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Original article

Evaluating the antioxidant and acetylcholinesterase inhibitory activities of some plants from Kohgiluyeh va Boyerahmad province, Iran

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Abstract

Background and objectives: Alzheimer's disease (AD) is a neurodegenerative disorder. Nowadays, many investigations are performed to find new drugs for AD and medicinal plants are considered as one of the most important sources for developing new drugs. According to the role of oxidant agents and acetylcholinesterase enzyme (AChE) in AD, plants with antioxidant and AChE inhibition properties could be good candidates for AD studies. In the present investigation, acetylcholinesterase inhibition (AChEI) and antioxidant effects of some plants from Kohgiluye va Boyerahmad province of Iran have been determined. **Methods:** The plants collected from Kohgiluyeh va Boyerahmad province (56 species) were extracted with methanol by using maceration method. AChEI activity of the extracts was determined using Ellman method in 96-well microplates. Antioxidant activity was determined using DPPH and FRAP methods. **Results:** The results showed that aerial parts of *Amygdalus scoparia* had the highest AChEI effect (50% inhibition in concentration of 300 µg/mL). The plant also demonstrated suitable antioxidant effects. *Epilobium minutiflorum* found to be the most potent species for DPPH inhibition and reduction of ferric-TPTZ complex (IC_{50} 3.6 µg/mL and FRAP value 335.0 mmol $FeSO_4.7H_2O$/100g Extract). **Conclusion:** Our results confirmed that almost all species with AChEI activity showed to be effective as potent antioxidant agents.

Keywords: acetylcholinesterase, antioxidant, DPPH, FRAP, Kohgiluyeh va Boyerahmad

Introduction

Alzheimer's disease (AD) is one of the most widespread degenerative illnesses of the human brain which involves multiple neuronal systems. In this case, a specific pattern of lesions evolves slowly over time and remains remarkably consistent across cases [1]. The pathogenesis of AD has not yet been elucidated. It is widely accepted that a combination of genetic susceptibility factors and environmental triggers are responsible for AD. It is proposed that beta amyloid protein, abnormal tau protein, oxidative damages, cholinergic deficit and slow inflammatory processes are possible mechanisms involved. No product with proven disease modifying properties is available yet, and current treatments offer symptomatic benefit

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only [2-6]. One of the ways to control AD is using acetylcholinesterase inhibitors (AChEIs) [7]. Because of low efficacy and side effects of the existing drugs, many researches being performed in order to find new drugs and plants are usually considered as good sources for drug discovery [8-12].

One of the causes of AD is oxidative destruction of brain neurons [3]. Free radicals are usually produced in human body during biochemical reactions and they are harmful for body cells; but there are several endogenic mechanisms to eliminate these radicals. If free radicals are produced more than toleration of body defense system, they cause damage to body. It has been established that free radicals can induce many diseases in human body such as AD, Parkinson's disease, inflammations, cardiovascular disorders, cancers, diabetes, *etc*; therefore, using antioxidant agents may prevent mentioned disorders. Many medicinal plants have antioxidant activity and could be used in AD [13-16].

Kohgiluyeh va Boyerahmad is a mountainous province in south-west of Iran. Lots of endemic plants grow in this area are used for prevention and treatment of disorders as folklore medicine [17]. Despite many medicinal herbs grow in this area, a few researches have been carried out about their properties. In the present investigation, in order to find medicinal plants for further AD studies, antioxidant and AChEI activities of 56 species of this area have been evaluated.

Experimental

Plant material

Fifty six species were collected from Kohgiluyeh va Boyerahmad province, Iran during 2009-2011. They were identified by Dr. H. Moazzeni and Dr. A. Pirani. A voucher specimen from each plant was kept at the Herbarium of TMRC, SBMU, Tehran, Iran.

