A comparative study on antioxidant and enzyme inhibitory activities of *Spartium junceum L.*

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Abstract

Spartium junceum L., a Fabaceae plant, is commonly known as "Spanish broom" and cultivated in the Mediterranean regions. It is used in various area in different part of the world. This study aims to investigate the chemical profile, antioxidant and different enzyme inhibitory activities (acetylcholinesterase, butyrylcholinesterase, α-glucosidase, α-amylase, and tyrosinase) of methanol, ethanol (70%) and aqueous extracts obtained from flowers and stems of S. junceum. Also, the phenolic composition of different extracts was analyzed by HPLC-DAD. Aqueous extract of stems showed the highest (63.94 mg GAE/g) phenolic content, it was followed by stem methanol extract (60.45 mg GAE/g), while aqueous extract of stems contained the highest (61.33 mg QE/g) flavonoid content, it was followed by stem methanol and ethanol extracts (39.81 mg GAE/g and 38.89 mg GAE/g, respectively). The stem methanol extract demonstrated highest DPPH radical scavenging (IC_{50}: 1.42 mg/mL) activity, while stem water extract exhibited strong ABTS radical scavenging activity (IC_{50}: 0.24 mg/mL). The tested extracts differ greatly in terms of enzyme inhibition activity. The flower ethanol extract showed highest Anti-acetylcholinesterase activity (IC_{50}: 0.72 mg/mL), however stem methanol extract exhibited strong anti-butyrylcholinesterase activity (IC_{50}: 0.08 mg/mL). The flower methanol extract was most active against α-glucosidase (IC_{50}: 0.44 mg/mL) enzyme. All extracts showed the least activity against α-amylase and tyrosinase enzymes. When the biological activity results are evaluated, it provides the evidence for the bioactivity-guided isolation studies on the active extracts of this plant.

Keywords: *Spartium junceum* • Antioxidant activity • enzyme inhibition • HPLC analysis

Introduction

Medicinal plants have been used in the treatment of various diseases since ancient times. The discovery of many drugs used in the clinic today is based on herbal resources [1]. The plant *Spartium junceum* is commonly known as "Spanish broom" or "Weaver's broom" in English, a perennial Mediterranean shrub species belonging to *Fabaceae* family [2]. *Spartium* is one of the few monospecific geniuses, closely related to the genera *Cytisus* and *Genista*. Today it has been used as composite material in automobile industries [3,4]. *S. junceum* L. has been reported to have sedative, diuretic, antiulcer, anti-inflammatory, analgesic and antitumor activities [2,5,6]. Phytochemical investigations revealed that *S. junceum* containing alkaloids, flavonoids, saponins and fatty acids [7–11]. The enzyme Acetylcholinesterase (AChE, EC 3.1.1.7) and Butyrylcholinesterase (BuChE, EC 3.1.1.8) are a specific important esterase that are responsible for hydrolyzes of different types of choline esters, one of them is Acetylcholine (Ach), the neurotransmitter mainly presents in the brain. The reduction of this enzyme regulation is associated with the pathology Alzheimers Disease (AD) [12]. Therefore, these enzyme inhibitors have been used as a therapeutic target in AD [13]. It was reported that there is a pathological link between AD and type-2 diabetes mellitus in the low-grade systematic inflammation [14].

Diabetes is a metabolic disorder results from insulin insufficient secretion or resistance, which is lead to increasing of blood glucose levels [15]. In diabetes, oxidative stress occurs due to the excessive number of free radicals in the body, which has harmful effects on the organs of diabetic patients. Diabetes is characterized by high concentrations of blood sugar levels, which can cause serious complications, such as organ failures and/or destruction of the kidneys, eyes, and various cardiovascular diseases. Therefore, the treatment methods mainly focus on reducing fluctuations in blood sugar levels and their related complications. One of the therapeutic approaches is to decrease the postprandial hyperglycemia by retarding the absorption of glucose through the inhibition of carbohydrate-hydrolyzing enzymes, such as α-amylase and α-glucosidase [16]. Tyrosinase, is a copper containing multifunctional enzyme that playing an important role on melanin synthesis. Therefore, it's inhibitors can be used for prevent unwanted browning in skin and foods [17]. Recently, many plants have been found to have anti-tyrosinase activity, which suggests that the plants are the good potential resources to look for new tyrosinase [18–21]. It was aimed to investigate the different solvent extracts (methanol, ethanol and aqueous) of *S. junceum* different parts (flowers and stems) for their chemical composition of phenolic and flavonoid compounds, antioxidant and enzyme inhibition activities in this study.

