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ORIGINAL ARTICLE

Anti-Alzheimer, antidiabetic and antioxidant potential of *Satureja cuneifolia* and analysis of its phenolic contents by LC-MS/MS

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Abstract Many chronic diseases such as diabetes and Alzheimer's disease are related to the type and quality of foods, which are consumed. Particularly, various plant origin products are stated as beneficial against such kind of chronic diseases with secondary metabolites such as their phenolic structures. *Satureja cuneifolia* is a plant, which is consumed as an herbal tea in some regions of Turkey and that's why investigate of its biological activity is important. In our study, the anti-diabetic and anti-Alzheimer potentials of the methanol and water extracts of *S. cuneifolia* plant were measured via some enzymes inhibition experiments as *in vitro*. The antioxidant ability of the same extracts was measured via radical scavenging and reducing power methods. Also, the total phenolics and flavonoids of the plant were identified. Finally, the extracts were analyzed by the LC-MS/MS analysis and the phenolic content of *S. cuneifolia* was clarified.

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1. Introduction

Satureja (Lamiaceae) is a genus, which is indigenous to South America, North Africa, North Asia, Canary Islands and Mediterranean Region. This genus of plants consists of fifteen different species of herbaceous, perennial and under-shrubs.

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Some of these species are endemic to Turkey. These species are used to make herbal tea and also consumed as a seasoning because of their aroma (Azaz et al., 2005). The plants of this genus are locally named as 'kekik', which means 'thyme'. Kekik is a general term which is not only used for plants which belong to the genus of Satureja, but also for plants belong to *Thymus, Origanum, Coridothymus* and *Thymbra*. The primary material of the study, *S. cuneifolia*, is a very important genus in Turkey among the species belongs to *Satureja* genus due to its natural spectrum (Tümen et al., 1998). Investigating the potential of the plant in terms of chronic diseases including diabetes and Alzheimer's disease (AD) has particular importance.

The global increase in the number of people with chronic diseases like diabetes and AD is a concerning issue which imposes a burden on countries' health systems and accordingly on national economies. It is well known that a number of chronic diseases are related to the type and quality of food, which are consumed (Tohma et al., 2019). Particularly, various plant origin products are stated as beneficial against such kind of chronic diseases with secondary metabolites such as their phenolic structures (Li et al., 2014).

Diabetes is a critically important metabolic disorder which causes different diseases seen in different organs such as eyes, kidneys and veins besides reducing the life quality of millions of people around the world (Ali et al., 2006; Tuomilehto et al., 2003; Türkan et al., 2018). Diabetes occurs due to a decrease in insulin production in the body (Type-1 diabetes mellitus) or due to the development of insulin resistance (Type-2 diabetes mellitus). One of the current approaches for Type-2 diabetes mellitus (T2DM) treatment is inhibition of carbohydrate-hydrolyzing enzymes including α-glycosidase and α-amylase. Thus, absorption of glucose units can be delayed, and in this way, postprandial plasma glucose levels can be reduced and postprandial hyperglycemia can be suppressed (Al-Goblan et al., 2014; Öztaşkın et al., 2019). T2DM is the most common type of diabetes and causes permanent hyperglycemia due to the fact that insulin is not sensitive to glucose overloading (Bursal et al., 2019; Zengin et al., 2018). The increase in the number of individuals with this disease is associated with a diet that may cause to obesity that is a key risk factor for rapidly increasing prevalence of T2DM, and also with social and cultural differences in lifestyles of people (Kuzu et al., 2019; Tobias et al., 2014). Recently, studies on identifying the anti-diabetic potential of various plants have been reported (Butala et al., 2017).

AD is described as a neurodegenerative disease, which develops progressively. The symptoms of Alzheimer progress to mild memory loss to serious dementia (Burmaoglu et al., 2019). Dementia is characterized by the sum of symptoms and findings arise with memory problems, linguistic problems, psychological and psychiatric changes and difficulties occur in daily life activities (Burns and Iliffe, 2009). Research studies show that acetylcholine (ACh) as a neurotransmitter decreases due to the decline in acetyltransferase activity and choline (Ch). Therefore, research studies conducted on the treatment of the illness have turned towards cholinesterase (ChE) inhibitive treatment as a symptomatic intervention (Kocyigit et al., 2018). As a result of these studies, inhibitive impacts of active natural compounds (ChE) existed in the extracts obtained from plant resources have been revealed, and it was reported that a number of plants include secondary metabolites that have ChE inhibition which can be utilized for the treatment of this illness (Mathew and Subramanian, 2014; Ozmen Ozgun et al., 2019).

