

A New Denfensin from Clitoria fairchildiana seeds

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INTRODUCTION. The expressive increase of mutant strains emerging as well antibiotic resistant bacteria aggravated infectious processes. New antimicrobial compounds are essential to novel antibiotics development. OBJECTIVE: To isolate and identify an antimicrobial peptide from sombreiro seeds (Clitoria fairchildiana), a species native to Brazil, founded in Cerrado biome, MATERIAL E METHODS: C. fairchildiana seeds were ground to fine flour, delipidated, and extracted in phosphate buffer, pH 7.6. The crude extract proteins were purified by Sephadex G-50 and C18-HPLC analytic column. The molecular weight of peptide of interest (CF-AMP) was determinate by 17% SDS-PAGE and MALDI-TOF techniques. The amino acid sequence of CF-AMP was obtained by Edman degradation method, and it was submitted to local alignment searching against the NCBInr using Blastp algorithm and the high identity sequence was identified as its correspondent isoforms. The theoretical three dimensional structure of CF-AMP was generated by Modeller 9v16 by through comparative modeling using 2GL1 (PDB ID) as the atomic coordinates template. Structural analysis of family conserved residues also exposed on CF-AMP was performed on PyMol 1.8. RESULTS AND DISCUSSION: In this work, one peptide lower than 6 kDa were purified and identified from sombreiro seeds by first using size-exclusion chromatography (SEC). The third, and last, SEC fraction counting small molecules was then fractioned by RP-HPLC, and CF-AMP fraction eluted at 30% acetonitrile showed molecular masses of 5156 Da. The complete sequence was determined showing 75% identity with a gamma thionin protein (or defensin) from Clitoria ternatea, translated from mRNA databanks. Defensins are well-known by its antimicrobial proprieties. Moreover, peptide net charge and hydrophobicity was in silico evaluated. Finally molecular modeling was also applied generating peptides three-dimensional structures, indicating a better explanation to probable mechanisms of action. CONCLUSION: CF-AMP here reported show remarkable potential to contribute in the development of novel antibiotics from natural sources.

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Key Words: Antimicrobial peptides, Plant Defensin, Protein Purification

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The post-translational modifications of SiAMP2 were evaluated by in silico experiment by several software and online prediction servers.