Material and methods

Plant materials

Aerial parts of *S. junceum* L. were collected from the fields surrounding Carpinis, judetul Timis, located in the western part of Romania (road sides, 950-1050 m, 10.04.2021) and identified by Prof. Dr. Diana Antal, Head of the Botanical department from the University of Medicine and Pharmacy "Victor Babes", Faculty of Pharmacy, Timisoara, Romania, to which we are grateful. The voucher specimen (4179) has been deposited in the Faculty herbarium.

Preparation of extracts

The plant material was dried at shade place for about two weeks and powdered by laboratory type mill. The different part (5 g) of *S. junceum* L. (flowers and stems) was macerated separately with methanol, ethanol and water at room temperature for 24 h. After filtration, the plant residue subjected to two time of extraction with same solvent. After combining, the solvent was evaporated under reduced pressure at 40 °C. The yield of extracts was given in Table 3. All the extracts were stored at -20 °C until experiments.

Chemical composition

The different extracts were analyzed by 1200 HPLC (Agilent technology, USA) coupled with a diode array detector (DAD). The phenolic compounds presented in the extracts were separated on a ACE C-18 (4.6 x 250 mm, 5 µm) column with a mobile phase flow rate of 0.8 $ml.min^{-1}$. The mobile phase comprised of solvent mixtures (A) ultra-pure water with 0.1% acetic acid, (B) ultra-pure water with 0.1% methanol and (C) ultra-pure water with 0.1% acetonitrile, respectively. The injection volume was 10 µL and detection wavelength was set at 280 nm. The column temperature was maintained at 40 °C. The gradient elusion was performed under the following program: 0-8 min 80:12:8 A: B: C. 75:15:10 at 8-10min, 70:18:12 at 10-24 min, 65:20:15 at 24-32 min, 50:35:15 at 32-40 min, 25:60:15 at 40-45 min and then back to 80:12:8 to recondition the column for 5 min. identification of the compounds was carried out by comparing the retention times (Rt) and the corresponding UV absorbance spectra with those of the single reference compounds.

In-vitro antioxidant activity

The antioxidant activity of the different extract was evaluated by DPPH and ABTS radical scavenging method, total phenol and flavonoid content, and iron chelating assay.

DPPH radical scavenging activity: The ability of extracts to scavenging 2,2'-di- phenyl-1-picrylhydrazyl radical (DPPH) was carried out according

to the method described previously [22]. The reaction mixture contained 180 µL of 0.1 mM DPPH solution freshly prepared in methanol and 40 µL of sample solutions at different concentrations. After 30 min, the absorbance was read at 517 nm. The values are presented as the means of triplicate analyses. The DPPH free radical scavenging potential was calculated using the following equation:

Inhibition % = (Acontrol-A sample)/Acontrol x100

ABTS radical scavenging activity: The ABTS radical scavenging activity was measured by adapting the colorimetric assay described before [23]. First, the $ABTS^{\cdot+}$ stock solution was produced by reacting 7 mM ABTS in H_2O with 2.45 mM potassium persulfate in the dark for 16 h. The working solution was prepared freshly from the stock by diluting with methanol to get an absorbance of 0.70 ± 0.02 at 734 nm. Then 180 µL of $ABTS^{\cdot+}$ solution was added to 20 µL of sample solution at different concentrations. After 10 min, the absorbance of the mixture was read at 734 nm.

Total phenol content: The concentrations of phenolic contents of the extract were estimated with Folin-Ciocalteu method and expressed as gallic acid equivalents (mg GAE/g) as dry weight basis and the values are presented as means of triplicate analyses [22].

Total flavonoid content: The concentrations of flavonoid contents of the extract were determined using aluminum chloride colorimetric method and expressed as gallic acid equivalents (mg GAE/g) as dry weight basis [24].