One of the health benefit effects of plants is prevention of chronic disorders. They are attributed to secondary metabolites of these plants, and particularly to the phenolic compounds that form an important part of these metabolites. These structures have an impact on biological functions in cells via mediums such as removing free radicals and inhibiting some metabolic enzymes (Bursal & Gülçin, 2011; Gülçin, 2012). Identifying the chemical structures of a plant after demonstrating its biological potential, and isolating these structures have an uttermost importance (Elmasri et al., 2014).

It is known that reactive oxygen species (ROS) are accumulated in living cells both as products of normal metabolism, and as exogenous products (Gülçin, 2011). In the case that accumulated ROS amount is not balanced by antioxidant structures and antioxidant enzymes (Altın et al., 2017; Köksal, 2011), it may pose a threat to human health. The given situation is associated with chronic problems such as cancer and cardiovascular diseases. Thus, a nutritious diet has an importance in terms of antioxidant compounds, and accordingly for health. With the purpose of identifying natural antioxidant structures, antioxidant potential of many plants which have been consumed as food have been investigated in the past 30 years, and the plants with potential have been identified (Altay et al., 2018; Tohma et al., 2016). In further studies, isolation of structures that demonstrate such kind of biological activity has been performed (Sağlam et al., 2015).

The aim of this study is to reveal the antidiabetic, anticholinergic and antioxidant potential of the plant *S. cuneifolia,* which is also consumed as herbal tea. In order to reach the specified purpose, stock solutions of the plant were prepared as the first step by obtaining water and methanol extracts. Inhibition studies were performed on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes to determine the anti-diabetic impact of water and methanol extracts. Similar studies were carried out to identify the anti-Alzheimer potential of the plant on α-amylase and α-glycosidase and the IC_{50} values were calculated for both extracts. Furthermore, to understand the antioxidant potential of the *S. cuneifolia* plant, FRAP, ferric ion reduction, copper ion reduction, DPPH removal, ABTS removal and DMPD removal tests were done, and the total phenolic and flavonoid amounts were determined for both extracts. Lastly, a phenolic compound analysis was performed via the LC-MS/MS method with the purpose of clarifying the chemical content that causes the biological activity of the plant.

2. Materials and methods

2.1. Plant material

S. cuneifolia was collected by Dr. Ömer Kılıç, between Antalya-Burdur road seventieth km, vicinity of Kızılkaya waist, stony, schist and limestone areas, at 1100–1200 m altitude, on July 2016. Identification of *S. cuneifolia* done with stereomicroscope by using seventh Flora of Turkey books (Davis, 1982), by taxonomist Ö. Kılıç from Technical Vocational College of Bingöl University. Plant sample is kept at the Technical Sciences Vocational High School of Bingöl University (Herbarium number: 5863).

2.2. Chemicals

Isorhamnetin (98%) and luteolin-7-O-glucoside (99%) were purchased from AppliChem and Genay-France, and used as standards at LC–MS/MS analysis. The other standard compounds (t-ferulic acid (99%), chlorogenic acid (95%), caffeic acid (98%), *p*-coumaric acid (98%), pyrogallol (98%), rutin (94%), Rosmarinic acid (96%), quercetin (98%), apigenin (95%), kaempferol (96%), luteolin (98%) and Fumaric acid (99%)] were obtained from Sigma-Aldrich.

HPLC grade methanol was procured from Merck and used to prepare calibration solutions in a linear range. The solutions of curcumin and *S. cuneifolia* were prepared as 100 mg/L were used in all experiments. Acetylcholinesterase and butyrylcholinesterase from Sigma and α-glycosidase and α-amylase from *Saccharomyces cerevisiae* were procured.

2.3. Preparation of extracts

In order to prepare the methanolic extraction of the plant, a 30 g *S. cuneifolia* was ground into a fine powder. The fine powder plant mixed with 0.6 L of methanol and after waiting for enough time, the methanol was removed by evaporation. The extraction process was continued until color disappeared and filtration was performed through Whatman No. 1 paper. Finally, the methanol was completely removed at 40 °C to obtain dry extract (Hatipoğlu et al., 2015; Koksal et al., 2011). The dried extract was kept at −20 °C.

In order to prepare the water extract of the plant, 25 g *S. cuneifolia* was ground into a fine powder. The fine powder plant mixed with 500 mL boiling water by magnetic stirrer for 20 min (Bursal et al., 2013). Then filtration and lyophilization were performed. The dried water extract was kept at −20 °C.

