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Determination of secondary metabolites of two *Satureja* species by LC- HRMS and evaluation of their antioxidant and anti-Alzheimer capacity

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Abstract: Plants are a significant source of antioxidants, including polyphenols, flavonoids, and vitamins with strong antioxidant qualities, and their use as a natural antioxidant source dates back centuries. *Satureja cuneifolia* Ten. and *Satureja hortensis* L. are two commercially important plant species consumed both as herbal tea and as spices in some regions of Türkiye. Therefore, it is very important to analyze the secondary metabolites of these plants and investigate their biological activities. In this study, the secondary metabolites of chloroform ($CHCl_3$) and methanol (MeOH) extracts of *S. cuneifolia* Ten. and *S. hortensis* L. were analyzed by LC-HRMS. Hesperidin (702.00 mg/kg extract) and fumaric acid (472.36 mg/kg extract) were found in the $CHCl_3$ extract of *S. hortensis*, whilst syringic and rosmarinic acid were the primary constituents of the MeOH extract of *S. hortensis* (44593.46 mg/kg extract, 37389.75 mg/kg extract, respectively) and the both extracts of *S. cuneifolia* (in $CHCl_3$ 926.44 and 825.42 mg/kg extract, in MeOH 55411.15 and 46045.31mg/kg extract, respectively). The extracts' antioxidant capacity was assessed using the reducing power and radical scavenging techniques, anti-Alzheimer potentials were measured by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes inhibition experiments as *in vitro*. It was determined that higher activity values were present in *S. cuinefolia* in direct proportion to its phenolic content.

Keywords: *Satureja*; *Satureja cuneifolia* Ten.; *Satureja hortensis* L.; LC-HRMS; secondary metabolite.

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1. Introduction

Türkiye is one of the countries with the highest number of species in the region in terms of members of the Lamiaceae family with strong aromatic properties. It hosts more than 600 species belonging to 45-47 genera of the Lamiaeae family. The endemism rate is approximately 45% [1]. In Türkiye, many species (aproximately 100 species) belonging to the genera *Origanum* L., *Satureja* L., *Thymbra* L., and *Thymus* L., (Lamiaceae), recognized for its strong aromatic structure and medicinal properties, are popularly known as 'thyme-kekik' and are widely used both as spices and teas [2, 3]. The genus *Satureja* is commonly known in Türkiye as "dağ kekiği", "taş kekiği", "kaya kekiği", "sivri kekik", and "çiprisa" [4-7]. Türkiye has the greatest diversity of species, with 18 species (7 of which are endemic) [4]. The species that are commonly utilized as spices and tea not only in Türkiye but also in the regions where they grow are commercially valuable; some species are harvested from their natural habitat, while others are cultivated [6, 8-11]. They have traditionally been used as carminatives, tonics, and muscle relaxants to treat cardiovascular problems, as well as stomach and gastrointestinal ailments such as cramps, nausea, indigestion, and diarrhea [12-17]. The aerial parts of these species are used not only as tea due to their different flavours, but also as a spice for stuffing, meat, chicken, pies and sausages [7, 18].

The two *Satureja* species studied in this study, *S. cuneifolia* Ten and *S. hortensis* L., are naturally grown and also cultivated in Türkiye and it is known that 90% of commercially collected *Satureja* species are *S. cuneifolia* species [7]. Various parts of these plants have been reported to be used to treat diseases: *S. hortensis* seeds are used for inflammatory diseases [15], as essential oil for colds and bronchitis [6], and above-ground parts of both species are used as tea for digestive, gastric problems, respiratory tract diseases and colds [3, 12, 15, 17]. *S. cuneifolia* is a unique plant that has been the focus of numerous studies in the fields of pharmacology, agriculture, and natural product research because of its diverse biological activity and rich chemical composition. The essential oil composition of *S. cuneifolia*, which usually grows in many regions of the Mediterranean Basin, has been the subject of many studies. Research on the chemical constituents of *S. cuneifolia* species has predominantly focused on essential oils, since it is widely used as tea. In Türkiye, the essential oils derived from this plant are characterized by the presence of compounds such as carvacrol and linalool, which contribute to its notable antimicrobial and antioxidant properties, followed by p-cymene and γ-terpinene, the biogenetic precursors of these molecules [3, 6, 9, 14, 18, 19-21] while similar results were obtained for *S. hortensis* [9-12, 14-16]. *S. cuneifolia* has been previously reported to exhibit in vitro anti-Alzheimer, antidiabetic, antimicrobial, antioxidant, antiurease, anticholinesterase and cytotoxic potential [22-25]. When the studies on *S. hortensis*, which has been listed as a medicinal plant since ancient times and traditionally used in the kitchens especially in southern Europe and the Mediterranean region, are examined it is seen that the studies focused on essential oil content and activity of the oil, and studies on phenolic content are becoming newly popular. In these studies, rosmarinic acid, caffeic acid and luteolin were determined as the main components in the extracts as a result of the examination of the extracts prepared from the above-ground part of the plant [10, 14-16, 26-27].

The antioxidant properties shown by the species have been largely attributed to the presence of phenolic compounds such as flavonoids and terpenoids [28-30], and antioxidants are known to reduce oxidative stress and exert neuroprotective effects [2, 31-32]. Reductions in oxidative stress and the antioxidant effect have been shown to affect apoptosis and cell viability in cells, which may have therapeutic implications in preventing neurodegenerative diseases such as Alzheimer's disease (AD) [33-34]. Progressive memory loss and cognitive deterioration are characteristics of AD, a common neurological illness. It is estimated that the disease will affect one in every 85 people worldwide and that by 2050, 43 percent of those affected will require intensive care [35]. The pathogenicity of Alzheimer's disease (AD) is multifactorial, involving vascular dysfunction, mitochondrial dysfunction due to overproduction of reactive oxygen species (ROS), and a combination of genetic and environmental factors. Some research has tried to explain the mechanism by which oxidative stress contributes to the development of AD. In one study, it was shown that oxidative stress causes the conversion of soluble amyloid into insoluble fibril form, which contributes to the progression of AD, and in another study, it

was shown that oxidation of Tau proteins, one of the characteristic features of AD, by free radicals in vitro may cause dimerisation and polymerisation of this protein [35-37].