Iron chelating assay: The ferrous ion chelating activity of the methanol extracts was evaluated by monitoring the decrease in absorbance at 562 nm of the iron (II)-ferrozine complex [25]. One milliliter of 0.125 mM $FeSO^4$ was added to 1.0 mL sample at different concentrations, followed by 1.0 mL of 0.3125 mM ferrozine. The mixture was allowed to equilibrate for 10 min before measuring the absorbance. The ability of the sample to chelate ferrous ion was calculated relative to the control.

Enzyme inhibition activity

Acetylcholinesterase and butyrylcholinesterase inhibitory activity: Anticholinesterase activity was measured by adapting the colorimetric assay described by Ellman et al. [26] with slight modification. All samples were dissolved in methanol or DMSO to prepared their stock solution at 5000 µg/mL concentration. Aliquots of 140 µL of 0.1 M phosphate buffer (pH 8.0), 20 µL sample solution at different concentration, and 20 µL of AChE solution (BChE) were mixed and incubated for 10 min at room temperature and 10 µL of 0.5 mM DTNB was added. The reaction was then initiated by the addition of 10 µL of 0.71 mM acetylthiocholine iodide (or 0.22 mM butyrylthiocholine iodide). After incubation for 10 min, the hydrolysis of the substrate was monitored using a Multiscango Elisa reader at 412 nm.

α-glucosidase inhibitory activity: α-glucosidase (*Saccharomyces cerevisiae*, EC 3.2.1.20, Sigma) inhibitory activity was evaluated by using 96-well plate method [27]. The enzyme solution (0.2 U/mL in 0.1M phosphate buffer, pH 6.8) was incubated with various concentrations of extract samples at 37 °C for 15 min. Then 20 µL of 3 mM PNPG (P-nitrophenyl-α-D-glucopyranoside) was added as substrate and the absorbance change was recorded at 405 nm after 30 min of incubation. Acarbose was used as positive control and buffer was used as control instead of sample.

α-amylase inhibitory activity: The α- amylase inhibitory activity of the extracts was evaluated using a method described by Özek et al. [28] with minor modifications. The reaction mixture containing 25 µL of the sample dissolved in DMSO / methanol at various concentrations and 50 µL of 20 mM phosphate buffer (pH 6.9) was added to 50 µL of α-amylase solution (from porcine pancreas, 0.8 U/mL in phosphate buffer) in a 96-well plate. The mixtures were pre-incubated for 10 min at 37 °C and then, 50 µL of %1 starch solution was added to start the reaction. The incubation was continued for 10 min and the reaction was terminated by the addition of 25 µL of 1 M HCl. Finally, 100 µL of I2/KI reagent was added to the mixture. The enzymatic activity was calculated versus control. Acarbose was used as a positive standard.

Tyrosinase inhibitory activity: Tyrosinase inhibitory activity was determined using the method described previously [29]. The extracts at different concentrations (20 µL) were mixed with 20 µL of 250 U/mL tyrosinase solution (from Mushrooms, sigma) and 100 µL of 100 mM phosphate buffer (pH 6.8) in a micro-well plate and incubated for 10 min at room temperature. Then the reaction was initiated by the addition of 20 µL of 3 mM L-tyrosine as a substrate. After 10 min, the absorbance was read at 492 nm. Kojic acid was used as positive control.

Statistical analysis

All the results are presented as mean values and standard deviations (SD) of three determinations. Statistical difference analyses between the extracts were performed using one-way analysis of variance (ANOVA). IC_{50} values were calculated determined by nonlinear regression analysis from the sigmoidal dose-response inhibition curve using Graphpad prism 8.0 software.

Results and Discussion

In this work, different biological activity of *S. junceum* L. extracts as well as chemical compositions were investigated. The chemical compositions of the methanol, ethanol and water extracts of *S. junceum* flowers and stems were determined by HPLC-DAD.

Table 1. Quantitative results of phenolic compounds in the methanol, ethanol, and water extracts of *S. junceum* different parts (mg/g, n=3).

