Conference paper

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Discovery of bioactive drug candidates from some Turkish medicinal plants-neuroprotective potential of *Iris pseudacorus* L.

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Abstract: Medicinal plants have an enormous potential for producing bioactive compounds of great benefit to mankind. There is a great scope for new drug candidates based on traditional medicinal plants throughout the world. The number of drugs derived from medicinal plants that are recently introduced into clinical use is increasing. Besides, numerous of standardized herbal extracts were also approved as phytomedicines by the health authorities to be used in phytotherapy. The drug discovery program from nature in our laboratory involves several steps from plant collection, extraction, HTS of the extracts by using *in vitro* enzyme inhibitory tests, bioassay-guided fractionation through the isolation and structure elucidation of bioactive compounds. Continuing our researches in the field of anticholinesterase activity, neuroprotective potential of *Iris pseudacorus* L. have presented in this article.

Keywords: anticholinesterase; biological activity; Eurasia 2018; *Iris pseudacorus*; isoflavonoid.

Introduction

Natural products have been recognized as an important tool in the drug discovery process throughout this century. Presently, over 100 natural product-derived pharmaceuticals are being used in modern medicine. Plants have been used as medicine by mankind to treat health-threatening diseases and still popular to obtain new drug candidates as it is the oldest medical practice for humans. It is worth saying that the number of drugs derived from medicinal plants that are recently introduced into clinical use is increasing. Additionally, several standardized herbal extracts were approved by the authorities to be used in therapy. These traditional medicines can serve as the source of potential new drugs and initial research focuses on the isolation of bioactive lead compounds for their ability to provide health benefits. Turkey is one of the rich countries in terms of bioresources depends on different climates, geographical location, ecological factors and aquatic environments as well as the passageway between Europe, Asia and Africa. Therefore, the floristic diversity provides a wide choice of species represented 12 000 taxa of which 3700 is endemic [1].

Among them, 1045 taxons belong to the class of geophytes having fleshy underground organs such as bulbs, corms, tubers, tuberous stems, tuberous roots, rhizomes and pseudobulbs [2]. Most of them require a "warm-cold-warm" sequence to complete their annual cycle. On the other hand, most of the geophytes contain beautiful and attractive flowers which also make them desired ornamental plants [3]. It is worth to mention that a good number of the geophytes species such as *Galanthus, Colchicum, Fritillaraia, Iris* etc. are considered

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as medicinal plants. Therefore, geophytes are well-known to possess economic and medicinal prominence. For this purpose, we initiated a huge project from different scientific disciplines relevant to geophytes growing all over the country in order to search and collect all data relevant to their botany, cultivation as well as chemotaxonomy and some biological activities. In this regard, neuroproprotective properties indicative of their role in neurodegenerative diseases of these taxons were investigated [4]. The genus *Iris* L. (Iridaceae) comprises of over 300 species distributed mainly in temperate regions of the Northern Hemisphere. This genus is represented by 56 taxons, 24 of which are endemic in the Flora of Turkey [1]. Most of the *Iris* species are cultivated for ornamental purposes worldwide. Recently, *Iris* species have gained great attention from the cosmetic and perfume industries due to the their violet-like smell caused by irone-type compounds. Phytochemical investigations on various species of *Iris* have resulted in the isolation of a variety of bioactive secondary metabolites [5]. Over 250 compounds have been reported from the genus *Iris*, which include flavonoids, isoflavonoids and their glycosides, benzoquinones, benzene derivatives, triterpenoids, steroids and stilbene glycosides [6–9]. Some of *Iris* species were reported to have various biological activities including potent antiulcer, anticancer and piscicidal activities [10]. There have been no previous phytochemical and bioactivity studies on *Iris germanica* and I. *soforana* growing in Turkey have investigated for their isoflavonoid glycosides [11–15]. There have been no previous cholinesterase inhibitory studies on *Iris* species growing in Turkey. Acetylcholinesterase (AChE), while butyrylcholinesterase (BChE, syn. pseudocholinesterase), its sister enzyme, has been stated to be involved in AD pathology and these inhibitors have become the most popular strategy for increasing cholinergic activity in the brain and have shown the most encouraging results as palliative therapy for Alzheimer's disease (AD) as a progressive neurological disease has become one of the major causes of death in the world, affecting the industrialized countries in particular. *In vitro* neuroprotective effect of extracts prepared from the bulbs of *Iris* species was determined by the Ellman method [16] using enzyme-linked immunoabsorbent assay (ELISA) microplate reader in comparison with galanthamine as the standard drug against AChE and BChE, which are the key enzymes mainly in pathogenesis of Alzheimer's disease. In this article, one of the genus named as *Iris* L. taxons of Turkish origin were investigated for their anticholinesterase activity.

