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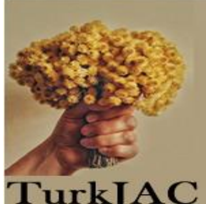

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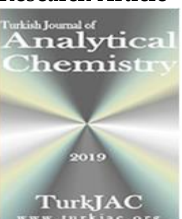

Determination of LC-MS/MS phenolic profile, antioxidant activity and *α*-glucosidase enzyme inhibition of *Linum mucronatum* Bertol. subsp. *armenum* (Bordz.) P.H.Davis

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Abstract

Plants include compounds having high antioxidant activity such as flavonoids, phenolics, and carotenoids. Antioxidant defense mechanisms play an important role in the prevention and treatment of oxidative stress diseases in humans. In the study, was performed to evaluate the antioxidant and *α*-glucosidase inhibitory effects of the flower and leaf parts of *Linum mucronatum* subsp. *armenum.* The antioxidant activities of the extracts were determined using six antioxidant activity determination assays (iron(III) reducing/antioxidant power (FRAP), DPPH radical scavenging activity, copper(II) reducing antioxidant activity (CUPRAC), ABTS radical scavenging capacity, total flavonoid content, and total phenolic content). While, the methanol extract showed the highest activity for the flower part, ethanol extracts of leaf part showed the highest antioxidant activity in the DPPH, FRAP and CUPRAC tests. The highest activity values in both flower and leaf parts were measured in acetone extract with SC_{50} = 0.287 mg/mL and SC_{50} = 0.163 mg/mL in ABTS test, respectively. Lowest activity values of solvent extracts were measured in hexane extracts in all tests. Phenolic compounds of the plant were identified using LC-MS/MS. These phenolics are kaempferol, vanillin, protecatechuic acid, caffeic acid, *p*-coumaric acid, *p*-OH benzoic acid, salicylic acid, quercetin and rutin. The leaf and flower parts have *α*-glucosidase enzyme inhibitor effect. It was determined that the leaf part of the plant (IC_{50} = 4.53 mg/mL) have higher enzyme inhibition than in the flower (IC_{50} = 6.10 mg/mL). As a result, it was determined that the plant showed the biological activity. The results will contribute to the studies on the biological activity of the other plant.

Keywords: Antioxidant activity, *α*-glucosidase, phenolic, *Linum*

1. Introduction

Oxidative stress, which take place because of an imbalance between antioxidants and free radicals in the human body, can negatively affect human health in many ways [1,2]. Especially, free radicals defect structure of cellular components such as nucleic acids, proteins and membrane phospholipids [3]. These radicals having unpaired electrons are highly unstable. To become stable, take electrons from other molecules and at the same time, oxidize the molecules. Thus, they cause the formation of another free radical [4]. Therefore, they are the major pathogens that cause various diseases in human such as diabetes, cancer, and neurodegenerative disorders [5]. Antioxidants known to prevent oxidative stress that may occur in the human body with the effect of free radicals [6]. There are mechanisms to prevent oxidative stress in the human body with antioxidants occurs endogenously or supplied externally from foods and supplements [7,8]. Recently, synthetic antioxidant compounds have been added to foodstuffs to protect food from reactive oxygen damages such as lipid oxidation during their processing and storage [9]. However, some synthetic antioxidant compounds such as BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) have been shown to have toxic effects on humans [10]. Today, because of that there is a greater tendency towards natural antioxidants rather than synthetic antioxidants that cause adverse health effects [11]. Plants are the most important source of natural antioxidants [12,13]. Antioxidants obtained from plants are effective in the prevention of many disorders such as diabetes and cancer [14]. One of the approaches used in the treatment of diabetes is to inhibit enzyme of alpha glucosidase for delay glucose absorption [15]. Plants

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contain hypoglycemic agents and 1200 plant species have been reported to have hypoglycemic activity. Therefore, it is important to use natural α-amylase and α-glucosidase inhibitors obtained from plant sources in the control of hyperglycemia [16]. One of the most common research topics is also the biological activities of plant compounds against various diseases, especially their antioxidant properties.

