation of biological activity potential of *Salvia cilicica* Boiss

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Plants are important natural resources used for many purposes. They especially stand out with their medical potential. In this study, the extract conditions showing the highest biological activity were optimized using the artificial intelligence application of *Salvia cilicica* Boiss. Then, the biological activities of the optimized extract were determined. Response Surface Method was used for optimization. Using the Box-Behnken experimental design, antioxidant capacities of the optimized extracts were evaluated through Rel Assay TAS and TOS kits, as well as DPPH and FRAP methods. Anticholinesterase activity was assessed by measuring acetyl- and butyrylcholinesterase enzyme activities. Total phenolic content was quantified using the Folin-Ciocalteu reagent, while total flavonoid content was analyzed via the aluminum chloride assay. The antiproliferative potential was examined against the A549 lung cancer cell line, and phenolic compounds were identified using an LC-MS/MS system. Optimization results indicated that the ideal extraction parameters were 45.890 °C, 7.730 h, and a 96.431 ethanol-to-water ratio. The extract obtained under these conditions exhibited a TAS value of 7.461 ± 0.065 mmol/L, a TOS value of 5.630 ± 0.163 µmol/L, and an OSI value of 0.075 ± 0.002. Total phenolic and flavonoid contents were measured as 97.681 ± 1.076 mg/g and 113.067 ± 0.621 mg/g, respectively. The FRAP value reached 70.669 ± 0.199 mg/g, while the DPPH value was determined as 53.208 ± 0.427 mg/g. Furthermore, anti-AChE and anti-BChE activities were recorded at 12.93 ± 0.72 µg/mL and 25.87 ± 1.44 µg/mL, respectively. The extract demonstrated notable cytotoxicity against the A549 cancer cell line. Additionally, 10 distinct phenolic compounds were identified in the plant. These findings highlight the significant biological potential of *S. cilicica* extract obtained under optimal conditions, suggesting its potential as a valuable natural resource in pharmacological applications.

Keywords Antioxidant, Antiproliferative, *Salvia*, Medicinal plants, Extract optimization

Since ancient times, humans have extensively utilized medicinal and aromatic plants. These plants play a crucial role in combating various diseases and are also valued for their aromatic properties, which stem from the bioactive compounds they naturally produce and their appealing fragrances[1]. Research has demonstrated that plants possess a wide range of biological properties, including anticancer, antidiabetic, anti-inflammatory, antiproliferative, antitumor, hepatoprotective, DNA-protective, antimicrobial, and antiallergic effects[2–6]. Understanding the biological properties of plants is essential for uncovering their pharmacological potential[7–10]. This research focused on identifying the biological activities of extracts from *Salvia cilicia* Boiss., which were obtained under optimized conditions designed to enhance their bioactivity through the use of artificial intelligence tools.

The genus *Salvia*, belonging to the Lamiaceae family, is the largest within the group, encompassing nearly 1,000 species of shrubs, herbaceous perennials, and annual plants. Commonly referred to as sage, *Salvia* species are well-known for their distinct aromatic characteristics. Since antiquity, they have been an integral part of traditional medicine, utilized for various applications such as managing snakebites, promoting fertility, acting as diuretics, providing local anesthesia, stopping bleeding, freshening indoor air, and treating flu-related illnesses[11]. *Salvia* species have a wide range of uses in traditional medicine. For example, in China, approximately 40 *Salvia* species are used to treat liver and kidney diseases, as well as cardiovascular and immune system disorders[12].

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Similarly, in South Africa, *Salvia* species are used to treat a variety of conditions, including microbial infections, cancer, malaria and inflammation[13]. In this context, the biological activities of *S. cilicica* extracts obtained under optimum conditions were determined in our study.

Materials and methods

The plant samples used in the study were collected from Kahramanmaraş (Türkiye). The plant identification was made using Flora of Turkey and the East Aegean Islands Volume 7 [14]. Herbarium Samples (Number: O.K-132) of the plant are kept in Osmaniye Korkut Ata University, Pharmacy Services Laboratory.

