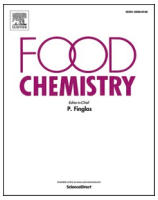
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Determination of the biological activities of endemic *Allium lazikkiyense* and its phytochemical profile by LC-MS/MS analysis

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ABSTRACT

This study investigated the phytochemical contents and *in vitro* antioxidant, enzyme inhibitory and anticancer properties of ethanol, methanol, and dichloromethane extracts prepared from aerial parts and onion peels of endemic *Allium lazikkiyense* Koçyiğit, Özhatay & E.Kaya. Ethanol extracts showed the highest antioxidant activity among the all extracts in terms of total phenolic and flavonoid contents as well as DPPH scavenging and iron reducing potentials. LC-MS/MS analysis results showed that aerial parts rich in fumaric acid, p-coumaric acid, ascorbic acid and hyperoside while onion peels rich in sarsasapogenin, caffeic acid and fumaric acid. Dichloromethane extracts showed a strong inhibitory effect on α-glucosidase, while all extracts exhibited low inhibitory activity on α-amylase, acetylcholinesterase and butyrylcholinesterase. Also, extracts from onion peels exhibited potent cytotoxic effect against MCF7 human breast cancer cells. The present study revealed that *A. lazikkiyense* could be a good natural source for antioxidant, α-glucosidase inhibitory, and anticancer activities.

1. Introduction

Oxidative stress is caused by an imbalance between reactive oxygen species (ROS) formation and its detoxification. Increase level of ROS in the body is harmful and has related to many situations such as aging, diabetes, carcinogenesis, and neurodegeneration (Yang et al., 2019). Antioxidants are known for their defensive property against ROS, and they are inhibiting free radicals that are constantly produced during oxidation processes. Fruits and vegetables are widely known for their good antioxidant property, consuming them protect the body against harmful reactions (Awad et al., 2017; Alfadda & Sallam, 2012).

Type 2 diabetes is one of the most common forms of diabetes mellitus and accounts for 90 % of all diabetic cases (Loizzo et al., 2020). Studies have shown that inhibition of α-glucosidase and α-amylase is a good approach for the treatment of Type 2 diabetes *via* delaying the digestion of carbohydrates. α-Glucosidase/α-amylase inhibitors such as acarbose, voglibose, and miglitol are among the most common antidiabetic drugs with the potential to improve glucose control but their use is limited due to high rates of side effects (Olokoba et al., 2012; Świerczewska et al., 2019). Therefore, searching for characterized digestive enzyme inhibitors from natural sources is important (Barakat et al., 2020).

Alzheimer's disease, the most common cause of dementia in older ages is a progressive neurodegenerative disease (Emir et al., 2020). Deficiency in levels of the neurotransmitter acetylcholine is one of the main causes of Alzheimer's disease. Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) is a good management for the treatment of Alzheimer's disease, as it increases the concentration of acetylcholine by inhibiting its hydrolysis (Atri et al., 2018; Aumeeruddy et al., 2018). Donepezil, rivastigmine and galantamine are licensed cholinesterase inhibitors for the symptomatic treatment of Alzheimer's disease (Marucci et al., 2021). Thus, investigating new compounds that have cholinesterase inhibitory potential from plants are an active research area.

Breast cancer is the most frequently diagnosed cancer in women and is the second leading cause of cancer-related death in women (Ahmad, 2019; Cheng et al., 2021). Currently, the standard methods for treating breast cancer are surgery followed by radiotherapy or chemotherapy. However, it is known that chemotherapeutic agents used to treat breast

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cancer such as paclitaxel, doxorubicin, and trastuzumab can cause acute and long-term adverse side effects on healthy organs of patients (Liyanage et al., 2019). In anticancer therapy, resistance against therapeutics is an important problem limiting the efficacy of cancer therapies. Therefore, finding alternative natural compounds is essential to prevent chemotherapy resistance (Cheng et al., 2021).

The genus *Allium* L., belonging to the family Amaryllidaceae, is economically important and includes several medicinal and edible species that have been used as food and therapeutics since ancient times. *Allium* species are a rich source of compounds that exhibit many bioactivities both *in vivo* and *in vitro*. It is known that compounds obtained from *Allium* species have a wide variety of effects such as antidiabetic, antioxidant, antihypertensive, anti-inflammatory, and anticancer. Studies have shown that many *Allium* species, both wild and cultivated, are rich in many phytoconstituents (such as alkaloids, flavonoids, essential oils, saponins, phytosterols, vitamins, and organosulphur compounds) with important pharmacological effects (Alam et al., 2022; Najeebullah et al., 2021). *Allium lazikkiyense* Koçyiğit, Özhatay & E.Kaya (Denizli onion) is a wild endemic *Allium* species that was introduced as a new species in 2014 (Özhatay et al., 2016). Wild *Allium* species are widely consumed for food, in addition to their use for health purposes in Türkiye. Fresh *Allium* leaves are collected from natural habitats regardless of species in early spring, are a sought-after ingredient in various culinary traditions. These leaves, offer a distinctive flavor and can be used in salads, pestos, or as a seasoning (Dogan, 2012; Ekşi et al., 2020).

To our literature research, no previous study has been found on the phytochemical content and biological effects of *A. lazikkiyense*. Based on the widespread ethnobotanical use of *Allium* species, this study investigated for the first time, the phytochemical contents, antioxidant and anticancer activities, inhibitory effects against some therapeutic enzymes (α-amylase, α-glucosidase, AChE, BChE) of ethanol, methanol, and dichloromethane extracts from aerial parts and onion peels of *A. lazikkiyense*.

