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Phytochemical characterization and bioactivity evaluation of extracts from different parts of *Salvia ceratophylla*

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Abstract

In this study, the phytochemical profile and biological activities of ethanol extracts of *Salvia ceratophylla* were comprehensively investigated. LC-MS/MS analyses identified numerous phenolic compounds, with rosmarinic acid determined as the dominant constituent across all extracts, reaching its highest level in the SCL extract (25140.57 µg/g). While hesperidin was consistently present in all extracts (336.37-361.91 µg/g), apigenin was found at notably high levels, particularly in the SCF extract (4720.14 µg/g). Additionally, malic acid (3430.91 µg/g, SCF) and caffeic acid (205.90 µg/g, SCL) were quantified at significant levels. GC-MS analyses revealed high concentrations of sterols and triterpenoids, including *β*-sitosterol (SCR: 5611.73 µg/g; SCM: 9733.81 µg/g), 7*α*-hydroxy-*β*-sitosterol (SCM: 8826.46 µg/g), oleanolic acid (38842.62 µg/g, SCM), and ursolic acid (74385.03 µg/g, SCM). These compounds are well-documented in the literature for their antioxidant, anti-inflammatory, and anticancer properties. When antioxidant activities were examined, the SCL extract exhibited the strongest effect in the DPPH (IC_{50}: 45.14 µg/mL), ABTS (IC_{50}: 5.68 µg/mL), and CUPRAC ($A_{0.5}$: 3.33 µg/mL) assays. It was found that all extracts generally showed no toxicity in the healthy cell line (PDF); in fact, some extracts were found to have a cell-proliferation-promoting effect. It was found that they did not exhibit any cytotoxic activity in the Caco-2 cell line. In contrast, the SCM fraction exhibited moderate cytotoxicity in the SkOV3 cell line, with a viability rate of 68.12%. Enzyme inhibition activities were generally weak; however, the SCR extract displayed significant inhibition against BChE (52.01±1.33%), while the SCM extract exhibited limited activity against urease (5.25±0.22%) and collagenase (8.01±0.15%). In conclusion, *S. ceratophylla* extracts possess strong antioxidant capacity due to their high phenolic and flavonoid contents, alongside a rich sterol and triterpenoid composition, highlighting their potential as a source of bioactive compounds warranting further investigation. These findings support the utilization of the species as a source of natural antioxidants and bioactive compounds in the food, cosmetic, and pharmaceutical industries.

Keywords: *Salvia ceratophylla*, LC-MS/MS, Rosmarinic acid, Oleanolic acid, Ursolic acid, Cytotoxic activity

INTRODUCTION

Natural products represent one of the most important sources of biologically active compounds and play a critical role in the development of new products in the pharmaceutical, food, and cosmetic industries (Tohma et al., 2016; Atanasov et al., 2021; Nasim et al., 2022). Within this context, the Lamiaceae family is noteworthy for its aromatic species widely utilized in both traditional and modern medicine (Chrysargyris, 2024; Cîmpeanu et al., 2026). Among its largest genera, *Salvia* is represented by approximately 900 species, with a broad geographical distribution including nearly 500 species in Central and South America, 250 species in Central Asia and the Mediterranean region, and 90 species in East Asia (Walker et al., 2004; Walker & Sytsma, 2007; Jeshvaghani et al., 2015).

The major phytochemical constituents of *Salvia* species include diterpenoids, phenolic acids, triterpenoids, flavonoids, and saccharides, which hold significant value in the cosmetic industry (Pitarevic et al., 1984; Bellomaria et al., 1992; Ahmed et al., 1994; Carta et al., 1996; Foray et al., 1999; Kaya et al., 2003; Zhou et al., 2009; Xu et al., 2018). Traditionally, *Salvia* species have been employed in wound healing, digestive disorders, and the treatment of infections (Kamatou et al., 2005;

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Kamatou, 2006). Modern pharmacological studies have further demonstrated that secondary metabolites of this genus exhibit anti-inflammatory, antioxidant, cytotoxic, and anticancer activities (Fang & Mc-Laughlin, 1990; Ulubelen et al., 1997; Son et al., 2005; Topcu et al., 2007; Topcu et al., 2008; Hao et al., 2009; Kolak et al., 2009; Tayarani-Najaran et al., 2013; Ozer et al., 2013; Akdeniz et al., 2021).

Within this broad biological diversity, *Salvia ceratophylla* L. emerges as a biennial, aromatic, lemon-scented species. Native to Turkey, Iran, and the Middle East, this species has been confirmed by LC-MS/MS and GC-MS analyses to be rich in phenolics, flavonoids, and terpenoids (Uysal et al., 2021). Recent studies have revealed that *S. ceratophylla* extracts possess strong antioxidant capacity, exhibit inhibitory effects on biologically critical enzymes such as cholinesterases (AChE, BChE), tyrosinase, and ACE, and display cytotoxic activity against cancer cell lines (Loizzo et al., 2014; Abu-Darwish et al., 2020). Ethnobotanical data indicate its traditional use in the treatment of inflammation, infections, and pain, while modern pharmacological research highlights its potential in managing chronic diseases such as diabetes, neurodegenerative disorders, and hypertension (Kasabri et al., 2014; Uysal et al., 2021). Consequently, *S. ceratophylla* represents a promising candidate for the development of new bioactive resources in the pharmaceutical, food, and cosmetic industries.

In this study, ethanol extracts prepared from the roots, branch, leaves, flowers, and mixed parts of *S. ceratophylla* were comprehensively evaluated for their phytochemical profile and biological activities. Phenolic and flavonoid contents were determined by LC-MS/MS, while terpenoid profiles were analyzed by GC-MS. Antioxidant capacities were assessed using ABTS, DPPH, and CUPRAC assays. Furthermore, the toxic effects of the extracts on healthy cell lines (PDF) and their cytotoxic activities against human colon (Caco-2) and ovarian (SkOV3) cancer cell lines were investigated. In addition, the inhibitory potentials of the extracts against biologically relevant enzymes, including cholinesterases (AChE and BChE), urease, tyrosinase, elastase, collagenase, and angiotensin-converting enzyme (ACE), were examined to elucidate the pharmacological significance of the species. Although previous studies on *S. ceratophylla* have focused on antioxidant, enzyme-inhibitory, and cytotoxic activities, comprehensive studies that comparatively evaluate the phytochemical composition and biological activities of the plant's different organs (root, branch, leaf, flower, and mixed parts) are limited. In particular, the quantitative analysis of phenolic compounds via LC-MS/MS, the determination of sterol and triterpene contents via GC-MS, and the evaluation of these in conjunction with antioxidant, cytotoxic, and enzyme-inhibitory activities have not been previously reported in detail. Therefore, the present study aims to comparatively elucidate the phytochemical and biological properties of different plant parts.