Because of that there are not enough studies on biochemical properties, The present study was focused some biochemical properties of the leaf and flower parts of *Linum mucronatum* Bertol. subsp. *armenum* (Bordz.) P.H.Davis (it is called sarıkamışketeni in Turkish) which is distributed in the Gümüşhane province of Türkiye. In the present study, it was aimed to identify the antioxidant activity potential in various solvent extracts of flowers and leaves of the plant. It was applied to determine antioxidant activity of the leaves, flower parts, tests having different mechanisms such as iron(III) reducing/antioxidant power (FRAP), $ABTS^{\bullet +}$ radical scavenging capacity, (DPPH) radical scavenging activity, copper(II) reducing antioxidant activity (CUPRAC), total flavonoid content and total phenolic content. Additionally, the phenolic profile of the plant parts was identified using LC-MS/MS and the inhibition of α-glucosidase enzyme were determined.

2. Material and methods

2.1. Preparation of extracts

L. mucronatum subsp. *armenum* was collected from Bağlarbaşı district of the Gümüşhane province in Black Sea Region of Türkiye in July 2021. It was stored in KTUB Herbaria (M. Gültepe 702). The plant is a non-shrub, herbaceous perennial with branching at the base. Flowering stems are erect or ascending, 10 – 35 cm long. These stems are keeled, spine straight and do not bear base leaves. Stem leaves oblong or oblanceolate, acuminate, 1 – 3 veined, 9 – 35 × 3 – 8 mm, stipules at leaf base. Inflorescence cymose, 7 and more flowered. Sepals lanceolate, 9 – 11 × 1.5 – 2.5 mm, keeled. It is membranous edged and ciliated at the tip. Petals yellow and base purple spotted, obovate, 19 – 33 × 8 – 11 mm, acute or obtuse. The filament tube is 3 – 4 mm and the filaments are 8–11 mm long at most. Anthers oblong 2 – 3 mm long, yellow in color. Staminode linear, up to 1 mm spherical ovary, linear stigma, capsule is 4 – 5 mm [17].

The flower and leaf parts of the plant were separated and dried at the room temperature. Then, the parts were powdered using a blender. Five solvents (methanol, ethanol, acetone, acetonitrile, and hexane) having different polarities were used for extraction. In the extraction process, 5 g of powdered both flower and leaf were weighed into a 100 mL beaker and 50 mL solvent was added to the onto the samples [18]. These mixtures were extracted using a magnetic stirrer for 2 hours. After that the extracts were filtered and the its solvents were removed in the rotary evaporator device (Heildoph, Germany). Their concentrations were determined by adding solvents. The prepared extracts were kept closed at +4 °C.

2.2. Analyses

2.2.1. Antioxidant activity

2.2.1.1. DPPH radical scavenging activity

The $DPPH^{\bullet}$ (1,1-diphenyl-2-picrylhydrazyl) radical assay is widely used in determining antioxidant activity of the substances [19]. In this method, antioxidants cause a decrease in intensity of the purple color from $DPPH^{\bullet}$. The antioxidant activity value is calculated based on this intensity decrease in the color. Firstly, 100 µM DPPH radical solution was added onto 750 µL of the sample. The solution was mixed with vortex, and it was kept at room temperature for 60 minutes. Then, Absorbances of the each mixture were determined at 517 nm with a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). For comparison, the activity values in different concentrations of trolox antioxidant standard (starting from 0.005 mg/ml) were also determined under the same conditions. The absorbances of DPPH radical in different sample concentration were measured and graph was plotted with the concentrations corresponding to these absorbances. In the $y = ax + b$ equation, the sample amount that cut in half $DPPH^{\bullet}$ concentration was determined in µg/mL and the results were expressed as the SC_{50} value. Lower SC_{50} values indicate higher radical scavenging potential.

2.2.1.2. Determination of iron (III) reduction / antioxidant power (FRAP)

In the method developed by Benzie and Strain [20], extracts of solvent of the leaf and flower parts were diluted at various concentrations (62.5 -125-250-500-1000 µM). It was applied same procedure the antioxidant standard Trolox. First, 50 µL of both sample and standard solution was pipetted to the tubes. In addition to this, 50 µL of sample solvent and sample solutions were added to the sample and reagent blank tubes respectively. Then, 1.5 mL of FRAP solvent was transferred to the blank tubes. After that FRAP reagent (1.5 mL) was pipetted to the all tubes excluding the sample blank tubes and were vortexed. At the end of a 20 min period, the values of absorbance were spectrophotometrically measured at 595 nm. The results were computed in µM TEAC by comparing the standard Trolox.