2. Materials and method

2.1. Chemicals

2,2′-diphenil-1-pycryl-hydrazyl (DPPH), 2,4,6-tri(2-pyrydyl)-1,3,5-triazine (TPTZ), 3,5-dinitrosalicylic acid (DNS), 5,5′-ditiobis(2-nitrobenzoic acid) (DTNB), acarbose, α-amylase, α-glucosidase, AChE, acetylthiocholine iodide (ATChI), BChE, butyrylthiocholine iodide (BTChI), catechin, dimethyl sulfoxide (DMSO), Folin-Ciocalteu reagent, galantamine hydrobromide, gallic acid, quercetin, p-nitrophenyl-α-D-glucopyranoside (pNPG), and starch were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle Medium/Nutrient Mixture F12 (DMEM-F12), Dulbecco's phosphate-buffered saline (DPBS), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Biosera (France). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Glentham Life Sciences (Corsham, UK). All other chemicals used were of analytical grade.

2.2. Plant material

An endemic *Allium* species, *A. lazikkiyense* (Fig. 1) was collected from Denizli, Çivril, Türkiye (Fig. 2) (38.211173 N, 29.596307 E), 850 m away from the its locus classicus in June 2019 and identified by Assoc. Prof. Mine Koçyiğit. The voucher specimen was then kept for later reference in the Herbarium of the Faculty of Pharmacy, Istanbul University (ISTE-102751).

2.3. Preparation of extracts

The aerial parts and onion peels of *A. lazikkiyense* were dried and grinded. For extraction, 10 g of aerial parts and onion peels were weighed and extracted with 100 mL of ethanol, methanol, and dichloromethane by using Soxhlet device for an average of 5 h. The mixtures were then evaporated using a rota-evaporator until dry. The obtained dry extracts were dissolved in ethanol for enzyme inhibition assays, and in DMSO for the analyses of antioxidant activity, total phenol/flavonoid content, and cytotoxicity.

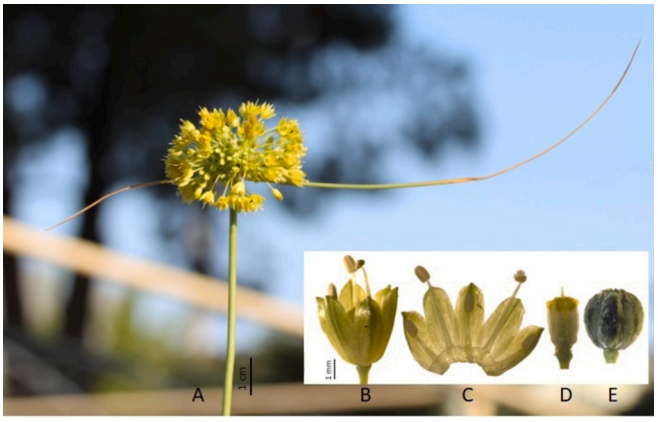


Fig. 1. *Allium lazzikiyense*; A. General view, B. Flower, C. Tepals and stamens, D. Ovary, E. Capsule.

2.4. Determination of total phenolic and flavonoid contents

The total phenolic content of *A. lazikkiyense* extracts were determined by using the Folin-Ciocalteu method (Slinkard & Singleton, 1977). Briefly, 5 μL of extract was added to each well of a 96-well plate containing 225 μL of distilled water. After a 3-min incubation, 5 μL of 2 N Folin-Ciocalteu reagent (diluted 1:3 in distilled water) and 15 μL of 2 % sodium carbonate solution were added to the mixture. The reaction mixture was incubated for 2 h. Subsequently, the absorbance of the mixture was measured at 760 nm against a blank. The results were expressed as mg gallic acid equivalents (GAE)/g extract using the obtained gallic acid standard curve ($y = 0.7855\times$-0.007, $R^2 = 0.999$).

The flavonoid contents of the extracts were determined by using aluminum chloride method (Sakanaka et al., 2005). To each well of a 96-well plate, 25 μL of extract was added followed by 125 μL of distilled water. Subsequently, 7.5 μL of 5 % sodium nitrite solution was added to the mixture. After 6 min, 15 μL of 10 % aluminum chloride solution was added. The mixture was allowed to stand for 5 min, and then 50 μL of 1 M sodium hydroxide solution followed by 27.5 μL of distilled water was added and mixed well. Finally, the absorbance of the mixture was measured at 510 nm against a blank. The results obtained were expressed as catechin equivalents (CE)/g extract using the standard curve ($y = 1.9905\times + 0.0087$, $R^2 = 0.9998$).

2.5. Determination of phytochemical content by LC-MS/MS analysis

The LC-MS/MS analysis was performed in Drug Application and Research Centre, Bezmialem Vakif University (Istanbul, Türkiye). The phytochemical contents of the extracts were quantitatively determined with an ultra-high performance liquid chromatography (UHPLC) system (UltiMate 3000, Thermo), coupled to a Thermo ORBITRAP Q-EXACTIVE mass spectrometer (MS) equipped with an electrospray ionisation (ESI) source. Extracts were dissolved in 1.5 mL methanol, centrifuged for 5 min, and the supernatant was used for analysis. A known quantity of an internal standard solution (100 mg/L) was added to the sample to yield a final concentration of 3 ppm. After filtration through a 0.45 μm pore size membrane, the filtered sample was injected into the LC-MS/MS system for subsequent analysis (Gülçin et al., 2010). Separation of the components was performed on a Fortis C18 reversed-phase column (150 × 3.0 mm, 3 μm; Fortis Technologies Ltd., UK) under isocratic conditions at

