Indian Journal of Traditional Knowledge
Vol 21(2), April 2022, pp 332-340

Chemical composition and inhibitory potentials of key-enzymes linked to neurodegenerative diseases of wild garlic: *Allium atrovioleceum* Boiss.

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Received 09 January 2020; revised 07 March 2022

Allium atroviolaceum is an edible plant and also known as wild garlic in Turkey, was collected from two different localities namely Kemalpaşa (AA-1) and Tire (AA-2). Phenolic constituents of the different parts of species were determined using LC-ESI-MS/MS, total phenolic (TPC), total flavonoid contents (TFC) and antioxidant activity of samples were performed by spectrophotometrically. Inhibitory potentials of samples against acetylcholinesterase, butyrylcholinesterase and tyrosinase were determined by a 96-well microplate reader. 28 compounds were quantified and naringenin (948.7 µg/g extract) as a flavonoid in bulb extracts of AA-2 and gallic acid (835.2 µg/g extract) as a phenolic acid in bulb extracts of AA-1 had the highest values. The flowers of AA-1 (23.72 mg GAE/g extract) had higher levels of TPC and was the most active sample for antioxidant assays. Cholinesterases and tyrosinase inhibitory activities activities were observed in all samples and the most potent was the bulbs (IC50 2.14, 1.98 µg/mL,) of AA-2 and flowers of AA-1 (62.53 µg/mL), respectively. Lastly, the data were investigated by principal component analysis for statistical analysis. Conclusively, this study determines the correlation between phenolic compounds, antioxidant and enzyme inhibitory activities of *A. atroviolaceum* as a functional food and contributes to detailed research of species belonging to the genus of *Allium*.

Keywords: *Allium*, Antioxidant, Enzyme inhibitory, Phenolics

IPC Code: Int Cl[22]: A61K 36/00, A61K 36/8962, A01N 65/42

Alzheimer's (AD) and Parkinson's (PD) diseases are the two most prevalent neurodegenerative diseases around the world and there were 46.8 million with AD and 6.1 million with PD patients in 2016[1]. Cholinesterase inhibitors, which enhance the level of acetylcholine (ACh) in the central nervous system are the major drugs used for symptomatic treatment of AD. However, these drugs have multifarious side effects, including nausea, vomiting, diarrhea, dizziness, abdominal pain and headache[2]. Tyrosinase is responsible for melanin biosynthesis in mammals so that it partakes skin pigmentation and other abnormalities and also creates the enzymatic browning of fruits and vegetables[3,4]. Moreover, the tyrosinase enzyme is associated with Parkinson's disease (PD) by oxidizing dopamine and levodopa to dopaquinone which causes more sensitive neurons towards cell damage due to their reactive properties. Neuromelanin interacts with α-synuclein protein which is considered to be managing for familial PD and also, making neurons in the substantia nigra pars compact more susceptible to toxic effects[5-7]. Thus, tyrosinase inhibition activity studies on natural products have become important in relation to PD. Phytoinhibitors are expectant agents for many diseases like AD and PD, thanks to their broad range of pharmacological activities and the minimal adverse impacts. For this reason, numerous types of investigation are increasing in recent years on the anticholinesterase and antityrosinase activities of the plants[8]. Furthermore, neurons are more susceptible to free radical damage and oxidative stress plays an important role in the occurrence of AD and PD as in many chronic diseases, so antioxidant agents are important to prevent the neurodegenerative effect of such oxidative stress in AD and PD[9].

The genus *Allium* L. (Amaryllidaceae) is represented by about 900 species, distributed across the northern hemisphere[10,11]. Many *Allium* species, particularly cultivated forms (*A. sativum* L. and *A. cepa* L., *A. porrum* L.) are widely used as vegetables, spices and folk remedies owing to their health beneficial properties[12,13]. Especially, these species have important effects on the cardiovascular system thanks to their hypotensive, hypolipidemic, platelet preventing and hypocholesterolemic effects[13]. *A. atroviolaceum*

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Boiss., known by the common name "wild garlic" is an ingredient of a traditional cheese of Turkey and the plant's onion and leaves are consumed raw or cooked in the flowering period and also, used instead of garlic in the eastern part of Turkey[14,15]. Moreover, this species had some biological activities such as cytotoxic, antiproliferative and apoptosis-inducing activities on breast, cervical and liver cell lines and antiplatelet activity similar to the garlic and onion[16,17].