2.4. Determination of antidiabetic potential

In order to determine the antidiabetic potential of the plant, inhibition properties of methanol and water extracts on α-glycosidase and α-amylase enzymes were investigated. The inhibitory efficacy of methanol and water extracts on α-glycosidase and α-amylase enzymes was applied using *p*-nitrophenyl-D-glucopyranoside (*p*-NPG) as described in previous study (Aktaş et al., 2019). Firstly, enzyme solution (20 µL) and the sample (10–100 µL) were mixed in 100 µL of buffer (pH 7.4). To determine the best enzyme inhibitory concentration different solutions were prepared. After that, p-NPG was added to the initiation of the reaction at 35 °C and the sample were incubated at same temperature for 12 min. Absorbances were followed at 405 nm. To determinate other inhibition parameters like the V_{max} and *Ki,* Lineweaver Burk graphs were used (Boztaş et al., 2015).

2.5. Determination of anti-Alzheimer potential

In order to determine the antidiabetic potential of the plant, inhibition properties of methanol and water extracts on acetylcholinesterase and butyrylcholinesterase enzymes were investigated (Yiğit et al., 2019). Butrylcholine iodide and acetylthiocholine iodide were used as substrates. Activities of AChE and BChE were designated using 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB). The solutions (50–200 µL) and the buffer solution (100 µL, pH 8.0, Tris-HCl, 1.0 M) were mixed to with acetylcholinesterase and butyrylcholinesterase enzymes solutions. After this step, the mixture was waited at 20 °C for 10 min. Then, 50 µL substrates and DTNB and were added to the mixtures and finally, the activity was measured at 412 nm (Biçer et al., 2019).

2.6. Determination of antioxidant potential

2.6.1. $DPPH^{\bullet}$ scavenging assay

The free radical scavenging potential of the extracts were evaluated using $DPPH^{\bullet}$ scavenging method (Blois, 1958). The method is rest on the removal of DPPH free radicals through antioxidants. The concentration of both extracts and standards were prepared as10–30 µg/mL. 1 mL of $DPPH^{\bullet}$ (0.1 mM) was added to tubes of each sample. These tubes were left in the dark at 25 °C for 30 min. The measurements were made at 517 nm. The potentials of samples on $DPPH^{\bullet}$ were determined and compared to the standards. In the end, IC_{50} values of all samples were calculated. The reduction in absorbance shows the DPPH free radical scavenging of samples capability.

2.6.2. $ABTS^{\bullet+}$ scavenging assay

In order to measure the free radical scavenging potential of the extracts $ABTS^{\bullet+}$ scavenging assay was used as a second radical scavenging method (Re et al., 1999) as described previously (Ak and Gülçin, 2008). According to this assay firstly, ABTS radical cation was generated. Thus, ABTS (7.0 mM) and $K_2S_2O_8$ (2.45 mM) were reacted. Before measurement, absorbance of the solution was adjusted to 0.750 ± 0.025 at 734 nm with buffer solution (pH 7.4, 0.1 M). Then, to 1 mL of $ABTS^{\bullet+}$ solution, 3 mL solution of *S. cuneifolia* extracts at different concentrations (10–30 µg/mL) was added. After half hour, the percent inhibition of $ABTS^{\bullet+}$ at 734 nm was measured for all samples. The reduction in absorbance shows the $ABTS^{\bullet+}$ free radical scavenging of samples capability.

2.6.3. $DMPD^{\bullet+}$ scavenging assay

$DMPD^{\bullet+}$ scavenging potential of *S. cuneifolia* extracts was detected according to previously reported technique by Fogliano et al. (1999) with slight modification. For this aim, 0.2 mL of 0.05 M $FeCl_3$ and 1 mL of DMPD solution were added to of buffer (100 mL, pH 5.3, 100 mM). The concentrations of the all samples were prepared as 10–30 µg/mL. The total volume was adjusted to 0.5 mL by water. An aliquot (1 mL) of $DMPD^{\bullet+}$ solution was transferred and absorbance was recorded at 505 nm after incubation for an hour.

2.6.4. FRAP assay

FRAP method is based on reduction of TPTZ-Fe^{3+} complex at acidic conditions. The increased absorbance was measured at 593 nm (Polat Köse et al., 2015). For this purpose, fresh TPTZ solution (10 mM) was prepared and mixed to buffer solution (pH 3.6, 0.3 M) and 20 mM $FeCl_3$ solution in water. Different concentrations (10–30 µg/mL) of *S. cuneifolia* extracts were dissolved in 5 mL of appropriate buffer, stirred and left at 37 °C for half hour. Finally, the absorbances were recorded at 593 nm (Benzie and Strain, 1998).

2.6.5. Cu^{2+} reducing assay

The reducing capacities of the *S. cuneifolia* extracts were evaluated using CUPRAC assay. In this method, neocuproine was used as a chromogenic oxidizing agent. Firstly, acetate buffer (1.0 M), $CuCl_2$ solution (10 mM), and neocuproine solution (7.5 mM) were added to each tube, as 1 mL and each tube were vortexed. The concentrations of the all samples were between 10 and 30 µg/mL and were added to tubes. The volumes of those tubes were completed to 4 mL with distilled water. The samples were kept at 25 °C for half hour. The absorbance was measured at 450 nm. The increased absorbance was defined as a rate of reducing ability (Gülçin et al., 2011).

