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Original article

Evaluating the antioxidant and acetylcholinesterase inhibitory activity of three *Centaurea* species

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Abstract

Factors such as oxidative stress and reduced acetylcholine level have been implicated in Alzheimer's disease (AD) pathology and recently there has been a trend towards natural product research to find potential sources of antioxidants and acetylcholinesterase inhibitors in the plants kingdom. *Centaurea* is a genus with about 500 species world wild, many of them have shown to possess biologic activity; *Centaurea albonites, C. aucheri and C. pseudoscabiosa* are three species which little investigation has been carried out about their biological properties. In the present study, the antioxidant and acetylcholinesterase inhibitory activity of the above mentioned species have been evaluated. The ability of the total extract and methanol fraction of the plants to scavenge free radicals has been assessed through DPPH radical scavenging assay, and the acetylcholinesterase inhibitory property has been evaluated by Ellman method. The total extract of all species exhibited moderate antioxidant activity whereas the extracts of *C. pseudoscabiosa* showed the strongest antioxidant property; its total extract also demonstrated the highest acetylcholinesterase inhibitory activity among the evaluated samples (19.2% inhibition). The results suggest the species as potential sources of natural antioxidants which could be focused in future studies of Alzheimer's disease.

Keywords: Acetylcholinesterase inhibitor**,** antioxidant, *Centaurea*, DPPH, Ellman

Introduction

Alzheimer's disease (AD) is the most prevalent form of dementia, arising as a result of malfunctions of different biochemical pathways. Multiple pathogenic factors including aggregated amyloid-β peptide (Aβ) and *tau* protein, excessive transition metals, oxidative stress and reduced acetylcholine level have been implicated in AD pathology [1]. Much research has been focused on finding new sources of antioxidants and acetylcholinesterase inhibitors (AChEIs) of natural origin and plants of different families have been the center of many of these studies.

About 500 species of *Centaurea* grow in the Mediterranean regions, Eurasia, North America and Australia. 74 *Centaurea* species grow in Iran among which 28 are endemic. Various species are grown as ornamentals around the world for their brightly colored, thistle like flowers [2-4], while others are used

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for treatment of ailments in some countries [5]. Several species of the genus *Centaurea* are well known for their traditional uses to treat diseases including bacterial infections, diabetes, diarrhea, fever, hypertension, malaria, rheumatism and tumors [6]. They are known for anti-inflammatory, choleretic, digestive, stomachic, diuretic, astringent, cytotoxic and antibacterial effects [7]. Phytochemical investigations have revealed that the compounds responsible for the pharmacological properties are flavonoids and sesquiterpene lactones predominantly germacranolides, eudesmanolides, elemanolides, and guaianolides [8]. *Centaurea albonites, C. aucheri* and *C. pseudoscabiosa* are three species with little or no history of previous biologic investigations. In the present study, the ability of the total extract and the methanol fraction of the three species to demonstrate antioxidant and AChEI activity have been investigated through DPPH and Ellman assays, respectively.

Experimental

Chemicals and Reagents

2,2-diphenyl,1-picrylhydrazyl (DPPH) free radicals and acetylcholinesterase (AChE) were purchased from Sigma (Germany). Acetylthiocholin iodide (ATCI) was prepared from Fluka (Germany). 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and other chemicals and solvents were provided from Merck (Germany).

Plant material

Centaurea albonites, C. aucheri and *C. pseudoscabiosa* aerial parts were collected from Hamedan province, Iran (June 2011). The species were authenticated by Mrs. Atefeh Pirani (Botanist), Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. A voucher specimen of each species is deposited at TMRC Herbarium for future reference. The aerial parts were dried in shade and ground prior to extraction.

Extraction

10 g of the dried aerial powder of each species was macerated with methanol 80% at room temperature for 3 days. Each day the solvent was replaced with fresh solvent. The filtrate was concentrated, dried and further used in DPPH and Ellman assays.

Fractionation

20 g of the dried powder of the species was macerated with *n*-hexane at room temperature for 3 days (the solvent was refreshed every day). Finally, the residue of the plant was extracted with chloroform and the same process continued for 3 days and also the same for MeOH. The concentrated extracts were used in the antioxidant and acetylcholinestarease inhibitory assays.

DPPH radical scavenging assay

The DPPH free radical method is based on the determination of the concentration of DPPH at steady state in a MeOH solution, after adding the antioxidants [9]. The principle of this spectrophotometric method is based on the intensity of the violet DPPH radical solution measurement at 520 nm. The radical is decolorized by compounds with antioxidant activity [10]. In order to determine DPPH radical scavenging activity of the extracts and fractions, 2 mL of a 100 μM DPPH methanol solution was added to 2 mL of various concentrations of the samples. The mixture was shaken vigorously and left to stand at room temperature for 30 min. The absorbance of the solutions was measured at 517 nm and the antioxidant activity was calculated using the following equation:

Scavenging capacity % = 100-[ABS of sample-ABS of blank)×100/ABS of control]. MeOH (2 mL) with the plant extract solution (2 mL) was used as blank, while DPPH solution (2 mL) with MeOH (2 mL) was applied as the negative control and Vitamin C as the positive control. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage *vs* concentration. The tests were performed in triplicate [11-13]