Plant phenolics are one of the most important groups of natural products and most investigated secondary metabolite groups owing to their wide range of pharmacological and biological actions. Many kinds of research on neurodegenerative diseases have shown that phenolic molecules cross the blood-brain barrier and create protective effects on neuron cells by different mechanisms[18-20]. In this context, *Allium* species are known to be rich in phenolic constituents together with organosulfur compounds which can attenuate neuroinflammatory responses in microglial cells through modulation of Nrf2-mediated signaling as well as other oxidative stress-related pathways. So, they possess neuroprotective efficacy by modulating cellular redox homeostasis and benefit brain health[21,22]. Also, neuroprotective effects of many *Allium* species were reported in the literature some of these have been used or suggested purpose of against neurodegenerative diseases in different regions[23-27]. In addition, several studies have been reported that *Allium* species had potent anticholinesterase, antityrosinase and antioxidant activities[28-30]. From this perspective, the phenolic profile screening with in vitro biological activities including acetylcholinesterase, butyrylcholinesterase, tyrosinase enzyme inhibitory, and antioxidant activity studies of *A. atrovioleceum*, were investigated for the determination potential of different parts against AD and PD.

Material and Methods

Chemicals

Reference standards used in the LC-MS/MS analysis and AChE (from *Electrophorus electricus*), BChE (from equine serum), Acetylthiocholine/ Butyrylthiocholine iodide, DTNB (Ellman's reagent) [5,5′-dithio-bis-(2-nitrobenzoic acid)], tyrosinase (from mushroom), galanthamine, L-Dopa and kojic acid were purchased from Sigma Aldrich.

Plant material

Allium atroviolaceum Boiss. was collected in 2018 from Kemalpaşa and Tire (İzmir/Turkey) in the flowering period. The plant was authenticated by Hasan Yıldırım. Voucher specimens (No's:1606 and 1611) have been kept in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

Sample preparation

The samples were prepared by the extraction of bulb, stem and flower parts of *A. atroviolaceum*. The extraction method was developed by our research group depends on shaking plant material with methanol[30]. Samples were preserved at 4°C and for LC-MS/MS analysis, they were filtered through a 0.22 μm PTFE syringe filter into a vial.

Total phenolic content (TPC)

A modified method was applied for the determination of the total phenolic content of extracts[31]. 1 mL of extracts (1 mg/mL), Folin–Ciocalteu reagent (diluted ten-fold) and 4 mL of Na_2CO_3 solution (7.5%) were mixed in a vial. The mixture was incubated at 25°C for 20 min and the absorbance was detected at 765 nm (Thermo Scientific-Evolution Array). Also, the calibration curve was defined with a similar procedure. Gallic acid was used as a positive control and outcomes of the assay were given as gallic acid equivalents. The analysis was performed in triplicate.

Total flavonoid content (TFC)

Total flavonoid content of extracts was evaluated by $AlCl_3$ method with slight modifications[32]. 2 mL of methanol extract, 0.1 mL of a 10% aluminum chloride solution, 0.1 mL of CH_3CO_2K (1 M) and 2.8 mL of distilled water were mixed in a vial. The mixture was incubated 30 min at room temperature and the absorbance was evaluated at 415 nm (Thermo Scientific-Evolution Array). Quercetin was used as a positive standard, and outcomes of the assay were given as quercetin equivalents. The analysis was performed in triplicate.

Identification of phenolic compounds

Phenolic compounds were quantified by liquid chromatography-mass spectrometry (TSQ Quantum™ Access MAX Triple Quadrupole Mass Spectrometer Thermo Scientific™). Chromatographic conditions and mass parameters were detailed in our previous study[30]. Quantification of molecules was achieved using an external standard method, the identification of the compounds in the samples was established by matching the retention times and MS/MS fragments with reference molecules. Mass parameters were given in Table 1. Outcomes of LC-MS/MS analysis were given as μg per gram of methanol extracts. The analysis was performed in triplicate.

Table 1 — Molecular formula, polarity, the m/z values of their precursor and product ions and collision energies used for fragmentation

