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Radical scavenging activity and cholinesterase inhibitory activity of *Leopoldia comosa* (L.) bulbs

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TITOLO
Attività radical scavenging e inibizione delle colinesterasi dei bulbi di *Leopoldia comosa* (L.)

KEY WORDS
Leopoldia comosa, bulbs, radical scavenving activity, Alzheimer's Disease, AChE and BChE inhibition

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Summary
Leopoldia comosa is a spontaneous plant growing in all Mediterranean area, including Calabria, where its bulbs (known as "cipudizze") were used in traditional gastronomy and used flavoured with garlic, chilli pepper, or fried with potatoes (1,2). Bulbs (300 g) were extracted with EtOH through maceration. In order to operate a separation of lipophilic compounds, the total extract was solubilized with MeOH/H_2O (8:2) and extracted with *n*-hexane. The ethanol extract showed a total phenols content of 56.6 mg chlorogenic acid equivalent *per* g of extract and a flavonoids content of 23.4 mg quercetin equivalent *per* g of extract. Ethanol extract demonstrated the strongest radical scavenging activity with an IC_{50} value of 40.9 μg/ml. Alzheimer's disease (AD) is the most common form of neurodegenerative disorders. Actually, AD treated by the use of agents which restore the level of acetylcholine through inhibition of both two major forms of cholinesterase: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). *n*-Hexane extract exhibited the highest bioactivity tested using Ellman's method (3) with IC_{50} values of 104.9 and 128.1 μg/mL for AChE and BChE, respectively.

Riassunto
Leopoldia comosa è una pianta spontanea dell'area Mediterranea che cresce anche in Calabria dove i suoi bulbi sono noti come "cipudizze" ed utilizzati nella tradizione gastronomica insaporite con aglio, peperoncino o fritte insieme alle patate (1,2). I bulbi (300 g) sono stati sottoposti ad estrazione per macerazione con EtOH. Con lo scopo di isolare la frazione lipofilica, l'estratto totale è stato solubilizzato in MeOH/H2O (8:2) e estratto con n-esano. L'estratto etanolico dei bulbi presenta un tenore in fenoli totali di 56.6 mg di equivalenti di acido clorogenico per g di estratto, mentre il contenuto in flavonoidi è di 23.4 mg di equivalenti di quercetina per g di estratto. L'estratto etanolico presenta la maggiore attività radical scavenging con un valore di IC50 di 40.9 μg/mL. Il morbo di Alzheimer (AD) è il più comune disordine neurodegenerativo dell'età senile. Ad oggi l'unico trattamento per l'AD è basato su farmaci che possano mantenere adeguati livelli di acetilcolina circolanti

e tali sostanze agiscono attraverso l'inibizione delle due maggiori forme di colinesterasi: acetilcholinesterasi (AChE) and butirilcholinesterasi (BChE). L'estratto in n-esano dei bulbi, testato attraverso l'Ellman's method (3), ha mostrato una potente attività inibitoria con valori di IC50 di 104.9 e 128.1 μg/mL per AChE e BChE, rispettivamente.

Introduction

Leopoldia comosa is a spontaneous plant growing in Calabria where its bulbs (known as "cipudizze") were used in traditional gastronomy and used flavoured with chilli pepper or fried with potatoes (1, 2). In recent years, the physiological functionality of food plant used in traditional cooking has received much interest. Oxidative damage, caused by the action of free radicals, may initiate and promote the progression of a number of chronic diseases, including Alzheimer's disease (4). Alzheimer's disease (AD) is the most common form of neurodegenerative disorders. In spite of the multi-factorial nature of AD, actually only the anticholinergic therapeutic approach is followed (5).

In our continuous search on bioactivity of plants food and spices, the aim of this study is to investigate the radical scavenging activity and the cholinesterase inhibitory properties of *L. comosa* bulbs extracts.

Materials and methods

Samples and extraction procedures

L. comosa bulbs (300 g) were bought in a local market in Cosenza (Italy). Bulbs were peeled and exhaustively extracted with ethanol through maceration. In order to operate a separation of lipophilic compounds, the total extract (yield 3.2%) was solubilized with MeOH/H_2O (8:2) and extracted with *n*-hexane (yield 0.1%).

Determination of total phenol and flavonoid content

The total phenols content of *L. comosa* ethanol extract was determined by the Folin-Ciocalteau method (6). Chlorogenic acid was used as a standard and the total phenols content was expressed as chlorogenic acid equivalents in mg *per* 100 g of extract. The flavonoids content of *L. comosa* was determined spectrophotometrically using a method based on the formation of a flavonoid-aluminium complex (6). Quercetin was chosen as a standard and the levels of total flavonoid content were determined in triplicate and expressed as quercetin equivalents in mg *per* 100 g of extract.

GC-MS analysis of n-hexane extract

The chemical profile of *L. comosa* *n*-hexane extract was evaluated by Gas Chromatography-Mass Spectrometry (GC-MS). GC-MS analyses were carried out as previously reported (7).