2.2.1.3. Copper (II) reducing antioxidant activity (CUPRAC)

In the CUPRAC method developed by Apak et al. [21] was made some modification and applied to the samples. First, the neocuproine alcoholic solution (96% ethanol), Cu(II) chloride solution, analysis solutions and ammonium acetate buffer (pH = 7) in equal volumes were transferred to the test tube. The volume of the final solution was set to 4.1 mL with adding pure water. These tubes were capped and stored at room conditions for 30 minutes and the values of absorbance were spectrophotometrically measured at 450 nm. As in other tests, antioxidant standard trolox was studied at 6 different concentrations. The antioxidant capacities of the samples were determined based on values from the trolox graph, as µM TEAC in trolox equivalent.

2.2.1.4. $ABTS^{•+}$ radical scavenging capacity

This method [22] is one of the commonly used methods by researchers for the determination of antioxidant activity. First, to prepare the stock solution of ABTS (7 mM), solvent mixture (water:ethanol 1:5) was prepared and ABTS solution was formed by dissolving ABTS reagent in the mixture. The solution was then mixed with a 2.45 mM solution of potassium persulfate prepared with water:ethanol (3:1). The mixture was kept in the dark at room conditions for 18 hours and $ABTS^{•+}$ was formed and diluted to 1/40. Thus, it was adjusted to absorbance of 0.07 at 734 nm. The standard antioxidant trolox solution used for comparison with sample extracts of different concentrations was pipetted in triplicate and the same procedure was applied to the sample and the reagent blank. These were left for 20 minutes at the room temperature. Finally, the values of absorbance were determined at 734 nm. Then sample amounts that cut in half the $ABTS^{•+}$concentration were computed in mg/mL and the results were reported as SC_{50}.

2.2.1.5. Total phenolic content (TPC)

The phenolic substance contents of the leaf and flower parts of *L. mucronatum* subsp. *armenum* extracted using different solvents were measured using Folin-Ciocalteu reagent based on Slinkard and Singleton method [23], with some modifications. First, 50 µL of extracts were diluted in distilled water (2.5 mL) and 0.2 N Folin-Ciocalteu (250 µL) of was transferred on the diluted extract. Then, 750 µL of Na_2CO_3 (7.5%) was pipetted onto the mixture and vortexed. After that the prepared tubes were stored for 120 min at room conditions and values of absorbance were measured at 765 nm spectrophotometrically.

Gallic acid antioxidant standard was prepared at various concentrations (62.5-125-250-500-1000 µg/mL). The phenolic compounds amounts were computes as gallic acid equivalent (GAE µg/ml) using the function of the line from the standard calibration graph.

2.2.1.6. Total flavonoid content (TFC)

The flavonoid contents of the leaf and flower parts of the *L. mucronatum* subsp. *armenum* were measured using the method developed by Fukumoto and Mazza [24]. As in the other tests, the measurements were carried out in triplicate. In addition, sample and reagent blanks were prepared. Samples in equal amounts (250 µL) were transferred into the tubes, then 2.1 mL of methanol was added to all the tubes. Finally, 50 µL of 1M CH_3COONH_4 (ammonium acetate) and 10% $Al(NO_3)_3.9H_2O$ (aluminum nitrate) were transferred to the tubes excluding the sample blank. After that, the mixtures were vortexed and stored at the room temperature for 40 min. The values of absorbance were read at 415 nm.

The antioxidant standard of quercetin was used simultaneously in the same conditions. Six different concentrations of quercetin (0.25 mg/mL) were prepared, and values of the absorbance were measured. Then, the standard calibration graph was drawn with the absorbance values corresponding to the concentration. According to the graph, the total flavonoid substance amounts of the samples were calculated in quercetin antioxidant equivalent (QAE mg/mL).