2.6.6. Fe^{3+} reducing assay

The reducing capacity potential of *S. cuneifolia* extracts were evaluated using Fe^{3+} reducing method as a different method from FRAP and CUPRAC methods. In this method, reducing amount was detected by the straight reduction of $Fe^{3+}(CN^{-})_6$. Then addition of excess ferric ions (Fe^{3+}) resulted the formation of the Perl's Prussian blue complex. For this purpose, 0.75 mL *S. cuneifolia* extracts, which including different concentrations of the (10–30 µg/mL) were added with $K_3Fe(CN)_6$ (1%, 1.25 mL) and buffer (1.25 mL, 0.2 M, pH 6.6) solutions. Then, the mixture was incubated at 50 °C for half hour. Then, 1.25 mL of trichloroacetic acid (TCA, 10%) and $FeCl_3$ (0.5 mL, 0.1%) was added to the mixture and the absorbance was read at 700 nm (Oyaizu, 2011).

2.7. LC–MS/MS analyses and instrumentation

Curcumin was used as internal standard in validation of experiments for all compounds. The validation parameters were determined to be repeatability, linearity, LOQ (limit of quantification) and LOD (limit of detection) experiments. The linearity for each compound for was determined by analyzing standard solution. The linearity ranges of each compound, linear regression equations of the compounds were given in previously (Gülçin et al., 2010). Accuracy of the method was determined by repeating the measurements of three concentrations for each compound. The accuracy was determined and the results were implemented to the uncertainty budget.

LOD and LOQ of the LC-MS/MS methods for the above compounds were calculated to be 0.5–50 mg/L. The LOQs were determined to be 10 times bigger than while the LODs were determined to be 3 times bigger than standard deviation. The concentration of each compound within the linear range and concentration of the reported method was obtained from the calibration curve given in previous studies (Kalin et al., 2015).

The EURACHEM/CITAC guide was used for evaluation of sources and quantification of uncertainty of LC-MS/MS method (EURACHEM/CITAC 2000). The calibration curve gives the maximum contribution come. More information about procedures of uncertainty evaluation is available in the literature (Gören et al., 2007; Köksal et al., 2017a,b).

2.8. Statistical analysis

Statistical analyses were used to evaluate anticholinergic, antidiabetic and antioxidant activity results by unpaired Student's *t*-test (GraphPad, La Jolla, CA. Software 7.0). All results were given as means with their standard deviation (SD). $p < 0.05$ was taken as the minimum level of significance.

3. Results and discussion

3.1. Antidiabetic potential

Diabetes has become a growing health problem for both developing and developed countries. Controlling hyperglycemia is one of the main challenges, which occur in terms of the follow-up process of this disease. In the year of 1980, 108 million people were diagnosed with diabetes, the number increased to 422 million in 2014, and the number is expected to rise up to 642 million in 2030 (Huseynova et al., 2018; Oyebode et al., 2018). This striking increase has aroused the attention and curiosity of scientists and many research studies were conducted on the issue. A method for testing the antidiabetic potential of a plant sample is measuring blood glucose levels in diabetic animals after applying different doses of plant extract. Despite the fact that various plant extracts are proved to be effective in lowering blood glucose levels via diabetic animal models that were created experimentally, the effect mechanism could not be completely presented (Aksu et al., 2018; Zhang et al., 2009). Another method that can be implemented for the same purpose is the investigation of inhibition characteristics of enzymes, which are existed in the human digestive system and responsible for carbohydrate hydrolization. Inhibition of digestive enzymes can delay carbohydrate intake in diabetic patients. In the study, the antidiabetic potential of both *S. cuneifolia* extracts were measured via α-amylase and α-glycosidase inhibition experiments. The results were presented in Table 1. Water and methanol extracts of *S. cuneifolia* exhibited IC_{50} values of 16.93 µg/mL (r^2: 0.9529) and 10.66 µg/mL (r^2: 0.9659) for α-glycosidase. This value was found as 9.11 µg/mL (r^2: 0.9677) and 18.23 µg/mL (r2: 0.9856) for α-amylase, respectively (Table 1). Also, Acarbose, which is a standard antidiabetic medicine, was used. The observations showed that methanol and water extracts inhibited both two enzymes significantly and at a level that is closer to the standard antidiabetic substance. The results presented in Table 1 demonstrate that methanol extract inhibited the α-glycosidase enzyme and water extract inhibited α-amylase enzyme better in comparison to the other one. Further studies which will be conducted on these two extracts, such as pure substance isolation, offer a possibility to discover new structures that have the potential of a showing the characteristics of a medicine.

