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Studies on Anticholinesterase and Antioxidant Effects of Samples from *Iris* L. Genus of Turkish Origin

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Abstract: The genus *Iris* L. (Iridaceae) is a member of geophytes with attractive flowers. There are about 56 *Iris* taxa growing in Turkey, 24 of which are endemic. A survey of the literature indicates that the research carried out on *Iris* species are focused on the flavonoid and volatile compounds of the plant.
In present study, the dichloromethane and methanol extracts prepared from the rhizomes of 47 *Iris* taxa growing in Turkey were investigated for their *in vitro* cholinesterase inhibitory effects against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which the enzymes linked to Alzheimer's diseases and antioxidant capacities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test as well.
The *Iris* extracts studied have been found more active against BChE than AChE. compared with 100 µg/ml galanthamine (89.29 ± 0.96 %) as reference, *Iris kerneriana* (coded as Y122) and *Iris pseudacorus* (coded as Y131) methanol extracts had significant BChE inhibition effect (respectively, 80.22 ± 1.04 % and 53.06 ± 1.13 %) at concentration of 200 µg/ml. Among tested samples, methanol extracts of *I. kerneriana*, *I. lazica*, *I. pseudacorus* and *I. suaveolens* have shown remarkable antioxidant activity at concentration of 2 mg/ml for DPPH compared with gallic acid.
Keywords: *Iris*, Anticholinesterase, Antioxidant, Activity

1. Introduction

Turkey is an important gen centers for biodiversity and it is known that Turkey possesses approximately 1045 geophyte taxons are economically important such as *Colchicum*, *Fritillaria,Hyacinthus*, *Lilium*, *Nectaroscordum*, *Polygonatum*, *Tulipa* and *Iris* species (Kaya, 2014). Among them, genus *Iris* (family *Iridaceae*) is represented by 56 species in Turkey, of which 24 are endemic (Güner, 2012). *Iris* species have gained great popularity in the perfume and cosmetic industries due to their sweet fragrance alongwith their ornamental purposes (Orhan et al. 2002; Atta-ur-Rahman et al. 2004; Sevim, 2018). *Iris* species have been previously recognized as rich sources of secondary metabolites and used in the treatments of cancer, inflammation and bacterial and viral infections (Wang et al. 2010; Singab et al. 2016). Previous phytochemical investigations on the *Iris* species have resulted in the isolation of a variety of compounds including flavonoids, isoflavonoids, isoflavonoid glycosides, benzoquinones, triterpenoids and stilbene glycosides and essential oils (Orhan et al. 2002; 2003, Atta-ur-Rahman et al. 2002; 2003; 2004).

The aim of the present study was to investigate the antioxidant capacities and anticholinesterase activities of 47 *Iris L.* species growing in Turkey in order to evaluate their medicinal value and to point to an easily accessible source of natural antioxidants that could be used as a possible food supplement in addition to cosmetic, and perfume industries.

2. Materials and Method

2.1. Plant material

The rhizomes of *Iris* L. species were collected from different locations in Turkey given in Table 1. Their identification was confirmed by Prof. Dr. Neriman Ozhatay and Prof. Dr. Adil Güner and preserved as *ex-situ* at Atatürk Horticultural Central Research Institute, Department of Ornamental Plant Breeding and Agronomy in Yalova, Turkey.