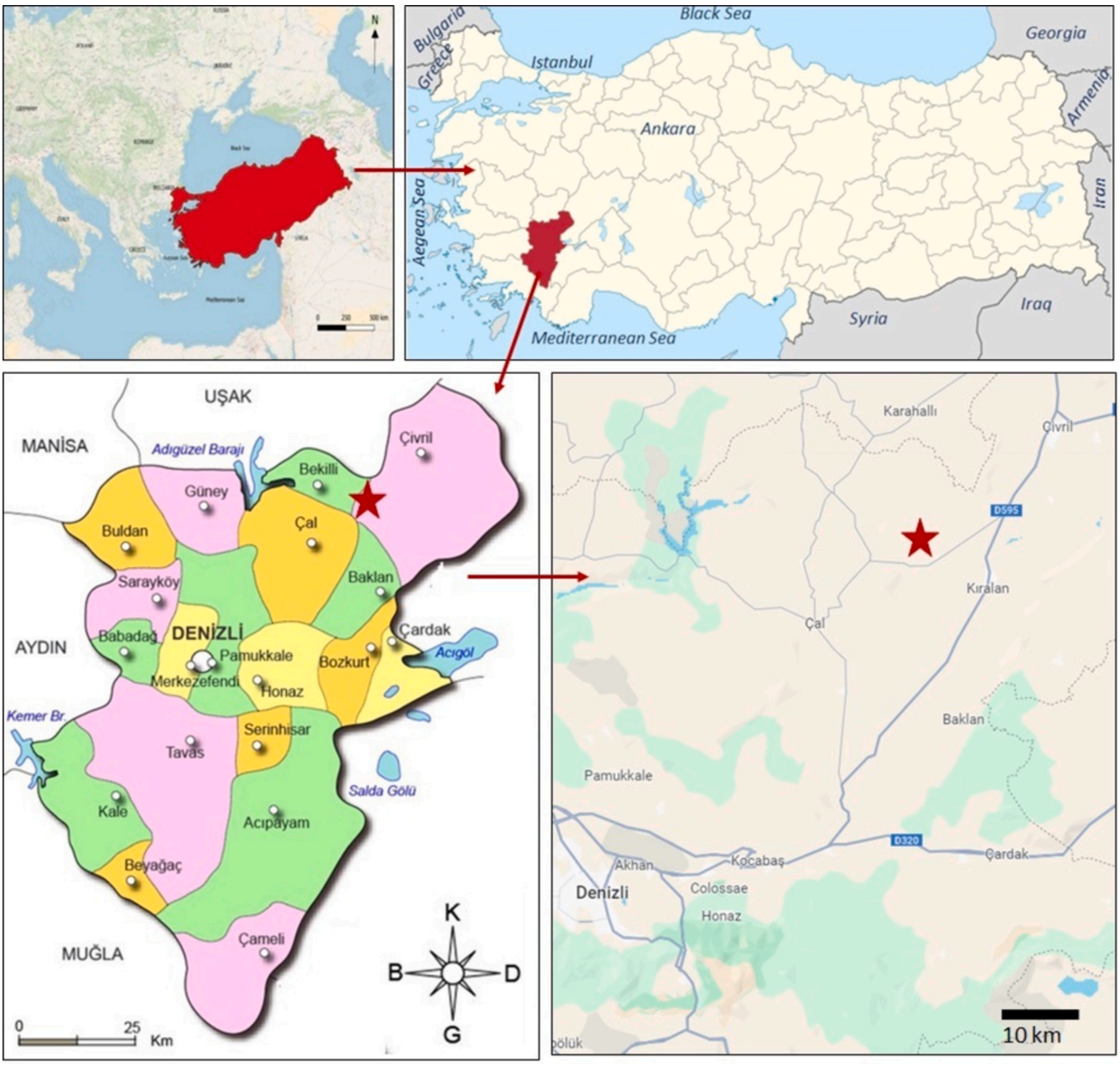


Fig. 2. Collected locality of *Allium lazzikiyense.*

25 °C. A sample volume of 1 μL was injected into the chromatographic system. The mobile phases used were (A) 1 % formic acid in water and (B) 1 % formic acid in methanol, delivered at a constant flow rate of 0.35 mL/min. A linear gradient was employed, initiating at 50 % solvent B for 1 min, subsequently increasing to 100 % solvent B over the next 2 min, maintaining a constant composition of 100 % solvent B for 3 min, and then returning to 50 % solvent B within 1 min. A 3-min equilibration period followed before the subsequent injection. Detection of the analytes was accomplished using mass spectrometry, operating in both positive and negative ESI modes. A mass range of 85 to 1500 *m/z* was scanned to identify the compounds of interest. The optimal operating conditions for the instrument were determined to be: sheath gas flow rate, 45 mL/min; auxiliary gas flow rate, 10 mL/min; spray voltage, 3.80 kV; capillary temperature, 320 °C; auxiliary gas heater temperature, 320 °C; and S-lens RF level, 50.0. The concentration of the analytes was determined using the external standard method. Calibration curves were generated by plotting the peak areas of known concentrations of standards against their corresponding concentrations, followed by linear regression analysis.

2.6. Determination of antioxidant activity

2.6.1. Determination of DPPH radical scavenging activity

The antioxidant property of the extracts was determined by using modified DPPH method (Brand-Williams et al., 1995). Briefly, 10 μL of various extract concentrations were mixed with 240 μL of 0.1 mM DPPH solution. The mixture was incubated in the dark at room temperature for 30 min. Subsequently, the absorbance decrease at 517 nm was measured against methanol to quantify the decolorization of DPPH from its purple radical form to its yellow form. Quercetin was used as a standard in the assay and the control was prepared without any inhibitor. The formula shown below was used to calculate the DPPH radical scavenging activity of the extracts as percent inhibition. Also, the results were expressed as IC_{50} value (half maximal inhibitory concentration).

$$\text{DPPH scavenging activity (\%)} = 1 - \left(\frac{\textit{Absorbance of the extract at 517 nm}}{\textit{Absorbance of the control at 517 nm}}\right) \times 100$$

2.6.2. Determination of ferric reducing antioxidant power (FRAP)

The reducing power of the extracts was determined by using modified FRAP method (Benzie & Strain, 1996). The FRAP reagent was prepared with a 1:1:10 mixture of three solutions: 20 mM ferric chloride, 10 mM TPTZ, and 300 mM sodium acetate buffer at pH 3.6. Briefly, 10 µL of extract was mixed with 20 µL of solvent, followed by 270 µL of freshly prepared FRAP reagent. The mixture was incubated at 37 °C for 10 min, and then increase in the absorbance at the 4th minute was read at 593 nm. Quercetin was used as a standard in the assay. Reducing power of the extracts was expressed as FRAP value (mM Fe^{+2}/mg) using equation of standard regression curve using $FeSO_4.7H_2O$ solution ($y = 0.631\times$-0.0954, $R^2 = 0.9801$).