3.2. Anti-Alzheimer potential

The number of individuals with Alzheimer's disease has been gradually increasing, similarly to the case of diabetes disease. According to worldwide research, the number of patients, which was recorded as 21.7 million in 1990, reached 46 million in 2015 (Taslimi et al., 2019). The 100% increase occurred in the numbers within a 15 years period gives rise to concerns. A growing number of epidemiological studies reveal that an unhealthy diet and nutrition may significantly augment the risk factors for this illness. It was revealed that a diet, which mainly includes vegetables, fruits and fish, such as Japanese and Mediterranean diet, created lower risk factors.

Table 1 The enzyme inhibition results (IC_{50} values; μg/mL) of *S. cuneifolia* against α-glycosidase, α-amylase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes.

Enzymes	*S. cuneifolia*-Water		*S. cuneifolia*-Methanol		Standards (μM)	
	IC_{50}	r^2	IC_{50}	r^2	IC_{50}	r^2
α-Glycosidase*	16.93	0.9529	10.66	0.9847	22.80	0.9922
α-Amylase*	9.11	0.9677	18.23	0.9659	10.01	0.9424
AChE**	93.58	0.9859	63.69	0.9821	0.124	0.9804
BChE**	53.72	0.9916	23.17	0.9757	0.101	0.9698

* Acarbose was used as positive control for α-glycosidase and α-amylase enzymes.
** Tacrine was used as positive control for AChE and BChE enzymes.

The aforementioned study also reported that consumption of antioxidant and phenolic-rich foods may be helpful in terms of AD (Erdemir et al., 2019). Therefore, considering the potential of plants that can be consumed by people in the form of herbal tea has an important role in terms of AD.

The inhibition impacts of water and methanol extracts of *S. cuneifolia* plant, which we aimed to determine its biological activity potential, on two different enzymes that are related with AD (AChE and BChE) were studied in different concentrations and IC_{50} values were calculated. The results were presented in Table 1. The inhibition data are summarized in Table 5. For evaluation of the effect of water and methanol extracts of *S. cuneifolia* on the indicated some metabolic enzymes, the following results had been found. Water and methanol extracts of *S. cuneifolia* had IC_{50} values 93.58 μg/mL (r^2: 0.9895) and 63.69 μg/mL (r^2: 0.9821) for AChE, respectively. Also, these values were calculated as 53.72 μg/mL (r^2: 0.9916) and 23.17 μg/mL (r^2: 0.9757) for BChE, respectively. On the other hand, tacrine as standard inhibitors for both cholinergic enzymes demonstrated Ki values of 124.0 nM (r^2: 0.9804) and 101.0 nM (r^2: 0.9698) toward AChE and BChE, respectively. During the measurements, Tacrine, which is a synthetic compound, was used as a positive control and IC_{50} values of this compound and of the plant extracts were compared. The findings showed that the anti-AD potential of the methanol extract of the plant was higher than the water extract, yet this potential was very low in comparison to Tacrine (Table 1). Particularly, the inhibition effect of the methanol extract on BChE enzyme (IC_{50}: 23.17 μg/mL) draws the attention and this result may guide higher-level research studies. Isolation studies, which will be conducted on the methanol extract on the plant, may result in new and natural inhibitors.

3.3. Antioxidant potential

Plants, vegetables and fruits are used at present as optimal sources of chemical constituents with antioxidant and antimicrobial activities. The used as supplementation of human diet with plants containing high amounts of compounds capable of deactivating free radicals may have beneficial effects. Generally, the extent of the antioxidant and antibacterial effects of the extracts could be attributed to their phenolic compositions (Ozcan and Juhaimi, 2011). Antioxidant potential of a plants can be defined as a level of preventing or inhibiting oxidation of biomolecules in the environment by the synergic effect of the antioxidant compound of a plant (Gülçin, 2012). It is expressed as a parameter that is used to indicate the quality of the plant or its benefits in the case that a plant is brewed as a tea or consumed as food such as salad. For this reason, plants with a nutritious value have drawn the attention of chemists, food engineers and pharmacists, and antioxidant activity potentials of these plants have been identified through different methods (Sehitoglu et al., 2015). In addition, presenting antioxidant potentials of these plants, which not consumed as food by people and obtaining secondary metabolites that cause to this activity is important in terms of obtaining new and natural antioxidant compounds. The reason for this situation is the argument, which implies that the use of synthetic antioxidant with the purpose of extending the shelf life of food may lead to negative health consequences (Balaydın et al., 2010).