Table 1. Population Number and Sample Codes of *Iris* Taxa

Sample Codes	Name of Taxa	Population Number
Y139	*Iris albicans* Lange	3505
Y103	*Iris aucheri* (Baker) Sealy	2105
Y111	*Iris bakeriana* Foster	4710
Y102	*Iris barnumiae* Foster & Baker	6507
Y112	*Iris caucasica* Hoffm. subsp. *caucasica*	2507
Y140	*Iris caucasica* Hoffm. subsp. *turcica* B. Mathew	2404
Y108	*Iris danfordiae* (Baker) Boiss. *	5104
Y119	*Iris elegantissima* Sosn.	3602
Y141	*Iris galatica* Siehe *	5201
Y113	*Iris gatesii* Foster	4702
Y115	*Iris germanica* L.	4802
Y114	*Iris germanica* L.	4605
Y142	*Iris histrio* Rchb. f.	2702
Y116	*Iris histrio* Rchb. f.	2704
Y117	*Iris histrioides* (G. F. Wilson) S. Arnott *	5304
Y120	*Iris junonia* Schott & Kotschy ex Schott *	0101
Y122	*Iris kerneriana* Ascherson & Sint. ex Baker *	3702
Y123	*Iris kirkwoodiae* Chaudhary	3106
Y124	*Iris lazica* Albov	5303
Y118	*Iris lycotis* Woron.	3001
Y126	*Iris masia* Dykes subsp. *masia*	6302
Y127	*Iris nectarifera* Güner var. *nectarifera* Güner *	4706
Y128	*Iris nezahatiae* Güner & H. Duman *	0802
Y129	*Iris orientalis* Miller	1001
Y130	*Iris pamphylica* Hedge *	0706
Y109	*Iris paradoxa* Steven f. *choschab*	6512
Y100	*Iris persica* L.	0201
Y131	*Iris pseudacorus* L.	3108
Y143	*Iris pseudacorus* L.	3405
Y101	*Iris pseudocaucasica* Grossh.	4406
Y110	*Iris pumila* L. subsp. *attica* (Boiss. & Heldr.)	1401
Y132	*Iris purpureobractea* B. Mathew & T. Baytop *	5401
Y104	*Iris reticulata* M. Bieb var. *reticulata*	2403
Y107	*Iris sari* Schott ex Baker *	1802
Y134	*Iris schachtii* Markgraf *	1804
Y144	*Iris sibirica* L.	7503
Y133	*Iris sintenisii* Janka subsp. *sintenisii*	3406
Y145	*Iris sprengeri* Siehe *	6805
Y135	*Iris spuria* L. subsp. *musulmanica* (Fomin) Takht.	2408
Y106	*Iris stenophylla* Hausskn. ex Baker subsp. *stenophylla* *	7003
Y105	*Iris stenophylla* Hausskn. ex Baker subsp. *stenophylla* *	0702
Y147	*Iris stenophylla* Hausskn. ex Baker subsp. *stenophylla* *	7005
Y137	*Iris suaveolens* Boiss. & Reut.	3401
Y146	*Iris taochia* Woronow ex Grossh. *	2505
Y136	*Iris unguicularis* Poir. subsp. *carica* (Wern. Schulze) var. *carica* *	0708
Y148	*Iris urminensis* Hoog	6505
Y138	*Iris xanthospuria* B. Mathew & T. Baytop *	4813

* Endemic taxa

2.2. Preparation of extracts

The washed with tap water, dried and powdered rhizomes (2 g) were extracted by maceration with dichloromethane at room temperature and concentrated under vacuum. Then residues were extracted by maceration with methanol and dried by rotary evaporator.

2.3. Cholinesterase inhibition assays

Extracts were investigated for their *in vitro* cholinesterase inhibitory activity at 200 μg/ml using ELISA microplate reader. AChE and BChE inhibitory activity was measured by slightly modified spectrophotometric method of Ellman et al. (Ellman et al. 1961). Electric eel AChE (Type-VI-S; EC 3.1.1.7, Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1.8, Sigma, St. Louis, MO, USA) were the enzyme sources used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as the substrates of the reaction. 5,5'-Dithio-bis(2-nitrobenzoic)acid (DTNB; Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All reagents and conditions were same as described in our previous publication (Sevim et al. 2013). Galanthamine (Sigma, St. Louis, MO, USA), the anticholinesterase alkaloid-type of drug obtained from the bulbs of Galanthus sp. was used as the reference. The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software (Sunnyvale, CA, USA). Experiments were run in triplicate and the results were expressed as average values with S.E.M.