MATERIALS AND METHODS

Plant Material and Extraction

Salvia ceratophylla L. specimens (Türkiye, B9 Van: Gürpınar district, Güzeldere passenger, roadside, 2450 m, 38°13′20″N, 43°54′02″E) were collected on 16 June 2024. Taxonomic identification was performed by Dr. Mehmet Fırat, Department of Biology, Faculty of Education, Van Yüzüncü Yıl University. The specimens were deposited in the herbarium under voucher number M. Fırat 40241 (VHLV). The plant material used in this study consisted of roots, branch, leaves, flowers, and mixed parts. These materials were shade-dried, ground, and subjected to maceration for ethanol extraction. The prepared extracts were coded as root (SCR), branch (SCB), leaf (SCL), flower (SCF), and mixed (SCM). The extraction procedure was based on the principle of solid-liquid extraction, using analytical-grade ethanol as the solvent. For each sample, 10 g of dried and powdered plant material was mixed with 50 mL ethanol and left at room temperature for 8 h. Subsequently, the samples were extracted in an ultrasonic bath at 40 kHz for 30 min. The resulting mixtures were filtered under vacuum, and the process was repeated three times for each sample to increase yield. The filtrates were concentrated by solvent evaporation at 40°C under reduced pressure (150 mbar) using a rotary evaporator. Finally, the crude extracts were transferred into pre-weighed tubes and stored at 4°C until further analysis (Akdeniz et al., 2021).

LC-MS/MS Based Quantitative Analysis

The phenolic and flavonoid constituents of *S. ceratophylla* extracts were determined using LC-MS/MS analysis. Prior to measurement, extract concentrations were standardized to 1000 mg/L to ensure methodological consistency. Quantitative analyses were performed on a Shimadzu Nexera ultra high performance liquid chromatography (UHPLC) system coupled with a Shimadzu LCMS 8040 triple quadrupole mass spectrometer, enabling accurate identification and quantification of target compounds. The UHPLC system was equipped with an LC 30AD binary pump, CTO 10ASvp column oven, DGU 20A3R degasser, and SIL 30AC autosampler. Chromatographic separation was achieved on an RP C18 Inertsil ODS 4 analytical column (100 mm × 2.1 mm, 2 µm), maintained at 35°C. The mobile phases consisted of eluent A (water containing 10 mM ammonium formate and 0.1% formic acid) and eluent B (acetonitrile). The gradient program was applied as follows: 0-10 min, 5-20% B; 10-22 min, 20% B; 22-36 min, 20-50% B; 36-40 min, 95% B; 40-50 min, 5% B. The flow rate was set at 0.25 mL/min, with an injection volume of 4 µL. Mass spectrometric detection was carried out using an electrospray ionization (ESI) source in negative ion mode. Operating parameters were as follows: interface temperature 350°C, DL temperature 250°C, heat block temperature 400°C, nebulizing gas (N_2) 3 L/min, and drying gas (N_2) 15 L/min. Data acquisition and processing were performed using LabSolutions software. Multiple reaction monitoring (MRM) was applied with two transitions per analyte, one for quantification and the other for confirmation. External calibration curves were constructed for each analyte at six concentration levels, with triplicate injections per level. All compounds exhibited correlation coefficients (R^2) ≥ 0.989. Accuracy and precision were assessed using the standard addition method, with intra day (n=6) and inter day (n=3, across three consecutive days) analyses to calculate %RSD and recovery values. Recovery

(%) was calculated as (measured amount - original amount)/spiked amount × 100. Limits of detection (LOD) and quantification (LOQ) were determined from ten replicate spiked samples at low concentration levels, using the formulas LOD = mean + 3 × SD and LOQ = mean + 10 × SD. Measurement uncertainty (U95) was derived from accuracy and precision data according to EURACHEM guidelines and reported at the 95% confidence level (k=2). Matrix effects were evaluated by comparing spiked and non spiked samples, revealing ion suppression or enhancement phenomena (Yilmaz et al., 2018; Yigitkan et al., 2022).

GC-MS Analysis for Di- and Tri-Terpenoid Contents

Diterpenoid contents were analyzed based on the method developed by Bakir et al. (2020). An Agilent 7890A GC/FID system coupled with an Agilent 5977B mass spectrometer was employed. Chromatographic separation was performed on a non-polar HP-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness), with helium as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature was initially set at 150°C, increased at a rate of 5°C/min to 300°C, and held for 20 min. Samples (2.0 µL) were injected in splitless mode. The injector and transfer line temperatures were maintained at 300°C, while the ion source temperature was set at 230°C. Electron impact (EI) ionization was carried out at 70 eV. Spectral data were acquired in full scan mode over the range of 100-600 m/z, and compound identification was confirmed by comparison with NIST and Wiley libraries as well as literature data. Quantification was performed using two characteristic ions of high abundance. Triterpenoid compounds (moronic acid, *α*-amyrin, oleanonic acid, oleanolic acid, betulinic acid, ursolic acid, and ursonic acid) were determined using a GC-MS method developed by our group (Akdeniz et al., 2024). To enhance volatility, derivatization was performed: samples were incubated with BSTFA containing 1% TMCS at 70°C for 120 min. The derivatized samples were subsequently injected into the GC-MS system. Analyses were conducted under the same instrumentation, with the oven temperature programmed from 200°C to 300°C at 10°C/min, held for 15 min, then increased to 310°C at 5°C/min and held for 2 min. The carrier gas flow rate was adjusted to 0.8 mL/min. MS data were collected in full scan mode over the range of 50-650 m/z, and quantification was achieved using two or three characteristic ions of high abundance. Quantification was performed for all compounds using an external calibration method based on authentic standards; no internal standard was employed. Calibration curves were constructed from peak areas of authentic standard solutions by linear regression, yielding correlation coefficients (r^2) in the range of 0.9991-0.9997. Calibration ranges and regression coefficients were reported as part of method validation. The repeatability and reliability of the method were supported by LOD/LOQ and recovery values. The LOD/LOQ values were determined as follows: *α*-amyrin TMS (335.71/389.85 mg/L), moronic acid TMS (484.57/575.49 mg/L), oleanonic acid TMS (493.52/569.16 mg/L), oleanolic acid 2TMS (504.40/589.80 mg/L), betulinic acid 2TMS (484.45/546.40 mg/L), ursolic acid 2TMS (459.09/506.43 mg/L), and ursonic acid TMS (484.09/572.27 mg/L). Recovery values ranged between 0.938 and 0.986, while %RSD values were found within 0.015-0.029.

Comprehensive Evaluation of Antioxidant and Cytotoxic Properties

The total phenolic contents of *S. ceratophylla* ethanol extracts (SCR, SCB, SCL, SCF, SCM) were calculated as pyrocatechol equivalents (PEs), while flavonoid contents were expressed as quercetin equivalents (QEs) (Slinkard & Singleton, 1977; Moreno et al., 2000). Phenolic content was determined using the Folin-Ciocalteu method at 765 nm, whereas flavonoid content was measured by the aluminum chloride colorimetric method at 415 nm. Antioxidant activities were assessed using DPPH and ABTS assays, with results expressed as IC_{50} values. In addition, the CUPRAC method was applied, and results were calculated as $A_{0.5}$ values. *α*-Tocopherol and BHT were used as reference standards in all assays (Blois, 1958; Re et al., 1999; Apak et al., 2004). The cytotoxic activities of the plant extracts were evaluated using the MTT cell viability assay. Healthy human dermal fibroblast (PDF), colon cancer (Caco-2), and ovarian cancer (SkOV3) cell lines were employed in the study. Cells were seeded into 96 well plates at densities of 1×10^4 cells/well for PDF and 5×10^3 cells/well for Caco-2 and SkOV3. To allow cell attachment, cultures were incubated for 24 h under humidified conditions at 37°C with 5% CO_2. Extracts were applied to the cells at a concentration of 50 µg/mL. For solvent control, an equivalent volume of ethanol used for extract dissolution was added to the cells. Following treatment, cells were incubated with the extracts for 48 h. At the end of the incubation period, 10 µL of MTT solution was added to each well, and cells were subjected to an additional 4 h incubation. To assess the potential direct interference of the extracts with the MTT reaction, parallel controls were performed in wells without cells. Absorbance was measured at 570 nm using an ELISA microplate reader. Cell viability was calculated as a percentage relative to the untreated control group. All experiments were conducted in three independent biological replicates, with three technical parallels for each concentration (Mojarraba et al., 2013).