Plants are a significant source of antioxidants, many of which are secondary metabolites like polyphenols, flavonoids, and vitamins that exhibit strong antioxidant activities [31-32, 38] and the use of plants as a source of natural antioxidants is as old as centuries. The aim of this study was to determine the phenolic profile, antioxidant capacity and cholinesterase enzyme activity of chloroform and methanol extracts prepared from two *Satureja* species that are widely used by people. The phenolic profile of the extracts was analysed using LC/HRMS. The antioxidant capacity was determined using the DPPH and CUPRAC methods. The AChE and BChE inhibition of the extracts was also determined.

2. Experimental

2.1. Chemicals

The comprehensive details on the origin and purity of the chemicals and reference materials utilized in the investigation is given in the supporting information.

2.2. Plant Material

Satureja cuneifolia Ten. species was collected from İzmir, Ödemiş (TD. 5218) while *Satureja hortensis* L. was collected from Erzincan, İliç (TD. 5173) during the flowering period by Prof. Dr. Tuncay Dirmenci (Balikesir University) in the year 2017.

The herbarium sample of this species was recorded and stored in Balıkesir University Necatibey Education Faculty Herbarium.

2.3. LC-HRMS Analysis

The LC-HRMS analysis was carried out by following our previous studies [38-40]. Secondary metabolites of the *Satureja* species were determined by using liquid chromatography-high-resolution mass spectrometry (LC-HRMS), which utilised an Orbitrap Q-Exactive mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled with a Troyasil (Istanbul, Turkey) C18 column (150 x 3 mm, 5 μm particle size). The method validation parameters used in this study were specificity, accuracy, precision, LOD and LOQ. The EURACHEM/CITAC guide and our previous studies were used to evaluate sources and quantify results [41]. Further information on the procedures for evaluating uncertainty can be found in the previous literature [38-44].

The specifity, linearity, accuracy, LOD and LOQ of the LC-HRMS method, uncertainty value of measurement results is described in Table S1 in supporting information [38-45].

2.4. Antioxidant Activity

2.4.1. Cupric Ions (Cu^{2+}) Reducing Ability Assay (CUPRAC)

The Cupric Ions (Cu^{2+}) Reducing Ability Assay (CUPRAC) is a widely used colorimetric method for evaluating the antioxidant capacity of various compounds. This assay measures the ability of antioxidants to reduce cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}), resulting in a colored complex, which gives maximum absorbance at 450 nm, that can be quantified spectrophotometrically. To carry out this experiment, 1 mL each of acetate buffer solution (1.0 M), neocuproine solution (7.5 mM) and $CuCl_2$ solution (10 mM) were transferred to test tubes and mixed using a vortex mixer. Then, samples were

added to the tubes at concentrations ranging from 15 to 45 µg/mL. The tubes were then filled to 1 mL with distilled water. The samples were then maintained at 25 °C for 30 minutes and the absorbance was recorded at 450 nm [3, 40, 44-46].

2.4.2. DPPH Radical Scavenging Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging capacity is a widely used method for evaluating antioxidant activity and is based on the principle of measuring antioxidant capacity through the change in color of the purple DPPH solution as a result of DPPH reacting with free radicals at a certain concentration. In this study the method was performed according to Blois method [47]. In summary, a solution of DPPH at a concentration of 0.1 mM in methanol was prepared, and 160 mL of this solution was added to 40 mL of sample solutions in methanol at different concentrations (10, 25, 50 and 100 mg/mL). These tubes were subjected to a 30-minute period of darkness. Subsequent to a period of 30 minutes, the measurements were conducted at 517 nm. In this study, the following compounds were utilised as standards: butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol. The potential of the samples on DPPH was determined and compared to the standards [47, 48].

2.5. Acetylcholinesterase (AChE)/Butyrylcholinesterase (BChE) Inbitition Assay

In order to ascertain the anti-Alzheimer potential, the acetylcholinesterase and butyrylcholinesterase enzyme inhibition values of the chloroform and methanol extracts of the species were determined using a slightly modified spectrophotometric method that was developed by Ellman et al. [49]. The following substances were utilised in the experiment: butrylcholine iodide and acetylthiocholine iodide, which served as substrates, galanthamine, which was used as positive control, and 5,5′-dithiobis(2-nitrobenzoic) acid (DTNB), which was used to measure anticholinesterase activity. All other reagents, conditions and calculations are available in our previous publications [38, 40, 45, 49-51].

2.6. Statistical Analyses

Triplicate analyses were averaged for the experiment. Data are presented as mean ± standard deviation. Variance ANOVA including one-way analysis was realized. Using Duncan's multiple range tests, significant differences between means were noted. $p < 0.05$ was regarded as significant, and $p < 0.01$ was very significant.

3. Results and discussion

3.1. LC-HRMS Analysis

Phenolics, the most abundant secondary metabolites of the plant kingdom, are ubiquitous in all organs of the plant and are therefore an integral part of the human diet. These compounds demonstrate a broad range of physiological properties, including anti-inflammatory, antimicrobial, antioxidant effects [50]. In this study, in order to determine the main constituents of the chloroform and methanol extracts of two *Satureja* species widely used by humans, pre-scan measurements were performed using a Thermo Orbitrap Q-Exactive LC-HRMS system in full scan mode with ESI source. The main peaks observed as a result of this procedure were compared with the available standards. It was then decided to validate the method for the compounds listed in Table 1. The details of the method validation procedure are given in

section 2.6 and the relevant data for the validation study are given in Supporting Information Table S1. The identified compounds and their percentages are given in Table 1.

