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Cytotoxicity and acetylcholinesterase inhibitory activity of an isolated crinine alkaloid from *Boophane disticha* (Amaryllidaceae)

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ABSTRACT

Ethnopharmacological relevance: *Boophane disticha* of the family Amaryllidaceae is used traditionally in southern Africa in the treatment of several neurological disorders.
Aim of the study: Although acetylcholinesterase (AChE) inhibitory activity has been reported for this plant, the aim of the study was to identify and characterise the compound responsible for this activity using bioassay guided fractionation. Toxicity of the isolated compound was also assessed.
Materials and methods: Bioassay guided isolation of the active compound from the methanol extract was carried out using column chromatography, TLC and preparative thin layer chromatography. Structural elucidation was carried out using high field 1D and 2D NMR and mass spectroscopy. AChE inhibitory activity was determined using the Ellman's colorimetric method. Cytotoxicity assessment was determined in human neuroblastoma (SH-SY5Y) cells using the MTT and neutral red uptake assays.
Results: The data obtained from the integration of the ^{1}H spectra confirmed the compound to be a 3:1 mixture of two epimers, with epimer A, 6α-hydroxycrinamine as the major epimer. The IC_{50} value for AChE inhibitory activity of the compound was 445 μM. The compound was observed to be cytotoxic in both the MTT and neutral red assays with IC_{50} values of 54.5 and 61.7 μM, respectively.
Conclusion: The present study describes for the first time, the isolation of 6-hydroxycrinamine, a crinine alkaloid, from the methanol extract of the bulbs of *B. disticha*. Although this compound possessed AChE inhibitory activity, it was found to be toxic to the neuroblastoma cells. Quantitative structure–activity relationship studies could be carried out to modify the structure in order to make it less toxic and improve its activity.

1. Introduction

Aging is one of the factors which results in the dysfunction of normal cellular regulation, affecting both central nervous and immune systems (Kawahami et al., 1999; Yu et al., 2005). It is an important risk factor of several neurodegenerative diseases including Alzheimer's disease (AD) (Yu et al., 2005). AD is a neurodegenerative disorder with increasing prevalence in the elderly population in the western world and the most common cause of age-related intellectual impairment occurring after the age of 60 (Silva et al., 2004). Treatment of AD has largely involved replacement of neurotransmitters that are known to be lacking, mostly based on the inhibition of acetylcholinesterase (AChE), an important approach that is founded on the cholinergic hypothesis for the disease (Francis et al., 1999; Konrath et al., 2012). The drugs approved so far for AD therapy act by counteracting the acetylcholine deficit and thus, improve levels of the neurotransmitter in the brain (Heinrich and Teoh, 2004; Ferreira et al., 2006). The molecular basis of the drugs used so far take advantage of their action as acetylcholinesterase inhibitors (Heinrich and Teoh, 2004). However, these drugs are extremely limited; possess considerable side effects related to cholinergic stimulation in brain and peripheral tissues and cause hepatotoxicity which is related to tacrine (Knapp et al., 1994; Thompson et al., 2004; Fang et al., 2008). Considering the complex pathology of AD, research is ongoing to develop new compounds and leads which are not toxic and have potential for use in the treatment of the disease.

Boophane disticha (L.f.) Herb. belongs to the family Amaryllidaceae. It is an attractive, deciduous bulbous plant with a thick covering of dry scales above the ground and is widely distributed in Africa, ranging from Sudan in the north to the Western Cape Province in the south (Wrinkle, 1984). Decoctions of bulb scales are given to sedate violent, psychotic patients (Van Wyk and Gericke, 2000) and bulb infusions are reported to be used to treat mental illness (Sobiecki, 2002).

Several alkaloids from this plant have been isolated and identified (Fig. 1) (Hautch and Stauffacher, 1961). Buphanidrine,

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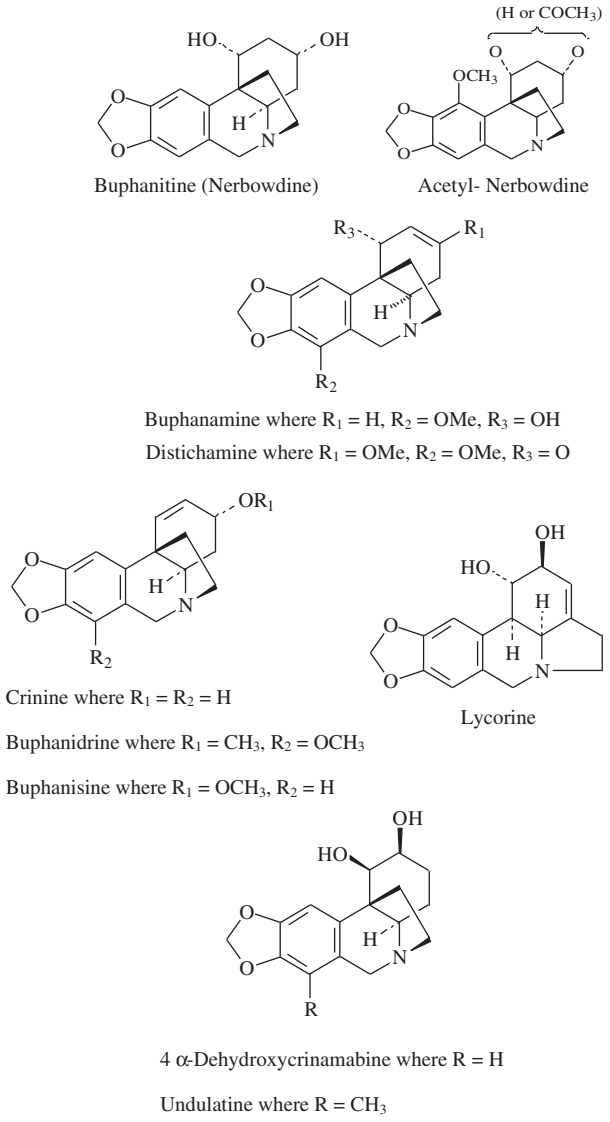