S. cuneifolia is a plant, which is consumed as a herbal tea in some regions of Turkey (Eminagaoglu et al., 2007). The antimicrobial properties of some aromatic plants and Satureja species were reported previously. It was reported *Satureja cuneifolia* had antifungal and antimicrobial effect (Biavati et al., 2004; Bagci et al., 2008; Ozkalp et al., 2009; Al Juhaimi et al., 2013). Its antioxidant potential was investigated in order to reveal its benefits for human health, and also to guide future research studies on discovering new and natural antioxidants. For the given purposes, three metal reduction methods (FRAP and reduction of Ferric and Copper ions) and three radicals (DPPH, ABTS and DMPD) elimination methods were implemented for both of the extracts.

The DPPH free radical scavenging activities of water and methanol extract of *S. cuneifolia* and positive antioxidants, such as BHA, BHT, α-Tocopherol and Trolox® were investigated as a main radical scavenging parameter. Additionally, we determined the IC_{50} values of both extracts and standards. The results are summarized in Table 2. The IC_{50} values of DPPH scavenging of both extracts and standard antioxidants decreased in following order: *S. cuneifolia*-Water (30.63 μg/mL; r^2: 0.9579), *S. cuneifolia*-Methanol (26.03 μg/mL; r^2: 0.9817), α-Tocopherol (15.37 μg/mL; r^2: 0.9684), BHT (11.01 μg/mL; r^2: 0.9810), BHA (10.66 μg/mL; r^2: 0.9508) and Trolox® (9.83 μg/mL; r^2: 0.9927). The lower IC_{50} values reflect an effective scavenged DPPH radical scavenging effect. It was observed that both *S. cuneifolia* extracts had the most effective DPPH radical scavenging activity when compared to the other samples.

ABTS radical scavenging assay can applicable for food and plant extracts including lipophilic and hydrophilic compounds. This assay is based on the inhibition of the absorbance of the radical cation $ABTS^{\cdot+}$ that has a characteristic wavelength showing absorption at 734 nm (Koksal et al., 2017). Both

Table 2 Determination of half maximal concentrations (IC_{50} values, μg/mL) of *S. cuneifolia* and standards for DPPH·, $ABTS^{\bullet+}$, and $DMPD^{\cdot+}$ scavenging activities.

Antioxidants	DPPH· scavenging	r^2	$ABTS^{\bullet+}$ scavenging	r^2	$DMPD^{\cdot+}$ scavenging	r^2
BHA	10.66	0.9508	8.07	0.9720	28.17	0.9618
BHT	11.01	0.9810	7.16	0.9955	33.81	0.9082
α-Tocopherol	15.37	0.9684	10.12	0.9429	40.62	0.9593
Trolox	9.83	0.9927	6.28	0.9112	31.18	0.9887
***S. cuneifolia*-Water**	30.63	0.9579	18.08	0.9627	38.16	0.9490
***S. cuneifolia*-Methanol**	26.03	0.9817	14.98	0.9528	35.27	0.9902

tested both *S. cuneifolia* extracts exhibited effective ABTS radical scavenging profile ($p > 0.001$). As seen in Table 2, water and ethanol extracts of *S. cuneifolia* effectively scavenged ABTS radicals in a concentration-dependent manner (10–30 μg/mL) and these differences were found statistically significant ($p < 0.001$). EC_{50} values for water and ethanol extracts of *S. cuneifolia* in this assay were determined as 18.08 μg/mL (r^2: 0.9627) and 14.98 μg/mL (r^2: 0.9528). Also, EC_{50} values were found as for 8.07 μg/mL (r^2: 0.9720) for BHA, 7.16 μg/mL (r^2: 0.9955) for BHT, 10.12 μg/mL (r^2: 0.9429) for α-Tocopherol and 6.28 μg/mL (r^2: 0.9112) for Trolox as a water-soluble analogue of α-Tocopherol.

The third evaluated radical scavenging assay is DMPD radical scavenging activity. The $DMPD^{\cdot+}$ scavenging assay had a very stable endpoint like ABTS radical scavenging assay (Koksal et al., 2017). As shown in Table 4, both *S. cuneifolia* extracts were an effective DMPD radical scavenging in a concentration-dependent manner (10–30 μg/mL). EC_{50} values of both water and methanol extracts of *S. cuneifolia* were calculated as 38.16 μg/mL (r^2: 0.9490) and 35.27 μg/mL (r^2: 0.9902). Whereas, EC_{50} values were found as for 28.17 μg/mL (r^2: 0.9618) for BHA, 33.81 μg/mL (r^2: 0.9082) for BHT, 40.62 μg/mL (r^2: 0.9593) for α-Tocopherol and 31.18 μg/mL (r^2: 0.9887) for Trolox. These results clearly shown that both *S. cuneifolia* extracts can easily transfer hydrogen atoms to DMPD radicals and quench the color and produce a decoloration of the solution.