2.4. Antioxidant capacity assay

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of the extracts was also tested at 2 mg/ml stock concentrations by ELISA microplate reader. It was measured by spectrophotometric method of Mardsen S. Blois which was modified by Hatano (Blois, 1958; Hatano, 1995). Gallic acid (Sigma, St. Louis, MO, USA) was employed as the reference. The measurements and calculations were evaluated by using Softmax PRO 4.3.2.LS software (Sunnyvale, CA, USA). Experiments were run in triplicate and the results were expressed as average values with S.E.M.

3. Results

The *in vitro* antioxidant and anticholinesterase activities of dichloromethane and methanol extracts prepared from the rhizomes of 47 *Iris* species collected from Turkey have reported for the first time in this study. Anticholinesterase activities and antioxidant capacities by using DPPH radical scavenging activity of dichloromethane and methanol extracts of *Iris* species were given in Table 2 and 3.

Table 2. AChE and BChE Inhibition (Inhibition % ± S.E.M.*) and DPPH Radical Scavenging Activity of (Scavenging activity % ± S.E.M.) of The Dichloromethane Extracts of *Iris* Taxa

Codes of Extracts	AChE Inhibition (% ± S.E.M.)	BChE Inhibition (% ± S.E.M.)	DPPH Radical Scavenging Activity (% ± S.E.M.)
	200 µg/ml [a]	200 µg/ml	2000 µg/ml [b]
Y100D	- **	10.46 ± 0.63	35.73 ± 1.88
Y101D	-	3.41 ± 1.05	29.35 ± 3.12
Y102D	-	-	24.98 ± 1.70
Y103D	-	-	30.72 ± 0.61
Y104D	-	11.42 ± 0.85	11.51 ± 2.37
Y105D	-	7.76 ± 0.16	27.79 ± 0.93
Y106D	-	-	31.51 ± 2.80
Y107D	-	11.65 ± 0.26	23.99 ± 0.66
Y108D	-	19.07 ± 3.20	8.21 ± 1.05
Y109D	-	15.05 ± 4.60	30.32 ± 1.88
Y110D	-	5.62 ± 0.32	9.10 ± 0.53
Y111D	7.81 ± 1.95	8.40 ± 0.19	19.56 ± 1.98
Y112D	-	9.60 ± 0.01	22.82 ± 1.47
Y113D	-	5.80 ± 0.74	15.05 ± 1.08
Y114D	-	-	30.30 ± 0.94
Y115D	-	-	22.38 ± 0.78
Y116D	-	22.92 ± 1.29	14.89 ± 1.57
Y117D	-	17.07 ± 5.43	8.92 ± 0.87
Y118D	5.14 ± 0.81	7.78 ± 0.68	10.04 ± 1.82
Y119D	11.74 ± 1.33	-	15.96 ± 2.33
Y120D	-	-	15.15 ± 2.43
Y122D	6.42 ± 1.89	-	25.52 ± 0.96
Y123D	-	1.76 ± 0.52	10.47 ± 2.68
Y124D	-	4.47 ± 0.73	52.09 ± 2.46
Y126D	6.99 ± 0.56	-	12.41 ± 1.23
Y127D	-	1.94 ± 0.73	29.28 ± 2.20
Y128D	-	-	7.49 ± 2.90
Y129D	-	-	10.94 ± 3.79
Y130D	-	9.72 ± 0.18	21.58 ± 0.82
Y131D	-	5.18 ± 0.18	30.36 ± 1.62
Y132D	-	-	57.91 ± 3.20
Y133D	-	10.34 ± 1.08	12.69 ± 1.23
Y134D	-	-	7.99 ± 1.88
Y135D	-	1.34 ± 0.55	11.41 ± 2.99
Y136D	-	10.58 ± 0.26	23.13 ± 1.02
Y137D	11.22 ± 0.99	6.18 ± 0.08	22.22 ± 0.63
Y138D	-	-	14.46 ± 1.26
Y139D	-	-	4.76 ± 1.10
Y140D	-	-	13.22 ± 1.37
Y141D	-	14.51 ± 1.74	24.15 ± 1.86
Y142D	13.49 ± 0.48	13.21 ± 0.87	9.89 ± 1.02
Y143D	8.41 ± 3.32	40.44 ± 0.12	63.46 ± 2.25
Y144D	-	6.39 ± 0.12	3.01 ± 2.20
Y145D	-	3.95 ± 2.16	11.71 ± 0.89
Y146D	-	4.45 ± 0.56	32.70 ± 0.34
Y147D	-	3.25 ± 2.26	7.87 ± 0.41
Y148D	-	-	21.19 ± 0.96
References			
G [1]	94.58 ± 0.82	89.29 ± 0.96	NT
GA [2]	NT ***	NT	91.56 ± 0.68