2.7. Determination of enzyme inhibitory activity

2.7.1. Determination of α-glucosidase inhibitory activity

Inhibition of α-glucosidase activity was carried out by using modifying Bothon et al.'s (2013) method. In this assay, the inhibitory activity is determined by a reaction in which α-glucosidase hydrolyzes pNPG resulting in the formation of a yellow-colored p-nitrophenol product. Briefly, 25 µL of extract was combined with 75 µL of 0.1 M sodium phosphate buffer (pH 6.8). Subsequently, 50 µL of α-glucosidase (from *Saccharomyces cerevisiae*) solution (1 U/mL) was added, and the mixture was preincubated at 37 °C for 10 min. Following this, 50 µL of 5 mM pNPG solution was added. The absorbance of released p-nitrophenol was read at 405 nm. Acarbose was used as a standard inhibitor and the control was prepared without any inhibitor. The formula below was used to calculate α-glucosidase inhibitory activity as percent inhibition and the results were expressed as IC_{50} value.

$$\text{Inhibitory activity (\%)} = 1 - \left(\frac{\text{Reaction rate of sample at 405 nm}}{\text{Reaction rate of control at 405 nm}}\right) \times 100$$

2.7.2. Determination of α-amylase inhibitory activity

α-Amylase inhibitory activity of the extracts was carried out by using modified DNS method (Ali et al., 2006). In this assay, α-amylase convert starch into reducing sugars (maltose) and released maltose is measure by the reduction of DNS. Briefly, 10 µL of extract was preincubated with 50 µL of α-amylase (from porcine pancreas) solution (3 U/mL) and 40 µL of 0.1 M sodium phosphate buffer (pH 6.8) at 25 °C for 10 min. Then 50 µL of 0.75 % starch solution was added to initiate the reaction, which proceeded for 5 min. The reaction was terminated by adding 75 µL of DNS color reagent (96 mM DNS, 5.31 M potassium sodium tartrate in 2 M NaOH). The mixtures were heated at 85 °C for 15 min, followed by dilution with distilled water (4-fold). The absorbance of the product was read at 540 nm. Acarbose was used as a standard inhibitor and the control was prepared without any inhibitor. The formula below was used to calculate α-amylase inhibitory activity as percent inhibition and the results were expressed as IC_{50} value.

$$\text{Inhibitory activity (\%)} = 1 - \left(\frac{\text{Absorbance of sample at 540 nm}}{\text{Absorbance of control at 540 nm}}\right) \times 100$$

2.7.3. Determination of AChE and BChE inhibitory activity

AChE and BChE inhibitory activities were carried by applying Ellman et al. (1961) method with some modifications. Briefly, 20 µL of extract was combined with 220 µL of Ellman solution (318 µM DTNB, 955 µM ATChI/BTChI in phosphate buffer, pH 7.5). Subsequently, 10 µL of AChE (from electric eel)/BChE (from equine serum) solution (0.5 U/mL) was added. Absorbance changes were monitored at 412 nm for 10 min. Galantamine was used as a standard inhibitor and the control was prepared without any inhibitor. The formula below was used to calculate AChE and BChE inhibitory activities (%). The results obtained were expressed as IC_{50} values.

$$\text{Inhibitory activity (\%)} = 1 - \left(\frac{\text{Reaction rate of sample at 412 nm}}{\text{Reaction rate of control at 412 nm}}\right) \times 100$$

2.8. Determination of anticancer activity

The cytotoxic activity of the extracts was investigated in the human breast cancer cell line (MCF7; HTB-22). The cell line was obtained from the American Type Culture Collection. MCF7 cell lines were grown in DMEM/F-12 medium supplemented with 10 % FBS and 100 U/mL penicillin and 100 µg/mL streptomycin. The cytotoxic activity was analyzed by using MTT method (Ganot et al., 2013). The cells (1×10^4 cell/90 µL) were plated and incubated at 37 °C for 24 h in 5 % CO_2 incubator (Nüve, Ankara, Türkiye). Then, 10 µL of extract was added and incubated again for another 72 h. After incubation, 20 µL of MTT solution (5 mg/mL) was added. The color intensity in the microplate wells was measured at 590 nm (670 nm reading was used as reference). The control was prepared without any inhibitor. The formula below was used to calculate the cytotoxic activity (%). The results obtained were expressed as IC_{50} values.

$$\text{Cytotoxic activity (\%)} = 1 - \left(\frac{\text{Absorbance of the extract at 590 nm}}{\text{Absorbance of the control at 590 nm}}\right) \times 100$$

2.9. Statistics analysis

The results were expressed as mean ± standard deviation (SD). The values of activities between two extracts were analyzed by an

Table 1
Total phenolic and flavonoid contents of the extracts from *A. lazikkiyense*.

	Aerial part			Onion peel		
	EtOH	MeOH	DCM	EtOH	MeOH	DCM
Phenol (mg GAE/g)	18.43 ± 0.81[a]	17.22 ± 0.73[a]	3.42 ± 0.39[b]	17.94 ± 0.45[a]	13.90 ± 0.46[c]	5.22 ± 0.31[d]
Flavonoid (mg CE/g)	7.50 ± 0.34[a]	5.82 ± 0.51[b]	2.66 ± 0.18[c]	7.06 ± 0.33[a]	5.59 ± 0.44[b]	3.71 ± 0.24[d]

Data are expressed as mean ± standard deviation ($n = 3$).
Different superscript letters in the same row indicate a significant difference ($p < 0.05$).
CE: Catechin equivalents, DCM: Dichloromethane extract, EtOH: Ethanol extract, GAE: Gallic acid equivalents, MeOH: Methanol extract.

independent-samples *t*-test by Microsoft® Excel® Microsoft 365 (Microsoft, USA). Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Total phenolic and flavonoid contents

The total phenolic and flavonoid contents of *A. lazikkineyse* extracts was shown in Table 1. Different values were obtained based on the solvents used for plant extraction. It was observed that extract with prepared high polarity solvents like methanol and ethanol contained higher amount of phenolics and flavonoids when compared to dichloromethane extracts. Similar results were obtained in a study conducted on *A. macrochaetum*, where it was reported that ethanol extracts contained higher amount of phenolics and flavonoids in both aerial and onion peels when compared to water extracts (Demirci Kayiran et al., 2019). Another study by Singh et al. (2009) showed that dichloromethane extracts from *A. cepa* had lower amount of phenolics and flavonoids, similar to that of our results.