Enzyme Inhibition Activity Analyses

The enzyme inhibition activities of the ethanol extracts of *S. ceratophylla* (SCR, SCB, SCL, SCF, SCM) were determined based on methods previously described in the literature. All extracts were tested at a fixed concentration of 100 µg/mL. Cholinesterase (AChE and BChE) inhibition was assessed using the spectrophotometric method of Ellman et al. (1961), with acetylthiocholine iodide and butyrylthiocholine iodide as substrates. Reaction mixtures were incubated for 15 min, and absorbance was measured at 412 nm; galantamine served as the positive control. Urease inhibition was evaluated according to the spectrophotometric method of Hina et al. (2015), using urea as substrate. Reaction mixtures were incubated for 30 min, and absorbance was recorded at 560 nm; thiourea was used as the positive control. Tyrosinase inhibition was determined following the method of Hearing & Jiménez (1987), with L-DOPA as substrate. Reaction mixtures were incubated for 20 min, and absorbance was measured at 475 nm; kojic acid was employed as the positive control. Elastase inhibition was assessed using the method of Kraunsoe et al. (1996), with N succinyl $(Ala)_3$ nitroanilide as substrate. Reaction mixtures were

incubated for 30 min, and absorbance was measured at 410 nm; oleanolic acid served as the positive control. Collagenase inhibition was determined according to the method of Thring et al. (2009), with N (3 [2 Furyl]acryloyl) Leu Gly Pro Ala as substrate. Reaction mixtures were incubated for 60 min, and absorbance was measured at 335 nm; epicatechin gallate was used as the positive control. ACE inhibition was evaluated following the method of Kwon et al. (2006), with minor modifications. Hippuryl histidyl leucine (HHL) was used as substrate, reaction mixtures were incubated for 30 min, and absorbance was measured at 228 nm; lisinopril served as the positive control. All experiments were performed in three independent biological replicates, with three technical parallels for each concentration. Blank corrections were carried out separately for each assay using wells containing extract and substrate but lacking enzyme. Possible solvent effects of the extracts were also controlled. Results were expressed as percentage inhibition values.

Statistical Method

All experimental data were obtained from three independent replicates, and results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied to evaluate statistical differences among groups, with a significance level set at $p < 0.05$. Statistical calculations were performed using Minitab 16.2.1 software.

RESULTS AND DISCUSSION

LC-MS/MS Analysis Results

LC-MS/MS analyses revealed the presence and quantitative distribution of numerous phenolic compounds in the ethanol extracts of *S. ceratophylla* (Table 1, Figure 1). Rosmarinic acid was identified as the dominant constituent across all extracts, with the highest concentration observed in the SCL extract (25140.57 µg/g). In addition, hesperidin was consistently detected in all extracts (336.37-361.91 µg/g). Apigenin was particularly abundant in the SCF extract (4720.14 µg/g). In addition, malic acid (3430.91 µg/g, SCF) and caffeic acid (205.90 µg/g, SCL) were quantified at noteworthy levels. Certain phenolic acids (e.g., gallic acid, ferulic acid, sinapic acid) were not detected, indicating a species-specific distribution pattern within the phenolic profile.

These findings demonstrate that *S. ceratophylla* possesses a phytochemical profile rich in phenolic compounds. The high rosmarinic acid content is consistent with previously reported levels in other *Salvia* species (Lu & Foo, 2002; Srećković, et al., 2022). The antioxidant and anti-inflammatory properties of rosmarinic acid are well-documented in the literature (Çulhaoğlu et al., 2015; Luo, et al., 2020). Elevated levels of flavonoids such as hesperidin and apigenin further support the pharmacological potential of the species. The notably high apigenin content in the SCF extract highlights the variability of compound distribution among plant organs, which may explain differences in biological activities across extracts. The presence of phenolic acids such as malic acid and caffeic acid reflects the metabolic diversity of the species, both of which are known contributors to antioxidant capacity (Re et al., 1999; Apak et al., 2004).

Although LC-MS/MS analyses of *S. ceratophylla* have not been previously reported, studies on other *Salvia* species corroborate these results. For instance, Bakir et al. (2020) reported high rosmarinic acid content (38035.7 µg/g) in *S. hyppargeia* leaf extract. Yilmaz et al. (2023) identified rosmarinic acid as a major compound in *S. wiedemannii*, *S. heldreichiana*, and *S. aucheri* subsp. *canescens*. Yıldız et al. (2025) detected hesperidin (6.465%), rosmarinic acid (5.248%), and fumaric acid (0.244%) in *S. aintabensis* methanol extract. Fotovvat et al. (2019) reported rosmarinic acid (4.25±1.04 mg/g) in *S. aethiopis* leaves, while Yigitkan et al. (2026) highlighted a remarkable rosmarinic acid content (34638.45 µg/g) in *S. cerino-pruinosa* var. *elazigensis*. In conclusion, the ethanol extracts of *S. ceratophylla* exhibit a diverse and phenolic-rich profile. The high levels of rosmarinic acid, hesperidin, and apigenin underscore the species' potential as a natural source of antioxidants and bioactive compounds for applications in the food, cosmetic, and pharmaceutical industries. The findings are consistent with phytochemical characteristics previously reported for other *Salvia* species.

GC-MS Analysis Results for Di- and Tri-Terpenoid Content

GC-MS analyses identified diterpenoid, triterpenoid, steroid, and flavonoid constituents in the ethanol extracts of *S. ceratophylla* (Table 2, Figure 2 and Figure 3). The presence of triterpenic acids at notably elevated concentrations was observed. Among sterol components, *β*-sitosterol was found in all extracts at high concentrations (SCR: 5611.73 µg/g; SCB: 1790.50 µg/g; SCL: 2746.96 µg/g; SCF: 1696.68 µg/g; SCM: 9733.81 µg/g). In addition, 7*α*-hydroxy-*β*-sitosterol was most abundant in the SCM extract (8826.46 µg/g). Within triterpenoids, oleanolic acid and ursolic acid were particularly prominent, with SCM extract containing 38842.62 µg/g oleanolic acid and 74385.03 µg/g ursolic acid substantially higher than levels measured in other extracts. Furthermore, ferruginol was detected as a diterpenoid at noteworthy concentrations (SCR: 4623.53 µg/g; SCM: 1667.49 µg/g).