Analyte	Retention time (min)	SJSM	SJSE	SJSA	SJFM	SJFE	SJFA
Gallic acid	4.69	------	------	0.114	------	0.08	0.143
3,4-dihydroxy benzoic acid	6.98	0.14	0.084	0.256	0.469	0.3	0.251
Catechine	7.97	0.351	0.773	0.061	1.045	0.85	0.077
Chlorogenic acid	8.79	0.077	0.127	0.162	0.058	0.07	0.043
4-hydroxy benzoic acid	10.65	0.084	0.206	0.478	0.186	0.08	0.155
1,2-dihydroxy benzene	11.09	0.233	0.259	0.171	0.105	0.06	0.087
Epicatechin	11.4	0.414	0.254	0.265	0.144	0.09	0.035
Vanilic acid	11.8	------	0.053	0.329	0.324	0.06	0
Caffeic acid	12.18	0.122	0.363	0.227	0.32	0.54	0.239
Vanillin	17.63	0.109	0.099	0.12	0.184	0.12	0.005
p-Coumaric acid	18.27	0	0.137	0.112	0.162	0.16	0.456
Sinapic acid	19.17	0.407	0.997	0.365	0.183	0.39	0
Trans-Ferulic acid	20.07	0.079	0.263	0.188	0.144	0.09	0.011
Elagic acid	21.17	0.969	0.711	1.286	0.666	1.09	0
Rutin	22.4	0.242	0.848	0.356	0.517	0.72	0.081
Salicylic acid	32.88	0.251	0.358	3.304	0.178	1.33	----
Quercetin	36.26	0.07	0.103	0.12	0.089	0.29	----
Campherol	39.97	0.098	0.074	0.116	0.128	0.11	0.052

Phenolic composition profile by HPLC

The characterization of phenolic compounds was performed by HPLC-DAD analysis, and data of retention time, tentative identification and concentration of phenolic acid derivatives and flavonoids are summarized in Table 1. The representative HPLC chromatogram of different extracts obtained from flowers and stems can be observed in Figures 1-2. In the current study, 18 of the phenolic compounds were quantified by the calibration curve of the corresponding authentic standard. According to HPLC analysis results as seen in Table 1-2 and Figures 1-2, the epicatechin and elagic acid were the predominant phenolic compounds in stem extracts, while 3,4-dihydroxy benzoic acid and caffeic acid were higher in flower extracts. In addition, the ethanol extract was containing higher amount of elagic and salicylic acid, while aqueous extract has higher amount of caffeic acid and p-coumaric acid.

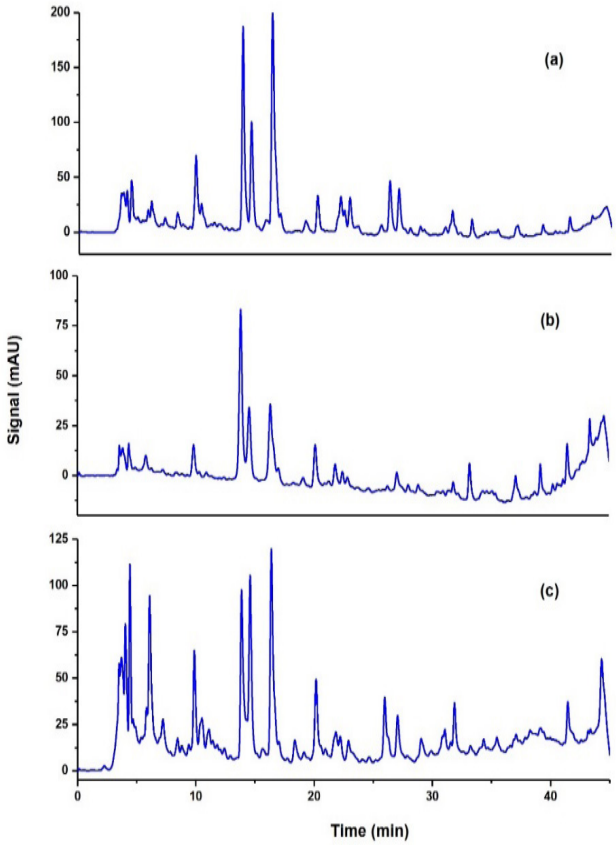

Figure 1. Representative HPLC chromatograms of phenolic compounds of stem extract of Spartium junceum in %70 EtOH (a), in MeOH (b), in H_2O (c)

Chlorogenic acid was found highest in the aqueous extract of S. junceum stems (0.162 mg/g), which was reported have anti-immunodeficiency and antidiabetic effect [30].