Compound	Molecular Formula	Collision energy (V)	Ion. mode	Precursor ion [m/z]	Product ion [m/z]
Gallic acid	$C_7H_6O_5$	15	Neg.	169.07	125.02
p-Coumaric acid	$C_9H_8O_3$	13	Neg.	163.01	119.08
3-Hydroxybenzoic acid	$C_7H_6O_3$	15	Neg.	137.18	93.18
4-Hydroxybenzoic acid	$C_7H_6O_3$	15	Neg.	137.15	93.15
Vanillic acid	$C_8H_8O_4$	7	Neg.	167.10	152.60
Ferulic acid	$C_{10}H_{10}O_4$	15	Neg.	193.04	134.10
Syringic acid	$C_9H_{10}O_5$	15	Neg.	19.11	182.18
Daidzein	$C_{15}H_{10}O_4$	34	Neg.	252.84	223.10
Chrysin	$C_{15}H_{10}O_4$	35	Neg.	253.18	143.06
Kaempferol	$C_{15}H_{10}O_6$	35	Neg.	285.10	227.01
Luteolin	$C_{15}H_{10}O_6$	30	Neg.	284.96	238.95
Fisetin	$C_{15}H_{10}O_6$	30	Neg.	285.10	135.01
Morin	$C_{15}H_{10}O_7$	23	Neg.	301.22	150.94
Quercetin	$C_{15}H_{10}O_7$	26	Neg.	301.20	150.94
3-*O*-methylquercetin	$C_{16}H_{12}O_7$	20	Neg.	315.10	290.99
Isorhamnetin	$C_{16}H_{12}O_7$	23	Neg.	315.09	300.02
Galangin	$C_{15}H_{10}O_5$	35	Pos.	271.20	153.11
Myricetin	$C_{15}H_{10}O_8$	25	Neg.	317.05	191.00
Vitexin	$C_{21}H_{20}O_{10}$	20	Neg.	431.29	310.96
Hesperidin	$C_{28}H_{34}O_{15}$	20	Neg.	609.40	300.88
3-Hydroxyflavone	$C_{15}H_{10}O_3$	34	Pos.	239.10	165.08
Naringenin	$C_{15}H_{12}O_5$	22	Neg.	271.05	151.01
Genistein	$C_{15}H_{10}O_5$	30	Pos.	271.09	153.11
Rutin	$C_{27}H_{30}O_{16}$	35	Neg.	609.24	299.91
Catechol	$C_6H_6O_2$	27	Neg.	109.22	108.10
(+)-Catechin	$C_{15}H_{14}O_6$	22	Neg.	289.10	203.07
(-)-Epicatechin	$C_{15}H_{14}O_6$	15	Pos.	291.22	139.10
(-)-Epigallocatechin gallate	$C_{22}H_{18}O_{11}$	15	Pos.	459.18	139.10

Limits of detection (LOD) and quantification (LOQ) were established at a signal-to-noise ratio (S/N) of 3 and 10, respectively. Also, analyses were experimentally determined by 10 injections of each analyte (Table 2).

Anticholinesterase activity

Anti-cholinesterase activities of the methanol extracts were evaluated by the described method, using 96-well ELISA microplate reader, modified from Ellman's method[33,34]. Concentrations were between 0.001–1000 mg/mL and also, galanthamine was used as a positive standard. The IC_{50} values of methanol extracts were determined with three independent experiments using the software GraphPad Prism V5.0 (GraphPad Software, San Diego, CA, USA).

Tyrosinase inhibitory activity

Anti-tyrosinase activities of methanol extracts was established using the modified dopachrome method with kojic acid as a positive standard[35]. Briefly, 1/15 M potassium phosphate buffer (pH 6.8) including 5% DMSO was used to prepare 7 different concentrations of methanol extracts (1000-1 µg/mL) and tyrosinase enzyme solution in the same phosphate buffer (46 U/mL) were mixed in a microtiter plate. After incubation at 25°C for 10 min, 2.5 mM L-Dopa substrate solution was added. And then, each well mixed and incubated at 25°C for 20 min. Subsequently, the absorbance was monitored at 475 nm. The percentage tyrosinase inhibition (I%) was calculated based on the following equation: (I%) = $\{(A_{control}-A_{sample}) / A_{control}\}$ x100, where $A_{control}$ clarifies the absorbance of the control in the presence of enzyme and A_{sample} is the absorbance of the sample. The assay was carried out by 96-well microplate using ELISA microplate reader and IC_{50} values of samples were determined using the software GraphPad Prism V5.0. The analysis was performed in triplicate.

Antioxidant activity

The antioxidant activities of samples were detected by DPPH and CUPRAC assays, according to Blois[36] and Apak *et al*[37] with slight modifications, respectively. 0.1 mM DPPH and the sample solution was mixed and the mixture was incubated for 30 min at room temperature. The absorbance was determined at

Table 2 — Analytical parameters of LC-MS/MS analysis.