2.2.2. Determination of plant phenolic substance content using LC-MS/MS

Determination of phenolic content by LC-MS/MS was performed at Scientific Technical Application and Research Center, Hitite University. Analysis of phenolic substance content in leaf and flower parts of the *L. mucronatum* subsp. *armenum* was using LC-MS/MS (Thermo Scientific/Dionex Ultimate 3000-TSQ Quantum). Ethanol solvent was used for the extraction of plant parts. Column (ODS Hypersil 4.6*250 mm, 5µm) were used in the sample analyzes and a gradient program were applied with formic acid (0.1% in deionized water) in A reservoir and methanol (100%) in B reservoir. In addition, it was optimized with the column temperature to 30 °C, the mobile phase flow to 0.7 mL/min and 20 µL of injection volume of standards and samples. The optimization of gradient program was carried out passing 100% in the A reservoir in 0–1 minutes, 5% A in 22 minutes for 3 minutes, and 100% in the B reservoir in 26 minutes for 8 minutes [17].

2.2.3. In vitro α-glucosidase enzyme inhibition

The *α*-glucosidase enzyme inhibitory activities of the ethanol extracts of the leaf and flower parts of *L.* mucronatum subsp. armenum were measured according to the modified method of Yu et al. [25].

Table 1. Antioxidant activities and total phenolic content and total flavonoid content in different solvent extract of leaves and flowers of *L. mucronatum* subsp. *armenum*

Plant parts	Solvent	DPPHSC$_{50}$ (mg/mL)	FRAPTEAC (μM)	CUPRACTEAC (μM)	ABTSSC$_{50}$ (mg/mL)	TPC (μg/mL GAE)	TFC (mg/mL QAE)
Flower	Methanol (M)	0.12 ± 0.08	643 ± 2.11	0.20 ± 0.12	0.56 ± 0.15	803 ± 2.86	0.10 ± 0.05
	Ethanol (E)	0.17 ± 0.10	372 ± 1.17	0.16 ± 0.08	0.88 ± 0.21	450 ± 1.55	0.07 ± 0.04
	Acetonitrile (ACN)	0.14 ± 0.09	572 ± 1.82	0.18 ± 0.09	0.82 ± 0.20	564 ± 1.76	0.03 ± 0.01
	Acetone (A)	0.20 ± 0.11	340 ± 1.02	0.11 ± 0.03	0.29 ± 0.13	391 ± 1.21	0.04 ± 0.01
	Hexane (H)	1.58 ± 0.25	44 ± 0.54	0.07 ± 0.02	10.17 ± 1.21	17 ± 0.23	0.002 ± 0.01
Leaf	Methanol (M)	0.03 ± 0.03	570 ± 1.76	0.16 ± 0.08	0.22 ± 0.09	821 ± 2.12	0.13 ± 0.03
	Ethanol (E)	0.02 ± 0.02	645 ± 2.05	0.16 ± 0.09	0.21 ± 0.09	827 ± 2.09	0.10 ± 0.02
	Acetonitrile (ACN)	0.05 ± 0.06	530 ± 1.55	0.15 ± 0.07	0.30 ± 0.11	787 ± 1.87	0.10 ± 0.02
	Acetone (A)	0.05 ± 0.06	512 ± 1.32	0.14 ± 0.05	0.16 ± 0.09	758 ± 1.45	0.10 ± 0.02
	Hexane (H)	0.36 ± 0.13	58 ± 0.83	0.07 ± 0.01	8.43 ± 1.04	23 ± 0.41	0.001 ± 0.01

In the test, 650 μL of phosphate buffer (0.1 M and pH: 6.8) was pipetted to the test tube. Then, 20 μL of sample and 30 μL of *α*-glucosidase enzyme (*Saccharomyces cerevisiae*, lyophilized powder, ≥ 10 units/mg protein – Sigma Aldrich) prepared in phosphate buffer were added on the solution. After, the mixture was kept at 37 °C for 10 minutes and 75 μL of substrate (4-Nitrophenyl-*α*-D-glucopyranoside) was added on to the mixture. Once again, it was incubated at 37 °C for 20 minutes, then 650 μL of 1M Na_2CO_3 was transferred to all tubes and the reaction was stopped. Absorbance values were measured at 405 nm at the UV/VIS spectrophotometer.