Fig. 1. Structures of several alkaloids previously isolated from the bulbs of *Boophane disticha*.

buphanamine and distichamine have been reported to have affinity to the serotonin transporter indicating their potential in treatment of depression and anxiety (Sandager et al., 2005; Neergaard et al., 2009). Also, acetylcholinesterase inhibitory activity has been reported for this plant (Adewusi, 2012). The aim of the study was to identify and characterise the compound responsible for the AChE inhibitory activity using bioassay guided fractionation. Toxicity of the isolated compound was determined using both the 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) and neutral red assays. The neutral red assay was included as it does not rely on a reduction reaction to determine viability, thereby reducing the possibility of obtaining false positive results in the presence of antioxidants or other reductive agents (Van Tonder, 2011).

2. Materials and methods

2.1. General

Nuclear magnetic resonance (NMR) spectroscopy was performed using a 600 MHz Varian NMR. Structural characterisations were carried out using a combination of one dimensional (1D) (^{1}H, ^{13}C) and various two dimensional (2D) experiments. The 2D experiments carried out included distortionless enhancement by polarisation transfer (DEPT), correlation spectroscopy (COSY), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond correlation (HMBC). Chemical shifts are reported in units of δ (ppm) and coupling constants (*J*) are expressed in Hz. UV–VIS detection was done on a WATERS PDA scanning from 200 nm to 600 nm. Mass spectrometry (MS) detection was performed using a WATERS SQD scanning from 100 to 1200 *m/z* with polarity (+/−)switching. Silica gel 60 (0.063–0.2 mm) was used for column chromatography, while pre-coated glass plates (Merck, SIL G-25 UV_{254}, 20 cm × 20 cm) were used for thin layer chromatography (TLC) and preparative TLC. Spots on the TLC plates were detected under UV light at short wave (250 nm) and long wave (365 nm) lengths, and by vanillin–H_2SO_4 and Dragendorff's reagent. Acetylthiocholine iodide (ATCI), acetylcholinesterase (AChE) and 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) purchased from Sigma, were used for the determination of AChE inhibitory activity while MTT and neutral red dye also purchased from Sigma were used for the cytotoxicity assays. Human neuroblastoma (SH-SY5Y) cells were purchased from American cell type collection culture (ATCC CRL-2266, Rockville, MD, USA).

2.2. Plant material

Bulbs of *Boophane disticha* (L.f.) Herb. (Amaryllidaceae) were a gift from the South African National Biodiversity Institute, Pretoria.

2.3. Extraction and bio-assay guided isolation of alkaloid

Plant material was cut into small pieces and air-dried at room temperature. Dried material was ground to a fine powder using an Ika Analytical Mill (Staufen, Germany), and stored at ambient temperature in the dark till use. 250 g of the powdered plant material was extracted with 2.5 l of methanol for 24 h while shaking. The extracts were filtered, concentrated using a rotary vacuum evaporator and further dried under reduced pressure. The residue (6 g) was subjected to column chromatography on silica gel by gradient elution with $CHCl_3$ and $CHCl_3$/MeOH mixtures starting from 100:0 until 70:30 as eluent to give 50 fractions. The fractions were pooled together based on the similarity in their R_f values on a thin-layer chromatography plate to give four sub-fractions. Each sub-fraction was tested for their inhibition of AChE on the TLC plate as described below. Sub-fractions 2 and 3 were the only active sub-fractions. Sub-fraction 2 was further chromatographed on a silica gel column using a stepwise gradient mixture of $CHCl_3$:MeOH starting from 95:5 until 75:25 as eluent to give another set of 30 fractions and tested for activity. Of this sub-fraction, only fractions 1, 4 and 8 were active.

Sub-fraction 3 was also further chromatographed on a silica gel column using a stepwise gradient mixture of $CHCl_3$:MeOH starting from 95:5 until 75:25 as eluent to give 23 fractions which were also tested for activity. Fractions 5, 14, 15 and 16 were active and had similar R_f values as fractions 1, 4 and 8 from sub-fraction 2, and so these seven fractions were combined and further purified. Preparative TLC yielded 6-hydroxycrinamine (20 mg), obtained as yellow crystals with a yield of 0.33%. The isolation of 6-hydroxycrinamine is summarised schematically in Fig. 2. The identity of 6-hydroxycrinamine was confirmed through NMR and MS analysis. Data obtained from mass spectroscopy was analysed using MassLynx 4.1 (SCN 704) software and the fragmentation patterns of the compounds isolated were identified with the Agilent ChemStation software which has a National