3.2. Phytochemical content by LC-MS/MS analysis

Phenolic compounds abundantly found in plants play an important role in various biological activities like antioxidant, antihypertensive, antidiabetic, anticancer, antifungal, and anti-inflammatory (Ambriz-Perez et al., 2016). Until present, all study conducted on *Allium* species show that they are rich phenolics (Alam et al., 2022). In the present study, the phytochemical profile of *A. lazikkiyense* was analyzed by the LC-MS/MS and determined compounds were presented in Table 2.

(+)-Catechin, dihydrocampherol, (−)-epigallocatechin gallate, (−)-epicatechin gallate, naringenin, nepetine, and salicylic acid were not found in any of the extracts. Among the identified compounds, fumaric acid, p-coumaric acid, ascorbic acid, and hyperoside were the major phytochemicals present in aerial parts and sarsapogenin, caffeic acid, and fumaric acid were in onion peels. p-Coumaric acid was found to be the major phenolic acid in ethanol (362.39 μg/g) and methanol (513.22 μg/g) extracts of aerial parts of *A. lazikkiyense*. In a similar study conducted by Demirci Kayiran et al. (2019) on *A. macrochaetum* (mountain garlic) that was collected from Kahramanmaraş, it was found that p-coumaric acid was one of the major compounds in ethanol (348.07 μg/g) and water (139.66 μg/g) extracts from aerial parts. In addition, many studies have shown that the aerial parts and bulbs of various *Allium* species contain high amounts of phenolic acids such as quinic, p-coumaric, vanillic, and caffeic acids (Emir et al., 2020; Izol et al., 2021). In an LC-MS/MS study conducted on *A. tuncelianum,* also known as Tunceli mountain garlic, by Takim et al. (2021), it was reported that the methanol extract from bulbs contained high amounts of fumaric acid (318.24 μg/g) and caffeic acid (237.52 μg/g). Similar results were obtained in the present study, where fumaric acid (141.14 μg/g) and caffeic acid (147.18 μg/g) were found to be major compounds in the methanol extracts from onion peels of *A. lazikkiyense*. In another study, it was reported that fumaric acid (5.92 mg/g) was the major compound in the methanol extract from the bulbs of *A. kurtzianum* (Yilmaz-Ozden et al., 2020). In the present study, it was found that rutin and hyperoside were present in all extracts, and the aerial part extracts contained higher amounts of these compounds when compared to the onion peel extracts. Similarly, it has been reported that rutin and hyperoside content of the extracts from aerial parts of *A. kurtzianum* was higher than that of the extracts from bulbs (Yilmaz-Ozden et al., 2020). Also, in a study by Simin et al. (2020), it was reported that *A. melayantherum, A. flavum, A. fuscum,* and *A. paniculatum* contained high amounts of rutin. The present study showed that the ethanol and methanol extracts from aerial parts of *A. lazikkiyense* contained significant amounts of ascorbic acids. Many studies show that most commonly consumed *Allium* species such as onion (*A. cepa*) and leek (*A. ampeloprasum* var. *porrum*) contain significant amounts of ascorbic acid (Bernaert et al., 2012; Upadhyay, 2016). Also, sarsasapogenin, a steroidal saponin, was also observed in high amounts in ethanol (2695.15 μg/g), methanol (3896.77 μg/g), and dichloromethane (1515.43 μg/g) extracts from onion peels. *Allium* species are known to be rich in steroidal saponins and these compounds have antifungal, cytotoxic and hypocholesterolemic effects (Sobolewska et al., 2016).

In the present study, it was observed that type of solvent used during extraction plays an important role in examining the amounts of phytochemical contents. The phytochemical contents of methanol extracts for both aerial parts and onion peels were the highest, while dichloromethane extracts were the lowest. It was concluded that high polarity solvents were more effective in obtaining phytochemical compounds of *A. lazikkiyense*. Similar results were obtained in a study on *A. cepa* showed that dichloromethane extracts exhibited the lowest phytochemical contents when compared to water, diethyl ether, ethyl acetate, and butanol fractions (Singh et al., 2009).

3.3. Antioxidant activity

DPPH radical scavenging activity and FRAP methods were used to determine the antioxidant potential of the extracts from *A. lazikkiyense*. DPPH is a standard method that is used to evaluate the antioxidant

Table 2
Amounts of the phytochemicals present in the extracts from *A. lazikkiyense* (μg/g extract).

		Aerial part			Onion peel		
Compound	MW (g/mol)	EtOH	MeOH	DCM	EtOH	MeOH	DCM
Ascorbic acid	176.12	234.12	406.98	<LOD	64.63	60.03	<LOD
Chlorogenic acid	354.31	7.94	1.12	4.59	6.49	23.15	2.66
Fumaric acid	116.07	8757.71	16,754.83	<LOD	172.95	141.14	<LOD
Orientin	448.4	<LOD	<LOD	<LOD	20.11	11.2	<LOD
Caffeic acid	180.16	11.57	22.31	<LOD	193.51	147.18	<LOD
Luteolin-7-rutinoside	594.5	1.36	2.81	<LOD	–	–	<LOD
p-Coumaric acid	164.16	362.39	513.22	<LOD	–	–	<LOD
Rutin	610.5	99.55	121.82	4.36	4.16	8.04	9.57
Hyperoside	464.4	220.51	334.34	3.9	6.43	4.4	6.38
Quercitrin	448.4	35.98	45.25	<LOD	2.65	5.36	<LOD
Quercetin	302.23	76	81.61	<LOD	<LOD	<LOD	<LOD
Campherol	286.24	28.49	27.27	<LOD	2.96	<LOD	<LOD
Hispidulin	300.26	1.23	1.03	7,11	10.91	5.77	22.34
Chrysin	254.24	3.81	–	23.85	4.48	17.99	55.85
Acacetin	284.26	4.99	14.21	31.65	7.44	16.14	49.47
Sarsasapogenin	416.6	<LOD	<LOD	<LOD	2695.15	3896.77	1515.43

DCM: Dichloromethane extract, EtOH: Ethanol extract, MeOH: Methanol extract, MW: Molecular weight, LOD: Limit of detection (Used for values below measurement limits).