Acarbose (positive control) were used in different concentrations as the standard inhibitor. The measurements were made in triplicate, including reagents and sample blanks. The IC_{50} values of acarbose and samples (sample concentration that cut in half the enzyme activity in the environment) were calculated. The lower the IC_{50} value of the sample, the more effective is in enzyme inhibition.

3. Results

3.1. Antioxidant activity

The antioxidant activities of the extracts prepared in 5 different solvents (methanol, ethanol, acetone, acetonitrile, hexane) of the leaves and flowers of the *L. mucronatum* subsp. *armenum* were determined using 6 different antioxidant activity determination methods (iron(III) reduction/antioxidant power (FRAP), DPPH radical scavenging activity, $ABTS^{•+}$ radical scavenging capacity, copper(II) reducing antioxidant activity (CUPRAC), total flavonoid content and total phenolic content tests) (Table 1). For DPPH test, while the methanol extract has the highest antioxidant activity (0.12 mg/mL) in the flower part, the highest value (0.017 mg/mL) in the leaf part were measured in ethanol extract. However, hexane extracts showed the lowest activity in both flower and leaf parts with 1.58 and 0.36 mg/mL, respectively. In addition, it was determined that the DPPH radical scavenging activity values of the leaf part were higher than the flower in all solvents.

The activity values measured in the FRAP test were similar to the DPPH test results. The methanol extract showed the highest antioxidant activity (643 μM TEAC) in the flower part, while hexane extract showed the lowest activity (44 μM TEAC). On the leaf part, the highest and lowest activity values were determined 645 μM TEAC in ethanol extract, 58 μM TEAC in hexane extract, respectively. As in DPPH and FRAP, the ranking of activity values measured in the CUPRAC test is M>ACN>E>A>H in flower and E>M>ACN>A>H in leaf.

In ABTS radical scavenging activity, lower SC_{50} values indicate higher radical scavenging potential. Unlike the other antioxidant activity tests, it is seen that the acetone extract has the highest antioxidant activity in both the flower (0.29 mg/mL) and leaf (0.163 mg/mL) parts. In addition, as in the other tests, hexane extracts of flower and leaf parts showed the lowest antioxidant activity as 10.17 mg/mL and 8.43 mg/mL, respectively.

Table 2. LC-MS/MS analysis of the phenolic compounds in the flower and leaf parts of *L. mucronatum* subsp. *armenum*

Phenolic Compounds	Flower (μg/mL)	Leaf (μg/mL)
Epicatechin	nd	nd
Catechin	nd	nd
Protocatechuic acid	0.41	nd
Protocatechuic aldehyde	nd	nd
Caffeic acid	0.86	0.22
Ferulic acid	nd	nd
Vanillin	0.34	0.44
Taxifolin	nd	nd
p-coumaric acid	1.41	1.92
Salicylic acid	0.45	nd
p-OH benzoic acid	0.39	nd
Rutin	1.19	0.42
Syringic acid	nd	nd
Quercetin	0.45	0.02
Rosmarinic acid	nd	nd
Kaempferol	0.26	nd
Resveratrol	nd	nd
Gallic acid	nd	nd
Ellagic acid	nd	nd
Oleuropein	nd	nd

nd: not detected

Unlike the others the ranking of activity values measured in the ABTS radical scavenging activity is A>M>ACN>E>H in flower and A>E>M>ACN>H in leaf.

The highest activity value of total phenolic content in the samples was measured in the methanol extract of flower part as 803 μg/mL GAE and in the ethanol extract of the leaf as 821 μg/mL GAE. In addition, hexane extract has the lowest amount of phenolic content in both flower (17 μg/mL GAE) and leaf (23 μg/mL GAE) parts. The ranking of activity values measured in the test was the same as in all other tests except ABTS radical scavenging activity. In determination of the total flavonoid content of the samples, it is seen that methanol extract has the highest activity in both the flower (0.098 mg/mL QAE) and leaf (0.126 mg/mL QAE) parts. Similar to all tests, the lowest activity values of flower and leaf were measured in hexane extract with 0.002 and 0.001 mg/mL QAE, respectively. The ranking of activity values of the solvent extracts was different from the other tests, and it was M>E>A>ACN>H in flower and M>E>A>ACN>H in leaf.

