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Original Article

Rhodolirium andicola: a new renewable source of alkaloids with acetylcholinesterase inhibitory activity, a study from nature to molecular docking

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ABSTRACT

Acetylcholinesterase is an important target for control of neurodegenerative diseases causing cholinergic signaling deficit. Traditionally, galanthamine has been used as an Amaryllidaceae-derived acetylcholinesterase inhibitor, although new Amaryllidaceae plants could serve as source for better acetylcholinesterase inhibitors. Therefore, the objective of this study was to characterize the alkaloid composition from bulbs of *Rhodolirium andicola* (Poepp.) Traub, a native Chilean Amaryllidaceae specie, and assess their inhibitory activity on acetylcholinesterase by *in vitro* and *in silico* methodologies. Alkaloidal extracts from *R. andicola* exhibited an inhibitory activity with IC_{50} values between 11.25 ± 0.04 and 57.78 ± 1.92 μg/ml that included isolated alkaloid, galanthamine (2.3 ± 0.18 μg/ml), Additionally, 12 alkaloids were detected using gas chromatography–mass spectrometry and identified by comparing their mass fragmentation patterns with literature and database NIST vs.2.0. To better understand the bioactivity of isolated compounds and alkaloidal extracts against acetylcholinesterase, a molecular docking approach was performed. Results suggested that alkaloids such as lycoramine, norpluvine diacetate and 6α-deoxy-tazettine expand the list of potential acetylcholinesterase inhibitors to not only galanthamine. The role of *R. andicola* as a source for acetylcholinesterase inhibitors is further discussed in this study.

Introduction

The enzyme acetylcholinesterase (AChE) is known by its rapid hydrolysis of neurotransmitter acetylcholine (ACh) in the cholinergic synapses (Barnard, 1974; Stryer, 1995). Inhibition of AChE is an important strategy for the treatment of diseases that involve cholinergic transmission deficit such as myasthenia gravis and Alzheimer's disease (AD) (Rahman and Choudhary, 2001; Mehndiratta et al., 2011). AD is the most common form of dementia in our society (World Alzheimer Report, 2015). Worldwide, it is currently estimated that 46 million people have AD or a related dementia, and considering that life expectancy will increase, it is estimated that people with AD will reach to 131.5 million by 2050 (World Alzheimer Report, 2015). These facts make AD one of the most investigated diseases throughout the world (Perry, 1986; Greig et al., 2001). Although AChE inhibition is an established therapeutic strategy to ameliorate cognitive dysfunction and memory loss associated with AD (Rahman and Choudhary, 2001), only a few compounds, such as tacrine, donepezil, physostigmine and galanthamine (Zarotsky et al., 2003) have been approved by the Food and Drug Administration (FDA) in the United States. However, several side-effect such as hepatotoxicity and problems associated with gastrointestinal symptoms, have been reported for the synthetic drugs tacrine and donepezil, respectively (Schulz, 2003; Mehta et al., 2012). In contrast, physostigmine and galanthamine, both from natural origin, have fewer side effects in patients with mild to moderate AD (Mehta et al., 2012). Consequently, many research groups have focused their studies on finding new renewable sources of compounds with acetylcholine esterase inhibitory activity (Mukherjee et al., 2007). In this regard, after the

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isolation of natural compound galanthamine, a long-acting, selective, reversible and competitive AChE inhibitor, approved in 2001 by FDA (Razadyne®), for clinical treatment of mild and moderate AD, several Amaryllidaceae species have been evaluated as new sources of galanthamine or other alkaloids with potential AChE inhibitory activity (López et al., 2002; Rhee et al., 2004; Ortiz et al., 2012). Although the chemical synthesis of galanthamine is available (Marco and Carreiras, 2006; Bulger et al., 2008), current pharmaceutical production of this compound is mainly limited to the extraction of natural populations of the Amaryllidaceae *Leucojum aestivum* and *Narcissus* spp. (Heinrich and Teoh, 2004).

In Chile, around 35 species of the Amaryllidaceae family are present covering a wide variety of eleven genera (Ravena, 2003). Particularly, *Rhodolirium andicola* (Poepp.) Traub, part of endemic Amaryllidaceae species growing in Chile, represents a potential source of alkaloids with AChE inhibitory activity. In this study, we describe the alkaloidal composition of *R. andicola* for the first time. We isolated three well-known alkaloids and evaluated their AChE inhibitory by the Ellman method (Ellman et al., 1961) as a first approach to probe *R. andicola* as a source of alkaloids. Additionally, we tested twelve other alkaloids identified by gas chromatography–mass spectrometry (GC–MS) by molecular docking using a crystal structure of AChE to propose new alkaloids as potential AChE inhibitors that could be used in further assays and likely treatments of neurodegenerative diseases.

Materials and methods

Chemicals

Silica gel 60 (Merck, 70–230 mesh) was used for column chromatography (CC) and silica gel 60 F_{254} for thin layer chromatography (TLC) analytical and preparative. MeOH and water (HPLC grade), $CHCl_3$, H_2SO_4, Et_2O, NH_4OH, hexane, BuOH, NH_3, EtOAc (analytical grade) were purchased from J.T. Baker (México). Acethyltiocholine iodide (ATCI), acetylcholinesterase (AChE) from *Electhrophorus electricus* (type VI-S lyophilized powder), 5,5-dithio-bis-2-nitrobenzoic acid (DTNB) and hydrocarbon mixture (C_6–C_{26}) (chemical purity >99%) were obtained from Sigma–Aldrich (St. Louis, MO, USA) whereas, Reminyl® (galanthamine hydrobromide salt) were purchased from Janssen-Cilag (Spain).