Determination of Radical Scavenging Activity

Radical scavenging capacity was determined according to the technique reported by Loizzo et al. (8). The DPPH radicals scavenging activity was calculated according to the following equation:

Scavenging activity =

$= [(A_0-A_1/A_0) \times 100]$

where A_0 is the absorbance of the control (blank, without extract)

and A_1 is the absorbance in the presence of the extract.

Cholinesterase inhibitory activities

Acetylcholinesterase and butyrylcholinesterase inhibiting activities were measured by method developed by Ellman et al. (7). The inhibition rate (%) was calculated by equation (Fig. 1).

Statistics

All experiments were carried out in triplicate. Data were expressed as means ± S.D. The concentration giving 50% inhibition (IC_{50}) was calculated by nonlinear regression with the use of Prism GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results and discussion

L. comosa bulbs were used in traditional gastronomy of South of Italy (1,2). Bulbs ethanol extract showed a total amount of the phenolic compounds with value of 56.6 mg chlorogenic acid equivalent *per* g of extract and a flavonoids content of 23.4 mg quercetin equivalent *per* g of extract. C16:0, C16:0 ethyl ester, C18:2ω6, and C18:2ω6 ethyl ester were identified as the main abundant compounds of the *n*-hexane extract. Both ethanol and *n*-hexane extracts demonstrated an interesting radical scavenging activity with IC_{50} values of 40.9 and 46.6 μg/mL, respectively (9). A strictly correlation between radical scavenging activity and level of phenolic components was previously re-

Figure 1

$$\text{Inhibition \%} = \frac{[(\text{Blank} - \text{Blank positive control}) - (\text{Experiment} - \text{Experiment control})]}{(\text{Blank} - \text{Blank positive control})}$$

Figure 2 - Cholinesterase inhibition of *Leopoldia comosa* bulbs extracts

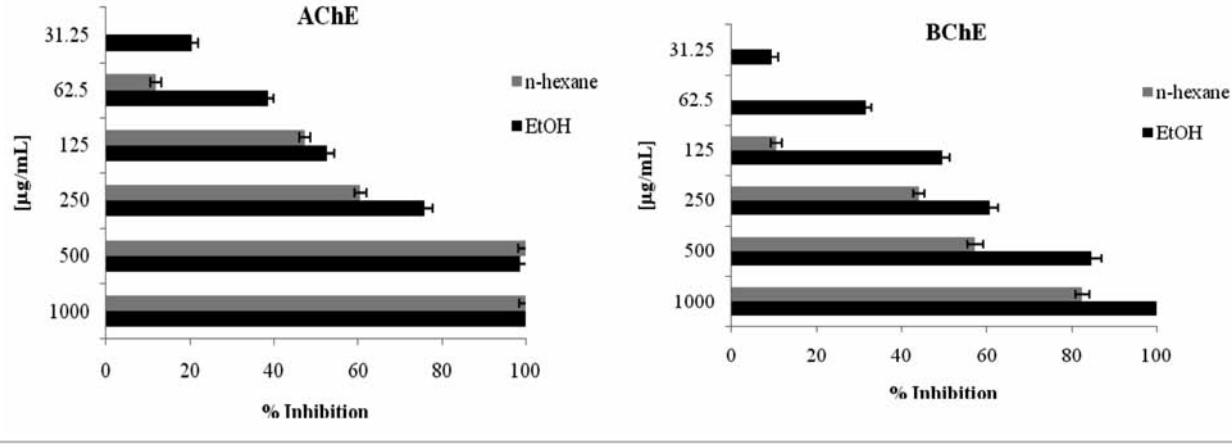

Table 1 - Comparative cholinesterase inhibitory activity of *L. comosa* extracts (IC_{50} μg/mL)

L. comosa	AChE	BChE
EtOH	131.0 ± 3.6*	282.9 ± 4.4*
n-Hexane	104.9 ± 2.5*	128.1 ± 2.7*
Physostigmine	0.17 ± 0.004	2.4 ± 0.02

IC_{50} values are mean ± S.D. (n=3); One-way ANOVA Analsyis: *** p< 0.0001; Dunnet's test: * p< 0. 01

ported (3, 10). Moreover, Richard et al. (11) suggesting that polyunsaturated fatty acid contributed to the antioxidant activity. This evidence could be partially explain our obtained results with *L. comosa* *n*-hexane extract since it is characterized by the presence of C18:2ω6 among other as major constituents.

In our continuous search of beneficial effects that some common consumed food plant and spice had on cholinesterase neurotransmission, herein we report the ability of *L. comosa* extract to inhibit AChE and BChE. A dose response curve was observed for all extracts (Fig. 2). Both extracts showed a stronger inhibition on AChE with IC_{50} values of 131.0 and 104.9 μg/mL, for EtOH and *n*-hexane extract, respectively (Tab. 1). Interesting was also the inhibitory activity of the *n*-hexane extract against BChE (IC_{50} value of 128.1 μg/mL), in fact in late stage AD, levels of AChE have declined by up to 85% and BChE represents the predominant ChE in brain. For this reason BChE inhibition have targeted as a new approach to intercede in the progression of AD.

Further works could be done on the identification of compounds responsible of the bioactivity of bulbs and how culinary process could influenced the bioactivity since this is may be of interest from functional point of view and for the revalorization of Calabrian traditional gastronomy plant food.

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