Although comprehensive studies on diterpenoid, triterpenoid, and steroid contents of *S. ceratophylla* are lacking, similar compounds have been reported in other *Salvia* species. For example, Akdeniz et al. (2022) documented high *β*-sitosterol content (27075.81 µg/g) in *S. cerino-pruinosa* chloroform extract. The same research group reported ferruginol, 6,7-dehydroroyleanone, and 7*α*-hydroxy-*β*-sitosterol in *S. kurdica*, *S. pseudeuphratica*, *S. rosifolia*, *S. siirtica*, and *S. cerino-pruinosa* varieties. Bakir et al. (2020) identified high levels of ferruginol (30787.97 µg/g), lupenone (23276.21 µg/g), and lupeol (20625.92 µg/g) in *S. hypargeia*. Akdeniz et al. (2021) reported *β*-sitosterol (27520 µg/g) in *S. multicaulis*, while Gören et al. (2002) confirmed the presence of ursolic acid, oleanolic acid, and *β*-sitosterol in acetone extracts of *S. ceratophylla* roots.

Table 1. Identification and quantification of phenolic compounds of ethanol extracts of *S. ceratophylla* species by LC-MS/MS

No	Analysts	RT[a]	Mother ion (*m/z*)[b]	Fragment ions (*m/z*)[c]	Quantification (µg analyte/g extract)[d]				
					SCR	SCB	SCL	SCF	SCM
1	Quinic acid	1.13	190.95	85.3-93.3	Nd	12.96	19.61	48.52	15.36
2	Malic acid	1.23	133.00	15.2-71.3	839.31	1756.89	856.23	3430.91	1377.95
3	Fumaric acid	1.48	115.00	71.4	87.66	256.19	225.36	142.85	130.84
4	Gallic acid	3.00	168.85	125.2-79.2	Nd	Nd	Nd	Nd	Nd
5	Protocatechuic acid	4.93	152.95	108.3	18.27	25.24	32.42	7.44	18.56
6	Pyrocatechol	6.48	109.00	108.35-91.3	Nd	Nd	Nd	Nd	Nd
7	Chlorogenic acid	7.13	353.15	191.2	Nd	Nd	Nd	Nd	4.60
8	4-OH Benzoic acid	7.39	136.95	93.3-65.3	Nd	Nd	Nd	Nd	Nd
9	Vanilic acid	8.57	166.90	152.3-108.3	Nd	Nd	Nd	Nd	Nd
10	Caffeic acid	8.80	178.95	135.2-134.3	79.33	106.19	205.90	56.91	134.02
11	Syringic acid	9.02	196.95	182.2-167.3	Nd	Nd	Nd	Nd	Nd
12	Vanillin	10.87	151.00	136.3-92.2	Nd	Nd	Nd	Nd	Nd
13	Salicylic acid	11.16	136.95	93.3-65.3	13.46	13.78	16.18	Nd	10.53
14	*p*-Coumaric acid	11.53	162.95	119.3-93.3	47.4	80.66	145.74	66.47	128.29
15	Rutin	12.61	609.05	300.1-271.1	7.01	6.16	7.72	Nd	9.66
16	Ferulic acid	12.62	192.95	178.3	Nd	Nd	Nd	Nd	Nd
17	Sinapinic acid	12.66	222.95	208.3-149.2	Nd	Nd	Nd	Nd	Nd
18	Hesperidin	12.67	610.90	303.1-465.1	349.52	343.02	349.87	336.37	361.91
19	Isoquercitrin	13.42	463.00	300.1-271.1	Nd	26.40	19.61	Nd	21.48
20	Rosmarinic acid	14.54	359.00	161.2-197.2	5943.83	11687.56	25140.57	6270.58	17757.65
21	Nicotiflorin	14.68	593.05	285.1-255.2	Nd	Nd	Nd	Nd	Nd
22	*o*-Coumaric acid	15.45	162.95	119.4-93.3	Nd	Nd	Nd	Nd	Nd
23	Rhoifolin	16.11	577.05	269.2-211.1	Nd	Nd	Nd	Nd	Nd
24	Quercitrin	16.41	447.15	301.1-255.1	Nd	Nd	Nd	Nd	Nd
25	Apigetrin	16.59	431.00	268.2-239.2	36.44	190.51	339.28	297.88	401.85
26	Coumarin	17.40	147.05	91.0-103.2	Nd	Nd	Nd	Nd	Nd
27	Myricetin	18.72	317.00	179.2-151.3	Nd	Nd	Nd	Nd	Nd
28	Fisetin	19.30	284.95	135.2-121.3	Nd	Nd	Nd	Nd	Nd
29	Cinnamic acid	25.61	147.00	103.15-77.3	Nd	Nd	Nd	Nd	Nd
30	Liquiritigenin	25.62	254.95	119.3-135.1	Nd	Nd	Nd	Nd	Nd
31	Quercetin	28.17	300.90	151.2-179.2	Nd	Nd	Nd	Nd	Nd
32	Luteolin	28.27	284.75	133.2-151.2	Nd	Nd	Nd	Nd	Nd
33	Naringenin	30.68	270.95	151.2-119.3	Nd	Nd	2.34	Nd	2.18
34	Apigenin	31.43	268.95	117.3-151.2	123.01	296.29	907.26	4720.14	1140.76
35	Hesperetin	31.76	300.95	164.2-136.2	Nd	Nd	Nd	Nd	Nd
36	Kaempferol	31.88	284.75	255.1-117.3	Nd	Nd	Nd	Nd	Nd
37	Chrysin	36.65	252.95	143.3-119.4	Nd	Nd	Nd	Nd	Nd

[a]RT: Retention time, [b]Mother ion (m/z): Molecular ions of the standard compounds (*m/z* ratio), [c]Fragment ions: MRM fragments for the related molecular ions, [d]Values in µg/g (w/w) of plant ethanol extract, Nd: Not detected.

These findings highlight the sterol- and triterpenoid-rich phytochemical profile of *S. ceratophylla*. The notably high levels of *β*-sitosterol, oleanolic acid, and ursolic acid are of particular pharmacological relevance. In the literature, these compounds have been associated with antioxidant, anti-inflammatory, and anticancer properties (Topcu et al., 2007; Çulhaoğlu et al., 2015). *β*-Sitosterol is well known for its role in membrane stability and cholesterol metabolism (Awad & Fink, 2000), while ursolic and oleanolic acids are recognized for their anti-inflammatory and hepatoprotective activities (Liu, 1995; Dzubak et al., 2006). The exceptionally high concentrations of these compounds in the SCM extract strongly support this species' potential for biological activity. As a result, GC-MS analyses have revealed that *S. ceratophylla* extracts are rich in diterpenoid, triterpenoid, and sterol components. Additionally, the SCM extract was found to contain higher levels of ursolic acid, oleanolic acid, and β-sitosterol compared to other extracts.