3.2. LC-MS/MS analysis

Phenolic profiles of the leaf and flower parts were identified quantitatively using LC-MS/MS device in their ethanol extracts. In total 20 of phenolic acids and flavonoids compounds were examined and 9 of them were identified in the flower part (caffeic acid, protocatechuic acid, vanillin, salicylic acid, *p*-coumaric acid, rutin, *p*-OH benzoic acid, quercetin and kaempferol) and 5 of them (caffeic acid, vanillin, *p*-coumaric acid, rutin and quercetin) were detected in the leaf (Table 2). The flower part has richer diversity and amount of phenolic compound than the leaf. In addition, caffeic acid, vanillin, rutin and quercetin were detected in both leaf and flower parts of the plant and the highest amount of phenolic compounds in both flower and leaf parts was measured for *p*-coumaric acid with 1.410 μg/mL and 1.923 μg/mL, respectively. Also, the rutin was dominant in the leaf (1.923 μg/mL). Finally, while the total phenolic content of the flower part of the plant was measured with 5.496 μg/mL, it was determined as 3.028 μg/mL in the leaf part.

3.3. *α*-Glucosidase inhibitor effect

The *α*-glucosidase inhibition activities (IC_{50}) of ethanol extracts of the flower and leaf parts of *L. mucronatum* subsp. *armenum* and were determined as 6.10 ± 0.21 and 4.53 ± 0.12 respectively (Fig. 1). I added, acarbose activity was measured as 0.70 ± 0.06 mg/mL. The lower the IC_{50} value is more effective the enzyme inhibition. Therefore, the enzyme inhibition of the leaf is higher than the flower.

4. Discussion

4.1. Antioxidant activity

Plants are rich in antioxidants, and it has further increased their importance in research in the field of health and functional food [26]. The activities of natural antioxidants are closely related to their functions. There are many applications for the use of plants with antioxidant activity in food and nutrition fields [27]. The studies about the determination of biological activity and chemical composition of members of the genus *Linum* generally focused on *Linum usitatissimum* L., which is called flaxseed and is the type species of the *Linum* genus [28–30]. However, phenolic content analysis, antioxidant activity determination and enzymatic activity studies have not been conducted on *L. mucronatum* subsp. *armenum.*

Antioxidant activities of solvents extracts in different polarities (methanol, ethanol, acetonitrile, acetone and hexane) of flowers and leaves of *L. mucronatum* subsp. *armenum* were determined using 6 methods. The methanol extract showed the highest antioxidant activity in flowers for all tests except ABTS radical scavenging activity. However, highest antioxidant activity in the leaves was determined in ethanol extract in all tests except tests of ABTS radical scavenging activity and total flavonoid content. In addition, different results were measured for each solvent in the same activity tests. The polarities of the solvents are effective on extraction of phytochemicals [31]. In a previous study, DPPH, ABTS, total antioxidant content, total phenol and flavonoid contents in methanolic extracts of leaf and fruit part extracts of *Linum arboretum* L. were carried out by Yıldız et al. [32] and IC_{50} values of leaf parts were calculated for DPPH and ABTS tests as 106.55 and 1144.8 μg/mL. In the same study, while the total phenol content was measured as 56.96 μg GA mg^{-1} and total flavonoid 426.49 μg catechin mg^{-1} in the leaf part.

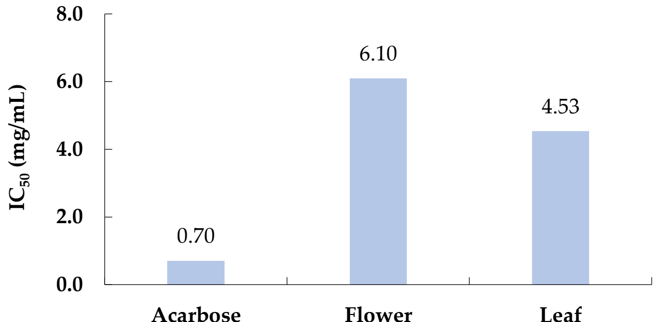

Figure 1. IC_{50} values of *α*-glucosidase enzyme inhibition of acarbose and flower and leaf parts of the *L. mucronatum* subsp. *armenum*