Experimental design

The Box-Behnken Design was used in planning experiments. Within the scope of the experimental design, experiments were conducted at three levels for each factor as low (-1), medium (0), and high (+1). The number of experiments was determined as 17 in accordance with the requirements of the design. First, experiments were conducted in the Soxhlet apparatus under extraction conditions of 40, 55 and 70˚C extraction temperature, 2-, 5- and 8-hours extraction time and 0, 50 and 100% ethanol/water ratio. Each experiment was repeated 3 times, and the response variable was measured. The obtained data was optimized by the response surface method (RSM)[15].

Optimization

In this study, RSM was used for optimization. Extraction temperature, extraction time and ethanol/water ratio were selected as the independent variables of the study. The response variable was determined as the total antioxidant activity (TAS) value of the obtained extract.

The optimization process was carried out using Design Expert 13 software with a second-order polynomial response as:

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

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 suitability of the model was tested using coefficient of determination (R^2), ANOVA analysis and *p*-values. Critical points were calculated from the derivatives of the model to optimize the response variable. In addition, three-dimensional surface plots were prepared to visualize the effects of independent variables. These plots were used to better understand the effects of variables on the response.

Extraction for bioactivity

The optimum conditions providing the highest biological activity were determined. As a result of the optimization analyses, it was determined that the optimum extract conditions were 45.890 ˚C temperature, 7.730 h and 96.431 ethanol/water ratio. In this context, the values closest to the optimum conditions were entered into the computer environment using the Gerhardt SOX-414 device and the extracts were obtained. All biological activity tests performed in the study were performed with extracts obtained under optimum extraction conditions. After the extracts produced under optimum conditions, the solvents were evaporated using the Buchi R100 Rotary Evaporator and crude extracts were obtained.

Antioxidant activity tests

Total antioxidant and oxidant analysis

Total antioxidant status (TAS) and total oxidant status (TOS) of plant extracts obtained under optimum conditions were determined using Rel Assay TAS and TOS kits. Analyses were performed according to the manufacturer's protocol provided with the kit. TAS values are expressed as mmol trolox equivalents/L. TOS values are reported as µmol hydrogen peroxide equivalents/L[16,17]. The OSI value was determined by calculating the percentage as the ratio of TOS values to TAS values[18].

DPPH free radical scavenging activity

1 mg/mL working solutions of plant extracts obtained under optimum conditions were prepared using DMSO. Then, 1 mL of this solution was mixed with 160 µL of DPPH solution (0.267 mM, 4 mL, 0.004% methanol solution) and incubated for 30 min at room temperature in the dark. Finally, absorbance was measured at 517 nm and the values were expressed as mg Trolox Equivalent/g extract[19].

Ferric reducing antioxidant power assay

A 100 µL stock solution was prepared from the plant extracts obtained under optimum conditions. Then, 2 mL of FRAP reactant was added to this stock solution. Then, 300 mM acetate buffer (pH 3.6), 40 mM HCl and 20 mM $FeCl_3$ $6H_2O$ solution was added to the prepared FRAP solution (10:1:1 ratio) by mixing with 10 mM 2,4,6-tris(2-pyridyl)-S-triazine solution and incubated at 37 °C for 4 min. Finally, measurement was made at 593 nm and the values were expressed as mg Trolox Equivalent/g extract[19].

Anticholinesterase activity tests

Anticholinesterase activities of plant extracts obtained under optimum conditions were determined using the Ellman method. Galantamine was used as a standard in the study[20]. working solutions between 200-3.125 µg/mL

were prepared from plant extracts. Then, 130 µL of 0.1 M pH = 8 phosphate buffer was added to the microplate. 10 µL of stock solution, 20 µL of enzyme (AChE or BChE enzyme solution) were added and incubated at 25°C in the dark for 10 min. Then, 20 µL of DTNB (5.5"-dithiobis-(2-nitrobenzoic acid), 6.8 µmol) solution and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added. Finally, measurement was made at 412 nm and IC50 values of percent inhibition of samples were expressed in µg/mL.

Antiproliferative activity tests

Antiproliferative activity of plant extracts obtained under optimum conditions was tested against A549 lung cancer cell line. For this purpose, working solutions were prepared from the extracts at concentrations of 25, 50, 100 and 200 µg/mL. The cells were allowed to reach 70–80% confluency. Then, dissociation was performed with 3.0 mL Trypsin-EDTA solution (Sigma-Aldrich, MO, USA). Then, the cells were plated and incubated. After 24 h incubation, working solutions were added and incubated for another 24 h. After incubation, the supernatants were dissolved with growth medium, 1 mg/mL MTT was added and incubation was carried out at 37 °C until purple precipitate formed. Finally, dimethylsulfoxide (DMSO) (Sigma-Aldrich, MO, USA) was added to the MTT solution and measured at 570 nm using an Epoch spectrophotometer (BioTek Instruments, Winooska, VT)[21].