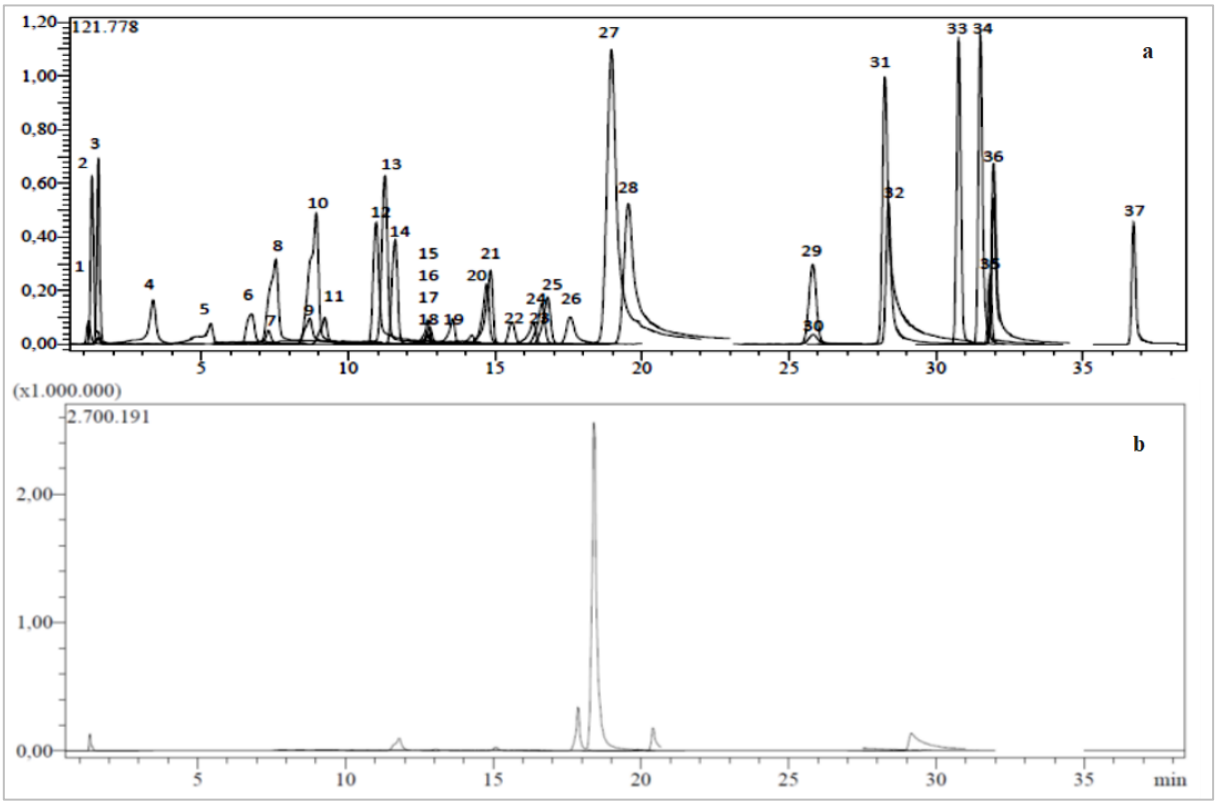

Figure 1. LC-MS/MS chromatograms **a:** TIC chromatogram of standard chemicals analysed by LC-MS/MS method. 1: quinic acid, 2: malic acid, 3: fumaric acid, 4: gallic acid, 5: protocatechuic acid, 6: pyrocatechol, 7: chlorogenic acid, 8: 4-OH-benzoic acid, 9: vanillic acid, 10: caffeic acid, 11: syringic acid, 12:vanillin, 13: salicylic acid, 14: p-coumaric acid, 15: rutin, 16: tr-ferulic acid, 17: sinapinic acid, 18: hesperidin, 19: isoquercitrin, 20: rosmarinic acid, 21: nicotiflorin, 22: *o*-coumaric acid, 23: rhoifolin, 24: quercitrin, 25: cosmosiin, 26: coumarin, 27: myricetin, 28: fisetin, 29: cinnamic acid, 30: liquiritigenin, 31: quercetin, 32: luteolin, 33: naringenin, 34: apigenin, 35: hesperetin, 36: kaempferol, 37: chrysin, **b:** LC-MS/MS chromatogram of mixed ethanol extract of *S. ceratophylla*

Table 2. Diterpenoid, triterpenoid, steroid and flavonoid contents of *S. ceratophylla* by GC-MS

No[a]	Compound	RT[b]	Mother ion (*m/z*)[c]	Fragment ions (*m/z*)[d]		Quantification (*μ*g analyte/g extract)[e]				
						SCR	SCB	SCL	SCF	SCM
	Diterpenoid, steroid and flavonoid contents									
3	Ferruginol	17.091	286.46 (95.7)	271(100)	189(63.5)	4623.53	Nd	Nd	Nd	1667.49
11	7*α*-Hydroxy-*β*-sitosterol	27.700	430.71 (2.5)	394(100)	135(75.7)	1373.54	874.48	1406.45	Nd	8826.46
13	Stigmasterol	30.052	412.36 (100)	255(87.1)	271(66)	1670.29	Nd	Nd	Nd	1698.94
14	*β*-Sitosterol	30.795	414.72 (100)	396(58)	303(53.2)	5611.73	1790.50	2746.96	1696.68	9733.81
	Triterpenoid contents									
1	*α*-Amyrin	17.99	498 (2.5)	218(100)	203(16.6)	Nd	Nd	Nd	Nd	Nd
2	Moronic acid	20.71	527 (21.1)	189(100)	203(40.3)	Nd	Nd	Nd	Nd	Nd
3	Oleanonic acid	20.96	527 (12.3)	203(100)	408(64.5)	Nd	Nd	Nd	Nd	Nd
4	Oleanolic acid	21.55	601 (2.3)	203(100)	189(31.5)	971.80	704.57	1311.73	872.50	38842.62
5	Betulinic acid	21.90	601 (4.9)	189(100)	203(34.5)	Nd	Nd	Nd	Nd	Nd
6	Ursolic acid	22.55	601 (2.3)	203(100)	189(32.9)	2173.83	908.45	1353.81	1353.81	74385.03
7	Ursonic acid	22.91	527 (9.5)	203(100)	320(60.4)	Nd	Nd	Nd	Nd	Nd

[a]Numbers on the far left raw indicate the standard phytochemical compounds. Undetected components: 1: sclareolide, 2: sclareol, 4: cryptanol, 5: 6,7-dehydroroyleanone, 6: suginal, 7: 9,10-dihydro-7,8-dimethyl-2-(1-methylethyl) phenanthrene-3-ol, 8: sugiol, 9: inuroyleanone, 10: 12-demethylmulticaulin, 12: salvigenin, 15: sinensetin, 16: lupenone, 17: *α*-amyrin, 18:lupeol, 19: 3-acetyl lupeol, 20: 1*α*,21*α*-dihydroxy-2-3-(1',1'-dimethyl-dioxymethylene) urs-9(11), 12-diene, 21: uvaol, 22: betulin, 23: pyxinol, 24: lup-(20)29-ene-2*α*-hydroxy-3*β*-acetate, 25: betulin 3*β*,28*β*-dicetate, 26: 21α-hydroxy,2*α*,3*β*-diacetoxy urs-9(11),12-diene. [b]RT: Retention time, [c]Mother ion (*m/z*): Molecular ions of the standard compounds (*m/z* ratio), [d]Fragment ions: MRM fragments for the related molecular ions, [e]Values in µg/g (w/w) of plant ethanol extract, Nd: Not detected.