The quantitative analysis of secondary metabolites from all extracts was performed with the use of thirty-two standards. This analysis resulted in the determination of thirty secondary metabolites. because (-)-epicatechin and caffeic acid phenethyl ester could not be determined in any extract. It was found that the methanol extracts of the plants contained more phenolic substances. This observation can be explained by the fact that phenolic compounds and methanol have close polarity index values. All extracts except chloroform extract of *S. hortensis* were rich in coumaric acids and derivatives (syringic acid and rosmarinic acid) and flavones (hesperidin, naringenin, acacetin). The structures of the most abundant phenolic compounds are given in Figure 1.

Table 1. The quantity of phenolic compounds determined in chloroform ($CHCl_3$) and methanol (MeOH) extracts of *Satureja cuneifolia* and *Satureja hortensis* (mg/kg extract) by LC/HRMS

No	Compound	*Satureja cuneifolia*		*Satureja hortensis*		U %
		$CHCl_3$	MeOH	$CHCl_3$	MeOH	(k=2)
1	Ascorbic acid	<LOD	1107.23	<LOD	354.82	11.07
2	Chlorogenic acid	6.16	301.34	<LOD	283.95	11.14
3	Fumaric acid	89.75	4157.77	**472.36**	**9685.80**	11.14
4	(-)-Epicatechin	<LOD	<LOD	<LOD	<LOD	11.91
5	(-)-Epicatechin gallate	<LOD	2.39	<LOD	2.65	11.21
6	Verbascoside	<LOD	14.18	<LOD	9.72	12.08
7	Orientin	<LOD	47.99	<LOD	34.23	11.47
8	Caffeic acid	45.73	548.45	<LOD	231.83	11.07
9	(+)-*trans* taxifolin	125.88	507.83	<LOD	59.51	11.19
10	Luteolin-7-rutinoside	38.14	1220.02	<LOD	285.73	11.45
11	Vanilic acid	59.52	1037.91	<LOD	744.68	11.61
12	Naringin	4.72	81.75	29.45	51.37	12
13	Luteolin 7-glucoside	4.24	186.20	<LOD	153.88	11.29
14	Hesperidin	442.46	**6074.31**	**702.00**	9466.63	11.15
15	Syringic acid	**926.44**	**55411.15**	<LOD	**44593.46**	12.37
16	Rosmarinic acid	**825.42**	**46045.31**	264.55	**37389.75**	11.63
17	Hyperoside	14.12	327.15	17.45	306.04	11.5
18	Dihydrokaempferol	528.53	631.21	<LOD	69.89	11.35
19	Apigenin 7-glucoside	<LOD	25.23	<LOD	37.10	11.9
20	Quercitrin	1.41	84.73	<LOD	81.69	11.69
21	Quercetin	6.78	143.42	<LOD	9.49	11.42
22	Salicylic acid	42.94	146.98	<LOD	49.47	11.4
23	Naringenin	**3889.55**	1841.13	316.36	1372.87	11.04
24	Luteolin	255.45	1011.25	<LOD	462.48	12.41
25	Nepetin	<LOD	9.87	<LOD	5.58	11.24
26	Apigenin	236.72	222.94	<LOD	28.19	11.54
27	Hispidulin	416.67	264.63	16.36	29.34	11.23
28	Isosakuranetin	96.41	61.73	<LOD	0.52	11.48
29	Penduletin	739.72	189.27	96.00	<LOD	11.81
30	Caffeic asit phenethyl ester	<LOD	<LOD	<LOD	<LOD	11.38
31	Chrysin	5.54	2.99	3.82	1.15	11.09
32	Acacetin	811.58	412.35	**333.82**	124.43	11.36

A total of thirty-two secondary metabolites were quantitatively determined from all extracts. The first three components of the extracts for *S. cuneifolia*, which are high in amount, are as follows: naringenin (3889.55 mg/kg extract), syringic acid (926.44 mg/kg extract) and rosmarinic acid (825.42 mg/kg extract) for extract $CHCl_3$. Syringic acid (6074.31 mg/kg extract), rosmarinic acid (55411.15mg/kg extract) and hesperidin (46045.31 mg/kg extract) for MeOH extract. In a previous study in which phenolic analyses of methanol and water extracts were performed for this plant, similar to our study, methanol extract was found to be richer in phenolic compounds, while rutin, kaempferol-3-O-rutinoside and fumaric acid were determined as the first three components [3]. Sezer (2023) determined rutin trihydrate as the main component in both methanol and ethanol extracts [22], while Koser et al. determined rosmarinic acid as the main component by HPLC analysis of water extract [56]. Hesperidin (702.00 mg/kg extract), fumaric acid (472.36 mg/kg extract), and acacetin (333.82 mg/kg ext ract) were the three most common chemicals found in the chloroform extract of *S. hortensis*. Following this, it was determined that only ten of the thirty-two standard compounds that were studied existed chloroform extracts. The main compounds found in the methanol extracts were characterized as syringic acid (44593.46 mg/kg extract), rosmarinic acid (37389.75 mg/kg extract), fumaric acid (9685.80.46 mg/kg extract). A review of the extant literature reveals that essential oils and extracts of *S. hortensis* are both abundant in polyphenols, including rosmarinic acid, caffeic acid, and flavonoids [8, 10-12, 14-16, 26-27]. It has been demonstrated that there is a correlation between the total phenolic content of *S. hortensis* and its antioxidant activity. This finding suggests that the potential of the plant for use in functional food applications may be enhanced [53-54].