Materials and methods

Chemicals

All solvents: methanol, acetonitrile n-hexan used for this work were of analytical grade and purchased from Merck (Darmstad, Germany), Formic acid (FA), MS-grade was purchased from Sigma-Aldrich (St. Louis, USA). The chromatographic stationary phases were purchased Silica gel 60 (60–200 mesh) from Sigma-Aldrich, (Steinheim, Germany).

Plant material

The rhizmoes *Iris pseudacorus* L. were colleced from Hatay and the voucher specimen (3108) have been preserved as *ex situ* in the Ataturk Hoticultural Central Research Institute of the Botanical Garden-Yalova-Turkey. The rhizomes were washed under tap water to remove soil and cut into approximately 1 cm pieces and dried. Then, the dried material was ground in the herbal mill for extraction and isolation procedures.

Liquid chromatography-mass spectrometry and high-resolution mass spectrometry

The optimization of LC_MS conditions was performed by using positive ion mode in the ESI. LCMS analyses were performed using an Agilent 6550 iFunnel Q-TOF LC/MS. Chromatographic analyses were performed on Agilent Zorbax Bonus-RP C18 column, (2.1 mm×150 mm, 5 mm), flow rate was 0.6 mL/min, analysis time

was 55 min, the injection volüme was 2 mL, the solvent system consisted of 0.1 % formic acid and 100 % acetonitrile.

Collision energy was set 10, 20 and 40 eV depending on the m/z of fragmented ions. The processing spectra was performed by using software. All analyses were conducted on in triplicate.

Procedure of activity-guided isolation

Three hundred fifty gram of powdered rhizomes were extracted three times with 1.5 L methanol by maceration at room temperature for three times. Combined filtered extracts were concentrated under reduced pressure, dissolved in 80 % aq. MeOH solution (v/v) and defatted in the separating funnel with n-hexane. Subsequently the obtained extract was lyophilized to obtain 87.75 g (D18RM). This extract was extracted with dichloromethane, ethylacetate and n-butanol respectively. Afterwards, the most BChE active extract (D18RM-D) (3.85 g) was applied onto open column chromatography on Silicagel by using dichloromethane-methanol (80:20) and 6 fractions were obtained as (N1-6). The most BChE inhibitory active fraction (N5, 1.32 g) was determined. This active fraction was applied on Flash chromatography (Combi falsh EZ-prep) on Silica gold and eluted with methanol (acidified with 0.1 % formic acid) at a flow rate of 5 mL min^{-1}. As a result, 5 compounds (DS 1–5) were obtained. Among the compounds, the most BChE inhibitory activity (DS-5) was found. The active compound (DS-5) were analyzed by LC-ESI-Q-TOF-MS/MS.

Microtiter assays for AChE and BChE enzyme inhibition

AChE and BChE inhibitory activity of the extracts was determined by modified spectrophotometric method of Ellman *et al.* [16]. Electric eel acetylcholinesterase (Type-VI-S, EC 3.1.1.7, Sigma) and horse serum butyrylcholinesterase (EC 3.1.1.8, Sigma) were used as the enzyme sources, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5,5′-Dithio-bis(2-nitrobenzoic) acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the cholinesterase activity. All the other reagents and conditions were the same as described in our previous publication 10. In brief, 140 µL of 0.1 mM sodium phosphate buffer (pH 8.0), 20 µL of 0.2 M DTNB, 20 µL of sample solutions (in a dilution series of 1–100 µg/mL) and 20 µL of 0.2 M acetylcholinesterase/butyrylcholinesterase solution were added by multichannel automatic pipette (Gilson Pipetman, France) in a 96-well microplate and incubated for 15 min at 25 °C. The reaction was then initiated with the addition of 10 µL of 0.2 M acetylthiocholine iodide butyrylthiocholine chloride. The hydrolysis of acetylthiocholine iodide/butyrylthiocholine chloride was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at a wavelength of 412 nm utilizing a 96-well microplate reader (VersaMax, Molecular Devices, USA). Galanthamine was used as reference and purchased from Sigma (St. Louis, MO, USA).