Compound	Ranges (µg/mL)	Linear equation	R^2	LOD (µg/mL)	LOQ (µg/mL)
Gallic acid	1-2000	y = 1.9844x - 16,915	0.9989	0.012	0.039
p-Coumaric acid	100-1000	y = 33.987x + 70,123	0.9983	0.023	0.061
Vanillic acid	1-500	y = 3.4917x - 0,0833	0.9995	0.019	0.055
Ferulic acid	1-2000	y = 7.037x - 7,5479	0.9988	0.031	0.096
Syringic acid	1-500	y = 2.0075x + 0,6098	0.9968	0.015	0.048
Daidzein	0.5-50	y = 200.61x - 44,783	0.9951	0.022	0.068
Chrysin	0.5-50	y = 144.38x + 7,0548	0.9991	0.018	0.055
Kaempferol	1-500	y = 1.0497x + 1,9539	0.9986	0.011	0.037
Luteolin	1-500	y = 407.05x - 224,2	0.9986	0.023	0.068
Fisetin	0.5-50	y = 39.924x - 1,6077	0.9977	0.030	0.096
Morin	0.5-250	y = 40.163x + 32,379	0.9979	0.017	0.054
Quercetin	0.5-250	y = 40.842x + 7,0164	0.9993	0.028	0.090
3-*O*-methylquercetin	0.5-250	y = 15.096x + 8,0153	0.9989	0.008	0.028
Isorhamnetin	1-2000	y = 215.42x - 567,56	0.9998	0.016	0.053
Galangin	10-500	y = 171.28x + 12,5	0.9976	0.025	0.081
Myricetin	0.5-50	y = 84.192x - 3,5256	0.9985	0.018	0.054
Vitexin	0.5-50	y = 279.22x + 2,1661	0.9976	0.009	0.031
Hesperidin	0.5-50	y = 127.82x + 20,836	0.9989	0.028	0.059
3-Hydroxyflavone	0.5-10	y = 6333.3x - 816,67	0.9965	0.019	0.061
Naringenin	1-2000	y = 752.9x - 20293	0.9985	0.027	0.088
Genistein	1-500	y = 183.64x + 505,51	0.9990	0.005	0.016
Rutin	0.5-100	y = 1985.8x + 1793,5	0.9992	0.015	0.049
Catechol	0.5-100	y = 3.1296x + 2,8607	0.9996	0.023	0.071
(+)-Catechin	0.5-100	y = 74.509x - 38,691	0.9988	0.006	0.022
(-)-Epicatechin	0.5-50	y = 95x – 26.15	0.9950	0.019	0.066
(-)-Epigallocatechin gallate	0.5-100	y = 4.0622x – 0.4726	0.9947	0.031	0.098

Table 3 — Sample ID, extraction yield, total phenolic and total flavonoid contents of extracts.

Collection site	Part used	Sample ID	Yield (%)	TPC (mg GAE[a]/g extract)	TFC (mg QE[b]/g extract)
Kemalpaşa/İzmir	Bulb	AA-1B	8.30	27.95 ± 0.9	6.21 ± 0.9
	Stem	AA-1H	10.99	21.22 ± 0.4	5.68 ± 0.6
	Flower	AA-1F	19.44	33.72 ± 1.4	8.63 ± 1.1
Tire/İzmir	Bulb	AA-2B	12.71	24.71 ± 0.5	9.17 ± 0.8
	Stem	AA-2H	11.55	20.66 ± 0.7	7.51 ± 0.8
	Flower	AA-2F	24.87	25.81 ± 1.8	6.93 ± 0.5

Values expressed are means ± S.D. of three parallel measurements. [a]Gallic acid equivalent; [b]Quercetin equivalent

517 nm (Thermo Scientific-Evolution Array) and α-tocopherol was used as a positive control.

For the CUPRAC assay, 10 mM $CuCl_2$, 7.5 mM neocuproine and solution of methanol extract were mixed in neutral pH (7). The mixture was incubated in the darkness for 30 min, the absorbance were determined at 450 nm and outcomes of assay were given Trolox equivalents/g of extract (mg TE/g extract). The analysis was performed in triplicate.

Statistical analysis

Principal component analysis (PCA) was carried out for decreasing the number of variables in the data matrix aiming to select the most selective parameters by SPSS 25 software. In this regard, the PCA visualized similarities and distinctions of samples. So, the most discriminating phenolics and biological activities and also the relationship between them in different parts of plants were emphasized.

Results and Discussion

An extraction method that is efficient, quick and with no changes in the structure of extracted compounds was applied to bulb, stem and flower parts of plants. Collection site, sample ID, extraction yield, the total phenolic and total flavonoid contents of extracts were shown in Table 3. All samples were found rich in phenolic composition and the highest

Table 4 — Concentrations of phenolic compounds (μg g^{-1} of extract) of samples.