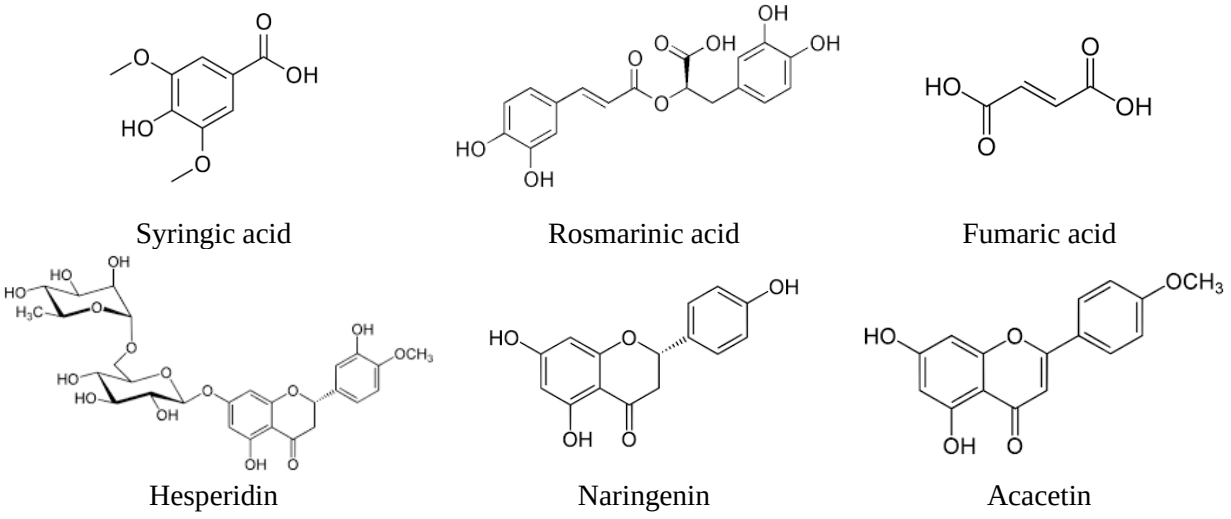

Figure 1. The most abundant phenolic compounds of *S. cuneifolia* and *S. hortensis* extracts

3.2. Bşological Activity

The antioxidant activities of the extracts were evaluated by DPPH free radical scavenging activity and copper ion reducing antioxidant capacity method. To determine the DPPH free radical scavenging activities, 10, 25, 50 and 100 µg/mL concentration series of the extracts were prepared, and α-tocopherol, BHA and BHT were used as positive controls. α-Tocopherol was also used as a positive control in the CUPRAC method. The result of DPPH (inhibition %) and CUPRAC (mmol TR/g) are given in Table 2 and Table 3, respectively.

Secondary metabolites of *S. cuenifolia* and *S. hortensis* and their biological activity

Phenolic compounds, which constitute the antioxidant potential of plants, have great structural diversity and different chemical compositions among plant-derived metabolites. Thanks to this structural diversity, they have the ability to prevent or inhibit the oxidation of various biomolecules in the environment. Studies show that phenolic substances, thanks to the phenolic and polyphenolic groups in their structures, can reduce oxidative stress by entering redox reactions that neutralise free radicals [54] and thus prevent cellular damage [29]. It has been reported that rosmarinic acid, hesperidin and naringin molecules, which are the main components of *S. cuneifolia* and *S. hortensis*, are highly active in terms of antioxidant activity [45].

When the antioxidant capacities of the extracts were analysed, methanol extracts, which are phenolic rich extracts of both plants, had higher activity values for DPPH, especially at 100 µg/mL concentration. For *S. cuneifolia*, DPPH scavenging capacity of both chloroform and methanol extracts were much better at all concentrations studied. According to the results of CUPRAC method, both chloroform (0.50± 0.02 mmol TR/g) and methanol extracts (0.51± 0.07 mmol TR/g) of *S. cuneifolia* had very high cupric ion reducing capacity. These results can be explained by the fact that *S. cunefolia* is richer in both composition and amount when the phenolic contents of the plant extracts are analysed. Research has demonstrated that extracts and essential oil of *S. cuneifolia* exhibits considerable antioxidant capacity, effectively scavenging reactive oxygen species and protecting cells from oxidative damage [2-3, 14-15, 22-25]. In one of the two studies on the phenolic content and activities of alcohol and water extracts prepared from the plant, the main components were found to be cyanidin chloride, fumaric acid, and chlorogenic acid. It was reported that the extract rich in phenolic compounds showed higher antioxidant capacity and AChE and BChE enzyme inhibition was relatively good [2]. In the other study, luteolin rutinoside and rosmarinic acid were identified as the more abundant components, and their significant cytotoxic activity against A549 cells was demonstrated [3]. In the literature, when the studies on the antioxidant capacity of *S. hortensis* species are examined, it is seen that the majority of the studies are on the activity of the essential oil of the plant. In these studies, it was reported that the essential oil of the species also showed a very high antioxidant capacity. Antioxidant capacity studies on the extracts generally focused on radical scavenging activity and it is emphasized that the main reason for the antioxidant capacity of this plant is due to the high content of rosmarinic acid [11-12, 26, 28, 53- 55].

Table 2. Inhibition % of the extracts and standards for DPPH· scavenging activity

	10 µg/mL		**25 µg/mL**		**50 µg/mL**		**100 µg/mL**	
Antioxidants	**MeOH**	**$CHCl_3$**	**MeOH**	**$CHCl_3$**	**MeOH**	**$CHCl_3$**	**MeOH**	**$CHCl_3$**
Satureja hortensis	5.0	0.0	16.3	0.0	29.7	0.0	61.8	1.5
Satureja cuneifolia	12.6	4.3	18.3	6.5	44.7	11.4	71.1	14.2
Blank	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
α-Tocopherol	63.0	60.1	85.9	86.4	86.2	87.5	86.1	87.0
BHA	82.1	82.1	85.8	85.8	86.3	86.3	86.7	86.7
BHT	40.8	40.8	59.3	59.3	65.1	65.1	79.0	79.0

*$CHCl_3$ chloroform; MeOH methanol.