Statistical analysis of data

Data obtained from *in vitro* enzyme inhibition experiments were expressed as means (±SD) from at least three independent experiments performed in triplicate. Statistical differences between the reference and the sample groups were evaluated by ANOVA (one way). Dunnett's multiple comparison tests was used as post hoc tests. $p < 0.05$ was considered to be significant.

Results and discussion

In this study, *Iris* L. species belonged to ornamental geophytes growing in Turkey were investigated for their *in vitro* cholinesterase inhibitory effects and antioxidant capacities. The dichloromethane and methanol

extracts prepared from the bulbs of 47 *Iris* species were screened by using modified Ellmann method. Among the tested extracts of *Iris* species growing in Turkey, the highest BChE inhibition was found to be caused with *Iris kerneriana* Asch. & Sint. ex Baker and *I. pseudacorus* L. (80.22 ± 1.04 % and 53.06 ± 1.13 %, respectively) at 200 μg/mL. (Reference: Galanthamine 89.29 ± 0.96 %). While *Iris kerneriana, an endemic species* showed the highest inhibitory activity, *I. pseudacorus* was selected for activity-guided fractionation and isolation studies. The powdered rhizomes of *I. pseudacorus* L. was extracted with *n*-hexane, dichloromethane, ethylacetate, *n*-butanol and water at room temperature.

The dichloromethane extract exhibited significant butyrylcholinesterase inhibitory activity (73.65 ± 2.06 %, ref. Galanthamine 80.02 ± 0.12 %). This extract was subjected to column chromatography and five subfractions were obtained. Among the subfractions coded as N5 was shown the significant butyrylcholinesterase inhibitory activity (93.78 ± 1.49 %, ref. Galanthamine 80.02 ± 0.12 %). This subfraction was separated using flash chromatography and the highest butyrylcholinesterase inhibitory activity of the separated compound 5 was determined as 94.00 ± 1.03 %, ref. Galanthamine 80.02 ± 0.12 %).

Compound DS-5 was analyzed by liquid chromatography-electron spray ionization-quadrupole/time-of-flight-mass spectrometry-mass spectrometry (LC-ESI-Q/TOF-MS-MS). In the MS/MS spectrum of the ion at m/z 477 (M + 1), the product ions at m/z 315 and 163 are formed and this fragmentation pathway of irisolidon-glucopyranoside (M^+ 476). The ion at m/z 315 (M + 1) and its product ions at m/z 300, 282 and 269. According these fragmentation pattern, this ion was characterized as irisolidon (M^+314) by the comparison with the mass spectra given in the literature [17–19].

Conclusions

Discoveries of lead compounds for the development of new drug candidates from bioresources can help to promote incentives for conservation by providing an economic return to innovative use of those sources. Screening of natural sources has had an impressive tool of determining active agents. The key to successfully discovering therapeutic agents from bioresources is based on bioassay-directed isolation techniques. HTS tests and mechanism-based screening protocols as well as information of folkloric utilization of plants have led to the discovery of lead compounds as drug candidates.

In this study, *Iris* L. species belonged to ornamental geophytes growing in Turkey were investigated for their *in vitro* cholinesterase inhibitory effects and antioxidant capacities. The dichloromethane and methanol extracts prepared from the bulbs of 47 *Iris* species were screened by using modified Ellmann method and the highest butyrylcholinesterase inhibitory effect was found in the dichloromethane extract of the bulbs of *I. pseudacorus* L. This active dichloromethane extract was subjected to fractionation on column and flash chromatographies and the activities of the fractions were tested. The determined active fraction was analyzed by using LC-ESI-Q/TOF-MS-MS technique. The responsible compound from the activity of this fraction was detected as irisolidonglucopyranoside as an isoflavonoid derivatives based on their mass data and by comparison with the references [17–19]. Depends on the less amount of the active compound, the synthesis of this compound is going on for *in vivo* studies.

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