Compound	AA-1B (μg/mL)	AA-1H (μg/mL)	AA-1F (μg/mL)	AA-2B (μg/mL)	AA-2H (μg/mL)	AA-2F (μg/mL)
Gallic acid	835.2 ± 3.14	415.2 ± 2.15	227.2 ± 1.69	99.2 ± 1.28	119.6 ± 1.77	127.2 ± 2.08
p-Coumaric acid	564.5 ± 2.07	674.2 ± 1.82	639.3 ± 0.18	273.4 ± 2.45	182.3 ± 1.42	173.1 ± 1.49
3-Hydroxybenzoic acid	323.3 ± 0.95	367.5 ± 3.69	759.2 ± 4.31	530.8 ± 1.07	299.0 ± 3.09	186.0 ± 3.56
4-Hydroxybenzoic acid	476.6 ± 1.52	388.2 ± 2.45	351.4 ± 2.46	379.0 ± 2.31	301.5 ± 2.55	280.6 ± 2.74
Vanillic acid	21.1 ± 0.43	29.1 ± 1.75	40.0 ± 3.15	82.3 ± 1.99	200.4 ± 3.89	194.6 ± 4.09
Ferulic acid	22.9 ± 2.76	56.6 ± 0.66	34.4 ± 0.77	508.7 ± 3.16	752.2 ± 4.16	469.2 ± 1.55
Syringic acid	47.5 ± 2.64	39.5 ± 0.79	68.0 ± 1.22	19.5 ± 1.54	30.5 ± 1.33	51.1 ± 1.79
Daidzein	3.1 ± 0.72	T	T	T	1.4 ± 0.22	ND
Chrysin	T	T	T	ND	ND	ND
Kaempferol	174.4 ± 1.89	48.0 ± 3.59	24.0 ± 1.67	314.4 ± 3.42	3.0 ± 1.09	198.2 ± 1.43
Luteolin	T	T	T	59.2 ± 2.19	5.2 ± 1.28	218.1 ± 0.88
Fisetin	25.3 ± 1.04	3.4 ± 0.68	3.0 ± 1.25	T	2.0 ± 1.49	T
Morin	3.3 ± 2.51	2.5 ± 0.11	1.5 ± 0.61	137.2 ± 4.08	7.5 ± 0.69	16.8 ± 1.15
Quercetin	2.8 ± 0.39	4.6 ± 2.91	5.9 ± 0.32	98.2 ± 1.52	7.7 ± 1.52	13.1 ± 1.68
3-*O*-methylquercetin	78.1 ± 0.17	105.2 ± 3.62	72.0 ± 1.47	18.3 ± 2.66	5.0 ± 0.76	20.3 ± 3.49
Isorhamnetin	10.6 ± 1.63	7.0 ± 0.64	5.0 ± 1.92	256.9 ± 1.89	52.0 ± 2.33	457.1 ± 1.98
Galangin	58.7 ± 2.44	81.6 ± 0.72	68.4 ± 2.09	93.2 ± 2.44	38.0 ± 1.84	26.1 ± 1.59
Myricetin	T	T	T	3.7 ± 1.02	T	T
Vitexin	1.1 ± 0.32	T	T	4.3 ± 0.59	T	1.6 ± 0.73
Hesperidin	1.0 ± 0.54	1.4 ± 0.88	1.7 ± 0.85	32.6 ± 1.32	2.2 ± 1.05	2.8 ± 0.66
3-Hydroxyflavone	8.1 ± 0.79	4.8 ± 0.94	5.2 ± 0.67	1.4 ± 0.24	1.4 ± 0.72	1.5 ± 0.24
Naringenin	16.1 ± 1.94	14.0 ± 0.36	14.5 ± 1.53	948.7 ± 4.11	32.9 ± 0.99	221.3 ± 1.69
Genistein	34.7 ± 2.01	31.4 ± 0.55	38.4 ± 2.79	363.3 ± 2.78	27.3 ± 1.76	430.5 ± 3.45
Rutin	ND	ND	T	83.4 ±1.62	T	T
Catechol	93.3 ± 1.65	23.3 ± 1.49	66.7 ± 3.06	32.5 ± 1.46	33.3 ± 1.55	47.5 ± 0.61
(+)-Catechin	T	1.2 ± 0.80	1.3 ± 0.55	98.2 ± 2.09	7.7 ± 0.93	13.1 ± 0.85
(-)-Epicatechin	3.6 ± 1.22	6.3 ± 1.44	4.3 ± 0.89	T	1.7 ± 0.28	T
(-)-Epigallocatechin gallate	17.1 ± 0.83	13.0 ± 4.03	15.2 ± 0.73	2.1 ± 0.81	11.9 ± 0.74	T