Table 3
The IC_{50} values for DPPH scavenging, α-glucosidase inhibitory, and cytotoxic activities of the extracts from *A. lazikkiyense*.

IC_{50}	Aerial part			Onion peel			Standard*
	EtOH	MeOH	DCM	EtOH	MeOH	DCM	
DPPH (mg/mL)	0.54 ± 0.017^{a}	0.68 ± 0.034^{b}	–	0.47 ± 0.009^{c}	0.71 ± 0.017^{b}	–	0.0049 ± 00001^{d}
α-Glucosidase (mg/mL)	0.98 ± 0.04^{a}	0.83 ± 0.02^{b}	0.39 ± 0.02^{c}	2.21 ± 0.10^{d}	2.55 ± 0.17^{e}	0.73 ± 0.03^{f}	0.12 ± 0.01^{g}
Cytotoxicity on MCF7 (mg/mL)	0.046 ± 0.003^{a}	0.55 ± 0.0004^{b}	0.362 ± 0.006^{c}	0.007 ± 0.0002^{d}	0.020 ± 0.001^{e}	0.037 ± 0.002^{f}	–

Data are expressed as mean ± standard deviation ($n = 3$).
Different superscript letters in the same row indicate a significant difference ($p < 0.05$).
The concentrations represent final concentrations in the reaction medium.
DCM: Dichloromethane extract, EtOH: Ethanol extract, MeOH: Methanol extract.
IC_{50} for DPPH scavenging activity: The inhibitory concentration that scavenges DPPH radical by 50 %.
IC_{50} for α-glucosidase inhibitory activity: The inhibitory concentration that inhibits activity of the enzyme by 50 %.
IC_{50} for cytotoxic activity: The inhibitory concentration that inhibits cell growth by 50 %.
MCF7: Human breast adenocarcinoma epithelial cell.
* The standard in the table represent quercetin for DPPH radical scavenging activity and acarbose for α-glucosidase inhibitory activity.

property of different compounds by measuring their free radical scavenging potential. DPPH radical scavenging activities of the extracts are presented as IC_{50} values in Table 3. The IC_{50} values of dichloromethane extracts could not be calculated as their scavenging activity was below 30 %. Both ethanol extracts were found to be strong DPPH radical scavenging activity. Similar results were obtained in a study conducted on *A. macrochaetum*, where the highest DPPH radical scavenging activity was found in ethanol extracts when compared to water extracts (Demirci Kayiran et al., 2019). A study on the methanol ($IC_{50} = 0.98 \pm 0.03$ mg/mL) and water ($IC_{50} = 0.86 \pm 0.01$ mg/mL) extracts of *A. mangolicum* (Wang et al., 2019) and another study conducted on ethanol ($IC_{50} = 0.85 \pm 0.01$ mg/mL), methanol ($IC_{50} = 0.63 \pm 0.01$ mg/mL), and water ($IC_{50} = 1.44 \pm 0.04$ mg/mL) extracts of *A. ursinum* (Pavlovic et al., 2017) were found to have lower DPPH radical scavenging activity than that of *A. lazikkyense* ethanol ($IC_{50} = 0.54 \pm 0.01$ mg/mL) and methanol ($IC_{50} = 0.68 \pm 0.03$ mg/mL) extracts from aerial parts.

The FRAP method is another standard method that is used to determine the antioxidant potential of compounds *via* measuring reducing power. In the FRAP method, compounds that contain antioxidant properties donate electrons to Fe^{3+}-TPTZ reducing it to Fe^{2+} complex (Benzie & Strain, 1996). The obtained FRAP values of the extracts in the present study were expressed as mM Fe^{+2}/mg and are shown in Table 4. It was observed that all ethanol extracts had the highest reducing powers when compared to methanol and dichloromethane extracts. Further, not much difference was observed in the methanol extracts from aerial parts and onion peels. On contrary, in a study on species *A. neapolitanum*, *A. subhirsutum,* and *A. roseum* the leaf and flower extracts of all the three species had higher FRAP values when compared to their bulbs extracts (Nencini et al., 2011). In a study by Singh et al. (2009) on *A. cepa*, it was shown that dichloromethane extracts had the lowest iron reducing power values.

As shown in many previous studies, extraction with polar solvents provides high yields of plant bioactive compounds with antioxidant properties such as phenolics (Khalili et al., 2022). Similarly in present work, it was observed that extracts of *A. lazikkiyense* prepared with high polar solvents such as ethanol and methanol exhibited potent antioxidant activities consistent with their phenolic content.

Also, in this study a strong correlation was observed between the antioxidant activity values (DPPH and FRAP, respectively) and major compounds found in the extract from aerial parts fumaric acid ($r = -0.834$; $r = 0.716$), p-coumaric acid ($r = -0.930$; $r = 0.844$), ascorbic acid ($r = -0.866$; $r = 0.757$) and hyperoside ($r = -0.907$; $r = 0.812$) as well as major compounds found in the extract from onion peels fumaric acid ($r = -0.997$; $r = 0.855$) and caffeic acid ($r = -0.999$; $r = 0.884$).

Table 4
FRAP values of the extracts from *A. lazikkiyense*.