Also, the total phenolics and flavonoids of the plants were identified. In order to compare the plant sample results of the six aforementioned methods, four different standards were used. The use of different methods in determining the antioxidant potential of a plant and the use of the highest number of standards which are possible will contribute to the evaluation of results as more significant (Tables 2 and 3). The approximate values of a plant sample to the standard values show that the level of high antioxidant potential of the plant. The results

Table 4 Total phenolic and total flavonoid content of water extracts of *S. cuneifolia*.

	S. cuneifolia-Water	*S. cuneifolia-Metanol*
Total phenols*	109.73	120.48
Total flavonoids**	83.08	88.12

* Determined as μg of gallic acid equivalent (GAE) in mg dried extracts.
** Determined as μg of quercetin equivalent (QE) in mg dried extracts.

of the all measurements performed in the study demonstrated that the activity displayed by methanol extract *S. cuneifolia* was higher than the water extract yet lower than the standard antioxidants; and particularly, it was approximate to α-Tocopherol and Trolox.

3.4. Phenolic compounds

Fruit, plants and have been widely used for the elaboration products rich in phenolic compounds. Phenolic compounds are bioactive secondary metabolites found in fruits and plants with potential beneficial effects on human health. They play a positive role on certain types of degenerative illnesses due to their properties associated with a regular consumption on the human diet. They are defined as a large secondary metabolite class, which shows various characteristics from basic structures such as phenolic acids to the polyphenols that combines a number of groups of different classes. These compounds are highly significant, not only for the quality of the products from plant origin but also for consumer health. The effects of phenolic structures on the taste and color of plant origin food have been previously reported (Cheynier, 2012). The health impacts of such kind of structures can be attributed to antioxidant

Table 3 Determination of reducing power of same concentration of *S. cuneifolia* and standard compounds by FRAP methods, ferric ions (Fe^{3+}) reducing and cupric ions (Cu^{2+}) reducing capacity by Cuprac method.

Antioxidants	Fe^{3+}-Fe^{2+} reducing		Cu^{2+}-Cu^{+} reducing		Fe^{3+}-TPTZ reducing	
	λ_{700}	R^2	λ_{450}	R^2	λ_{593}	R^2
BHA	2.404 ± 0.013	0.9622	2.398 ± 0.020	0.9588	2.733 ± 0.017	0.9629
BHT	2.307 ± 0.009	0.9902	2.568 ± 0.011	0.9362	2.809 ± 0.012	0.9788
α-Tocopherol	1.644 ± 0.017	0.9118	1.371 ± 0.009	0.9277	2.327 ± 0.001	0.9998
Trolox	2.177 ± 0.007	0.9736	1.282 ± 0.008	0.9811	2.432 ± 0.015	0.9611
***S. cuneifolia*-Water**	1.244 ± 0.010	0.9962	1.167 ± 0.004	0.9398	1.509 ± 0.016	0.9737
***S. cuneifolia*-Methanol**	1.344 ± 0.008	0.9808	1.203 ± 0.005	0.9690	1.594 ± 0.010	0.9183

Table 5 LC-MS/MS parameters of selected compounds.

	Compounds	Parent ion	Daughter ion	Collision energy (V)
1	Quercitrin	471.9	309.9	16
2	Gallic acid	168.6	124	13
3	Epigallocatechin	305	125	18
4	Epicatechin	289	245	14
5	Cyanidin-3-O-Glu	449	287	16
6	Cyanidin chloride	611.5	287	28
7	Catechin	289	245	15
8	Ascorbic acid	175	114.6	12
9	Apigenin	269	151	22
10	Caffeic acid	179	135	10
11	Chlorogenic acid	353	191	14
12	Ellagic acid	301	150.5	10
13	Kaempferol	287	152.3	30
14	Salvigenin	329	295.8	15
15	Fumaric acid	115	71	8
16	Pyrogallol	125	80	16
17	t-Ferulic acid	193	133	15
18	Luteolin	285	132	30
19	Isorhamnetin	315	300	15
20	Quercetagetin-3,6-dimethylether	345.1	329.5	16
21	Rosmarinic acid	359.2	160.5	15
22	Luteolin-7-O-Glucoside	447	284.5	14
23	Luteolin-5-O-Glucoside	447	289.5	20
24	Kaempferol-3-O-Rutinoside	593	284.4	18
25	Rutin	609	301	16
26	Curcumin*	369.3	176.9	20

* It was used as internal standard.

characteristics that are mediated by different mechanisms such as the elimination of ROS, enzymes inhibition, chelation of certain metal ions and related to oxidative stress (Altay et al., 2018; Dangles, 2012; Köktepe et al., 2017). Furthermore, it is known that phenolic compounds, which are received via daily diet, play a role in more complex mechanisms including modulation of cell signaling and regulation of gut flora. All of these characteristics depend on the structure and number of the phenolics; however, they can be also altered with reactions and interactions that occur in the digestive system (Cheynier, 2012). A huge variety of biologically active phenolics containing one or more aromatic rings are found naturally in plant foods, where they provide much of the flavour, colour and texture.