Values expressed are means ± S.D. of three parallel measurements. ND: not detected, t: trace amounts.

values were detected in AA-1F (33.72 mg GAE/g extract) and AA-2B (9.17 mg QE/g extract) for TPC and TFC, respectively. This technique, due to its high selectivity and sensitivity, is considered more advantageous compared with other LC methods. The amounts of 28 compounds were determined in different parts of the plants and also Table 4 shows the content of the phenolic compounds in the samples. The LC-MS/MS data revealed that the phenolic contents of the samples are quite different from each other, although they are the same species collected from different localities. Generally, the amounts of the phenolic acids were found higher levels in AA-1 samples than AA-2, but this situation was opposite for flavonoids. 2 compounds in AA-1F and AA-2B, 3 compounds in AA-1H and AA-2F, 7 compounds in AA-1B and 10 compounds in AA-2B were the major phenolics of all extracts. Also, 3-hydroxybenzoic acid (530.8 μg/g extract) as a phenolic acid and naringenin (948.7 μg/g extract) as a flavonoid were the major constituents of AA-2B. TIC chromatograms and mass fragmentations of major compounds of AA-2B were shown in Figure 1. Further, chrysin had the lowest levels in all extracts which was not detected in AA-2 samples and was in trace amounts in AA-1 samples. A remarkable point is that rutin was found as 83.4 μg/g extract with a high concentration in AA-2B, while presented in trace amounts or not detected in other samples.

Methanol extracts from bulb, stem and flower parts of plants were tested for their AChE and BuChE inhibitory activities (Table 5) and galanthamine was used as a positive standard. IC_{50} values of galanthamine were calculated as 0.106 μg/mL and 1.04 μg/mL, respectively. Also, all samples showed AChE and BuChE inhibitory activities and the most potent sample was AA-2B (IC_{50} 2.14 and 1.98 μg/mL respectively). Phenolic compounds with higher concentrations may well contribute to the anticholinesterase inhibitory activity. Kaempferol, luteolin, quercetin, galangin, naringenin, genistein, morin and rutin were reported as potent anticholinesterase molecules[38-40].

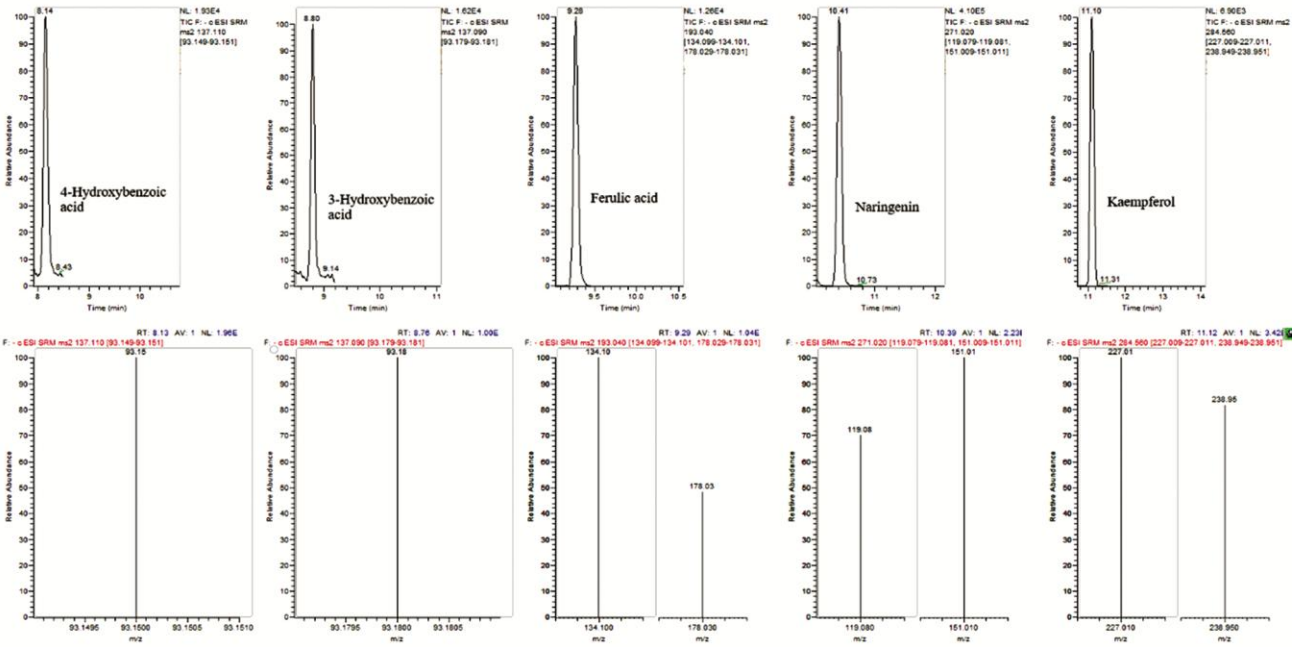

Fig. 1 — Chromatograms and ms fragmentations of major compounds of AA-2B.