Total phenolic and total flavonoid analysis

1 mg/mL working solutions were prepared from plant extracts obtained under optimum conditions. 1 mL of Folin-Ciocalteu reactant (1:9, v/v) was added to this solution and mixed. Then, 0.75 mL of 1% Na2CO3 solution was added and incubated for 2 h at room temperature. Finally, measurement was made at 760 nm. Total phenolic contents were expressed in mg/g according to the calibration curve of gallic acid standard solution[22].

Total flavonoid levels of the extracts were measured by aluminum chloride test[23]. For the test, 0.1 mL of 10% $Al(NO_3)_3$, 0.1 mL of 1 M NH_4CH_3COO (Ammonium acetate), 4.3 mL of methanol, 0.5 µg/mL of quercetin and 0.5 mg/mL of plant extract were mixed. The mixture was then incubated for 40 min. Then, absorbance was measured at 415 nm. Total flavonoid content was expressed in mg/g.

Phenolic analysis with LC-MS/MS

The analysis of phenolic compounds in the plant extract was carried out using LC-MS/MS system. A total of 26 different phenolic compounds were used as reference standards in the study. Analyses were carried out on Shimadzu Nexera HPLC instrument integrated with tandem mass spectrometry (MS/MS). The liquid chromatography system used consisted of LC-30AD binary pump, DGU-20A3R degasser, CTO-10AS vp column oven and SIL-30AC automatic sampler. The separation process was carried out using a reverse phase C18 analytical column (Inertsil ODS-4, 150 mm × 4.6 mm, 3 µm) and the column temperature was fixed at 40 °C. Water with 5 mM ammonium formate containing 0.1% formic acid (Phase A) and methanol with 5 mM ammonium formate containing 0.1% formic acid (Phase B) were used as the mobile phase. The gradient elution program was optimized by considering the concentration changes of phase B over time (t (min): B%: 0.40, 20.90, 23.99, 24.40, 29.40). The flow rate of the mobile phase was determined as 0.5 mL/min, and the injection volume for each sample was set as 4 µL.

Statistical analysis

All experiments were performed in triplicate to ensure the reliability and reproducibility of the results. In the statistical evaluation of all analyses performed in this study, 'SPSS 21.0 for Windows' program was used. In tests with more than three groups, Simple Variance Analysis (BVA) was applied to determine the differences between the groups; Duncan test ($\alpha = 0.05$ confidence level) was used to determine the differences between the groups. In the anticholinesterase test, t-test was applied because there were two different groups.

Results and discussions

Optimization of extraction conditions

In this study, one of the ways to benefit from the *S. cilicica* plant with maximum efficiency was investigated. For this purpose, an experimental design was made according to the Box-Behnken design, extracts were taken under the extraction conditions obtained in this design and the TAS values of these extracts were determined. Using the obtained data, extraction conditions were optimized to maximize the total antioxidant potential. TAS values obtained from the experimental study are given in Table 1.

When Table 1 is examined, at lower temperatures (e.g. 40 °C), TAS values are generally lower (e.g. 5.326 mmol/L). Medium temperatures (55 °C) generally gave higher results in terms of TAS values (e.g. 6.968 mmol/L, 7.370 mmol/L). Higher temperatures (70 °C) tend to decrease TAS values (e.g. 5.110 mmol/L, 5.601 mmol/L). This shows that high temperatures may cause degradation of antioxidant compounds. Short-term extractions (2 h) generally gave lower TAS values (e.g. 5.036 mmol/L, 5.110 mmol/L). Long-term extractions may have provided more dissolution of antioxidant compounds. TAS values obtained with the use of 50% ethanol remained at medium levels (e.g. 5.896 mmol/L, 5.843 mmol/L). With the use of 0% ethanol (only water), TAS values decreased (e.g. 5.036 mmol/L, 5.299 mmol/L). Ethanol seems to be an effective solvent in increasing the solubility of antioxidant compounds. As seen in Table 1, the difference between the studied groups was found to be statistically significantly different ($p < 0.05$). The obtained data were optimized with the RSM method and as a result of the optimization analyzes, it was seen that the optimum extract conditions were 45.890 °C temperature, 7.730 h and 96.431 ethanol/water ratio.