Plant material

Rhodolirium andicola (Poepp.) Traub, Amaryllidaceae, bulbs were collected during the flowering season in December 2016 from National park Conguillio, Araucanía Region, Chile (S 38°44,426′ WO 72°38,887 height: 1389 m.a.s.l.). The plant was identified by Dr. Marcelo Baeza and deposited at the herbarium of Universidad de Concepción, Concepción, Chile (voucher no. CONC 182466).

Alkaloid extraction and fractionation

Dried bulbs (2.5 kg) were extracted three times with MeOH (1 g of dry sample by 10 ml of solvent) at room temperature for one week (Rhee et al., 2004). The solution was filtered and the solvent was evaporated under reduced pressure on a rotary evaporator (40 °C). The residue (150 g) was dissolved in water (250 ml) and acidified to pH 2 with H_2SO_4 (2%, v/v). The acid solution was defatted with Et_2O (5 × 100 ml) and $CHCl_3$ (5 × 100 ml). Then, the acid solution was basified with 25% ammonia solution up to pH 9–10 and the alkaloids were extracted with hexane (5 × 100 ml), $CHCl_3$ (5 × 100 ml) and BuOH (5 × 80 ml), to obtain the hexanic (0.27 g), chloroformic (1.3 g), and buthanolic alkaloidal extracts (2.5 g) respectively (Ortiz et al., 2012; Sheng-Dian et al., 2013; de Andrade et al., 2016). The hexanic alkaloidal extract was roughly separated by column chromatography on 10 g of silica gel 60 (Merck, 70–230 mesh) using a gradient with hexane (100%), gradually enriching with $CHCl_3$ (0 → 100%) and subsequently increasing the polarity with EtOAc, and finally increasing it with MeOH (0 → 50%) (de Andrade et al., 2014; Ortiz et al., 2016) to give five fractions (I–V). Column fractions were monitored by TLC, and similar ones were combined and evaporated to dryness. Fractions I and II were combined and subjected to preparative TLC, (silica gel 60 F_{254} with $CHCl_3$/hexane/MeOH, 5:2:3, in NH_3 atmosphere) to give the compound-A (10 mg). Column chromatographic on Sephadex LH-20 of fractions III–V in MeOH gave three subfractions. The second subfraction, positive to Dragendorff reagent, was subjected to preparative TLC, (silica gel 60 F_{254} with $CHCl_3$/MeOH, 9:1, in NH_3 atmosphere) to give the compound-B (15 mg). Whereas, the separation of compounds from chloroformic alkaloidal extract (1.3 g) was performed by preparative column chromatography on 50 g of silica gel 60 (Merck, 70–230 mesh), as stationary phase (Ortiz et al., 2012). The elution started with chloroform increasing the polarity with methanol, enriched gradually with 10% methanol up to 100% methanol (Elisha et al., 2013) to give one hundred fractions of 10 ml. Fractions with similar profiles based on visualized under ultraviolet light (254 nm), and analysis by Dragendorff reagent were combined and evaporated to dryness. Column chromatographic on Sephadex LH-20 of fractions 60–100 in MeOH gave four subfractions. The third subfraction, positive to Dragendorff reagent, was subjected to preparative TLC, (silica gel 60 F_{254} with $CHCl_3$/MeOH, 9:1, in NH_3 atmosphere) to give the compound-C (20 mg) and compound-D (40 mg) respectively.

GC/MS analysis

The extracts were analyzed by coupled gas chromatography–mass spectrometry (GC–MS) with electron impact ionization (70 eV) using an Agilent, model 7890A chromatograph equipped with a HP-5ms capillary column (30 m × 0.25 mm × 0.25 μm; J&W Scientific) with helium carrier gas. The GC oven was programmed to ramp from 100 °C (for 3 min) to 280 °C at 10 °C/min and held for 19 min. The injector and transfer line temperatures were 250 °C and 285 °C respectively. The alkaloid compounds were identified by comparing their GC mass spectra with data from the NIST MS Search 2.0 library, Kovats indices (RI) and mass spectra reported in the literature (Mukherjee et al., 2007; Ortiz et al., 2016). The Kovats retention indexes of the compounds were recorded with standard of an *n*-hydrocarbon mixture (C_9–C_{26}). The proportion of each alkaloid in the basic extracts is expressed as a percentage of ion current (TIC).

Acetylcholinesterase inhibitory activity

Inhibition of AChE by alkaloidal extracts and isolated compounds was determined using the spectrophotometric method according to Ellman et al. (1961) and modified by Ortiz et al. (2012). Fifty microliters of AChE (0.25 U/ml) in phosphate buffer saline (8 mM KH_2PO_4, 2.3 mM Na_2HPO_4, 0.15 M NaCl, pH 7.5) and 50 μl of the samples dissolved in the same buffer were added to the wells. The plates were incubated for 30 min at 25 °C before the addition of 100 μl of the substrate solution (0.04 M Na_2HPO_4, 0.2 mM, 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB), 0.24 nM acethyltiocholine iodide (ATCI) in HPLC grade water). The absorbance was read in a microplate reader (Varioskan™ Flash) at 405 nm after 5 min. Inhibition of enzyme was calculated as a percentage compared with an assay using a buffer without any inhibitor. The IC_{50} values were the means ± SD of three determinations. Reminyl® was used as positive control.