Table 3. Cupric ions' (Cu^{2+}) reducing capacity of the extracts by CUPRAC method.

	$TEAC_{CUPRAC}$ (mmol TR/g)	
Antioxidants	**MeOH**	**$CHCl_3$**
Satureja hortensis	0.41± 0.02	0.30± 0.01
Satureja cuneifolia	0.51± 0.07	0.50± 0.02
Negative control	0.11± 0.01	0.16± 0.01
α-Tocopherol	0.95± 0.14	0.95± 0.06

In the treatment of AD, acetylcholinesterase inhibitor tablets which can have negative side effects, are used. To avoid these side effects, natural chemicals with antioxidant and anticholinesterase properties are preferred [56]. It has been suggested that antioxidants may play an important role as neuroprotective

agents in the early stage of AD [57] and *Satureja* species are important sources of antioxidant phenolic compounds. Therefore, it can be said that the antioxidant potential of *Satureja* species can be used to prevent the development of Alzheimer's disease and neuronal degeneration [58-59].

Similar to the antioxidant capacity, when the cholinesterase enzyme inhibition capacities of the extracts were examined, it was observed that *S. cunefolia* showed a relatively better inhibition for both AChE and BChE enzymes, yet this potential was very low in comparison to galanthamine. The results of inhibition values can be seen in Table 4.

There are two studies examining the inhibition effect of *S.cuneifolia* species on AChE and BChE enzymes, one of which examined the inhibition capacities of essential oil [34] and the other of water and methanol extracts [2]. Taslimi et al. (2020) reported that the inhibition value of the methanol extract against BChE enzyme (IC_{50}: 23.17 mg/mL) was remarkable [2], while Orhan et al. (2008) found that the essential oil obtained from *S. cuneifolia* exhibited a very significant inhibitory activity against both enzymes (AChE: 86.5%, BChE:98.4%) [34]. In the study investigating the inhibition of amyloid beta protein, which has preventive and/or therapeutic potential for AD, it was emphasised that *S. hortensis* showed a significant inhibition among the other Lamiaceae species studied (EC_{50}: 0.049 ± 0.056 mg/ml) and rosmarinic acid isolated from the ethanol extract was the most important reason for this inhibition [57].

Table 4. Acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes' inhibition (%) of the extracts (mg/mL)

	AChE (inhibition%)		**BChE (inhibition%)**	
Sample Name	**MeOH**	**$CHCl_3$**	**MeOH**	**$CHCl_3$**
Satureja hortensis	25.3±2.4	19.3±2.8	1.1±0	4.8±2.9
Satureja cuneifolia	41.4±2.1	22.5±3.1	2.0±1.1	30.5±1.9
Galantamine	96.8±1.3		83.3±0.7	

This study is the first study in which the inhibition value of *S. hortensis* against AChE and BChE enzymes was determined, and it was concluded that *S. hortensis* has inhibitory properties against the AChE enzyme, albeit weakly. In the study in which the AChE enzyme inhibition effect and antioxidant capacity of Lamiaceae species growing in Croatia were investigated, the inhibition values of the most common hydroxycinnamic acid derivatives (such as caffeic, ferulic, p-coumaric and chlorogenic acid, rosmarinic acid) were also examined, and it was shown that those rich in these components showed higher inhibition and higher antioxidant capacity value [60]. In parallel with this study, more remarkable results were obtained in our study in terms of both antioxidant and enzyme inhibition values of *S. cuneifolia*, which contains more hydroxycinnamic acid derivatives (Table 1).

4. Conclusions

In conclusion, this study investigated the antioxidant and anti-Alzheimer's capacities of methanol and chloroform extracts of two of the most widely used and traded *Satureja* species. Since it has been reported in many studies that secondary metabolites, especially phenolic compounds, carried by plants are mainly responsible for these two activities, the phenolic compound contents of these species were analyzed by LC/HRMS. The method used for this analysis was validated and its parameters were determined. Since methanol dissolves a wide range of bioactive substances, including phenolics, flavonoids, alkaloids, and terpenoids, methanolic extracts of plants are extensively researched for their antioxidant qualities. Medium- to low-polarity biocomponents can be extracted from plants using low-polarity solvents such as petroleum ether and chloroform. It contains mostly fatty acids, some alkaloids, phenolic derivatives, and terpenoids. For this reason, their activity results are not as high as polar extracts. The high effectiveness of the chloroform extract in the activity results obtained in this study showed that

the nonpolar components contained in this plant are also important. This is the first report for the phenolic component analysis of chloroform extracts of the studied species and it was found that this extract was richer in phenolic components, especially for *S. cuneifolia* species. For the methanol extracts, a more detailed phenolic component determination was provided by using different standards in addition to the components determined in previous studies. According to the results obtained, both extracts of *S. cuneifolia* were found to be rich in phenolics, especially the potential BChE enzyme inhibition value and cupric ion scavenging value of the chloroform extract were found to be remarkable. The anti-Alzheimer capacity of *S. hortensis* was also determined for the first time against AChE and BChE enzymes. Both species should continue to be used as rich phenolic sources and potential antioxidant support in our daily diets. Especially the potential of *S. cuneifolia* against AD should be investigated in more detail, and studies including isolation-structure-activity are necessary for both species, which are rich sources of phenolic compounds.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/ journal-of-chemical-metrology

