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AFFINITY SELECTION MASS SPECTROMETRY FOR IDENTIFICATION OF ACETYLCHOLINESTERASE LIGANDS IN STRYCHNOS PECKII

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Strychnos species are recognized for biosynthesizing a wide range of alkaloids with proven activity on the central nervous system. *Strychnos peckii* is traditionally used as a component of *curare*, a preparation employed by Indigenous natives from northern Brazil for hunting and warfare. Due to its use in paralytic preparations, the aqueous extract of *S. peckii* was selected to search for acetylcholinesterase (AChE) ligands using affinity selection mass spectrometry (AS-MS), via enzyme immobilization on magnetic particles. Untargeted dereplication was initially carried out resulting in 46 annotated metabolites of which 33 were alkaloids[1]. For the AS-MS assay, 200 µg of *eel*AChE was immobilized on 10 mg of amino-functionalized magnetic beads through Schiff's base, using glutaraldehyde as the linker.[2] Control samples were prepared under identical conditions, with enzyme inactivation achieved using 100% MeOH (t = 60 min). Choline formation was monitored to assess enzymatic activity. The four stages of the assay were modulated using galantamine as the standard ligand: incubation, separation, ligand dissociation, and ligand identification by LC-HRMS. An affinity ratio (AR) of 1.3 was determined for galantamine. The affinity selection assay was thus carried out under these modulated conditions. Dissociated ligands were analyzed by LC-HRMS, and the data were processed using MZmine for ion alignment, deconvolution, and peak area calculation. The metabolites with AR > 1.3 were identified as AChE ligands and annotated as: (**1**) *lyaloside* (3.73), (**2**) *10-hydroxygeissoschizol* (3.69), (**3**) *camptothecin derivative* (2.51); (**4**) *5-carboxy strictosidine* (2.05); (**5**) *corynantheol* (2.04); (**6**) *isodolichantoside* (1.90); (**7**) *derivative* (1.68); (**8**) *strictosidine* (1.66); (**9**) *corynantheidal* (1.63); (**10**) *desoxycordifoline* (1.57); (**11**) *kaempferol-3-O-rhamnoside* (1.36); (**12**) *kaempferol* (1.35); (**13**) *topotecan derivative* (1.29); (**14**) *harman-3-carboxylic acid* (1.27). Among them, compounds (**1**), (**3**), (**7**), (**8**), (**10**-**12**), and (**14**) have already been described as AChE ligands in the literature. The metabolites (**4**), (**8**), (**10**) and (**11**) have been isolated and are currently undergoing orthogonal assays for structural confirmation and biochemical characterization.

[1]Cassas F, Santos CLG, da Silva FMA, Cass QB. Strychnos peckii B.L. Rob.: secondary metabolite annotation by liquid chromatography tandem high-resolution mass spectrometry with insights from biogenesis. Anal Bioanal Chem. 2025 Jul 1. doi: 10.1007/s00216-025-05967-0;

[2]Lima, Juliana M.; Leme, Gabriel M.; Costa, Emmanoel V.; Cass, Quezia B. LC-HRMS and acetylcholinesterase affinity assay as a workflow for profiling alkaloids in Annona salzmannii extract. Journal of Chromatography B-Analytical Technologies in The Biomedical and Life Sciences, v. 1164, p. 122493, 2021. doi. 10.1016/j.jchromb.2020.122493.

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