Table 5 — Enzyme inhibitory and antioxidant properties of the samples.

Sample	AChE Inhibition (IC_{50} µg/mL)	BuChE Inhibition (IC_{50} µg/mL)	Tyrosinase Inhibition (IC_{50} µg/mL)	DPPH (IC_{50} µg/mL)	CUPRAC (mg TE/g extract)
AA-1B	51.33±0.11	40.51±0.09	67.40±0.05	51.93±2.54	124.81±0.95
AA-1H	119.45±0.09	4.90±0.01	78.83±0.16	85.31±2.03	71.52±1.16
AA-1F	65.87±0.03	34.48±0.12	62.53±0.08	42.66±1.38	182.92±2.43
AA-2B	2.14±0.02	1.98±0.04	120.66±0.24	58.27±0.72	131.77±0.83
AA-2H	96.25±0.16	73.22±0.21	95.61±0.17	97.58±1.73	68.44±1.75
AA-2F	4.31±0.04	2.41±0.07	147.1±0.08	62.44±0.94	109.25±2.09
Galanthamine	0.106±0.01	1.04±0.01	-	-	-
Kojic acid	-	-	7.9±0.02	-	-
α-Tocopherol	-	-	-	9.33±0.47	-

Values expressed are means ± S.D. of three parallel measurements. std: Standard

The tyrosinase inhibitory potentials of the extracts (Table 5) were determined spectrophotometrically, using a microplate assay with a 96-well microplate reader and kojic acid was used as a positive control (IC_{50} 7.9 µg/mL). IC_{50} values of the samples were found to be high, compared to the cholinesterase inhibitory activity. The highest tyrosinase inhibitory activity was observed in AA-1F and the lowest was in AA-2F, interestingly. Phenolic compouds such as 4-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, (-)-epigallocatechin gallate, kaempferol, catechol and catechin may have contribute to the anti-tyrosinase activity of the extracts. Among these molecules, 4-hydroxybenzoic acid, vanillic acid, (-)-epigallocatechin gallate and kaempferol were found to be strong anti-tyrosinase compounds[41]. In addition, catechol and catechin with the dihydroxyphenol structure, and *p*-coumaric acid with the similar chemical structure of L-tyrosine, are the substrate molecules for tyrosinase enzyme[42,43].

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method was carried out for the antioxidant activity of extracts (Table 5). The highest activity was found in AA-1F (42.66 µg/mL) sample and the lowest was in AA-2H (97.58 µg/mL) and so that a prominent correlation was observed with TFC. For the reducing power capacity of samples, CUPRAC assay was utilized and AA-1F (182,92 mg TE/g extract) was the most potent sample, similarly DPPH assay.