Antioxidant activity

Phenolic and flavonoids are important secondary metabolites with known antioxidant activity [31, 32]. Although phenolic have been proven to have many biological activities in previous studies, these bioactivities cannot be evaluated separately from their antioxidant properties. In this respect, the use of antioxidants in the treatment of chronic diseases caused by oxidative stress helps the treatment. Therefore, in vitro antioxidant capacity was usually used to investigate whether the plant has medicinal value. Several methods have been developed to study the antioxidant activity of plant extracts or substances. In this work, DPPH, ABTS, iron chelating activity methods were applied to evaluate the antioxidant activity of the different extracts obtained from *S. junceum* stems and flowers.

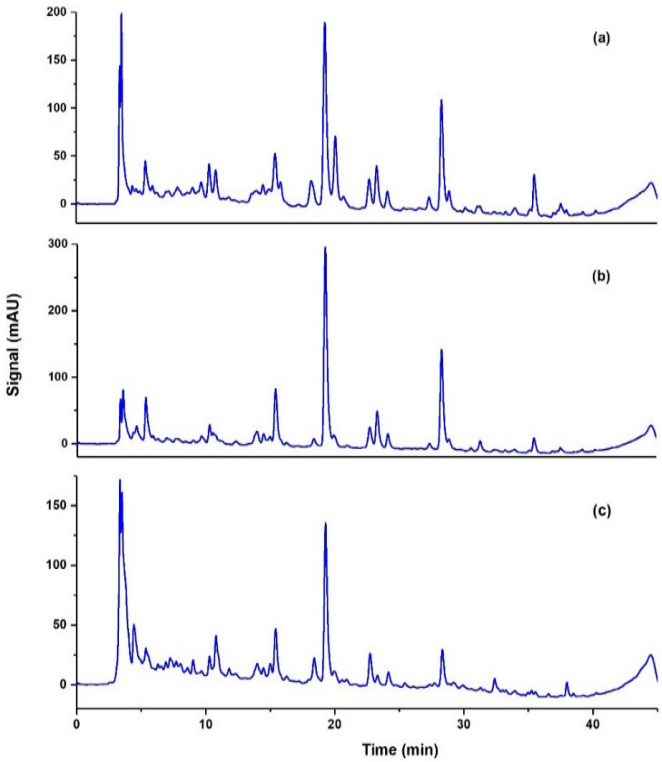

Figure 2. Representative HPLC chromatograms of phenolic compounds of flower extract of Spartium junceum in MeOH (a), in %70 EtOH (b), in H2O (c)

Table 2. Statistical analysis for the calibration of phenolic compounds.

Analyte	Equation of the line	Correlation coefficient (R^2)	Linearity Range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	y = 31.253x + 12.544	0.9945	20-Jan	1.462	4.429
3,4-dihydroxy benzoic acid	y = 18.77x - 13.155	0.9987	20-Jan	0.709	2.147
Catechine	y = 17.596x - 29.243	0.9849	20-Jan	1.914	5.799
Chlorogenic acid	y = 27.171x - 2.8747	0.9957	30-Jan	1.684	5.104
4-hydroxy benzoic acid	y = 20.954x - 8.7103	0.9933	20-Jan	1.624	4.922
1,2-dihydroxy benzene	y = 19.068x - 5.5374	0.9961	20-Jan	1.226	3.714
Epicatechin	y = 15.533x - 14.341	0.9897	20-Jan	2.012	6.098
Vanilic acid	y = 51.22x - 28.146	0.9978	20-Jan	0.921	2.792
Caffeic acid	y = 4.3809x + 9.5062	0.9895	30-Jan	6.658	20.175
Vanillin	y = 53.104x - 11.503	0.9977	20-Jan	0.941	2.851
p-Coumaric acid	y = 106.46x - 21.757	0.9985	20-Jan	0.767	2.324
Sinapic acid	y = 11.042x + 0.3898	0.9969	20-Jan	1.093	3.313
trans Ferulic acid	y = 42.376x - 8.6912	0.9992	20-Jan	0.562	1.702
Elagic acid	y = 9.1482x + 14.667	0.9992	Jan-50	4.619	13.998
Rutin	y = 34.986x - 35.717	0.9906	20-Jan	2.163	6.554
Salicylic acid	y = 5.8716x + 8.6391	0.9949	1 - 111	4.342	13.157
Quercetin	y = 36.023x - 0.5746	0.9991	20-Jan	0.585	1.772
Campherol	y = 21.813x - 6.7027	0.999	30-Jan	0.803	2.433

Table 3. Antioxidant activity of different extracts of *Spartium junceum L*.