	Aerial part			Onion peel			Standard
	EtOH	MeOH	DCM	EtOH	MeOH	DCM	Quercetin
FRAP (mM Fe^{+2}/mg)	13.69 ± 0.25[a]	11.86 ± 0.43[b]	7.18 ± 0.38[c]	14.59 ± 0.21[d]	11.34 ± 0.40[b]	9.73 ± 0.41[e]	1754.50 ± 57.39[f]

Data are expressed as mean ± standard deviation (n = 3).
Different superscript letters in the same row indicate a significant difference ($p < 0.05$).
The concentrations represent final concentrations in the reaction medium.
DCM: Dichloromethane extract, EtOH: Ethanol extract, FRAP: Ferric reducing antioxidant power, MeOH: Methanol extract.

3.4. α-Glucosidase and α-amylase inhibitory activity

Diabetes mellitus is one of the most common metabolic conditions caused due to rise in sugar level in blood. α-Glucosidase/α-amylase inhibitors are among the most common antidiabetic drugs to have the potential to improve glucose control (Olokoba et al., 2012; Świerczewska et al., 2019). High dietary intake of *Allium* vegetables like *A. cepa, A. sativum*, and *A. porrum* result a lower incidence of hyperglycemia (Melino et al., 2019). Studies show that *Allium* species are widely consumed both in Türkiye and in many parts of the world due to its lowering effect on hyperglycemia (Aslan et al., 2010; Najeebullah et al., 2021). In the present study, the *in vitro* hypoglycemic activities of *A. lazikkyense* was determined, by measuring α-glucosidase and α-amylase inhibitory effects. It was observed that dichloromethane extracts from both aerial parts and onion peels exhibited a higher amount of α-glucosidase inhibitory activity followed by methanol and ethanol extracts (Table 3). On contrary, none of the extracts were found to have an effective inhibitory activity on α-amylase and the values obtained were way too low when compared to its standard values as shown in Table 5. Nickavar and Yousefian (2009) reported that no α-amylase inhibition was observed on 6 different *Allium* species (*A. cepa, A. sativum, A. akaka, A. ampeloprasum, A. hirtifolium, and A. porrum*) grown in Iran. In addition, in another study on α-glucosidase inhibitory activity of *A. commutatum* ethanol extract from aerial parts ($IC_{50} = 0.325$ mg/mL) was found to be similar to that of our present results of aerial parts of dichloromethane extracts ($IC_{50} = 0.390$ mg/mL) (Loizzo et al., 2020). In a study conducted by Yilmaz-Ozden et al. (2020), it was reported that the methanol extracts from aerial parts and bulbs of *A. kurtzianum* did not show any inhibitory activity on both α-glucosidase and α-amylase. In a study on *A. consangenium* (Mahnashi et al., 2022), it was reported that the plant inhibited α-glucosidase ($IC_{50} = 85.54$ μg/mL) and α-amylase ($IC_{50} = 61.19$ μg/mL).

There are many studies in the literature reported the α-glucosidase inhibitory activities of various plant extracts prepared by solvents with different polarity. These studies revealed that appropriate solvent for extracting compounds exhibiting α-glucosidase inhibitory activity varies according to the plant species (Chen et al., 2022; Lin et al., 2022). In our study, it was determined that the extract from *A. lazikkyense* prepared

Table 5
Inhibitory activities of the extracts at a concentration of 0.4 mg/mL on α-amylase, AChE, and BChE.

	Aerial parts			Onion peels			
Inhibition (%)	EtOH	MeOH	DCM	EtOH	MeOH	DCM	Standard*
α-Amylase	12.16 ± 0.86^{a}	$11.90 \pm 1.41^{a,b}$	$12.46 \pm 0.98^{a,b}$	$15.23 \pm 1.60^{b,c}$	15.63 ± 0.63^{c}	–	82.32 ± 0.76^{d}
AChE	25.78 ± 1.84^{a}	22.07 ± 2.18^{a}	15.94 ± 1.69^{b}	31.66 ± 1.44^{c}	22.76 ± 1.48^{a}	13.73 ± 1.67^{b}	75.36 ± 1.21^{d}
BChE	17.14 ± 1.63^{a}	$29.57 \pm 1.83^{b,d}$	20.53 ± 1.39^{a}	$21.38 \pm 2.06^{a,c}$	$25.41 \pm 1.90^{c,d}$	9.80 ± 1.78^{e}	68.87 ± 1.88^{f}

Data are expressed as mean ± standard deviation (n = 3).
Different superscript letters in the same row indicate a significant difference ($p < 0.05$).
Concentrations represent final concentrations in the reaction medium.
DCM: Dichloromethane extract, EtOH: Ethanol extract, MeOH: Methanol extract.
* The standard in the table represents acarbose for α- amylase inhibitory activity and galatamine for AChE/BChE inhibitory activity. Inhibitory activity (%) of acarbose at 0.04 mg/mL is presented in the Table. The table presents the inhibitory activities (%) of galantamine at concentrations of 0.002 mg/mL for AChE and 0.02 mg/mL for BChE.

with dichloromethane, which less polar than methanol and ethanol, is more suitable for α-glucosidase inhibition.

When the relationship between the phytochemical content of the *A. lazikkiyense* extracts and IC_{50} values of α-glucosidase inhibitory activity was evaluated high correlation was observed for ethanol and methanol extracts. However, dichloromethane extracts from both aerial parts and onion peels showed the highest inhibition on α-glucosidase despite containing extremely low levels of phytochemicals.