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

Experiment number	Extraction temperature (°C)	Extraction time (h)	Ethanol/water ratio (%)	TAS (mmol/L)
1	55	8	100	7.370 ± 0.026^{l}
2	40	8	50	6.871 ± 0.026^{j}
3	55	5	50	5.896 ± 0.022^{fg}
4	40	5	0	5.449 ± 0.029^{c}
5	55	5	50	5.824 ± 0.020^{f}
6	40	2	50	5.326 ± 0.047^{b}
7	55	5	50	5.843 ± 0.011^{f}
8	70	2	50	5.110 ± 0.014^{a}
9	55	5	50	5.982 ± 0.041^{g}
10	70	8	50	5.601 ± 0.031^{d}
11	40	5	100	6.540 ± 0.025^{i}
12	55	2	0	5.036 ± 0.006^{a}
13	55	8	0	6.968 ± 0.035^{k}
14	70	5	0	5.299 ± 0.037^{b}
15	55	2	100	6.284 ± 0.017^{h}
16	55	5	50	5.731 ± 0.020^{e}
17	70	5	100	5.348 ± 0.036^{b}

Table 1. TAS values of the extracts of *S. Cililica*. *Different exponential letters indicate that the difference between the groups is statistically significant according to Duncan's multiple range test ($p < 0.05$).

$$TAS = 5.86 - 0.353\ X_1 + 0.631\ X_2 + 0.348\ X_3 - 0.263\ X_1X_2 - 0.260\ X_1X_3 - 0.211\ X_2X_3 - 0.441\ X_1^2 + 0.313\ X_2^2 + 0245\ X_3^2$$

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

The next stages of the study were completed using the extract under optimum conditions recommended by the RSM method.

Antioxidant activity of optimized extract

Free radicals are produced as a result of routine metabolic activities in living organisms. Depending on the severity and continuity of external factors, free radical levels may increase. In these cases, serious cellular damage may occur. The antioxidant defense system is activated and reduces or suppresses the effects of free radicals. In cases where the antioxidant defense system is inadequate, oxidative stress occurs[24]. As a result of oxidative stress, serious diseases such as cancer, diabetes, Alzheimer's, Parkinson's can be seen in humans. Extra antioxidants can be used to reduce the effects of oxidative stress[25]. With these properties, plants stand out as important natural resources. In our study, antioxidant potentials of optimized extracts of *S. cilicica* were determined. Findings are presented in Table 2.

In the literature, it has been reported that *S. cilicica* has antioxidant potential using different methods[26,27]. In our study, antioxidant activities of *S. cilicica* extracts produced under optimum conditions that maximize its biological activity were determined. Contrary to literature data, in this study, TAS, TOS and OSI values of *S. cilicica* were determined for the first time using Rel Assay kits. In studies performed on different plant species, 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 [28]. In another study, 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 [29]. In another study, the TAS value of Sinapis arvensis was reported as 5.232 mmol/L, TOS value as 7.564 µmol/L and OSI value as 0.146 [30]. 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[31]. The TAS value of *Rumex scutatus* was reported as 8.656 mmol/L, TOS value as 4.951 µmol/L and OSI value as 0.057[32]. Compared to these studies, the TAS value of the optimized extract of *S. cilicica* used in our study is lower than *H. spectabile*, *A. cotula* and *R. scutatus*, and higher than *S. arvensis* and *A. kurdica*. The TAS value is an indicator of the total antioxidant components found in the plant[30]. It was determined that *S. cilicica* used in our study has antioxidant potential. In addition, the TOS and OSI values of the optimized extract of *S. cilicica* are lower than *H. spectabile*, *A. cotula*, *S. arvensis* and *A. kurdica*, and higher than *R. scutatus*. The TOS value is an indicator of the oxidant components found in the plant. The OSI value shows how much the oxidant components detected in the plant are suppressed by endogenous antioxidant components[30]. It was observed that the optimized extract of *S. cilicica* used in our study had low oxidant component levels. In addition, the low oxidative stress index of *S. cilicica* is an important indicator of the plant's potential to suppress oxidant compounds. In this context, it was determined that the antioxidant potential of *S. cilicica* extract produced under optimum conditions was high.