Ellman assay

Ellman assay was stablished by Ellman *et al.*, 1961 which can assess the cholinesterase activity *in vitro* [14]. It is based on the reaction of thiocholine (one of the products of enzymatic hydrolysis of ATCH) with DTNB (Ellman's reagent) forming a yellow product (5-mercapto-2-nitrobenzoic acid and its

dissociated forms) at pH 8 which can be detected at 405 nm. [15]. We have conducted the experiment according to the above assay with some modifications (Houghton *et al*.) in a micro plate [16,17]. The concentration of 3000 µg/mL of every sample was prepared by dissolving the material in methanol. 125 µL of 3 mM DTNB, 25 µL of 15 mM ATCI, 50 µL of phosphate buffer (pH 8), and 25 µL of the sample dissolved in methanol were added in wells of 96-well plates. The absorbance was recorded at 405 nm every 13 s for 65 s. 25 µL of 0.22 U/mL of AChE enzyme was then added and the absorbance was again measured every 13 s for 104 s using a TECAN micro plate reader at 405 nm. Absorbance *vs* time was plotted and the rate of enzyme activity was compared to an assay using methanol without inhibitor. Any increase in absorbance due to the spontaneous hydrolysis of substrate was corrected by subtracting the rate of the reaction before adding the enzyme from the rate after adding the enzyme. Inhibition percentage was obtained by comparing the rates of the sample to the blank (MeOH). Donepezil was used as the positive control.

Results and Discussion

The *n*-hexane and chloroform fractions of the species did not dissolve in MeOH, therefore, only the activity of the total extract and the MeOH fractions were evaluated. The results of the DPPH and Ellman assays are demonstrated in figures 1 and 2, respectively.

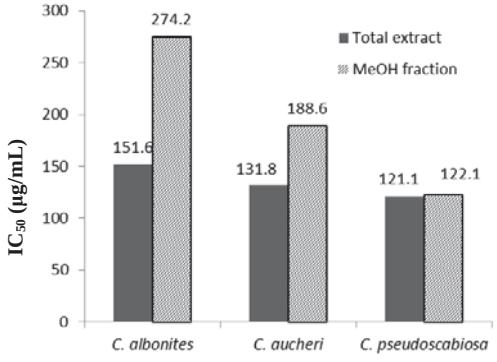

Figure 1. DPPH free radical scavenging activity of total extracts and methanol fractions of *C. albonites, C. aucheri and C. pseudoscabiosa*

Overproduction of free radicals has been implicated in various chronic diseases, such as cancer, atherosclerosis, diabetes and inflammatory diseases and also in aging.

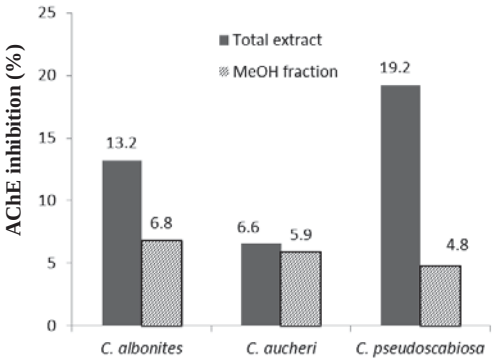

Figure 2. AChEI activity of total extracts and methanol fractions of *C. albonites, C. Aucheri and C. pseudoscabiosa*

Therefore, external supply of free-radical-scavengers is often necessary to maintain good health and prevent diseases [6].

The oxidative stress (the undue oxidation of biomolecules leading to cellular damage), promotes many studies of antioxidants in the prevention of AD [18].

AD is accompanied by dysfunctions in cholinergic neurotransmission of the central nervous system thus, cholinesterase inhibitors may act as potential leads in the discovery of therapeutics for such nervous system disorders [20]. *Centaurea* species are known for their anti-diabetic, anti-diarrhetic, anti-rheumatic, anti-inflammatory, choleretic, digestive, stomachic, diuretic, menstrual, astringent, hypotensive, antipyretic, cytotoxic, antibacterial properties and are used single or mixed [7]. The constituents of *Centaurea* are mainly terpenoids, flavonoids, acetylenic compounds and lignans [11] and *Centaurea* species have been found to be potential sources of natural antioxidants [4,21-23].

The results of the present study are in agreement with previous works about the antioxidant activity of the genus *Centaurea* and demonstrate moderate DPPH radical scavenging activity of the total extract and methanol fractions. The IC_{50} values for the MeOH fractions of *C. albonites* and *C. aucheri* [compared to Vit C (2.3 µg/mL)], were higher than the IC_{50} of their total extract while these values were almost the same for *C. pseudoscabiosa*. This might suggest that the compounds responsible for the antioxidant activity of the latter were mostly the polar components which were present in the MeOH fraction while the lower IC_{50}values of the total

extracts of *C. albonites and C. aucheri* implied that some other semi polar or even non polar compounds could have participated in the antioxidant property. Proposing polar constituents to be responsible for the demonstrated antioxidant activity is of no surprise, since secondary metabolites like flavonoids have been previously reported from different species of *Centaurea* [24-29] and flavonoids are famous compounds for possessing antioxidant property.

C. pseudoscabiosa total extract presented the strongest AChEI activity and was also found to be the most potent antioxidant among the evaluated species, this could imply a relation between the antioxidant and AChEI activity and a promising natural source for AD but further studies to confirm the exact mechanism and the responsible constituents are necessary. Although the AChEI activity of the species is weak, it might be a good candidate for further investigations and there is a possibility of isolating potent compounds that are present in trace amounts. Coming to a conclusion, *C. albonites, C. aucheri and C. pseudoscabiosa* are three *Centaurea* species which could be natural sources for antioxidant constituents, while *C. psudoscabiosa* is of special attention for its AChEI activity.

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