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References

[1] F. Celep and T. Dirmenci (2017). Systematic and bio-geographic overview of Lamiaceae in Turkey, *Nat. Vol. Essent. Oil*, **4**(**4**),14–27.
[2] P. Taslimi, E. Köksal, A. C. Gören, E. Bursal, A. Aras, Ö. Kılıç, S. Alwasel and İ. Gülçin (2020). Anti-Alzheimer, antidiabetic and antioxidant potential of *Satureja cuneifolia* and analysis of its phenolic contents by LC-MS/MS, *Arab. J. Chem*. **13**, 4528–4537.
[3] G. Yıldız, S. İlgün, G. Şeker Karatoprak, Y. B. Köse, F. Göger, H. E. Temel and B. Demirci (2024). Chemical profile, in vitro pharmacological activity and *Satureja cuneifolia* Ten. evaluation of essential oil based on distillation time, *Int. J. Environ. Health R.* **34**(**4**), 1944-1960.
[4] H. Duman, T. Dirmenci and T. Özcan (2023). A new annual *Satureja* (Lamiaceae) species from Turkey with molecular evidence, and lectotypification of two species, *Turk. J. Bot.* **47**, 61-72.
[5] S. Selvi, R. Polat, U. Çakılcıoğlu, F. Celep and T. Dirmenci (2022). An ethnobotanical review on medicinal plants of the Lamiaceae family in Turkey, *Turk. J. Bot*. **46** (**4**), 283-332.
[6] S. Carikci, A. C. Goren, T. Dirmenci, B. Yalcinkaya, A. Erkucuk and G. Topcu (2020). Composition of the essential oil of *Satureja metastasiantha*: a new species for the flora of Turkey. *Z. Naturforsch. C*, **75**(**7-8**), 271-277.
[7] F. Satıl, T. Dirmenci, G. Tümen and Y. Turan (2008). Commercial and ethnic uses of *Satureja* (Sivri Kekik) species in Turkey, *Ekoloji* **17**(**67**)**,** 1-7.
[8] J. Rocha, R. Leandro, R. Direito, M. Gonçalves, M. P. Duarte, A. Fernandes, B. Sepodes and M. E. Figueira (2020). Attenuation of colonic injury and inflammation by administration of a phenolic extract of summer savory (*Satureja hortensis* L.) in experimental inflammatory bowel disease in mice, *Appl. Sci.* **10**(**23**), 8465.
[9] O. Gedik, Y. Z. Kocabas and O. Cinar. (2022). Determining the essential oil components and plant nutrient element values of some *Satureja* genotypes, *S. Afr. J. Bot.* **149**, 297-302.
[10] A. R. Ladan Moghadam (2015). Antioxidant activity and essential oil evaluation of *Satureja hortensis* L. (Lamiaceae) from Iran, *J. Essent. Oil Bear. Plants*, **18**(**2**), 455-459.
[11] V. Hajhashemi, A. Ghannadi and S. K. Pezeshkian (2002). Antinociceptive and anti-inflammatory effects of *Satureja hortensis* L. extracts and essential oil, *J. Ethnopharmacol.* **82**(**2-3**), 83-87.

[12] F. Jafari, F. Ghavidel and M. M. Zarshenas (2016). A critical overview on the pharmacological and clinical aspects of popular *Satureja* species, *J. Acupunct. Meridian Stud.* **9**, 118-127.

[13] A. C. Gören, G. Bilsel, M. Altun and F. Satıl (2003). Fatty acid composition of seeds of *Satureja thymbra* and *S. cuneifolia*, *Z. Naturforsch. C*, **58**(**7**-**8**), 502-504.

[14] B. Tepe and M. Cilkiz (2016). A pharmacological and phytochemical overview on *Satureja*, *Pharm. Biol.* **54**(**3**), 375-412.

[15] S. Saeidnia, A. R. Gohari, A. Manayi and M. Kourepaz-Mahmoodabadi (2015). *Satureja*: Ethnomedicine, phytochemical diversity and pharmacological activities, SpringerBriefs in Pharmacology and Toxicology, Springer International Publishing, Germany. doi:10.1007/978-3-319-25026-7

[16] S. Momtaz and M. Abdollahi (2010). An update on pharmacology of *Satureja* species, *Int. J. Pharmacol.* **6**, 454-461.

[17] T. Baytop (1999) Türkiye'de Bitkiler ile Tedavi, Nobel Tıp Kitap Evleri, Ankara, Türkiye.

[18] F. Oke, B. Aslim, S. Ozturk and S. Altundag (2009). Essential oil composition, antimicrobial and antioxidant activities of *Satureja cuneifolia* Ten, *Food Chem.* **112**(**4**), 874-879.

[19] M. Altun and A. C. Goren (2007). Essential oil composition of *Satureja cuneifolia* by simultaneous distillation-extraction and thermal desorption GC-MS techniques, *J. Essent. Oil-Bear. Plants*, **10**, 139-144.

[20] T. Taskin, M. Dogan, M. E. Cam, T. Sahin and I. Senkardeş (2020). In vitro anti-urease, antioxidant, anticholinesterase, cytotoxic and in vivo anti-inflammatory potential of *Satureja cuneifolia* Ten. *Not. Sci. Biol*. **12**, 222-232.

[21] B. Yayli, G. Tosun, M. Karaköse, G. Renda and N. Yayli (2014). SPME/GC-MS analysis of volatile organic compounds from three Lamiaceae species (*Nepeta conferta* Hedge and Lamond, *Origanum onites* L. and *Satureja cuneifolia* Ten.) growing in Turkey, *Asian J. Chem*. **26**(**9**), 2541-2544.

[22] E. N. Ş. Sezer (2023). Phenolic screening and anticancer potential of various wild savory extracts, *Emir. J. Food Agric.* **35**, 271-279.

[23] H. D. Dorman, O. Bachmayer, M. Kosar and R. Hiltunen (2004). Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey, *J. Agric. Food Chem.* **52**(**4**), 762-770.