The antioxidant activities of various *Salvia* species (such as *S. blepharochlaena*, *S. euphratica* var. *leiocalycina*, and *S. verticillata* subsp. *amasiaca*) have been evaluated in the literature, with the use of dichloromethane, methanol, and water extracts. These studies utilized DPPH and FRAP assays to assess the antioxidant potential. According to the findings, the DPPH assay results ranged from 3.76 to 382.76 mg/g, while the FRAP assay results ranged from 14.53 to 560.38 mg/g[33]. When compared to previous research, the antioxidant potential of the optimized extract of *S. cilicica* used in our study showed similar results in both DPPH and FRAP assays. These

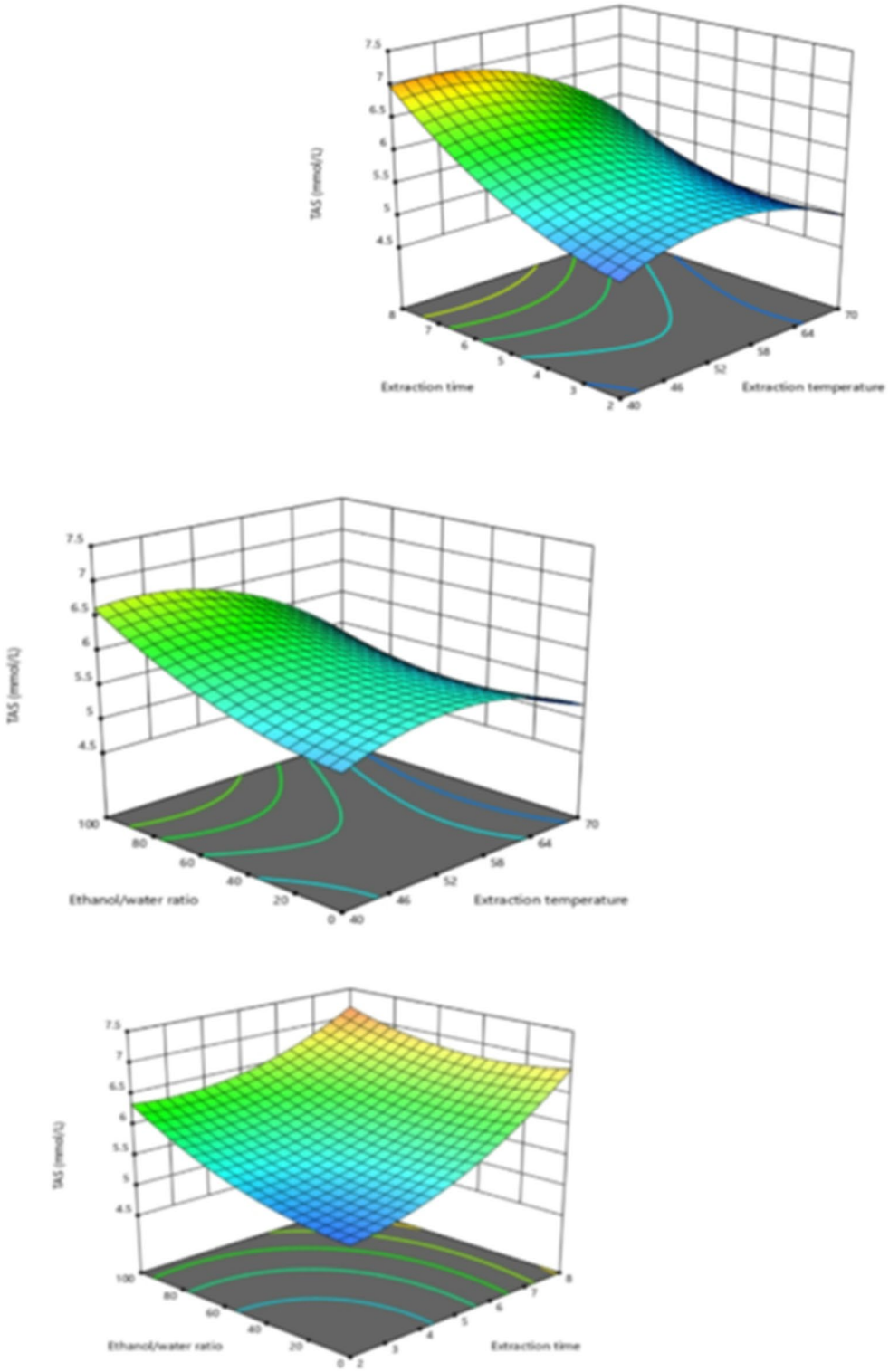

Fig. 1. Response surface plots of TAS of *S. cilicica.*

findings confirm that the extract of *S. cilicica*, produced under optimal conditions, exhibits a significant level of antioxidant activity.

Plants possess a variety of biological activities due to the phenolic and flavonoid compounds they contain. In this study, we assessed the TPC and TFC of the *S. cilicica* extract, produced under optimal conditions. The results are presented in Table 2. According to previous studies, the total phenolic content of methanol, dichloromethane, and water extracts of species such as *S. blepharochlaena*, *S. euphratica* var. *leiocalycina*, and *S. verticillata* subsp. *amasiaca* ranged between 12.42 and 119.11 mg/g, while the total flavonoid content ranged

Parameters	Values
TPC (mg/g)	97.681 ± 1.076
TFC (mg/g)	113.067 ± 0.621
OSI (TOS/(TAS*10))	0.075 ± 0.002
TOS (µmol/L)	5.630 ± 0.163
TAS (mmol/L)	7.461 ± 0.065
FRAP (mg Trolox Equi/g)	70.669 ± 0.199
DPPH (mg Trolox Equi/g)	53.208 ± 0.427

Table 2. Antioxidant activity data of *S. Cilicica*.

Sample	AChE (µg/mL)	BChE (µg/mL)
Optimize extract	12.93 ± 0.72^{b}	25.87 ± 1.44^{b}
Galantamine	7.64 ± 1.17^{a}	17.28 ± 0.43^{a}

