Indole Alkaloids from *Tabernaemontana australis* (Müll. Arg.) Miers that inhibit acetylcholinesterase enzyme

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**Introduction**

Alzheimer’s disease (AD) is the most common neurodegenerative disorder of this century and the most prevalent cause of dementia with ageing. Symptomatic pharmacological treatment of AD is mainly based on the use of acetylcholinesterase inhibitors (AChEI) (e.g. donezepil, rivastigmine and galanthamine), which have beneficial effects on cognitive, functional, and behavioural symptoms of the disease as well as undesired side effects.

Monoterpenoid indole alkaloids have been extensively investigated for a wide variety of pharmacological effects, such as anticontraceptive, antitumor, anti-inflammatory, antimalarial, bactericidal and leishmanicidal activities, as well as stimulatory or toxic effects on central nervous system.⁴ Although the CNS activities reported, up to date, only monoterpenoid indole alkaloids from the Apocynaceae *Haplophyton crooksii* have been assayed as cholinesterase inhibitors.⁵

The species *Tabernaemontana australis* (Müll. Arg.) Miers (sin. *Peschiera australis*) (Apocynaceae), which flourishes in Brazil, Argentina, Uruguay, and Paraguay, has been poorly investigated with regard to its chemical composition and specific pharmacological activities. Recent studies reported the antileishmanial activity of the ethanolic extracts and of the isolated monoterpenoid indole alkaloid coronaridine (1).³ TLC chromatography of the crude stalk chloroform extract indicated the presence of alkaloids coloured by Dragendorff’s reagent and bioautography using the modified Ellman’s method⁴ revealed the presence of acetylcholinesterase inhibitors among the alkaloids.

**Methods**

**Plant Material.** *T. australis* was collected at Botanical Garden of Rio de Janeiro where a voucher specimen is deposited as ICN-68457.

**Extraction and isolation of alkaloids.** Dried stalk (631g, 80 °C for 7 days) was extracted on a Soxhlet apparatus with EtOH to give 53g of a brown residue after reduced pressure concentration. The ethanolic extract was suspended in 5% HCl, extracted with CHCl₃ and the pH of the aqueous acidic fraction was adjusted to 9 with NH₄OH. After extraction with CHCl₃ and reduced pressure concentration, 2.6 g of a crude chloroform extract were obtained. Silica gel open column chromatography of 1.5g in a gradient elution of cyclohexane: ethyl acetate: methanol afforded 9 fractions, from which were isolated: coronaridine 1 (14 mg), voacangine 2 (29 mg), hydroxyindole voacangine 3 (25mg) and rupicoline 4 (5 mg) identified on the basis of its spectral data and comparison of literature.³ GCMS of the third fraction showed the presence of voachalotine (8) and oxyindole voachalotine (9). Other fractions were obtained as mixtures.

**Screening for AChE activity (bioautography):** Acetylcholinesterase inhibitory activity was determined using the modified Ellman’s method.⁴ Briefly, crude extract or pure compounds were diluted in CH₂Cl₂ at a concentration of 10 mg/ml or 0.01 and 0.1 mM, respectively. A volume of 2.5 μL
of each sample was spotted on the silica gel TLC plate and developed with the solvent hexane:ethyl acetate, 1:1; 2.5 µl of 0.1 and 0.01 mM physostigmine and galanthamine solutions in methanol were also spotted as reference compounds. After developing the TLC plate, enzyme inhibitory activities of the developed spots were detected by spraying the substrate, dye and enzyme. TLC analysis without solvent development was also used to test the enzyme inhibition in different pure compounds concentrations.

The presence of cholinesterase inhibitory activity was determined by the formation of well-defined white spots made visible by spraying with DTNB, which gives a yellow background.

**General.** GC analyses were carried out on an Agilent GC 6890 gas chromatograph equipped with a fused silica DB1 (J & W, 25 m x 0.25mm x 0.25 µm) capillary column directly coupled to a quadrupole mass spectrometer Agilent 5973. EI-mass spectra were recorded at 70 eV. Conditions: injector (split mode, 1:30) at 250 °C; oven temperature: 150 to 290 °C (5 min) at 4 °C. min⁻¹, He as carrier gas at 1 mL. min⁻¹. The NMR spectra (δ ppm and J in Hz) were recorded on a Bruker DRX-300 spectrometer in CDCl₃ as solvent and as internal reference. FTIR spectrum with a KBr disc was recorded on a Nicolet AVATAR-FTIR spectrometer.

**Discussion**

GCMS analysis of the crude chloroform extract indicated the presence of at least 8 alkaloids with molecular weight ranging from 280 to 384. Analysis of the fragmentation pattern of the alkaloids in MS, associated with the Wiley 275 MS library, comparison with literature data and standards co-injection suggested the presence of coronaridine (1), voacangine (2), hydroxyindoleine voacangine (3), rupicoline (4), ibogamine (5), ibogaline (6), and desethyl-voacangine (8).

Silica gel open column chromatography of the crude chloroform extract afforded 9 fractions. Fractions 1-4 were eluted in silica gel chromatography with a gradient of cyclohexane and ethyl acetate to give 1, 2, 3 and 4 identified by spectroscopic analyses by 1D and 2D NMR, IR and MS and comparison with literature. Alkaloids 5-10 were obtained as mixtures after column chromatography.

Compounds 1, 2 and 3 and 4 were submitted to evaluation of their acetylcholinesterase (AChE) inhibitory activities. Using bioautography in silica gel TLC all the tested compounds were found to inhibit AChE in the same concentration of physostigmine and galanthamine detection limits (0.01 mM).

**Conclusion**

This is the first report of the anticholinesterasic activity of alkaloids 1-4, which contain a different carbon framework from ibogaine type anticolinesterasic alkaloids from *Haplophyton crooksii*. These results indicate that TLC assay based on Ellman’s method is the most simple, fast and cheap method to screen new anticholinesterasic inhibitors from plants.

**Acknowledgements**

PIBC-UFF, CNPq and FAPERJ

**References**


