



## CYTOTOXIC ACTIVITY OF THE METHANOLIC EXTRACT AND FRACTIONS FROM *DIDEMNUM CINERACEUM* ASCIDIAN

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**Abstract:** *Didemnum cineraceum* belongs to Didemnidae family that includes 578 species and is the most chemically productive ascidian family with 375 known compounds<sup>1</sup>. The *Didemnum* genus has attracted the attention of researchers for interesting metabolites as alkaloids, lipids, peptides and macrolides<sup>2</sup>. *D. cineraceum* was collected in Ilhabela– SP litoral. The methanolic crude extract was obtained and submitted to the cyclic loading protocol using HP-20 resin. The extract was passed through the column resulting six fractions: A-100% H<sub>2</sub>O, B-20% Me<sub>2</sub>CO, C-40% Me<sub>2</sub>CO, D-60% Me<sub>2</sub>CO, E-80% Me<sub>2</sub>CO and F-100% Me<sub>2</sub>CO. The fractions were analyzed by HPLC-DAD, TLC and <sup>1</sup>H NMR. The methanolic extract and the obtained fractions were also evaluated against normal human lung fibroblasts (GM07492A). The D-60% Me<sub>2</sub>CO fraction (m= 110.2 mg) presented IC<sub>50</sub> 140.9 µg/mL in the cytotoxic activity and was selected for chemical study. The D-60% Me<sub>2</sub>CO fraction was cyclic loading again with HP-20ss resin using MeOH and H<sub>2</sub>O as mobile phase. Ten fractions were obtained. The fractions G-60% MeOH (m= 4.6 mg) and H-70% MeOH (m= 10.0 mg) were selected to <sup>1</sup>H and <sup>13</sup>C NMR experiments and MS analysis. The <sup>1</sup>H and <sup>13</sup>C NMR of the G-60% MeOH and H-70% MeOH fractions were similar. Regarding the mass spectrum of G-60% MeOH and H-70% MeOH fractions it was possible to note the presence of two main compounds with [M-H] 513.3159 and [M-H] 527.3317, respectively. The resonance data together with the HR-ESI/MS data suggest the presence of polyketides in these fractions. The Biselide A (C<sub>25</sub>H<sub>33</sub>ClO<sub>10</sub>), [M+Na]<sup>+</sup> 551.1660 is a representative compound of this chemical class that has been previously isolated from *Didemnum* sp. However further studies are underway in order to obtain pure substances and accomplish the structural elucidation.

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### References:

- [1] Schmidt, E.W., Donia, M.S., McIntosh, J.A., Fricke, W.F., and Ravel, J. 2012. Origin and Variation of Tunicate Secondary Metabolites. *J. Nat. Prod.* 75: 295-304.
- [2] Mohamed, G.A., Ibrahim, S.R.M., Badr, J.M., Youssef, D.T.A. 2014. Didemnaketals D and E, bioactive terpenoids from a Red Sea ascidian *Didemnum* species. *Tetrahedron.* 70: 35-40.