Table 3. Anticholinesterase activity of *S. Cilicica*. *Means followed by different letter(s) differ significantly at $p < 0.05$ (according to t-test).

from 4.57 to 22.79 mg/g[33]. Compared to this study, the total phenolic content of the optimized extract of *S. cilicica* used in our study showed similar results, while the total flavonoid content was determined to be higher.

As a result, it was determined that the optimized extracts of *S. cilicica* had high antioxidant activity and rich biological contents when compared to the extracts of other *Salvia* species reported in the literature. The results obtained in the optimization process showed that the antioxidant potential was at a remarkable level in DPPH and FRAP tests. While similarity was observed with other Salvia species in the literature in terms of total phenolic content, higher values were obtained in terms of total flavonoid content. Thanks to the optimum extraction conditions, it was revealed that *S. cilicica* is a rich source of phenolic and flavonoid compounds and the effects of these compounds on biological activities can be increased. These findings strengthen the potential of the optimized extract in various industries such as functional food, pharmaceuticals and cosmetics.

Anti-Alzheimer activity

Alzheimer's disease is known to affect 10–30% of individuals over the age of 65, with a 1–3% prevalence rate in the general population[34]. It is a neurodegenerative disorder that leads to cognitive decline and can manifest in both amnestic and non-amnestic forms. Several natural compounds derived from plants, animals, and microorganisms have demonstrated positive effects in managing Alzheimer's disease[35]. In this study, we evaluated the anticholinesterase activity of the *S. cilicica* extract produced under optimized conditions. The IC50 values obtained are presented in Table 3.