Chemicals

Acetylthiocholine iodide (ATCI) was obtained from Fluka (Germany). Acetylcholinesterase enzyme (AChE) from bovine erythrocytes and 2,2-diphenyl-1-picryl hydrazyl (DPPH) were prepared from Sigma (Germany). 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) and TPTZ (2,4,6-tri-(2-pyridyl)-1,3,5-triazine) were purchased from Merck (Germany). Methanol and all other solvents were provided from Merck (Germany).

Plants extraction

Fifty g of each plant powder was macerated with methanol for 24 h. The mixture was filtered and concentrated using rotary evaporator and dried. It was kept in refrigerator before test.

Acetylcholinesterase inhibitory assay

AChEI activity was determined using a microplate reader based on Ellman's method [8,18,19]. Briefly, 125 µL of 3 Mm DTNB, 25 µL of 15 mM ATCI and 50 µL of phosphate buffer pH 8, and 25 µL of sample (were dissolved in methanol 3 mg/mL) were added to 96-well plates. The absorbance was measured at 405 nm every 13 sec for 65 sec. 25 µL of 0.22 U/mL of AChE enzyme was then added and the absorbance was recorded every 13 sec for 104 sec. The absorbance was plotted against time and the enzyme activity was calculated. Any increase in the absorbance due to the non-enzymatic hydrolysis of substrate was corrected by subtracting the rate of reaction before addition of the enzyme from the rate after addition of the enzyme. Percentage of enzyme inhibition was calculated by comparing the rates for the sample to the blank (using methanol without extract). Donepezil was used in the experiment as the positive control and its IC_{50} was calculated.

2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay

This technique is one of the most widely used methods for evaluating antioxidant activity of samples with natural sources. The method is based on scavenging of DPPH free radicals by antioxidants which induce a decrease in absorbance at about 520 nm. When a DPPH solution is mixed with a hydrogen donating substance, the reduced form of DPPH radical is generated accompanied by loss of color [20]. In the current experiment, in order to determine DPPH radical scavenging activity of samples,

100 µL of 100 µM DPPH methanol solution was added to 100 µL of various concentrations of the extracts. The mixture was shaken and left at room temperature for 30 min. Then, the absorbance of the solutions was measured at 517 nm by an ELISA reader and antioxidant activity was calculated using the following equation:
Scavenging capacity % = 100-[(ABS of sample-ABS of blank)×100/ABS of control]. Mixture of 100 µL methanol with 100 µL of the plant extract solution was used as the blank, while 100 µL DPPH solution plus 100 µL methanol was used as the negative control. Butylated hydroxytoluene (BHT) was used as positive control as well. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration [11,15]. The tests were performed in triplicate .

Ferric reducing ability of plasma (FRAP) assay
The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form, in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 mL of a 10 mmol/L TPTZ solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3.6H_2O$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.6) which was prepared freshly and warmed at 37 ºC. Alliquots of 40 µL of sample were mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent and were incubated at 37 ºC for 10 min. The absorbance of the reaction mixture was measured at 593 nm. Calibration curve of $FeSO_4.7H_2O$ was plotted by using different concentrations. FRAP value was determined for each solution and expressed as mmol $FeSO_4.7H_2O$/100 g extract [14,21,22].