The DPPH values in leaf part of *L. arboreum* were similar to the values (124 µg/mL) in methanolic extracts of *L. mucronatum* subsp. *armenum* (Table 1). However, in the present study, values of ABTS (555 µg/mL) and total phenolic content (803 µg GAE mL) were significantly higher than the values measured in *L. arboreum*, while the total flavonoid content value (98 µg/mL QAE) was determined in the very low amount. In another study, DPPH (IC_{50}) values, total phenol and flavonoid contents of *L. arboreum* were determined as 85.1 µg/mL, 40.7 and 55.4 µg/mg in the herbal methanolic extract [33]. While DPPH values were similar to the results in the present study, the total phenolic and flavonoid content values were significantly lower. Many factors are effective on antioxidant activity values in plants; For example, individual genetic diversity, part of the plant analyzed, post-harvest handling, stage of maturity, variety, climatic conditions, environmental modification processing, and storage [18,34]. FRAP, CUPRAC, total phenolic content and total flavonoid content values measured in same solvent extracts of leaf and flower parts were determined similar to each other. However, this situation was not observed in both DPPH and ABTS measurements. For example, while the DPPH value is 0.17 mg/mL in leaf ethanol extracts, it is 0.02 mg/mL in flowers. Likewise, the ABTS value ethanol extracts, was measured as 0.88 mg/mL in the leaf and 0.21 mg/mL in the flower. It can be explained by the fact that the flowers have high antioxidant activity due to their more pigmentation [35].

4.2. LC-MS/MS analysis

Phenolic compound composition and amounts in the flower and leaf parts of *L. mucronatum* subsp. *armenum* were determined using LC-MS/MS device. The total phenolic content and phenolic composition of the flower was higher than the leaf based on used the phenolic standards (Table 2). *p*-coumaric acid, an organic compound belonging to the hydroxycinnamic acid class [36] was the phenolic compound having the highest amount in both flower and leaf parts with 1.41 and 1.92 µg/mL, respectively. *p*-coumaric acid is mostly found in the cell wall of grasses, fruits and vegetables in the form of esterified or free acid [37,38] In the development of therapeutic drugs, the main aim of most research groups is to use natural and organic compounds, which are known to have no harm or side effects against the environment, humans and all other organisms. *p*-coumaric acid inhibits the growth of bacterial pathogens [37,39] and can be used for these purposes. In addition, the antimelanogenic effects (natural skin lightening) of *p*-coumaric acid from plants have been demonstrated in a variety of experimental investigations, also including human studies [40]. In addition, rutin was determined by the highest amount in flower with 1.19 µg/mL. It is also known as quercetin-3-O-rutinoside, and vitamin P is a flavonoid found in many foods, beverages, and vegetables. It has biological activity effects such as antioxidant, anti-inflammatory, anti-diabetic, nephroprotective, gastroprotective, neuroprotective and hepatoprotective [41,42]. In both leaf and flower parts of the plants, other phenolic compounds such as caffeic acid having various bioactivity, which is present in many food sources including blueberry, coffee drinks, apple and cider [43,44] and vanillin the main component of natural vanilla, which is common used as an aroma and flavor enhancer in foods [45], and quercetin, which is a powerful antioxidant that protects the plant against biotic and abiotic stress factors were also detected. *p*-coumaric acid and vanillin in both flower and leaf ethanolic extracts of *L. mucronatum* subsp. *armenum* were also detected in *L. usitatissimum* [46].