No relevant data could be found in the existing literature regarding the anticholinesterase activity of *S. cilicica*. However, studies on various *Salvia* species native to Türkiye have investigated their anticholinesterase properties. For instance, extracts from species such as *Salvia albimaculata*, *Salvia cyanescens*, *Salvia frigida*, and *Salvia migrostegia* have shown anticholinesterase activity[36]. Furthermore, *Salvia cryptantha* has also been explored for its anticholinesterase effects[37]. In our study, we assessed the inhibitory effects of *S. cilicica* on both acetylcholinesterase and butyrylcholinesterase. The results revealed that *S. cilicica* displayed lower activity compared to the standard galantamine. Identifying enzymes involved in the development of diseases and inhibiting their action is crucial for disease management, particularly for neurodegenerative disorders such as Alzheimer's disease. Enzymes like acetylcholinesterase and butyrylcholinesterase are central to the pathophysiology of these diseases, as they play a role in the breakdown of neurotransmitters essential for brain function. By inhibiting these enzymes, it may be possible to reduce cognitive decline and improve quality of life for individuals affected by such conditions[38]. In our study, it was determined that the anticholinesterase activity of *S. cilicica* extracts was lower compared to the standard galantamine used. However, it is thought that this activity obtained provides an important basis for the potential effects of the bioactive compounds found in the plant. In particular, inhibition of cholinesterase enzymes is a critical strategy in the treatment of neurodegenerative disorders such as Alzheimer's disease, and it is important to investigate naturally derived inhibitors targeting this enzyme. In conclusion, this study reveals that *S. cilicica* may contribute to anticholinesterase activity and that this plant should be examined in more detailed biochemical and pharmacological studies to be conducted in the future. In addition, these findings show that *S. cilicica* can be evaluated as a natural inhibitor source in drug development processes.

Antiproliferative activity

The antiproliferative activity of plants is associated with their ability to hinder the growth and proliferation of cancerous cells. The observation of antiproliferative effects in various plant species suggests that these plants harbor biologically active compounds with potential anticancer properties. Such plants could present alternative or complementary therapeutic strategies in cancer treatment by suppressing cancer cell growth and

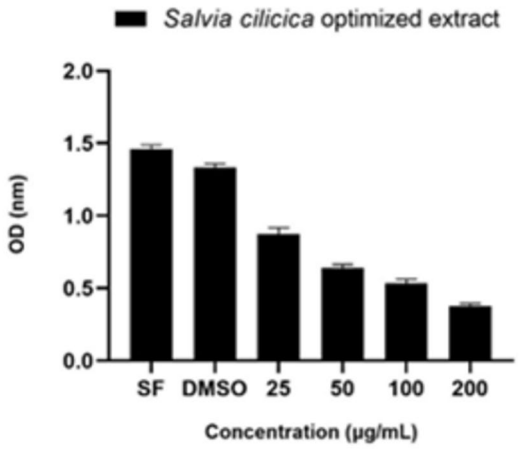

Fig. 2. Antiproliferative activity of *S. cilicica* extract. (SF: Serum-Free medium; DMSO: Group to which medium and DMSO were applied; Group to which the extract was applied at concentrations of 25, 50, 100 and 200 µg/mL, respectively).

Phenolic compounds	Ret. time	m/z	Values (mg/g)
Acetohydroxamic acid	0.460	76.15 > 58.00	4.52
Fumaric acid	0.859	115.30 > 71.20	5.30
Gallic acid	1.483	169.10 > 124.90	0.70
Protocatechuic acid	2.060	152.80 > 108.00	1.16
4-Hydroxybenzoic acid	2.550	137.20 > 93.00	0.59
Caffeic acid	2.850	178.80 > 134.90	115.40
Naringenin	3.960	271.20 > 150.90	2.37
Quercetin	4.000	300.80 > 150.80	6.68
Luteolin	4.122	285.20 > 133.00	9.79
Kaempferol	4.222	287.00 > 153.00	13.56

Table 4. Phenolic compounds of *S. Cilicica*.

proliferation[39]. In our research, we assessed the effect of the optimized extract of *S. cilicica* on the A549 lung cancer cell line, with the results presented in Fig. 2.