Results and Discussion
The results of AChEI and antioxidant activities of 56 species collected from Kohgiluyeh va Boyerahmad province along with their family and parts used have been shown in table 1. The species belonged to different families. The most plants were from Apiaceae (9 species); while Lamiaceae (7 species), Asteraceae (6 species), Fabaceae and Rosaceae (4 species each) were in the next orders. The results showed that all Rosaceae species had antioxidant and AChEI activities. Nineteen species showed AChEI properties more than 10%, among them *Amygdalus scoparia* aerial parts showed the most considerable effect (50% inhibition at 300 µg/mL). This plant widely grows in Iran [23] but a few studies have been performed on this species. An investigation has been performed on the seeds of *A. scoparia* and it has been established that they contained palmitic, palmetoleic, stearic, oleic and linoleic fatty acids [24]. This plant has shown antioxidant activity as well. Regarding AChEI and antioxidant activities and distribution of the plant in Iran, it could be considered as a good candidate for AD researches. *Alhagi pseudoalhagi*, *Hypericum perforatum*, *Glycyrrhiza glabra*, *Datura innoxia*, *Sanguisorba minor* demonstrated AChEI activity as well. Moreover, plants with AChEI ability have been mostly found in Rosaceae family in the present study. *Epilobium minutiflorum* showed the most antioxidant activity in FRAP method (335.0 mmol $FeSO_4.7H_2O$/100g Ex.) which was more than BHT as positive control. *Thymus daenensis*, *Rosa canina*, *Sanguisorba minor* and *Hypericum perforatum* showed antioxidant activity almost equal to BHT. In DPPH assay, some of the species showed considerable radical scavenging activity even more than BHT. Among the examined samples, *Epilobium minutiflorum* exhibited the most activity (IC_{50} 3.6 µg/mL) which was more potent than BHT. These results introduced this species as a good antioxidant agent. This species might be promising for further isolation of potent antioxidant compounds. *Ferulago angulata*, *Rosa canina*, *Hypericum perforatum*, *Teucrium polium*, *Thymus daenensis*, *Nepeta glomerulosa*, *Sanguisorba minor* and *Amygdalus scoparia* are in the next orders. Regarding the results, most of the plant which demonstrated AChEI properties, showed antioxidant activity as well. These include *Thymus daenensis, Rosa canina, Sanguisorba minor, Stachys pilifera, Hypericum perforatum, Ferulago angulata, Glycyrrhiza glabra, Alhagi pseudoalhagi, Amygdalus scoparia* and *Cerasus microcarpa. Datura innoxia* was an exception which showed

Table 1. AChEI and antioxidant activities of some plants from Kohgiluyeh va Boyerahmad province