Sample	Extract yield (%)	DPPH· assay[a] IC_{50} (mg/mL)	$ABTS^{\cdot+}$ assay[a] IC_{50} (µg/mL)	Total phenol content (mg GAE/g)	Total flavonoid content (mg QE/g)	Iron ion chelating activity[a] IC_{50} (µg/mL)
Flower MeOH extract	29.34	2.18 ± 1.35	0.42 ± 4.18	37.85±1.07	29.94±5.54	4.75± 2.33
Flower EtOH extract	39.02	2.37 ± 1.56	0.46 ± 2.00	32.99±1.47	27.43±2.75	2.52 ± 3.79
Flower H_2O extract	11.80	3.37 ± 0.91	0.30 ± 2.20	41.27±2.86	35.91± 4.89	1.11± 4.58
Stem MeOH extract	69.34	1.42 ±1.93	0.39 ± 5.16	60.45±1.78	39.81±1.57	1.39± 3.69
Stem EtOH extract	14.94	1.82 ± 3.28	0.33 ± 1.95	39.03±3.93	38.89±4.06	1.29 ± 1.29
Stem H_2O extract	6.46	1.69 ± 1.22	0.24 ± 2.99	63.94±5.99	61.33±5.46	0.66 ± 1.33
Quercetin[b]		0.047± 0.69	-	-	-	-
BHT[b]		-	0.042± 0.91	-	-	-
EDTA[b]		-	-	-	-	0.050±1.35

[a]IC_{50} values represent the mean ± SD of three parallel measurements ($p<0.05$). [b]Reference compounds. NT: not tested

Table 4. Enzyme inhibition activity of different extracts of *Spartium junceum L*.

Plant parts	Extracts	Acetylcholinesterase[a] IC_{50} (mg/mL)	Butyrylcholinesterase[a] IC_{50} (mg/mL)	α-glucosidase[a] IC_{50} (mg/mL)	α-amylase[a] IC_{50}(mg/mL)	Tyrosinase[a] IC_{50}(mg/mL)
Flower	MeOH	1.09 ± 7.56	0.22 ± 5.20	0.44 ± 4.07	3.09 ± 2.77	1.42 ± 0.68
	EtOH	0.72 ± 5.79	0.62 ± 8.20	na	2.24 ± 5.19	0.99 ± 0.29
	H_2O	4.61 ± 4.84	4.16 ± 6.36	5.36 ± 2.51	3.75 ± 2.96	0.99 ± 1.08
Stem	MeOH	2.04 ± 5.04	0.08 ± 5.90	1.75 ± 3.72	2.6 ± 3.19	1.35 ± 0.53
	EtOH	1.99 ± 8.76	0.52 ± 7.42	na	2.20 ± 4.14	1.12 ± 1.48
	H_2O	8.63 ± 7.89	8.78 ± 4.70	3.97 ± 1.68	3.08 ± 3.06	1.05± 1.31
Galanthamin[b]		0.038 ± 0.28	0.042 ± 0.12	-	-	-
Acarbose[b]		-	-	0.86± 1.37	0.37 ± 1.75	-
Kojic acid[b]		-	-	-	-	0.31 ± 2.24

[a]IC_{50} values represent the mean ± SD of three parallel measurements ($p<0.05$). [b]Reference compounds. ¸ NA: not active.