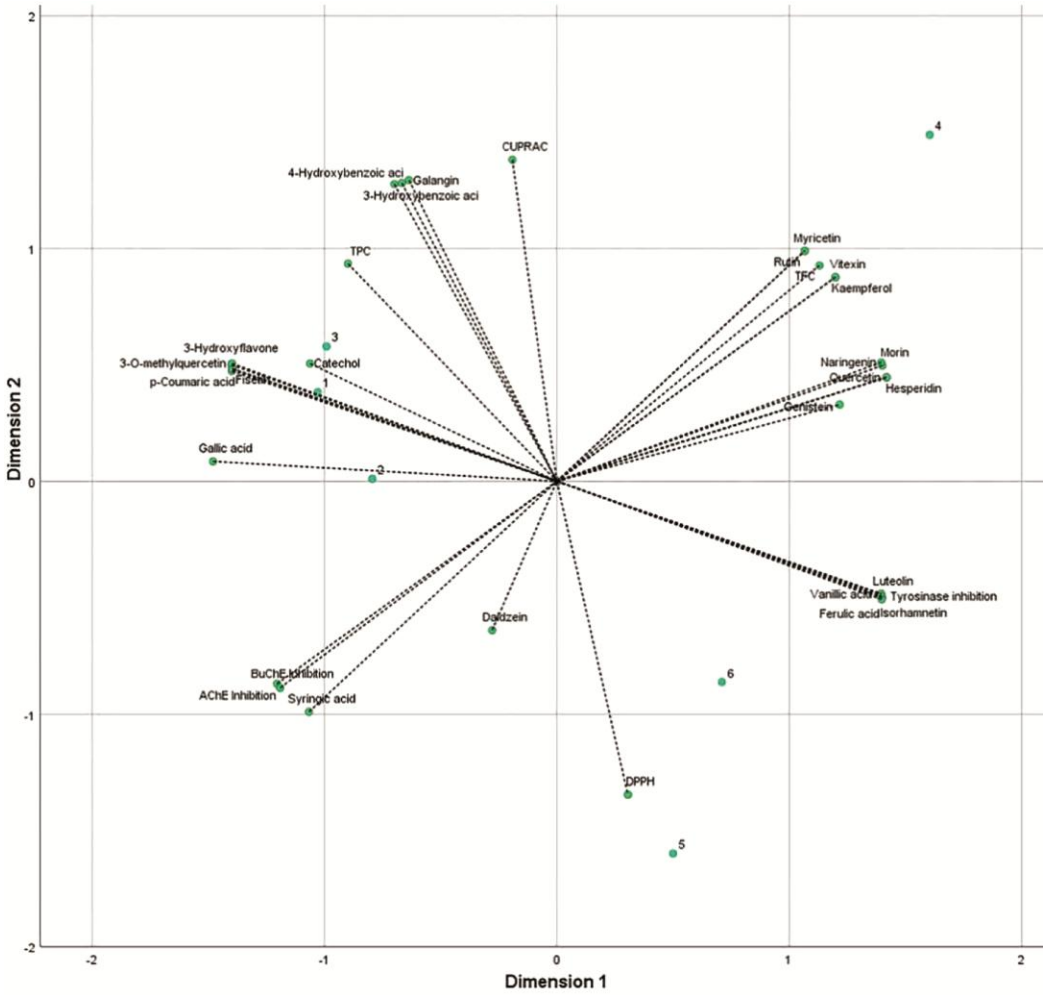

Fig. 2 — Principal component analysis of phenolic compounds, antioxidant activity and enzyme inhibitory potencies in samples. 1; AA-1B, 2; AA-1H, 3; AA-1F, 4; AA-2B, 5; AA-2H, 6; AA-2F.

To interrelate and to easily visualize the samples of the outcomes of this investigation, PCA was performed and a two-dimensional PCA scatter plot (based on two first PCs) was constructed (Fig. 2). First of all, it should be noted that the IC_{50} values and activity potency of samples are inversely proportional. Therefore, when these activities are evaluated statistically with samples or data of these samples, the negative correlation should be taken into consideration. The angle between the cholinesterase inhibitory activity and the AA-2B is the highest, thus the correlation is the greatest. Similar situation was detected for anti-tyrosinase activity with AA-1 samples. The total variance explained by the two principal components (PC1 and PC2) and data resulted in 93.05. The first (PC1) and second principal component (PC1) were explained as 65.17% and 27.88% of the variability, respectively. Loading plot of the variables demonstrated that, phenolic acids other than ferulic acid and vanillic acid were found to be discriminating for AA-1 samples and myricetin, vitexin, kaempferol and rutin were observed as the most distinguishing compounds for AA-2B.

Conclusion

Bulb, stem and flower extracts of *A. atroviolaceum*, collected from different localities, were investigated in terms of the phenolic composition, anti-cholinesterase, anti-tyrosinase and antioxidant activities. Our findings demonstrated that the concentrations and distributions of phenolic compounds of extracts are quite different from each other and phenolic acids are primary phenolics of the samples. All samples showed distinctive cholinesterase and tyrosinase inhibitory and antioxidant activities and a correlation between phenolic content and biological assays was detected. Owing to possessed

diverse bioactive compounds and so showed different biological activities, *A. atroviolaceum* may be a functional food and natural source for advanced *in vivo* research on Alzheimer's and Parkinson's diseases as an *Allium* species.

Acknowledgments

Authors acknowledge the financial support for this study by TUBITAK (217S341).

Conflict of Interest

The authors declared no conflict of interest in the manuscript.

Authors' Contributions

Conceptualization; AE, Formal analysis; AE, Funding acquisition; AE and CE, Resources; AE, Software; AE, Biological analyses; CE, Supervision; AE, Writing; AE.

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