No.	Scientific name	Family	Parts used	AChEI (%)±SD	FRAP value±SD (mmol $FeSO_4.7H_2O$/ 100g Ex.)	IC_{50} (µg/mL)±SD in DPPH assay
1	*Acanthophyllum bracteatum* Boiss.	Caryophyllaceae	Aerial parts	0.0	55.0±1.3	89.7±7.2
2	*Achillea wilhelmsii* C. Koch	Asteraceae	Aerial parts	15.6±1.6	52.7±1.0	44.7±2.7
3	*Alhagi pseudoalhagi* (M. B.) Desf.	Fabaceae	Aerial parts	37.6±2.0	140.7±5.7	33.7±4.0
4	*Amygdalus scoparia* Spach	Rosaceae	Aerial parts	50.0±1.1	140.0±5.0	10.2±0.7
5	*Arctium minus* (Hill) Bernh.	Asteraceae	Aerial parts	0.0	41.1±0.7	36.8±2.3
			Rhizomes		110.7±0.7	11.2±1.6
6	*Astragalus fasciculifolius* Boiss.	Fabaceae	Aerial parts	0.0	53.1±1.1	132.8±6.1
7	*Capparis spinosa* L.	Capparidaceae	Aerial parts	0.0	46.0±0.4	41.4±2.1
8	*Carthamus oxyacantha* M. B.	Asteraceae	Aerial parts	9.8±0.1	52.6±0.1	50.8±1.2
9	*Cerasus microcarpa* (C. A. Mey.) Boiss.	Rosaceae	Young branches	23.2±1.5	136.0±5.0	11.1±1.0
10	*Chaerophyllum macropodum* Boiss.	Apiaceae	Aerial parts	0.0	33.7±1.0	159.0±13.3
11	*Chenopodium foliosum* (Moench) Aschers.	Chenopodiaceae	Aerial parts	6.6±1.3	42.1±0.8	74.7±10.6
12	*Cichorium intybus* L.	Asteraceae	Aerial parts	0.0	59.4±0.6	142.1±2.0
13	*Daphne mucronata* Royle	Thymelaeaceae	Aerial parts	8.2±0.8	144.0±1.4	34.2±1.0
14	*Datura innoxia* Miller	Solanaceae	Aerial parts	34.2±6.0	45.2±1.4	58.6±0.5
15	*Descurainia sophia* (L.) Schur	Brassicaceae	Aerial parts	16.0±1.6	51.4±1.6	47.4±1.4
16	*Dorema aucheri* Boiss.	Apiaceae	Tubers	0.0	42.4±0.6	119.0±2.8
17	*Epilobium minutiflorum* Hausskn.	Onagraceae	Whole plant	9.0±0.8	335.0±6.8	3.6±0.5
18	*Eremostachys adenantha* Jaub. & Spach	Lamiaceae	Aerial parts	0.0	71.4±1.7	39.4±0.7
19	*Eremostachys macrophylla* Montbr. & Auch.	Lamiaceae	Aerial parts	0.0	116.4±2.6	13.0±0.32
20	*Eryngium billardieri* F. Delaroche	Apiaceae	Aerial parts	8.0±2.1	40.0±1.1	86.5±6.4
21	*Ferula assa-foetida* L.	Apiaceae	Tubers	8.2±0.4	39.0±1.1	282.0±12.1
22	*Ferulago angulata* (Schlecht.) Boiss.	Apiaceae	Whole plant	23.2±5.8	196.2±9.1	4.1±0.7
			Aerial parts	10.1±0.0	37.1±0.7	12.8±1.5
23	*Foeniculum vulgare* Miller	Apiaceae	Aerial parts	0.0	58..3±1.2	20.2±1.0
24	*Fraxinus rotundifolia* Miller	Oleaceae	Aerial parts	0.0	157.4±8.0	15.5±1.0
25	*Gentiana olivieri* Griseb.	Gentianaceae	Whole plant	0.0	34.6±0.8	130.7±7.2
			Roots		17.7±0.4	-
26	*Glycyrrhiza glabra* L.	Fabaceae	Aerial parts	36.9±0.4	160.0±3.0	11.4±1.4
			Roots	23.2±2.2	128.2±0.0	19.5±2.7
27	*Gypsophila polyclada* Fenzl ex Boiss.	Caryophyllaceae	Aerial parts	0.0	53.3±1.0	38.4±4.5
28	*Haussknechtia elymaitica*	Apiaceae	Tubers	19.0±3.0	35.0±0.2	285.3±2.7

