Studies on Anti cholinesterase 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 (Güler, 2012). *Iris* species have gained great popularity in the perfume and cosmetic industries due to their sweet fragrance along with their ornamental purposes (Orhan et al. 2002; Atta-ur-Rahman et al. 2002; 2003; 2004). Previous phytochemical investigations on the *Iris* species have resulted in the isolation of a variety of compounds including flavonoids, isoflavonoids, *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, iso flavonoid 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 barmuniae Foster & Baker                      | 6507              |
| Y112         | Iris caucasia Hoffm. subsp. caucasia               | 2507              |
| Y140         | Iris caucasia Hoffm. subsp. turcica B. Mathew     | 2404              |
| Y108         | Iris danfordiae (Baker) Boiss. *                   | 5104              |
| Y119         | Iris elegantissima Sosn.                          | 3602              |
| Y141         | Iris galatica Siehe *                              | 5201              |
| Y113         | Iris gatasi 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 Allov                                 | 5303              |
| Y118         | Iris lycots 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 pampylica 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 pseudocaucasia 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 *                          | 2004              |
| Y144         | Iris sibirica L.                                   | 7503              |
| Y133         | Iris sintenisii Janka subsp. sintenisii            | 3406              |
| Y145         | Iris sprengerieri Siehe *                          | 6805              |
| Y135         | Iris spuria L. subsp. musubanica (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 uinguicularis Poir. subsp. carica (Wern. Schulze) var. carica * | 0708              |
| Y148         | Iris arminensis 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′-Dithiobis(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 4.3.2.1 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 4.3.2.1 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.
| 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 b                 | 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                 | 27.90 ± 0.93                |                                              |
| Y106D -           | -                           | 31.51 ± 2.80                |                                              |
| Y107D -           | 11.65 ± 0.26                | 23.99 ± 0.66                |                                              |
| Y108D -           | 19.07 ± 3.20                | 8.21 ± 0.85                 |                                              |
| 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**

G¹ 94.58 ± 0.82 89.29 ± 0.96 NT
GA² 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)
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. schachttii were also shown low activity against AChE and BChE (Hacibekiroğlu 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-picylhydrazyl (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 acid (Table 3.). Therefore, the aforementioned Iris species have been deserved further searches for theirs high BChE inhibition and antioxidant potential.

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