Table 6 Amount of secondary metabolites in extracts mg/kg concentration.

Phenolic Compounds	*S. cuneifolia*-Methanol	*S. cuneifolia* - Water
Gallic acid	4.18 ± 0.29	2.74 ± 0.19
Epicatechin	14.67 ± 1.49	128.37 ± 13.01
Cyanidin chloride	553.23 ± 37.25	–
Catechin	12.51 ± 0.84	–
Apigenin	89.66 ± 7.22	42.61 ± 3.43
Caffeic acid	15.15 ± 30	12.04 ± 2.38
Chlorogenic acid	115.55 ± 16	23.69 ± 3.28
Ellagic acid	1.63 ± 0.11	–
Kaempferol	80.94 ± 5.71	74.12 ± 5.23
Salvigenin	15.29 ± 1.04	–
Fumaric acid	277.98 ± 19.28	369.2 ± 25.60
Pyrogallol	17.7 ± 1.18	11.01 ± 0.73
t-Ferulic acid	4.56 ± 0.32	–
Luteolin	172.12 ± 44.21	73.98 ± 19.00
Quercetagetin-3.6-dimethylether	242.44 ± 45.39	–
Rosmarinic acid	6.64 ± 0.51	–
Luteolin-7-O-Glucoside	5 ± 0.51	–
Luteolin-5-O-Glucoside	2.19 ± 0.14	–
Kaempferol-3-O-Rutinoside	49.62 ± 4.48	615.14 ± 55.59
Rutin	782.58 ± 51.26	–

The present study determined total phenolic and flavonoid amounts (Table 4) and identified the phenolic compounds existed in the structure of *S. cuneifolia* plant. To identify the content, methanol and water extracts of the plant, which was used in the biological activity studies, were analyzed by the LC-MS/MS (Table 6). For the analysis, 25 phenolic compounds were used as a standard and chromatogram was prepared. The first three most frequent compounds existed in the structure of the plant were rutin, kaempferol-3-O-rutinoside and fumaric acid. It was identified that 10 of the 25 standard compounds (gallic acid, epicatechin, apigenin, caffeic acid, chlorogenic acid, kaempferol, fumaric acid, pyrogallol, luteolin, and kaempferol-3-O-rutinoside) that were studied existed both in methanol and water extracts, five of them (quercitrin, epigallocatechin, cyanidin-3-O-glucoside, ascorbic acid, and isorhamnetin) were not identified in both extracts, and the compound with the lowest amount among the phenolic structures which could be identified was found as ellagic acid (1.63 ± 0.11 mg/kg). The results showed that 10 standard compounds (cyanidin chloride, catechin, ellagic acid, quercetagetin-3,6-dimethylether, t-ferulic acid, rosmarinic

acid, luteolin-5-O-glucoside, luteolin-7-O-glucoside, and rutin) existed only in the methanol extract. There was no standard compound, which was found only in the water extract (Table 5). The results revealed that the methanol extract was richer in terms of phenolic compounds. This situation can be attributed to the approximate polarity index values of phenolic compounds to methanol. The fact that phenolic structures are molecules, which have a first-degree responsibility in antioxidant activities of plants, is widely known and the present study has confirmed the given fact once again. The methanol extract of the *S. cuneifolia* plant, which had more phenolic compounds, displayed a higher antioxidant behavior activity than the water extract of the same plant in six different antioxidant identification tests, which were studied. The interest has been resulted from the encouraging results of the antioxidant and antiradical properties of plants and their phytochemicals, such as phenolic compounds. Phytochemicals can improve the effectiveness of chemotherapeutic agents, while decreasing the side effects of the standard chemotherapeutic agents (Alibakhshi et al., 2016).

4. Conclusion

This study revealed the antidiabetic and anti-Alzheimer effects of the methanol and water extracts of *S. cuneifolia* plant. Moreover, the phenolics that existed in the structure of the plant and have the potential to show such kind of effects were also identified. The results of the study showed that *S. cuneifolia* plant has significant potential in terms of both diseases. The study highlighted the phenolic content of the plant; yet, apart from the standards used in this study, it is possible to find certain structures, which have not been identified until today in the extracts of *S. cuneifolia* plant. Therefore, it is important to specify the structures of secondary metabolites existed in *S. cuneifolia* plant by isolating them through the activity-driven isolation method, and to study the structures which will be obtained in terms of the given three potentials.

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