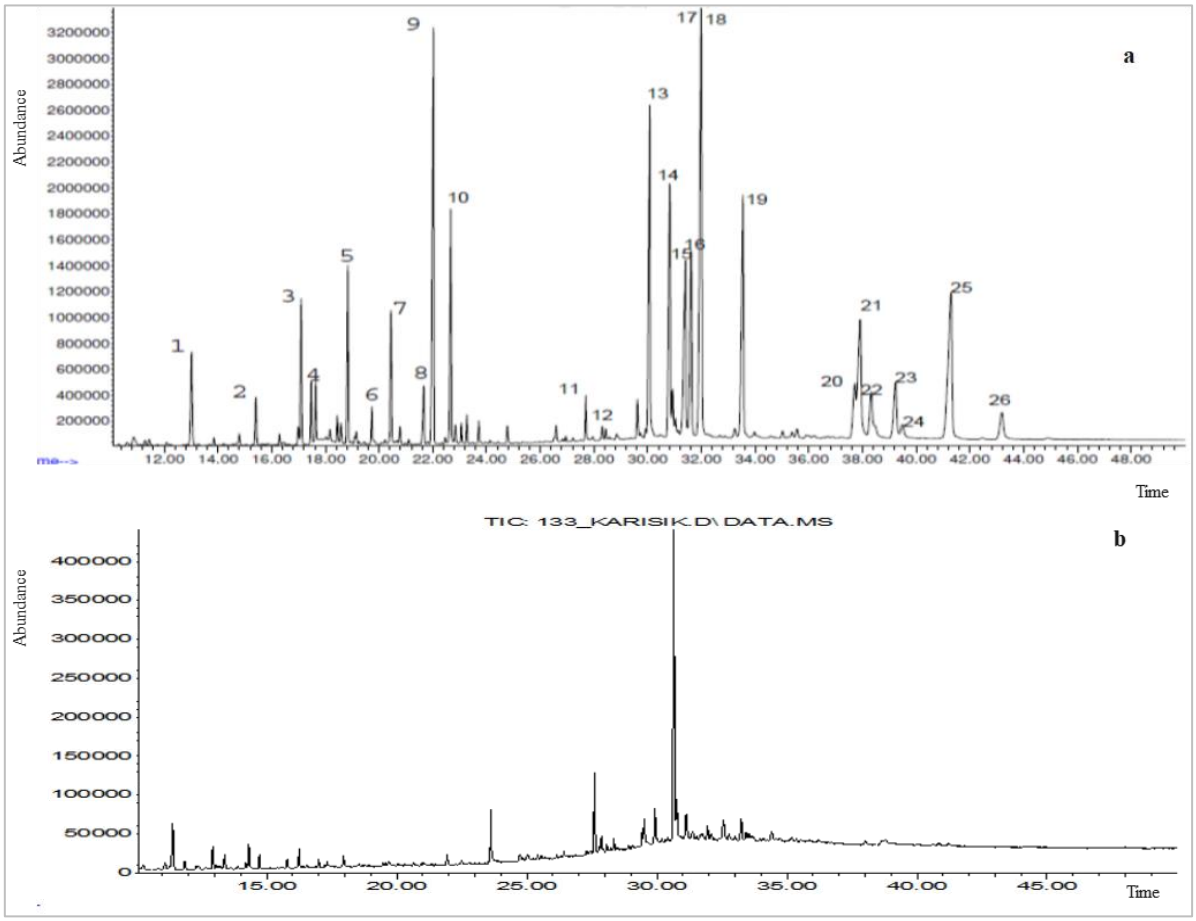

Figure 2. GC-MS chromatograms **a:** TIC chromatogram of standard chemicals analysed by GC-MS method. 1: sclareolide, 2: sclareol, 3: ferruginol, 4: cryptanol, 5: 6,7-dehydroroyleanone, 6: suginal, 7: 9,10-Dihydro-7,8-dimethyl-2-(1-methylethyl) phenanthren-3-ol, 8: sugiol, 9: inuroyleanone, 10: 12-demethylmulticauline, 11: 7-*α*-hydroxy-*β*-sitosterol, 12: salvigenin, 13: stigmasterol, 14: *β*-sitosterol, 15: sinensetin, 16: lupenone, 17: *α*-amyrin, 18:lupeol, 19: 3-acetyl lupeol, 20: 1*α*,21*α*-dihydroxy-2-3-(1'1'-dimethyl-dioxymethylene)urs-9(11),12-diene, 21: uvaol, 22: betulin, 23: pyxinol, 24: Lup-(20)29-ene-2*α*-hydroxy-3*β*-acetate, 25: betulin 3*β*,28*β*-dicetate, 26: 21-*α*-hydroxy, 2*α*,3*β*-diacetoxy urs-9(11),12-diene. **b:** GC-MS chromatogram of mixed ethanol extract of *S. ceratophylla*

Analysis Results for Total Flavonoid-Phenolic Content, Antioxidant Activity, and Cytotoxic Effects

The total phenolic and flavonoid contents, as well as the antioxidant and cytotoxic activities of *S. ceratophylla* extracts, were evaluated (Table 3). The highest total phenolic content was observed in the SCL extract (73.30±1.04 µg PEs/mg), while the highest flavonoid content was also found in the SCL extract (42.71±0.98 µg QEs/mg). Antioxidant activity assays revealed that the SCL extract exhibited the strongest effects in DPPH (IC_{50}: 45.14 µg/mL), ABTS (IC_{50}: 5.68 µg/mL), and CUPRAC ($A_{0.5}$: 3.33 µg/mL) tests. These findings confirm that *S. ceratophylla* extracts are rich in phenolic and flavonoid compounds.

The literature supports these results. Poyraz et al. (2017) reported high phenolic contents in methanol and ethanol extracts of *S. ceratophylla* (168.35±1.97 and 330.14±2.28 mg GAE/g, respectively). Loizzo et al. (2014) documented 32.7 mg GAE/g phenolic and 27.0 mg GAE/g flavonoid contents in methanol extracts. Uysal et al. (2021) reported 72.50 mg GAE/g phenolic and 43.77 mg RE/g flavonoid contents in methanol extracts. Additional studies by Orhan et al. (2007), Iravani et al. (2020), and Gursoy et al. (2012) also demonstrated strong radical scavenging capacity in this species. Antioxidant activity findings are consistent with the present study: Uysal et al. (2021) reported high DPPH and CUPRAC capacities in hydro-methanolic extracts; Orhan et al. (2007) observed 84.8% inhibition against DPPH radicals in methanol extracts; Iravani et al. (2020) identified strong antioxidant activity (IC_{50}: 30.08 µg/mL) in essential oils from Iranian samples; Gursoy et al. (2012) reported 62.84% DPPH radical scavenging capacity; and Loizzo et al. (2014) confirmed high antioxidant activity in DPPH, ABTS, and FRAP assays.