[24] O. Eminagaoglu, B. Tepe, O. Yumrutas, H. A. Akpulat, D. Daferera, M. Polissiou and A. Sokmen (2007). The *in vitro* antioxidative properties of the essential oils and methanol extracts of *Satureja spicigera* (K. Koch.) Boiss. and *Satureja cuneifolia* Ten, *Food Chem.* **100**(**1**), 339-343.

[25] S. Jennan, R. Fouad, A. Nordine, A. Farah, B. Bennani, S. Moja, H. Greche and F. Mahjoubi (2018). Chemical composition and antibacterial screening of aerial parts of essential oils of three *Satureja* species (*Satureja briquetti*, *Satureja atlántica* and *Satureja alpina*) growing wild in the middle Atlas Mountains of Morocco, *J. Essent. Oil-Bear Plants*, **21**(**3**), 741-748.

[26] A. Ejaz, S. Waliat, M. S. Arshad, W. Khalid, M. Z. Khalid, H. A. Rasul Suleria, M.I. Luca, Luca, C. Mironeasa, A. Batariuc, M. Ungureanu-Iuga, I. Coţovanu and S. A. Mironeasa (2023). A comprehensive review of summer savory (*Satureja hortensis* L.): promising ingredient for production of functional foods, *Front. Pharmacol*. **14**, 1198970.

[27] I. Fierascu, C. E. Dinu-Pirvu, R. C. Fierascu, B. S. Velescu, V. Anuta, A. Ortan and V. Jinga (2018). Phytochemical profile and biological activities of *Satureja hortensis* L.: A review of the last decade, *Molecules* **23**(**10**), 2458.

[28] O. Blokhina (2003). Antioxidants, oxidative damage and oxygen deprivation stress: A review, *Ann. Bot.* **91**(**2**), 179-194.

[29] J. Dai and R. Mumper (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties, *Molecules* **15**(**10**), 7313-7352.

[30] P. Lai, X. Li, S. Qiu and S. Song (2024). Chemical composition and evaluation of the antibacterial, synergistic antibacterial, antioxidant and cytotoxic activities of the essential oil of *Macrothelypteris torresiana* (gaudich.) Ching, *Rec. Nat. Prod.* **18**(**5**), 538-543.

[31] H. Karageçili, T. Polat, M. A. Yılmaz, M. Fidan, M. C. Karaismailoğlu and İ. Gülçin (2024). Evaluation of the antioxidant, antidiabetic and anti-Alzheimer effects of *Capsella bursa-pastoris*-Polyphenolic profiling by LC-MS/MS, *Rec. Nat. Prod.* **18**(**6**), 643-662.

[32] H. Karageçili, M. Yılmaz, S. Alwasel, M. Arık and I. Gülçın (2023). Comprehensively revealing the profile of *Pistacia vera* l. cv. Siirt Turpentine - antioxidant, antidiabetic, anti-Alzheimer, and antiglaucoma effects, *Rec. Nat. Prod.* **17**(**5**), 918-937.

[33] O. A. Ojo, T. C. Maduakolam-Aniobi, G. A. Gyebi, T. O. Soyinka, O. F. Ejiogu, A. B. Ojo, M. Alruwaili, N. H. Ali, S.A. Alnaaim, B. A. Alsfouk and G. El-Saber Batiha (2025). Experimental and computational analyses of the anti-alzheimer and antidiabetic effects of flavonoid-rich extract of avocado seeds (*Persea americana* Mill.), *Nutrire* **50**(**1**), 1-27.

[34] I. Orhan, M. Kartal, Y. Kan and B. Şener (2008). Activity of essential oils and individual components against acetyl and butyrylcholinesterase. *Z. Naturforsch C*, **63**(**7-8**), 547-553.

[35] S. Singh and R. K. Singh (2025). Recent advancements in the understanding of the alterations in mitochondrial biogenesis in Alzheimer's disease, *Mol. Biol. Rep*.**52**(**1**), 1-18.

[36] A. I. Abushouk, A. Negida, H. Ahmed and M. M. Abdel-Daim (2017). Neuroprotective mechanisms of plant extracts against MPTP induced neurotoxicity: Future applications in Parkinson's disease, *Biomed. Pharmacother.* **85**, 635-645.

[37] M. Saeedi, Y. Vahedi-Mazdabadi, A. Rastegari, M. Soleimani, M. Eftekhari, T. Akbarzadeh (2020). Evaluation of *Asarum europaeum* L. rhizome for the biological activities related to Alzheimer's disease, *Res. J. Pharmacogn.* **7**(**3**), 25-33.

[38] H. Kızıltaş, Z. Bingöl, A. C. Gören, S. Alwasel and I. Gülçın (2021). Anticholinergic, antidiabetic and antioxidant activities of *Ferula orientalis* L. determination of its polyphenol contents by LC-HRMS, *Rec. Nat. Prod*. **15**(**6**), 513-528.

[39] Z. Özer, S. Çarıkçı, T. Kılıç, S. Selvi and A. C. Gören (2024). Determination of the effect of different drying methods on secondary metabolites of *Lavandula pedunculata* (Mill.) Cav. subsp. cariensis (Boiss.) Upson & S. Andrews by LC-HRMS, *J. Chem. Metrol.* **18**(**2**), 124-133.

[40] B. K. Kınoğlu, T. Dirmenci, S. H. Alwasel, İ. Gülçin and A. C. Gören (2023). Quantification of main secondary metabolites of *Satureja icarica* PH Davis (Lamiaceae) by LC-HRMS and evaluation of antioxidant capacities, *J. Chem. Metrol*. **17**, 199-214.

[41] Eurachem/Citac Guide CG4 (2000). Quantifying uncertainty in analytical measurements, 2nd Edition.

