



NOVEL CYCLOTIDES FROM *NOISETTIA ORCHIDIFLORA* (RUDGE) (VIOLACEAE)

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Cyclotides are small disulfide-rich peptides that are characterized by a head-to-tail cyclized peptide backbone and a knotted arrangement of three conserved disulfide bonds. Their primary function in plants is thought to be as defense agents, based on their potent insecticidal activity, but they also have a range of other biological activities, including insecticidal, nematocidal, molluscicidal, antimicrobial, anti-HIV, and antitumor activities. They are present in many plants from Violaceae, Rubiaceae, Fabaceae and Cucurbitaceae families, despite this, the work involving Brazilian plants are scarce. To address these deficiencies, our group has been dedicated to study these class of interesting natural products in Brazilian plants and this work describes the isolation of three cyclotides from *Noisettia orchidiflora* (Rudge) collected on Atlantic Forest. For isolation, crude plant extract were prepared using MeOH:H₂O (6:4; v/v) solvent extraction overnight followed by filtration. After addition of CH₂Cl₂, the aqueous phase was separated and applied to solid-phase extraction C₁₈ cartridges. A peptidic fraction was collected and freeze-dried. Prior to the purification procedure, the tissues were analyses by MALDI-TOF, using reduction and alkylation reactions to confirm the presence of 6 cysteines. For MALDI-TOF was possible to find several masses in the range of 2500 to 4500 Da, indicative of cyclotides. Thus, this fraction were analyzed by preparative RP-HPLC using linear acetonitrile gradients and the pure compounds were sequencing by digestion with different enzymes (Glu-C, Trypsin and Chymotrypsin) and MS/MS. In conclusion, we characterized three cyclotides: two novel, Nor B and Nor C, with molecular mass of 3180 and 3444 Da respectively and the known circulin B, which has molecular mass 3282 Da.

References:

- [1] Daly, N.L., Rosengren, K.J., Craik, D.J. 2009. Discovery, structure and biological activities of cyclotides. *Advanced Drug Delivery Reviews*. 61: 918-930.
- [2] Burman, R., Gunasekera, S., Strömstedt, A.A., Göransson, U. 2014. Chemistry and biology of cyclotides: Circular plant peptides outside the box. *Journal of Natural Products*. 77: 724-736.