* Standard error mean (n=3), ** No activity, *** Not tested, a Final concentration, b Stock concentration, D: Dichloromethane, 1 Galanthamine (100 µg/ml), 2 Gallic acid (2000 µg/ml)

Table 3. AChE and BChE Inhibition (Inhibition % ± S.E.M.*) and DPPH Radical Scavenging Activity of (Scavenging activity % ± S.E.M.) of The Methanol Extracts of *Iris* Taxa

Codes of Extracts	AChE Inhibition (% ± S.E.M.)	BChE Inhibition (% ± S.E.M.)	DPPH Radical Scavenging Activity (% ± S.E.M.)
	200 µg/ml [a]	200 µg/ml	2000 µg/ml [b]
Y100M	- **	7.53 ± 1.45	4.79 ± 1.84
Y101M	-	10.84 ± 1.06	7.51 ± 0.96
Y102M	-	2.54 ± 0.84	65.28 ± 1.77
Y103M	-	14.07 ± 2.20	12.57 ± 0.68
Y104M	-	9.28 ± 1.17	4.84 ± 2.10
Y105M	-	15.96 ± 2.68	8.55 ± 2.37
Y106M	-	6.52 ± 1.48	5.04 ± 0.48
Y107M	-	15.36 ± 1.61	40.26 ± 0.70
Y108M	-	19.81 ± 0.84	3.55 ± 1.27
Y109M	-	-	39.50 ± 2.69
Y110M	-	23.23 ± 4.25	12.99 ± 2.52
Y111M	-	37.63 ± 0.02	9.26 ± 0.66
Y112M	-	4.06 ± 0.41	7.71 ± 0.73
Y113M	-	17.02 ± 2.82	55.49 ± 1.34
Y114M	-	-	15.58 ± 1.46
Y115M	-	16.64 ± 3.74	21.90 ± 1.44
Y116M	-	12.92 ± 1.47	5.15 ± 0.55
Y117M	-	4.19 ± 0.58	2.42 ± 0.66
Y118M	-	22.41 ± 1.39	37.70 ± 2.91
Y119M	-	12.22 ± 2.63	42.33 ± 2.89
Y120M	-	10.57 ± 2.51	24.26 ± 0.74
Y122M	**40.40 ± 3.30**	**80.22 ± 1.04**	**91.33 ± 0.05**
Y123M	-	10.73 ± 3.17	44.85 ± 2.42
Y124M	-	15.37 ± 4.09	**90.42 ± 0.40**
Y126M	-	28.40 ± 1.34	29.45 ± 1.46
Y127M	-	2.98 ± 1.49	44.68 ± 1.36
Y128M	-	3.26 ± 1.75	2.58 ± 0.66
Y129M	-	7.12 ± 1.62	7.25 ± 1.16
Y130M	-	13.03 ± 0.51	4.92 ± 0.21
Y131M	9.89 ± 0.52	**53.06 ± 1.13**	**91.61 ± 0.58**
Y132M	-	6.40 ± 3.38	9.54 ± 0.19
Y133M	-	22.00 ± 2.20	12.44 ± 1.27
Y134M	-	3.45 ± 1.91	41.35 ± 0.39
Y135M	-	-	8.20 ± 0.22
Y136M	-	-	55.72 ± 1.09
Y137M	-	-	**84.31 ± 0.63**
Y138M	-	6.33 ± 0.46	5.67 ± 1.55
Y139M	-	11.13 ± 1.47	12.28 ± 1.07
Y140M	-	4.28 ± 0.26	12.92 ± 0.28
Y141M	-	1.93 ± 0.10	11.98 ± 0.47
Y142M	1.43 ± 0.43	6.36 ± 0.98	5.68 ± 0.79
Y143M	22.56 ± 1.86	5.86 ± 2.71	64.02 ± 14.37
Y144M	-	16.92 ± 1.76	13.43 ± 3.33
Y145M	-	5.13 ± 0.49	16.69 ± 1.99
Y146M	-	2.34 ± 1.16	14.65 ± 1.73
Y147M	-	8.06 ± 0.93	9.90 ± 0.06
Y148M	-	4.72 ± 2.22	52.05 ± 1.90
References			
G [1]	94.58 ± 0.82	89.29 ± 0.96	NT
GA [2]	NT ***	NT	91.56 ± 0.68