Table 1. Continued

No.	Scientific name	Family	Parts used	AChEI (%)±SD	FRAP value±SD (mmol $FeSO_4.7H_2O$/ 100g Ex.)	IC_{50} (µg/mL)±SD in DPPH assay
	Boiss.		Leaves	0.0	56.0±0.4	46.1±2.4
29	*Helichrysum oligocephalum* DC.	Asteraceae	Whole plant	0.0	62.0±0.4	22.5±1.7
30	*Hyoscyamus reticulatus* L.	Solanaceae	Aerial parts	0.0	60.8±1.2	59.1±8.3
31	*Hypericum perforatum* L.	Hypericaceae	Whole plant	37.3±0.1	239.2±3.53	5.5±0.6
32	*Juniperus excelsa* M. B.	Cupressaceae	Aerial parts	0.0	135.7±3.3	13.0±1.6
33	*Lepidium draba* L.	Brassicaceae	Whole plant	11.6±0.5	40.0±0.5	113.2±1.8
34	*Lonicera nummulariifolia* Jaub. & Spach	Caprifoliaceae	Young branches	9.1±1.3	137.6±0.7	14.8±1.0
35	*Malva parviflora* L.	Malvaceae	Whole plant	0.0	47.0±1.2	308.2±3.5
36	*Mindium laevigatum* (Vent.) Rech. F. & Schiman-Czeika	Campanulaceae	Aerial parts	12.4±1.5	35.3±0.5	116.6±12.0
37	*Nasturtium officinale* (L.) R. Br.	Brassicaceae	Whole plant	0.0	46.5±0.7	86.8±8.5
38	*Nepeta glomerulosa* Boiss.	Lamiaceae	Aerial parts	0.0	135.4±3.4	7.2±1.0
39	*Nerium indicum* Miller	Apocynaceae	Aerial parts	4.3±1.2	114.7±2.8	129.6±2.5
40	*Prangos uloptera* DC.	Apiaceae	Aerial parts	8.5±1.3	52.6±1.0	41.5±1.5
41	*Ricinus communis* L.	Euphorbiaceae	Aerial parts	0.0	114.2±3.2	19.0±1.0
42	*Rosa canina* L.	Rosaceae	Fruit	28.7±5.5	236.0±6.0	4.6±0.3
43	*Salvia sclarea* L.	Lamiaceae	Aerial parts	9.8±0.6	79.0±1.0	12.0±1.0
44	*Sanguisorba minor* Scop.	Rosaceae	Whole plant	32.4±0.2	227.3±3.0	8.4±0.3
45	*Scrophularia striata* Boiss.	Scrophulariaceae	Aerial parts	7.6±2.5	45.2±0.5	140.0±6.0
46	*Silene chlorifolia* Sm.	Caryophyllaceae	Aerial parts	0.0	58.8±1.0	69.1±7.6
47	*Silene propinqua* Schischk.	Caryophyllaceae	Whole plant	0.0	58.4±1.4	139.3±6.3
48	*Stachys pilifera* Benth.	Lamiaceae	Aerial parts	24.5±1.8	115.0±4.0	37.0±2.8
49	*Tanacetum polycephalum* Schultz-Bip.	Asteraceae	Aerial parts	13.1±0.7	110.7±3.8	28.1±3.2
50	*Teucrium polium* L.	Lamiaceae	Whole plant	0.0	177.0±3.6	6.0±0.6
51	*Thymus daenensis* Celak	Lamiacea	Whole plant	23.5±2.7	213.2±2.6	6.1±0.2
52	*Turgenia latifolia* (L.) Hoffm.	Apiaceae	Whole plant	0.0	40.7±0.2	62.3±6.0
53	*Urginea maritima* (L.) Baker	Liliaceae	Bulbs	0.0	27.7±0.5	350.0±8.2
54	*Verbascum songaricum* Schrenk ex Fisch. & C. A. Mey.	Scrophulariaceae	Aerial parts	0.0	147.7±4.6	14.1±1.4
55	*Vicia variabilis* Freyn & Sint.	Fabaceae	Aerial parts	0.0	30.8±0.3	175.5±12.6
56	*Vitex pseudo-negundo* (Hausskn.) Hand-Mzt.	Verbenaceae	Leaves and flowers	0.0	107.5±1.4	21.7±3.2
57	Donepezil	-	-	IC_{50}: 0.003 µg/mL	-	-
58	BHT	-	-	-	265.2±0.4	9.34±1.14

AChEI without antioxidant activity in FRAP assay. A previous investigation on the plants of Kohgiluyeh va Boyerahmad established that *Eryngium billardieri*, *Hypericum perforatum*,

Nerium indicum, Thymus daenensis, Urgenea maritima, Acanthophyllum bracteatum and *Datura innoxia* were cytotoxic on MDBK cell line [25,26]; while *Eryngium billardieri, Haussknechtia elymatica, Hypericum perforatum, Satureja bachtiarica, Turgenia latifolia* showed prophage induction ability in Escherichia coli K-12(λ) through induct test which shows their potential to interact with DNA [27]; thus, it is recommended that these plants not to be considered as the first choice in AD researches. Unless, further investigations have been carried out about their toxicity. Several investigations have established that many medicinal plants were demonstrated antioxidant activity but a few of them have shown AChEI property, therefore focusing on the plants with both effects might shorten the way to find new drugs for AD.

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Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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