4.3. α-Glucosidase inhibitor effect

Enzymes, consist of a long chain of amino acids joined by peptide bonds, are biological catalysts in the protein structure responsible for numerous biochemical reactions occurring in the cell [47,48]. Therefore, enzymes are compounds that are necessary for the survival of organisms. Although enzymes are only synthesis in cells, many of them can leave cells and continue to function *in vitro*. Because of these properties, enzymes are used in industry and food production processes, bioremediation and medicine. For example, α-glucosidase, an important enzyme in the treatment of diabetes, catalyzes the last step of the digestion of carbohydrates [15]. Therefore, α-glucosidase inhibitors are a class of oral drugs. Thus, in type 2 diabetes, the absorption of carbohydrates from the intestine is reduced by the inhibitor effect and it slowed down the rate of glucose pass into blood in the postprandial state [49]. These type inhibitors have been reported from plant and microbial sources [50]. Plants have an important place among all organisms in terms of showing α-glucosidase enzyme inhibition. Benella et al. [49] emphasized that natural products isolated from medicinal plants that inhibit α glucosidase strongly. Within the genus *Linum*, enzyme activity determination studies were mostly carried out on *L. usitatissimum* which is widely known and cultivated. Some of the enzymes studied in this species are as follows: Alkalase [51], β-glucosidase enzyme [52], α-amylase and α-glucosidase [53], However, there are no studies on the inhibition of α-glucosidase enzyme of *L. mucronatum* subsp. *armenum*. In the present study, *In vitro* α-glucosidase enzyme inhibition was investigated. It was determined that the enzyme inhibition IC_{50} value of the leaf (4.53 mg/mL) was higher than in the flower (6.10 mg/mL). Many studies have been carried out to determine the α-glucosidase enzyme inhibition of plants.

Benella et al. [49], 47 of plant species belonging to 29 families were evaluated in terms of *α*-glucosidase enzyme inhibition, and it was reported that their IC_{50} values varied between 0.9 μg/mL and 17 mg/mL. In other a study, Lawag et al. [54], inhibitor activities of 6 plant species for the same enzyme were reported IC_{50} values (0.08 and 519.86 μg/mL). Assefa et al. [55] classified the natural *α*-glucosidase inhibitor compounds with IC_{50} values according to the data obtained from the literature and it was reported that quercetin (IC_{50}: 7 μM) and protecatechuic acid (IC_{50}: 85.1 μg/mL). These both the compounds were identified in leaf and flower ethanolic extracts of the *L. mucronatum* subsp. *armenum*. In addition, it was reported that *p*-coumaric acid [56], caffeic acid [57], rutin [58] phenolics showed significantly *α*-glucosidase inhibitor activity. These compounds were identified both the leaf and flower parts of *L. mucronatum* subsp. *armenum*

5. Conclusions

Antioxidant activities of the extracts of the leaf and flower of *L. mucronatum* subsp. *armenum* prepared in five different solvents were measured using six different antioxidant activity tests. Extracts have high antioxidant activity. Activity values of leaf and flower parts were different from each other in all solvent extracts due to solvent polarity. In the flower part of the plant, the activity values of the methanol extracts are the highest in all tests except ABTS test (highest value in acetone solvent). The highest activity values in the leaf part were measured in ethanol extracts for all tests, except ABTS (highest value in acetone solvent) and total flavonoid content (highest value in methanol solvent). However, the lowest activity values of both leaf and flower were measured in hexane solvent extract in all tests. When the activity values of the leaf and flower parts were evaluated together, the activity of the leaf part was determined to be higher in all tests except CUPRAC. Vanillin, quercetin, caffeic acid, rutin, *p*- and coumaric acid phenolics were identified in LC-MS/MS analyzes of ethanol extracts of both leaf and flower parts. In addition, *p*-coumaric acid was the compound with the highest amount in both parts. Finally, the total phenolic content of the flower part and leaf part was measured using LC-MS/MS as 5.496 μg/mL and 3.028 μg/mL, respectively. The *α*-glucosidase inhibition activities (IC_{50}) of ethanol extracts of the leaf and flower parts of *L. mucronatum* subsp. *armenum* were determined as 4.53 ± 0.12 and 6.10 ± 0.21 respectively. The results indicate that, leaf and flower parts of the *L. mucronatum* subsp. *armenum* have antioxidant activity and *α*-glucosidase enzyme inhibition. Therefore, *L. mucronatum* subsp. *armenum* can also be evaluated as antibacterial, antifungal, cytotoxic and other enzyme activities. Determine the biological activities of other species, subspecies, and varieties in *Linum* genus, which has a rich taxon diversity in our country will contribute to the design of new biotechnological products.

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