* Standard error mean (n=3), ** No activity, *** Not tested, a Final concentration, b Stock concentration, M: Methanol, 1 Galanthamine (100 µg/ml), 2 Gallic acid (2000 µg/ml)

4. Discussion

Oxidative stress is known to play an important role in pathogenesis of several diseases such as diabetes mellitus and neurodegenerative disorders (Howes ve ark. 2003; Sevim, 2018). On the other hand, one of the hypothesis that has been proposed to restrain the cholinergic function is the inhibition of AChE and BChE for the elevation of acetylcholine level for treatment of AD. Depends on side effects of available drugs used for AD have resulted in continuing our researches to determine AChE inhibitors from geophytes.

During this extensive study, the extracts of 47 *Iris* taxa have been screened for their antioxidant and anticholinesterase effects due to their rich phenolic compounds. From these species, *Iris kerneriana* and *I. pseudacorus* have been found the highest BChE inhibitory effects. In the previous researches on the anticholinesterase activity of *I. suaveolens, I. albicans* and *I. schachtii* were also shown low activity against AChE and BChE (Hacıbekiroğlu ve Kolak, 2011; 2015; Mocan et al. 2018). In regarding radical scavenging effect of *Iris kerneriana, I. lazica, I. pseudacorus* and *I. suaveolens* have been determined above 90 % as similar standard compound used as gallic acid. These results indicated that the highest antioxidant activity was exhibited for methanolic extracts contained polar compounds.

5. Conclusions

Iris species are cultivated on a commercial scale as ornamental plants. In this study, the dichloromethane and methanol extracts prepared from the rhizomes of 47 *Iris* taxa growing in Turkey were investigated for their *in vitro* cholinesterase inhibitory effects against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) which the enzymes linked to Alzheimer's diseases and also antioxidant capacities using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test. The samples have been found more active against BChE than AChE. Compared with 100 µg/ml galanthamine (89.29 ± 0.96 %) as reference, *Iris kerneriana* (coded as Y122) and *Iris pseudacorus* (coded as Y131) methanol extracts had significant BChE inhibition effect (respectively, 80.22 ± 1.04 % and 53.06 ± 1.13 %) at concentration of 200 µg/ml (Table 3). In addition, methanol extracts of *I. kerneriana, I. lazica, I. pseudacorus* and *I. suaveolens* have shown remarkable antioxidant activity at concentration of 2 mg/ml for DPPH compared with gallic asid (Table 3.). Therefore, the aforementioned *Iris* species have been deserved further searches for theirs high BChE inhibition and antioxidant potential.

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