There is limited data in the literature on the effect of *S. cilicica* on the A549 lung cancer cell line. In our study, it was observed that the optimized S. cilicica extract showed significant antiproliferative effects and this effect increased in parallel with the concentration. As seen in Fig. 2, when the viability rate of A549 cells was examined, it was understood that the concentrations of the extract decreased the cell viability and this effect became stronger as the concentration increased. Literature reports on the antiproliferative properties of various Salvia species indicate that these plants show notable activity against multiple cancer cell lines. For instance, *S. sclarea*, *S. spinosa*, *S. lanigera*, *S. palaestina*, *Salvia dominica*, and *S. menthaefolia* have been shown to exert antiproliferative effects on colorectal adenocarcinoma (WiDr and HT-29), choriocarcinoma (JEG-3), glioblastoma (DBTRG-05MG, T98G, U-87MG), prostate adenocarcinoma (MDA Pca2b), B lymphoblast (CIR) and endometrial adenocarcinoma (HEC-1 A) cell lines[40]. In conclusion, in our study, it was determined that the optimized extract of *S. cilicica* exhibited a strong concentration-dependent antiproliferative activity against the A549 lung cancer cell line. Although there is no evidence in the literature regarding the activity of *S. cilicica* against this cell line, it has been reported that other *Salvia* species exhibit significant antiproliferative effects on different cancer cell lines. The results obtained indicate that *S. cilicica* is a promising plant for the development of natural anticancer agents. However, advanced cellular and molecular studies are required to better understand the biological mechanisms of this effect and to evaluate its clinical applicability. These findings reveal that *S. cilicica* has a natural potential that can contribute to new strategies in cancer treatment.

Phenolic contents

Plants synthesize a wide range of bioactive compounds, which play a crucial role in various fields such as health, medicine, and industry. These compounds are part of the plant's natural defense system against environmental stresses and are known for their diverse biological activities[41]. In this study, the phenolic composition of the optimized *S. cilicica* extract was analyzed using the LC-MS/MS device. The results are presented in Table 4.

In our study, the phenolic composition of the *S. cilicica* extracts, obtained under the optimal conditions that maximized biological activity, was analyzed. A total of 10 compounds were identified, including luteolin, acetohydroxamic acid, protocatechuic acid, kaempferol, fumaric acid, gallic acid, caffeic acid, quercetin, 4-hydroxybenzoic acid, and naringenin. The other 14 compounds screened have not been detected. The phenolic profiles of several *Salvia* species have been documented in the literature. For example, *S. officinalis* has been reported to contain gallic acid, ferulic acid, caffeic acid, rosmarinic acid, genkwanin, luteolin, naringin, carnosol, luteolin-7-O-glucoside, apigenin-7-glucoside, p-hydroxybenzoic acid, methyl carnosate, apigenin, and carnosic acid[42]. Another study identified luteolin-7-O-glucoside, gallic acid, o-coumaric acid, p-OH-benzoic acid, luteolin caffeic acid, and rosmarinic acid in *S. halophila* and *S. virgata*[43]. In conclusion, in our study, phenolic compounds of the extracts of *S. cilicica* produced under optimum extraction conditions exhibiting the highest biological activity were determined comprehensively. As a result of the analyses, 10 different phenolic compounds were identified, including acetohydroxamic acid, kaempferol, fumaric acid, gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, naringenin, quercetin and luteolin. Studies on the phenolic contents of *Salvia* species in the literature reveal that these plants have rich biological potential. For example, species such as *S. officinalis*, *S. halophila* and *S. virgata* have been reported to contain similar phenolic compounds such as caffeic acid, gallic acid and luteolin[43]. In addition to these studies, the phenolic content profile of *S. cilicica* was also revealed and it was determined that optimized extracts could be a source of natural bioactive compounds. These results show that optimized extraction conditions are an important factor in increasing biological activity and that *S. cilicica* can be considered as a natural source of phenolic compounds. These findings support the potential use of extracts obtained from the plant in various areas such as functional food, medicine and cosmetics and emphasize the importance of investigating the biological richness of this species in depth with further studies.

Conclusion

Our study examining the biological activities of *S. cilicica* extract and using extracts produced under optimized extraction conditions revealed that this plant has high antioxidant, anti-Alzheimer and anti-proliferative properties. In particular, the effective anti-proliferative activity of optimized extracts against cancer cells indicates that *S. cilicica* can be evaluated as a potential natural treatment agent. These effects are thought to be strongly related to the rich phytochemical profile of the plant and the high biological activity components obtained through optimized extraction process. In addition, the obtained data indicate that this plant may also be beneficial on neurodegenerative disorders such as Alzheimer's disease. Our study emphasizes the positive effects of optimized extraction conditions on biological activities and reveals that *S. cilicica* is a promising candidate for both pharmacological research and natural product-based treatments, indicating that the biological effects of this plant should be investigated in more detail.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Author contributions
The experimental studies, analysis, writing and review of this manuscript weremade by O. K.

Declarations

Competing interests
The authors declare no competing interests.

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