The toxic effects of *S. ceratophylla* ethanol extracts (SCR, SCB, SCL, SCF, SCM) were assessed in PDF (healthy) cell lines, while cytotoxic effects were evaluated in Caco-2 (colon cancer) and SkOV3 (ovarian cancer) cell lines (Table 3). Upon evaluation of the cytotoxic activity results, it was found that none of the extracts exhibited toxic effects in the PDF cell line. Upon examination of the cytotoxicity profiles, it was observed that none of the fractions exhibited cytotoxic activity in the Caco-2 cell line. In contrast, a different pattern emerged in the SkOV3 cell line. The SCR, SCB, SCL, and SCF fractions exhibited no cytotoxic effects with high viability (86-97%), while the SCM extract exhibited moderate cytotoxic activity at 68.12%.

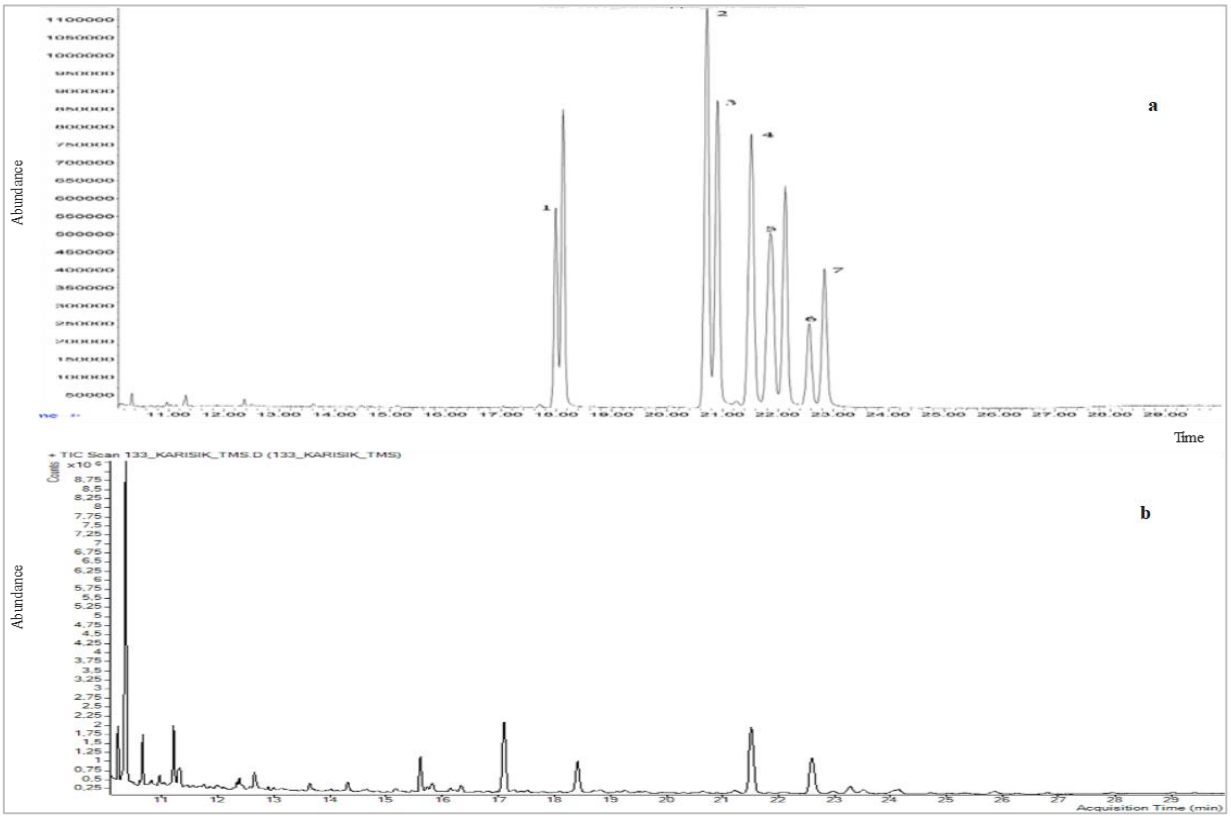

Figure 3. GC-MS chromatograms, **a:** TIC chromatogram of standard chemicals analysed by GC-MS method. 1: *α*-amyrin, 2: moronic acid 3: oleanonic acid, 4: oleanolic acid, 5: betulinic acid, 6: ursolic acid, 7: ursonic acid, **b:** GC-MS chromatogram of mixed ethanol extract of *S. ceratophylla*

The results obtained demonstrate that *S. ceratophylla* fractions are generally non-toxic and preserve or enhance viability in normal cell lines. These findings suggest a favorable safety profile under the tested conditions.

In the literature, Abu-Darwish et al. (2020) reported that the hexane extract of *S. ceratophylla* exhibited mild cytotoxic activity against the SkOV3 cell line. Loizzo et al. (2014) demonstrated that the species exerted inhibitory effects on C32 and ACHN cell lines, but did not show marked cytotoxicity in the Caco-2 cell line. Conversely, Kasabri et al. (2014) indicated that the ethanol extract displayed strong cytotoxic activity in the Caco-2 cell line (IC_{50}: 9.2±0.5 µg/mL). For this reason, conflicting results have been reported in the literature regarding the effects of *S. ceratophylla* extracts on the Caco-2 cell line. This inconsistency may be attributed to differences in extraction methods and in the phenolic/flavonoid composition of the extracts, which can lead to varying biological activities. In general, *S. ceratophylla* extracts have been shown to possess high phenolic and flavonoid content and strong antioxidant capacity.

Table 3. Total phenolic and flavonoid content, antioxidant activity, and toxic/cytotoxic activity of *S. ceratophylla*

Samples[a]	Total phenolic (*µ*g PEs/mg)[b]	Total flavonoids (*µ*g QEs/mg)[c]	Antioxidant activity (µg/mL)			Toxic/cytotoxic activity (vitality%, at 50 µg/mL)		
			DPPH (IC_{50})	ABTS (IC_{50})	CUPRAC ($A_{0.5}$)	PDF	Caco-2	SkOV3
SCR	23.16 ± 0.58	20.16 ± 0.45	105.71 ± 1.24	46.05 ± 0.99	49.23 ± 0.69	88.67 ± 1.01	101.83 ± 1.23	87.91 ± 0.89
SCB	38.58 ± 0.46	35.83 ± 0.49	67.50 ± 1.11	10.23 ± 0.07	28.75 ± 0.54	104.25 ± 1.21	119.71 ± 1.58	97.35 ± 1.05
SCL	73.30 ± 1.04	42.71 ± 0.98	45.14 ± 0.56	5.68 ± 0.08	3.33 ± 0.01	118.38 ± 1.45	115.74 ± 1.66	86.99 ± 1.09
SCF	39.78 ± 0.75	28.00 ± 0.93	72.00 ± 0.98	22.45 ± 0.17	48.33 ± 0.36	277.24 ± 5.23	100.47 ± 1.44	88.12 ± 0.99
SCM	35.48 ± 0.43	21.53 ± 0.48	65.73 ± 0.78	37.50 ± 0.68	36.25 ± 0.47	198.81 ± 2.48	112.60 ± 1.35	68.12 ± 0.58
BHT	-	-	55.79 ± 1.02	16.76 ± 0.67	9.29 ± 0.27	-	-	-
α-TOC	-	-	12.45 ± 0.21	7.55 ± 0.18	16.39 ± 0.56	-	-	-

[a]The results are presented as IC_{50} values, which are the mean and standard deviation of three parallel measurements.
[b]Phenolic content equivalent to pyrocatechol.(y = 0.0734 (µg) + 0.0419 (r^2: *0.9987*)
[c]Flavonoid content equivalent to quercetin. (y = 0.0387 (µg) + 0.0007 (r^2: *0.9951*)

Enzyme Inhibition Activity Results

The enzyme inhibition activities of *S. ceratophylla* extracts were evaluated (Table 4), revealing limited but selective effects on different enzymes. The SCR extract exhibited notable inhibition against BChE (52.01±1.33%), while showing low activity against collagenase (6.97±0.21%) and ACE (7.29±0.96%). The SCM extract displayed weak inhibition of BChE (10.06±0.17%), urease (5.25±0.22%), and collagenase (8.01±0.15%). The SCL extract demonstrated only weak activity against urease (6.24±0.25%). No significant activity was observed in SCB and SCF extracts.