DPPH radical scavenging activity

The radical scavenging activities of different extracts from *S. junceum* evaluated by a stable radical DPPH are presented in Table 3. All the extracts exhibited DPPH radical scavenging activities in a concentration-dependent manner, especially the methanol extract of stems showed strong DPPH radical scavenging activity than the other extracts. The IC_{50} values of stem extracts were in order of methanol extract 1.42 ±1.93 > aqueous extract 1.69 ± 1.22> ethanol extract 1.82 ± 3.28 mg/mL. The different extracts from flowers exhibited lower DPPH radical scavenging activity than stem extracts, which may be due to the difference of chemical compounds containing in the extracts.

ABTS radical scavenging activity

The ABTS radical scavenging results of different extracts from *S. junceum* L. are shown in Table 3. All of the samples showed good and almost similar ABTS radical scavenging activities at the same concentrations in a concentration- dependent response. Among the extracts, the aqueous extract from stems demonstrated higher ABTS radical scavenging activity than others (IC_{50} value: 0.24 ± 2.99 µg/ml), it was followed by aqueous extract from flowers (0.30 ± 2.20 µg/ml). Since ABTS is a polar radical, it is mostly scavenging by polar compounds. In this study, the ABTS radical scavenging activity of water extract was higher than that of other extracts, which shows that the results are reasonable.

Iron chelating activity

The chelating of Fe^{2+} by extracts was estimated by the colorimetric method, in that ferrozine quantitatively make a ferrous-ferrozine complex. When iron chelating agent presents in the medium, it is possible to delay the formation of this complex, detection of the reduction in color strength allows to estimation of the chelating activity [33]. The metal ions play an important role in many radical reactions. The stem aqueous extract was the most active iron chelator with the IC_{50} value of 0.66 ± 1.33 µg/mL, which is lower than the positive standard EDTA (IC_{50} value 0.050 ± 1.35 µg/mL).

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

The results of the total phenolic and flavonoid content are presented in Table 1. The content of total phenols in extracts, expressed as gallic acid equivalents (GAE), varied between 32.99 ± 1.47 and 63.94 ± 5.99 mg/g of dry extract. The content of total flavonoids in extracts, expressed as quercetin equivalents (QE), varied between 27.43 ± 2.75 and 61.33 ± 5.46 mg/g of dry extract. The highest quantity of flavonoid compounds was found in aqueous extract of stems.

Enzyme inhibitory activities

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities: In the *in vitro* assays, AChE and BChE efficiently catalyze the hydrolysis of their respective natural substrate-acetylthioholine (AcSCh) and butyrylthiocholine (BuSCh), to produce acetate/butyrate and thiocholine. The last one generates the yellow 5-thio-2-nitrobenzoate anion by reacting with DTNB, which can be quantified at 405 nm [34]. The ethanol extract of flowers showed strong AChE inhibitory activity (IC_{50} value was 0.72 ± 5.79 mg/mL) than other extracts. However, a strong BChE inhibitory activity observed for methanol extracts from stems (IC_{50} value was 0.08 ± 5.90 mg/mL).

α-glucosidase and α-amylase inhibitory activities: In order to investigate the possible antidiabetic activity of *S. junceum* L. extracts, the inhibition effect on two enzymes, α-glucosidase and α-amylase, which play an important role in the metabolism of polysaccharides, were investigated. The methanol extract from flowers showed strong α-glucosidase inhibitory activity (IC_{50} value: 0.44 ± 4.07 mg/mL), which was found to be more effective than the acarbose used as a positive control drug (IC_{50} value: 0.86 ± 1.37 mg/mL) (Table 4). However, all the extracts showed weak α-amylase inhibitory activities compared with acarbose.

Tyrosinase inhibitory activity: The tyrosinase inhibitory effects of tested extracts increased in a linear concentration –dependent manner. The ethanol and aqueous extracts from flower were the most active with IC_{50} values of 0.99 mg/mL.

Conclusion

The present study reports for the first time the *in vitro* antioxidant and five different enzyme inhibitory activities of *S. junceum* as well as phenolic profiling by HPLC. According to the results in this work, the *S. junceum* extracts possess variable enzyme inhibitory and antioxidant activity. The ethanol extract of flowers shows Anti-AChE activity and the methanol extract of stems exhibited strong Anti-BChE activity. The results of this research represent a good preliminary examination for further analysis on *S. junceum* to discovering of new and potential enzyme inhibitors as well as food preservatives.

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