3.5. AChE and BChE inhibitory activity

Alzheimer's disease, one of the most common neurodegenerative diseases has mainly been associated with memory loss in old age. Cholinesterase inhibitors are used in the treatment of the early and middle stages of the disease (Vafadarnejad et al., 2018). The *in vitro* AChE and BChE inhibitory activities of *A. lazikkiyense* obtained in the present study are shown in Table 5. IC_{50} values could not be calculated since all extracts showed less than 50 % of inhibition and the results were expressed as percent inhibition at 0.04 mg/mL extract concentration for each enzyme. In the present study, ethanol extracts from onion peels exhibited higher inhibition on AChE while dichloromethane extracts shows lowest inhibitory effect. As for the BChE inhibitory activities of the extracts, it was found that all extracts showed low inhibition on BChE (9.80 %–29.57 %). In the present study, the obtained values of galantamine, that was used as a positive control, was found to have an $IC_{50} = 0.96 \pm 0.04$ μg/mL for AChE and $IC_{50} = 8.57 \pm 0.23$ μg/mL for BChE, respectively. In a study conducted by Izol et al. (2021) on ethanol extracts of the aerial parts and roots of 12 different *Allium* species grown in Türkiye (*A. subakaka, A. atroviolaceum, A. chrysantherum, A. kharputense, A. rhetoreanum, A. scabriscapum, A. schoenoprasum, A. scorodoprasum* subsp. *rotundum, A. shatakiense, A. shirnakiense, A. tripedale,* and *A. vineale*), in consistent with the present study's results, it was reported that the extracts have low (5 %–20 %) AChE and BChE inhibitory activities. In another study conducted with extracts from bulbs of *A. tuncealinum*, it was reported that methanol, hexane, chloroform, butanol, and water extracts showed low inhibitory (2.56 %–23.56 %) activities on AChE and BChE, whereas the ethyl acetate extract showed an average inhibition (47.33 %) on BChE (Karakaya et al., 2019). In the present study, both aerial parts and onion peels extracts exhibited similarly low cholinesterase inhibitory activities. On the other hand, Emir et al. (2020) showed that the aerial parts of *A. nigrum* extracts for both AChE ($IC_{50} = 6.1$ μg/mL) and BChE ($IC_{50} = 3.27$ μg/mL) had more effective inhibition when compared to our aerial parts values at 0.4 mg/mL concentration. In addition, Rocchetti et al. (2022) stated that terpenoids and anthocyanins contained in *Allium* species are the most effective compounds in the inhibition of AChE and BChE. *A. lazikkiyense* may not have sufficient amounts of these compounds, hence, this could be the reason behind low inhibition of AChE and BChE in the present study.

3.6. Anticancer activity

Breast cancer is the second most common type of cancer after lung cancer and has been the leading cause of death among women (Ahmad, 2019). Both *in vivo* and *in vitro* research show that consumption of *Allium* species reduces the risk of breast cancer (Miraghajani et al., 2018). The presence of phenolics, flavonoids, and saponins in *Allium* species may be beneficial in preventing breast cancer. Quite in many studies, a lot of *Allium* species have widely been investigated for their anticancer potentials (Asemani et al., 2019). The present study reported anticancer potentials of *A. lazikkiyense* on MCF7 human breast cancer cell line and obtained IC_{50} values were shown in Table 3. The results showed that the extracts from onion peels especially the ethanol extract exhibited higher cytotoxic effect on MCF7 cells compared to the aerial parts. Similarly, in a study by Isbilen et al. (2018) on *A. autumnale* and another study on *A. willeanum* (Isbilen & Volkan, 2021) both reported that ethanol extracts from bulbs have strong antipoliferative and cytotoxic effect on breats cancer cell lines (MCF7 and MDA-MB-231). In another study on ethanol extract from leaf of *A. paradoxum* (Gholipour et al., 2018) and on methanol extract from flowers of *A. atroviolaceum* (Khazaei et al., 2017) have also been reported to have effective cytotoxic effect on MCF7 cells. In another study conducted by Mskhiladze et al. (2015) on *A. gramineum* reported that the flower extract showed strong cytotoxic effet with an $IC_{50} = 4.8$ μg/mL. It was stated that the main contributions to highest cytotoxic effect could be due to the presence of high amount of the saponins. As for results of the present study, it was observed by LC-MS/MS analysis that extracts from onion peels of *A. lazikkiyense* are rich in sarsasapogenin (a steroidal saponin) and this could be the source result to high cytotoxic activity in onion peels of the plant.

In addition, the present study confirmed the strong negative correlation among the IC_{50} values of cytotoxic activity and major compounds found in the extract from aerial parts fumaric acid ($r = -0.867$), p-coumaric acid ($r = -0.951$), ascorbic acid ($r = -0.897$), and hyperoside ($r = -0.932$) as well as major compounds found in the extract from onion peels fumaric acid ($r = -0.958$) and caffeic acid ($r = -0.973$). However, the IC_{50} values were moderately correlated with sarsasapogenin ($r = -0.548$).

4. Conclusion

In the present study, phytochemical contents, antioxidant and anticancer activities, inhibitory effects against some therapeutic enzymes (α-amylase, α-glucosidase, AChE, BChE) of aerial parts and onion peels from endemic *A. lazikkiyense* were revealed for the first time. Our results demonstrated that aerial parts of *A. lazikkiyense* are richer in phytochemical compounds apart from sarsasapogenin that showed highest in onion peels. Ethanol and methanol extracts of both aerial parts and onion peels showed strong antioxidant activities while dichloromethane extracts showed high α-glucosidase inhibitory effect. In particular, the extracts from onion peels exhibited potent cytotoxic effect against MCF7

human breast cancer cells. All extracts showed low cholinesterase and α-amylase inhibitory activity.

Overall, the results suggest that *A. lazikkiyense* extracts possess various *in vitro* bioactivities such as antioxidant, α-glucosidase inhibitory, and anticancer with the type of solvent used for extraction significantly influencing the content of bioactive compounds and their associated activities. However, some limitations need to be addressed in future studies. Firstly, further studies are required to identify and quantify the major bioactive compounds responsible for the observed activities. Secondly, *in vivo* models are necessary to confirm the efficacy of the extracts and elucidate their mechanisms of action. In conclusion, this study represents the first comprehensive investigation into the potential health benefits of *A. lazikkiyense* extracts, providing novel insights into its phytochemical profile and biological activities.

CRediT authorship contribution statement

Gozde Hasbal-Celikok: Methodology, Investigation, Formal analysis, Data curation, Writing – review & editing. **Faiza Tabassum Azami:** Investigation, Formal analysis, Writing – original draft. **Yasin Celikok:** Formal analysis. **Servet Duranay:** Formal analysis. **Mine Kocyigit:** Project administration, Writing – original draft. **Tugba Yilmaz-Ozden:** Conceptualization, Project administration, Methodology, Supervision, Investigation, Formal analysis, Data curation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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