Compared with reference compounds, the extracts exhibited substantially lower inhibition values. Galantamine showed strong inhibition of AChE (93.70±0.60%) and BChE (78.41±1.03%), thiourea nearly complete inhibition of urease (99.79±1.68%), kojic acid potent inhibition of tyrosinase (83.82±1.25%), oleanolic acid moderate inhibition of elastase (46.11±0.87%), epicatechin gallate moderate inhibition of collagenase (46.77±0.98%), and lisinopril strong inhibition of ACE (97.88±1.12%).

Overall, the results indicate that *S. ceratophylla* extracts exhibit generally low levels of enzyme inhibition, though certain extracts demonstrated selective activity, particularly against BChE. This suggests that while the phenolic and flavonoid contents strongly contribute to antioxidant capacity, enzyme inhibition activities remain limited.

These findings are partially consistent with previous reports. Orhan et al. (2007) noted that *S. ceratophylla* extracts prepared with different solvents generally exhibited low AChE inhibition, though chloroform extracts showed higher BChE inhibition. Uysal et al. (2021) reported that hydro-methanolic extracts displayed significant inhibitory effects on tyrosinase, *α*-amylase, and *α*-glucosidase, but were inactive against AChE and BChE. Such discrepancies are likely attributable to differences in plant parts, extraction solvents, and applied concentrations. Other studies on *Salvia* species also emphasize the critical role of solvent choice and extraction conditions in determining enzyme inhibition outcomes (Orhan et al., 2012; Çulhaoğlu et al., 2015).

In summary, although GC-MS and LC-MS/MS analyses confirmed that *S. ceratophylla* extracts are rich in phenolics and flavonoids, it was found that the enzyme inhibitory activities of these extracts are limited. The SCR extract exhibited good inhibitory activity against the BChE enzyme, while the SCM extract demonstrated moderate-to-low inhibitory activity against urease and collagenase enzymes. While the findings highlight the species' strong antioxidant potential, they also indicate that its enzyme inhibitory properties require further support through biochemical and pharmacological studies.

Table 4. Enzyme inhibition activities of *S. ceratophylla* species

Samples	Enzyme inhibition% ± S.D.[a] at 100 µg/mL[a]						
	AChE	**BChE**	**Urease**	**Tyrosinase**	**Elastase**	**Collagenase**	**ACE**
SCR	Na	52.01 ± 1.33	Na	Na	Na	6.97 ± 0.21	7.29 ± 0.96
SCB	Na	Na	Na	Na	Na	5.92 ± 0.10	-
SCL	Na	Na	6.24 ± 0.25	Na	Na	Na	-
SCF	Na	Na	Na	Na	Na	Na	-
SCM	Na	10.06 ± 0.17	5.25 ± 0.22	Na	Na	8.01 ± 0.15	Na
Galanthamine[b]	93.70 ± 0.60	78.41 ± 1.03	-	-	-	-	-
Thiourea[b]	-	-	99.79 ± 1.68	-	-	-	-
Kojic acid[b]	-	-	-	83.82 ± 1.25	-	-	-
Oleanolic acid[b]	-	-	-	-	46.11 ± 0.87	-	-
Epicatechingallate[b]	-	-	-	-	-	46.77 ± 0.98	-
Lisinopril[b]	-	-	-	-	-	-	97.88 ± 1.12

[a]Values expressed are means ± S.D. of three parallel measurements and values were calculated according to negative control.
[b]Standard compound
[c]Not active

CONCLUSION

This study comprehensively revealed the phytochemical profile and biological activities of ethanol extracts of *S. ceratophylla*. LC-MS/MS analyses demonstrated that the species is rich in phenolic compounds, particularly rosmarinic acid, hesperidin, and apigenin, which were detected at high levels. These findings confirm the presence of phenolic and flavonoid constituents that significantly contribute to the antioxidant capacity of the species. GC-MS analyses further highlighted the abundance of sterols and triterpenoids, including *β*-sitosterol, oleanolic acid, and ursolic acid. The SCM extract contained higher levels of 7*α*-hydroxy-*β*-sitosterol, *β*-sitosterol, oleanolic acid, and ursolic acid than other extracts.

Antioxidant tests revealed that the SCL extract exhibited the strongest activity in the DPPH, ABTS, and CUPRAC assays; these results may be attributed to the extract's high phenolic and flavonoid content. Cytotoxicity tests determined that *S. ceratophylla* extracts did not exhibit toxic effects on the PDF cell line. In fact, some fractions exhibited a cell-viability-enhancing effect. It was found that none of the extracts exhibited cytotoxic activity on the Caco-2 cell line. In contrast, on the SkOV3 cell line, only the SCM fraction exhibited moderate cytotoxicity with a viability rate of 68.12%. These findings indicate that different parts of the *S. ceratophylla* species may exhibit varying effects on the SkOV3 cell line. Therefore, further detailed *in vitro* and *in vivo* studies are required to evaluate this species as a potential candidate for cancer treatment using natural products.

Enzyme inhibition assays showed generally low activity, though the SCR extract exhibited good inhibition against BChE, and the SCM extract demonstrated limited effects on urease and collagenase.

In general, *S. ceratophylla* extracts are characterized by their high content of phenolics and flavonoids, their strong antioxidant capacity, and their significant content of sterols and triterpenoids. The phytochemical diversity, supported by antioxidant activity and limited enzyme inhibitory activities, underscores the need for further biochemical and

pharmacological studies to fully elucidate the species' biological potential. These findings are consistent with the phytochemical and biological properties reported for other *Salvia* species. To robustly demonstrate the pharmaceutical value of *S. ceratophylla*, these findings should be supported by more detailed *in vitro* and *in vivo* studies.

Ethical Statements and Declarations

Conflict of Interest

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Authorship Contribution

Conceptualization: AE, İY, MÇ, MFD, İA, MF; methodology: AE, İY, MÇ, MFD, Nİ; software: AE, İY, MÇ, MFD, Nİ; validation: AE, İY, EÇK; formal analysis: İY, MFD, EÇK, Nİ, SİK; investigation: AE, İY, MFD, EÇK, MÇ, İA; resources: İY, MÇ, MFD, İA, Nİ ; writing-original draft preparation: AE, İY, MÇ, MFD, Nİ; writing-review and editing; AE, İY, MÇ, Nİ, MFD, İA, SİK; supervision: AE, İY.

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