[42] M. Mutlu, Z. Bingöl, E. M. Uç, E. Koksal, A. C. Gören, S. H. Alwasel and İ. Gülçin (2023). Comprehensive metabolite profiling of cinnamon (*Cinnamomum zeylanicum*) leaf oil using LC-HR/MS, GC/MS, and GCFID: determination of antiglaucoma, antioxidant, anticholinergic, and antidiabetic profiles, *Life* **13**(**1**), 136.

[43] H. Kızıltaş, Z. Bingol, A. Goren, S. Alwasel and İ. Gülçin (2023). Analysis of phenolic compounds by LC-HRMS and determination of antioxidant and enzyme inhibitory properties of *Verbascum speciousum* Schrad. *Rec. Nat. Prod*. **17** (**3**), 485-500.

[44] S. Carikci, A. C. Goren and T. Kilic (2020). Diterpenoid and phenolic contents of *Sideritis hololeuca* Boiss & Heldr. Apud Bentham with antioxidant and anticholinesterase activity, *Z. Naturforsch. C*, **75**(**5-6**), 161-169.

[45] B. K. Kınoğlu, I. Gülçin and A. C. Gören (2024). Quantification of secondary metabolites of *Satureja pilosa* (Lamiaceae) by LC-HRMS and evaluation of antioxidant and cholinergic activities, *Rec. Nat. Prod.* **18**(**6**), 674-686.

[46] I. Gulcin (2008). Measurement of antioxidant ability of melatonin and serotonin by the DMPD and CUPRAC methods as trolox equivalent, *J. Enzyme Inhib. Med. Chem*. **23**(**6**), 871–876.

[47] M.S. Blois (1958) Antioxidant determinations by the use of a stable free radical, *Nature*. **26**,1199.

[48] Z. Özer, A. C. Gören, T. Kılıç, M. Öncü, S. Çarıkçı and T. Dirmenci (2020). The phenolic contents, antioxidant and anticholinesterase activity of section Amaracus (Gled.) Vogel and Anatolicon Ietsw. of *Origanum* L. species, *Arab. J. Chem.***13**(**4**), 5027-5039.

[49] G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherston (1961). A new and rapid colorimetric determination of acetylcholinesterase activity, *Biochem. Pharmacol*. **7**, 88–95.

[50] S. Çarıkçı (2020). Antioxidant and anticholinesterase properties of *Sideritis perfoliata* subsp. *athoa* (Papan. &Kokkini) Baden and *Sideritis trojana* Bornm. teas from Mount Ida-Turkey and their phenolic characterization by LC-MS/MS, *J. Turk. Chem. Soc. Sect. A Chem.* **7**(**2**), 617-634.

[51] L. Güven, A. Ertürk, M. Yılmaz, S. Alwasel and İ. Gülçin (2024). *Alchemilla pseudocartalinica* Juz: phytochemical screening by UPLC-MS/MS, molecular docking, anti-oxidant, anti-diabetic, anti-glaucoma, and anti-Alzheimer effects, *Rec. Nat. Prod.* **18**, 251-272.

[52] M. Kosar, H. J. D. Dorman, O. Bachmayer, K. H. C. Baser, R. Hiltunen (2003). An improved on-line HPLC-DPPH method for the screening of free radical scavenging compounds in water extracts of Lamiaceae plants, *Chem. Nat. Compd.* **39**, 161–166.

[53] M. Ghojavand, P. Kasraie, H. R. Tohidi-Moghadam, M. Nasri and H. R. Larijani (2024). Morphological and physiological responses of summer savory (*Satureja hortensis* L.) to azotobacter, mycorrhiza, and salicylic acid under drought stress conditions in Rey Region, *Proc. Indian Natl. Sci. Acad.* **90**(**1**), 132-144.

[54] J. M. Mašković, V. Jakovljević, V. Živković, M. Mitić, L. V. Kurćubić, J. Mitić, and P. Z. Mašković (2024). Optimization of ultrasound-assisted extraction of phenolics from *Satureja hortensis* L. and antioxidant activity: Response surface methodology approach, *Processes* **12(9)**, 2042.

[55] I. Chkhikvishvili, T. Sanikidze, N. Gogia, T. Mchedlishvili, M. Enukidze, M. Machavariani, Y. Vinokur and V. Rodov (2013). Rosmarinic acid-rich extracts of summer savory (*Satureja hortensis* L.) protect Jurkat T cells against oxidative stress, *Oxid. Med. Cell. Longev*. 456253.

[56] N. Balasundram, K. Sundram and S. Samman (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses, *Food Chem.* **99(1)**, 191-203.

[57] Y. Shigaki, H. Tanaka, H. Akama, T. Ogara, K. Uwai and K. Tokuraku (2013). A microliter-scale high-throughput screening system with quantum-dot nanoprobes for amyloid-β aggregation inhibitors, *Plos One* **8**(**8**), e72992.

[58] M. Öztürk (2012). Anticholinesterase and antioxidant activities of Savoury (*Satureja thymbra* L.) with identified major terpenes of the essential oil, *Food Chem*. **134**, 48–54.

[59] F. V. M. Silva, A. Martins, J. Salta, N. R. Neng, J. M. F. Nogueira, D. Mira, N. Gaspar, J. Justino, C. Grosso, J. S. Urieta, A. M. Palavra, A. P. Rauter (2009). Phytochemical profile and anticholinesterase and antimicrobial activities of supercritical versus conventional extracts of *Satureja montana*, *J. Agric. Food Chem*. **57**,11557–11563.

[60] S. Vladimir-Knežević, B. Blažeković, M. Kindl, J. Vladić, A. D. Lower-Nedza, A.H. Brantner (2014). Acetylcholinesterase inhibitory, antioxidant and phytochemical properties of selected medicinal plants of the Lamiaceae family, *Molecules* **19**(**1**), 767-782.